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AN OUTLINE OF THE MORPHOLOGY AND LIFE HISTORY OF *CRITHIDIA LEPTOCORIDIS*, SP. NOV.

BY

IRENE McCulloch
UNIVERSITY OF CALIFORNIA PUBLICATIONS

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I. INTRODUCTION

1. OCCURRENCE

The discovery of immense numbers of parasitic flagellates in the intestinal tract of the box-elder bug, *Leptocoris trivittatus*, so commonly found about the buildings on the Campus of the University of Kansas, led to a study of the structure and life-cycle of the parasite. This new flagellate now under investigation is here called *Crithidia leptocoridis*. The reasons for this will be deferred to a later
paper in connection with a discussion of the group, Hcrptomonas, Crithidia, and Trypanosoma.

The type is in the protozoological collections of the Zoological Laboratory of the University of California and a cotype has been deposited in the United States National Museum at Washington.

2. Importance

The importance of such a study is clear when it is taken into consideration that a thorough investigation of the life-cycle of Crithidia will be essential in order to explain the complete life-cycle of Trypanosoma. The material of Crithidia leptocoridis is invaluable for such a study. In the first place, the box-elder bugs can be obtained readily throughout the year and the mature insects show a hundred per cent infection; secondly, multiple fission forms of this parasite, which may be intracellular, have been found here, indicating that the life-cycle of Crithidia leptocoridis is very similar to that of Trypanosoma lewisi recently described by Minchin and Thomson (1915); thirdly, the crithdial stages of Schizotrypanum cruzi as figured by Chagas (1909) are almost identical with certain stages in the life-cycle of Crithidia leptocoridis.

3. Methods

The material used in this investigation includes the living material, stained smears and sections, cross and longitudinal, of the entire bug and of the digestive tract.

For the study of the living material, the intestinal tract was removed in one of two ways. For ordinary work the tip of the abdomen was clipped off, the contents pushed out with a needle upon a slide and covered with a drop of Ringer's or normal salt solution. For more careful work the digestive tract was dissected out in Ringer's solution and then transferred to a slide. By pressing down the cover slip firmly and examining under a microscope, the parasites could be seen in situ through the intestinal wall.

In determining the localization of the parasites, the digestive tract was cut carefully into its several parts, the stomach, mid-intestine, ilium, colon, and rectum. Each part was then teased out with needles and mounted under a cover glass. Such preparations were frequently sealed with vaseline and studied for a period of five or six days. More permanent cultures were prepared by making a hanging-drop culture.
on a cover glass and sealing the cover glass over a hollow side with vaseline. However, bacterial infection in such cultures prevented a successful study for any length of time.

For about one-half of the preparations observations of the living material were made and recorded, then the material on both cover glass and slide was fixed and stained. Frequently the smear on the cover slip was stained with iron haematoxylin and the smear on the slide with Giemsa, making a total of eight or ten preparations from a single bug. Other preparations were fixed at once without allowing any time for exposure, in hot Schaudinn's fluid and stained with iron haematoxylin. Dobell's iron haematein method was also of great value.

4. Acknowledgments

To Miss Nadine Nowlin, Assistant Professor of Zoology, University of Kansas, through whose efforts and assistance I was able to undertake this work, I wish to acknowledge my deepest gratitude, and to Dr. C. A. Kofoid, Professor of Zoology, University of California, I wish to express my sincere thanks for many helpful suggestions and much valuable criticism during the past two years.

I wish also to take this opportunity to publicly thank the several people who kindly collected box-elder bugs for me in different parts of the country.

II. Morphology

In the vegetative stage (pl. 2, figs. 54–56; pl. 3, figs. 57–62 and text fig. A) *Crithidia leptocoridis* is a flagellate with a relatively long, slightly flattened body, tapering gradually both anteriorly and posteriorly to fine points. In the central part of the hyaline body there is a large vesicular nucleus connected directly with the extranuclear organelles, the rhizoplast, "kinetonneucleus," flagellum, basal granule and the "axostyle." (text fig. A).

The cytoplasm of this vegetative form is very hyaline and does not stain uniformly. Around the "kinetonneucleus" (pl. 3, figs. 57–60) there is a light-staining area which extends forwards along the flagellum (pl. 2, fig. 55). The crithidial forms of *Schizotrypanum cruzi* (Chagas, 1909) show a similar area. Patton also figures such a region in *Crithidia gerridis* (1908). Among the herpetomonads this area is still more prominent. In *Herpetomonas luciliae* Strickland (1911) and in *Herpetomonas muscae domesticae* Prowazek (1904)
and Wenyon (1913) call this region the cytopharynx. Wenyon describes an additional structure, a slit-like groove leading into the so-called cytopharynx; this he calls a cytostome, believing that bacteria pass through the cytostome and cytopharynx into the endoplasm of the posterior part of the body. Such a structure and function has not been noted in the study of *Crithidia leptocoridis*.

The nucleus (trophonucleus) of the flagellate of the vegetative phase has a diameter from 1 to 1.5 μ, almost equal to the width (pl. 3, figs. 58–62) of the body and lies in a central position or just above the center in the anterior half of the organism (pl. 3, fig. 62). A distinct nuclear membrane (pl. 3, figs. 57, 59, and 62) is always present. The distribution of the chromatin material within the nuclear membrane is subject to much variation in the elongate flagellates of the vegetative phase. Probably the most characteristic disposal of the chromatin is in the form of a single central granule or karyosome (pl. 2, figs. 54–56; pl. 3, figs. 57, 58). As seen in plate 3, figures 60 and 62, the chromatin is in three elongate granules. Patton (1908) and Porter (1909) found the chromatin in some forms of *Crithidia gerridis* in eight chromatin granules or chromosomes. This will be discussed in more detail later in connection with longitudinal division.

The extranuclear organelles, as previously mentioned, consist of the “kinetomemucleus,” rhizoplast, flagellum, basal granule, and the “axostyle” (text fig. A). The “kinetomemucleus” has always been found anterior to the nucleus. In the vegetative phase the distance between the nucleus and the “kinetomemucleus” varies from 1.5 μ (pl. 2, figs. 54 and 55) to 5 μ (pl. 3, figs. 57–62). In stained preparations the “kinetomemucleus” presents an exceedingly clear-cut outline, since it stains deeply and lies in an area of lightly stained cytoplasm (pl. 2, fig. 55). It is made up of two relatively large chromatin granules lying in close proximity (pl. 2, figs. 54–56). Looking from a certain angle these two chromatin granules have the appearance of a single

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**Fig. A.** Lateral view of *Crithidia leptocoridis* to show the nucleus and extranuclear organelles. X 1800. *ax.*, axostyle; *bas. gr.*, basal granule; *chr. gr.*, chromatin granule; *fl.*, flagellum; *n.*, nucleus; *rh.*, rhizoplast; *und. m.*, undulating membrane; *vac.*, vacuole-like area about the “kinetomemucleus.”
chromatin bar lying in a transverse position with respect to the long axis of the body. At still another angle the "kinetonucleus" may present the appearance of a single round chromatin mass. There is a definite connection between the "kinetonucleus" and the basal granule at the base of the flagellum in the form of numerous fine unstained fibers which form a cone-shaped structure at the base of the flagellum (pl. 2, fig. 54). Chagas (1909, pl. 13, figs. 13-18) figures a similar structure in the crithidial stages of *Schizotrypanum cruzi*. Since these fibers do not stain with Giemsa or iron haematoxylin it was some time before the connection was observed.

At the apex of these fibers there is a very small basal granule (pl. 3, figs. 57-62) or a slight enlargement of the base of the flagellum. In many instances it is exceedingly difficult to demonstrate the presence of this structure.

The rhizoplast is a faint line connecting the "kinetonucleus" with the nucleus. In the vegetative forms this line does not usually pass from the central karyosome of the nucleus to the "kinetonucleus," as seen in plate 2, figure 54. In plate 2, figure 55, the rhizoplast can be traced from the nuclear membrane of the nucleus to the chromatin mass of the "kinetonucleus." There is still another method of attachment on the part of the rhizoplast; it may come from the basal granule and pass around the side of the "kinetonucleus" (pl. 3, figs. 57, 60).

An "axostyle" is present in *Crithidia leptocoridis* apparently in the form of a chromatic thread extending from the basal granule to the posterior end of the body, where it may terminate in a small chromatin granule (pl. 3, figs. 58-59). This "axostyle" is most readily seen in the living flagellates of the vegetative phase. Such stains as Giemsa and iron haematoxylin show it. A certain per cent of the flagellates in the prepared material show an additional structure parallel with the "axostyle" line in the form of an extension of the light or unstained area about the "kinetonucleus" to the posterior end of the body (pl. 4, fig. 75). Mackinnon (1910), Wenyon (1913), and other investigators have described such a structure in *Herpetomonas muscae domesticae*. In *Crithidia leptocoridis* in a few instances it has the appearance of a "longitudinal canal" outlined on the one side by the "axostyle" thread and on the other by a similar thread, less distinct (pl. 4, fig. 75), which connects with the flagellum. The relation of this "axostyle" to the rhizoplast in this form is not clear. If this be an axostyle, the homology with respect to certain structures in
other flagellates is very uncertain. In a few instances there is a distinct rhizoplast in addition to the "axostyle" connecting the "kinetonneuleus" with the nucleus (pl. 38, fig. 58b); in the larger number of these long flagellates, however, there is only one line. In this case there is a possible fusion of the rhizoplast and "axostyle" as far as the nucleus and the axostyle extends thence from the nucleus to the posterior end of the body. Occasionally there are other lines (text fig. A; pl. 2, figs. 52, 54), possibly myonemic fibers in the periplast of the body, which show distinctly in iron haematoxylin preparations. These lines are connected directly with the flagellum (pl. 2, figs. 52, 55).

The flagellum extends forwards as a single heavy line from the basal granule along the anterior projection of protoplasm for about one-half of its length in the flagellates of the vegetative phase (pl. 3, figs. 57-62). This flagellum is readily stained with Giemsa and iron haematoxylin.

The flagellum is in the margin of the undulating membrane, which shows no specialized structure in the elongate flagellates found in the rectum. It is merely a prolongation of the protoplasm and plays a very small part in the movement of the organism.

III. MOVEMENT

The characteristic, vigorous movement of the elongate flagellates indicates at once their presence in any living material. There are flashes of indistinct forms and a big disturbance of the cellular debris in the field. When the flagellates become more quiet it is observed that the flagellum, body, and undulating membrane all assist in progression. There is a very rapid vibration of the rigid body from side to side, combined with the spiral movements of the flagellate end of the body either to the right or to the left. Then an instant follows in which the spiral movement alone is in evidence. An instant later the flagellate end whips around and the organism darts off in another direction. In comparison with Crithidia gerridis and several other species, Crithidia leptocoridis has a very rigid body. This rigidity is noticeable in the movements. On several occasions, an intestinal preparation of living material showed the rigid flagellates which had escaped from the colon in the act of boring their way into the Malpighian tubules at certain points. Many had penetrated at least one-third of the body-length. By careful focusing it was determined that the parasites had actually bored their way thus far.
IV. LONGITUDINAL DIVISION

In the vegetative phase of the life-cycle the method of reproduction is longitudinal binary fission. A study of the living flagellates shows that the first indication of such a process is a slight increase in the width of the elongate forms together with the appearance of two flagella and a subsequent splitting of the protoplasm along the median plane beginning at the anterior end. The stained preparations tend to show that the "'kinetonucleus'" (pl. 3, fig. 70) divides first. In a few instances there is evidence that the division of the nucleus precedes that of the "'kinetonucleus'" (pl. 3, fig. 69). Normally the basal granule, flagellum, and "'kinetonucleus'" divide simultaneously. The splitting of the flagellum proceeds anteriorly while the rhizoplast and axostyle split posteriorly (pl. 3, fig. 69). Along with the above processes the division of the nucleus to form two daughter nuclei takes place. There is some evidence that at least a promitotic or possibly a mitotic process is present in such a division. As has already been pointed out, plate 3, figures 60 and 62, show three elongate chromatin granules. On the same plate, figures 64, 65 and 66, there are spindle-like formations with chromosomic granules not unlike the structures found in the mitotic process of trichomonad flagellates (Kofoid and Swezy, 1915). On the other hand, no centrosome structure has been demonstrated at the poles of the spindle referred to above in plate 3, figures 64, 65 and 66, as found in the trichomonad flagellates. Further investigation may yet reveal such a structure, but since such structures in *Crithidia leptocoridis* are so small it becomes very difficult to interpret them with any degree of accuracy. In regard to the number of the chromosome-like granules, no general conclusion can be made as yet; however, there are some indications that the number is four large or eight small ones (pl. 3, figs. 64–68). If a chromatin granule is present at the posterior end of the axostyle there is likewise a constriction of it into two parts when the axostyle divides.

The question whether the flagellum splits in *Crithidia leptocoridis* to form two as found by Porter (1910) in *Crithidia melophagia*, or whether there is a new outgrowth from the basal granule as described for *Herpetomonas muscae domesticae* by Wenyon (1913), can not be answered definitely as yet. Observations of the living material would go to show that it is a process of splitting rather than a new outgrowth. The stained preparations also tend to confirm this.
The dividing flagellate moves rapidly and vigorously throughout the process of division. As soon as the two flagella are present the longitudinal division of the protoplasm is hastened by the antagonistic movements on the part of the two daughter individuals.

V. LIFE-HISTORY

The life-history of Crithidia leptocoridis as outlined in plates 1-4 has been based largely upon a study of the living material supplemented by stained preparations. However, the interpretation of the life-history of such a parasitic flagellate is subject to much error since it is largely a matter of conjecture owing to the fact that continuous development can not be followed step by step. It must also be considered, in the first place, that some of the parasites may be degenerating forms, secondly that fixation and staining have distorted the structure, and thirdly that the life-cycle of several parasites could be readily confused.

For these reasons it is with some hesitation that a great number of doubtful forms have been included in this paper, namely, two different types of preflagellates and the so-called multiple fission forms of the life-cycle. Fragmentary references and figures in the papers of prior investigators to such doubtful forms have occurred, as will be pointed out in a later paper.

At the present time it seems best to follow the old system of nomenclature for the several stages of the crithidial life cycle, namely, preflagellate, flagellate and postflagellate. The terms stomach and rectal phase (Minchin and Thomson, 1915), together with the term vegetative phase would seem also to be appropriate in the light of the present investigations of this flagellate.

The life-cycle of Crithidia leptocoridis is divided up into three stages: the preflagellate (pl. 1, figs. 1-28; pl. 2, figs. 24-33) which includes the stomach phase of the life-cycle; the flagellate (pl. 2, figs. 34-56; pl. 3, figs. 57-73), which includes the flagellates in the vegetative phase as found in the mid-intestine, ilium and colon; and the postflagellate (pl. 4, figs. 74-108) which includes the rectal forms.

2. Preflagellate (Stomach Phase)

The preflagellate forms (pl. 1, figs. 1-28; pl. 2, figs. 24-53) are found in the stomach of young nymphs. Immediately after the ingestion of the spores, which are small, round, or oval forms from 1.5
to 2 μ in diameter (pl. 1, fig. 1), there is obviously a period of rapid growth. The spores are well protected by a thick, light brown membrane and it is only with difficulty that the nuclear structure can be observed in the stained material. There is some evidence, very slight to be sure, that two developmental forms occur in *Crithidia leptocoridis*, one a non-flagellated form (pl. 1, figs. 4–7) enclosed in a capsule-like structure, and a flagellated form which develops from the small, round spores (pl. 1, figs. 1–2).

The non-flagellated forms (pl. 1, figs. 3, 7, 10, 14b, 15b) are readily destained. The nucleus is usually found in the posterior part of the body. The chromatin stains deeply and may be in the form of one or more granules (pl. 1, figs. 7, 10, 16b, 21b). From the central chromatin mass there is a network extending out to the nuclear membrane (pl. 1, fig. 6) in the form of radiating fibers. This is a very characteristic structure and often serves as a means of identification of *Crithidia leptocoridis*.

These non-flagellated forms do not show all the extranuclear organelles found in the mature flagellates. The "kinetosome" is not clearly shown in the early stages. Later two chromatin granules lying in close proximity are present. The rhizoplast is readily distinguished in some forms (pl. 1, figs. 17b and 19b) in which it passes forward from the nucleus to the kinetosome and thence out to the anterior end of the body. The presence of an "axostyle" has not been observed as yet in these forms.

The flagellated forms come from the oval spore forms (pl. 1, figs. 1, 2, 8, 9, and 11). These are at once characterized as small forms vigorously rotating about by means of a well-developed flagellum and undulating membrane (pl. 1, fig. 11). Like the non-flagellated forms of the preflagellate stage, they occur in the stomach of the young nymphs and the infection is usually light. The cytoplasm shifts and in a short time there is formed a pear-shaped organism. These forms are not unlike some of the "Initialformen" figured (pl. 11, fig. 48) by Chagas (1909) in the life-cycle of *Schizotrypanum cruzi*. The comparison might be carried still further, pointing out the presence of two types among his "Initialformen" (pl. 11, figs. 49–60) found in the mid-intestine, a flagellated, and a non-flagellated form.

The nuclear structure of the flagellated forms is essentially the same as that of the non-flagellated forms. The nucleus is relatively large and is located in the extreme posterior end of the body at first. Later it becomes more central. Here again the chromatin material is
found in a single central or in several irregular granules (pl. 1, figs. 11–16). The nuclear membrane is always present and the nuclear network within can usually be demonstrated. The kinetosome stands out very prominently as compared with that of the non-flagellated forms.

In connection with the preflagellate stage some interesting forms have been found (pl. 1, figs. 14–24) wherein there is an elongate flagellate bearing a small, round, non-flagellated form near the end of the flagellum. No explanation of these forms can be made as yet, but it is strongly suggestive of sexual reproduction. Careful observation of the living material does not indicate that we have here a process of unequal longitudinal division. A parallel case has been observed in the living material of *Herpetomonas muscae domesticae*. Patton (1908) also shows elongate flagellates with several smaller oval forms in a similar position (pl. 1, figs. 9–10) with the explanation that the figures are a result of the way in which multiplication has taken place.

In addition to the method of movement described for the elongate flagellates, a flagellate such as is seen in plate 1, fig. 13, progresses with a serpentine movement with the posterior end directed forwards. Such a flagellate has been observed to bore its way into cellular masses in the living material, the posterior end entering first.

Following the appearance of the preflagellate forms just described, there are great masses of Leishmania-like forms found in the lumen of the stomach. The evidence points to the fact that the flagellates described above undergo a multiple fission process, either intracellular or extracellular. Direct evidence of the intracellular forms has not yet been found in sections of the epithelial lining of the stomach, but the so-called tailed and tailless spheres (Minchin and Thomson, 1915) have been found in cellular masses on smear preparations. As seen on plate 1, figure 26, there is a flagellate rounding up to form a tailed sphere. The tailed spheres have been studied in the living material also. The movement is slow. The flagellum of the parent lashes about feebly while within there are found a variable number of almost mature flagellates wriggling about. Whether the organisms in plate 2, figures 29–32, can be interpreted as tailless spheres is somewhat doubtful, but this is the most plausible explanation. Upon development they seem to give rise to a large plasmodial mass in which are the small merozoites (pl. 2, fig. 37). Frequently a large number of the small multiple fission forms, or merozoites, are found free from the plasmodium (pl. 2, figs. 38–45). On the one hand, development
may take place within the plasmodial mass. If a plasmodial mass remains intact until the flagellates reach maturity, this might be one explanation of the aggregation rosettes found in *Crithidia leptocoridis*. On the other hand, such an aggregation rosette can be looked upon as an epithelial cell which, upon being separated from the rest, rounded up and the great mass of flagellates still remain attached as when it was a part of the lining of the digestive tract.

In these multiple fission forms or merozoites the nucleus is always present in the extreme posterior end and the "kinetosome" lies a little anterior within the characteristic light area. The flagellum is present, being coiled back upon the body to which it adheres for some time. Later the cytoplasm parts and the flagellum straightens out. At first, there are slow contractions of the body, but the flagellum soon becomes more active and the organism darts off across the field after many ineffectual efforts. The rhizoplast can be demonstrated without difficulty in these forms, but there is no additional structure which might be looked upon as an "axostyle." Among these merozoites longitudinal division probably occurs as seen in plate 2, figures 28 and 46.

3. The Flagellate Stage (Vegetative Phase)

The flagellates arising from the multiple fission forms and likewise from preflagellate forms which do not undergo the multiple fission process, migrate posteriorly into the mid-intestine, ilium, colon, and rectum. These are the flagellates of the vegetative phase, and since their structure has already been described only some of the important variations in size will be taken up here.

The flagellates vary in length from 20 to 40 μ and from 1.5 to 3 in width (pl. 2, figs. 54, 56; pl. 3, figs. 57–62). The extremely long forms (pl. 3, figs. 60–62) are always found in the ilium and colon. In a few instances these have been found attached to the wall of the colon for a time.

4. Postflagellate Stage (Rectal Phase)

The postflagellate forms are found in the rectal region. Soon after the migration of the flagellates posteriorly to the mid-intestine, ilium and colon, there are a few which make their way at once into the rectum. As the insects become mature the rectum is apt to be lined with attached flagellates and the lumen filled with a different type of the parasites, the free forms.
The attached forms (pl. 4, figs. 93-99) are flagellates which upon becoming attached to the wall of the rectum undergo rapid longitudinal division until the whole rectal region is lined with a compact layer of parasites. Longitudinal sections of the rectal region show that while the upper part is lined with elongate flagellates (pl. 2, figs. 54-55) the lower, more posterior part is covered with small oval forms (pl. 4, figs. 93-99). The small oval forms show certain changes. The length of the body decreases, while the width increases, and the slightly flattened body becomes round. Both ends of the body become blunt (pl. 4, fig. 99) and the undulating membrane tends to disappear (pl. 4, fig. 97). The change in size at this time is evidently due to a process of binary fission rather than to any shrinking on the part of the organism. Along with the changes taking place in the external form, there are changes going on in the nucleus and extranuclear organelles. The nucleus is relatively much larger (pl. 4, fig. 97) in respect to the size of the body than in the elongate flagellate stage. The vesicular character becomes more pronounced (pl. 4, figs. 94, 95) and the chromatin in the form of karyosomes which vary in size and staining capacity. The kinetonucleus comes to lie close to the nucleus (pl. 4, fig. 95) but shows no tendency to migrate posterior to the nucleus. There is no evidence of an "axostyle" present, but the rhizoplast can be readily observed in these forms as a faint line connecting the nucleus directly with the kinetonucleus (pl. 4, fig. 96).

The free forms (pl. 4, figs. 74-86) in the rectum are of great interest because of their varied size and shape. The width of the body increases at the expense of the length, as seen in plate 4, figures 74-86. Some forms become almost spherical (pl. 4, figs. 85-92). The movement of such forms is very slow. The undulating membrane is very much in evidence (pl. 4, figs. 77, 78-81) in connection with the spiral movements of the flagellate and in those to the right or to the left. Such a flagellate as that seen in plate 4, figure 81, is similar to Trypanosoma rotatorium in movement. The broad, flat forms as seen on plate 4, figures 77 and 78, are not due to drying in the process of fixing and staining. They are found in the living material. It is possible that these forms may be degenerate ones or that they may be rounding up to form spheres in preparation for multiple fission. In support of the last hypothesis merozoites have been found in the rectal region.

The nucleus of the free forms of the rectal phase is also relatively large (pl. 4, figs. 74 and 78). The chromatin frequently indicates
degeneration by staining very lightly in comparison with that of the "kinetonucleus." The "kinetonucleus" retains its normal size and lies near to the nucleus. It is in such a parasite as that seen in plate 4, figure 74, that the clear, threadlike fibers which connect the "kinetonucleus" with the basal granule can be most readily demonstrated. The rhizoplast is very distinct owing to the tendency of these large forms to become very vacuolate (pl. 4, figs. 85, 86 and 89). The axostyle also can be seen readily in such forms as shown on plate 4, figures 74 and 81.

Under certain conditions the attached forms in the rectum have been observed to become free from the rectal wall and to begin encystment at once. The flagellum coils about the body and coalesces with it (pl. 4, figs. 85, 87, 89 and 91). A reduction in size of the encysting form follows (pl. 4, figs. 102-104). Only one nuclear mass becomes visible (pl. 4, figs. 106-107) and a thick protective covering forms about the spore (pl. 4, fig. 108). The spores thus formed pass out with the excreta in great numbers.

VI. RELATION OF PARASITE TO HOST

The box-elder bugs are without doubt casually infected by means of spores or moist excreta taken up with the food. The exact conditions under which these insects become infected are not definitely known as yet. The box-elder bugs are vegetable feeders, sucking the juice of the box-elder and maple trees and the fruit of the raspberry. On a few occasions, under laboratory conditions of extreme hunger, several of the more vigorous insects were observed to suck the contents from the digestive tract of another insect in a weakened condition. This was not observed to happen in the presence of an abundance of food.

Investigations were carried on to determine whether hereditary infection occurs in the box-elder bug, Leptocoridis trivittatus. The ovaries were examined in both the living and sectioned material, and negative results were obtained in every instance. Many nymphs were examined. A majority of the wild nymphs showed infection in the stomach. The percentage of infection increased with the age of the host until mature insects showed 100 per cent infection. Such an infection is also widespread; in these investigations infected insects were obtained from Kansas, California, Washington, Utah and New Mexico. Numerous eggs were obtained and hatched in the laboratory.
All of the laboratory nymphs examined were free from parasites, but it was impossible to keep the laboratory nymphs alive long enough to get conclusive evidence. The contents of eggs were examined but no parasites were found.

The regions of infection are the stomach, mid-intestine, ilium, colon, and rectum. Examination of the salivary glands, Malpighian tubules, reproductive organs and body cavity of the box-elder bug for Crithidia have always yielded negative results.

VIII. LITERATURE CITED

CHAGAS, C.
1909. Ueber eine neue Trypanosomiasis des Menschen. Mem. Inst. Oswaldo Cruz, 1, 159-218, pls. 9-13, 10 figs. in text.

KOFORD, C. A., and SWEEZY, O.

MACKINNON, D. L.
1910. Herpetomonas from the alimentary tract of certain dung-flies. Parasitology, 3, 255-274, pl. 19, 4 figs. in text.

MINCHIN, E. A., and THOMSON, J. D.

PATTON, W. S.

PORTER, A.
1909. The morphology and life-history of Crithidia gerridis, as found in the British waterbug, Gerris paludum. Parasitology, 2, 348-366, pl. 4.

PROWAZEK, S.

STRICKLAND, C.
1911. Description of a Herpetomonas parasitic in the alimentary tract of a common green bottle fly, Lucilia sp. Parasitology, 4, 222-236, pls. 8-9, 2 figs. in text.

WENTON, C. M.

Transmitted May 21, 1915.

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University of California.
All figures were outlined with a camera lucida, using 1/16 Leitz objective and Zeiss ocular 12. The magnification is in all cases approximately 2880. Unless otherwise stated, all figures in the plates were drawn from preparations fixed with hot Schaudinn’s fluid and stained in iron haematoxylin.

EXPLANATION OF PLATE 1

_Crithidia leptocoridis_ sp. nov., preflagellate stages

Fig. 1. Spore forms from the stomach with only one nuclear mass showing.

Fig. 2. Developing spores from stomach, nuclear structure indefinite, but probably two chromatin masses present.

Figs. 3-7. Non-flagellates of the stomach phase in several stages of development. Nucleus and kinetonucleus are shown clearly in figure 7. Capsule-like covering is still present in figures 6 and 7.

Figs. 8-9. Flagellated forms of the stomach phase. The flagellum first appears, then the undulating membrane. The nuclear structure is not clear until further development takes place.

Fig. 10. Non-flagellated form of the stomach phase, showing the nucleus, "kinetonucleus," rhizoplast and basal granule.

Figs. 11-13. Flagellated forms of the stomach phase developing into pear-shaped organisms which move with posterior end directed forwards and enter cellular masses in this way. They show the nucleus, "kinetonucleus," rhizoplast, basal granule, flagellum and undulating membrane.

Figs. 14-24. Preflagellate forms found in the stomach. Peculiar relation of two flagellates which cannot be explained as yet, but it is strongly suggestive of sexual reproduction and apparently not due to a process of division or an "accidental attachment" of the two forms.

Figs. 25-26. Flagellates in the stomach preparing to round up to form tailed spheres. Large vesicular nuclei and well developed "kinetonuclei" are present.

Figs. 27-28. Flagellates rounding up to form spheres in the stomach. These are extracellular forms.
EXPLANATION OF PLATE 2

**Crithidia leptocoridis** sp. nov., preflagellate stages

Figs. 29-32. Multiple fission forms of the stomach phase which are intracellular apparently and show the probable origin of the merozoites.

Fig. 33. An early plasmodial form in the stomach.

Figs. 34-36. Plasmodial forms containing very small merozoites. Each merozoite shows the nucleus in posterior end and the "kinetonucleus" in the anterior end.

Fig. 37. An unusually large plasmodial form from the stomach. Not all of the merozoites are the same size. The more mature ones show the vesicular nucleus in the posterior end and the "kinetonucleus" surrounded by a light area in the anterior end. The rhizoplast can also be seen in the larger merozoites.

Fig. 38. A merozoite which has escaped from plasmodium and has undergone a rapid longitudinal division apparently.

Figs. 39-45. Merozoites in different stages of development. With one exception all these show both the nucleus, "kinetonucleus," rhizoplast and the characteristic light area about the "kinetonucleus."

Fig. 46. A peculiar structure which is seemingly due to rapid longitudinal division and a subsequent failure on the part of the resulting forms to separate at once.

Figs. 47-51. A series of flagellates from stomach showing the flagellum present which is just beginning to separate from body proper and to straighten out. Figure 50 shows the flagellum bent back upon the body almost the entire length. An "axostyle" is present in figure 51.

Fig. 52. Almost mature flagellate from stomach. Nucleus with karyosome, rhizoplast, "axostyle" and a myonemic fiber connected with the flagellum.

Fig. 53. Almost mature flagellate from stomach. Nucleus with three large chromosome-like granules. "Axostyle" present with several chromidia in close proximity.

Fig. 54. A more elongate flagellate, network in nucleus together with a central karyosome, rhizoplast and "axostyle." A myonemic fiber lateral to "kinetonucleus" connected with the flagellum.

Fig. 55. Mature flagellate from mid-intestine. Light area about the nucleus extended forward along flagellum and posterior to the nucleus, myonemic lines outline this area. "Axostyle" present.

Fig. 56. Mature flagellate from mid-intestine. "Axostyle" has a wavy appearance and this is an edge view of the parasite.
EXPLANATION OF PLATE 3

*Crithidia leptocoridis* sp. nov., flagellate stages

Fig. 57. Elongate flagellate from the colon. No direct rhizoplast present but a fusion of rhizoplast and "axostyle" from base of flagellum to nuclear membrane.

Figs. 58 and 58b. Elongate flagellates from colon, vegetative phase. Prominent "axostyle" with chromidia present. The attachment of rhizoplast and axostyle the same as in figure 57. In 58b there is an extension of clear area about "kinetonucleus" to the nucleus.

Fig. 59. An elongate flagellate from colon. Body somewhat wider. Two chromosomes present in posterior end. Chromatin of nucleus stained lightly.

Figs. 60 and 62. Extremely long, thin flagellates of colon. Three elongate granules present in nucleus. Edge view of body showing the "axostyle" clearly.

Fig. 61. Another long flagellate of colon, showing a chromidial granule in posterior part of the body.

Fig. 63. Early longitudinal division stage. The "kinetonucleus" has divided but the nucleus shows no constriction yet.

Fig. 64. Early longitudinal division stage, "kinetonucleus" already divided with a spindle-like formation within nuclear membrane of the nucleus.

Fig. 65. Division stage showing splitting of the rhizoplast and "axostyle." A spindle-like structure is present within the nuclear membrane. Five chromosome-like bodies are present, three large and two small ones.

Figs. 66-68. Division forms, showing some irregular structures in connection with the nuclei. In each there are four large chromosome-like granules.

Fig. 69. A division form from the colon showing that nucleus has divided before the "kinetonucleus." A chromatin granule is present at the base of the "axostyle" and it is about to divide. The light area in the center indicates the plane of division.

Figs. 70-71. Division forms. Figure 70 shows a granule at the base of the "axostyle" which has divided also.

Fig. 72. More advanced division form. Cytoplasm dividing longitudinally in a median plane. Chromatin in irregular granules.

Fig. 73. Advanced division form. "Axostyle" could not be traced posterior to the chromatin granule. Chromatin material in the nucleus in irregular masses.
EXPLANATION OF PLATE 4

*Crithidia leptocoridis* sp. nov., postflagellate stages

Fig. 74. Free rectal form, showing great width of body, nuclear network and chromosome-like granules in the nucleus, rhizoplast, and a myonemal fiber running along the nuclear membrane, thence to flagellum.

Fig. 75. Free rectal form, showing a so-called longitudinal canal, a rhizoplast and "axostyle" are both present. There is also a chromatin granule at the base of the "axostyle."

Fig. 76. Free rectal form preparing for division. Nucleus shows two chromatin masses and a spindle-like structure, within the nuclear membrane.

Figs. 77-79. Large, spherical, rectal forms with vesicular nucleus. In figures 77 and 78 the chromatin material stains lightly, indicating possibly a degenerate form.

Fig. 80. A free rectal form undergoing division. Cytoplasm is vacuolated.

Fig. 81. A free rectal form. Nucleus is elongated, posterior end of body is very blunt and the "axostyle" line extends around the posterior end.

Figs. 82-86. Free rectal forms. Figure 83 shows many small granules within the nucleus. All these forms show the vesicular nucleus with a rhizoplast connecting the nucleus and the "kinetomucleus."

Figs. 87-90. Free rectal forms preparing for encystment. The flagellum is coalescing with the body and later disappearing. A shrinking of these forms also shown here.

Figs. 91-92. Free rectal forms, showing the flagellum almost entirely absorbed, especially in figure 92.

Figs. 93-97. Attached flagellates of the lower part of the rectum. Reduced size due to continued longitudinal division and to a change in shape. Undulating membrane almost absent. Rhizoplast clearly shown but "axostyle" can not be demonstrated. Nucleus relatively large.

Figs. 98-99. Attached rectal forms which have become free just prior to encystment.

Figs. 100-104. Free rectal forms encysting. The rhizoplast is still present and flagellum is rapidly disappearing. The nucleus is relatively large and vesicular.

Figs. 105-108. Final stages in spore formation in rectum. Spores have a thick protective covering and apparently a single chromatin mass in the center.
ON GIARDIA MICROTI SP. NOV., FROM THE MEADOW MOUSE

BY

CHARLES ATWOOD KOFOID AND ELIZABETH BOHN CHRISTIANSEN

ON BINARY AND MULTIPLE FISSION IN GIARDIA MURIS (GRASSI)

BY

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ON GIARDIA MICROTI SP. NOV., FROM THE MEADOW MOUSE

BY CHARLES ATWOOD KOFOID AND ELIZABETH BOHN CHRISTIANSEN

The systematic examination of a series of rodent hosts for the detection of Giardia muris (Grassi) has brought to light in Microtus californicus californicus (Peale) a species differing in appearance, proportions, and stainability from the widely prevalent G. muris, and not possessing the characteristics of any known species of the genus. The hosts were taken in traps set in Strawberry Cañon on the University campus. Of the six hosts examined three only were infected with Giardia, two males and one female, the parasites occurring in the duodenum, ilium, and colon, and in one instance in the rectum also. This species was not detected in 98 culture mice, both white and gray, in 42 culture rats, in 12 Belgian hares, in 59 Peromyscus maniculatus gambeli (Baird) trapped in the same general region as the host Microtus, nor in two coyotes (Canis ochropus ochropus Escholtz) trapped in the same cañon with Microtus and presumably feeding upon them and often tearing open or carrying off our traps. Nor was G. muris found in any of the six Microtus examined.

They cause an inflation of the intestine, whose walls become thin and flaccid, and assume a yellowish-orange color in the infected region. An abundance of material was secured in various stages of binary and multiple fission and in encystment, including the so-called "copulation cysts," and cysts in which multiple fission had produced a 16-nucleate plasmodium or somatella.

The material has been prepared by the wet method from smears well rubbed out from the intestinal epithelium, fixed in hot Schaudinn's fluid, and stained in iron haematoxylin.
The type and eotypes are deposited in the collections of the protozoological laboratory of the Department of Zoology of the University of California. One eotype slide is deposited in the United States National Museum.

**Giardia microti** sp. nov.

*Diagnosis.*—Length of body 1.83–2.47 transdiameters, widest 0.25–0.35 of total length from anterior margin, slender tapering posteriorly, parabasals slender, curved awl-shaped, usually oblique, 0.5 transdiameter in length. Length 10–16 μ, transdiameter, 5–7 μ. Host, *Microtus californicus californicus* (Peale), in intestine from duodenum to rectum.

*Description.*—Body elongate pyriform in outline in dorsal or ventral view, with semicircular anterior outline, widest 0.25–0.35 of total length from the anterior end towards the posterior margin of the cytostome. The region posterior to this is tapering conical 32°–55° with slightly concave, rarely convex sides. The cytosome (*cyt.*, fig. A) occupies the anterior 0.3–0.5 of the flattened ventral surface and follows closely the anterior outline of the body somewhat more than in *G. muris*.

The organism is a binucleate somatella of usual *Giardia* type. The two nuclei (*nuc.*, fig. 1) are ellipsoidal in the prophase, 2–2.5 μ in length.

*Giardia microti* has the same extranuclear organelles as other species of the genus. The neuromotor apparatus consists of a rhizoplast passing from the karyosome (*intranuc. rhiz.*) through the centrosome (*cent.*) at the anterior pole of the nucleus, thence to the blepharoplast (*l. bleph.*) of its side of the axostyle. From, or near the blepharoplasts arise all of the flagella. Of these there are three pairs, bilaterally arranged with respect to the major axis of the body.

The antero-lateral flagellum (*ant. lat. fl.*) passes anteriorly within the cytoplasm in a sweeping curve to the opposite side of the body, meeting its mate of the opposite side in the anterior chiasma (*ant. chiasma*), a node in the two fibers in the median plane, and runs thence in the rim of the cytostome (*intracyt. lat. fl.*) parallel to the anterior peristome as a somewhat thicker strand usually obscured by the peristome, and emerges from a small basal granule (*lat. bas. gr.*) as a free flagellum (*ant. lat. fl.*)
Fig. 1. Ventral view of Giardia microti sp. nov., in early prophase of mitosis, somewhat diagrammatic. X 5100

Abbreviations: ant. chiasma, anterior chiasma; ant. lat. fl., anterior lateral flagellum; ant. perist., anterior peristome; ax., axostyle; kary., karyosome; cent., centrosome; cyt., cytostome; fr. vent. fl., free ventral flagellum; intracyt. lat. fl., intracytoplasmic part of the antero-lateral flagellum; intracyt. post. lat. fl., intracytoplasmic part of the postero-lateral flagellum; intranuc. rhiz., intranuclear rhizoplast; lat. bas. gr., lateral basal granule of antero-lateral flagellum; l. bleph., left blepharoplast; nuc., nucleus; par. b., parabasal body; post. bas. gr., posterior basal granule of posterior flagella; post. fl., posterior flagella; post. lat. fl., posterior lateral flagellum; post. perist., posterior peristome; rhiz., rhizoplast joining nucleus to blepharoplast; tri. halo, triangular halo about posterior part of the axostyle.
The postero-lateral flagellum (*post. lat. fl.*) arises from the blepharoplast, passes posteriorly in the cytoplasm (*intracyt. post. lat. fl.*) close to and parallel with the axostyle on its ventral side to about the level of the middle of the nucleus, then diverges about 15°–25° from the axostyle along the outer margin of the triangular halo (*tri. halo*) to emerge from the cytoplasm as a free flagellum (*post. lat. fl.*) at a little more than midway between the points of emergence of the antero-lateral and the posterior flagella. The lower end of the intracytoplasmic part of the flagellum is often somewhat thickened and there is sometimes a small basal granule at the point of emergence not found in *G. numis*.

The posterior flagellum (*post. fl.*) springs from the posterior granule (*post. bas. gr.*) on the tip of the axostyle which represents its intracytoplasmic part fused with that of its mate.

The free ventral flagellum has no prolonged intracytoplasmic part and cannot be traced to the blepharoplast. It emerges from a vaguely and somewhat diffusely stained axostyle, and usually extends posteriorly, but has great freedom of action and may extend anteriorly. The two ventral flagella often act in unison and often lie parallel to one another or to the postero-lateral flagella.

The flagella are long, about 1.5 the length of the body, and are subequal in length. They serve not only for locomotion but also to keep the surrounding medium in motion when the parasite is fixed upon the epithelial cells of its host by the ventral sucker-like cytostome.

The axostyle (*ax.*) is a single organ (splitting into two in the prophase of mitosis, as in fig. 1), axially located, and extending along the ventral surface from near the center of the cytostome to the posterior tip where it ends in a chromatic basal granule. It is somewhat denser than the surrounding cytoplasm, somewhat chromatic but less so than other parts of the neuromotor apparatus. It is homogeneous throughout and clearly delimited from the adjacent cytoplasm. It represents the intracytoplasmic part of the two posterior flagella (see Kofoid and Swezy, 1915 a, b).

It bears on each corner of its anterior end the deeply stained ellipsoidal blepharoplast (*l. bleph.*) imbedded in its substance and directly connected with the nucleus and centrosome and karyosome by the rhizoplast. It also gives rise directly to the antero-lateral and postero-lateral flagella, and, by way of the axostyle, to the posterior
flagella. Connections with the free ventral flagella are not demonstrable, but may be expected. The axostyle in *G. microti* is more slender than in *G. muris* and is less expanded anteriorly.

The parabasal bodies (par. b.) are two slender, tapering, curved, deeply staining rods lying in the cytoplasm dorsal to the axostyle, in the posterior half of the body. Their length is about 0.5 the transdiameter of the body and the greatest diameter is 0.05–0.10 their length. They usually lie parallel to one another and trend obliquely posteriorly either to the right or left of the median plane. No chromatic fibrils connecting these with the axostyle (blepharoplast?) have as yet been detected in *G. microti*, though the presence of such fibrils in *G. muris* is presumptive evidence that their detection may be expected in *G. microti* also, in favorably located individuals.

The peristome is a deeply chromatic strand in the rim of the cytostome. It consists of two parts, an anterior arc (ant. perist.) parallel to and often obscuring the intracytoplasmic part of the antero-lateral flagellum, and a posterior part (post. perist.) passing in an arc convex posteriorly from the lateral basal granule towards the median plane to the region of the intracytoplasmic part of the postero-lateral flagellum. The peristome is subject to considerable variation in volume and position and tends to disappear in stages of multiple fission and encystment.

**Dimensions.**—Length, excluding flagella, 10–16 μ; transdiameter 5–7 μ; length of cysts 11–14 μ, diameter 5–7 μ.

**Habitat.**—Duodenum, ileum, colon, and rectum of *Microtus californicus californicus* (Peale) from Berkeley, California.

**Comparisons.**—This species is nearest to *Lambilina cuniculi* Bensen—the correct name of which is *Giardia duodenalis* (Davaine)—in general proportions, but differs from it in the more tapering posterior cone, shorter "tail," and in the more slender, more elongated parabasals. It is also possible that the cytostome in *G. microti* more nearly reaches the margin of the body than in *G. duodenalis*, though the great mobility of this organ makes this character of less certainty than the others above noted.

It differs strikingly from the stout, rotund *G. muris* (Grassi) in its more tapering, less convex posterior cone whose angle in *G. microti* is 32.5–55° and approaches 90° in *G. muris*. The parabasals in *G. muris* are ellipsoidal bodies about half the length and twice the width of those in *G. microti*. This difference alone is clearly diagnostic.
The cytosome in *G. muris* is also less distended to the periphery of the body than in *G. microti*.

It differs from *Lamblia intestinalis* (Lambl) in the somewhat smaller size, 10–16 μ as compared with 10–25 μ and in the marked difference in the parabasals, which are slender and curved in *G. microti*, but much larger and massive and apparently fused in one body, according to Bensen (1908) in the former, though Prowazek and Werner (1914) figure a single "Kolbenkörper" stating erroneously that it is on the ventral side of the axostyle. They mention its division "in mehrere Spangen" in old individuals. The figures of Rodenwaldt (1912) leave little doubt that there are at times, at least, two stout, brush-shaped parabasals in an obliquely curved position dorsal to the axostyle. These are very much like those of *G. microti* except that they are a little stouter and less curved.

Since the specific name *intestinalis* was preoccupied in the genus *Cercomonas* by Diesing's (1850) reference of *Bodo intestinalis* to that genus, Lambl's (1859) use of the name for the species from man is invalid and no other available name remains. To preserve as far as may be, a clue to its past nomenclatural history, the name *Giardia lamblia* Stiles in litt. is here proposed for the species hitherto generally known as *Lamblia intestinalis* (Lambl) from man.

From *G. agilis* Kunstler (including *G. alata* Kunstler and Gineste) it differs in the shorter, stouter body and less constriction of the region of the cytosome from that of the elongated body behind it. The ventral cup forms only 0.20–0.25 of the total length in *G. agilis* and nearly 0.5 in *G. microti*.

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BENSEN, W.

DIESING, K. M.
1850. Systema Helminthum. Samptibus Academiae Caesareae scientiarium, Vindobonae (C. Gerold’s Sohn, Wien), v. 1, xvi + 680; v. 2, vi + 596.

KOFOID, C. A., AND SWEZY, O.

LAMB, W.

Prowazek, S. V., AND WERNER, II.

RODENWALDT, E.
1911. "'Flagellaten *(Trichomonas, Lamblia)*' in Prowazek, "'Handbuch der pathogenen Protozoen,'" 1, 78-97, pl. 3, 9 figs. in text.
ON BINARY AND MULTIPLE FISSION IN

GIARDIA MURIS (GRASSI)

BY

CHARLES ATWOOD KOFOID AND ELIZABETH BOHN CHRISTIANSEN

In the last edition of Kolle and Wassermann’s Handbuch der Pathogenen Mikroorganismen, Jollos (1913) states that division stages of Lamblia (= Giardia Kunstler) have never been observed among the great numbers of individuals found as parasites in the intestinal lumen of mammals. “so dass eine Vermehrung auf diesem Stadium kaum anzunehmen ist.” He casts doubts upon Noe’s (1909) reported binary and multiple fission as not well grounded, but cites the oft-observed multiplication in cysts as insufficient to explain the enormous numbers of the parasites. Alexeieff (1914) and Schaudinn (1903) described as copulation cysts those containing two individuals, a view to which Wenyon (1907) adheres, though Bohne and Prowazek (1908) distinguish certain cysts as multiplication cysts and others as copulation cysts, and Rodenwaldt (1911) is skeptical as to the reliability of these earlier interpretations, especially as to copulation and autogamy, as was also Dobell (1909) before him, on other material, however. Alexeieff (1914) explicitly states “pas de division à l’état libre,” and Prowazek and Werner (1914) in their very recent summary of the literature and their reinvestigation of the structure and development of Lamblia state emphatically that multiplication by binary, and occasionally multiple fission occurs only in the cysts, though they find one case of spindle formation in the free stage.

This question as to the limitation of reproduction to the encysted stage is of more than technical interest, since it might appear, if true, to limit the extent of infection to the number of ingested spores. In
any event it has an important bearing on the extent and rate of increase of the parasites and thus upon their significance in human and comparative medicine.

It is the purpose of the present paper to describe binary and multiple fission in both free and encysted *Giardia*, to describe mitosis in the cells of this minute organism, to add to the scepticism regarding autogamy and an *Octomitus* stage, and to bring some slight but inconclusive evidence for maturation and conjugation.

This paper is based on the examination of these parasites in gray and white culture mice, field and meadow mice, rats, rabbits, and coyotes which feed upon meadow mice, as tabulated below. In all 220 mammals were examined between August 26, 1914, and July 20, 1915. Of these, twenty-nine, or thirteen per cent, were obviously infected with *Giardia*. It is possible that more complete examination would have revealed them in some hosts in which they were not found. Their seemingly entire absence in culture rats and in the rabbits examined is noteworthy in view of the previous reports of the occurrence of *Lamblia* in these hosts. The absence in rats may be due to the age of the hosts, most of those examined being old individuals. *Giardia* is rare in old mice.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number examined</th>
<th>Number infected</th>
<th>Gray</th>
<th>White</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td>Culture mice</td>
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<td>22</td>
<td>76</td>
<td>22</td>
<td>62</td>
<td>36</td>
</tr>
<tr>
<td><em>Peromyscus maniculatus gambeli</em> (Baird)</td>
<td>59</td>
<td>4</td>
<td>...</td>
<td>...</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>Microtus californicus californicus</em> (Peale)</td>
<td>6</td>
<td>3</td>
<td>...</td>
<td>...</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
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<td>0</td>
<td>8</td>
<td>34</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Belgian hares</td>
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<td>0</td>
<td>9</td>
<td>3</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><em>Canis ochropus ochropus</em> Eschsholtz</td>
<td>2</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The distribution of infection by color, sex, and organ is indicated below:

<table>
<thead>
<tr>
<th>Host</th>
<th>Oesophagus</th>
<th>Stomach</th>
<th>Jejunum</th>
<th>Ilium</th>
<th>Caecum</th>
<th>Colon</th>
<th>Rectum</th>
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</thead>
<tbody>
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<td><strong>Culture mice</strong></td>
<td>14</td>
<td>8</td>
<td>15</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td><em>Peromyscus</em></td>
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<td>...</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Microtus</em></td>
<td>3</td>
<td>...</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>8</td>
<td>20</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>23</td>
</tr>
</tbody>
</table>

The region of frequent and maximum infection is the small intestine in the duodenum and occasionally near the caecum, though both above and below the latter it is often severe. Heavily infected hosts
can be detected on opening the abdomen by the yellowish translucent color and flaccid consistency of the wall at the point of infection. In the case of infection by *Trichomonas muris* the intestine is less inflated, the color somewhat duller, and the contents less gelatinous and viscid. The two parasites occasionally occur together, but a heavy infection by the one is not as a rule accompanied by a heavy infection by the other. No cases of intestinal cancer coincident with infection were noted, a single instance of axillary cancer being the only one detected.

The material has been prepared by smearing deeply from the fresh intestinal wall, fixing in warm Schaudinn’s fluid by the wet method and staining in Heidenhain’s iron haematoxylin. Cross-sections of the infected intestine prepared with the same fixer and stain have also been used. We attribute our success in finding the fission stages to the use of smears well rubbed out to yield the deeply seated parasites from the crypts, and from the epithelium itself, and also to thorough search of the material.

**Morphology**

The actively swimming trophozoite (pl. 5, figs. 1, 4, and text fig. A) is broadly pear-shaped in outline with semicircular anterior, and convex-conical (70°–90°) posterior outline. Its total length, excluding flagella, is 1.25–1.50 of its greatest transverse diameter, which is about 0.4 of the total length from the broadly rounded anterior end. Posteriorly it tapers to an acute or even acuminate tip containing the axostyle which does not protrude as in *Trichomonas*. In lateral view (pl. 5, fig. 5; pl. 8, fig. 53) the ventral surface in the anterior 0.6 is flattened, and more or less deeply cupped in a large ventral cup, or so-called mouth, the cytostome (*cyt.*, fig. A) which occupies nearly the anterior half of the ventral surface and extends nearly from side to side. Its outline and depth vary with the contraction of the encircling peristome (*ant. perist.*, *post. perist.*, fig. A) and with the distention of the body. In some cases (pl. 5, fig. 2) it extends posterior to the peristome forming a recess, or may be very considerably reduced (pl. 5, fig. 4), or fades out posteriorly as in some phases of mitosis (pl. 6, figs. 16, 17), or disappears entirely as in multiple fission in cysts. The dorsal outline in lateral view is much elevated, to a height even exceeding a hemisphere or the transverse diameter, and is often much higher than figured (pl. 5, fig. 5, and pl. 8, figs. 53, 54), forming
Fig. A. Diagrammatic ventral view of active trophozoite of *Giardia muris*. X 5100.

Abbreviations: ant. chiasma, anterior chiasma; ant. halo, anterior halo; ant. lat. fl., anterior lateral flagellum; ant. perist., anterior peristome; ar., axostyle; cary., karyosome; cary. halo, karyosome halo; cent., centrosome; cyt., cytostome; fr. vent. fl., free ventral flagellum; intracyt. lat. fl., intracytoplasmic part of the postero-lateral flagellum; intranuc. rhiz., intra-nuclear rhizoplast; lat. bas. gr., lateral basal granule; nuc., nucleus; par. b., parabasal bodies; post. bas. gr., posterior basal granule; post. fl., posterior flagellum; post. lat. fl., postero-lateral flagellum; post. perist., posterior peristome; l. bleph., left blepharoplast; rhiz., rhizoplast; tri. halo., triangular halo.
in life a crystal-clear vesicle on the outer ends of the epithelial cells. The posterior end tapers down abruptly to a short, very mobile tail having in it the tip of the axostyle. Its mobility is indicated by its various positions in our figures (pl. 5, figs. 3, 5, 11).

This organism is one of the so-called diplozoic flagellates consisting of two unparted cells in a condition such as might occur if mitosis of a unicellular flagellate with accompanying division of all cell organelles except the axostyle should take place without subsequent plasmotomy. It is in reality a binucleate somatella with all organelles in duplicate (except the axostyle and cytostome), but with these organelles in a bilateral arrangement about a single axostyle. The cytoplasm shows no duplicity of external form. It is one individual, not a colony of similar cells, nor two incompletely divided ones, but two mutually interadapted cells forming a bilaterally symmetrical organism.

The organelles (fig. A) of this simplest (from the standpoint of numbers only) possible multicellular organism consist of two nuclei (\textit{nuc.}), two blepharoplasts (\textit{bleph.}) from both of which arises one interconnecting neuromotor system joining all of the organelles, one axostyle (\textit{ax.}), two parabasal bodies (\textit{par. b.}), and one large ventral cup or cytostome (\textit{cyt.}).

The two nuclei (\textit{nuc.}) are ellipsoidal bodies, with distinct nuclear membranes, subcentral karoyosomes, sometimes with surrounding halo, and remarkably little chromatin as a rule outside of the karyosome. They are about 1 by 2.5\textmu in length and transverse diameter respectively, and lie near the ventral surface, symmetrically placed near the middle of the cytoplasmic mass, with their long axis diverging 5°–20° posteriorly from the main axis of the organism. From the karyosome of each a rhizoplast (\textit{intranuc., rhiz.}) passes to the nuclear membrane and thence (\textit{rhiz.}) to the blepharoplast of its side at the head of the axostyle, forming as it passes the nuclear membrane, an anterior membrane granule. It is possible that this represents the centrosome (\textit{centl., fig. A}). There is a corresponding granule, the posterior membrane granule at the opposite pole in some nuclei (pl. 5, figs. 1, 2) which does not seem to be a second centrosome, judging from the fact that it may appear when no indications of mitosis are visible.

These rhizoplasts arising from the central karyosomes are connected with the blepharoplasts, the centers from which starts the complicated
neuromotor apparatus of these organisms. This apparatus is continuous as one structure throughout, and consists of the two blepharoplasts (bleph.) from which radiate the two rhizoplasts (rhiz.), the anterior or lateral (lat. fl.), postero-lateral (post. lat. fl.) and the free ventral (fr. vent. fl.) flagella, and the axostyle (axr.) terminating posteriorly in the posterior flagella (post. fl., fig. A).

The blepharoplasts are two large, deeply staining, spheroidal bodies symmetrically placed at the right and left sides of the anterior end of the axostyle. They are joined by a slender, deeply staining transverse fiber or commissure, which cannot be the homologue of the paradesmose joining blepharoplasts in binary fission of trichomonad flagellates (Kofoid and Swezy, 1915 a, b) for this joins centrosomes when they emerge from the blepharoplasts in Trichomonas, and does not unite them here. Three flagella and one-half of the axostyle, terminating in one posterior flagellum take their origin or radiate from each blepharoplast and each is connected with one nucleus.

This neuromotor system is also joined to the parabasal bodies, that is, to the pair of large, subellipsoidal, deeply staining masses sometimes fused into one, lying dorsal to the middle of the axostyle. The connecting fibers (pl. 5, fig. 5; pl. 8, figs. 53, 56) to the parabasals are made out with great difficulty and have been found by us only in several favorably located individuals. They join the dorsal side of the axostyle and we are not able to follow them separately to the blepharoplasts, nor to be certain of their constant or general occurrence because of the great technical difficulty of detecting such small structures in the mass of stained material above or below them in all except lateral views.

These bodies have been regarded by previous investigators prior to Alexeieff (1914) as "rätselhafte Körper" of unknown significance and morphological relationship. We believe, because of their stainability, connection with the neuromotor apparatus (blepharoplast?), and because of the evident relationship of Giardia to the trichomonads, that this body is homologous with the parabasal of Devescovina of Janicki (1911) and with the chromatic basal rod or parabasal (see Kofoid and Swezy, 1915 a, b) of Trichomonas. This homology was first tentatively suggested by Alexeieff (1914).

The flagella are eight in number, all trail posteriorly, and emerge on the ventral side in four bilaterally placed pairs, the antero-laterals (ant. lat. fl., fig. A), postero-laterals (post. lat. fl.), free ventrals (fr.
vent. fl.), and the posteriors (post. fl.). The antero-lateral flagellum (ant. lat. fl.) arises from the blepharoplast, crosses obliquely anteriorly to the opposite side, crossing its mate of the opposite side in the middle line and enlarging at their junction in an anterior chromatic granule (ant. chiasma). It follows the curvature of the ventral margin and adjacent anterior peristome to the posterior end of that structure, where it enlarges in a basal granule (lat. bas. gr., fig. A) at the point of its emergence from the cytoplasm. The intracytoplasmic part of this flagellum (intracyt. lat. fl.) is easily confused with the adjacent heavy peristome which it parallels and by which it is usually obscured. It is more deeply stained than the exposed free flagellum.

The postero-lateral flagellum (post. lat. fl., fig. A) passes through the cytoplasm posteriorly from the blepharoplast (pl. 5, fig. 8) parallel to the axostyle along its ventral surface to about the level of the posterior border of the nucleus where it crosses the posterior peristome. Here it swings obliquely outward 15°–20° from the axostyle, becomes heavier and stains more deeply, and emerges from the cytoplasm at or usually somewhat within the postero-lateral margin. Its varying point of emergence is doubtless due to the mobility of this region. There is no basal granule here as a rule, though in some individuals (pl. 5, fig. 3) a small one is present.

The free ventral flagellum (fr. vent. fl., fig. A) emerges on the ventral face shortly behind the blepharoplasts on the ventral side of the axostyle. A darker region (pl. 5, fig. 8) is often present on the base of each flagellum at this point parallel to the axostyle. It does not seem to be a thickened part of an axostyle as figured by Wenyon (1907) and Bensen (1908), but rather independent of, but ventral to it. We are unable to trace its connection thence with the blepharoplast as in the ease of the other flagella. These flagella are stated to be heavier and more active by Wenyon (1907). We find no increased size, though their position gives a greater freedom of movement and they are more active than the other flagella, except the posteriors, and are sometimes seen anteriorly directed (pl. 6, figs. 28, 29).

The posterior flagellum (post. fl., fig. A) is a direct continuation of the axostyle. In the ease of the single axostyle prior to the approach of mitosis there is one flagellum from each side of its tip (pl. 5, fig. 1) at which there may be a basal granule, but this is not always present. After the division of the single axostyle to form two (pl. 5, fig. 6), one flagellum and one basal granule go with each daughter
axostyle, and later in the metaphase (pl. 6, fig. 26) the new flagellum grows out, forming a pair at the tip of each daughter axostyle. These flagella are the most active ones in the set, a condition paralleled by the intense activity of the non-flagellated projecting tip of the axostyle in *Trichomonas*. We may therefore interpret the axostyle here as in trichomonad flagellates (see Kofoid and Swezy, 1915 a, b) as the intracytoplasmic part of a flagellum, greatly expanded and therefore staining less deeply than the intracytoplasmic parts of the other flagella in *Giardia*.

The points of emergence of the two flagella of a pair are usually bilaterally symmetrical, but may be shifted, probably by contraction. The axostyle (a.r., fig. A) is a flexible, hyaline, more or less deeply staining axial rod bearing on both the right and left corners of its enlarged anterior end a large blepharoplast. It tapers distally to 0.5 its anterior diameter and terminates at the cytoplasmic margin in posterior basal granule (*post. bas. gr.*, fig. A) from which spring the two posterior flagella. The axostyle has been figured by Wenyon (1907), Bohne and Prowazek (1908), Bensen (1908), Rodenwalt (1911), and Prowazek and Werner (1914) as double. It is double early in binary fission (pls. 5, 6, figs. 6–17) and such stages are abundant in our material. They are, however, early stages in mitosis and are preceded by a stage in which there is structurally a single axostyle with two blepharoplasts. This is followed by a stage with two axostyles and four blepharoplasts, and it appears that it is these later stages which have been utilized inadvertently as typical of the trophozoite. We regard the stage prior to this with one axostyle and two blepharoplasts as the typical one prior to mitotic conditions.

The cytostome (*cyt.*, fig. A) is the ventral cup, bordered by the deeply staining peristome consisting of two regions on either side, an antero-lateral arc (*ant. perist.*) and a posterior one (*post. perist.*). The former are continuous anteriorly, being connected by a thinner bridge across the gap above the anterior chiasma. The posterior peristome is weaker, curves abruptly inwards from near the basal granule of the antero-lateral flagellum with an arc convex posteriorly and disappears near the axostyle without cross-connection to the peristome of the opposite side. The cup is single, but its fundamentally double origin or fate is indicated in the anterior break in size of the peristome and in the posterior interruption. The chromatic peristome is a deeply staining, very heavy strand. We are unable to
connect it structurally by attachment to the neuromotor apparatus, but it lies in close conjunction with it, usually so as to obscure it, and stains in the same manner. Its position, the varying shapes and dimensions it assumes in life and in stained preparations (pl. 5, figs. 1, 4) indicate a neuromotor function.

Two areas distinguished by relative lack of stainability are to be noted, an anterior halo (*ant. halo, fig. A*) in the median line above and anterior to the blepharoplasts, and a triangular halo (*tri. halo*) about the axostyle posteriorly between the intracytoplasmic parts of the postero-lateral flagella. Their function, if any, and significance are wholly problematical.

**Binary Fission**

This process occurs in both encysted and non-encysted individuals. It is probable that many of the incipient phases observed may be antecedent in some cases to multiple mitosis, following binary mitosis. The occurrence of two mobile detached individuals in one cyst is open to the interpretation as the result of binary fission. Binary fission is abundant in our slides in the non-encysted individuals, especially in its early phases. It seems probable, therefore, that the previous failure to detect it is not due to its non-occurrence. Binary fission consists of two processes, mitosis, involving the nucleus and the extra-nuclear neuromotor apparatus, and plasmotomy, independent of and subsequent to mitosis, involving the cytoplasm.

**Mitosis**

This process involves blepharoplasts, axostyle, and flagella, as well as the nucleus. The division of each follows no fixed chronological sequence, multitudinous minor variations in the relative order of division being apparent in our material. In a general way the sequence of events is as follows: Division of the blepharoplasts, of the axostyle, of the anterior flagella, enlargement of the karyosome, appearance of eight chromatic masses, their arrangement in the form of a split skein, the division of the centrosome and formation of the paradesmose between the daughters, the fusion of the eight chromatic masses into four masses or chromosomes, the appearance of these in the equatorial plate of the intranuclear spindle, migration of daughter chromosomes towards the poles of the spindle, division of the parabasals, nuclear constriction, and much later, plasmotomy. The formation of the other new flagella is subject to considerable variation in relative chronology.
The prophase may be regarded as the period prior to the formation of the equatorial plate, the metaphase as the separation of chromosomes in this plate, the anaphase as the period of their polar migration, and the telophase as that of nuclear reconstruction. We will now follow the history of each organelle through these periods.

The blepharoplasts divide (pl. 5, figs. 8, 7, 6) simultaneously, spreading the head of the axostyle and forming between the migrating daughters a new chromatic band. These lie athwart the heads of the daughter axostyles as they part and at no time take any part in spindle formation as does the paradesmose in trichomonads.

The axostyle divides immediately after the blepharoplasts and splits lengthwise from the anterior end posteriorly, splitting the basal granule, and each daughter taking one of the two posterior flagella (pls. 5, 6, figs. 6-17). This process completes the organelles of the two component cells, but it is also the initial step in the division of the individual. It is the last organ to divide in the trichomonads and its behavior here is an indication of the evolution of *Giardia* from a unicellular type of trichomonad affinities, in which a binucleate somatella with undivided axostyle has come to be the normal trophozoite individual.

The flagella arise, not by splitting, but by new outgrowth from the blepharoplast, though none has been figured. The new flagellum follows the path established by the intracytoplasmic shaft of the old one and emerges at its side. It is difficult to determine whether or not this is outgrowth or splitting, but it appears to be the former. The intracytoplasmic part of the posterior flagella, the axostyle, is reproduced by splitting. The distinction between the two methods seems to have no morphological significance. The first flagellum to divide is the antero-lateral which forms a new anterior chiasma, the inner flagellum being the new one in each case (pl. 5, figs. 5, 6, 7). The emergence of the new flagellum at the surface seems to be delayed till after nuclear division.

No case of the division or new outgrowth of the intracytoplasmic part of the postero-lateral flagellum has been observed in our material, nor has the formation of new ventral flagella been found in progress. They are fully formed prior to plasmodium (pl. 7, fig. 33). The posterior flagella, originally a pair from the tip of the single axostyle, become doubled sooner or later during mitosis (pl. 5, fig. 10), but this may be long delayed (pl. 6, fig. 29).
The method by which the new cytostome is formed is by no means clear. The posterior peristome is often much thickened at the metaphase (pl. 6, fig. 26) and may be even heavier than the anterior peristome. We find no evidence of the division of either of these chromatic lines. They are apparently formed de novo on the new side of the daughter somatella. The four instances (pl. 7, figs. 31-34) of plasmotomy following mitosis (pl. 6, fig. 30) are not wholly in agreement in their evidence on this point. The peristomal margins on the upper left and lower right side of the figure are heavier (older) in both zooids in figure 31, as though twisting at the cytoplasmic bridge had placed the parental peristome on opposite sides. In figure 32 (dorsal view) the outer peristomes, right on the lower and left on the upper, are heavier and presumably ancestral, while the inner are faint or missing and are apparently in the process of formation. In figure 33 both are heavy on the lower zooid, and absent or lightly developed in the upper, as though one daughter had taken the whole peristomal equipment and the other was forming it anew. In figure 34 the anterior peristome alone is present and in both daughters is rather light.

The behavior of the parabasals during the process of mitosis and binary fission is still somewhat problematical. It is doubtless complicated by multiple fission and the possibility of moribund or pathological conditions. We have one clear case of its duplication, presumably by division in the metaphase, since the two pairs of parabasals are of equal size and adjacent (pl. 6, fig. 28). In other comparable stages of the same or later phases the pair of parabasals cannot be found (pl. 6, fig. 30), and in some instances scattered granular chromidial masses are found in the cytoplasm in the region where the parabasals normally occur. It is also absent in encysted stages (pl. 7, figs. 34-43). Its disappearance may therefore be incidental to the process of encystment and be correlated with changing conditions of metabolism.

The process of nuclear mitosis is carried on wholly within the persistent nuclear membrane as in the trichomonad flagellates. The successive phases established for metazoan mitosis are approximately recognizable in this protozoan nucleus. The prophase is introduced by the intranuclear chromidial cloud (pl. 5, figs. 4, 6) whose subsequent disappearance (pls. 5, 6, figs. 8-17) is accompanied by an increased stainability of the cytoplasm (figs. 14, 15) with slight trace of extranuclear chromidia, and by increased stainability of the peristome.
There is some evidence, resting on several preparations (pl. 5, figs. 6-8) that the granule on the nuclear membrane at the entrance of the rhizoplast is the centrosome which divides to form the polar centrosomes with extranuclear paradesmose between (fig. 8).

A considerable elongation of the nucleus follows this stage and in some of such nuclei (pl. 6, fig. 20; pl. 8, fig. 59) a centrosome is seen at either pole. In other cases it is revealed at the upper end, but the lower is obscured by the chromatic posterior peristome. In some (pl. 5, fig. 11) it is not visible at either pole. Spindle fibers are not visible till the metaphase and then but faintly. No external astral rays and no centrosphere were found and the paradesmose does not persist as in *Trichomonas* (Kofoid and Swezy, 1915 a, b).

The organization of the chromosomes, however, follows somewhat the same history as in *Trichomonas*. The stage of encrusted nuclear membrane (pl. 5, fig. 3) if followed by that of the intranuclear chromidial cloud with halo about the karyosome (pl. 5, fig. 4), the enlargement of the karyosome (pl. 8, fig. 55), its breaking up into scattered granules (pl. 5, figs. 8-11), their subsequent arrangement in a split skein (pl. 5, figs. 13, 12; pl. 8, fig. 58) from which there later emerge four larger chromosomes (pl. 5, figs. 14, 15; pl. 6, figs. 16, 17). This stage is certainly subsequent to that of more (eight) scattered granules (figs. 8-11) as proved by its later phase of division in the axostyle. We interpret this earlier phase (figs. 8-13) as one of precocious splitting of the chromosomes or chromatic thread, followed by a subsequent fusion as in *Trichomonas* (Kofoid and Swezy, 1915 a, b), prior to the appearance of the definitive number in the equatorial plate, and their final division in the metaphase. Traces of their bivalent character appear in some nuclei (pl. 6, fig. 17).

The formation of the "amphiaster" stage (pl. 6, figs. 18-22) with the chromosomes in the equatorial plate follows the refusion. There are two rather different types of spindles present, one (figs. 18-20, 22) in which the spindle is shrunken away from the membrane, possibly an artefact or pathological. In this the chromatin in the plate is small in amount, the chromosomes indistinct, and the centrosomes somewhat enlarged. In the other (pl. 6, fig. 21) the spindle is plump, fills the nucleus, the chromatin is larger in amount, the chromosomes more distinct, and general appearance more normal.

In the metaphase (pl. 6, figs. 23, 24) the chromatin masses elongate, constrict in the middle, and part. We are unable to see any connec-
tion between this plane of constriction and the previous one of pre-
cocious splitting except in figure 26 in which there are four pairs,
possibly not yet arranged in the equatorial plate. The cleft in these
is perpendicular to the equator. The number of chromosomes at this
stage is always four and there is little differentiation among them and
no marked lagging pair as in Trichomonas.

The anaphase (pl. 6, figs. 27-29) is brief. In this the chromosomes
move apart toward, but not to, the pole, either in one plane (fig. 27),
or irregularly (fig. 28), and tend to group themselves in two coalescing
pairs (fig. 29) suggestive of two ancestral sources as in some Metazoa.

The telophase is accomplished after the constriction of the nucleus
(fig. 29) into two spherical nuclei (fig. 30) within which the four
chromosomes mass themselves into the central karyosome.

Plasmotomy or constriction of the cytoplasm occurs after mitosis
is completed (pl. 7, figs. 31-34). It is possible that these figures
represent the first division of a phase of multiple mitosis since in each
case the axostyles and nuclei are in the prophase of an ensuing division.
We have little evidence as to the direction of the plane of division
having found only one (fig. 32) stage intermediate between the com-
pletion of mitosis (fig. 30) and the late stage of plasmotomy (figs. 31,
33, 34) in which the two zooids have so far pulled asunder that their
anterior ends are nearly 180° apart. As shown in the discussion of
the division of the peristome, there is a little evidence that the plane
is longitudinal, parting the peristome in the median line. Such a plane
in the middorsal-ventral position would part not only the peristome
but the daughter axostyles and leave sister nuclei in each of the result-
ing organisms. In figure 32 the left sister has moved anteriorly,
detaching itself from the right along such a plane. Persistence of a
connecting strand of cytoplasm at the posterior end and a shifting of
the daughters to an end to end position as in Trichomonas is suggested
by figures 31, 33, 34.

Our material has not furnished a full series of stages illustrating
both the development and disintegration of the multinuclear plas-
modium such as was found in Trichomonas (Kofoid and Swezy, 1915
b), but enough of the stages have been found to determine that this
organism also forms an 8-zooid plasmodium, which because of the
binucleate nature of the individual will in this species contain sixteen
instead of eight nuclei. A 4-nucleate stage in the prophase of the
next division leading to the 8-nucleate with four potential individuals
is perhaps to be seen in plate 7, figure 31. A completed 8-nucleate stage is shown in figure 48, and the nuclei of this are in the prophase for the next division. A group of four small individuals probably in a late stage of disintegration (pl. 7, fig. 49) of the plasmodium were found which have about the size that two have in the preceding stage (fig. 48). They suggest the completion of the 8-zooid, 16-nucleate plasmodium or somatella, and its subsequent disintegration into single binucleate individuals of small size.

The small free zooids resulting from multiple fission are not to be confused with the small Hexamitus muris Grassi (see Wenyon, 1907) which occurs in the same hosts with Giardia muris. It is, as Wenyon (1907) has stated, a distinct species. Hartmann’s (1910) suggestion that it is a phase of the life history of Giardia muris is entirely unsupported by our observations on the life-history of Giardia and the structure of Hexamitus muris.

This method in which the form of the original parent individual is lost after the first mitosis is not, however, the only method of formation of a multinucleate somatella. Another type (pl. 7, figs. 45–47) occurs among non-encysted individuals, in which the parental form is preserved during the multiplication of nuclei, and the extranuclear neuromotor apparatus does not keep pace in its divisions with nuclear duplications, there being for example eight nuclei and but two axostyles, and not as yet two full sets of flagella.

An individual which has reached the 8-nucleate stage (4-zooid) is shown in plate 7, figure 45, and in the prophase of the next division leading to the 16-nucleate (8-zooid) in plate 7, figure 46. The nuclei are symmetrically arranged in the second, but are scattered without reference to bilateral symmetry in the first. In figure 47 we have apparently a moribund individual approaching the 8-nucleate stage, in which the cytoplasm is full of deeply staining chromidial masses and the nuclei are passing through the second division leading to the 8-nucleate plasmodium. It shows two nuclei in recent constriction and two not yet constricted. Evidences of four chromosomes are present in figures 45 and 46. The main distinction between this method of multiple fission and the one first described lies in the delay in the division of extranuclear organelles in the latter. The individuation of the potential organisms is less advanced in the cytoplasmic region than it is in the nuclear, possibly as the result of external conditions acting on one or both these regions.
Multiple Mitosis in Cysts

Multiple mitosis also occurs in encysted individuals and is presumably followed by multiple fission. All stages in the formation of a 16-nucleate somatella, including the 2-, 4-, 8-, and 16-nucleate cyst have been found (pl. 7, figs. 35-44). Four chromosomes occur here as elsewhere at mitosis though they are somewhat more fused in pairs. The daughter nuclei retain only approximately a bilateral grouping and ultimately spread through the cytoplasm from end to end (pl. 7, fig. 44). The axostyles do not exceed two in any of our 16-nucleate (8-zooid) cysts. The chromatic margins of the peristome and the parabasals disappear, diffuse deeply staining masses appear in their places, and the two halos disappear. The posterior peristome forms a deeply stained thick semicircle (pl. 7, fig. 38) attached to the posterior ends of the intracytoplasmic part of the anterior flagella which persists after the rest of the extensions of the neuromotor apparatus has disintegrated, and finally breaks up into a thin granular line (pl. 7, fig. 42). The intracytoplasmic ends of the pos tero-laterals persist for some time as dark lines (pl. 7, fig. 41).

The cyst wall is thin, hyaline, double-contoured, and is generally closely applied to the body, though in some cases (pl. 7, figs. 38, 39) possibly due to shrinkage, there is an intervening space. No empty cysts have been found, so that presumably the final phase of multiple fission, the detachment of eight fully equipped small individuals successively from the escaped 16-nucleate plasmodium, or their escape singly from the cyst, takes place outside of the host in which they are produced, or possibly in a new host.

Encystment

Three types of cysts (which may be reducible to two) occur. (1) Cysts in which multiple fission is in progress or completed (pl. 7, figs. 35-37, 41-44). (2) So-called copulation cysts containing two detached individuals (pl. 8, fig. 61). (3) Possible maturation stages in these "copulation" cysts. Such a cyst in G. microti (pl. 8, figs. 62-63) shows evidence of the two individuals, apparently back to back, and with the anterior ends at opposite poles as in the earlier (gametocyte?) stage (fig. 61) and in certain free stages (pl. 8, fig. 55). It is not certain that these are maturation stages rather than multiple fission of two individuals in the stage of advanced plasmodity (figs. 31-34) which have swung into a back-to-back position and encysted (fig. 61).
The evidence indicative of maturation is restricted to (1) progressive fusion (figs. 61-63), (2) differences in sizes of nuclei suggesting reduction (figs. 62, 63), and (3) possible reduction of the amount of chromatin. There is no satisfactory evidence of a reduction of the number of chromosomes from four to two (fig. 63). If this interpretation is correct, figure 61 represents encysted gametocytes, figure 62 gametocytes II after the first maturation division with marked nuclear reduction in size in the first polar nuclei, and figure 63 the completion of maturation in the upper pair of nuclei with two pairs of smallest nuclei representing the divided first polar nuclei, two larger nuclei representing the second polar nuclei and the two gametic nuclei still with four chromosomes each. The lower individual is still in the stage of gametocyte II. It is obvious that gametes thus formed would be binucleate and that a double zygote would result from gametic union. This fact and the apparent absence of reduction of chromosomes militate against the sexual interpretation.

The final and only conclusive proof of this interpretation will be the observed sexual behavior of the resulting gametes. Of this we have no evidence. The possibility of multiple fission (cf. figs. 63 and 64 noting distribution of nuclei in both) is open, and the differences in nuclear size (figs. 62, 63) might be merely pathological and the occurrence of free pairing individuals (fig. 55) mere accidental artefacts. The internal evidence (except in the matter of a reduction division) in favor of the sexual interpretation is both normal and consistent as far as it goes, but is very incomplete and therefore inconclusive. The occurrence of sexual reproduction in *Giardia muris* and *G. microti* is therefore very tentatively suggested. We have no satisfactory evidence that the maturing “gametocytes” are sister cells or that autogamy occurs.

**Summary**

*Giardia muris* occurs in culture mice (gray and white), and in *Peromyscus*, but was not found in *Microtus*; neither it nor other species were found in culture rats, Belgian hares, or coyotes, feeding where *Peromyscus* was taken. It causes chronic enteritis, especially in young hosts, with inflation and yellowish color of intestine.

The normal trophozoite has a single axostyle, not two as heretofore described, an integrated neuromotor apparatus with fibrillar connections joining the karyosomes, centrosomes, blepharoplasts, flagella.
parabasal bodies, and axostyle of the two cells in one more or less continuous system.

Binary fission occurs abundantly and normally, with delayed plasmotomy. Normal mitosis occurs with intranuclear spindle, and four chromosomes in two groups. Precocious splitting of chromosomes in the prophase is followed by their fusion into a split skein from which the four chromosomes emerge on the equatorial plate. The blepharoplast and axostyle lead in mitosis.

Multiple fission is of three types: (1) Free individuals form a plasmodium-like somatella of eight fully equipped zooids, in the formation of which the duplication of organelles keeps pace with nuclear multiplication. (2) Free individuals form eight zooids but nuclear multiplication precedes the division of the organelles. Encystment may follow. (3) Encysted single individuals form 8-zooid, 16-nucleate plasmodial masses with chromatic disintegration of organelles, the axostyles persisting longest. The small free zooids are not to be confused with Hexamitus muris Grassi, which is a distinct species. We find no evidence of an "Octomitus" stage of Giardia.

There is tentative evidence of the fusion of two free individuals and also of copulation cysts which may be derived therefrom with the two gametocyte individuals back to back and of their maturation by two divisions. Chromosome reduction has not been detected in these divisions.

The most striking feature of the development of the free, 16-nucleate, 8-zooid plasmodium, or somatella, is the preservation in each successive step of the process, of the fully equipped binucleate individuals. The individuality of the potential zooids is morphologically established and maintained, and there is evidence also of their functional independence in the independent motor struggles of each which result ultimately in plasmotomy. In the cases of multiple fission in cysts and free individuals in which nuclear multiplication outruns that of the other organelles, this individuality is more or less disrupted, or even lost. The possibility that some of these at least may represent involution or pathological states on the part of the parasite itself, should be borne in mind in all attempts to unravel the baffling significance of these protean aspects of this most interesting, suggestive, small, but by no means simple organism.

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LITERATURE CITED

BENSEN, W.
1908a. Die Darmprotozoen des Menschen. Arch. f. Schiff's u. Tropenhyg., 12, 661-676, 7 figs. in text.

BOHNE, A., AND PROWAZEK, S. v.

DOBELL, C. C.

JOLLOS, V.
1913. Darmflagellaten des Menschen in Kolle und Wassermann "Handbuch der pathogenen Mikroorganismen, 7, 687-702, 15 figs. in text.

NOC, F.

KOFOID, C. A., AND SWEZY, O.

RODENWALDT, E.
1911. Flagellaten (Trichomonas, Lamblia) in Prowazek, "Handbuch der Pathogenen Protozoen," 1, 78-97, pl. 3, 9 figs. in text.

SCHAUDINN, F.

WENYON, C. M.
EXPLANATION OF PLATES

All figures of *Giardia muris* (Grassi) drawn with camera lucida from smear preparations fixed in warm Schaudinn’s fluid and stained in Heidenhain’s iron haematoxylin, unless otherwise stated. × 2720. All from culture mice unless otherwise stated. Full length of flagella not shown in any figures.

PLATE 5

Figures 1-6, 8-15 are of *Giardia muris* (Grassi).

Figure 7 is of *Giardia microti* Kofoid and Christiansen.

Fig. 1. Dorsal view, trophozoite with quiescent nucleus, ventral cytostome coincident with posterior peristome.

Fig. 2. Dorsal view, the same, ventral cytostome extended posteriorly beyond posterior peristome.

Fig. 3. Dorsal view, first indications of division, nuclear membrane heavily incrusted, intranuclear rhizoplast has disappeared, axostyle widening, body rounded up.

Fig. 4. Dorsal view, early prophase, peristome contracted, or unusually small, nucleus rounded up, filled with intranuclear chromidial cloud, karyosome halo distinct, parabasals enlarged, cytoplasmic chromidia abundant. Note chromatic intracytoplasmic part of postero-lateral flagella.

Fig. 5. Lateral view of trophozoite showing dorsal position of parabasals in the dorsal hump and ventral position of blepharoplasts, of anterior and posterior peristome, and of the intracytoplasmic parts of the lateral flagella.

Fig. 6. Dorsal view of early prophase. Body rounded up, nuclear membrane faint, intranuclear cloud present, blepharoplast, anterior chiasma, axostyle, posterior axostylar granule, posterior chromatic lines, and posterior lateral flagella divided. Karyosome enlarged, intranuclear chromidia disappearing, centrosomes dividing (?).

Fig. 7. Early prophase, blepharoplasts dividing, anterior peristome and intracytoplasmic part of antero-lateral flagella very distinct, centrosomes divided (?), somewhat abnormal.

Fig. 8. Late prophase, axostyle dividing, blepharoplasts divided, centrosomes divided, in polar position, with paradesmose between on the outside of the nuclear membrane, karyosome breaking up.

Fig. 9. Ventral view of late prophase, blepharoplasts divided, axostyle in division, no flagella divided. Nuclear membrane incrusted, eight chromatin granules in nucleus.

Fig. 10. Dorsal view of a later stage of prophase, axostyle and posterior flagella completely divided.

Fig. 11. The same of similar stage, blepharoplasts only divided, the eight chromatin granules arranged in two rows of four each. From *Peromyscus*.

Fig. 12. The same, of similar stage, chromatin in two parallel granular masses, chiasma and blepharoplast divided, axostyle in division, ventral flagella thrown forward. From *Peromyscus*.

Fig. 13. Dorsal view of late prophase, blepharoplasts and axostyle divided. Chromatin in two sinuous finely granular, subparallel masses.

Fig. 14. Dorsal view of similar stage, blepharoplasts and chiasma, axostyle dividing, chromatin in four masses, peristome thickened.

Fig. 15. Dorsal view of similar stage, blepharoplasts and axostyle divided. Posterior peristome faded out. From *Peromyscus*. [48]
Mitosis in Giardia muris (Grassi), × 2720, from culture mice unless otherwise stated.

Fig. 16. Late prophase. Blepharoplasts, axostyle, and posterior flagella divided, chromatin in four masses, posterior peristome faded out, cytoplasm abnormal, anterior halo displaced.

Fig. 17. Dorsal view of later stage, axostyles diverging, duplex nature chromatin masses indicated.

Fig. 18. Metaphase, intranuclear spindle (shrunken?) without distinct chromosomes, nuclear membrane and peristome heavily chromatic. From Peromyscus.

Fig. 19. Dorsal view of a similar stage.

Fig. 20. The same stage, with heavy chromatic masses at poles of spindle.

Fig. 21. Dorsal view of the same stage, spindle not shrunken, chromosomes emerging.

Fig. 22. The same stage as figure 20, with heavy polar masses.

Fig. 23. Later metaphase, four chromosomes elongating and constricting in the middle.

Fig. 24. Close of metaphase, chromosomes parted in the right nucleus, parting in the left.

Fig. 25. Dorsal view of anaphase, chromosomes moving to poles of spindle.

Fig. 26. Chromatin masses irregularly placed on the spindle, apparently splitting parallel with spindle fibers. From Peromyscus.

Fig. 27. Ventral view of late anaphase, chromosomes regularly grouped in two sets at about equivalent levels.

Fig. 28. Dorsal view of anaphase, chromosomes irregular in distribution, two sets of parabasal bodies present (divided?).

Fig. 29. Late anaphase, constriction of nuclear membrane to form daughter nuclei.

Fig. 30. Division completed in Giardia microti Kofoid and Christiansen. Posterior peristomes detached, enlarged, parabasals massed below axostyle, large chromatic masses in posterior half.
Binary and multiple fission in *Giardia*. All figures of *G. muris* (Grassi) from culture mice unless otherwise stated. × 2720.

Fig. 31. Early phase of multiple fission, in late prophase of second division. Daughter zooids with full equipment of organelles, blepharoplasts and axostyles divided for second division, chromatin in split skein condition. Probably twisted at the protoplasmic bridge.

Fig. 32. Dorsal view of about the same stage but of much smaller size.

Fig. 33. The same stage, protoplasmic connection reduced.

Fig. 34. The same in late prophase. From *Peromyscus*.

Fig. 35.—Cyst with one individual with nuclei showing four chromosomes, no parabasals, peristome disappearing, and chromidial masses appearing in the cytoplasm. The intracytoplasmic parts of the antero-lateral and postero-lateral flagella are still visible, especially the former.

Fig. 36. The same stage. Chromatin massed in bifid central karyosome, chromidia increased, peristome gone, antero-lateral flagella displaced.

Fig. 37. Anaphase of first division of encysted individual, four chromosomes in two masses at either pole, parabasal persisting, small amount of chromidia.

Fig. 38. First division of *G. microtis* completed, posterior peristomes and intracytoplasmic parts of antero-lateral and postero-lateral flagella persisting but displaced, quadriripartite central karyosome, few chromidia. From *Microtus*.

Fig. 39. Later stage with extranuclear organelles in dissolution, with considerable chromidial accumulation in the cytoplasm. From *Peromyscus*.

Fig. 40. Problematical stage, possibly pathological, in anaphase of second division, no cyst wall, organelles in dissolution, much chromidial material. From *Peromyscus*.

Fig. 41. "Conjugation cyst," evidently containing two individuals with ends in reversed position in each of which the nuclei have completed the first division, and in which a second division is approaching. The difference in appearance of the nuclei is indicative of position only, not of structure. From *Peromyscus*.

Fig. 42. Cyst with sixteen nuclei bilaterally arranged. Axostyles, intracytoplasmic parts of antero-lateral and postero-lateral flagella persisting but disorganized, some chromidial material, peristome gone. Four chromosomes.

Fig. 43. Cyst with sixteen bilaterally grouped nuclei each with very small central karyosome, all cytoplasmic organelles gone except axostyles and postero-lateral flagella, many rounded chromidial masses. From *Peromyscus*.

Fig. 44. Cyst with sixteen nuclei each showing four chromosomes, bilateral arrangement not evident, blepharoplasts, axostyles, and intracytoplasmic parts of antero-lateral and postero-lateral flagella persisting, two masses of chromidial spheres. From *Peromyscus*.

Fig. 45. Free individual of *G. microtis* in early stage of multiple fission, all organelles intact, no chromidia, four axostyles indicated and eight nuclei present, bilateral arrangement of nuclei disturbed. From *Microtus*.

Fig. 46. The same stage of *G. microtis* with two axostyles and nuclei in bilateral arrangement, two chromidial masses present. From *Microtus*.

Fig. 47. An earlier stage of multiple fission in free individual of *G. muris* in prophase (lower nuclei) and anaphase (upper nuclei) of second division, two axostyles present, posterior peristome gone, anterior peristome degenerate, diffuse chromidial masses present.

Fig. 48. Somatella of *G. muris* in 8-nucleate, 4-zooid stage, with nuclei in late prophase of third division, the two zooids to the left in end-to-end position, the two to the right parallel to each other. From *Peromyscus*.

Fig. 49. Somatella of four small zooids, probably a disintegrative phase of a 16-nucleate, 8-zooid stage. From *Peromyscus*.
PLATE 8

*Giardia muris* (Grassi) and *G. microti* Kofoid and Christiansen. X 2720.
All figures from smear preparations.

Fig. 50. *Giardia muris* in prophase, nuclei with eight chromatin masses, abnormal condition in anterior halo in cytoplasm. From culture mouse.

Fig. 51. Optical projection of *G. muris* from anterior end. Note ventral location of antero-lateral flagella (intracytoplasmic part) and peristome, and halo about axostyles and parabasals. From culture mouse.

Fig. 52. Dorsal view of late anaphase in *G. muris* showing spindle fibers between diverging chromosomes. From culture mouse.

Fig. 53. Lateral view of *G. muris* showing a fiber passing from each parabasal to dorsal side of the axostyles and a problematical faintly granular line, found only in this preparation, passing from parabasal region towards the blepharoplast. From culture mouse.

Fig. 54. Lateral view of *G. muris* in situ on detached epithelial cells of intestinal wall of host. From culture mouse.

Fig. 55. Two free individuals of *G. microti* in end-to-end position similar to that found in so-called conjugation cysts. From *Microtus*.

Fig. 56. *G. muris* in late prophase showing the two parabasal bodies with chromatic fibers passing to the dorsal side of the axostyle. From culture mouse.

Fig. 57. *G. microti* in late prophase of binary fission with large karyosome, divided blepharoplasts and dividing axostyle. Note slender parabasals. From *Microtus*.

Fig. 58. Later prophase of *G. microti* with split skein. From *Microtus*.

Fig. 59. Metaphase of *G. microti* with blepharoplasts and anterior chiasma divided, axostyle more deeply cleft, daughter centrosomes in polar positions, four small chromosomes in equatorial plate in two distinct groups. From *Microtus*.

Fig. 60. Two free individuals of *G. microti* in end-to-end position, the upper one (in figure) with the first division completed and cytoplasmic organelles disintegrating, the lower is apparently in a late phase of total disintegration, even the nuclei taking on a diffuse appearance. From *Microtus*.

Fig. 61. "Conjugation cyst" of *G. microti* with two normal individuals in back-to-back position with ends reversed. From *Microtus*.

Fig. 62. The same in a later phase. Each nucleus has completed one division. In each "conjugant" there are two large nuclei with a large amount of chromatin (possibly parents of gametic nuclei) and two smaller ones with less chromatin (possibly first polar nuclei). From *Microtus*.

Fig. 63. The same in a still later phase. The lower "conjugant" is still in about the same phase as those in figure 62. The nuclei in the upper have all divided again. The first polar nuclei have each given rise to two equal small nuclei, and the other two each to one large nucleus with abundant chromatin in four chromosomes (the possible gametic nuclei?) and one smaller one with little chromatin but still with apparently four chromosomes (second polar nucleus?). From *Microtus*.

Fig. 64. Cyst of *G. microti* with sixteen equal nuclei, two degenerating axostyles and peristomes, the result of multiple fission.

[54]
THE CULTIVATION OF TISSUES FROM AMPHIBIANS

BY

JOHN C. JOHNSON
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THE CULTIVATION OF TISSUES FROM AMPHIBIANS

BY

JOHN C. JOHNSON

This paper gives the results of studies and experiments made during the last eight months on the cultivation of tissues in vitro, principally from amphibians; together with a slight modification in technique adopted in order to get a greater percentage of more active and even outgrowths from these tissues. In general, the technique used was that as first devised by Professor Ross G. Harrison in his successful cultivation of nerve tissue, modified and improved by Dr. Alexis Carrel and Montrose T. Burrows of the Rockefeller Institute for Medical Research. The usual thick slides with deep cups were used, over which were placed cover-slips closely sealed by vaseline. No. 1 coverslips proved to be the best, as with these the oil-immersion lens could be used to greater advantage.

Tissues were cultivated in plasma, lymph, blood serum, and blood serum mixed with Grübler's gelatine of different proportions and strengths. Lymph often gave good results, but the fluid drawn after the first few minutes generally became too thin to coagulate and thus was of little value. Pure plasma behaved in just the reverse manner, coagulating so readily as to be of great inconvenience. Best results were obtained by a medium first devised by Professor S. J. Holmes, consisting of blood serum and a two or three per cent Grübler's gelatine solution mixed about half and half. The reason for such a mixture rather than pure blood or blood serum is that the fluid can be kept, it seems, almost indefinitely. Serum two weeks old was just as effective as newly prepared material.
The amphibians used in these cultures were frogs of different kinds, *Plethodon, Siren lacertina,* and *Dicemycythus torosus.* The extreme smallness of *Plethodon,* allowing only two or three drops of blood, forbade its use. *Siren lacertina* proved too hard to sterilize, due to its slimy skin; frogs were not readily obtained, so in the majority of the work *Dicemycythus torosus* was used, and this proved to be very favorable material. *Dicemycythus* adults appear here at the beginning of the rainy season in December, and lay their eggs beginning in January and extending into March. In order to keep embryos inside of the gelatinous covering, and thus free from infection, until the latter part of April, the refrigerator was used.

In passing, mention should be made of attempts to cultivate goldfish tissue. Entirely negative results were obtained, due to the practical impossibility of getting non-infected blood serum, and even if this was obtained the tissues themselves were nearly always infected. Often nematodes were embedded in the muscular tissue. The fins and tail parts could not be successfully disinfected without killing the tissue itself.

The spherical form of the gelatinous egg capsules of *Dicemycythus* makes it very easy to wash and disinfect them in corrosive sublimate, without injuring the inner eggs or embryos in the least. Embryos giving best results were those about ten millimeters long, although any stage previous to breaking through the egg-case, gave good results. About eighty per cent of the cultures made from embryonic tissue showed very visible outgrowths.

Nerve outgrowths were more rare than any other type, yet possibly most interesting. One tissue was most vigorous, sending out twenty-six distinct fibers, some of them thirty-two micromillimeters in length. Harrison, in his first memorable work on nerve outgrowths, was first to demonstrate that the protoplasmic strand theory of nerve origin is entirely incorrect, that instead there is an active outgrowth of the nerve itself. Several observations were made to discover the precise method of nerve outgrowth. Under the oil-immersion lens could always be found an expanded bulb with two or more small amoeboid pseudopods at the tip of each nerve. Nearly always there was a dominant larger pseudopod which would determine the direction of growth. Increase in length of nerve was caused by these pseudopods creeping along, always with the very tips attached to the under surface of the cover-glass, producing here apparently a sort of rolling
motion from the surface away from, to the surface attached to the glass. Lack of dark granules here of some kind made it very difficult, in fact impossible, to trace the current of the protoplasm further, yet the above fact, coupled with the fact that always just behind the enlarged bulb the fibers are thinner than at any other point, seemed to show very definitely that there is an out-pulling of nerve tissue rather than an out-pushing (see figs. 1 and 2).

Another very interesting feature is the extreme frequency with which tissues of the head region formed gill-like structures. These thumb-like processes are very similar to the regular gill structures themselves, although generally smaller and often not as regular in outline.

A modification of technique generally used seems to me to be quite essential in order to get a greater percentage of immediate active outgrowths. This modification is the transition drop. I have often noticed that media in which tissues were placed failed to coagulate, particularly close around the tissue itself, although I knew the media to be coagulable previous to putting on the cover-slip. Failure to solidify around the tissue is always detrimental to outgrowth, at least slowing it down considerably as has been demonstrated by others, and verified in my own experiments many times. This failure to become solid I found to be due to the fact that no matter how small the instrument used upon which I transferred tissues from Ringer's solution to serum mixture on the cover-glass, there was always a considerable film of the solution transferred with tissues at the same time to the serum, thus allowing the tissue to float somewhat loosely rather than having the necessary solid support. To overcome this difficulty the tissue was always placed in a drop of same serum mixture where it was rinsed thoroughly of Ringer's solution and from here transferred to the drop of serum mixture on the cover-glass. In ease of retransference of tissues, individual drops have to be used in order to prevent the infection of one tissue by another. The only objection

Fig. 1. Outline of one nerve fiber as viewed under oil-immersion lens.
to this transition drop is that it requires nearly twice as much serum, which when dealing with very small animals has to be considered. Experiments with tissues from the same embryo in which the transition drop was used in one case and not in the other always showed that the former tissues gave outgrowths more frequent and more vigorous.

It was nearly always found that the flat drop proved more satisfactory than the deeper hanging drop, since it brings the tissue in contact with the cover-glass, upon which all tissues seem to develop best; also it enables the use of the oil-immersion lens which is so essential for detail work.

Some very large hanging-drop cultures were made by using a common Stender dish. The cover was coated with vaseline on the edge, and in the bottom of the dish was placed moist cotton to keep the serum from drying up. Although more outgrowths were noticeable, no particular advantage resulted, as the thickness of the lid forbids a close study by the high-power lens.

Accidentally in transference of a tissue which had sent out some vigorous epithelial outgrowths, bacteria were introduced which in a day developed large independent colonies. As one of these colonies

Fig. 2. Entire nerve outgrowths of one tissue as viewed with low-power lens. Tissue from anterior dorsal part of embryo.
enlarged it touched about midway an outgrowth, causing its activity immediately to be increased very greatly. This part of the outgrowth was more slender than its base. As there is no difficulty in performing this experiment it was tried several times, with always the result that if bacteria attacked the outgrowth other than at tip they acted as a stimulant for increased growth for about a day.

Many cultures were made in order to determine somewhat accurately the proper length of time that should elapse on the average between retransplantation of tissue from one drop of serum mixture to another. This was done by taking careful observation of the appearance of the cells; by noting the rapidity of reawakening of activity after being left different lengths of time in a drop of serum mixture, and lastly by observing the beating heart tissue.

The first two observations showed that two to four days is the proper length of time, but varying slightly with kind of tissue and temperature. The heart tissue observations perhaps gave more definite light along this line. The number of beats I thought would be lowered with the increased toxic condition of the medium resulting from katabolism. The temperature was kept to see if parts of heart tissue of the embryo responded to heat as does the adult heart. Results showed that there is a close relationship between the condition of the blood serum, temperature and rate of the heart beat. On February 21 at 4:30 p.m., heart tissue put up four days previously was noticed beating at the rate of 29 times per minute and regularly; at 8:30 a.m. of the next day it had dropped to 16 times per minute but still beating regularly; at 11:30 a.m. of the same day it had slowed down to 14 times per minute and was beating very irregularly, varying from three to six seconds between beats. This tissue was bathed in Ringer’s solution for fifteen minutes and retransferred for the first time at about noon. By 8:00 a.m. of the next day it had gained to 34 times per minute, beating regularly; by 9:30 a.m. of the following day it had increased to 38 times per minute; at 11:30 a.m. to 42 times per minute. Here I think it would have reached its maximum rapidity had the temperature remained the same in afternoon as in morning. At 1:30 p.m. it had decreased to 40+ times per minute, but the decline was counteracted by the rise of temperature in the afternoon of about twelve degrees. This increase of heart beat was evident each afternoon. According to this, heart tissue at least, and possibly most others also, ought to be washed in Ringer’s solution and changed to a new drop
of serum about every forty-eight hours if the temperature is around 60° Fahrenheit. Allowing tissue to remain one day longer in same medium, however, showed no decided retrogression of activity, although it could be noticed. The following table shows the complete life-history in serum of above discussed tissue. The tissue was prepared in forenoon of February 17; the first noticed heart action was on February 21 at 4:30 p.m.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Beats per minute</th>
<th>Regularity</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 21</td>
<td>4:30 P.M.</td>
<td>29</td>
<td>Regular</td>
<td>Evidently slowing down.</td>
</tr>
<tr>
<td>Feb. 22</td>
<td>8:30 A.M.</td>
<td>16</td>
<td>Regular</td>
<td>Less tissue active.</td>
</tr>
<tr>
<td>Feb. 22</td>
<td>11:30 A.M.</td>
<td>14</td>
<td>Irregular</td>
<td>Small amount of tissue active.</td>
</tr>
<tr>
<td>Feb. 22</td>
<td>12:00 m.</td>
<td>(Washed in Ringer's solution; transferred to new drop of serum.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb. 23</td>
<td>8:00 A.M.</td>
<td>34</td>
<td>Regular</td>
<td>Increasing amount of tissue active.</td>
</tr>
<tr>
<td>Feb. 24</td>
<td>9:30 A.M.</td>
<td>38</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 24</td>
<td>11:30 A.M.</td>
<td>42</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 24</td>
<td>1:30 P.M.</td>
<td>40</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 24</td>
<td>3:30 P.M.</td>
<td>39</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 24</td>
<td>5:30 P.M.</td>
<td>41</td>
<td>Regular</td>
<td>Increase with afternoon temperature.</td>
</tr>
<tr>
<td>Feb. 24</td>
<td>7:30 P.M.</td>
<td>42</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 25</td>
<td>8:30 A.M.</td>
<td>39</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 25</td>
<td>10:30 A.M.</td>
<td>39</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 25</td>
<td>1:30 P.M.</td>
<td>39</td>
<td>Regular</td>
<td>Slightly less tissue active.</td>
</tr>
<tr>
<td>Feb. 25</td>
<td>3:30 P.M.</td>
<td>41</td>
<td>Regular</td>
<td>Increase with afternoon temperature.</td>
</tr>
<tr>
<td>Feb. 25</td>
<td>5:30 P.M.</td>
<td>41</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 26</td>
<td>8:00 A.M.</td>
<td>34</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 26</td>
<td>10:00 A.M.</td>
<td>34</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 26</td>
<td>12:00 m.</td>
<td>35</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 26</td>
<td>2:00 P.M.</td>
<td>35</td>
<td>Regular</td>
<td>Very much less tissue active.</td>
</tr>
<tr>
<td>Feb. 26</td>
<td>4:00 P.M.</td>
<td>36</td>
<td>Regular</td>
<td>Increase with afternoon temperature.</td>
</tr>
<tr>
<td>Feb. 26</td>
<td>8:00 P.M.</td>
<td>36</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 27</td>
<td>9:30 A.M.</td>
<td>33</td>
<td>Regular</td>
<td>Only small part tissue active.</td>
</tr>
<tr>
<td>Feb. 27</td>
<td>11:30 A.M.</td>
<td>32</td>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>Feb. 27</td>
<td>12:30 P.M.</td>
<td>28</td>
<td>Irregular</td>
<td>Just outer tip active. Very weak.</td>
</tr>
</tbody>
</table>

The tissue was washed in Ringer's solution for fifteen minutes but the heart action failed to revive, although outgrowths resulted later. The tissue was purposely allowed to dwindle in activity (and amount of tissue active) to see how great the revival would be, but it was left a trifle too long. To avoid possible error in counting the beats, three-minute records were always taken, counting the first fraction of a beat as one and not the last fraction, and then dividing by three.

Often individual cells separated from the rest of the tissues would occasionally send out small strands of protoplasm, but the activity
was not as great or as long continued, as in cells attached to the tissue itself.

On several occasions vigorous tissue showing gill-like outgrowths were tested as to their response to heated pins placed just above and near them on the top of the cover-glass. Always vigorous contractions resulted if the pins were placed directly over them; if the pins were placed to one side the outgrowths would bend away from the stimulus until they came up against the main body of the tissue; if the hot pins were then placed on the opposite side this same gill-like structure would retreat in the other direction. This response continued for several times, when apparently from exhaustion, or too great heat, it gradually ceased.

Dozens of cultures were kept active from fifteen to twenty days; several from twenty to thirty days; three for thirty-three days, and two thirty-six days and forty-one days respectively. Both these latter ones were connective tissues and accidently dried up from failure to seal them up tightly by vaseline. The indications are that they would otherwise have continued activity for a much longer period.

SUMMARY


2. Gill-like structures appear on tissues from the head region of the body.

3. Flat drops of serum produced better results than deep rounded drops, causing tissue to be up against the solid cover-slip; they afford also better conditions for study with an oil-immersion lens.

4. A common Stender dish with moist cotton in the bottom offers a good way for cultivating a large quantity of tissue.

5. At other points than at the tips of outgrowths, bacterial infection at first increases activity.

6. *Dicyclophus* tissues thrive best when washed in Ringer's solution and transferred about every two days to a fresh medium.

7. Increased toxic condition of serum and tissue causes decreased heart activity. The heart beat increases in proportion to the increase of temperature.
8. Gill-like outgrowths can be moved in any desired direction by changing the position of a hot pin applied to the outer surface of the cover-slip.

9. Connective tissue was kept in a healthy growing condition for forty-one days, when it was accidently killed.

This work was carried on in the laboratory of Professor S. J. Holmes, whose criticisms and suggestions are sincerely appreciated.

*Transmitted September 10, 1915.*

**IMPORTANT REFERENCES**

**Carrel, A.**


**Carrell, A. and Burrows, M. T.**


1911b. An addition to the technique of the cultivation of tissues in vitro. *Jour. Exp. Med.*, 14, 244-247, pls. 25-27.

**Ebeling, A. H.**


**Harrison, R. G.**


**Holmes, S. J.**


**Sundwall, J.**

NOTES ON THE TINTINNOINA

1. ON THE PROBABLE ORIGIN OF Dictyocysta Tiara Haeckel

2. ON Petalotricha Entzi sp. nov.

BY

Charles Atwood Kofoid

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8. Distribution of River Otters in California, with Description of a New Subspecies, by Joseph Grinnell. Pp. 305-310, plate 14. October, 1914 ........................................ .05

NOTES ON THE TINTINNOINA

1. ON THE PROBABLE ORIGIN OF *DICTYOCYSTA TIARA* HAECKEL

2. ON *PETALOTRICHA ENTZI* SP. NOV.

BY

CHARLES ATWOOD KOFOID

1. ON THE PROBABLE ORIGIN OF *DICTYOCYSTA TIARA* HAECKEL

We owe to Professor Ernst Haeckel (1873) our first adequate information regarding the structure and relationships of those minute protozoans of the high seas known as the Tintinnoina, which build for themselves beautiful vase- or bell-shaped houses or loricae of delicate texture, elaborate patterns, and wide range of form. Among the species which he described was one from off Lanzerotte, in the Canary Islands, which because of its mitre-like structure he named *Dictyocysta tiara*. He published (1873, pl. 27, fig. 7) a figure of this species drawn in the flowing lines for which his facile hand is famous. We have reproduced this in our text-figure 1. The noteworthy features of this species as compared with those of all others described in *Dictyocysta* are the marked zone of contraction at the base of the oral fenestrae, and the very much contracted aboral region. There is less of this suboral constriction in other species of *Dictyocysta*, and none has the pointed tapering aboral end, but rather a hemispherical or at most convex-subconical one.

Since Haeckel’s (1873) discovery of this species no one has seen it, although Cleve (1901) ransacked hundreds of samples from the surface waters of the tropical and semitropical Atlantic and Brandt (1906, 1907) and Laackmann (1910) have monographed the group with extensive collections from these regions.
It is the general experience that pelagic protozoa are cosmopolitan and are found widely and quite generally in large numbers. There are, to be sure, especially among the highly differentiated and minute dinoflagellates of the family Dinophysidae, not a few instances in which only a very few individuals of a species have ever been seen. This may be due to escape through the meshes of the silk net on the one hand and thus not necessarily to rarity in nature, or on the other to actual rarity which is not unknown in nature among highly specialized tropical species of plants and animals, as among orchids, birds of paradise, and species of cowries (Cypraea). A classic instance in recent literature of a persistently rare species is Oenothera lamarckiana, and other cases are not unknown among fresh-water rotifers. However, in the case of Dictyocysta the genus is not extremely specialized and the other species are all fairly abundant and of wide distribution in all warm or temperate seas. The occurrence of a rare species in this genus is therefore to be looked upon with suspicion and some other explanation than rarity in nature sought for absence of records of its reappearance.

Brandt (1907) in his monograph of the Tintinnina of the Plankton Expedition reduces Haeckel’s species to Dictyocysta templum var. tiara with the comment: "Ich halte D. tiara nur für eine allerdings sehr sonderbare Formvarietät von D. templum und bezweifle, dass die Figur richtig ist. Sie gehört wohl—wie manche der anderen von Haeckel selbst gezeichneten Abbildungen—zu den ‘Kunstformen der Natur.’" The last reference is to the well-known art work in which Haeckel has assembled and portrayed, not always with scientific accuracy, the beautiful and bizarre forms of life, including many from the pelagic organisms of the sea.

The opportunity which my investigations of the past fifteen years have given me of becoming acquainted with micro-organisms of the pelagic life of the sea under various conditions, and especially my contact with the Tintinnina, has brought that experience which enables the investigator to detect the abnormal from the normal, or at least
to be cautious about single instances of unusual form or structure among organisms. Brandt's suggestion that Haeckel's *Dictyocysta tiara* is a "sehr sonderbare Formvarietät" has therefore my full accord. Furthermore, certain experiences with the species of *Dictyocysta* have afforded a clue to the probable source of Haeckel's *D. tiara*. It may be noted in passing that the correct name for *Dictyocysta templum* Haeckel is *D. lepida* Ehrenberg.

When the formalin in sea water in which the plankton containing *Dictyocysta* is permitted to evaporate under the cover glass the lorica undergoes a peculiar shrinkage, which distorts it as in Haeckel's (1873)

![Figure 2](image1.png)  
**Fig. 2.** *Dictyocysta lepida* Ehrenberg var., on uncontracted lorica. × 500. The loricae shown in figures 2 and 3 belong to *D. templum* var. *b*. Brandt.

![Figure 3](image2.png)  
**Fig. 3.** *Dictyocysta lepida* Ehrenberg var. × 500. Original showing contracting aboral region due to shrinkage.

figure. A lorica of *D. lepida* which is thus distorted is shown in text-figure 2. The same symmetrical collapsing of the aboral region which appears in *D. tiara*, reducing it to a tapering form much narrower than in the uncontracted stage (fig. 3), occurs also in this lorica as dessication ensues. We conclude therefore that Haeckel's (1873) figure of *D. tiara* represents only a large lorica of *D. lepida* with ten instead of the usual eight fenestrae which had shrunken in the aboral region as the result of dessication, perhaps on the plankton net in the interval between one collection and another, or on the side of the container, or in the course of examination upon the slide. Haeckel's lorica measures 100\(\mu\) in length, a size almost attained (95\(\mu\)) by *D. lepida grandis* Brandt.
Our figures are made from one of the smaller (55μ), stouter varieties. Had they been made from a more slender one such as Brandt’s *D. templum* var. *f.* or his var. *g. grandis* the resemblance to *D. tiara* would doubtless be more striking than in our figures.

2. ON *PETALOTRICA ENTZI* SP. NOV.

In the course of a revision of the genus *Petalotricha* Kent there have come to our attention two figures assigned among others by Entz, Jr. (1905, p. 131, figs. 30-32) to *Petalotricha ampulla* (Fol) Daday. These figures differ so strikingly from the others of this species reproduced in Entz’s paper and from material in our hands of this and all known species of the genus that their separate characterization as a distinct species seems desirable. These figures also differ from all published accounts and figures of species of *Petalotricha* or related *Tintinnoina*.

Entz’s figures of this form here described as new and of *Petalotricha ampulla ampulla* (Fol) Kent from the Adriatic at Quarnero are here reproduced for comparison.

*Petalotricha entzi* sp. nov.

Figures 4-6

*Diagnosis.*—Lorica cup-shaped, its length equaling diameter to edge of oral shelf, wall of nuchal region greatly thickened, aboral end hemispherical, oral rim, edge of oral shelf and nuchal ledge serrate, lower bowl with longitudinal striae. Length, about 100μ. Adriatic.

*Description.*—Lorica flaring cup-shaped, rotund aborally, its length 1.28 oral diameters, equaling that of the oral shelf. Bowl, collar, and oral shelf hidden externally by the thickening of the wall so that the nuchal constriction visible externally in all other species is here completely lost except for a slight nuchal concavity. The oral shelf thus exposes only an upper surface which slopes downward towards the lumen about 25° for about 0.11 oral diameter, where it meets the low oral rim. Its upper surface is somewhat concave and fluted, each ridge corresponding to a marginal serration on the edge of the oral shelf and an inner but smaller one in the oral rim (fig. 6). There are about 55 serrations in the circumference. Although the nuchal constriction is masked externally, it has a well-defined internal ledge which constricts the lumen to 0.8 the oral diameter and forms a shallow
gutter above its projecting angle. Its inner margin bears about 20 saw-tooth serrations pointed in the clockwise direction. It may be significant that this corresponds approximately to the number of membranelles described by Fol (1881) for *Petalotricha ampulla*. Short

Fig. 4. *Petalotricha entzi* sp. nov. After Entz, Jr. (1905, figs. 30–32). Figure 4 is a full lateral view showing optical section of the wall, fig. 5 a tilted lorica showing the fluted upper surface of the oral shelf, and the distinctly serrate angle formed by the inwardly projecting nuchal ledge, and longitudinal striae on the lower bowl. Figure 6 is an optical section through the oral region showing the 2–4 layers of alveoli between the inner and outer lamellae.

Fig. 7. *Petalotricha ampulla ampulla* (Fol) Kent, an unusually elongated lorica. Figure 8, the same, normal proportions. These two figures magnified about 333 diameters (based on Reichert obj. 8, comp. oc. 4, used by Entz).
striae pass from the depressions between the ridges on the oral shelf aborally on the inner sloping face of the collar for a short distance. The limits of the collar are visible in optical section or inner view only. It is 0.2 oral diameter in height and forms a cone of 30° contracting aborally.

The bowl is rotund, its length below the collar being 0.88 oral diameter, with only the least trace of external constriction below the level of the inner nuchal ledge. Its diameter is equal to the oral diameter, and its aboral end is almost hemispherical. From near the equator of the bowl there run posteriorly, apparently on its outer surface, parallel, equidistant faint lines. These appear to be shallow depressions and to be almost as many (24 on one face) as the flutings (27) on the oral shelf. Flutings and striae of this sort have not been described in any other species of *Petalotricha*.

The wall is composed of an inner and an outer lamella enclosing 2-4 layers of alveoli in the nuchal and collar regions and decreasing to one below the nuchal ledge. The greatest thickness at the nuchal ledge is 0.15 oral diameter. Below the ledge it decreases to 0.05. The usual band of circular or elliptical fenestrae, with their long axes vertical, is found on the upper half of the bowl. There are 2-3 irregular rows of areas of unequal size, none over 0.08 oral diameter in greatest diameter, and about 25 across one face. There are indications of a row of horizontally placed fenestrae in the oral rim.

*Dimensions.*—Entz, Jr. (1905) does not give measurements for these figures separately. Employing the manufacturer's statement of the magnification of "obj. 6, comp. oc. 4" used by Entz, we arrive at the following: Length, total, 100μ; collar, 18μ; bowl, 82μ; diameter oral, 87μ; oral shelf, 98μ; nuchal ledge, 62μ; bowl, 80μ.

*Comparisons.*—This species is wholly distinct from the others and is peculiar in the submerging of the oral shelf by the thickened wall of the nuchal region, in the fluted oral shelf and ridged bowl. In its morphological components in the nuchal region and structure of the wall it is, however, clearly a *Petalotricha*. The thickened wall is suggestive of the heavy wall of *P. capsu* Brandt, and the form of bowl and serrations of some of the varieties of *P. ampulla* figured by Brandt (1906), especially var. *b*, and, in the matter of serrations, vars. *d* and *e*.

*Transmitted September 28, 1915.*

Zoological Laboratory,
 University of California.
LITERATURE CITED

BRANDT, K.

CLEVE, P. T.

ENTZ, G., Jr.
1905. A Quarnero Tintinnidai. All. Köz., 3, 121–133, 36 figs. in text.

FOL, H.

HAECKEL, E.

LAACKMANN, H.
6. BINARY AND MULTIPLE FISSION IN

_Hexamitus_

BY

OLIVE SWEZY

7. ON A NEW TRICHOMEONAD FLAGELLATE,

_Trichomitus parvus_, FROM THE

INTESTINE OF AMPHIBIANS

BY

OLIVE SWEZY
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BINARY AND MULTIPLE FISSION IN

HEXAMITUS

BY

OLIVE SWEZY

Historical.—The generic name Octomitus was proposed by Prowazek (1904) for a flagellate with eight flagella so minute that, until within quite recent years, its form and number of flagella have been matters of great uncertainty. A review of the earlier literature on this genus has been given by Dobell (1909) and is not repeated here, beyond a few facts necessary to justify the name I have given to it.

The generic name, Hexamita, was proposed by Dujardin (1841) and was later slightly modified by Bütschli (1878) to Hexamitus. These organisms, though figured by both Dujardin and Bütschli as possessing six flagella, have since been conceded by most investigators to be identical with the eight-flagellated form as it is now described. The type form is H. inflatus Dujardin, a free-living flagellate.

As Dobell (1909) has so well pointed out, the various names which have been applied to this flagellate are not available, and there remains only the original name, Hexamitus, and the one proposed by Prowazek, Octomitus, to consider.

Prowazek (1904) created the genus Octomitus for a flagellate from the rat, but his organism was very evidently only a form of what had already been described as Hexamitus muris Grassi.

Dobell (1909), recognizing Hexamitus inflatus Duj. as the type species of Hexamitus, claims that the parasitic forms must have a different generic name, and therefore proposes to recognize Prowazek’s name Octomitus, thus making habitat and not morphological characters the basis of generic distinction. The accident or preference of habitat is hardly one which can safely be used with these organisms
as a generic character, unless this has so modified the structure of the organism that it has also become morphologically differentiated and significant. The ability to live in one medium rather than another, as in fresh water or the intestinal fluid, is dependent on chemical reactions and differences, and is often acquired as a secondary modification, as shown in the possibility of both free and parasitic life by the same organism, as in the case of trichomonad flagellates (Kofoid and Swezy, 1915b).

Alexeieff has added more confusion to the already existing chaos. In describing the flagellate from Motella tricirrata and M. mustela he (1910) uses Moroff’s name, Urophagus, rejecting Hexamitus, “parce qu’il exprime un caractère basé sur une observation inexacte (en réalité il y a 8 flagellés).” In 1911 Alexeieff describes the same or a similar flagellate from species of Triton and Axrotil and designates it as Octomitus intestinalis Prow, without reference to his previous acceptance of the name Urophagus intestinalis Moroff. Again in 1912 he figures Hexamitus parvus Alex. (“H. intestinalis Duj. pro parte = Octomitus dujardini Dobell pro parte”), and gives no reason for using a name which he had previously discarded. In 1914 he says, “Contrairement à l’opinion de Dobell et de Minchin on ne peut pas, pour agir conformément aux règles de la nomenclature, changer le nom Hexamitus pour l’Octomitus, malgré que ‘Hexamitus’ consacre une erreur d’observation (en réalité il y 8 flagellés et non 6),” and in the following paragraph gives both Hexamitus and Octomitus as two good genera of the family Hexamitidae.

In view of the fact that habitat alone cannot be used as a generic character, and that all the species thus far described are morphologically similar, different at the most only in specific characters, it is evident that the name Octomitus must be discarded in favor of the older term Hexamitus. That inaccurate observation was the basis of the first description and generic designation cannot be given as a reason for discarding the original generic name, as applicability from the standpoint of description is not a basis for testing the validity of generic names. This principle, if adopted, would throw out many generic names from the list of protozoan genera.

For these reasons we use, on the grounds of priority, as the generic name for the eight-flagellated protozoan, both parasitic and free-living, Hexamitus Dujardin, recognizing as the type species H. inflatus Dujardin, the first species in Dujardin’s (1841) paper.
Material and Technique

Occurrence.—Species of Hexamitus have been figured from a wide variety of hosts, including nearly all species of Amphibia which have been examined, as well as from fishes, snakes, tortoises, rats, and mice, and it is quite probable that further investigations will reveal a still greater number of hosts. One species, *H. inflatus*, has been figured from stagnant water.

The observations which follow were based on examination of *Hexamitus* from a variety of amphibians, *Diemyctylus torosus* Esch., *Ancides lugubris* (Hallowell), *Plethodon oregonensis* Girard, *Batracoscoops attenuatus* Esch., *Rana boylei* Baird, *R. draytoni* Baird, all obtained in and around Berkeley, California, and *Rana pipiens* Shreber from Illinois.

The region of infection has been the same in all the hosts examined, the rectum and large intestine, with special concentration about the point of junction of the large and small intestine. The entire length of the intestinal tract has been examined repeatedly, without showing the presence of flagellates elsewhere, except those very probably introduced with the instruments used.

Wet fixation for permanent preparations was used exclusively, hot Schaudinn’s fluid and Flemming’s solution giving the best results. Heidenhain’s iron haematoxylin was used for most of the work, though many other stains were tried with varying results.

Study of the living animal was made possible by sealing down the cover glass with vaseline, after adding a few drops of normal salt solution to the material from the intestine. *Intra-vitam* staining was tried with neutral red, methylene blue N, new methylene blue GI and Janus green, prepared with normal salt solution.

In spite of the wide range of hosts, one species, *Hexamitus intestinalis* Duj., seems to be the one commonly met with. It retains its specific characters through all the great diversity of environmental conditions. It is frequently the only protozoan found in the intestine and is then generally present in vast numbers. Sometimes associated with it, or sometimes alone, are other species, or it may be only varieties, which occur in much smaller numbers. The possibility of these latter being only developmental forms of *H. intestinalis* can be decided, as in the case of the other flagellates considered, only after an investigation of the complete life-cycle of these organisms has established the fact that the life-cycle here is not a simple, direct one as
data at the present time would seem to indicate. Until this has been
done they may each be ranked as distinct species. One of these forms,
Hexamitus ovatus sp. nov., is considered in the following pages, to-
gether with H. intestinalis Duj. and H. batarachorum sp. nov.

These investigations were begun at the suggestion of Professor
C. A. Kofoid, whose help and encouragement have been unfailing
throughout the course of the work.

**Hexamitus ovatus** sp. nov.

This flagellate has been found in abundance in only one host, *Dic-
myctylus torosus*, occurring only sparingly in the other amphibians
examined.

**Morphology**

The form of this flagellate is ellipsoidal to ovoidal, with the broader
end anterior and 6 to 8μ in length. The posterior end may be slightly
pointed, but generally it is more or less rounded. At the anterior
end is a mass of chromatin, the blepharoplast complex, which consists
of two granules (pl. 9, fig. 1), closely packed together so as to appear
as one in most cases. From these arise the six anterior flagella, three
from each granule. The flagella are usually from 1.5–2 times the
length of the body, but are frequently much longer.

Arising from the blepharoplast, one from each granule, and ex-
tending backwards through the cytoplasm to the posterior end of the
body in a curve parallel to and rather near the periphery, are two slender
axostyles, so narrow as to appear at times as scarcely more
than a line. Arising from the distal ends of these are two trailing
posterior flagella. The axostyles are very flexible and often appear
twisted owing to the movements of the body (pl. 9, fig. 2).

The nuclei are two in number and are situated immediately behind
the blepharoplasts, each being connected with a single granule of the
blepharoplast complex. The nuclei are elongated, rounded at both
ends and about 1.5 by 2 or 3μ in size (pl. 9, fig. 1).

The chromatin consists of one large, elongated, centrally located
mass, apparently continuous at the anterior end with the blepharoplast.
This chromatin mass is often curved or club-shaped and is nearly as
long as the nucleus. A very definite nuclear membrane is present
which, especially prior to division, usually takes a heavy stain with
iron haematoxylin (pl. 9, fig. 1).
The cytoplasm is granular and vacuolated, with no definite periplast and no distinction between ectoplasm and endoplasm. *Intra-vitam* staining with neutral red shows, with *H. ovatus* as well as *H. intestinalis*, the presence of a few, usually three or four, deeply staining granules in the cytoplasm of the posterior third of the body. In the living protoplasm a number of more or less highly refractive bodies or granules are visible, scattered through the cytoplasm. In spite of the lack of a structurally differentiated periplast, the body is notably uniform in outline, exhibiting few or no amoeboid tendencies.

**Binary Fission**

The splitting of the axostyles is the first sign of division in the trophozoite and is accompanied by a more or less rounding up of the body. The splitting is longitudinal, beginning at the anterior end and including the posterior flagella (pl. 9, fig. 3), at the same time the granules of the blepharoplast complex separate. The nuclei begin to round up, as do also the chromatic masses which come to lie in the centers of the nuclei, losing their connections with the blepharoplasts (pl. 9, fig. 4). The entire structure appears at this time as two large vesicular nuclei, each with a very large central spheroidal karyosome. With the division of the blepharoplasts of each nucleus the two daughter blepharoplasts, each with one of the daughter axostyles, move 180° apart to opposite poles of the nucleus, remaining connected by a slender, darkly staining fibril, the paradesmose (pl. 9, fig. 4). One flagellum is retained by one daughter blepharoplast, the other two going with the other daughter blepharoplast. In this as well as in the other forms of *Hexamitus* under observation a striking decrease in the amount of chromatin material in the blepharoplast complex takes place before the division of that body and the migration of the daughter blepharoplasts to the poles of the nuclei. No chromatin seems to appear, as such, in the cytoplasm at this time, the extruded material probably being absorbed.

When the new positions have been taken up by the daughter blepharoplasts, the karyosomes begin to assume an irregular appearance (pl. 9, fig. 5), and soon break up into a number of granules which later form a segmented spireme or skein (pl. 9, fig. 6). In just what way this changes into chromosomes has not been observed.

During this process the spindle fibers begin to form between the daughter blepharoplasts, which here function as centrosomes. The
spindle is composed of faintly staining fibers, usually few in number, formed inside the nuclear membrane. It is to be noted that the para-
desmose is outside the nuclear membrane and takes no part in the
formation of the spindle (pl. 9, figs. 7, 8). During this time also the
new flagella make their appearance as new outgrowths.

With the appearance of the spindle fibers the chromatin becomes
massed into two large granules, which apparently split (pl. 9, fig. 7)
before taking a position on the spindle. The number of chromosomes
is two, as shown by numerous figures in the late anaphase and telophase
stages. In the equatorial plane the chromatin can very seldom be
resolved into individual chromosomes, but appears as undifferentiated
masses (pl. 9, figs. 8, 9). Division is not always synchronous in both
nuclei, since one may lag somewhat behind the other (pl. 9, fig. 8).

In the anaphase, as the chromosomes move towards the pole, inter-
zonal spindle fibers can still be seen stretched between the chromosomes
as well as connecting them with the blepharoplasts or centrosomes (pl. 9,
figs. 8, 9). These interzonal fibers later disappear (pl. 9, fig. 10) and
the only parts of the spindle remaining are the short fibers connecting
the centrosomes with the blepharoplasts. These become darker appar-
ently through chromatin moving out along them from the chromo-
somes (pl. 9, fig. 10). In the reorganization of the nucleus in the
telophase this migration of chromatin takes place to a greater extent,
the amount of chromatin becoming greater at the same time, until the
large, club-shaped karyosome again appears (pl. 9, fig. 1) and the
nucleus is reconstituted.

There is no constriction and division of the nuclear membrane, but
instead this gradually fades out and disappears while two new mem-
branes are formed inside the old (pl. 9, figs. 10–12). The new mem-
brane, at its first appearance, is stained but faintly, gradually be-
coming darker until, after the complete disappearance of the old
membrane, it takes a black color with iron haematoxylin (pl. 10, fig. 13).
The paradesmose persists throughout these stages, disappearing only
with the reorganization of the nucleus.

Multiple Fission

In common with most of the members of the Polymastigina, as
well perhaps as in the majority of Protozoa, it has been found that
multiple fission is prevalent among the Hexamitidae, though whether
preceded by conjugation, or not, is as yet undetermined. Its occur-
ence in these flagellates has not heretofore been described as multiple fission, yet multinucleate forms, the product of multiple fission, are almost as frequently met with in smears from the intestinal wall as are stages in binary fission.

The process of multiple fission is accomplished by quickly repeated mitoses without synchronous division of the cytoplasm, thus resulting in a multinucleated plasmodium or somatella. Following the first division of the nuclei and the completion of the attendant organelles, the second division is initiated in the same way by the splitting of the blepharoplasts and axostyles (pl. 10, fig. 16). The next step, the moving apart of the blepharoplasts to take up polar positions 180° apart on each nucleus (pl. 10, fig. 17), is identical with the corresponding process in binary fission (pl. 9, fig. 4), save only in the increased number of nuclei in the organism as a whole.

The third division, giving rise to the nuclei and extranuclear organelles adequate for eight individuals, was not followed out fully. Figure 18, plate 10, with its ten nuclei, representing five individuals, shows that it had taken place in the earlier history of that somatella.

Division of the somatella, or plasmatomy, consists in the liberation of one individual at a time, in the manner described for the trichomonads (Kofoid and Swezy, 1915b).

The flagellates move about actively throughout the whole process of multiple fission. No evidence has been found thus far to indicate that multiple fission ever takes place while the flagellate is encysted. The constant lashing about of the flagella gives to the organism something of the rolling motion of Volvox. There seems to be no constant appreciable increase in size of the forms undergoing multiple fission as compared with those dividing by simple binary fission.

**Hexamitus batrachorum** sp. nov.

This flagellate is often present in the greatest abundance, and frequently individuals of this species may be found in hosts where the predominating species is one of the larger forms like *H. intestinalis*, though this is not always the case.

It has occurred in *Rana pipiens*, in *Batracoseps attenuatus* and sparingly in the other amphibians examined. It resembles, in its nuclear structure, a *Hexamitus* figured by Alexieff (1912) from a tortoise, *Nicoria trijuga*, in Ceylon, which he designates as *H. parvus*. His figures are accompanied by no description, however, but seem to
present distinctive characters which separate it from the flagellate upon which these observations are based. These characters are the point of origin of the flagella which as figured in H. parvus arise laterally in two groups widely separated from one another, while in the flagellate from amphibians they are anterior and closely connected. The extranuclear chromidial bodies (parabasals?) are of a definite shape and position in both forms, in H. parvus having a circular form and occupying positions between the axostyles, while in our species they are situated on the axostyles and nearer their posterior extremities than in H. parvus. Further investigation on both flagellates may reveal greater similarities, but for the present it seems best to treat them as separate species. I have, therefore, applied the name Hexamitus batrachorum to the flagellate described below.

On account of its size, it may be confused with the smaller forms of H. intestinalis. It is distinguished from this, however, principally by the structure of its nucleus, which is unlike that of the other species of Hexamitus.

Morphology

Hexamitus batrachorum is small, seldom exceeding 5 or 6 \( \mu \) in length by 3 or 4 \( \mu \) in width, and in general shape is ellipsoidal, rounded at both ends (pl. 10, fig. 21). The three pairs of flagella at the anterior end arise from two chromatic granules, the blepharoplast complex, which are often massed together indistinguishably. These rest upon the nuclear membranes and also give rise to the two slender axostyles which pass through the center of the cytoplasm, convex outwardly, to the posterior border of the cell, giving rise there to the two posterior flagella. Near the posterior ends of the axostyles two groups of chromatic granules are usually found, consisting of a common mass at the periphery, and a granule on each axostyle a short distance above the point of emergence.

The two rather large circular nuclei have very distinct membranes, which is one of the distinguishing characteristics of this species, as with the exception of H. batrachorum and possibly one other the nuclear membrane, if present, seems not to be distinct in Hexamitus. The chromatin is arranged in a number of small granules or clumps, sometimes one or two situated centrally, frequently one in the center and four or five lying on the nuclear membrane (pl. 10, fig. 21). The remainder of the nucleus seems to be devoid of chromatin. The axostyles
can be seen to pass over or under the nuclei to reach the blepharoplasts, and seem in no way to be connected with the nuclei.

The cytoplasm is granular, sometimes more or less vacuolated, and, in general, contains no food inclusions other than fluid-filled vacuoles.

Fission

The process of fission, binary and multiple, was not observed beyond the occurrence of numerous forms which showed that multiple fission takes place here as well as in the other species described. The forms noted (pl. 10, figs. 22, 23) are similar to the corresponding stages of *H. ovatus* and *H. intestinalis*.

**Hexamitus intestinalis** Dujardin

This is a variable form both as to size and general appearance, and yet it is quite evident from an examination of the figures given by different investigators that more than one species has been described under this name.

**Morphology**

*Hexamitus intestinalis* varies in size from 9 to 12μ in length and 5 to 8μ in width, though forms both above and below these limits are occasionally met with. In general outline the body is ovoidal, tapering more or less toward the posterior end (pl. 11, fig. 25), which may occasionally be metabolic in its appearance (pl. 11, fig. 27). Individuals are frequently met with which are rounded at the posterior end (pl. 11, fig. 26), thus showing three quite distinct bodily forms or changes. In hosts where the rounded, oval individuals are present the majority of the flagellates seem to belong to that type. When forms having the posterior extremity metabolic are found that type will be predominant in the preparations made, and the same thing is true of the third type with pointed posterior end. These changes do not seem to indicate any specific differentiation, but are rather different responses of one species to changes of medium, due to slight environmental changes. This, however, is merely a suggestion from observations. No attempt has been made to prove it by experiment.

The three pairs of flagella arise at the anterior end from two pairs of basal granules, the central ones of which may become fused and appear as one granule (pl. 11, fig. 25). One flagellum arises from each of the outermost granules and two flagella from each of the inner
granules of this double blepharoplast complex. The apparent sizes and positions of these granules vary greatly. Sometimes they are separated by distinct spaces and again they are massed into one granule from which all the flagella appear to arise (pl. 11, fig. 27). The flagella are equal in length and are frequently two or even three times the length of the body.

Extending backwards from the blepharoplast complex are two slender, outwardly convex, hyaline axostyles, which terminate at the posterior border of the body in small chromatin granules from which arise the two posterior trailing flagella (pl. 11, fig. 25). The axostyles show a clear, homogenous structure and appear to have a very definite boundary. They may be more or less widely separated in the body and are frequently crossed in certain aspects. They are not rigid structures, but are very flexible, turning and bending easily with the movements of the protoplasmic body.

The most characteristic structures of *H. intestinalis* are the two nuclei. These are situated in the anterior part of the body, immediately behind the blepharoplast complex (pl. 11, fig. 25). In the ordinary trophozoite these show no definite structure and seem also to be devoid of a nuclear membrane, consisting only of a large club-shaped mass of chromatin material, from 3 to 4 or 5 μ in length. The anterior ends of these are often massed together with the blepharoplast complex, exhibiting together the general shape of a horseshoe (pl. 11, fig. 27). The proximal ends of the axostyle unite with each other and then pass forward to the blepharoplast complex in a rather broad band which divides again, one half going to each half of the blepharoplast (pl. 11, fig. 40). The two nuclei are attached to the blepharoplast complex, one on either side of the axostyles to which they are not attached.

The precise relations of the neuromotor apparatus cannot be made out in the ordinary preparations, but in many cases what appears to be cytoplasmic degeneration has resulted in the loss of all the surrounding cytoplasm, leaving the nuclei with the attached motor apparatus intact, as shown in figure 40, plate 11. These appearances indicate that the neuromotor apparatus is a structural unit (Kofoid and Swezy, 1915a, b).

The protoplasm is alveolar with granules closely packed between the alveoli. In very many of the smaller individuals the alveoli are but indistinctly marked off and the granular structure is more conspicuous. The occurrence is often noted of two large vacuoles in the anterior part, closely pressed between the axostyles and the blepharo-
plast complex. What special significance these may have could not be determined. Their persistence in degenerated forms where the cytoplasm has entirely disappeared would suggest that they were not mere protoplasmic vacuoles (pl. 11, fig. 41).

No differentiation of the cytoplasm into ectoplasm and endoplasm has been observed, the body being covered, apparently, by a very thin periplast.

**Binary Fission**

Binary fission in *Hexamitus intestinalis* follows the same general process already outlined for *H. ovatus*. The early prophase shown in figure 28, plate 11, shows the completion of the splitting of the blepharoplasts, axostyles and posterior flagella and the beginning of the migration of each daughter group to opposite poles of the nucleus. The great diminution of the chromatin material in the blepharoplast complex is even more striking here than in *H. ovatus*. The early prophase shown in figure 29, plate 11, where the large mass of chromatin is broken up and only a few granules remain in the nucleus, may or may not have some significance in the process, though it is hard to correlate it with the earlier and later stages. It is quite probable that it is the result of some abnormality or some phase of degeneracy.

The apparent lack of a nuclear membrane is here quite striking, the spindle evidently lying entirely free in the cytoplasm (pl. 11, figs. 30, 31).

Here, as also in *H. ovatus*, multiple fission is a common mode of multiplication at some period in the life cycle (pl. 11, figs. 37, 38, 39) and probably results in a somatella of eight pairs of nuclei, i.e., potentially eight trophozoites.

**Discussion**

The first attempts to portray this process of multiple fission in *Hexamitus* were made by Foà (1904) and Wenyon (1907), but a few stages only were figured. These agree with certain stages abundant in my own preparations. Dobell (1909) refers to the figures given by these investigators as "merely degenerate and fused forms which have nothing whatever to do with division." His own explanation of division as consisting of the absorption of both axostyles and caudal flagella, the division of the nucleus and the appearance of new axostyles
and flagella, the caudal ones being either a new outgrowth or formed by a drawing out of the axostyles at the point of severance, is unconvincing in the extreme. Alexeieff (1911) in Hexamitus from Triton cristata gives a more connected series of division figures which are in accord with those given here. Details of chromatin and nuclear division he has not figured, however.

The two types of division shown here differ only in that the spindle formation is intranuclear in Hexamitus ovatus and no membrane is apparent in H. intestinalis, with the lack also of membrane formation in the telophase stage of division in the latter species. The whole process presents many points of similarity to that figured for the trichomonad flagellates (Kofoid and Swezy, 1915a, b), notably in the formation of a skein or spireme, a constant number of chromosomes and their division before taking a position on the spindle, and the relation of the flagella to the centrosome or blepharoplast during division. The dissolution of the old nuclear membrane and the formation of two entirely new ones may be taken as a step forward, similar to the process evolved in metazoan mitosis.

The idea has already been brought forward (Kofoid and Swezy, 1915a, b) that the axostyle of Trichomonas represents an intracytoplasmic flagellum, one of the accessory motor organelles of the body. The behavior (during division) of the axostyles in Hexamitus, homologous organs, is strongly corroborative of that interpretation. At this time they may be distinguished from the flagella only by their greater thickness, great motility being shown in the constant change of position to which they are subject.

**Summary**

Cell division in Hexamitus is a simple form of mitosis, initiated by division of the blepharoplasts, followed by longitudinal splitting of both axostyles. Four chromosomes are found on the mitotic spindle, two going to each daughter nuclei. New nuclear membranes are formed inside the old one, which fades out and disappears before the completion of the process of cell division.

Multiple fission takes place in the encysted forms by a series of successive divisions of the two nuclei and the accompanying motor apparatus without corresponding division of the cell body, forming a somatella of eight undivided binucleate individuals. These later break
up by successive splitting off of one individual at a time. The binuclear structure of the potential individuals is maintained throughout the process.

The processes of both binary and multiple fission are similar throughout in at least two species, *Hexamitus ovatus* and *H. intestinalis*. Multiple fission has been observed in a third species, *H. batrachorum*.

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**LITERATURE CITED**

ALEXEIEFF, A.


1912. *Sur quelques protistes parasites intestinaux d'une tortue de Ceylon (Vicoria trijuga).* Zool. Anz., 40, 97-105, 3 figs. in text.


BÜTSCHELL, O.


DOBELL, C. C.


DUJARDIN, F.


Fol, A.


KOFORD, C. A., and SWEZY, O.


PROWAZEK, S.


WENYON, C. M.

1907. *Observations on the Protozoa in the intestine of mice.* Arch. Prot. Suppl., 1, 169-197, pls. 10-12, 1 fig. in text.
EXPLANATION OF PLATE 9

All figures were drawn by camera from preparations fixed in hot Schaudinn's fluid and stained with iron haematoxylin.

Binary fission in Hexamitus ovatus, sp. nov. from Diemyctylus torosus. \( \times 2583 \).

Fig. 1. Trophozoite showing six anterior and two posterior flagella, blepharoplast, nuclei and axostyles.

Fig. 2. The same, axostyles twisted.

Fig. 3. Prophase of division, showing splitting of the axostyles.

Fig. 4. Prophase of division: blepharoplasts and motor apparatus occupying polar positions in relation to the nuclei, connected by paradesmoses.

Fig. 5. Prophase of division with the karyosome breaking up into granules.

Fig. 6. Prophase with spireme formation.

Fig. 7. Prophase with choromosomes emerging from spireme (?)

Fig. 8. Metaphase of division, two chromosomes visible in upper nucleus.

Fig. 9. Early anaphase of division, chromosomes moving towards poles.

Fig. 10. Early telophase: beginning of formation of new nuclear membranes, chromosomes visible.

Fig. 11. The same: old nuclear membrane beginning to fade.

Fig. 12. The same stage. Chromosomes distinct.

[84]
EXPLANATION OF PLATE 10

Binary and multiple fission in *Hexamitus ovatus* sp. nov. (figs. 13–20) and *H. batrachorum* sp. nov. (figs. 21–24). × 2583.

Fig. 13. Completion of division of organelles; note persistence of paradesmose.

Fig. 14. A later stage: paradesmose has disappeared.

Fig. 15. Complete reorganization of nuclei preparatory to final separation of two individuals.

Fig. 16. Beginning of multiple fission: axostyles divided; karyosome breaking up.

Fig. 17. Migration of blepharoplasts to opposite sides of nuclei before spindle formation. Paradesmoses formed.

Fig. 18. Somatella of five sets of organelles of individuals the product of multiple fission and subsequent disintegrative separation of a part of the trophozoites.

Fig. 19. The same, with four individuals.

Fig. 20. The same stage; note variation in size.

Figs. 21–24. *Hexamitus batrachorum* sp. nov. from *Rana pipiens*.

Fig. 21. Trophozoite showing characteristic nuclear appearance.

Fig. 22. Somatella with four pairs of nuclei, product of multiple fission.

Fig. 23. The same, with three pairs of nuclei.

Fig. 24. The same, with two pairs of nuclei.
EXPLANATION OF PLATE 11

Figs. 25-41. *Hexamitus intestinalis* from *Dicymycytlus torosus*. × 2583

Fig. 25. Trophozoite with oval form; note characteristic nuclei without nuclear membrane and with large blepharoplast complex.

Fig. 26. Trophozoite with rounded extremities; axostyles crossed.

Fig. 27. Trophozoite with metabolic posterior extremity. Blepharoplast and nuclei fused.

Fig. 28. Early prophase of division; separation of blepharoplasts with their attendant motor organelles.

Fig. 29. Prophase of division; unusual appearance of chromatin.

Fig. 30. Prophase; spireme with spindle fibers appearing.

Fig. 31. Prophase with spindle formation.

Fig. 32. Prophase with equatorial plate.

Fig. 33. Metaphase of division.

Fig. 34. Anaphase of division.

Fig. 35. Telophase of division with beginning of reorganization of nuclei.

Fig. 36. Beginning of final separation of daughter individuals.

Fig. 37. Somatella, the product of multiple fission with seven pairs of nuclei.

Fig. 38. Somatella with six pairs of nuclei.

Fig. 39. Somatella with three pairs of nuclei.

Fig. 40. Neuromotor apparatus after degeneration of cytoplasm, showing connection of nuclei and axostyles with blepharoplasts.

Fig. 41. The same, showing the presence of two large vacuoles between the nuclei.
ON A NEW TRICHOMONAD FLAGELLATE,  
*TRICHIOMITUS PARVUS*, FROM THE  
INTESTINE OF AMPHIBIANS  

BY  
OLIVE SWEZY  

In the course of a series of investigations of the parasitic Protozoa found in amphibians a hitherto undescribed form has been met with which has undoubted trichomonad affinities. Since, however, it lacks two of the most striking features of that group, the axostyle and cytostome, it must be placed in a separate genus. The name *Trichomitus parvus* is proposed for this new genus and species.

It has been found in abundance in *Batracoseps attenuatus* Eschs. and more rarely in *Diemycylus torosus* Eschs., from near Berkeley, California, and in *Rana pipiens* Shreber from Illinois. The place of occurrence of this parasitic flagellate was the same in all these forms, that is, the upper part of the large intestine.

**Morphology**

*Trichomitus parvus* is a small spheroidal flagellate, almost elliptical, sometimes nearly circular in outline, varying from 5 to 10 μ in length and from 4 to 8 μ in width. The living animal presents the appearance of a minute ball and moves with a rapid rotating motion.

The comparatively large nucleus is situated at the anterior end, immediately behind the blepharoplast and touching it (pl. 12, fig. 1). It is globular in shape and in staining with iron haematoxylin always, or nearly always, shows a darkly staining border, the nuclear membrane. Part of the chromatin is sometimes massed around the periphery in blocks, usually about five in number, with a central karyosome (pl. 12, fig. 11). In many other cases nearly all of the chromatin is massed in a single central karyosome (pl. 12, fig. 2).
Lying against the anterior surface of the nuclear membrane is the rather large blepharoplast from which the motor apparatus arises. This consists of three equal anterior flagella, about 2.5 times the length of the body (pl. 12, fig. 4) in length, together with an undulating membrane which passes backwards on the surface to the posterior end of the body and terminates in a trailing flagellum. The membrane is well developed, often having a width of 2 or 3μ, and is bordered along both edges by deeply staining lines. The outer or chromatic margin is very slender, and the one at the line of attachment to the body, the chromatic basal rod, or parabasal body, is slightly thicker and more conspicuous by reason of its deeper stain. The membrane is without granulations or apparent structure. The outer margin greatly exceeds the inner one in length and is thrown into 5–10 ripples or folds (pl. 12, fig. 2).

The membrane is in constant motion in the living animal, undulations passing from the anterior end posteriorly through the length of the membrane. The whole structure closely resembles in its details the same organelle in Trichomonas, and in the living condition the animal is easily mistaken for a species in that genus.

The protoplasm is alveolar and shows no differentiation into ectoplasm and endoplasm, neither is there any definite, structurally differentiated pellicle present. The contour of the body is quite regular, with few or no amoeboid tendencies which might be expected from the lack of a definite pellicle. Such movements are very pronounced in Trichomonas (Kofoid and Swezy, 1915b). No indications of food particles have been observed in the protoplasm. There are, however, numerous fluid-filled vacuoles ranging in size from 0.2–2μ crowding the protoplasm at all stages except in a narrow zone about the nucleus. These are smaller and less distinct during multiple mitosis (pl. 12, figs. 12–17).

**Mitosis**

Owing to the extremely minute size of Trichomitus pareaus, the process of binary fission is very difficult to follow in all its details. A sufficient number of stages have been found, however, to indicate that division is by a simple form of mitosis comparable with that described for Trichomonas (Kofoid and Swezy, 1915a).

The beginning of the process is the division of the blepharoplast (pl. 12, fig. 4), and probably of the undulating membrane, though no actual evidence on this latter point has been obtainable thus far. As
the daughter blepharoplasts move apart both undulating membranes are found well developed and nearly equal in size (pl. 12, fig. 5). The behavior of the chromatic basal rod or parabasal body has not been quite clear, but since the second one, when first observed in the dividing cell, does not take a dark stain with iron haematoxylin, this reaction appearing later, it is probably a new outgrowth, as is the case with the same organelle in the trichomonads.

The new flagella begin to make their appearance as new outgrowths as soon as the blepharoplast has divided (pl. 12, fig. 5), two of the old flagella going to one daughter blepharoplast and one to the other.

With the beginning of mitosis a rearrangement of the chromatins takes place (pl. 12, figs. 5, 6). The karyosome breaks up into a number of granules (fig. 4) and these later become arranged into a short skein or spireme (fig. 5). The breaking up of this into a definite number of chromosomes was not very clear, but indications of this are found in figures 6 and 7. As shown in the late telophase stage of division (figs. 9, 12), the number of chromosomes is apparently two. Some indication of a larger number is found in figure 7, but it is quite possible that this indicates rather a precocious splitting of the chromosomes as in Trichomonas (see Kofoid and Swezy, 1915a).

Division of the nucleus takes place within the nuclear membranes. The formation of a definite spindle has not been observed, but a comparison of the several stages shown with the corresponding stages found in Trichomonas and allied forms (Kofoid and Swezy, 1915b) will at once show the very close resemblance of the process in Trichomonas to that described for other trichomonads and is suggestive that the missing stages will be found to resemble the corresponding stages of the other species. This point, however, is not insisted upon. It is sufficient to call attention to the striking similarities in the division cycles of all the members of the three genera referred to, namely, Trichomonas augusta, T. muris, Tetratrichomonas provazcki, and Entrichomonatia lacertae, and leave it to future investigation to supply the missing details.

Throughout mitosis and even after the completion of the division of the nucleus and the separation of the two daughter nuclei the blepharoplasts remain connected by a darkly staining line, the paradesmose (pl. 12, fig. 9). It lies outside the nuclear membrane and is apparently attached only to the blepharoplasts. This line gradually loses its staining reactions and disappears.
Each chromosome is connected with the blepharoplast by a slender fibril (pl. 12, fig. 9), apparently the remains of a central spindle. These fibrils later disappear and the chromatin becomes distributed in the usual manner of the resting nucleus of the trophozoite (pl. 12, fig. 11).

The final division of the protoplasm does not take place immediately upon completion of nuclear division, as binucleated forms are generally quite abundant in those preparations in which division is found to occur.

**Multiple Fission**

In addition to the process of simple binary fission described above multiple fission also takes place. What relation this process has to the complete life cycle can only be conjectured and its solution must await further investigation.

The process of multiple fission (pl. 12, figs. 12–16) takes place without the individual becoming encysted. Whether or not it is preceded by conjugation has yet to be determined. Multiple fission consists essentially of repeated divisions of the nucleus and its associated motor apparatus, by the process already outlined for simple binary fission (pl. 12, fig. 12), but without the synchronous division of the cytoplasm, and followed by disintegrative plasmotomy. Division takes place three times, resulting in an organism or somatella containing eight nuclei and their attendant motor organelles (pl. 12, fig. 13). This somatella is globular in shape and moves with a rolling motion in no constant direction, owing to the incessant lashing of the forty-eight flagella on all sides.

Plasmotomy or division of the somatella into single individuals takes place with comparative slowness, one individual being budded off at a time, resulting successively in 7-, 6-, 5-, 4- (pl. 12, figs. 14, 15), 3- (pl. 12, fig. 16), and 2-cell (pl. 12, fig. 17) stages. Active locomotion continues throughout this disintegrative phase of the somatella.

The question might be raised as to the possibility of *Trichomitus parvus* being only a developmental form of *Trichomonas*. So far there has been no evidence brought forth to show that *Trichomonas* passes through a developmental cycle which includes so great a difference of structure as exists between these two forms. On the other hand, evidence seems to point to the fact that *Trichomitus* passes through a cycle, including binary and multiple fission comparable to that already described for *Trichomonas*. This fact, in itself, would not preclude
the possibility of such a relation existing, but the entire lack of any transitional stages would suggest that it is, for the present at any rate, only a remote possibility. In view of these considerations and also because there is no genus in which it can consistently be placed, it is proposed it establish for it a new genus, \textit{Trichomitus}, belonging near \textit{Trichomonas} among the Tetramitidae. The generic characters consist of the following:

\textbf{Trichomitus} \textit{gen. nov.}

A motor apparatus consisting of three equal anterior flagella arising from a single blepharoplast, with a well-developed undulating membrane extending posteriorly along the surface of the body and terminating in a posterior trailing flagellum. The membrane is bordered by a chromatic margin and has at its base the chromatic basal rod or parabasal body. The nucleus is placed anteriorly, immediately behind the blepharoplast. There is neither cytostome nor axostyle present. The type species of this genus is \textit{Trichomitus parvus}. The type slide is in the protozoological collections of the Department of Zoology of the University of California, and a cotype slide has been sent to the United States National Museum.

Grateful acknowledgments are due to Professor C. A. Kofoid for the help and encouragement given throughout these investigations.

\textit{Transmitted September 30, 1915.}

\textbf{Zoological Laboratory.}
\textbf{University of California.}

\textbf{LITERATURE CITED}

\textit{Kofoid, C. A., and Swezy, O.}


EXPLANATION OF PLATE 12

*Trichomitus parvus* gen. nov., sp. nov.

All figures from preparations fixed with hot Schaudinn’s fluid and stained with iron haematoxylin, drawn with camera lucida and Zeiss 2 mm. apochromatic oil immersion. × 2583.

Fig. 1. Trophozoite, showing nucleus, blepharoplast, three anterior and one posterior flagella and undulating membrane with its chromatic margin and chromatic basal rod or parabasal body.

Fig. 2. Large trophozoite, showing variation in size.

Fig. 3. Trophozoite, showing variation in nuclear structure (central karyosome).

Fig. 4. Prophase of division; with splitting of the blepharoplast and outgrowth of one new flagellum.

Fig. 5. Prophase with the daughter blepharo-plasts on opposite sides of the nucleus, spireme formation of the chromatin, and extra nuclear paradesmose.

Fig. 6. Slightly later stage of the same.

Fig. 7. The same stage, with possibly a differentiation of the chromatin into (splitting?) chromosomes.

Fig. 8. End of prophase with chromatins massed at the center of nuclei. "Amphinaster" stage.

Fig. 9. Telophase with two chromosomes bound to the blepharo-plasts by short fibrils: outgrowth of full complements of flagella.

Fig. 10. Later stage, showing reorganization of nuclei: note persistence of paradesmose connecting daughter nuclei and blepharoplasts.

Fig. 11. Stage preparatory to final separation of the daughter organisms.

Fig. 12. Second division of the nucleus in process of multiple fission. Division not synchronous.

Fig. 13. Eight-nucleated somatella, showing division of nuclei completed, each with full set of organelles.

Fig. 14. Stage in plasmotomy showing four-nucleated organism.

Fig. 15. The same, showing variation in size.

Fig. 16. Three-nucleated somatella.

Fig. 17. Final stage in plasmotomy.
ON *BLEPHAROCORYS EQUI* SP. NOV., A NEW CILIATE FROM THE CAECUM OF THE HORSE

BY

IRWIN C. SCHUMACHER

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ON BLEPHAROCORYS EQUI SP. NOV., A NEW CILIATE FROM THE CAECUM OF THE HORSE

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IRWIN C. SCHUMACHER

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INTRODUCTION

During the spring of 1915 while studying the ciliates from the caecum of the horse I became particularly interested in one of a rather bizarre shape. Upon consulting the literature on this subject I found that Fiorentini (1890) and Bundle (1895) had described certain forms apparently closely allied to the one in question. But on more detailed investigation I noticed differences of such a nature as to convince me that I was dealing here with a form not described by either of the above mentioned authors or by others.

The aim of the present paper is to give the general morphology of this form and to compare it with the closely allied forms described by Fiorentini (1890) and Bundle (1895).

ACKNOWLEDGMENTS

This work was carried on at the University of California, at Berkeley, under the direction of Professor C. A. Kofoid. Whatever
there be in it of merit or value is due chiefly to his kind and helpful suggestions and to the interest he always showed therein. My acknowledgments and thanks are also due Mrs. Purington for her valuable help with my drawings and to Mr. E. B. Talt of Albany for his kindness and thoughtfulness in aiding me to obtain the material for this work.

Technique

The material used in this work was obtained at Mr. Talt’s ranch at Albany, thirty minutes from the laboratory by car. Because of the difficulty of obtaining material at frequent intervals and because of the large amount that could be obtained from one horse, the caecum of which frequently contained as much as five gallons of semi-fluid food, it was both necessary and possible to obtain material for study in the living and fixed condition from the same host. The methods of procuring the material are essentially the same as those described by Dr. R. G. Sharp (1914) in his paper on Diplodinium caudatum.

In every case extreme care was taken to avoid any fall in the temperature of the caecal fluid between the time of killing the horse and studying the living material. This was equally true of the fixed material. That the material was well fixed was shown by the fact that protozoans were obtained with cilia extended and without contractions or contortions of the body.

Schaudinn’s alcoholic sublimate and Zenker’s fixing fluids, used hot, gave uniformly the best results. When followed by Heidenhain’s iron-alum haematoxylin or Delafield’s haematoxylin, with a counter-stain such as eosin, acid fuchsin or erythrosin. in toto mounts were obtained which gave a clear differentiation of the nuclear and cytoplasmic structures.

In my study of the ciliates from the caecum of the horse I have identified the following forms previously reported from Italy or Germany: Cycloposthium bipalatum (Fiorentini) Bundle, Parasotricha colpoidea Fiorentini, Didemis ovalis Fiorentini, and Didemis quadrata Fiorentini.

I have found no forms corresponding to the following species: Blepharocorys uncinata (Fiorentini) Bundle, Blepharocorys valvata (Fiorentini) Bundle, Blepharocorys unifasciculatum (Fiorentini) Sharp, and Blepharocorys jubata Bundle (1895).

However, as stated above, I find a form which combines the generic characters as described by Bundle (1895) for Blepharocorys, but does
not agree with any of the above. This new species I call *Blepharocorys equi*.

Of the ciliates occurring in the cæcum of the horse, this was one of the most difficult to study, both on account of its small size and its rather complicated structure. It was always found in large numbers and in four out of the five horses examined. Its absence in this one case may be correlated with the fact that the host was in an abnormal condition both as regards temperature, which was high, and abnormal intestinal conditions, due to enteritis.

**Morphology**

Seen from the right side (pl. 13, fig. 1), this animal has approximately the shape of a kernel of rice, somewhat asymmetrical, the dorsal side being convex, the ventral side slightly concave, and the ends rounded.

The body, which is constant in form, is stiff, non-contractile, inelastic and about three times as long as it is wide. It is thickest in the region through the anterior end of the macronucleus (*mac.*, pl. 13, fig. 2). It decreases slowly in thickness from here posteriorly, but anteriorly more rapidly. The greatest width is in a plane passed through the body about midway between the dorsal and ventral surfaces.

The anterior end, as seen in side view (pl. 13, figs. 1, 3), contains a large vestibule (*vest.*, pl. 13, figs. 1, 3, 5), the lower wall of which is formed by a ventral lip (*v. l.*). Its dorsal wall is formed by the frontal cap (*fr. c.*). This frontal cap is covered dorsally and partly continuous laterally with two dorsal plates (*r. d. pl., l. d. pl.*). Leading from the vestibule posteriorly into the body beyond its center and showing clearly both the living and preserved animals is a large gullet or oesophagus (*ocs.*). The anus (*an.*), situated at the posterior extremity of the body a little to the ventral side. Dorsal plates, oesophagus, oral region, ventral lip and anal region are ciliated as described later.

In size this *Blepharocorys* stands about midway in the series of the ciliates from the cæcum of the horse. The following table gives the dimensions of several members of this species:
Animal measured | L. | T. | Animal measured | W. |
--- | --- | --- | --- | --- |
1 | 49 | 22 | 7 | 14 |
2 | 50 | 22 | 8 | 16 |
3 | 50 | 22 | 9 | 14 |
4 | 50 | 25 | 10 | 14 |
5 | 50 | 22 | 11 | 18 |
6 | 50 | 22 | 12 | 11 |
Average | | 22 |  | 14 |

L.—Length of body, without anterior process, in microns.
T.—Thickness of body through the region of the macronucleus, in microns.
W.—Width of body at level of the micronucleus, in microns.

Measurements of the anterior process give a mean value of 9–11 μ for that part projecting from the body.

The ectoplasm is a firm, thin and refractive peripheral layer of homogeneous appearance beneath the cuticle. The entoplasm shows under high magnification (2500) no definite structures, but appears to be a more or less homogeneous mass containing small particles of food. So far as could be determined no concretion vacuoles were ever present.

The whole body is covered by a thin, tough, cuticle of high refractive index. There are no surface markings or striations of any kind.

From the dorsal part of that end pointed anteriorly in locomotion there projects a corkscrew-like structure, the anterior process (ant. pr., pl. 13, figs. 3, 5). This process makes two turns, is rounded on its anterior end and the terminal portion lies in a plane at right angles to the long axis of the body. Posteriorly, it passes in an irregular path through the outer covering of the body and, gradually diminishing in thickness, passes along in the ventral part of the frontal cap, then dorsal to the oesophagus and finally bending slightly toward the ventral side ends just posterior to the end of the oesophagus. It is clear, highly refractive, homogeneous in appearance, and of marked rigidity. Its path through the entoplasm can be traced as a light line. Its length in all is not quite that of the entire animal; that part which projects anteriorly from the body is about one-fourth the total length of the process.

What its function is I cannot say. At least two explanations may be suggested: (1) that it is a result of transverse fission, and (2) that it serves as a hook with which the ciliate may attach itself to other objects, such as the intestinal epithelium. I have never seen it used in this manner, nor as an organ of offense or defense, nor have I seen the final stages in fission.
That it is a very definite and constant structure is shown (1) by the fact that in no case could a ciliate of this form be found in which it was lacking, (2) by its great rigidity, and (3) by the fact that it is always wound the same way, i.e., so as to form a right-hand screw.

In the anterior ventral region of the body there is a deep impo
ceting resulting in the formation of the vestibule (vest., pl. 13, figs. 1, 3, 5) mentioned above. This vestibule extends the entire width of the body. Its lower wall is made up of a ventral lip or mentum (v. l., pl. 13, figs. 1, 2), thickest in the mid-ventral region, thinning out dorso-
laterally on each side until it reaches almost to the mid-lateral lines and extending about one-fifth of a transdiameter farther posteriorly on the left side than on the right side of the body.

The dorsal wall of the vestibule is formed by the frontal cap (fr. c., pl. 13, figs. 2, 5), a prolongation of the body anteriorly and dorsally to the mouth (or., pl. 13, figs. 1, 3). It is thickest in the middle and extends laterally on each side to form the dorso-lateral edges of the vestibule.

Arising in common from the mid-dorsal line of the frontal cap throughout its entire length are two plates, known respectively as the right and left dorsal plates (r. d. pl. l. d. pl., pl. 13, figs. 1, 2), the left one being much the larger of the two. Neither plate lies wholly in one plane. In its anterior part (pl. 13, figs. 1, 2, 5) the left dorsal plate extends laterally and ventrally around the end of the frontal cap and ends at a point just to the right and posterior to the corre-
sponding end of the right dorsal plate. The main part of the plate arches away from the body slightly, curves ventrally around the left side of the body and extends along its whole ledge over the left margin of the ventral lip (v. l., pl. 13, figs. 1, 2), thus forming a lateral wall for the vestibule on the left side of the body. Posteriorly, however, its margin does not extend as far back as the posterior lateral edge of the vestibule; there is left in this region consequently an opening into the vestibule posterior to the left dorsal plate (pl. 13, fig. 3).

The right dorsal plate, like the left, extends laterally and ventrally around the end of the frontal cap. The remaining part simply arches away from the body laterally and, unlike the left dorsal plate, bears little relation to the vestibule.

Situated on the anterior dorsal side of this ciliate and best seen in a dorsal view (pl. 13, fig. 4) is a zone of cilia, the dorsal ciliary zone. These cilia (d. cil., pl. 13, figs. 2, 4, 5), while not broad enough to be called membranelles, are fully as long as the adoral membranelles of
the ventral lip. Seen in side view (pl. 13, figs. 1, 3, 5), they appear to arise from the entire dorsal surface of the dorsal plates. This impression is still further strengthened while observing the living animals, for the cilia then part to the sides of the anterior process and hang down over each side. That they do not arise in such a manner is, however, clearly shown in a dorsal view. Here they are seen to take their origin in a gradual curve, convex anteriorly, extending from the middle of the dorsal surface of the left dorsal plate to the extreme right side of the body.

On the ventral side of the ventral lip (c. l., pl. 13, fig. 2), extending obliquely from the extreme anterior mid-ventral point to the point where the lip meets the left side of the body and then passing over its margin just to the edge of the vestibule, is a row of adoral membrandelles (ador. m., pl. 13, figs. 2, 5). The longest of these membranelles is a little less than one-fourth of a transdiameter of the whole body. Their free ends point anteriorly and ventrally in the fixed material.

The mouth or oral opening (or., pl. 13, figs. 1, 3) is an irregular, poorly defined opening leading directly into the oesophagus. It is situated at the left posterior end of the vestibule close to the ventral side of the body.

The oesophagus (oes., pl. 13, figs. 1, 3, 5) lies in an oblique position with reference to the long axis of the body, its anterior end connecting directly with the oral opening in the left ventral part of the body, its posterior end dorsal and on the right side of the body. The right wall of the anterior one-half of the oesophagus forms a concavity in the endoplasm, around which fits the left wall of the macronucleus. The posterior end, beginning at a point about seven-eighths of its length from the anterior end, bends sharply to the ventral side. The oesophagus is essentially round in cross-section and at its anterior end is about one-sixth of a transdiameter wide. The lumen gradually narrows as the posterior end is approached and disappears at a point in the endoplasm approximately midway between the macronucleus and the posterior end of the body. The oesophagus is ciliated along its dorsal wall with fine cilia, increasing in size and continuous anteriorly with the oral membranelles.

The oral membranelles (or. m., pl. 13, figs. 1, 5) are small blade-like structures, the longest being about one-half the length of an adoral membranelle. They arise in a line from the dorsal wall of the vestibule just anterior to the oral opening. Posteriorly they decrease in length and are continuous with the cilia of the oesophagus.
The macronucleus \((\text{mac.}, \text{pl. 13, fig. } 2)\) is situated in the anterior one-half of the body, dorsal and to the right of the oesophagus. In general the macronucleus has a rather constant size, shape, and position in the animal. In side view (pl. 13, fig. 1) it is heart-shaped, with the point extending posteriorly. Seen in a dorsal or ventral view \((\text{mac.}, \text{pl. 13, figs. } 2, 4)\), its shape is more irregular, and it appears blunt and rounded on both ends. Its diameter remains fairly constant until just posterior to the micronucleus, where it is greater. On its dorsal surface near the right anterior side there is a small invagination in which the micronucleus partly lies, and, as mentioned above, in its left side there is a pronounced groove, along which the right wall of the oesophagus runs. The macronucleus is distinctly granular. After iron-alum haematoxylin stain these granules stand out very distinctly and clearly, but owing to the thickness of the nucleus it was impossible to count them in toto preparations.

Measurements of several macronuclei yield the following figures:

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<th>Animal measured</th>
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<tr>
<td>6</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

Average, 13 9 6

\(L.\)—Greatest length in microns.
\(W.\)—Greatest width in microns.
\(T.\)—Greatest thickness in microns.

The micronucleus \((\text{mic.}, \text{pl. 13, figs. } 2, 5)\) may be clearly distinguished even in the living non-stained animals. Here it appears by transmitted light as a shining little body situated in a depression of the right dorsal surface of the macronucleus, close to its anterior end. It appears finely granular in structure and refracts light more strongly than does the macronucleus. In the stained material it is seen to be an ellipsoidal structure, finely granular, staining deeply, and separated from the macronucleus by a clear space.

The single contractile vacuole \((c. \ v., \text{pl. } 13, \text{figs. } 3, 4, 5)\) is situated in the posterior ventral part of the body in very close relation to the anal tube. It frequently reaches the size of a sphere with a diameter equal to one-half a transdiameter of the whole body.
The anal opening (an., pl. 13, figs. 3, 5) lies at the posterior end of the body slightly to the ventral side. It is more easily distinguished in a view from the left (an., pl. 13, fig. 3), where it is seen to be connected with an anal tube (an. t., pl. 13, fig. 3) which extends into the body to a point on the posterior dorsal side of the contractile vacuole. This suggests the idea that the contractile vacuole stands in connection with the anus through the anal tube. Whether this is really the case I am unable to say.

The posterior end of the body around the anal opening is covered with cilia (an. cil., pl. 13, figs. 2, 3, 4). In length these stand between those of the dorsal ciliary zone and the adoral membranelles, on the one hand, and the oral membranelles and the cilia of the gullet on the other.

Reproduction, so far as I have observed, is by transverse fission, one stage of which is shown in figure 4, in which the micronucleus has divided and the new gullet and oral region are forming.

Observations on the Living Material

Observations on the living animals made under conditions as nearly normal as possible show that they travel at a rate of speed less than that of the other ciliates from the caecum of the horse. The normal course taken by this animal in forward movement is not in a straight line, but is more or less that of a spiral, with frequent turnings about the long axis of the body. This turning is due in part to the asymmetrical arrangement of the adoral membranelles and in part to the curvature of the anterior process.

The movement of the adoral membranelles is wave-like, beginning at the extreme anterior end of the zone and extending slowly along its whole length to the posterior end.

The cuticle shows clear and transparent, with no striations. Ectoplasm and entoplasm are clearly defined, the latter much darker in color and filled with fine particles of food. At certain levels the macronucleus and micronucleus can be easily distinguished, the macronucleus having a grayish granular appearance and the micronucleus appearing as a bright, round, more highly refractive body. The contractile vacuole shows up very clearly in the living animals. The contractions are regular; but instead of being sudden, as in Paramecium, are slow and more of the nature of true pulsations.
Systematic Status

The genus Blepharocorys as established by Bundle (1895) included the following species:

- *Blepharocorys uncinata* (Fiorentini) 1890, Bundle, 1895.
- *Blepharocorys valvata* (Fiorentini) 1890, Bundle, 1895.
- *Blepharocorys unifasciculatum* (Fiorentini) 1890, Sharp, 1914.
- *Blepharocorys jubata* Bundle, 1895.

The first two of these species had been described by Fiorentini (1890) under the names of *Diplodinrum uncinatum* and *Entodinium valvatum*. Bundle (1895) showed that these two species were undoubtedly referable to his genus *Blepharocorys*. And, moreover, on the basis of their assigned characteristics they could not be correctly referred to the genera *Diplodinium* and *Entodinium*, or even to the family Ophryoscolecidae.

Bundle (1895) does not discuss *Diplodinium unifasciculatum* Fiorentini; but it can be seen at a glance that this animal cannot be correctly assigned either to the genus *Diplodinium* or to *Entodinium*. In my opinion it may be referred to the genus *Blepharocorys* Bundle (see Sharp, 1914), hence its name becomes *Blepharocorys unifasciculatum* (Fiorentini) Sharp. It lacks (?), however, the spiral anterior process. A glance at the animal Fiorentini has figured as *Diplodinium unifasciculatum* shows that it could never be confounded with *Blepharocorys cqui*.

*Blepharocorys cqui* may be distinguished from *Blepharocorys uncinata* (Fiorentini) by the shape of its frontal cap, which in *Blepharocorys cqui* is covered by a right and left dorsal plate and by a zone of cilia on its dorsal surface. In *Blepharocorys uncinata* (Fiorentini), as described and figured by Bundle, there are no such plates present and the dorsal part of the frontal cap, i.e., the "Sternkuppe" of Bundle, is not ciliated. Furthermore, there is present in *Blepharocorys cqui* a ventral zone of adoral membranelles; this zone is not present in *Blepharocorys uncinata* (Fiorentini).

*Blepharocorys cqui* differs greatly from *Blepharocorys valvata* (Fiorentini) and *Blepharocorys jubata* Bundle by the fact that in both of these latter species an anterior process is lacking, whereas it is one of the most constant characteristics of *Blepharocorys cqui*.

The most important characteristics of *Blepharocorys cqui* may be summed up as follows:

Outer covering of body stiff, inelastic and noncontractile. Body about three times as long as wide, a slender corkscrew-shaped anterior
process projecting therefrom. Dorsal surface more or less convex; ventral surface slightly concave. Right and left dorsal plates on an interior prolongation of the body, the frontal cap. No retractile peristome. Mouth a simple more or less circular opening situated in the left posterior end of a vestibule near the ventral side of the body. Oesophagus funnel-shaped, ciliated along its dorsal surface. Cilia on dorsal plates, in gullet and anal region; membranelles in oral and ventral lip regions. Rest of body naked. Large heart-shaped macro-nucleus and a single small micronucleus. Anus in the posterior end of the body. A single large contractile vacuole in the anal region. Locomotion slow, with frequent turning about the long axis of the body. Food consisting of bacteria and fine pieces of fodder. Length, 50μ. Found in the caecum of the horse.

Transmitted September 30, 1915.

Zoological Laboratory, University of California.

LITERATURE CITED

Fiorentini, A.
1890. Intorno ai protisti dell'intestino degli equini (Pavia, Bizzoni). 24 pp., 5 pls.

Bundle, A.

Sharp, R. G.
EXPLANATION OF PLATE 13

*Blepharocorys equi*

Camera lucida drawings; structures underlying surface shown in outline. X 1875.

Fig. 1. View of right side of body.
Fig. 2. Ventral view of body.
Fig. 3. View of left side of body.
Fig. 4. Dorsal view of body.
Fig. 5. Transverse division stage. View of right side of body.

ABBREVIATIONS

ador. m.—adoral membranelles.
an.—anal opening.
an. cil.—anal cilia.
an. t.—anal tube.
ant. pr.—anterior process.
c. v.—contractile vacuole.
D.—dorsal side of body.
d. cil.—dorsal cilia.
fr. c.—frontal cap.
L.—left side of body.
l. d. pl.—left dorsal plate.

mac.—macronucleus.
mic.—micronucleus.
oes.—oesophagus.
or.—oral opening, mouth.
or. m.—oral membranelles.
R.—right side of body.
r. d. pl.—right dorsal plate.
V.—ventral side of body.
vest.—vestibule.
v. l.—ventral lip.
THREE NEW HELICES FROM CALIFORNIA

BY

S. STILLMAN BERRY

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S. STILLMAN BERRY

In the course of a somewhat extended study having in view an attempted correlation of the life-zones now recognized as holding for plants and most of the higher vertebrates with the distribution of some favorable group of invertebrates, considerable material has been collected which requires consideration that the results may be utilized as an aid in the further work. The present paper, so far as the two Southern California species are concerned, is therefore to be regarded as one of a preliminary series which will be issued at irregular intervals as the material collected seems to require. Two species and one subspecies of land snails are here described as new, all of them well-marked and interesting forms.

Epiphragmophora petricola, new species

Shell depressed, conspicuously umbilicate, the umbilicus having a diameter of about one-ninth the greater diameter of the shell. Color a warm golden brown, becoming a little paler and yellower on the base, and with a broad and very conspicuous dark chestnut-brown band on the shoulder, bordered above and below by a slightly narrower band of a tint lighter than the body of the shell; the color in dead shells soon bleaching to a more yellow tone, becoming a dead white upon the final loss of the epidermis. Lip (except in old specimens) but little thickened and only slightly reflected save at the pillar, where it tends to cover the edge of the umbilicus.

Epidermis somewhat glossy, more so in shells found dead, but which have not become bleached. Whorls 5½; neanic whorls showing a very fine granulation, with numerous minute radial wrinkles superimposed; three or four spirally disposed series of small, distinct, quite regularly spaced, elongate tubercles appear likewise, the latter almost
quincunxially arranged, so that they appear ranked in oblique as well as spiral series, but on the later whorls the number of tubercles increases so rapidly and irregularly that the oblique arrangement is soon lost, the lines of growth at the same time becoming more prominent, and the fine underlying granulation less distinct or even obsolete; sculpture of penultimate whorl transitional toward that of the last whorl, where the tubercles have given way to a series of rather weakly incised spiral lines, more or less interrupted where they cross the lines of growth, and becoming obsolete below the shoulder.

Spire varying from very depressed to low conic; whorls convex, the last whorl descending in front. Aperture ample and very oblique. Edges of peristome somewhat converging and connected by a very thin, transparent, parietal callus.

On the penultimate whorl a rather high power reveals in certain lights a number of exceedingly delicate, sharp, distantly spaced, oblique, incised lines, intersecting the lines of growth almost at right angles.

Greater diameter of shell, 29.5 mm.; lesser diameter, 23 mm.; height from umbilicus to apex, 13 mm.

**Type.**—Cat. no. 3480 of the writer's collection; paratypes in the collections of the University of California, and the private collection of Mr. Allyn G. Smith.

**Type Locality.**—A rocky talus slope on the southeast wall of Mill Creek Canon, San Bernardino Mountains, California, near the old road, about 1 ½ miles from the cañon mouth, altitude about 3250 feet; ten dead shells. A. G. Smith and S. S. Berry, Jan. 7, 1914; three living specimens. A. G. Smith, May 12, 1914; one living specimen. S. S. Berry, April 8, 1915.

**Remarks.**—This fine helicoid, one of the largest of the southern fauna, is distinguished by the aforementioned characters from all others known to me. It perhaps resembles a very large and extremely flattened form of *E. traski* more than any of the other Californian species, and I believe the two species to be rather nearly allied, though the situations in which they are respectively to be found are very dissimilar. *E. pelvicola* was first discovered while quarrying through a rocky slide in the possible hope of obtaining *Micraria anta* or *Sonorella*, genera as yet unknown from the San Bernardino Range. The species does not seem to be an abundant one, and several hours' arduous labor in turning over large blocks of stone and clearing out the detritus.
repeated on several occasions, have yielded to date only a single adult living specimen, all the remainder being immature or merely dead shells. While probably occurring all through Mill Creek Cañon, and perhaps neighboring parts of the range in favorable situations, only the one slide of the few so far examined has yielded specimens. A find by Mr. Smith of several shells on or near the surface leads to the belief that the species is not always, if ever, of strictly subterranean habit, at least not in the same sense as *Somarcella*.

**Epiphragmophora tudiculata rufigera**, new subspecies

Shell low conic, rounded, narrowly umbilicate. Color a warm brown, the apical whorls a little paler, with a broad (1.5-2 mm.), conspicuous, very dark, chestnut-brown band on the shoulder, bordered above and below by a slightly narrower band much lighter and yellower in tint than the body of the shell. Peristome moderately thickened, scarcely reflexed above, but more so below, and deflected over the umbilicus so as sometimes nearly to cover it.

Surface very glossy, but roughened by the occasionally quite conspicuous lines of growth and by the very fine, copious, even malleation, which covers almost the entire shell; the malleation scarcely developed on the apical whorls or the region immediately adjacent to the umbilicus. Neanic whorls very finely radially wrinkled and sparsely quincuncially papillose; earlier succeeding whorls with traces of a delicate incised spiral sculpture.

Whorls about 5½, the last descending in front. Aperture oblique, the end of the peristome somewhat converging and connected by a thin, transparent, whitish callus.

Greater diameter of largest shell, 28 mm., of type, 27 mm., of smallest, 22.5 mm. Lesser diameter of largest shell, 22 mm., of type, 21 mm., of smallest, 17.5 mm. Height (umbilicus to apex), of largest shell, 14.5 mm., of type, 13 mm., of smallest, 11.0 mm.

Type.—Cat. no. 3481 of the writer's collection; paratypes in the collections of the University of California and the California Academy of Sciences.

Type Locality.—Among leaves, shrubbery, and piles of lumber, and under boards, near the southeastern entrance to Cañon Crest Park, Redlands, California; S. S. Berry, 1911 to 1915, thirty-five specimens.

Remarks.—Pilsbry has given a brief summary of the described races of *E. tudiculata*, the common chaparral snail of Southern California, in *The Nautilus*, vol. 27, p. 49, but the present handsome form
is similar to none of them except the E. t. subdolus of Hemphill. Specimens from Hemphill's original material are now before me, and undeniably evidence a close relationship, but the Redlands specimens differ very constantly in their warmer, brighter coloration, much broader and darker color band, swollen base, obese spire, and less spreading outline. A few of the shells show a tendency to approach subdolus in one or more of these features, but the majority of these are immature or appear more or less pathologic in some way. Normal specimens so far as seen are quite uniform.

This is the common Helix of the hills about Redlands, being especially abundant in the Heights region of the city, where the orange orchards cease and the chaparral begins, but from other localities I have not seen it. The race of ludiculata found in the neighboring mountain canyons appears subspecifically different, and, curiously enough, seems to resemble the typical coast form much more nearly than it does either rufiterra or subdolus.

Polygyra pinicola, new species

Shell small, roundly conic, thin, covered copiously with numerous small, slender, epidermal hairs, arranged almost quincuncially in lines oblique to the very weak lines of growth. Embryonic whorls at first nearly smooth, then finely radially wrinkled, and showing a sculpture of small elongate granules. Spire low, somewhat convex, sutures impressed. Whorls about 5½. Body whorl almost angled at the first third, deeply constricted back of the peristome, the base swollen and rounded; very slightly descending in front. Lip white, thickened and reflexed, but not very wide; narrowed below the pillar, then again flaring slightly so as partially or even entirely to close the minute, scarcely permeable umbilicus; lip sometimes with a slight extra thickening at the base, otherwise without evidence of teeth; upper and lower ends connected by a barely perceptible wash of callus. Color a light brownish horn, without trace of a band.

Greater diameter of largest shell, 13 mm., of type, 12.5 mm., of smallest, 11 mm. Lesser diameter of largest shell, 11.5 mm., of type, 11 mm., of smallest, 9 mm. Height (umbilicus to apex), of largest shell, 6.5 mm., of type, 6.7 mm., of smallest, 5 mm.

Type.—Cat. no. 3482 of the writer's collection; paratypes in the collections of the University of California and the California Academy of Sciences.
Type Locality.—Under logs in the pine and oak woods just back of Pacific Grove, Monterey County, California; S. S. Berry, April, 1908, seventeen specimens.

Remarks.—A shell of this species taken by me in the summer of 1906 was then reported (Nautilus, vol. 22, p. 40) as P. columbiana armigera Aney. Armigera, however, as evidenced by one of Aney’s original specimens now in my possession, is quite a different thing. P. pinicola is well characterized by the complete absence of even traces of teeth, its almost imperforate umbilicus, narrow peristome, thin transparent shell, and small size. A few years since it would surely have been ranked as a subspecies of P. columbiana, and such may well prove its proper position, but the very large series of western Polygyras studied by me affords absolutely no intergrades, and the weight of all the evidence seems to be almost wholly in favor of recognizing the distinctness of the two forms. Phylogenetically, pinicola undoubtedly represents an extreme southern outpost of columbiana, which has been in isolation long enough for the development and fixation of its own special characters.

Shells of the usual type found in the neighborhood of Santa Cruz, just across Monterey Bay, are widely different, having less similarity to pinicola than some of the more northern forms.

As indicated by the given measurements, the species is remarkably constant in size, as well as in its other characters.

Transmitted December 29, 1915.
ON *TRYPANOSOMA TRIATOMAE*, A NEW FLAGELLATE FROM A HEMIPTERAN BUG FROM THE NESTS OF THE WOOD RAT *NEOTOMA FUSCIPES*

BY

CHARLES ATWOOD KOFOID AND IRENE McCULLOCH

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I. INTRODUCTION

The discovery by Chagas (1909) in Brazilian human trypanosomiasis of a trypanosome which passes one stage in an hemipteran bug, Conorhinus megistus, allied to the so-called "kissing-bug", has placed all biting Hemiptera which infest mammals under suspicion. For a number of years reports have come to us from time to time, especially from prospectors in the desert regions of California and Arizona, of certain black bugs which infest their shacks and bite the
occupants at night. The bites are annoying, cause local inflammation, and general malaise, but no permanent ill effects so far as is known. Among the various insects, principally Coleoptera, which have been sent in to us as the supposed disturbers were a few individuals of Triatoma protracta Uhler.

We are indebted to Mr. E. P. Van Duzee for its identification, and also to him and to Mr. W. S. Wright for a supply of bugs which have been secured from the nests of the wood rat, Neotoma fuscipes.

It is the purpose of this present preliminary paper to place on record the discovery, in the digestive tract of this bug from the nest of the wood rat, of a trypanosome in various stages of development, which, since it is associated in the stomach of the bug with the remnants of mammalian blood corpuscles, is presumably a parasite of the blood of the wood rat. We hope to be able to elaborate its life-history more fully in a future paper.

The most completely known life-history of a trypanosome is that of Trypanosoma lewisi of the rat, which has the flea for its invertebrate host. We owe to Minchin and Thomson (1915) our fullest account of the stages in the invertebrate host. Since the evidence at hand indicates that the form we have found in Triatoma is in some important particulars much like those which these investigators found in the flea, rather than like the stages described by Chagas (1909) in Conomphalus for Schizotrypanum cruzi, we shall follow the terminology of the former authors rather than that of the Brazilian investigator, with the important exception that we will call the kinetosome the parabasal body (see Kofoid, 1916). Our paper should therefore be compared with that of Minchin and Thomson and especially with their diagrammatic presentation of the life-cycle in the flea, as shown in their plate 45.

In brief, the cycle which they have found is as follows: The trypanosome ingested by the flea with the blood of the rat passes through a stomach, and a later rectal phase. In the former it retains its trypanosome characters. In the early stomach phase the nucleus and parabasal are far apart, the latter being well towards the posterior end of the body. It then enters an epithelial cell of the stomach wall, undergoes multiple fission therein, and emerges as a smaller, more slender merozoite with the parabasal somewhat nearer the nucleus. These merozoites may repeat the process of intracellular multiple fission in the stomach, or may pass into the rectum, where they transform at once into the erythridial phase marked by the migration of
the parabasal body anteriorly to a position in front of the nucleus. In this erithidiial stage binary fission occurs and the flagellates become either stout haptomonads attached by their flagella to the surface of the rectal epithelium, or slender motile nectomonads which swim about freely and may re-enter the pylorus, where they apparently may give rise to haptomonads, to rounded-up forms (latent bodies?), or to the terminal little trypaniform stages which pass out through the rectum. As in the pylorus, so also in the rectum, the termination of the process appears to be the small trypaniform stage, though here, too, the small rounded-up form is also found. No stages of sexual reproduction have been detected by these authors at any stage.

The material of Trypanosoma triatomae at our disposal does not enable us to outline its full life-history in the bug, but such stages as we find appear to fit admirably into the scheme of development as determined by Minchin and Thomson (1915) in the flea. We will now proceed with a brief account of these and a comparison of them with the corresponding phases of T. lewisi.

Trypanosoma triatomae sp. nov.

II. THE TRYpanosome Phase

1. The early stomach phase.—There was but a single bug in our material which had recently fed. Its stomach contained a mass of half-digested blood swarming with trypanosomes of large size (pl. 14, figs. 1–8). In these stages the body is 20–30 μ in length and 2–3 μ in width. The body has about two undulations, the posterior one occupying more than two-thirds of the total length. The anterior 0.2 of the body tapers abruptly into the short flagellum, which is also less than 0.2 of the body in length. The nucleus is 3–6 μ in length and 0.6–1.6 μ in diameter. It lies characteristically at one side against the concave surface a little behind the middle of the body. It is diffusely granular in our one lot of material of this stage. The flagellum continues as a chromatic marginal thread posteriorly along the convex side of the body to the parabasal body, with which it appears to merge in most cases. The blepharoplast is everywhere small in this species and may be found on closest inspection as a small granule at the base of the flagellum (pl. 14, figs. 6, 7). From it there runs toward the parabasal body in some instances (fig. 6) a fan-shaped suspensory body or parabasal rhizoplast. In a number of instances of Giemsa-stained individuals a chromatic thread could be traced posterior to
the parabasal as a slender strand terminating in a small chromatic granule. This extension is possibly an axostyle and the terminal granule is not to be regarded as the blepharoplast.

The parabasal body, or so-called kinetonucleus, is found in these early stomach phases at varying levels from the extreme posterior tip (pl. 14, fig. 1) to a level half way from the tip to the posterior end of the nucleus (fig. 8). It varies much in size, shape, and degree of stainability or of extraction of the stain. It may be spheroidal (fig. 1), ellipsoidal (fig. 5), trilobed (fig. 8), or even irregular (fig. 7) in shape. It is intensely stained in some instances (fig. 7), shows a central dark granule and a peripheral lighter zone (fig. 6), or may have a light center and dark ends (fig. 1). These facts tend to support the view advanced by us (Kofoid, 1916) that the "kinetonucleus" is in reality the parabasal body or kinetic reservoir, fluctuating in the volume of its substance with the changing internal conditions and motor activities.

The position of this parabasal body is not fundamentally and morphologically an axial one at the base of the flagellum, but rather a lateral one, attached to the blepharoplast and pendant by the fan-shaped parabasal rhizoplast. In certain views it appears to be axial (pl. 14, figs. 8, 18). In others (figs. 5–7), even in the typical trypanosomes of the early stomach phase, it appears to be laterally attached. This spatial relation is most clearly demonstrable, however, in the crithidial phases (pl. 14, figs. 14, 15).

The cytoplasm in our material of the early stomach phase stains rather deeply, leaving a clearer zone running lengthwise below the marginal flagellum in the undulating membrane. The stainability of both nucleus and cytoplasm and the variability of the parabasal are indicative of a physiological state in the organism verging on degeneration, though not far advanced in any of the individuals.

2. Late stomach phase.—Minchin and Thomson (1915) find that intracellular multiple fission ensues rather quickly, intracellular stages appearing as early as eight hours after the flea had fed upon an infected rat and continue for as much as five days, and possibly longer. None of our material has been examined within two days after possible opportunity to feed on the wood rat, so that the possibility that some at least of the trypanosomes had already passed an intracellular stage is at least open. We have not as yet seen, however, any intracellular stages. Nevertheless we interpret certain stomach stages (pl. 14, figs. 9–12) found in a bug which was removed
from the rat's nest four days prior to the examination, as merozoites, or stomach phases resulting from multiple fission. The stomach of this bug contained digesting blood and the trypanosomes were of a smaller, narrower, often straighter type (compare figs. 1–8 with 9–12). These differences are of the same general type of change as that found by Minchin and Thomson (1915, pl. 45) in T. lewisi in the flea after intracellular multiple fission.

The merozoite (pl. 14, figs. 9–12) is characterized by shorter body (less than 20μ), less diameter (not over 2μ), more elongate, narrower nucleus, more rigid body (note more flowing curves), and more attenuate proportions. It does not seem advisable to use the term "crithidiomorphic" forms for these, as Minchin and Thomson (1915) have done in this phase of T. lewisi, since the parabasal body and blepharoplast in the merozoites of T. triatomae are characteristically located at the extreme posterior tip of the body.

III. THE CRITHIDIAL PHASE

We find in our material a very characteristic occurrence of large crithidial forms in the stomach (pl. 14, figs. 13–19, pl. 15, figs. 20–28), while those of the rectum (pl. 15, figs. 29–47) are on the whole in the material we have examined distinctly smaller.

These size distinctions raise the question whether or not the crithidial phase in Trypanosoma triatomae may not play a larger part than in T. lewisi, and at least suggests the possibility that it may arise regularly in the stomach rather than in the rectum. Minchin and Thomson (1915) explain the presence of crithidial forms in the stomach in the flea as due to the backward migration of these stages from the rectum after transformation therein from the trypanosome phase.

Our grounds for suggesting the possibility of the initial normal development of crithidial stages in the stomach of Triatoma are (1) their abundance, (2) their larger size generally in the stomach than in the rectum, and (3) the occurrence of rolled-up stages, which in the trypanosome phase of T. lewisi in the flea are premonitory of the impending entrance of the parasite into the epithelial cell for multiple fission. Let us now consider these stomach phases of the crithidial forms of T. triatomae.

1. Transition forms.—When ingested with blood the organism is a typical trypanosome with the parabasal posterior to the nucleus
(pl. 14, figs. 1-8) and this relation is retained in the subsequent merozoite phase resulting from multiple fission (?) (pl. 14, figs. 9-12). In the crithidia form, on the other hand, the parabasal is anterior to the nucleus (pl. 14, figs. 14-18, pl. 15, figs. 20-31).

The transition appears to result from two processes. The first is a posterior migration of the nucleus (compare figs. 13 and 18), and the second an anterior migration of the parabasal body to a position anterior to the nucleus (figs. 19-23). Quite a variety of pictures are obtainable of this process, so that it is probable that there is no rigid sequence of movements of the organs concerned.

In addition to the change in position of the two organs named, there is also a structural metamorphosis of each. In this connection we must take the occasion to point out the fact that our conclusion that the trypanosome of the stomach and the crithidia form found therewith are identical species rests merely on similarities to the cycle of Trypanosoma lewisi, and not as yet upon experimental feeding and infection. While this hypothesis of identity is a reasonable one and proof of it by experiment is to be expected, the possibility remains of the occurrence here of two specifically distinct organisms. The transition from the condition in figures 11 and 12 to that in figure 14 or figure 22 is a considerable one, which our material imperfectly bridges. It is more fully bridged in Minchin and Thomson’s (1915) account of T. lewisi.

The metamorphosis of the nucleus is profound. It changes from the elongated, slender, asymmetrical, granular type (pl. 14, figs. 9-12) to the spheroidal, symmetrically located, vesicular type (pl. 14, figs. 14-19, pl. 15, figs. 20-32). These two types are consistently maintained throughout the individuals of the trypanosome and crithidia stages respectively, in our material. In both kind and degree the change is not unlike that which Chagas (1909) finds in Schizotrypanum cruzi in Conorhinus megistus, but it is considerably greater in both respects than Minchin and Thomson found in T. lewisi in the flea.

The parabasal also undergoes a metamorphosis in several particulars. It is (1) more distinct from the blepharoplast, (2) has a more distinct fan-shaped suspensory apparatus, and (3) forms a transversely located bar or bilobed structure located, as a rule, immediately anterior to the nucleus.

The process of transition from the trypanosome type to the crithidia one is suggested by the anterior progression in location of the parabasal in the early stomach phase (pl. 14, figs. 1-8), and by the
occurrence in the same stomach with those of an occasional individual with an oval, but still diffusely granular, nucleus and a parabasal which had almost reached the anterior position characteristic of the crithidial phase (pl. 14, fig. 13).

2. The structure of the crithidial form.—The crithidial form has a length of 8-40μ and a diameter of 1.8-2.5μ, rarely as much as 4μ. It is widest posteriorly and the posterior end is more or less abruptly subconical. Anteriorly the body is slender and tapering (pl. 14, figs. 14-19, pl. 15, figs. 22-26). It is less sinuous in locomotion, the posterior part being more rigidly held in one form than in the trypanosome stage; hence in the pictures which it affords the body is more nearly straight, or more regularly curved than in those of the trypanosome phase. It also moves with a more darting motion. The undulating membrane is much reduced and no differentiated zone is evident along the flagellum.

The nucleus is spherical, posteriorly located, 1.2-2μ in diameter, with chromatin-encrusted membrane, central karyosome in diameter about 0.35 that of the nucleus. The flagellum terminates in a very minute blepharoplast (pl. 14, fig. 19), immediately above the level of the bilobed, transversely located parabasal, whose length is 0.5-0.7 the diameter of the nucleus. The parabasal often lies adjacent to the nuclear membrane, and appears to be suspended from the blepharoplast by a fan-shaped parabasal rhizoplast. The parabasal is often seen to be distinctly lateral in attachment (pl. 14, figs. 14, 15). In rare cases a nuclear rhizoplast appears to run from the blepharoplast to the nuclear membrane (pl. 15, fig. 23), or even to the central karyosome (figs. 21, 22). Occasionally traces of a chromatic fiber run posteriorly from the blepharoplast to the posterior end as a sort of a feebly developed axostyle (pl. 14, figs. 14, 15, pl. 15, figs. 23, 29). Traces of a myoneme anterior to the nucleus are sometimes to be found (pl. 14, fig. 14, pl. 15, figs. 22, 23).

There appears to be among the crithidial forms in the stomach two types representing two tendencies. The first is a progressive rolling up (or unrolling?) of the flagellate and the second may include a progressive decrease (or increase?) in size. We will now consider these two series.

3. The rolled-up type.—There appears among the crithidial forms of the stomach a series of large individuals 20-30μ in length with claviform body and posteriorly located nucleus (pl. 14, figs. 16-19, pl. 15, figs. 20, 21). It is not unlike, in its progress and result, the
rolled-up form of the trypanosome phase of *T. lewisi* prior to its entrance into the epithelial cell for multiple fission. We have no evidence, however, that the crithidial phase of *T. triatomae* enters the cells and undergoes multiple fission in that phase, but the possibility of this is certainly suggested by the sequence of elavate to rolled-up individuals found in the stomach of *Triatoma protracta*. This series as represented in our material seems to involve a progressive enlargement of the posterior region at the expense of the anterior (pl. 14, figs. 14–19), the bending of the posterior end around anteriorly (pl. 15, fig. 20) and the fusion of the two limbs of the U-shaped body into one oval structure (pl. 15, fig. 21), in which the only trace of its method of origin is the bent chromatic axis of the flagellum. This oval stage was found by us in smears. No trace of its intracellular occurrence could be detected in our smear preparations. There is the possibility that the process may be one of unrolling rather than of rolling up, but the parallel in *T. lewisi* suggests the probability of the former, with the corollary of intracellular invasion and possibly even of multiple fission in this crithidial phase.

4. *The stout type.*—This is similar in proportions to the haptomonad forms in *T. lewisi*, which attach themselves by their flagella to the rectal surface and undergo there repeated binary fissions. One instance of spindle formation in a short, stout form (similar to that in pl. 15, fig. 28) has been found in our preparations. The degree of haptomonad attachment among the rectal forms as observed in living material through the wall was much less in our restricted material of *T. triatomae* than it was in *Crithidia leptocoridis* McCulloch (1915), or than in *T. lewisi* as described by Minehin and Thomson (1915).

It seems probable, however, that the series of forms of decreasing size and increasing stoutness shown in our figures 23–30, plate 15, represents a transition to the haptomonad phase, or at least to one corresponding to that stage in *T. lewisi*. However, all of the members of this series, from the very large individual with central median nucleus (pl. 15, fig. 23) to the short stumpy form (fig. 28), are found in the stomach and only the smaller ones in the rectum. The nucleus does not progress so far posteriorly here as in the rolled-up type and the parabasal becomes somewhat more condensed as compared with the earlier crithidial types, and thus more like that of the rectal trypanosome forms (cf. figs. 30 and 41).
These facts seem to indicate that in *Triatoma* the transformation of the early crithidial phase into the haptomonad phase may take place largely in the stomach, or at least that the transition forms invade the stomach freely.

5. *The slender type.*—Minchin and Thomson describe as nectomonad forms certain slender, active, crithidial flagellates of the rectum which return to the pylorus and there give rise to the terminal stumpy trypanosome of the rectal type or remigrate to the rectum to carry through the same process. These are slender, active forms with ellipsoidal, deeply and diffusely stained nuclei. We have not found individuals with this type of nucleus. If nectomonads occur in *T. triatomae*, as we have figured it, they may be represented by the slender type of our figure 22.

IV. THE RECTAL TRYPANOSOME PHASE

The cycle in the intestine of the invertebrate host terminates in an infective form, a minute trypanosome which results from the reverse transformation of the crithidial type back again to that of the trypanosome. In *Triatoma protracta* these small trypaniform flagellates have been found in great abundance in the rectum, where they are in active locomotion.

They are somewhat smaller than the merozoites of the stomach, though attaining their size in some instances (cf. pl. 14, figs. 9–12, and pl. 15, figs. 33–43). The transition from the crithidial phase to the trypaniform is accomplished in the stumpy, rectal, crithidial forms by the migration of the parabasal from its anterior position to one posterior to the nucleus (pl. 15, figs. 31–33), and the change in form of the nucleus from a spheroidal to an ellipsoidal one. This process continues with the gradual fading out and dissipation in a granular chromidial cloud of the chromatic nuclear membrane and the central karyosome (pl. 15, figs. 33–37), and the subsequent development of the typical elongated, asymmetrical, granular nucleus shifted to one side of the body (pl. 15, figs. 38, 39). The parabasal body in the meantime decreases in size and migrates to the extreme posterior end of the body (pl. 15, figs. 33–43).

Minchin and Thomson (1915) include among the haptomonad types of the rectal phase numerous small pyriform to spheroidal forms in which there is no free flagellum but only a short intraerytoplasmic shaft of that structure. We find also in the rectum of *Triatoma*
protracta rounded-up forms (pl. 15, figs. 45–47), with some evidence for their activity in rounding up, with the flagellum peripheral as in the rounding-up phase in the stomach. The authors quoted regard these forms as a normal part of the haptomonad phase. The possibility of cysts or latent bodies may well be kept open.

V. Comparison With Schizotrypanum cruzi Chagas

The species we have here described as Trypanosoma triatomae has much in common with the form previously described by Chagas (1909) as Schizotrypanum cruzi from Conorhinus megistus Burmeister from Brazil. It resembles it in the trypanosome, erithidial, and trypaniform stages. It resembles the trypanosome stage in its asymmetrical, elongated nucleus, but may be a trifle larger and stouter. The erithidial stages are quite similar in the spherical, vesicular nucleus with radial chromatic strands from the central karyosome, in the transverse parabasal, and in the fan-shaped parabasal rhizoplast. It may be somewhat blunter and more clavate posteriorly. The rectal trypaniform stages are, so far as known, more divergent. They appear to have more elongate nuclei and to be more slender in proportions. In view of these differences, it seems best to give separate specific status to the form from Triatoma protracta. We know too little of the life-history in both this form and of Schizotrypanum cruzi to stress the apparent differences between the two. We have found as yet no forms in the body cavity or the salivary glands. It is also obvious, in the light of Minchin and Thomson’s (1915) failure to find sexual reproduction in Trypanosoma lewisi, that a re-examination of Schizotrypanum is desirable, especially since the distinctions between this genus and Trypanosoma are less obvious now than formerly.

VI. Summary

1. Triatoma protractus Uhler, a hemipteran bug found in the nests of the wood rat, Neotoma fuscipes, harbors in its digestive tract a trypanosome, Trypanosoma triatomae sp. nov., which is found in its stomach with blood possibly derived from the wood rat.

2. The digestive tract of the bug also contains erithidial and trypaniform stages which are probably later forms in the cycle in the invertebrate host.

3. The trypanosome and erithidial stages are remarkably similar to the corresponding stages in the cycle of Schizotrypanum cruzi
Chagas in *Conorhinus magistus* Burm. from Brazil, which is the etiological factor in South American human trypanosomiasis.

4. Stages occur which are comparable to those described by Minchin and Thomson (1915) for *Trypanosoma lewisi*. We find the early stomach phase, or recently ingested trypanosome, the late stomach phase, or merozoite, following a probable multiple fission in the epithelial cells of the stomach, a crithidial phase of large size in the stomach, with a "rolled-up" stage suggestive of intracellular multiple fission in the crithidial stage, and a transition series leading to small, stout crithidial forms. These small crithidial forms probably become the smaller haptomonad types which we find undergoing binary fission in the rectum. The final trypaniform stages are apparently different from those of *Schizotrypanum* in some minor details. They occur in numbers in both transition and final stages in the rectum.

5. The crithidial stage appears to be more extended in this species than in *Trypanosoma lewisi* and to run a cycle of larger forms in the stomach than in the rectum.

6. The structure of the so-called "kinetoflagellum" supports the interpretation that it is in reality the parabasal body.

*Transmitted December 16, 1915.*

Zoological Laboratory,
University of California.

VI. LITERATURE CITED

Chagas, C.

Kofoid, C. A.

McCulloch, Irene.

Minchin, E. A., and Thomson, J. D.
VII. EXPLANATION OF PLATE 14

_Trypanosoma triatomae_ sp. nov., from stomach of _Neotoma fuscipes_.

All figures were outlined with a camera lucida using a 1/16 Leitz objective and Zeiss ocular 12. The magnification is in all cases approximately 3195.

Figs. 1–8. Large, broad trypanosomes from stomach of a bug about four days after possible infection. Giemsa stain, wet method.

Fig. 1. Parabasal in extreme posterior end showing a broad, bar-shaped structure stained deeply at ends. Blepharoplast small. Nucleus elongate, in characteristic position at concave side close against the edge, numerous heavily stained granules within nuclear membrane. Prominent undulating membrane the entire length of the flagellum.

Fig. 2. Large, broad trypanosome having large, uniformly stained parabasal a short distance from posterior end. The fan-shaped suspensory structure, or parabasal rhizoplast, quite clearly shown, nucleus elongate, granular, asymmetrical. Small blepharoplast. Paint chromatic line (axostyle?) extends from blepharoplast to posterior end.

Fig. 3. More slender trypanosome, with small parabasal and extremely long nucleus.

Fig. 4. The same as figure 2, with myoneme present.

Fig. 5. Shorter, broad form showing evidence of the shifting of the blepharoplast and parabasal anteriorly. The parabasal shows unequal staining in periphery and center. Small blepharoplast and clearly defined parabasal rhizoplast.

Fig. 6. The same, with a chromatic line extending from blepharoplast to posterior end, terminating in a very small granule.

Fig. 7. Large, slender trypanosome with a large, square, parabasal with fan-shaped rhizoplast and slightly elongate nucleus.

Fig. 8. An exceedingly large trypanosome. The blepharoplast and parabasal have migrated about one-half of the distance between posterior end and nucleus.

Figs. 9–12. Merozoite-like forms from the stomach. Bodies more elongate and narrower. Nuclei narrower, showing great numbers of small granules. Parabasals small, compact, deeply stained; blepharoplasts and parabasal rhizoplasts not distinguishable. Schaudinn-iron haematoxylin, wet method.

Fig. 13. Crithidial form from stomach, showing transformation from trypanosome to crithidial form. Note trypanosome-like shape of body, but blepharoplast and parabasal body have migrated to a position opposite that of the nucleus. Nucleus broadly ellipsoidal but still diffusely stained; a distinct parabasal rhizoplast. Paint axostylar thread along edge of body. Giemsa stain, wet method.

Figs. 14, 15, 16, 18. Large crithidial stages from stomach. Conical or blunt posterior end. Large, vesicular nucleus, with distinct nuclear membrane, central karyosome and radiating fibers. Parabasals relatively small, having a slight bilobed appearance on upper edge. Parabasal rhizoplasts distinct. Small blepharoplasts. Figures 14, 15, 16 show axostyles extending from blepharoplast to posterior end. Figure 16 also shows distinct rhizoplast connecting blepharoplast with karyosome of nucleus. Note lateral attachment of parabasal to blepharoplast in figures 14 and 15. Schaudinn-iron haematoxylin, wet method.

Figs. 17, 19. Crithidial forms in stomach leading toward rolled-up stage. Fig. 17—Body bent, but cytoplasm has not yet massed about nucleus. Fig. 19—Massing of cytoplasm about nucleus as result of the progressive bending of body.

[124]
EXPLANATION OF PLATE 15

All figures from material fixed in Schaudinn's fluid and stained by the wet iron-haematoxylin method. × 3195.

Figs. 21–28, inclusive, from the stomach; figs. 29–47, from the rectum.

Fig. 21. Rolled-up crithidial stage in U-shape with central lacuna and long, free flagellum.

Fig. 22. Later phase of rolled-up crithidial stage, suggesting preparation for intracellular multiple fission.

Figs. 22–28. Transition from crithidial forms in the stomach to the haptomonad crithidial forms in the rectum.

Fig. 22. Small crithidial form suggestive of the nectomonad phase, with nucleus well forward.

Fig. 23. Very large crithidial type with nucleus far forward, rhizoplast extending to nuclear membrane with granules on membrane, and granular axostyle.

Figs. 24–26. Slender crithidial types with posterior nucleus, attenuate anterior end, and small nuclei.

Figs. 27, 28. Stout crithidial types approaching the haptomonad phase.

Figs. 29–30. Small, stout, rectal haptomonads, granular axostyle in figure 29.

Figs. 31–33. Transition forms from haptomonad phase to the rectal trypaniform phase, showing progressive lateral and posterior migration of the parabasal.

Fig. 31. With axostyle, figure 32 with myonemes.

Figs. 34–43. Rectal trypaniform stages, showing transformation of the vesicular nucleus to the elongate, granular, asymmetrical nucleus. Note small but variable parabasal.

Figs. 45–47. Rounded-up haptomonad stages from the rectum.
THE GENERA MONOCERCOMONAS
AND POLYMASTIX

BY
OLIVE SWEZY

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THE GENERA MONOCERCOMONAS AND POLYMASTIX

BY

OLIVE SWEZY

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INTRODUCTION

The genus Monocercomonas was founded by Grassi in 1881 for a flagellate which he had found parasitic in the larvae of Gryllotalpa and Melolontha, and designated Monocercomonas insectorum. In an earlier memoir (1879) he described a flagellate from the larva of Melolontha as Schedaeocercomonas melolonthae, and later in the same year one from the larva of Gryllotalpa as S. gryllotalpae. These flagellates, on further investigation, proved to be the same form, and with his redescriptions of them in 1881 he proposed for them the new name, Monocercomonas insectorum.

The uncertainty of his information regarding their structure will be evident from the fact that in 1879 he gave the name Trichomonas, and in 1888 that of Trichomastix as synonyms for the forms which he had observed and earlier designated as Monocercomonas. These three names were confused by him and used interchangeably. In 1883 he described a flagellate from man which he called Monocercomonas hominis and which is undoubtedly a trichomonad.
The flagellate from insects has come to be generally accepted as the type species of the genus, with the name *Monocercomonas melolonthae*. This is a flagellate about 12 to 15 μ long and 8 to 11 μ wide, ovoidal in outline, with the broader end anterior, and more or less pointed at the posterior end (pl. 16, fig. 3). Forms are occasionally met with which are spheroidal in outline. The four equal flagella extend anteriorly, arising at the anterior extremity of the body, usually in two groups of two flagella each from two blepharoplasts sometimes more or less widely separated from each other. Arising from one blepharoplast is a slender, usually darkly-staining axostyle which curves around the nucleus and passes backward to the posterior end of the body. This differs from the axostyle in *Trichomonas* (Kofoid and Swezy, 1915, a, b) and *Hexamitus* (Swezy, 1915a) as well as that in *Giardia* (Kofoid and Christiansen, 1915) in that it seems to occupy a position along the surface of the body and not in the center of the protoplasm. It does not project beyond the body but terminates in the periplast at the posterior extremity (pl. 16, fig. 1). Its position, relations, and structure would strongly suggest the correctness of the interpretation which has been placed upon the axostyle in *Trichomonas* by Kofoid and Swezy (1915b) namely, that it is an intraeytoplasmic flagellum, which in *Monocercomonas* has not yet migrated to the deeper portions of the cytoplasm.

The large vesicular nucleus is situated in the anterior part of the cell, immediately behind the blepharoplasts which rest upon the nuclear membrane. The chromatin is massed in a large karyosome centrally placed, often with smaller granules occupying a position against the nuclear membrane (pl. 16, fig. 2). The nuclear membrane itself often takes a dark stain with iron haematoxylin.

There is neither cytostome, undulating membrane, nor trailing flagellum present.

A flagellate has been figured by MacKinnon (1912) from the larva of the crane-fly *Tipula*, which she considers identical with the species described by Grassi as *M. melolonthae*. Jollos (1911) describes a flagellate from the larva of *Cetonia* which he places in the genus *Monocercomonas* as *M. cetoniae* Jollos.

França (1913), also working on insect larvae, figures an organism from the larvae of *Phyllognatus* and *Oryctes* which shows the generic characters of *Monocercomonas* and evidently belongs to that genus, probably to the species *melolonthae*. 
These two forms comprise the only well-authenticated species in this genus, namely, *Monocercomonas melolonthae* (Grassi) and *M. cetoniae* Jollos.

The life-history of these flagellates is practically unknown. A few stages in the division of *M. cetoniae* have been figured by Jollos (1911), but these are inadequate to give a fair idea of the actual process. Some cases have been figured in which the axostyle is absent (França, 1913). Whether this is due to faulty technique alone or to the probability that it may be absent during some stages in the life-cycle, more extended observation must determine.

Another flagellate which has been placed in this genus is that described by Dobell (1909) as *Monocercomonas bufonis* from the intestine of frogs and toads. This organism has a more slender body, is somewhat longer than *M. melolonthae*, does not possess an axostyle, has a comparatively small nucleus with the chromatin massed in a number of granules or diffused (pl. 16, fig. 6) in a cloud. These facts would make its inclusion with this genus doubtful. Alexeieff (1911) figures the same form from *Triton* and *Axololl* and finds that it possesses a "chromidial" body below the nucleus (pl. 16, fig. 7), a fact still further separating it from *Monocercomonas*.

During my own investigations on the flagellate parasites of amphibian hosts, including frogs and toads, as well as newts and salamanders, my attention was directed to this flagellate, which is nearly always present and frequently is very abundant. My first observation led me to agree with Alexeieff (1911) in regard to the structure of the organism. A further acquaintance with it, however, soon convinced me that a very important and constant part of its structure had been entirely overlooked by both these investigators. This was the very definite cuticle, or pellicle, which is obliquely striated (pl. 16, fig. 4). This gives to the body a fairly rigid contour. The presence of a definite cuticle or pellicle is further shown in specimens which are rather overstained, by a dark line around the periphery of the body. The striations are regular, parallel, and about the same distance apart in most individuals.

This fact in its structure still further separates it from *Monocercomonas*, and at the same time suggests its relationship with another genus, *Polymastix*.

**The Genus Polymastix**

The genus *Polymastix* was established by Bütschli (1884) for a small flagellate parasitic in the larva of *Melolontha vulgaris*, oval in
outline, with four equal flagella at the rounded anterior end. The distinguishing characteristic was that "auf der Körperoberfläche bemerkt man eine verschiedene Anzahl dunkler und verschieden langer Striche, die Grassi für triboccystenartige Gebilde zu halten geneigt, während sie Kunstler für Rippen der Oberfläche erklärt."

Mackinnon (1912) describes a flagellate from the larva of the crane-fly *Tipula* which she places in the genus *Polymastix*. In this the periplast is raised up in longitudinal ribs which in the living organism appear to run almost unbroken from one end of the flagellate to the other, but are found to be discontinuous on staining.

Hamburger (1911) describes the flagellate from the larva of *Melolontha*, the periplast of which is raised into longitudinal ribs extending from one end of the organism to the other. Both Hamburger and Mackinnon figure forms in which the "ribs" of the periplast become detached from the body and apparently fall off. The suggestion has been made, however, that this appearance may be due to bacteria. It does not occur in the species which I have had under observation.
França (1913) figures a *Polymastix* from the larva of *Oryctes* which presents the same characteristics as those previously described by Mackinnon and Hamburger.

It is thus evident that some clear distinctions exist between the flagellates placed in the genus *Polymastix* by these investigators, and the form from frogs and salamanders. Another feature is the presence of the "siderophile" body of Alexeieff (1911), or parabasal body. The homologue of this body may, however, be the extra-nuclear siderophilous granules figured in the *Polymastix* from *Tipula* by Mackinnon (1912).

These distinctions do not seem to warrant the creation of a new genus for the flagellate from frogs and salamanders. It is therefore proposed, provisionally at least, to place it in the genus *Polymastix* as *P. bufonis* (Dobell).

**Morphology of Polymastix Bufonis**

This flagellate is small, 10 to 15 μ in length and 5 to 8 μ in width. In outline it is more or less oval or pyriform (pl. 16, fig. 8), somewhat irregular in contour but never ameboid. The posterior end may be rounded, blunt or pointed.

The slightly thickened periplast of the body is marked by striations which extend obliquely across the body (text-fig. A), or in a nearly longitudinal direction (pl. 16, fig. 9). There appear to be thickened ridges or very slight folds in the periplast, which do not show any definite staining reactions with iron haematoxylin. In many specimens these striations may escape observation altogether. Some time had elapsed after my first observation of this flagellate before I became aware of this peculiarity in its structure. Going back over my earlier material, however, I found that they could be demonstrated in nearly all cases. This fact confirms my belief that the flagellate is identical with the one described by Dobell (1909) and Alexeieff (1911), and not a new form as might appear from the figures shown.

The four equal flagella arise at the anterior extremity of the body, usually in one group (text-fig. A) or in two groups of two flagella each (pl. 16, fig. 8), though this latter case may be only an early prophase of division. The flagella originate in a single granule, the blepharoplast (text-fig. A, *bleph.*). Alexeieff (1911) figures the blepharoplast as a number of minute granules. This was not found to be the case in any specimens in my material.
The nucleus is comparatively large, with a very distinct nuclear membrane and the chromatin more or less diffused throughout its extent (text-fig. A), less frequently with a karyosome (pl. 16, figs. 4, 7), or granules lying on the periphery. In some specimens a slender rhizoplast may be noted connecting the nucleus and the blepharoplast (text-fig. A, nuc. rhiz.) This structure, as in the case of *Trichomonas* (Kofoid and Swezy, 1915b), and perhaps most other flagellates, is probably normally present but, owing to its lack of affinity for most of the stains used, is difficult to demonstrate.

Below the nucleus and partly surrounding it, is the parabasal body (text-fig. A, par. b.). This is quite variable in its appearance, as is evident from the figures shown. The form which it assumes in most of the individuals is that of a band extending partly around the cell immediately below the nucleus. Figure 6, plate 16, looking at the flagellate from the anterior end, shows the entire structure. This exhibits a heavy band across the flagellate below the nucleus when viewed from one side (pl. 16, fig. 7) or, viewed from the opposite side, two shorter bands (pl. 17, figs. 10, 13). Extending upward from one end of the parabasal body is the slender rhizoplast of this structure (text-fig. A, par. rhiz.) which joins the blepharoplast. This like the nuclear rhizoplast, is usually difficult to demonstrate, yet it is very definite in a number of cases (pl. 16, figs. 5, 7; pl. 17, figs. 13, 15).

The parabasal body was not figured by Dobell (1909) in his description of this flagellate as *Monocercomonas bufonis*. Alexieff (1911) figured this structure, terming it the "corps sidérophile," and later Janicki (1915) homologized it with the parabasal bodies of the Trichonymphida, a conclusion which I had reached before the appearance of his paper (Swezy, 1915b).

The protoplasm is more or less alveolar, sometimes filled with large vacuoles (pl. 16, fig. 4; pl. 17, fig. 20), but more frequently these are small and indistinctly marked off (pl. 16, fig. 5).

There is no cytostome present nor are food bodies commonly found in the cytoplasm, though not infrequently vacuoles are observed which have a minute granular mass in the center, suggesting partly digested food substance (pl. 17, fig. 19).

**Mitosis**

Mitosis in *Polymastix bufonis* is initiated by a splitting of the blepharoplast (pl. 16, figs. 8, 9). At the same time the chromatin of the nucleus becomes condensed into a number of granules, the intra-
nuclear spaces losing their staining reactions (pl. 16, fig. 8). The
daughter blepharoplasts move apart (pl. 17, fig. 11), but never sepa-
rate very widely and take no further part in the division of the
nucleus or cell. Two new flagella appear as new outgrowths from
each blepharoplast.

No definite spireme seems to be formed by the chromatin, but a
varying number of granules appear in the nucleus (pl. 16, fig. 8; pl.
17, figs. 10–12). These appear to coalesce in pairs until but two pairs
remain (pl. 17, fig. 12).

A primitive spindle arrangement is noted with spindle fibers very
feebly developed. At the time of the equatorial plate formation (pl.
17, fig. 13), as well as in later stages, no centrosomes are found, but a
small mass of chromatin is present at the poles and extends a short
distance along the spindle fibers (pl. 17, figs. 13–15), forming a pole
plate.

As the chromatin mass on the equatorial plate separates and moves
toward the poles it is found that four chromosomes are present, two
going to each pole (pl. 17, figs. 14, 15). As the chromosomes near the
poles they apparently fuse with the pole plate (pl. 17, fig. 16), the
number of chromosomes remaining distinct until after the formation
of the new nuclear membrane (figs. 17, 18).

The whole process of nuclear division takes place within the
nuclear membrane, including the formation of the new nuclear mem-
branes (pl. 17, figs. 17, 18). There is no constriction of the old mem-
brane, new membranes being formed around each group of chromo-
somes after they have reached the poles. This at first appears as a
very faint line but later, when the old nuclear membrane begins to
fade out, the new membranes become thicker and more distinct until
they have taken on the usual appearance with complete obliteration
of the old membrane (pl. 17, fig. 19). A similar process in nuclear
membrane formation is found in *Hexamitus ovatus* (Swezy, 1915a).

With the reorganization of the nucleus the final steps in the process
of cell division take place. The parabasal body elongates with the
drawing apart of the daughter nuclei (pl. 17, fig. 20) and divides by
a simple constriction with no suggestion of mitosis in it. At the same
time the cytoplasm begins to divide at the anterior end (pl. 17, figs.
20–21), the cleft extending backward until the posterior end of the
body is reached and the organism separates into two daughter flagel-
lates. In these later stages the rhizoplasts connecting the blepharo-
plasts with the nuclei are frequently found, but the exact origin of
these fibrils could not be traced. Their absence during the stages of mitosis of the nucleus would indicate their origin as later outgrowths from either the nucleus or the blepharoplast. Some suggestions of splitting of the parabasal rhizoplast are found in figures 13 and 15, plate 17.

The only division stages of this flagellate heretofore described have been those figured by Alexeieff (1911), two figures similar to ones shown herewith (pl. 17, figs. 16, 20), differing in no essential details beyond the four and six blepharoplasts which he figures.

Summary

The generic characters of Monocercomonas are as follows: four equal anterior flagella, arising from one or more blepharoplasts or basal granules, a large vesicular nucleus situated at the anterior end, a slender axostyle arising in the blepharoplast and terminating in the periplast at the posterior extremity of the body. The only authentic species are M. melolonthae (Grassi) and M. ectori Jollos.

The genus Polymastix differs from Monocercomonas mainly in the absence of an axostyle, the presence of striations on the definite periplast, and, in most cases, the extranuclear chromidial bodies or parabasal bodies.

Monocercomonas bufonis Dobell possesses the generic characters of Polymastix, hence should be placed in that genus as P. bufonis (Dobell).

The process of division in Polymastix bufonis is a simple form of mitosis, exhibiting two chromosomes, pole plates instead of centrosomes and the formation of new nuclear membranes inside the old one which disappears. The parabasal body divides by a simple constriction. The division of the cytoplasm begins at the anterior end and proceeds posteriorly.

This forms part of a series of investigations on protozoan parasites carried on in the protozoological laboratory of the University of California under the direction of Professor Charles Atwood Kofoid, to whom the writer is much indebted for helpful suggestions and criticisms.

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LITERATURE CITED

ALEXEIEFF, A

BÜTSCHLI, O.

DOBELL, C. C.

FRANÇA, C.

GRASSI, B.

HAMBURGER, C.

JANICKI, C.

JOLLOS, V.

KOFOID, C. A. AND CHRISTIANSEN, E. B.
1915. On binary and multiple fission in Giardia muris (Grassi). Univ. Calif. Publ. Zool., 16, 30-54, pls. 5-8, 1 fig. in text.

KOFOID, C. A. AND SWEZY, O

MACKINNON, D. L.
1912. Protista parasitic in the larva of the crane-fly, Tipula sp. Parasit., 5, 175-189, 27 figs. in text.

SWEZY, O.
EXPLANATION OF PLATE 16

All figures were drawn with the camera from preparations fixed in hot Schaudinn's fluid and stained with iron haematoxylin. \( \times 2480 \).

Figs. 1 to 3. *Monocercomonas melolonthae* (Grassi) from the intestine of *Plethodon oregonensis*.

Fig. 1. *Monocercomonas melolonthae*, showing the typical axostyle, four equal anterior flagella, and vesicular nucleus.

Fig. 2. The same, with chromatin granules lying against the nuclear membrane.

Fig. 3. The same, showing variations in size and contour.

Figs. 4 to 9. *Polymastix bufonis* from the intestine of *Diemyctylus torosus*.

Fig. 4. Ordinary trophozoite with large vacuoles in the cytoplasm and a karyosome in the nucleus.

Fig. 5. Trophozoite showing the rhizoplast connecting the parabasal body and the blepharoplast.

Fig. 6. View of trophozoite from the anterior end, showing the entire structure of the parabasal body.

Fig. 7. Large trophozoite with vesicular nucleus and large parabasal body.

Fig. 8. Early prophase of division.

Fig. 9. Early prophase of division. The blepharoplast has divided and the chromatin has become condensed into granules.
EXPLANATION OF PLATE 17

Figures of Polymastix bufonis (Dobell)

Fig. 10. Early prophase of division, with outgrowth of two new flagella.
Fig. 11. The same.
Fig. 12. Prophase, with chromatin condensed into two pairs of granules.
Fig. 13. Equatorial plate formation and pole plates occupying the position of centrosomes.

Fig. 14. Metaphase, showing the two pairs of chromosomes moving apart.
Fig. 15. The same. Note parabasal rhizoplasts.
Fig. 16. Telophase, with coalescence of pole plates and chromosomes.
Fig. 17. The same, with the beginning of the formation of the new nuclear membranes.

Fig. 18. The same.
Fig. 19. Beginning of the reorganization of the nuclei; complete disappearance of the old nuclear membrane.
Fig. 20. Beginning of division of the cytoplasm; parabasal not yet divided.
Fig. 21. The same, with the separation of the parabasal body into two daughter parabasal bodies.
NOTES ON THE SPINY LOBSTER (*PANULIRUS INTERRUPTUS*) OF THE CALIFORNIA COAST

BY

BENNET M. ALLEN
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Nos. 5 and 6 in one cover. April, 1914


NOTES ON THE SPINY LOBSTER (PANULIRUS INTERRUPTUS) OF THE CALIFORNIA COAST

BY

BENNET M. ALLEN

This account is based upon work done under the auspices of the California Fish and Game Commission from June to January, 1911, and in the summers of 1912 and 1913. The investigations were chiefly along economic lines, hence these notes explaining the scientific results of the work must be considered merely as a by-product of the main work. It is unfortunate that these observations do not extend through the entire year.

Throughout the course of the work the Scripps Institution for Biological Research of the University of California has been my headquarters, and the splendid facilities for work there have been freely placed at my disposal. I am deeply indebted to Dr. William E. Ritter for hearty encouragement and help. He has given many valuable suggestions.

SIZE

Specimens of the spiny lobster weighing over ten pounds are very uncommon, although those of four, five, and six pounds weight are not infrequently met with. I myself saw a specimen caught at Catalina Island that weighed seventeen pounds and I have heard reports of others that were said to weigh twenty pounds. These large ones are usually males and are commonly called "bulls," although I have seen a female weighing six and one-half pounds and eighteen inches in length, and have been told of others that reached still greater size. Practically nothing is known about the length of life of these animals or their rate of growth.
There is a large range of color variation from almost black through shades of dark mahogany, reddish purple to a light red color. Fishermen speak of albino specimens. This variation in color is probably due to individual variation alone. It is certainly neither a mark of sex nor of age. Some fishermen assert that it is correlated with the environment from which the specimens are caught. There is, however, no uniformity in these views. In examining traps laid among the kelp all shades were found in the same trap. Similar results are found to obtain in catches made from shore.

The numerous spines with which these animals are armed serve as an admirable defense. They are especially well developed on the carapace and antennae, where they point forward, thus being directed toward intruders, as the animal lies backed into a crevice between the rocks. The eyes are protected by a pair of especially well-developed overlying spines. The sides of each joint of the abdomen are produced into sharp spine-like points. These are brought into play by the sharp and strong ventral flexion of the abdomen. This is a very serious matter for the unwary person who would take hold of that portion of the body.

As regards the habitat of this crustacean, it may be said with certainty that its home is among the rocks. It occurs in water of varying depths, from shallow tide pools exposed at low tide, to kelp beds at a depth of from three to eighteen fathoms. It is well known that the kelp is attached to rocks, beneath and in the crevices of which the spiny lobster hides. It offers additional protection besides that afforded by the rocks. Spiny lobsters cross sandy stretches only at night in going from one rocky area to another. They are frequently caught at depths of fifteen to twenty fathoms and I have been told of a case where they were caught at a depth of thirty-five fathoms between Santa Cruz and Santa Rosa islands. Mr. O. M. Seeley, from whom I have this information, states that he has himself caught them at approximately this depth along the coast of Lower California. On August 10-12 I had a trap set in water thirty-seven fathoms deep, with rocky bottom and at a distance of one and one-quarter miles
west by a trifle south of the south end of the south island of the Coronado group. It was baited in the regular way but contained no spiny lobsters when examined on the mornings of August 11 and 12.

Fishermen all agree in saying that the spiny lobster is strictly nocturnal in its habits. It is even claimed very positively and by most reliable observers that they are caught in but small numbers on moonlight nights, the only exceptions being in those cases where traps are set in deep shadow.

**FOOD**

The spiny lobster uses a great variety of food. It takes flesh of all kinds. Fishermen usually bait their traps with fish meat. Many claim with apparent justification that badly decayed fish attracts them most of all and the practice is to set aside the fish caught for bait for several days before using it in the traps. Abalone meat is often used, and flesh of sea-lions, domestic animals, etc.

Examination of stomach contents shows most varied material, such as broken parts of their own species, sponge material, fish scales, etc. Many fragments of kelp leaves are often found, together with remains of other algae.

**ENEMIES**

The spiny lobster has many enemies. The chief are the sheephead, conger-eel, jew-fish and devil-fish. I have frequently seen remnants of the spiny lobster in the stomachs of sheephead. Sheephead and conger-eels are often caught in lobster traps. In such cases, a large proportion of the spiny lobsters are found to be mutilated by having legs, antennae and portions of the trunk bitten off. Jew-fish caught by fishermen have been found to carry as many as ten or twelve spiny lobsters in the stomach.

**MOVEMENTS**

The spiny lobster usually walks forward, although it may walk sidewise or backward if necessary. In swimming, it moves backward, making strong movements with a flexion of the abdomen. One specimen was seen by the writer slowly swimming backward at the surface of the water. I have talked with several fishermen who claim to have seen schools of spiny lobsters swimming near the surface. This
should be accepted with caution. One hears frequent accounts of the replenishment of spiny lobster fishing-grounds within a few months or a year after they have been practically fished out. Nearly all fishermen state that there must be considerable migrations of spiny lobsters because they may sometimes find them wholly absent from a place where they may later occur in plenty. Changes in the feeding conditions may in part account for their seeking the bait more at one time than at another, but I feel that such an explanation would hardly account for these fluctuations in the size of the catch, and such replenishment of localities must be considered as due to haphazard or concerted migrations from without.

Some experiments were carried out in the winter of 1911 with an aim to determining the direction and extent of these movements. It was decided that the region of Santa Barbara and the outlying islands was especially well adapted for the carrying out of the experiment. The mainland affords sixty miles of rocky coast with an almost continuous barrier of kelp. While Santa Barbara is not in the center of this region, it is so situated as to afford an opportunity for the spiny lobsters to travel long distances in each direction along a coast where intensive fishing is being carried on. Santa Rosa and Santa Cruz islands are about thirty and twenty-five miles distant from the mainland respectively, while Anacapa is about twenty miles from Ventura. Spiny lobster fishing is widely practiced in all this region with the exception of Santa Rosa Island, where the owners will not permit fishermen to land. At times, however, fishing operations are carried on there from boats. These three islands are separated from one another by channels about five miles wide. It would be possible for a spiny lobster to travel between Santa Cruz and Santa Rosa islands without reaching a depth greater than twenty-four fathoms and between Santa Cruz and Anacapa Islands a depth of thirty-three fathoms. Many of the fishermen took an intelligent interest in the experiment. Among the first to respond by tagging lobsters were the members of the Japanese Asahi Company on Santa Cruz Island.

I used a tag of somewhat unusual kind. It was thin and light, and had stamped upon it directions sufficiently full to indicate its purpose. It was thought that this would gain returns from it in the unlikely event of any tagged specimens falling into the hands of fishermen not instructed regarding the experiment. I made a very thorough canvass of the territory covered by the experiment and per-
sonally met practically all of the men engaged in fishing there. Each tag was numbered. This type of tag has some advantages. It is light, and can be bent to lie close to the part to which it is attached. It is made of brass, which renders it inexpensive, resistant to the action of the water, and at the same time conspicuous to the fisherman catching the tagged specimens. The tag was fastened by means of soft brass No. 24 wire. The metal of wire and of tag must be the same, in order to avoid galvanic action.

After a good deal of consideration, I decided to fasten these tags to the thick stump to which the feeler is attached. This stump remains attached to the lobster when the feeler itself is broken off. It is beset with spines that prevent the wire fastenings from slipping. It is held well above the ground and is not often brought in contact with objects, however much that is true of the feeler attached to it. Since lobsters are handled by the feelers, these stumps are conspicuous and a tag fastened to one would almost certainly attract notice. One difficulty is found in the fact that the tag, to a certain extent, binds the action between the second and third segments of the stump. This is reduced to a minimum by fastening the tag to the underside. Since the hinge movement between these segments is from side to side it is not seriously interfered with by the method of attachment of the tag.

The purchase of lobsters is expensive and I bought about 200 only, which I myself tagged and put into the water at certain points. The points chosen for this were Frey's Harbor and Prisoner's Harbor on Santa Cruz Island, a point on the south side of Anacapa Island, and the entrance to Santa Barbara Harbor. I purchased marketable specimens for this work in the main, but used some twenty undersized ones on Anacapa Island because they chanced to be available.

Certain of these tags were sent to each fisherman in the region, who was engaged in catching spiny lobsters. With them was a circular giving full directions for affixing the tags and for keeping proper records. Post cards were furnished at the same time. These had on their backs maps of the coast region along which each fisherman was operating. On one half the face was printed the address and on the other half were spaces for data regarding the length and sex of the spiny lobster released, the number of the tag used and the date of affixing. These cards were used for recording the data regarding the tagging of spiny lobsters while similar cards were furnished for making reports of their capture.
REPORT ON EXPERIMENTS TO DETERMINE THE DISTANCES TRAVELLED BY THE SPINY LOBSTER

I personally released tagged lobsters in the following numbers at the places indicated:

<table>
<thead>
<tr>
<th>Location</th>
<th>Number</th>
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</thead>
<tbody>
<tr>
<td>Anacapa Island</td>
<td>46</td>
</tr>
<tr>
<td>Santa Cruz Island</td>
<td>74</td>
</tr>
<tr>
<td>Santa Barbara</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
</tr>
</tbody>
</table>

The accompanying table gives a detailed account of the numbers of the tags affixed at the various places, both by myself as indicated above and by various co-operating fishermen who affixed 129 tags to my knowledge. There were possibly a slightly larger number of which I have not been notified.

It will be seen in the tables that the maximum distance travelled was 9.6 miles in 28 days' time. Another specimen traveled 6 miles in 14 days, making the maximum rate of .43 miles per day. Both of these were caught by the Vidovich Brothers, Slavonian fishermen whom I know personally and in whom I have full confidence. The tag that was carried 9.6 miles (No. 434) was one that I myself had affixed. Mr. Vidovich sent it to me after recovering it. It might be further stated that Japanese fishermen on Santa Cruz Island caught a specimen tagged by me, that had travelled 31/4 miles. Most of the spiny lobsters travelled much shorter distances:

- Eleven travelled less than one mile in an average of 18 days.
- Eight travelled more than one mile in an average of 27 days.
- Of the remaining seven the records are not complete.
- The total average distance travelled was 1.4 miles.
- The total average time in the water was 22 days.

The accompanying map (fig. 1) shows that various directions of dispersal were followed from a given point. This is well illustrated in the course followed by the five recovered specimens of the fifty-three spiny lobsters released east of Prisoner's Harbor, on Santa Cruz Island. Their directions of dispersal can be best understood by reference to the map. Three went east, one went west and one remained at the same point where it was liberated. The last-mentioned had remained at the same point for sixteen days, while No. 152, released at the same time and place, had travelled three and one-quarter miles.
Fig. 1. Distances and directions traveled by Spiny Lobsters released from Santa Cruz Island.
<table>
<thead>
<tr>
<th>Name of Fisherman</th>
<th>Place</th>
<th>No. of Tags</th>
<th>Date</th>
<th>Total No. Tagged</th>
<th>Caught</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorge</td>
<td>Canada del Capitan</td>
<td>249-250</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Self</td>
<td>Santa Barbara</td>
<td>401-484</td>
<td>Nov. 12</td>
<td>84</td>
<td>5</td>
</tr>
<tr>
<td>Vidovich Bros.</td>
<td>Below Santa Barbara</td>
<td>371-379</td>
<td></td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

**SANTA CRUZ ISLAND**

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<th>Name of Fisherman</th>
<th>Place</th>
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<th>Date</th>
<th>Total No. Tagged</th>
<th>Caught</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watanabe</td>
<td>Cochie Point</td>
<td>201-210</td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Takamatsu</td>
<td>Cochie Point</td>
<td>91-99</td>
<td></td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Laro &amp; Eaton</td>
<td>Scorpion Cove</td>
<td></td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Erickson</td>
<td>Yellow Banks</td>
<td>521-528</td>
<td></td>
<td>8</td>
<td>1 caught twice</td>
</tr>
<tr>
<td>Johnson</td>
<td>Yellow Banks</td>
<td>511-512, 515, 517-520, 620, 622-625, 630</td>
<td></td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Hansen</td>
<td>Bine Banks</td>
<td>561, 63-64, 68-69, 70</td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Asanuma</td>
<td>Blue Banks</td>
<td>571-580</td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Niedever</td>
<td>Gall Island and Alamo</td>
<td>62-70</td>
<td></td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Miyo</td>
<td>Forney's Cove</td>
<td>51-60</td>
<td></td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>&quot;Greeks&quot;</td>
<td>Hazard's Cove</td>
<td>31-40</td>
<td></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Newton</td>
<td>Ladies' Harbor</td>
<td>1-6</td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Self</td>
<td>Frey's Harbor</td>
<td>100-122</td>
<td>Nov. 1</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Asahi Pelican Bay</td>
<td>Pelican Bay</td>
<td>11-15, 15, 16, 18-20, 81-90</td>
<td></td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Self</td>
<td>Prisoner's Harbor</td>
<td>123-175</td>
<td>Nov. 3</td>
<td>52</td>
<td>5</td>
</tr>
</tbody>
</table>

**ANACAPA ISLAND**

<table>
<thead>
<tr>
<th>Name of Fisherman</th>
<th>Place</th>
<th>No. of Tags</th>
<th>Date</th>
<th>Total No. Tagged</th>
<th>Caught</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self</td>
<td>Anacapa Island</td>
<td>176-200, 301-315, 291-292, 396-399</td>
<td>Nov. 6</td>
<td>46</td>
<td>1</td>
</tr>
</tbody>
</table>

|             |             |             |       | 334             | 27     |
### Table: California Coast Lobster Data

<table>
<thead>
<tr>
<th>Location</th>
<th>Male</th>
<th>Female</th>
<th>Not Known</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>East of Point Arena Bay</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Point Arena</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Sand Harbor</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Harbor</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Santa Cruz Island</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CAYDETTE</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>28</td>
</tr>
</tbody>
</table>

### Notes
- The table represents the distribution of male and female Spiny Lobsters (Panulirus interruptus) caught in different locations along the California Coast.
- The data includes counts from various locations such as East of Point Arena Bay, Point Arena, and Sand Harbor, among others.
- The total count of lobsters caught is 28 with a breakdown of 14 males and 8 females.
- The table also notes the presence of "not known" entries, indicating uncertainty in some data points.
in seventeen days. Their movements were apparently haphazard, and yet there may be some significance in the fact that the three that travelled over three miles did so in an easterly direction. It would be quite hazardous, however, to draw conclusions from such slender data.

One curious result of the work is seen in the repeated capture of certain specimens. Of course, all of the specimens were first caught before they could be tagged. Of the twenty-seven specimens caught the greater portion were at my request thrown back again into the water. Of these five were caught once more—the third time in all. It is really quite surprising that one-fifth of those caught twice should be thus caught a third time. The data are too meager to warrant a positive conclusion, but it would appear that there are certain individuals more susceptible to trapping than others. At least they do

Fig. 2. Distances and directions traveled by Spiny Lobsters released at Santa Barbara.

not learn by experience to avoid being caught. No. 475 travelled a distance of one and two-fifths miles from shallow to deeper water. Since it was released on November 12, the time when the alleged migration to deeper water is taking place, this case would tend to substantiate the occurrence of such a migration process.

Data regarding the size and sex of tagged lobsters seem to show that these factors have no bearing upon either the direction or extent of migration.

A very important by-product of this experiment is the light that it throws upon the intensity of the fishing operations, and the consequent depletion of the species. We have records of 334 tagged lobsters. Several fishermen given tags no doubt affixed them without sending a record of it. They would probably also fail to send a record of the capture of tagged spiny lobsters. The isolation and illiteracy of many would be a factor in this connection, as would also
the ignorance of the English language on the part of many foreign fishermen. I have been told of fishermen who caught tagged spiny lobsters and failed to report them. This was especially true in the vicinity of Santa Barbara.

Taking the figures as we have them, of 334 spiny lobsters that were tagged, twenty-seven were caught and recorded. This is 8 per cent of the whole. The experiment, roughly speaking, was carried on for a period of 100 days, or two-thirds of the lawful fishing season. Since the great bulk of the season's catch is made during the first month or two of the season, it is fair to say that had the experiment run through the entire extent of the fishing season, fully twice as many tagged spiny lobsters would have been caught as our figures show. They were tagged at points so chosen as to give a fair indication of the average intensity of fishing operations. We would thus be justified in asserting that the yearly catch probably amounts to at least 16 per cent of the total number of spiny lobsters in the localities where fishing operations are carried on. There are so many other factors involved that the estimate should be accepted only tentatively.

If the spiny lobster is just able to hold its own against its natural enemies this additional destruction due to fishing operations might suffice to bring about extinction if carried on long enough. If the rise of price entailed by increasing scarcity should tempt fishermen to redouble their efforts there would be a dangerous acceleration of the process of extinction. This should be watched from year to year. Of course, the size of the catch each year would serve as a rough index of the abundance; but estimates so formed would not be reliable because they would not show how far this decreased catch might be due to the number of fishermen employed, the number of traps operated, or the efficiency of the fishermen engaged. These are all extremely variable factors.

Within certain limits it should be possible to make a fairly accurate spiny lobster census of the entire coast line from Point Conception to San Diego by these tagging experiments. The expense of such work should be comparatively slight.

**REPRODUCTION**

The very small coral-red eggs are produced in greatest numbers near the first of May, although some scattering individuals spawn as early as March 1. The maximum number of spawn-bearing females
is observed at about the last of June. Even before this time some have shed their young. They continue to hatch in large numbers through July and well on into August. Here and there one may find cases in which they have not hatched until the middle of August. Roughly the breeding season may be said to extend through May, June and July. A set of eggs probably requires from nine to ten weeks for hatching. These statements are based upon personal observation only as regards the termination of the spawning season. It has been necessary to accept the statements of the more intelligent and reliable fishermen as to the time of its commencement.

The eggs are attached to the hairs upon the pleopods of the female. At first they are of a scarlet color but change later to maroon and dark brown as the larvae develop.

There is no difficulty in securing the young. It is only necessary to impound spawn-bearing females. The young hatch very readily even after the spawn-bearing parent has been kept in captivity for weeks. Attempts to rear them, however, proved futile. Their extreme delicacy and pelagic habit make their culture an especially difficult problem.

A few observations were made to determine the amount of spawn carried by the females of different size. They resulted as follows:

<table>
<thead>
<tr>
<th>Length of female</th>
<th>Weight of spawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 inches</td>
<td>5.115 grams</td>
</tr>
<tr>
<td>9(\frac{1}{2}) inches</td>
<td>13.422 grams—average of 3 specimens</td>
</tr>
<tr>
<td>13(\frac{1}{2}) inches</td>
<td>52.930 grams</td>
</tr>
<tr>
<td>14 inches</td>
<td>67.845 grams</td>
</tr>
</tbody>
</table>

These weight determinations were made after drying the superficial moisture from the eggs by spreading them out upon filter paper. The results give a fair basis of comparison because the spawn was in each case treated uniformly. A careful estimate of the number of eggs carried by the fourteen-inch female showed it to be approximately 500,000. This was based upon a careful count of a weighed portion of the mass.

Examination of the catches of spiny lobsters made during the breeding season leave no doubt that, in general, they spawn each year. It is always possible to find some adult females that do not bear spawn, even at the height of the breeding season, but the proportion is always small at that time and may be chiefly explained by
the occurrence of scattering cases of very early or very late spawning that do not come within the period of maximum spawn bearing.

Insemination takes place from a putty-like mass of sperm material placed upon the ventral surface of the female's thorax between the last three pairs of appendages. This mass contains contorted tubular cavities in which the spermatozoa lie. It is at first white and soft but in a short time turns black and becomes hard. It comes to resemble whalebone in consistency.

Spawn-carrying females are said to seek sheltered spots in the lee of islands or points of land and to take refuge in sheltered crevices of rocks along shore. There are many rumors of favorite spawning grounds. They are all sheltered and close to shore.

EXUVIATION

There are quite conflicting reports from fishermen regarding the shedding of the shell. My observations have been made in the summer and early fall. It is possible to find spiny lobsters in process of shedding their shells at all times during this period; but they are most common in August, September and October. Some fishermen state that shedding takes place most commonly in the spring. It is quite probable that it may occur to a certain extent at all times of the year.

SEX RATIO

Examination of spiny lobsters brought from Mexican waters in July showed a great preponderance of males. This was to be explained by the fact that the females still carrying spawn are by law excluded from the markets. In August and September, after the young have hatched, the proportion is reversed, because the males have become scarce through long fishing while the females protected during the spawn-bearing period are then available for market use. These factors make sex counts of such catches made in Mexican waters of little value. Since no spiny lobsters of either sex can be legally taken along the California coast during the spawning season this selective fishing does not take place. Conditions were especially favorable when these observations were made because they were carried out at the resumption of spiny lobster fishing after a two-year period during which it was entirely forbidden.
The following table shows the sex ratios noted at various times and places:

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 20 San Juan Point</td>
<td>136</td>
<td>147</td>
<td>283</td>
</tr>
<tr>
<td>Sept. 22 San Juan Point</td>
<td>67</td>
<td>91</td>
<td>158</td>
</tr>
<tr>
<td>Sept. 23 San Juan Point</td>
<td>55</td>
<td>104</td>
<td>159</td>
</tr>
<tr>
<td>Sept. 29 Santa Barbara</td>
<td>14</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Oct. 3 Santa Barbara</td>
<td>36</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>Oct. 7 Santa Barbara</td>
<td>57</td>
<td>29</td>
<td>86</td>
</tr>
<tr>
<td>Oct. 11 San Pedro</td>
<td>39</td>
<td>57</td>
<td>96</td>
</tr>
<tr>
<td>Oct. 16 Santa Cruz Island (Yellow Banks)</td>
<td>32</td>
<td>47</td>
<td>79</td>
</tr>
<tr>
<td>Nov. 1 Santa Cruz Island (Ladies' Harbor)</td>
<td>9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>Nov. 3 Santa Cruz Island (Pelican Bay)</td>
<td>30</td>
<td>22</td>
<td>52</td>
</tr>
<tr>
<td>Nov. 6 Anacapa Island (North Side)</td>
<td>41</td>
<td>18</td>
<td>59</td>
</tr>
<tr>
<td>Nov. 12 Santa Barbara</td>
<td>68</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>584</strong></td>
<td><strong>573</strong></td>
<td><strong>1157</strong></td>
</tr>
</tbody>
</table>

It will be seen that the sex ratio varies greatly but not in such a manner as to be attributable to the date when made. It is quite impossible for me to explain this upon any grounds other than chance. The final averages in the count of the sexes show them to be of practically even number.

Much more work should be done upon the spiny lobster in order to make it as well known to us as the true lobster of our Atlantic coast. It is quite important from an economic standpoint that we have the fullest information about this rapidly diminishing form.

*Transmitted November 23, 1915.*
NOTES ON THE MARINE FISHES OF CALIFORNIA

BY

CARL L. HUBBS

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NOTES ON THE MARINE FISHES OF CALIFORNIA

BY

CARL L. HUBBS

This paper is based in part upon several small collections made by the author during the last two years. In addition, notes are presented of fishes; first, in the Scripps Institution for Biological Research of the University of California, at La Jolla; second, in the collections of Stanford University; third, in the museum of the Los Angeles High School, and fourth, from the Aquarium at Avalon, Santa Catalina Island.

A new genus, Lestidiops, and two new species, L. sphyraenopsis and Otophidium scrippsi, are herein described.

I am indebted to Professor W. E. Ritter for permission to report upon certain fishes in the Scripps Institution, and for kindness shown me during my brief stays at the Institution. I also desire to thank Dr. Charles F. Holder, of Pasadena, and Professor George B. Culver, of the Los Angeles High School, for assistance in obtaining specimens. This paper has been prepared in the Zoology Laboratory of Stanford University, and the kind suggestions of Dr. Charles H. Gilbert, of that institution, have been of material assistance in the preparation of this report.

Raja inornata Jordan and Gilbert

This ray is common off Redondo and Coronado, near shore.

Ophichthus triserialis (Kaup)

This species is common off the west coast of Mexico, and has previously been recorded from the California coast only from Monterey Bay. It is now recorded for the first time from Southern California, the record being based upon two specimens from off San Pedro, and
upon two from off La Jolla. The northern known limit of the distribution of this species is now extended to Tomales Bay, from which locality Mr. N. B. Scofield has sent a specimen to Stanford University.

The eye of the adult is contained five times in the gape.

**Ophichthys zophochir** Jordan and Gilbert

Two specimens of this el, hitherto known from Panama to Guaymas, Mexico, were caught with hook and line off the wharf at Playa del Rey, Los Angeles County. Length 493 and 443 mm. The teeth on the vomer and jaws of the smaller specimen are in two rather irregular rows, while those of the larger specimen are in narrow bands. The origin of the dorsal in this species is subject to some variation, being over the middle or the tips of the pectoral rays. Head 3 in trunk; depth of body 2.5 and 3 in trunk (in larger and smaller specimens, respectively); gape 3 and 2.7 in head; gill openings about one-third wider than eye; eye 1.5 in snout; pectoral 2.4 and 2.6 in head.

**Anchovia compressa** (Girard)

This anchovy is abundant in the sloughs at Anaheim Landing. Anal rays 39 to 35. There is a wide variation in form, the depth of the body varying from 3.9 to 4.5. The most robust and the most slender specimens are of very different appearance.

Genus *Lestidiops*, new (*Paralepididae*)

Body elongate and compressed, entirely naked, excepting a series of about 120 concealed scales along the lateral line, which rapidly decrease in size posteriorly; a single pore at the end of a long curved tube above and below each scale. Dorsal well behind middle of body, entirely behind ventrals; anal well before dorsal: adipose dorsal short, near caudal; anus midway between ventral and dorsal. Belly with a fleshy keel. Head deep, compressed. Premaxillaries with a pair of anterior canines, followed by a series of short retrorse teeth; mandibular teeth in two series, those of the outer series small, one opposite each inner canine; palatine teeth similar to mandibular teeth; no teeth on the vomer, nor on the broad, free tongue. Gillrakers short and spinous.

*Lestidiops* is closely related to the genus *Lestidium,* differing in the absence of teeth on the tongue; in the much more attenuate body posteriorly; and in the character of the lateral line. The pores are

---

1 Gilbert, Bull. U. S. Fish Comm. for 1903, part II, p. 608, fig. 236.
single, instead of 3 in number, above and below each concealed scale; these scales are smaller, 120 instead of 68; and the lateral line terminates opposite the middle, rather than the end, of the anal fin.


*Lestidiops sphyraenopsis*, new species

Plate 18

The type-specimen was seined near shore in Avalon Bay, Santa Catalina Island, California, and was procured from the Aquarium. It is deposited in the fish collections of Stanford University (no. 22597).

Body extremely elongate, deepest at the nape, attenuate posteriorly. Snout deep and long; anterior orbital margin behind middle of head; interorbital space flattish, the sides curved gently inward; two low interorbital ridges, slightly convergent posteriorly; premaxillaries forming margin of upper jaw; maxillaries very slender, with an acuminate tip not reaching to below orbit; preorbital groove extended past anterior margin of orbit; premaxillaries with an anterior pair of moderate, curved, depressible canines, about half as long as the mandibular canines; premaxillaries arched and toothless before and between the canines, canine of each side followed by a toothless space, as long as the canine, this space followed by a single series of small retrorose teeth, evenly decreasing in size to the end of the premaxillary; mandible with a double series of teeth, 7 in each series, the inner series composed of depressible canines, one-third as long as orbit; the outer series composed of short fixed teeth, one opposite each canine; vomer and tongue toothless; palatine teeth similar to those of mandible, with 5 depressible canines, and a series of fixed teeth, one opposite each canine, the outer series continued behind the canines, along the trenchant edge of the palatine, extending as far back as the premaxillary. Four long gills; the slit behind the last about as wide as pupil; gill-rakers very short and spinous; pseudobranchiae developed; 7 branchiostegals. Photophores not evident on type specimen.

Pectoral inserted slightly below axis of body; ventrals a little behind middle of body; dorsals inserted well behind ventrals, the interspace longer than the snout; origin of anal but little nearer origin of dorsal than base of caudal; anal base half as long as the distance between anal and ventrals; ventrals short, reaching about half the distance from their base to anus, which is about midway between insertion of anal and origin of dorsal.
Color faded as a result of preservation in strong formaldehyde; no evident markings excepting a dark occipital spot; snout and jaws with traces of dark pigment; vertical fins punctate.

Vertebræ, as counted through the translucent body, without dissection, more than 90: 49, including hypural, behind vertical from origin of dorsal.

This species is apparently not closely related to Sudis ringens Jordan and Gilbert, as that species was described as having large scales. Sudis ringens also has a shorter head; shorter mandible; shorter snout; the maxillaries reaching to below eye; and a greater distance between dorsal and anal fins, owing to the more posterior position of the dorsal.

Only the type known.

COMPARATIVE TABLE OF MEASUREMENTS IN HUNDREDTHS OF LENGTH TO BASE OF CAUDAL*

<table>
<thead>
<tr>
<th></th>
<th>Sudis ringens</th>
<th>Lestidium nudum</th>
<th>Lestidiops sphyraenopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length to base of caudal, inches</td>
<td>6.25</td>
<td>......</td>
<td>9</td>
</tr>
<tr>
<td>Length to base of caudal, mm.</td>
<td>......</td>
<td>200</td>
<td>230</td>
</tr>
<tr>
<td>Greatest depth of body</td>
<td>6</td>
<td>8.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Least depth of body</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Depth, between dorsal and anal</td>
<td>......</td>
<td>6.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Length, caudal peduncle</td>
<td>6</td>
<td>......</td>
<td>5.5</td>
</tr>
<tr>
<td>Length, head</td>
<td>16.5</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Length, maxillary</td>
<td>9</td>
<td>10.5</td>
<td>9</td>
</tr>
<tr>
<td>Length, mandible</td>
<td>11.5</td>
<td>......</td>
<td>13</td>
</tr>
<tr>
<td>Length, snout</td>
<td>8</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Length, orbit</td>
<td>......</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>Width, interorbital</td>
<td>......</td>
<td>2.7</td>
<td>2</td>
</tr>
<tr>
<td>Length, base of dorsal</td>
<td>5.5</td>
<td>3</td>
<td>4.5</td>
</tr>
<tr>
<td>Length, base of anal</td>
<td>14</td>
<td>18</td>
<td>13.5</td>
</tr>
<tr>
<td>Anus to origin of anal</td>
<td>......</td>
<td>18.5</td>
<td>21</td>
</tr>
<tr>
<td>Tip of snout to ventrals</td>
<td>57</td>
<td>61</td>
<td>65</td>
</tr>
<tr>
<td>dorsal</td>
<td>80</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>anal</td>
<td>8</td>
<td>(0.4 head)</td>
<td></td>
</tr>
</tbody>
</table>

Fin-rays:

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>dorsal</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>anal</td>
<td>26</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>ventral</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>pectoral</td>
<td>13</td>
<td>11–12</td>
<td></td>
</tr>
</tbody>
</table>

From figure.

* Measurements based on type-specimen of each species.

Hubbs: Marine Fishes of California

Lestidium nudum has been recorded by Waite from Sandy Island, South Pacific. His three specimens gave the following counts:

- Dorsal rays: 12, 10, 10
- Anal rays: 29, 31, 32
- Scales: 68, 68, 68

Lestidium japonicum has 42 to 49 anal rays.

Fundulus parvipinnis Girard

Exceedingly abundant in all bays and sloughs; ascending the San Gabriel and San Diego rivers to fresh water. I have also collected this species at Playa del Rey, Anaheim Landing, Mission Bay, San Diego Bay, and in sloughs at the mouth of Tia Juana River, near the Mexican border.

Cololabis saira (Brevoort)

Scombresox saira Brevoort, Perry’s Expedition to Japan, 1856, p. 281, pl. VII, fig. 4.
Cololabis brevirostris Jordan and Evermann, Fishes of North and Middle America, I, p. 726, 1896.

I am unable to separate our specimens of Cololabis from California (called C. brevirostris) from specimens of the Japanese species, C. saira.

Table of Measurements in Hundredths of Total Length to Caudal Base

<table>
<thead>
<tr>
<th>Monterey Bay</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, mm.</td>
<td>173</td>
</tr>
<tr>
<td>Length, eye</td>
<td>4</td>
</tr>
<tr>
<td>Width, bony interorbital</td>
<td>3.7</td>
</tr>
<tr>
<td>Length, snout</td>
<td>8.5</td>
</tr>
<tr>
<td>Length, maxillary</td>
<td>5.5</td>
</tr>
<tr>
<td>Length, mandible</td>
<td>11</td>
</tr>
<tr>
<td>Length to anus</td>
<td>68</td>
</tr>
<tr>
<td>Depth, body</td>
<td>12</td>
</tr>
<tr>
<td>Width, body</td>
<td>6</td>
</tr>
<tr>
<td>Depth, caudal peduncle</td>
<td>2.5</td>
</tr>
<tr>
<td>Snout to dorsal</td>
<td>72</td>
</tr>
<tr>
<td>Length, dorsal base</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Table of Measurements in Hundredths of Total Length to Caudal Base—(Continued)

<table>
<thead>
<tr>
<th></th>
<th>Monterey Bay</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, dorsal fin</td>
<td>5.5 6 6</td>
<td>5.5 5.5 6 6</td>
</tr>
<tr>
<td>Height, first dorsal finlet</td>
<td>3.5 4 3.5</td>
<td>3.2 3.5 ...... 3.5</td>
</tr>
<tr>
<td>Length, anal base</td>
<td>10 10 9</td>
<td>10.3 11 10 11</td>
</tr>
<tr>
<td>Height, first anal finlet</td>
<td>3.2 3.5 3.2 3.5</td>
<td>3.2 3.5 3.2 3.5</td>
</tr>
<tr>
<td>Length, pectoral</td>
<td>7 7 7</td>
<td>7 7 7 8</td>
</tr>
<tr>
<td>Ventral to anus</td>
<td>14 13 13.5 13</td>
<td>13 11.5 12 13</td>
</tr>
<tr>
<td>Length, ventral</td>
<td>6.5 6 6 6</td>
<td>6.2 6.5 6.5 6</td>
</tr>
</tbody>
</table>

Table of Scale, Fin, and Gill-Raker Counts

<table>
<thead>
<tr>
<th></th>
<th>Monterey Bay</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale rows</td>
<td>121 117 119</td>
<td>121 115</td>
</tr>
<tr>
<td>Dorsal rays</td>
<td>9 10 10</td>
<td>10 11 11</td>
</tr>
<tr>
<td>Dorsal finlets</td>
<td>6 5 6</td>
<td>6 6 6</td>
</tr>
<tr>
<td>Anal finlets</td>
<td>6 6 6</td>
<td>7 6 6</td>
</tr>
<tr>
<td>Pectoral rays</td>
<td>13 13 13</td>
<td>13 14 14</td>
</tr>
<tr>
<td>Gill-rakers; first arch</td>
<td>9 + 38</td>
<td>...... 8 + 37</td>
</tr>
</tbody>
</table>

Syngnathus californiensis Storer

A male specimen, 224 mm. long, was collected on August 8, 1913, among a large quantity of kelp on the beach at Coronado. Head 9; D. 43; rings 19 + 48; dorsal on 1 + 9 rings; pouch (containing eggs) on 23 caudal rings; eye 8.2; snout 2; body 2.4 in tail; depth 3.5 in head; pectoral 6 in head; caudal 4.2; height of dorsal 5.5 in its length; base of dorsal 1/4 longer than head. Color olive-green anteriorly, shading into brown on tail; pouch unmarked; sides anteriorly with gray rosettes between rings. Head sparingly spotted with gray.

A specimen in the Scripps Institution was taken at a surface haul off La Jolla. D. 43; rings 21 + 47; dorsal on 1 + 9 rings; snout 1.8; eye 8.

Both of these specimens were taken from the kelp beds, while none were found in the bays, where the following species is abundant.

Syngnathus leptorhynchus Girard

This is the commonest pipe-fish in the bays and sloughs. Specimens were obtained in the sloughs at Anaheim Landing, Orange County, and in San Diego Bay.

The specimens from Anaheim Landing show some variations from current descriptions. The adult length is greater, 10 instead of 6.
inches. The pouch extends over 20½ to 22 caudal rings, instead of 19. The dorsal fin rays are more numerous, 31 to 35 instead of 30 to 32; and the fin covers from $1 + 7$ to $0 + 8$ rings. The caudal rings are more numerous, 39 to 43, instead of 36 to 41.

Males taken in January at Anaheim Landing contained eggs in the pouch; those taken in April contained young; a female, 165 mm. long, collected on August 6, contained large eggs; a male, 127 mm. long, taken on the same day, had the pouch empty.

In young specimens 4 types of coloration were found. Some were entirely green, corresponding to the color of the eel-grass in which the fish were taken; others were entirely grayish-brown, corresponding closely to the color of some growth on the algae; others were green, with a grayish-brown stripe along the dorsal surface; still others were green, with horizontal bars of grayish-brown. It may be of interest to note that these four types of coloration were also found in prawns taken with the pipe-fish.

**Syngnathus griseolineatus** Ayres

A female specimen, from Santa Cruz, 210 mm. long (without caudal). D. 40, on $1 + 8$ rings; rings $19 + 43$; eye 8.2; snout 1.7. Another specimen, a female 215 mm. long, was collected by Mr. N. B. Scofield in San Francisco Bay. D. 41, on $1 + 8$ rings; rings $18 + 42$; eye 8.7; snout 1.6.

**Syngnathus barbarae** (Swain)

Dr. C. H. Gilbert has re-examined the type of this species, and permits the writer to present the following from his notes:

Dorsal, 35.

Species certainly distinct from *griseolineatus* or *leptorhynchus*, with which the type has been directly compared.

It is probable that the tail is abnormally shortened in this specimen. The caudal is irregularly attached, the joint being abnormal and not as small as usual, the caudal peduncle not normally tapering. The presence of but 10 rings behind the caudal pouch also suggests an early accident and the formation of a new caudal fin. The dorsal completely covers 8 rings, the first of which contains the anus.
Measurements in Hundredths of Total Length to Vent; 1, the Type of
*S. barbarae; 2, S. griselineatus; 3 and 4, S. leptorhynchus*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length in mm.</td>
<td>91</td>
<td>99</td>
<td>70</td>
<td>53</td>
</tr>
<tr>
<td>Head</td>
<td>30.5</td>
<td>31.1</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Snout</td>
<td>14.9</td>
<td>17.1</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Greatest depth snout</td>
<td>5.1</td>
<td>4</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Least depth snout</td>
<td>3</td>
<td>2.6</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Eye</td>
<td>2.8</td>
<td>3.5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Greatest depth trunk</td>
<td>9</td>
<td>7.7</td>
<td>7.5</td>
<td>6</td>
</tr>
<tr>
<td>Base of dorsal</td>
<td>29</td>
<td>30</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Length of brood pouch</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Syngnathus auliscus** (Swain)

Collected with *S. leptorhynchus* at Anaheim Landing. The color is plain brown, sometimes with lighter rings. The specimens show the same differences from current descriptions that were noted by Starks and Morris. Two specimens collected in San Diego Bay on February 14, 1914, are 100 mm. long, one a male with eggs in the pouch, the other a female with mature ova.

**Syngnathus arctus** (Jenkins and Evermann)

Two specimens were taken with *S. leptorhynchus* in the eel-grass of Spanish Bight, San Diego Bay, by Mrs. Frank Stephens.

**Leuresthes tenuis** (Ayres)

Specimens were bought from a fisherman, who had seined them between Newport and Laguna. Dorsal IV or V–I, 8 to 11.

Several specimens of this species were sent to Stanford University by Mr. J. B. Joplin, of Santa Ana, who described the breeding habits of this fish. He wrote:

"It is the peculiar nature of these fish which attracts our attention. Three months during the year, usually March, April and May, on the second, third and fourth nights after the full moon, at full tide, great schools of them come out in the breakers, at the mouth and for half a mile on each side of where a small fresh water stream flows into the ocean, for the purpose of depositing its eggs or spawn in the sand. The water recedes and when the fish are not disturbed they wiggle tails down in the sand, as far out as the force of the water will carry them, both males and females together—sometimes as many

---

as eight or ten together—where the crust of the sand has been broken. Sometimes only one female is found, with just her head visible.

"Why they come out at night, which is usually from 10 to 1 o'cloak, and the run usually lasts three hours or longer, is one question.

"I have been observing them for thirty years and the time of their coming is so regular that during that time I have rarely missed them."

A detailed study of these interesting habits, or a confirmation of them, is highly desirable.

**Icosteus aenigmaticus** Lockington

A specimen in the Scripps Institution, from the Cortez Banks, off San Diego, was collected in June, 1913, at a depth of 80 to 90 fathoms. This species has heretofore not been recorded south of Monterey Bay.

**Lepidopus xantusi** Goode and Bean

Two more specimens of this species have been taken in southern California. One, from off La Jolla, has 79 dorsal spines; the other, from Avalon, has 80.

**Paralabrax maculatofasciatus** (Steindachner)

This bass is common along the Southern California coast northward to Redondo.

**Sebastodes goodei** Eigenmann and Eigenmann

A specimen in the Scripps Institution has the anal rays III, 9.

**Sebastodes aurora** (Gilbert)

A small specimen in the Scripps Institution was dredged in 150 to 185 meters, off La Jolla. Dorsal, XIII, 13; anal, III, 6; pores, 29; eye, 3.1; maxillary, 2.1; 19 gill-rakers on the lower limb of the outer arch, the anterior ones small, the longest gill-raker 2.2 in eye.
Sebastodes diploproa (Gilbert)

A specimen, 125 mm. long without caudal, in the Scripps Institution, was dredged off La Jolla, at a depth of 292 meters. Dorsal, XIII, 13; anal, III, 7; 37 pores in lateral line; scales deciduous, much more numerous than the pores; premaxillaries anteriorly forming the two projecting, dentigerous lobes diagnostic of the species; preorbital with two strong spines; gill-rakers 11 + 24, those at both ends of the arch small, the longest 3.5 in postorbital length of head; orbit, 2.8; interorbital width, 2.25 in orbit. The eye is larger, and the interorbital narrower, than usual in this species.

A small specimen, 67 mm. long, was dredged off La Jolla, at a depth of 185 meters. Dorsal, XIII, 13; anal, III, 7; head, 2.5; depth, 2.9. Another small specimen, 41 mm. long, was obtained off La Jolla, at a depth of 160 fathoms. Dorsal, XIII, 12; anal, III, 7; head, 2.33; depth, 3. A dark spot present on anterior soft rays of dorsal and anal fins.

### Table of Measurements of Paratypes of S. diploproa.

<table>
<thead>
<tr>
<th>Orbit in head</th>
<th>Interorbital in orbit</th>
<th>Orbit in head</th>
<th>Interorbital width</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.9</td>
<td>3.2</td>
<td>1.8</td>
</tr>
<tr>
<td>3.1</td>
<td>1.8</td>
<td>3</td>
<td>1.85</td>
</tr>
<tr>
<td>3.1</td>
<td>1.95</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1.95</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>3.05</td>
<td>2</td>
<td>2.9</td>
<td>2</td>
</tr>
<tr>
<td>3.33</td>
<td>1.7</td>
<td>2.8</td>
<td>1.85</td>
</tr>
<tr>
<td>3.2</td>
<td>1.75</td>
<td>2.8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1.75</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3.2</td>
<td>1.9</td>
</tr>
<tr>
<td>3.1</td>
<td>2</td>
<td>....</td>
<td>......</td>
</tr>
</tbody>
</table>

Sebastodes saxicola (Gilbert)

Plate 19

Three specimens in the Scripps Institution were dredged off La Jolla, at a depth of 185 meters. They differ somewhat from the type description, but the differences are apparently due to individual variation. Body deeper; depth, 2.66 to 2.75; caudal peduncle also deeper. Two specimens have 8 soft anal rays. Pores, 35 to 37 in the lateral line, 45 in type description, 36 and 41 in two specimens from Albatross Station 3677. Anal spines shorter, the second 3 in head. About 24 gill-rakers on lower limb of outer arch.
Measurements in Hundredths of Length to Base of Caudal

<table>
<thead>
<tr>
<th>Off La Jolla</th>
<th>Alb. Sta. 3677</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, mm.</td>
<td>103 94 90</td>
</tr>
<tr>
<td>Length, head</td>
<td>40 42 39.5</td>
</tr>
<tr>
<td>Length, orbit</td>
<td>14.5 15 13.5</td>
</tr>
<tr>
<td>Length, snout</td>
<td>9 9 8</td>
</tr>
<tr>
<td>Length, maxillary</td>
<td>18 19 19</td>
</tr>
<tr>
<td>Width, interorbital</td>
<td>8 7.5 7</td>
</tr>
<tr>
<td>Depth, body</td>
<td>37 40.5 37</td>
</tr>
<tr>
<td>Depth, caudal peduncle</td>
<td>10 10.5 10</td>
</tr>
<tr>
<td>Width, body</td>
<td>18 20 17.7</td>
</tr>
<tr>
<td>Height, dorsal spines</td>
<td>14.5 16 16.5</td>
</tr>
<tr>
<td>Height, soft dorsal rays</td>
<td>15 16 15.5</td>
</tr>
<tr>
<td>Height, first anal spine</td>
<td>7 7.5 7.5</td>
</tr>
<tr>
<td>Height, second anal spine</td>
<td>...... 15 17</td>
</tr>
<tr>
<td>Height, third anal spine</td>
<td>12 13 13</td>
</tr>
<tr>
<td>Height, soft anal rays</td>
<td>14 16 15</td>
</tr>
<tr>
<td>Length, pectoral rays</td>
<td>28 30.5 29.5</td>
</tr>
<tr>
<td>Length, ventral spine</td>
<td>15.5 16 16.5</td>
</tr>
<tr>
<td>Length, ventral rays</td>
<td>21 22 21.5</td>
</tr>
<tr>
<td>Dorsal soft rays</td>
<td>12 12 12</td>
</tr>
<tr>
<td>Anal rays</td>
<td>8 7 8</td>
</tr>
<tr>
<td>Pectoral rays</td>
<td>16 16 16</td>
</tr>
<tr>
<td>Pores in lateral line</td>
<td>35 36 37</td>
</tr>
</tbody>
</table>

**Sebastodes elongatus** (Ayres)

A specimen with 14 dorsal spines was collected in the San Diego market.

**Scorpaena guttata** Girard

Very common in Santa Monica, San Pedro, and San Diego Bays. Forty-five to 55 pores in the lateral line. Spines of head variously developed; the opercular spines straight and weak in some specimens, curved and strong in others.

**Zonogobius zebra** (Gilbert)

Twenty-four specimens in the Scripps Institution, from San Clemente Island, show some differences from the type description, probably due to the fact that the type was only 12 mm. long. Some of these discrepancies were corrected by Snodgrass and Heller⁶, who reported on a specimen taken by R. C. McGregor at Todos Santos Island, Mexico. This specimen agrees in all respects with those from

San Clemente Island. The measurement of the spinous dorsal in the table of Snodgrass and Heller is of the second ray, the first and longest ray being broken; the pectoral fin is also broken, the fin being much longer than indicated.

Scales with a row of apical spines; no scales on head, belly, nor along base of spinous dorsal. Cross bars half as wide as interspaces in the largest specimens, but relatively much wider in those as small as the type; the bars in alcoholic specimens are olive brown, with darker streaks along each border and near the center. Last soft ray of dorsal and anal longest. In the adult, but not in the young, the first dorsal spine is filamentous. Teeth in a wide mandibular band; and in a narrow maxillary band, the outer series of which is enlarged. The teeth of the lower jaw are not in 2 series, as stated by Snodgrass and Heller.

**Measuresments of Five Specimens**

<table>
<thead>
<tr>
<th></th>
<th>Length</th>
<th>39</th>
<th>38</th>
<th>33</th>
<th>27.5</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length to base of caudal, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of head</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.9</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Depth, body</td>
<td>4.4</td>
<td>4.9</td>
<td>4.7</td>
<td>4.4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Length, eye</td>
<td>4.25</td>
<td>4.1</td>
<td>4</td>
<td>4.2</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Length, maxillary</td>
<td>2.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.3</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Cross bars behind eye</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>First D. spine in length of body</td>
<td>2</td>
<td></td>
<td>2.6</td>
<td></td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Second D. spine in length of body</td>
<td>2.8</td>
<td>3.6</td>
<td>4</td>
<td></td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Third D. spine in head</td>
<td>1.6</td>
<td>1.65</td>
<td>1.6</td>
<td>1.7</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Soft rays of dorsal</td>
<td>1.6</td>
<td>1.55</td>
<td>1.6</td>
<td>1.55</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Length, caudal</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, anal</td>
<td>1.8</td>
<td>1.5</td>
<td>1.4</td>
<td>1.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Length, ventral</td>
<td>1.33</td>
<td>1</td>
<td>1.2</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Length, pectoral</td>
<td>.95</td>
<td>1</td>
<td>.8</td>
<td>.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dorsal rays</td>
<td>VI</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Anal rays</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

**Gillichthys mirabilis Cooper**

This curious mud-burrowing goby was found abundantly in bays and sloughs at Ventura, Playa del Rey, San Pedro, Anaheim Bay, Mission Bay, and in sloughs at the mouth of Tia Juana River, near the Mexican border.

**Clevelandia ios (Jordan and Gilbert)**

This little goby, together with the related species, *Quilenta y-cauda* and *Hypnus gilberti*, is common about the sloughs, especially in the small pools left on the mud flats by the retreating tide. They have
similar burrowing habits to those of the larger *Gillichthys*, but frequently merely bury themselves with mud or sand by a lateral movement of the body and fins. *C. ios* ascends the San Gabriel River to fresh water. A specimen in the Stanford University Museum was collected in San Bartolome Bay, Lower California.

**Typhlogobius californiensis** Steindachner

Two specimens of the Blind Goby, hitherto known from Point Loma, Laguna Beach, and from Dead Man's Island and Point Fermin, near San Pedro, were collected in the rock pools of Rocky Point, near Redondo. Another specimen, in the Scripps Institution, was collected in the rock-pools near La Jolla.

**Echineis remora** Linnaeus

Two specimens, from Santa Catalina Island, have 16 or 17 laminae.

**Neoclinus satiricus** Girard

Fairly common at Redondo and at Avalon, Santa Catalina Island. The membrane connecting the maxillaries with the lower jaw is green in life, abruptly edged with yellow.

**Hypsoblennius gentilis** (Girard)

Two blennies of this species, from San Diego Bay, show some variations from current descriptions. Head, 3.7 and 4, respectively; dorsal, XIII, 15 and XIII, 16; maxillaries extending in one specimen to below middle of eye, in the other to below posterior margin of eye; length of maxillary, 2.6 and 2.3; lateral line developed in one specimen beyond the anterior straight part, its tip being pointed downward.

**Cryptotrema corallinum** Gilbert

One specimen, in the Scripps Institution, was dredged off La Jolla. Length, 44 mm., without caudal; head, 3.8; depth, 6; dorsal, XXVII. 12; anal, H, 26; interorbital, 2.1 in orbit; 45 scales along straight part of lateral line, 6 on oblique portion, 19 on posterior straight part, 70 in all.

**Anarrhichthys ocellatus** (Ayres)

One specimen, in the Museum of the Los Angeles High School, was caught by fishermen off Redondo. This is the first record south of Santa Barbara.7 Length, about 1255 mm.; head, 9.2; depth, 1.3;

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longest dorsal ray, 1.3; pectoral, 1.55; eye, 8.9 in head; snout, 4.75; maxillary, 2.25; interorbital, 6.2; width of head, 2.2

Maynea californica Gilbert

Five specimens in the Scripps Institution, from off La Jolla.

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Otophidium taylori Girard

Chilara taylori Jordan and Evermann, Fishes of North and Middle America, III, 1898, p. 2489.

Professor J. O. Snyder has called my attention to the fact that the so-called foramen of the air-bladder, the absence of which has been supposed to separate Chilara from Otophidium, is sometimes present in this species, its form and size subject to great variation. The genus Chilara is apparently untenable.

Two small unsotted specimens, in the Scripps Institution, were collected off La Jolla, at a depth of 55 meters. Head 5.9 and 6 in length without caudal; depth, 10 and 11.2; gill-rakers, x + 7 to 9. One large spotted, and two small unsotted specimens, from Santa Catalina Island, have 8 gill-rakers on the lower limb of the outer arch.

Otophidium scrippsi, new species

Plate 20


The type, deposited in the Scripps Institution, La Jolla, California, is a specimen 168 mm. long without caudal. It was dredged on September 1, 1908, near Cerros Island, off the coast of Lower California: lat. 28° 2' 1" N.; long. 115° 6' 0" E.; depth, 73 meters; bottom, green
"O. scrippsi is not closely related to *O. taylori*, having fewer gill-rakers, a longer head, a more robust form, and plain coloration. It is more closely related to *O. galeoides* (Gilbert) and to *O. indefatigabile* Jordan and Bollman, but differs from them in the longer head, smaller eye, plain coloration, and the long, slender gill-rakers. There are 5 instead of 4 gill-rakers on the lower limb of the first arch, and the longest is more than one-third as long as eye; while the short, broad gill-rakers of the related species are contained about 5.33 in the eye.

Form of body as in related species, the depth 5.7 in length without caudal; greatest depth at origin of dorsal; body moderately compressed anteriorly, rather sharply compressed posteriorly.

Upper profile of head convex, not greatly curved from eye to occiput; snout moderately blunt, 4.75 in head to upper angle of gill opening; head, 4.5 in length without caudal; eye nearly round, 4.2 in head; interorbital space flattish, widening rather abruptly posteriorly, its least width 1.5 in eye; lower jaw included; maxillary, 2.75 in head; preorbital width, 2.6 in eye. Teeth rather small, in narrow bands on jaws and palatines, and in a triangular patch on vomer; the outer premaxillary series a little enlarged. Nostrils without conspicuous flaps, the two nearly evenly spaced between margin of eye and tip of snout; opercle ending in a sharp, flat, concealed spine; gill-rakers rather slender, the longest, at angle, nearly as long as pupil, almost one-third as long as eye; 3 tubercles on upper limb of gill arch, 5 well developed gill-rakers on lower limb; pseudobranchiae small, but evident, the filaments about half as long as pupil; 6 branchiostegals.

Scales small, somewhat imbedded, non-imbricate, widely spaced on belly, their longer axes frequently at right angles. Head and fins naked. Lateral line high, its distance from dorsal base .2 its distance above anus.

Fins as in other species, except that the caudal is emarginate. Pectoral, 2.3 in head; ventral, 2.2.

Air bladder short and thick in type, especially hardened and thickened in a very definite median horizontal band, this band divided posteriorly by a deep constriction, not quite as deep as diameter of pupil; distance from end of air-bladder to anus, 2.4 in head; no posterior foramen.

Back light dusky; sides lighter; belly and under side of head pale. Body without dark spots or other markings. Dorsal light, margined with blackish; anal similar to dorsal, the margin widening posteriorly; paired fins pale.
A small specimen was taken by Snodgrass and Heller in the Galapagos Archipelago, "from the stomach of a tunny at Tagus Cove, Albermarle Island," and referred by them to *C. taylori*. It has 5 gill-rakers on one side and 4, with 2 rudiments, on the other side and certainly is not *O. taylori*. The gill-rakers are longer than in *O. indefatigabile* or *O. galeoides*, resembling those of *O. scrippsi*, of which species it may be the young, although the teeth are relatively larger. (Named for Miss Ellen B. Scripps, whose generous gifts to the Scripps Institution have been a great help in the study of the zoology of Southern California).

**Hippoglossina stomata** Eigenmann and Eigenmann.

One specimen was purchased in the San Diego market. Eyes sinistral. Vertebrae 11 + 27, with hypural; the anterior 5 vertebrae much smaller than those following, the length of the second only half that of the eleventh; these anterior 5 vertebrae directed slightly downward.

**Lyopsetta exilis** (Jordan and Gilbert)

Three young specimens, 73, 76 and 96 mm. long, without caudal, in the Scripps Institution, were taken at a depth of 292 meters off La Jolla.

**Pleuronichthys verticalis** Jordan and Gilbert

This species is common in the San Diego market. A specimen 190 mm. long, without caudal, was collected in the San Francisco markets by Dr. D. S. Jordan. It is dark brown on the eyed side; a black spot, not ocellated, on lateral line near middle of its length; indistinct darker spots on each side of caudal peduncle, and elsewhere on body, corresponding to certain of those on the other specimen obtained; vertical fins with darker bars, and with a very narrow white border, widest anteriorly; dorsal fin light anteriorly; extreme tips of rays of paired fins white. Another specimen, 210 mm. long, was collected with the specimen just described. Its coloration is highly variegated: the large median spot is strikingly ocellated by a white ring, and smaller white spots are scattered over the body, surrounded by dark rings, which form reticulations about the white spots anteriorly. Other specimens vary from this ornate type of coloration to a perfectly plain type.
Pleuronichthys ritteri Starks and Morris⁹

One specimen from off San Pedro.

Young specimens have a variegated coloration. A specimen 61 mm. long, without caudal, from off La Jolla, has the 3 ocellated spots very distinct; in addition there are 2 others posteriorly along the bases of the dorsal and anal. Body mottled with dark and light brown; a conspicuous square dark blotch, wider than eye, between median ocellated spot and caudal, others, less distinct, before and behind it; body covered with mottled areas which are frequently bordered with narrow dark lines. Dorsal and anal variegated, darker anteriorly, with a small dark spot opposite the anterior ocellated spot along the base of the fin. Caudal dark, with about 5 vertical bars, and with a whitish edge. Pectoral with 3 broad bars. Ventral light brown. All fins colorless on the blind side, except the decurrent part of the dorsal.

Pleuronichthys coenosus Girard

One specimen, in the Scripps Institution, was taken off La Jolla on July 10, 1914. Several like specimens, with highly variegated coloration, were seen in the aquarium at Avalon, Santa Catalina Island.

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Transmitted September 2, 1915.
Leithophthalus subpteropterus (x 77)

Drawn by W. S. Allison from the type-specimen from Avalon.
Sebastodes saxatilis (Hildebrand)}

Drawn by W. S. Allison from a specimen taken in 125 fathoms off La Jolla.
Ophichthus scriptus (x 11)

Drawn by W. S. Akenson from the type specimen from Cerros Island.
THE FEEDING HABITS AND FOOD OF PELAGIC COPEPODS AND THE QUESTION OF NUTRITION BY ORGANIC SUBSTANCES IN SOLUTION IN THE WATER

BY

CALVIN O. ESTERLY
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THE FEEDING HABITS AND FOOD OF PELAGIC COPEPODS AND THE QUESTION OF NUTRITION BY ORGANIC SUBSTANCES IN SOLUTION IN THE WATER

BY

CALVIN O. ESTERLY

It is well known how some of the animals that feed on the minute organisms of the plankton obtain their food. For example, the particles ingested by the appendicularians are gathered by means of a screen-like arrangement that separates them from a current of water. Or, as in the case of the Salps, the food material is entangled in mucus and then carried to the opening of the esophagus.

The matter is different, however, when such forms as the Copepoda are considered. These animals are provided with well-developed appendages which have a varied equipment of bristles. The copepods feed upon the minute animals and plants of the plankton (Peck, 1896; Dakin, 1908), and it is a rather interesting question how the complex appendages are used in getting food. Such organisms as diatoms are too small to be grasped or manipulated by the bristles of the mouth parts, yet the copepods ingest diatoms in large numbers.

The literature, so far as I have been able to discover, contains no accurate information as to the method by which the pelagic copepods get their food. Dakin (1908, p. 776) speaks of "the food-matter caught by the mouth appendages and introduced into the mouth by the mandibles," but he does not say much more about the process. Lohmann (1911, p. 11) states casually that a large part of the copepods make the same filtration experiment as the appendicularians,
but he does not tell how the filtering is accomplished or what organs take part.

The method of food-getting by the Ostraeoda appears to have received more attention, and since it is similar to that in the copepods, it may be well to make note of it here. Biedermann (1911, p. 651) gives this account for the Daphnids:

Die betreffenden Körperchen werden durch Bewegungen der Beinanhänge herbeigestrudelt und schließlich zu einem Bissen geformt, der dann durch peristaltische Bewegungen des Oesophagus rasch in den Mitteldarm befördert wird. Mittels der reusenartigen Fortsätze ihrer beständig auf und ab bewegten Gliedmassen vermögen die Daphnien grosse Wassermengen durchzusiehen.

Woltereck (1908, p. 872) makes very similar statements.

I can state, from observations upon Calanus finmarchicus and Eucalanus elongatus, that the copepods get their food in essentially the same manner as the ostraeods. I have watched many living specimens of Calanus get carmine grains in what appears to be necessarily the method by which they obtain organisms for food from the sea water.

When a living Calanus is put into a small amount of water, the animal nearly always scurries about, ventral side up. Under these conditions it is possible to see how solid particles are concentrated and taken into the mouth. The necessary preliminary to actual ingestion is the production of currents which transport the particles toward the body. The movement of the water is due to the activity of the appendages, chiefly those of the head. It is not possible to state just how the currents are set up, because of the great rapidity with which the appendages move. It is easy to determine that the bits of carmine are brought from considerable distances toward the body; they come from in front of the animal and pass between the bases of the anterior antennae, or from behind the bases of the posterior maxillipeds. In both cases the particles are driven toward the mouth. At times, however, the carmine is driven in all directions from the body, but one cannot see how this is brought about.

While some of the appendages play an active part in the process of food-getting, others with their bristles assume a passive but important role. This consists in directing the moving particles definitely toward the mouth, and it is brought about chiefly by the long spinose bristles of the anterior maxillipeds. As indicated in figure 1, they are so arranged that those of each appendage form the side of a trough or funnel which narrows at the anterior end. The long bristles do not
fit closely together, but the spaces between them are reduced by the interlacing spinules, or 'cilia.' The spinules all point toward the tip of the bristles so that the progress of solid particles along the bristles is not retarded. The grains of ink or carmine strike the sides of the funnel and are carried along until they reach its apex, where they are formed into a little ball or pellet.
The mouth opening (figs. 1, 2, mth.) lies anterior and dorsal to the margin of a prominent scoop-shaped lip (fig. 2), and the tips of the long bristles that form the sides of the trough reach beyond the lateral margins of the scoop so that the anterior end of the funnel is completed on the ventral side by the lip (fig. 1). The cluster of grains is held immediately behind the mouth, in the pocket formed by the ventral and lateral portions of the lip and the wall of the body dorsally. The pellet is taken through the mouth into the gut when the upper end of the esophagus is widened by the action of dilator muscles, as noted by Dakin (1908, p. 776), and when in the stomach it is carried back and forth by peristalsis.

Fig. 2. A view, from the right side, of the head of Eucalanus. No appendages are shown; the prominent lip appears beneath the head, and the opening of the mouth (mth.) is indicated (above the lip) from which leads the esophagus (oes.).

The stiff, heavy bristles on the inner lobe of the maxilla (fig. 1, mxl.), are used to crowd the mass more closely under the lip. They do not move very often, but it is not difficult to determine what their part is. They act much like fingers in keeping the pellet in position.

After the pellet has been formed and held in position, it often happens that it is broken up and the particles are scattered widely. This is accomplished by a backward sweep of the bristles in the sides of the trough, aided to some extent by a movement of the bristles on the inner lobe of the maxilla in the same direction. I have not observed that the anterior maxillipeds make any other movements than those that lead to the dispersal of the pellet. I have not been able to determine what the jaws of the mandibles do, though it seems probable that they are used to break up objects that are too large to be taken through the mouth.
It seems clear that the free-swimming copepods must obtain their food in the general way set down above, but some individuals will not ingest particles of carmine under the conditions that others do. All the animals in a watch-glass are in constant activity with the characteristic jerky movements, but not all of them will show the carmine in the digestive tract. This suggests that the movements of the appendages that lead to locomotion are not necessarily the ones that cause solid particles to gather in the mouth region. There may be special movements of certain appendages or of parts of them that cause the pellets to form. There is a possibility, however, that the pellets are constantly formed and as regularly rejected by the animals that do not take in the carmine.

The formation and ingestion of the particles takes place rapidly. If specimens of Calanus are taken from clean water and put into a watch-glass at the edge of the stage of the microscope and carmine is added, the coloring matter will have been ingested within the few seconds that elapse before the animals can be brought under the objective, and the particles can then be seen as they move back and forth in the digestive tract. On the other hand, specimens have been kept in good condition for three or four hours in water containing carmine, and in that time most of the animals did not show the color in the tube. Attention may be called to the similarity between the process of food-getting just described for the Copepoda, and that in Paramecium, where the oral groove and cilia take the place of the bristles and complex appendages.

While I have observed the actual ingestion of solid particles only in Calanus, I have often seen the pellets formed by Eucalanus, but they were not taken into the mouth. The figures shown are of Eucalanus, but the structures are so similar in Calanus that the one will do for the other. The essential occurrences are the same in both these forms, so far as I have been able to determine, though there are certain structural differences in the appendages.

The question of how marine animals are nourished has caused a good deal of discussion within the last few years. This seems to have been brought about largely by the introduction of Pütter's ideas. This investigator (Pütter, 1907 a, b, 1909), as is well known, believes there is evidence that the food requirements of many forms can not be met if the animals are dependent on organic material actually ingested. If this is true, there is some other source of utilizable food, and it is found, according to Pütter, in the organic compounds in solution in
the water. Pütter (1909, p. 4 ff.) gives figures which, he states, indicate the amount of food needed by various forms, and he shows (1909, pp. 105-107) that there is a much larger quantity of soluble organic compounds in a volume of water than there is of solid food as found in the plankton organisms. He shows, furthermore (1909, p. 49), that to satisfy the food requirements by the ingestion of plankton organisms, it would be necessary for an animal to filter an unbelievably large volume of water if the volume of the animal itself is considered.

It follows, therefore, that the substances in solution, though but slightly concentrated, form an additional source of food, and Pütter (1909, pp. 44-53) gives instances to show that such substances really are used. His general conclusion is as follows (1909, p. 147):

Die Ernährung eines grossen Teiles der Formen aller Stämme vollzieht sich nicht in der Weise, wie man bisher, in grober Analogie mit den Säugetieren und Vögeln annahm; d. h. dass geformte Nahrung aufgenommen, durch die Verdauung gelöst und gespalten und in diesem Zustände resorbiert wird, sondern eine grosse Anzahl von Tieren, speziell die absolut kleinen Formen aller Stämme, nehmen, so weit sie im Wasser leben, ihre Nahrung direkt in gelöster Form aus dem Wasser.

This view was apparently retained in 1913 (Pütter, 1913).

While Pütter's presentation of data is impressive, his contention as to dissolved food substances is not supported by subsequent investigation. The recent studies of Moore, Edie, Whiteley and Dakin (1912, 1913) led them to agree with Pütter so far as to say that uniformly distributed plankton is not sufficient to maintain nutritive equilibrium. These investigators found (Moore et al., 1912, pp. 260, 281) that the organic material contained in the plankton obtained by passage through a no. 20 net and a Chamberland filter is, on the average, one part in a million. Under such conditions, only forms with slow metabolism (such as sponges or ascidians) can possibly be nourished upon the plankton. Pütter's results as to the amount of dissolved organic compounds are not confirmed, however, and Moore and his co-workers attribute his high figures to faulty methods of analysis, their results (p. 280) being that the soluble material does not exceed one part in a million. Pütter (1909, p. 145) gives 10 to 20 mg. per litre as a rough estimate of the average content of sea water in dissolved organic compounds.

The answer of Moore and the others (1912, p. 260) to the question, What is the food supply, then? is that it is the plankton but only when it occurs in rich shoals; that is, the swimming plankton feeders must seek the best grounds in order to get enough food to maintain nutritive equilibrium.
It must be kept in mind, however, that there are large quantities of plankton consisting of organisms so small that the finest nets do not capture them. This is the so-called nanno- or centrifuge plankton of Lohmann (1911), and Lohmann believes that organisms like these comprise most of the food of such forms as the microcrustacea and the appendicularians. Unless special methods of collecting are employed, this part of the plankton will be neglected in an estimate of the amount of food available.

This paper is not intended as a review or criticism of Pütter’s work, although it must necessarily be mentioned. One way of answering in a measure the questions he raises, as he himself suggests (Pütter 1909, p. 51), is by determining, through an examination of the contents of the intestine, what organisms and how many of them are eaten by the plankton feeders.

The important group of the Copepoda has been examined in this way by Dakin (1908), who studied the contents of the gut in several species. He found that diatoms are the most abundant organisms in the tract, especially specimens of *Thalassiosira* or *Coscinodiscus* so small that they would pass through a no. 20 net. *Peridinium divergens* and *P. cerasus* were next in frequency. Silico-flagellates did not occur in large numbers, the Tintinnidae were rare and *Ceratium* appeared to be absent (Dakin, 1908, pp. 777–779). Dakin noted that in many cases the tract was full of a green mass, as Hensen (1893, p. 94) had found, and the former states (Dakin, 1908, p. 777) that, in his opinion “it is the micro-organisms, many of which pass through even a no. 20 ‘Müllergaze’ that play the most important role.”

So far as I am aware, Dakin’s paper is the only one that deals especially with the food of marine copepods. Hensen (1893, p. 94) states that repeated searching revealed only one shell of a *Cyclorella*, although the tract was always full of a green mass which he regarded as derived from diatoms.

I have recently examined the intestinal contents of several hundred copepods of different species, taken at different seasons and at different depths. All had been preserved in formalin. The animals were divided with a scalpel into right and left parts, the section being a little to one side of the middle line. The entire digestive tract was removed by means of a needle, and mounted by itself in glycerine. Such preparations are very easy to make, and can be examined with the oil-immersion.
Most of the specimens examined belonged to *Calanus finmarchicus* and the facts about the intestinal contents of that form, as I found them, are as follows. It happens that in a third of the cases the tube had no contents at all. Practically all of the other specimens, however, showed a green or greenish-yellow mass of varying extent. In some it filled the intestine, in others there was only a small bit, but the characteristic color was present. The colored mass, even under the oil-immersion, was homogeneous, except for broken bits of diatom shell and particles of detritus and mineral matter.

The recognisable remains of organisms in the gut of *Calanus* consist chiefly of the shells of diatoms, of which *Coscinodiscus* is the most abundant. Broken or entire shells occur in most cases when diatoms are present at all. It is hard to estimate the number of specimens when the shells are broken; I saw one or two entire tests, and fragments are more abundant. In three cases there were masses of broken *Coscinodiscus* shells, and evidently the plants had been ingested in large numbers. Usually, however, the number of pieces, even, is under ten. Another diatom that occurred frequently was one of the *Thalassiothrix* type, but the shells were not found as often as those of *Coscinodiscus*. In several instances the intestine was packed full of pieces of *Thalassiothrix*. The diatoms *Planktoniella* and *Navicula* occurred, also, the former in one copepod and the latter in two.

Eleven specimens of *Calanus* contained fragments of a silicoflagellate closely resembling *Dictyocha* (if not that form) but not over four of the pieces were seen in one animal.

It is interesting to find that *Calanus* ingests the Coccolithophores. Gran (1902, p. 163) observed the plates in the fecal capsules of copepods, and Murray and Hjort (1912, p. 382, 719) also mention that the plates occur in the capsules. Nineteen specimens of *Calanus* that I examined had the coccoliths in the intestinal contents. The numbers varied widely; in some the intestine was literally packed with them, but usually there were not many. There were twenty entire organisms in one copepod, nine in another. The coccolithophores belong almost entirely to that group which has the perforated coccoliths (Lohmann, 1902, p. 127), but I saw two with the rodlike coccoliths. The one commonly found is much like the drawing of *Pontosphaera huxleyi* as shown in plate 4, figure 3, of Lohmann's paper (1902).

Other organisms were found to occur in the gut of *Calanus*. A species of *Dimorphysis* was found in five specimens, one of which contained two of the cells. *Prorocentrum* (probably *P. micans*) was
found in seven specimens, one of which contained 63 of the protozoans and another twelve; the intestine in the former case was closely packed with the cells. *Peridinium* was recognised in seven, two of them in one copepod; in this particular case the cells had retained their shape but were without contents, while in the others the tests were wrinkled and distorted yet recognisable as those of some *Peridinium*. Mangin (1911, p. 45) has noted the effect of the digestive processes of various animals on *Peridinium*. One *Calanus* contained the skeleton of a nauplius, and in three instances there were the broken bristles of some crustacean.

*Eucalanus clongatus.*—This copepod is abundant in the San Diego region, ranking next to *Calanus*. *Eucalanus* is exceedingly transparent, so that the entire digestive tract can be seen through the exoskeleton and muscles. I have examined numbers of this copepod both living and in serial sections, and have never seen the recognisable remains of any ingested organism. Most specimens, in fact, are devoid of any intestinal contents, though in two cases I have seen a fecal capsule in the posterior part of the tract; the capsule, however, contained only a colorless granular mass of homogeneous character.

*Scoleithrix persecans.*—This copepod is notable because in all of the seventeen specimens the intestinal contents were abundant. All of the animals contained crustacean bristles, and in some instances the gut was packed with them. Many of the bristles were those of some copepod, but it is certain that there were the remains of other crustacea. The tract of several specimens were filled with a red-brown mass of finely granular material in which the bristles were contained. Two specimens of this copepod had ingested *Coscinodiscus* in small numbers.

*Pleuromamma quadrungulata.*—Fifteen specimens were examined. In six the tract was empty except for a few scattered mineral particles. Of the others, one contained a piece of a radiolarian test, three had broken bristles of some sort, and five showed traces of the greenish material.

*Pleuromamma abdominalis.*—Out of fifteen specimens, there were seven whose intestinal tracts were empty, while six had more or less of the green color. Among the organisms recognised were the diatoms *Synecria, Thallasithrix, Fragilaria* and *Coscinodiscus*, all as fragments; *Dietyocha; Globigerina*; broken bristles of a copepod. In no case was the tract even moderately full.
Gaidius pungens.—Sixteen were examined, and only two of them were entirely empty. Eight showed the green color. The organic remains consisted of broken Coscinodiscus, Dictyocha and a radiolarian; in addition nine of the animals contained broken bristles, but I could not determine their source. On the whole, judging from these specimens, this copepod may be said to get an abundance of food.

Undeuchaeta bispinosa.—Six of the twelve specimens had nothing in the tract; four of the others showed a bit of green material, one contained several copepod feet, and the remaining one had a Chaetoceras bristle and a large amount of stuff with the appearance of mineral debris.

Since the copepods form an important group because of their numbers in the plankton and because they are used as food by higher organisms (Peck, 1896, p. 353), the food of the copepods is of more than incidental interest. It seems plain enough that the copepods feed principally upon the small forms of the plankton, such as the diatoms, so far as one can judge from an examination of the intestinal contents. But, as has been pointed out by Lohmann (1909), and Biedermann (1911, pp. 650, 651), this method involves a certain amount of error, which may be considerable. Only the indigestible portions of the food can be recognised, and it is certain that forms are ingested which do not have shells or skeletons. To determine accurately what is eaten and how much, it is necessary to be able to observe the animals alive and while feeding (Lohmann, 1909, p. 26). This would permit one to determine how much food material passes through the gut in a given time, which, as Biedermann has pointed out (1911, p. 650), it is more important to ascertain than to estimate from the intestinal remains how much was eaten.

It is interesting to note, however, that many of the intestinal canals examined were devoid of contents. We may reasonably assume, under such conditions, that food either has not recently been ingested or that it consists of exceedingly delicate forms that are soon broken up without leaving traces. The amount of material in the intestine ought to furnish some clue as to the amount of food eaten. The specimens of Gaidius and of Scolecithrix perseccans which I saw appeared to have had an abundance, but only one Calanus could be said to be in that condition and it had over sixty cells of Prorocentrum. Eucalanus never has solid contents in the tracts so far as my observation goes.

With these matters before us, it is interesting to consider the figures given by Pütter (1909, p. 63) as to the needs of a Calanus.
He has estimated that a "Calanus spec." will require 15,800 Coscinodiscus of medium size daily, or 9,750,000 Thalassiosira nana, to cover its food needs. If these figures are even approximately correct, it must be evident that the needs of a Calanus in the San Diego region are either far from satisfied or else there is some other source of food than that which appears in the intestinal contents. Dakin (1908, p. 777) counted 200 diatoms as a maximum number, while many animals did not have anything in the alimentary canal, and in a review of Pütter's work (Dakin, 1910, p. 214) he states that if the figures given by Pütter for the food requirements of copepods are correct, he does not see how they get enough food in diatoms or flagellates. He will be ready to accept, then, Pütter's theory that dissolved substances form an additional source of food.

If the unicellular plants and animals comprising the centrifuge plankton are taken in large enough numbers, the problem is solved so far as the copepod is concerned, and such organisms will not leave definite traces. But if the green matter noted so often is derived from such food organisms, they are not taken in large numbers in these waters, if one may judge from the amount of that material that shows in the tracts of animals preserved in formalin.

Although the part taken by the centrifuge plankton in the nutrition of the marine copepods is not known, it is probable that those unicellular forms are most important, and that the ingested numbers could be ascertained from a study of living copepod material. Lohmann (1909, p. 24) states that the forms of the nannoplankton like chrysomonads or Rhodomonas can be recognised in living Tintinnidae or naked ciliates, while in preserved material only the skeletons of silico-flagellates and diatoms or the remnants of Peridinium can be seen. The same may be true of the intestinal contents of the copepods. As things are, it is largely a matter of opinion what forms other than those with skeletons are present.

In the case of some of the fresh-water Cladocera, however, Woltereek (1908) showed that the centrifuge plankton has an important role. He found that these crustaceans are better nourished in the upper lake at Lunz than in the lower lake, though the net-plankton is much greater in the latter. The upper lake, however, contains a richer centrifuge plankton, and these are the organisms that are regarded as of first importance in the nutrition of the Cladocera.

The answer to the important questions raised by Pütter's investigations will be most readily obtained, probably, by ascertaining what
organisms are used as food and in what numbers. If we can discover how much food is taken by Calanus, even as a very rough average from a large series of observations, we shall have to be satisfied that the food requirements are met, whether the actual results conform to the theoretical needs or not.

SUMMARY

1. Particles that float (such as grains of carmine) are carried toward the mouth by water currents set up by movements of the head appendages. The particles are definitely directed by means of the sides of a sort of trough formed by the long bristles of the anterior maxilliped. These appendages are stationary most of the time.

2. There is formed a little pellet, which is held immediately behind the mouth and then taken in when the esophagus is dilated.

3. It appears that the ordinary movements of locomotion are not necessarily those that cause the formation of the pellets, since animals may be kept for hours in water to which carmine has been added without ingesting the particles, while in other cases the color appears in the intestinal tract in a few seconds.

4. An examination of the digestive tracts of several species of marine copepods shows that diatoms are the organisms whose remains appear most often, but in many cases the tracts are empty or contain only bits of debris and more or less of a green mass.

5. The amount of food that is indicated by the intestinal contents is surprisingly small in the majority of cases, and especially so when the figures given by Pütter as to the needs of a copepod are considered.

6. It is likely, however, that the food of these animals consists to an important degree of organisms of the centrifuge plankton which are without shells and do not leave recognisable remains. At any rate, this possibility needs careful investigation before Pütter's theory of nutrition through dissolved organic material is accepted.

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LITERATURE CITED

BIEDERMANN, W.

Dakin, Wm. J

Gran, H. H.

Hensen, V.

LoHMANN, H.
1902. Die Coccolithophoridae, eine Monographie der Coccolithen bildeten Flagellaten, zugleich ein Beitrag zur Kenntnis der Mittelmeerauftriebs. Arch. f. Protistenk., 1, 89-165, Taf. 4-6, 5 Tab.

Mangin, L.

Moore, Benjamin, Edward D. Ede, Edward Whiteley, W. J. Dakin.

Murray, John and Hjort, Johan.

Peck, J. I.
Pütter, A.
1907b. Der Stoffhaushalt des Meeres. Ibid., 321–368, 14 tables.

Woltereck, R.
THE KINETONUCLEUS OF FLAGELLATES AND
THE BINUCLEAR THEORY OF HARTMANN

BY

OLIVE SWEZY

This series contains the contributions from the Department of Zoology, from the Marine Laboratory of the Scripps Institution for Biological Research, at La Jolla, California, and from the California Museum of Vertebrate Zoology in Berkeley.


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THE KINETONUCLEUS OF FLAGELLATES AND THE BINUCLEAR THEORY OF HARTMANN

BY

OLIVE SWEZY

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INTRODUCTION

The much discussed question of binuclearity among the Flagellata has unfortunately depended for its answer upon the conditions found among the smallest members of that group, which because of their small size present peculiar difficulties to the investigator. Added to the very great difficulty of working with organisms so near the limits of microscopical vision, is the fact that, at the present time, no critical stain for chromatin and chromatin alone has been found.

All members of the so-called Binucleata are parasitic flagellates, and in that fact lies another danger which has not been fully enough appreciated. Parasitic forms, and this is especially true of those
inhabiting the blood and tissues, are peculiarly subject to reactions to the toxins of their hosts and possibly to endotoxins of their own production, and on this account often present pathological conditions of the cytoplasm and more especially of the nucleus.

Another difficulty arises when the attempt is made to state in exact terms the meaning of the word nucleus. In general the nucleus of the Protozoa is a very definite structure which can be pointed out with certainty in almost every case, having a definite degree of organization that readily distinguishes it from extra-nuclear chromidial bodies. This is far from being the case, however, with the so-called second nucleus of the Binueleata of Hartmann.

The presence of extra-nuclear chromidia or chromatin in a cell, even when having a definite position and persisting from one cell-division to the next, cannot be considered as indicative of a binucleate condition unless other facts, such as mitosis during division, point to the nuclear behavior of the chromidia.

Since it is known that some cytoplasmic structures, as the undulating membrane and axostyle of Trichomonas, the axostyle of Hexamitus and Giardia, divide during the process of cell-division, the division of an extra-nuclear chromidial body loses much of its significance. In how far that division can indicate nuclear function is a question that can be answered only by the most careful experiments and study of a wide range of material. In the present state of our knowledge of the chemical and physiological reactions of the various cell constituents of the Protozoa, the wide application of conclusions which at the best must be tentative, is to be avoided.

In the course of a series of investigations carried on during the past two years on the protozoan parasites of various amphibian, reptilian, and mammalian hosts, the subject of extra-nuclear chromatic structures and chromidia, and their function in the cell, has claimed a considerable amount of my attention. Certain new facts have been thus brought to light in my investigations which seemed to demand, for their explanation, a complete revision of the present status of extra-nuclear chromatin structures and chromidia, and also of the binuclear theory, so closely connected therewith.

This has become a much discussed question within recent years, and a large amount of literature has accumulated on the subject. It is a question, however, on which theories may easily outrun facts. The influence of Weismann's ideas on the germ plasm has been largely the cause of the attempt to find in these simple organisms the anlage of the
"idioplasm." This has resulted in the separation of the chromatin of the protozoan cell into so-called "generative" and "vegetative" chromatin, corresponding respectively to the terms used in the metazoan cell by Weismann.

The writer wishes to take this opportunity to acknowledge her great indebtedness to Professor Charles Atwood Kofoid, at whose suggestion the work was begun, for the kindly interest and helpful criticisms which have meant so much for its progress.

B. HISTORICAL

The present binuclear theory of Hartmann is the direct product of the earlier theories concerning nuclear relations of the cell. One of the first to attempt a formulation of these relations was Bütschli, whose work on the diatoms (1891) opened up a fruitful field for speculation. With his discovery of the centrosome of the diatom Surirella he attempted to homologize it with the micronucleus of the Ciliata. A further extension of this idea was made by Lauterborn (1893), working on the same subject. In 1894 Heidenhain carried it still farther, homologizing these two structures with the centrosome of the metazoan cell, making that organelle a derivative of the micronucleus of the Ciliata, and the metazoan nucleus a derivative of the macronucleus. In thus tracing out the line of development, the condition found in the Ciliata is taken by Heidenhain as the primitive one from which other forms, having a more specialized centrosome, have arisen, finally culminating in the production of the metazoan cell. This is an inversion of what seems to be the natural order. The Ciliata evidently stand at the end of a highly specialized line rather than at the beginning of the whole protozoan and metazoan development.

The hypothesis advanced by R. Hertwig in 1892 was very similar to that put forth by Bütschli the preceding year regarding the origin of the centrosome. He suggested that it might be derived from one of the nuclei of a binucleated cell which had lost its chromatin but retained the active kinetic element required in division. The other nucleus retains its chromatin but loses its active kinetic element.

Lauterborn, in another memoir (1896), gives as the starting-point for the development of the centrosome, not the Ciliata, but Amoeba binucleata, a cell containing two equal nuclei. According to him two lines of development took place here, one producing the two differen-
icated nuclei of the Ciliata and the other resulting in the metazoan nucleus and centrosome.

Closely connected with these theories are two other conceptions first formulated by R. Hertwig in 1902-03, as the chromidial hypothesis and the nucleo-cytoplasmic ratio. The latter is the more important generalization, and has been far-reaching in its scope, spreading widely in the domain of metazoan as well as protozoan cytology.

Briefly stated it may be given thus: The normal continuance of the vital functions of a cell is dependent upon a certain quantitative relation of nucleus and cytoplasm. Any disturbance of this ratio, as, for example, an increase in the nuclear size without a corresponding cytoplasmic growth, leads to degeneration and death. The extrusion of chromidia from the nucleus may be one means of preserving this ratio.

The chromidial hypothesis was closely interwoven with his nucleocytoplasmic ratio, but it received its fullest amplification at the hands of Goldschmidt (1904-1905), and Schaudinn (1896-1903).

Goldschmidt, from his work on nematodes and Protozoa, came to the conclusion that every cell is primarily binucleate, containing a somatic and a generative nucleus. These may be united into a single structure, the amphinucleus, or may be completely separated, as in the ciliates. A separation may also take place by the formation of chromidia at the time of gametogenesis. This view has led to the homologizing of widely different structures in both metazoan and protozoan cells, much of which, in the opinion of Dobell (1909), "greatly exceed the limits of legitimate inference."

Building on this foundation, Schaudinn (1896-1903) worked out a theory of binuclearity which differs somewhat from the earlier views. The occurrence of a typical centrosome in the Heliozoa led him to the conception of its origin from a second cell nucleus which became differentiated early in the phylogenetic history of the Protozoa. The earliest forms in this evolutionary transformation would be that in which two nuclei of equal value were present, and this condition he found in Amoeba binucleata, with its two nuclei equal in structure and function, which divide by a primitive mitosis. He says, however, that "Zugleich scheint an den flachen Polen die Membran sich etwas zu verdicken, so dass es hier, wie bei Actinosphaerium, schon auf so frühem Stadium zur Ausbildung des sogenannten Polplatten kommt die Function der hier fehlenden Centrosomen mit ihren Strahlen-
systemen erfüllen." He does not state whether, before reaching the next stage of evolution, the nucleus is dropped out, leaving the pole-plate as the centrosome or central spindle, or *vice versa*, or whether both structures are transformed into one organelle.

A second step is reached in *Paramoeba cilhardi*, in which the "Nebenkörper" functions as a central spindle, as does also the "centralkörper" of the diatoms. A still further advance is found in the trypanosomes with their tropho- and kinetonuclei. As a result of his work on these forms Schaudinn came to the conclusion that a condition of binuclearity was found in all cells, metazoan as well as protozoan.

These theories of Schaudinn's were further elaborated and amplified by his followers. Foremost among these investigators has been Hartmann, whose work has resulted in a new system of classification for some groups of the Protozoa. In connection with Prowazek (1907), he expanded this theory to its utmost capacity, finding a second nucleus in most forms of the Protozoa where, to the unaided imagination, but one such structure is actually present. In such forms as the *Amoeba*, for example, the central karyosome of the nucleus becomes the second nucleus.

In his system of the Protozoa (1907) he creates a new order, the Binucleata, for all those forms which have a second nuclear structure. This order includes the Haemosporidia as well as the Haemoflagellata.

No attempt has been made in the following discussion to deal with the earlier questions that have been thus briefly touched upon here, except in so far as they relate to the present binuclear theory of Hartmann. This theory has been a gradual outgrowth and transformation of the earlier ones, many parts of which have been discarded in the light of newer observations and facts.

An attempt will be made, however, to estimate the present value of this theory in its relation to the more recent investigations on these flagellates, as well as to give different interpretation to some of the earlier observations on which this theory was founded.

C. THE ORDER BINUCLEATA

The forms included by Hartmann in the order Binucleata cover a wide range of protozoans, apparently differing in phylogenetic derivation as well as in structure. The basic principle of his classification is nuclear dimorphism as exhibited in the trypanosomes, which he also claims to have established in the Haemosporidia as well. The accept-
ance of this classification would remove the latter group from the Sporozoa altogether and place it with the Haemoflagellata.

A careful examination of the known facts regarding the life-cycle, as well as the morphology, of these two groups, reveals striking differences and few similarities, too few indeed to be of the taxonomic value assigned to them by Hartmann. An attempt will be made in the following discussion to point these out, and also to indicate the lines along which their relations may be better sought.

I. The Binuclear Theory

Nuclear dimorphism is not an uncommon condition in the Protozoa. The presence of the macro- and micronuclei have been quite clearly established in many of the Ciliata, and these relations will probably hold for most members of that class. The macronucleus controls the trophic or vegetative functions and the micronucleus the generative functions of the cell. At the time of conjugation the macronucleus can be seen to degenerate in situ, with no trace of its material passing to the gametic nuclei or to the other conjugant, thus definitely showing that it takes no part in this process.

Nowhere else among the Protozoa has nuclear dimorphism of this type been thus far established.

The attempts of Schaudiinn to establish it among the Rhizopoda have not been upheld by all of the facts as exhibited in his own investigations. He found (1903) that the gametic nuclei of the foraminiferan Polystomella crispa and the rhizopod Centropyxis aculeata were formed from the chromidia scattered through the cytoplasm. A certain amount of chromidia is given out by the nucleus during the growth period, but before the beginning of the formation of gametes the amount is greatly increased by nuclear disintegration. From these chromidia a number of small nuclei are formed, after which the cytoplasm breaks up into a number of small individuals. This does not take place, however, until after the complete disappearance of the original vegetative nucleus, or rather its change into chromidia or "generative" chromatin.

There is no evidence here of two kinds of chromatin substance. On the contrary, facts seem to point to the conclusion that the terms "vegetative" and "generative" merely designate the successive states or phases of the same substance, there being no indication of a loss of any material in the change from a nuclear to a cytoplasmic position.
Nor can I agree with Calkins (1909) when he states that Schaudinn has "correctly compared it (chromidia) with the micronuclei of the Infusoria." The two structures seem to be fundamentally and morphologically different, as well as varying greatly in their behavior.

In an earlier memoir (1895), Schaudinn discusses reproduction in the Foraminifera Patellina corrugata and Discorbina globularis. In all the specimens he observed there was but one nucleus in the early stages. As the reproductive phase comes on, the nucleus becomes segregated into a number of parts, usually seven or ten, scattered through the cytoplasm. Some of these may later subdivide in the same way until as many as thirty nuclei may be present. The protoplasm becomes divided up about these nuclei, forming new individuals which escape from the parent body. In this case also the original nucleus is considered the vegetative, and the parts into which it becomes divided the generative, chromidia or nucleus. There is no indication in this process of a nuclear dimorphism, though that is claimed for it.

It is comparable with the successive divisions of the nucleus found occurring in Amoeba, Trichomonas, etc., by which multinucleated individuals are formed in the process of multiple fission.

Swareczewsky (1909, pl. 19, figs. 13, 14, 35-39) describes a process in the formation of gametes in Allagromia ovoides comparable to that given by Schaudinn. The nucleus casts out its chromatin until only the reticulate structure remains, which then gradually disappears. These chromatin particles wander out to the periphery of the cell and ultimately form the gametic nuclei. This is a case comparable with the ones cited above, and also with other cases of chromidia formation claimed by Schaudinn, Goldschmidt, and Hartmann as examples of nuclear dualism. Swareczewsky, however, points out the fact that it cannot be so interpreted in Allagromia ovoides, since one mass of chromatin performs both functions in the cell, first in its position in the definitive nucleus, and second, by wandering out into the cytoplasm.

The interpretation which Schaudinn has put upon these and similar phenomena is open to certain cogent objections. In the first place, he claims the chromidia in the cytoplasm as sexual or generative chromatin, forming the generative nucleus. The original nucleus is formed of vegetative chromatin and is the vegetative nucleus, according to his interpretation. A change of position and form is here, apparently, the only real distinction between the two types of chromatin. Trophochromatin and generative chromatin can be distin-
guished as different substances only by their ultimate behavior during gametogenesis, as may be done in the Ciliata, already mentioned above. In the cases cited here it is impossible to distinguish between the chromidia formed by the nucleus during the growth period of the cell and that formed by the extrusion of chromatin in the degeneration of the nucleus, whereby the amount of chromidia in the cytoplasm is greatly increased. No disintegration and disappearance of any material and the preservation of another material distinct from it can be shown to take place, as is the case in Paramaecium, where the macronucleus dwindles and fades away during the process of conjugation. The facts, then, as brought out in the investigations of these observers do not substantiate their claims for the presence of two different kinds of chromatin. The alternative explanation above proposed seems to adhere to the evidence more closely.

In Plasmodiaphora brassicace (Prowazek, 1905b) the gametic nuclei are formed from the nucleus and the extruded chromidia are absorbed in the cytoplasm. The chromidia in this case formed the "vegetative" chromatin and the nucleus the "generative" chromatin, just the reverse of the relation assigned to them by the proponents of the binuclear theory in Patellina.

Another example which is a combination of the two methods noted above is that described by Bott (1907, pl. 3, figs. 2–10) in the common Pelomyxa at the time of gamete formation. The chromidia cast out by the nucleus form secondary nuclei which in their turn undergo a process of so-called "reduction" by casting out part of their chromatin, after which the gamete nuclei are formed (Bott, 1907, pl. 3, figs. 12–16). The chromidia in this case are said to contain both vegetative and generative chromatin (Minchin, 1912).

It may be pointed out, however, that the so-called "reduction," as well as the casting out of "vegetative" chromatin, is also found in the ordinary binary fission process of Trichomonas (Kofoid and Swezy, 1915a, b) and prior to the encystment of some amoebae (Wilson, 1915) which have no known relation to gamete formation. In these cases it can hardly be called a differentiation of vegetative and generative chromatin. It is more probably a phase of metabolism rather than of segregation of hypothetical idiomplasm.

The casting out of material from the nucleus prior to gametogenesis is not limited to the Protozoa. According to Kellieott (1913), "the chromatin of the nucleus which is not included in the spireme, often indeed the greater part of the whole amount of this material, is
thrown out into the cytoplasm and dissolves; the more fluid parts of
the nucleus are also thrown into the cytoplasm by the dissolution of
the nuclear membrane." In _Aequorea_ Häcker (1892) has described a
process whereby the nucleolus is cast out into the egg-cytoplasm,
where it degenerates. In many forms among the Metazoa it is cast
out during the formation of the polar bodies or it may fade away _in situ_ (Wilson, 1911). The nucleolus of the metazoan cells presents a
variety of staining reactions, those of the echinoderms often staining
deeply with chromatin stains.

Häcker (1892) has made the suggestion that, in some cases at least,
the nucleoli are accumulations of by-products of nuclear activity, de-


erived from the chromatin and representing passive material which is

of no further direct use in the nucleus.

It seems not improbable that we are dealing here with the same

sort of substance in the Protozoa. The period prior to gamete for-

mation is usually one of great metabolic activity and growth, and conse-

quently an excess of nuclear material may result which is of no further

use in forming nuclei and cells, and is cast out to degenerate in the
cytoplasm. This explanation fits the conditions as found in _Amoeba_

and _Trichomonas_ as well as those forms of the Foraminifera and

_Heliozoa_ in which a residue of material may remain after the for-

mation of the gamete nuclei. It certainly seems a more natural explana-

tion than does the view of tropho- and generative chromatin which

has usually been applied to these conditions, based on the supposition

that two substances were here involved, each playing different parts in

the activities of cell life. No differentiation of these substances has

thus far been made. It seems more probable, indeed, that it is one

and the same substance, combined with secretion products as the re-

sult of great metabolic activity at some periods in the life-cycle.

Hartmann, in his further elaboration of Schaudienn's binuclear

theory, advanced the idea that all cells of the Protozoa as well as

Metazoa were binucleated, possessing a locomotor-generative nucleus,

known as the centrosome in the Metazoa, the "blepharoplast" or

"kinetonneuleus" in the trypanosomes, the "central grain" in the

Heliozoa, the "Nebenkern" in _Paramoeba_, etc., and also possessing a
trophogenerative nucleus forming the definitive nucleus (Hartmann

and Prowazek, 1907). Further investigation revealed, as he claimed

(Hartmann and Chagas, 1910), that both the "kinetonneuleus" and

the trophonucleus possessed their own centrosomes (Cytozentrum) as

well as generative chromatin. To meet this condition he proposed the
new names "lokomotorische-generative und idiogenerative" chromatin.

In the latest exposition of his views Hartmann (1911) has modified his earlier ideas on the general prevalence of the binnuclear condition and limits it to those organisms which possess a second "nucleus" which arises by an actual division of the "Hauptkern." "Von einer eigentlichen Doppelkernigkeit dagegen kann nur dann gesprochen werden, wenn durch eine polare Teilung des individualisierten Centriols, sei sie homopol oder heteropol, zwei distinkte Kernindividuen gebildet werden." All the forms in which he considers this to actually take place he includes in the order Binucleata, as follows: Herpetomonas, Crithidia, Trypanosoma, Trypanoplasma, Prowazekia, Leishmania, Halteridia, Haemogregarinida, Piroplasmina and Plasmodiida.

The work of Werbitzki (1910) in proving that the "kinetonucleus" of the trypanosomes is not composed of nuclear chromatin, by showing that it may be dissolved out by chemicals which have no action on the nucleus, has shown the need of further investigations before claiming for any extra-nuclear chromidial body, because of its staining reactions, the specific properties of nuclear chromatin.

II. CRITICAL DISCUSSION OF THE BINUCLEATA

The followers of Schaudinn have upheld the views and theories regarding binuclearity which he put forth and have made constant and repeated endeavors to confirm them, with the result, it must be confessed, of sometimes seeming to rely on his authority rather than critically to discuss the alternative possibilities raised by the actual conditions and new discoveries. This has resulted in the seeming confirmation of some views which, in the hands of other less partisan observers, have proven to be errors of interpretation or observation. A notable instance of such error is the work of Schaudinn (1904) himself on the parasites of the little owl (Athene noctuae). In spite of the strong doubts cast upon the correctness of these observations by the results of the work of Novy and McNeal (1906), the Sergants (1906), Woodcock (1910), and Minchin and Woodcock (1911), as well as others, this work has been used by Hartmann as one of the foundation stones for his later modification of the binnuclear theory, and the creation of the order Binucleata.

In this, as in the earlier theory, the starting-point is the nuclear nature of the "kinetonucleus" or the parabasal body, a word formed
by Janicki (1911), and one by which we shall designate it in this paper. In proof of this Hartmann cites the so-called "reducing division" of the parabasal body and the fusion of the two resulting parabasal bodies at the time of conjugation in Trypanosoma noctuae as figured by Schaudinn (1904).

As an additional proof of the validity of his contention he further states that "jeder Zweifel an der Kernnatur des Blepharoplasten wird aber durch den von Rosenbusch einwandfrei erbrachten Nachweis be- seitigt dass er sich regelrecht mitotische teilt, indem Polkappen, Chromosomenplatte und Spindle gebildet werden" (Hartmann and Jollos, 1910).

In the following discussion of the value of these claims, conditions as found among the Haemoflagellata will be considered first, followed by a comparison of these forms with the Haemosporidia.

1. THE HAEMOFLAGELLATA

The work of Schaudinn (1904) on the life-cycle of the blood parasites of the little owl has been discussed and its validity questioned and disproved in important particulars: this is so well known that the criticisms need not be repeated here.

There is one point, however, which so far has not received much adverse attention, namely, that the parabasal body arises by actual division of the nucleus. Schaudinn's figures on this point fail to show any conclusive evidence to bear out the statements to that effect made in his discussion. If critically examined these will show that another interpretation may be used.

Figure 1 may represent some phase of the Halteridium nucleus, but there is no evidence to show that it is connected in any way with the figures following. The fibrils connecting the nucleus, blepharoplast and parabasal body, the so-called "centrodesmoso" (figs. 3-5) are not indicative, in the slightest degree, of the origin of the one by the division of the other. On the contrary, this connecting rhizoplast is present in well-stained specimens of some trypanosomes (Chagas, 1909), in Crithidia (fig. 15), in Trypanoplasma (figs. 9, 10) and it is quite possible that it is the general condition throughout the whole group. In many forms, as in Trichomonas, the rhizoplast connecting the nucleus and blepharoplast is a structure which takes stain very lightly, for the most part, and this fact probably accounts for its non-appearance in most of the figures given of haemoflagellates by various authors.
In figure 3 the division of the parabasal body is shown, and again, as in the preceding form, there seems to be no necessary connection between it and the figure following. To call the structure in figures

Figs. 1–3. Trypanosoma noctuae (Halteria noctuae), from Minchin (1912, figs. 30a-b-c) after Schaudinn (1904, figs. 1c-d-e). Fig. 1. Nucleus of the "ookinete" is dividing into two unequal halves; the divided centriole is connected by a "centrodesmose." Fig. 2. Division of the nucleus completed; "centrodesmose" uniting the two daughter nuclei. Fig. 3. The smaller nucleus is dividing to furnish a third nucleus. The second nucleus probably represents pigment granules common in this species.

Figs. 4–6. Trypanosoma noctuae, from Minchin (1912, figs. 30d-e-f) after Schaudinn (1904, figs. If-g-h). Fig. 4 (?). The third nucleus is dividing to furnish a proximal and a distal centriole. Fig. 5. Further division of the third nucleus, with the fibrils of the achromatic spindle forming the myonemes, the "centrodesmose" the flagellum. Fig. 6. Fully developed trypansome. The "centrodesmose" represents the rhizoplast joining the nucleus and the related organelles.

Abbreviations: bg., smaller nucleus dividing to form a third nucleus; bg.1 bg.2 proximal and distal centrioles formed by division of the third nucleus, bg.1 becomes the blepharoplast of flagellum; my., myonemes formed by achromatic spindle; N., trophonucleus; n., "kinetonucleus;" P., pigment granules.

4 and 5 a spindle is quite conjectural, though it may represent a simple outgrowth of the flagellum and membrane. The figures throughout seem, in the light of our fuller knowledge of the structure of haemo-
flagellates today, to stand in no necessary chronological sequence, and not to have the mitotic significance assigned to them by Schaudinn.

The main evidence rests on the presence of the "centrodesmose" or "central spindle," which is neither more nor less than the connecting fibril between the nucleus and blepharoplast found throughout a number of the Mastigophora. In no well-founded case has the connecting fibril been shown to be formed by a centrodesmose. Its connection with the karyosome also is a not uncommon occurrence (Hartmann and Chagas, 1910; Chagas, 1909; Kofoid and Christiansen, 1915). If this course of reasoning holds we should expect to find that the blepharoplast of Spongonomas uvella, Cercomonas parva (Hartmann and Chagas, 1910), Trichomonas (Kofoid and Swezy, 1915a), and many other forms, would also arise by a "heteropole division" of the nucleus, with the central spindle remaining as the connecting rhizoplast, a conclusion which is, on the face of it, absurd, and for which there is no satisfactory evidence.

Indeed, Hartmann, in his attempt to confirm these observations of Schaudinn's, has even claimed that "die Geisseln der Binucleaten werden, wie Schaudinn und Prowazek zuerst für Trypanosomen gezeigt haben und Hartmann, Rosenbusch und Chagas vollauff bestätigen durch heteropole Mitose des Blepharoplasten gebildet."

The developmental forms of trypanosomes as they have been figured by many investigators (Dolfein, 1910, Minehin and Thomson, 1915), show the presence of both nucleus and parabasal body in minute forms resulting from schizogony, as indeed throughout the complete life-cycle. This is also the case in the cultural forms obtained by Rosenbusch (1909). Prowazek's work (1905) on Trypanosoma lewisi and T. bruci, though based entirely on the unreliable Giemsa method and with many of his figures somewhat difficult to interpret, in nearly every case shows the presence of both structures.

There is no evidence in these many figures of a single instance of the origin of a parabasal body by heteropole division of the nucleus. It is rather a permanent cell organ. Its origin after the still much sought sexual stage is as yet unknown.

Chagas (1909) claims to have found sexually differentiated forms in Schizotrypanum cruzi. In one form the blepharoplast and flagellum are lost before the organism begins to round up at the beginning of the process of schizogony. In the other case it unites with the nucleus. His figures, however, do not bear out these conclusions. His plate 10, figures 1–3, with merozoites in the blood corpuscles, which do not
presumably have a parabasal body, still show two structures which
may be nuclei and parabasal body. The same may be said of the
forms in schizogony (Chagas, pl. 11, figs. 41–44, pl. 13, figs. 27–29).
In forms so minute, if a few of the merozoites exhibit both structures
it is safe to assume, at least as an alternative proposition, that they
are present in the other individuals as well, though they may be
obscured by their temporary position or conditions of staining. In
the closely allied but phylogenetically earlier forms, Herpetomonas
and Crithidia, Patton (1908), Porter (1909), and Wenyon (1913),
found the same conditions, that is, no stages in which the parabasal
body is habitually absent and no instance of its origin by heteropolar
mitosis of the nuclei. Prowazek (1904) describes a process of par-
thenogenesis with degeneration of the parabasal body in Herpeto-
monas muscae domesticae, but his figures are unconvincing in the ex-
treme. Results so entirely at variance with the observations of later
investigators cannot carry much weight.

In Crithidia leptocoridas McCulloch (1915) includes some doubt-
ful forms with the suggestion that there is some slight evidence of the
occurrence of two developmental forms not unlike those figured by
Chagas (1909) in the life-cycle of Schizotrypanum cruzi. As pointed
out above, the figures of Chagas do not bear out the interpretation
which he has placed upon them, and the case is not made stronger by
the comparison with the figures of Crithidia. The two developmental
forms of that flagellate, including the very questionable uninucleated
spores, must remain, for the present at least, as “doubtful forms,”
as Miss McCulloch has termed them. Further investigation may, per-
haps, reveal closer relations between them and the other stages, which
the data at hand fails to establish.

The solution of the question of sexually differentiated gametes and
of syngamy in these forms has thus far proven elusive, and the re-
corded occurrence of bisexual gametes and sexual reproduction, as in
the case of Trypanosoma lewisi as recorded by Prowazek (1905), may
be received with much skepticism. The fact that it has not been
found beyond dispute is not, of course, conclusive evidence against
its occurrence, but it is conclusive evidence in so far as the so-called
nuclear behavior of the parabasal body during conjugation is con-
cerned. The well-known instance described by Schaudinn in “Try-
panosoma” noctuae, cited by Hartmann, is very plainly the result of
a peculiar arrangement of pigment granules so common in these
forms.
In the opinion of the majority of investigators on these forms, the process of division is initiated by the splitting of the basal granule and the appearance of the new flagellum, and in this they agree with what is accurately known of the higher Mastigophora. In the figures of Rosenbusch (1909, pl. 25, figs. 6, 7; pl. 27, figs. 60–63, 73–74), however, which show a mitotic spindle in the parabasal body, no other sign of division in the cell is apparent, both blepharoplast and flagellum appearing as undivided. This makes it seem extremely probable that these "spindles" are only accidental appearances of the parabasal body which may be pathogenic and not of general and normal occurrence, and have nothing whatever to do with division.

On the other hand, those forms which show an undoubted division of the blepharoplast and flagellum (Rosenbusch, pl. 25, figs. 12, 15; pl. 27, figs. 65, 67, 76) also show what can logically be interpreted only as mere pulling out and constriction of the parabasal body. This interpretation agrees with what has been found by Minchin and Thomson (1913) and many others in trypanosomes. As has been pointed out by Wenyon (1913), "it is quite possible to pick out from preparations isolated instances of nuclear division which can be interpreted by the desiring mind as stages in a mitosis." These appearances, which may be quite accidental, are apt to give a misleading idea of the actual process.

*Trypanoplasma*, with its parabasal body exceeding the nucleus in size, is very similar in its behavior to the trypanosomes and other haemoflagellates. The division of the parabasal body as figured by Martin (1910, 1913) shows a striking similarity in its behavior to the same structure in *Polymastix*, and will be discussed below in connection with mitosis in *Polymastix bufonis*.

*Prowazekia* is another form in which the parabasal body has been figured. The process of mitosis as described by Bělař (1914) is initiated, as in the majority of flagellates, by a splitting of the blepharoplast and the appearance of the new flagellum. The next step in the procedure may vary somewhat, the parabasal body appearing to divide before the nucleus, sometimes simultaneously with it. The first step appears to be invariable. As figured by this investigator, with the moving apart of the daughter blepharoplasts (his pl. 9, figs. 18, 23, 25, 28), the parabasal body elongates and gradually assumes a dumbbell shape and is constricted in the middle. Following established authority, however, Bělař calls this mitotic division with spindle formation. His figures do not give the slightest indication of the
presence of a typical spindle, while they do support the alternative hypothesis that the process is a simple "amitotic" constriction of the parabasal body.

The figures of Hartman and Chagas (1910) also "für Prowazekia gezeigt, dass der Blepharoplast mitotisch teilt. Seine Kernmatur kann danach nicht mehr in Frage gezogen werden." Two figures only are given to substantiate this emphatic statement, both of which are open to question. In their figures 6, 7, 8, of plate 4, the nuclear division of Cercomonas parea is shown and is described thus: "Sie erscheint scheinbar als eine einfache Durchschnüring (sog. Amitose)." On plate 8, figure 64, the division of the parabasal body of Prowazekia cruzi is shown. This differs in no wise from the nuclear division of Cercomonas parea, and yet in this case it is called mitotic division. That figure 64 shows an actual division of the parabasal body might be inferred from the fact that the blepharoplast has already divided. Figure 65 is probably an accidental appearance of the parabasal body, as no other signs of division are apparent in the cell; neither can it be correlated with any other figure given for Prowazekia by these authors, nor with my own observations.

The undoubted presence in Leishmania, at one stage of the life-cycle, of a motor apparatus connected with a blepharoplast and parabasal body, indicates its relation to Crithidia and Herpetomonas. Its life-cycle also can be correlated with that of these flagellates, so far, at least, as they are accurately known. Here also, as in the other forms, the same conditions are found with respect to the nuclear nature of the parabasal body.

Franchini (1912), investigating the Leishmania of the digestive tract of Anopheles maculipennis, showed the presence of both parabasal body and nucleus in all developmental stages figured. There is no evidence given to indicate its heteropole origin, or that its behavior during division is anything but a simple constriction.

In the facts as they have been presented here, there has been no undoubted evidence to support the claim made by the upholders of the binuclear theory that the parabasal body of the haemoflagellates is nuclear in nature, arises by a heteropole division, or any other mitotic division, of the nucleus, and is mitotic in its division. On the contrary, the whole evidence seems to show that it is a constant cell organ, with no facts pointing to its origin and that it divides by a simple constriction.
2. THE HAEMOSPORIDIA

The evidences upon which are based the claims for haemoflagellate affinities of the Haemopiridia are slight in the extreme. The occasional and accidental presence of minute granules which take up nuclear stain has been hailed by Hartmann and his followers as the sought-for second nucleus. A granule which may appear in one individual among many, which is at no time connected with a motor apparatus, and which neither is related to the division of any part of the cell, nor has ever shown any signs of division itself, requires a great deal of imagination to transform it into the homologue of the permanent cell organ of the trypanosomes.

Woodcock, in his earlier work (1909, 1910) on the Halteridium of the little owl, was inclined to agree with Hartmann’s ideas of the flagellate affinities of these forms, but as the result of further investigations (1912, 1914) his views on this subject have entirely changed. In his opinion the extra-nuclear body, prominent during some periods of the life-cycle on the periphery of the nucleus, is the karyosome of the nucleus and not the homologue of the parabasal body of the trypanosomes. Its function is not that of a locomotor component, nor is there any evidence that it stands in any relation to the kinetic activities of the cell, a point which is of very great importance.

As the same author has shown (1912), the nuclear conditions found in Halteridium are present also in Karyolosus lacertae and Leucocytozoon ziemanni.

A criticism might be made here which applies equally well to much of the literature relating to these forms, that is, the uncritical use of terms taken from the cytology of the metazoan cell, where definite structures with definite functions are denoted, and applied to all sorts of vague organelles in the Protozoa which, neither by analogy nor homology, have a well-proven relation to the organs denoted by those names in the metazoan cell. The application of the word centrosome to any fibril connecting two granules which are not in any way related to the division of the cell, is an example of this. This word was coined by Heidenhain (1894) to designate the achromatic element in which the centrosome or centriole in the metazoan cell is imbedded, and which connects the daughter centrosomes at the time of division, presumably forming part of the central spindle. It is as permanent as the centrosome and takes an active part in the division of the cell and its nucleus.
In the publications of Woodecock, Berliner (1909), Hartmann, and many others, this term is applied to any fibril connecting two granules, with little regard to the origin, function, or fate of the structure. In fact, much of the proof for the so-called binuclear condition of the Haemosporidia rests on the demonstration of this fibril, and a fair example of its use is afforded by the memoir of Woodecock (1912). In his plates 9 and 10, Woodecock has figured two granules which sometimes are equal. When these are connected by a darkly staining line it is termed the centrodesmose and a process of division is supposed to have taken place. As noted above, to function as a true centrodesmose it must take part in a division of the nucleus and cell, and evidence of this is totally lacking throughout the entire series. Indeed division of the Halteridium during the stage of the life-cycle figured by Woodecock has never been known to occur. An elaborate process of division, involving a centrosome, centrodesmose and central spindle, has before this time not been found necessary to account for the extrusion of a granule of chromatin from the nucleus. Hartmann and Jollos (1910, pl. 10, figs. 5a, 7) figure the same condition as evidence for the origin of the parabasal body by division of the nucleus in Babesia and Plasmodium. Similar figures of Berliner (1909) of Haemoproteus noctuae are cited by Hartmann as proof of the haemoflagellate affinities of that species.

On the grounds stated above it is impossible to justify the use of the terms centrodesmose and central spindle as applied here. It is equally impossible to postulate that the mere chance appearance of a granule, which is not constant in position or occurrence, is of great significance from a taxonomic standpoint.

As Woodeock (1912, 1914) and Minchin (1912) have pointed out, the relations of Halteridium are rather with the Coccidia than with the Haemoflagellata, judging by the life-cycle as a whole, and by their morphology.

The linking of the Haemogregarina with the Haemoflagellata has rested on the same sort of evidence as in the case of Haemoproteus. Minchin and Woodeock (1910) have pointed out the essential differences between the nuclear conditions of the haemogregarines and the trypanosomes. Reichenow (1910) and Robertson (1910) have given accounts of the life-cycles of these forms which agree in all essential details, and which also separate these organisms very clearly from the flagellates. The importance of considering the life-cycle as a whole in the systematic placing of any organism has come to be a truism in
these days, and yet it is frequently disregarded, as in the present instance, where the haemogregarines are considered closely related to the haemoflagellates.

Among the Piroplasmida and Plasmodiidae even less foundation can be found to support the claims for a binnuclear condition. In one form, at least, of this group the life-cycle has been worked out with a fair degree of accuracy and thoroughness, by a number of investigators whose results correspond in all essential details. I refer to the well-known life-cycle of the malarial parasite. The general concurrence of opinion among the majority of investigators leaves no doubt as to the non-occurrence of a flagellate stage in these forms. Hartmann (1907) figures such a stage in *Protesoma*. Attention must be called to the striking resemblance between his figure and that given by Neumann (1908, pl. 5, fig. 51), which the latter interprets as the fertilization of the macrogamete by the microgamete. An examination of Neumann's other figures bears out this interpretation. Possibly the form figured by Hartmann represents the same process.

The heteropole division of the nucleus to form the parabasal body in *Plasmodium* from the monkey, as figured by Berenberg-Gossler (1909), is a very far-fetched interpretation of facts. His figures 12 and 13, plate 16, are shown as the building of the "Nebenkern," a structure which, judging from his figures, the organism possesses only in this, the dividing state. These figures differ in no essential details from figures 14 and 17 on the same plate, which he interprets as division of the nucleus, and which do not show the presence of the previously divided-off parabasal body. In fact, this latter structure ceases to exist, apparently, after it has been formed by an unequal division of the nucleus. The division of the nucleus in figure 17 is also unequal, but in both cases there seems to be an equal distribution of the chromatin material.

The interpretation of this granule as the parabasal body seems to rest solely on the fact that it is formed, apparently, by an unequal division of the nucleus. It does not appear in the stages immediately following this, where the nuclei divide several times in the process of schizogony. This would strongly suggest, if these were actually successive stages, that the granule in question was merely a bit of extruded chromatin which later disappeared in the cytoplasm. At any rate, Berenberg-Gossler's figures prove that a parabasal body is not a permanent cell structure in this species of *Plasmodium* and that it is not even found constantly in any one stage of the life-cycle.
Piroplasma has been described by Nuttall and Graham-Smith (1906, 1907) as possessing, in many individuals, a blepharoplast, so-called. This is a transient structure with no relation to a motor apparatus nor to the division process. Neumann (1909) figures, from the bat-mite, a species of Achromaticus which becomes a flagellate form in its development. As already pointed out by Minchin (1912), a trypanosome from the same host has probably been confused with the Piroplasma in this case. A comparison of his figures and Giemsa-stained preparations of the small forms of trypanosomes becomes very instructive in the light of this suggestion.

The figures of flagellated forms of Babesia canis observed by Breinl and Hindle (1908) in the blood of dogs dying from piroplasmosis have attached to them the strongest doubts. They are very possibly intestinal flagellates such as may be occasionally found in the blood under pathological conditions, and may be in no way related to the life-cycle of Babesia canis.

Finally, Hartmann’s contention (1910) that the microgametes of Protosoma possess an undulating membrane rests on unverifiable evidence and is not supported by the results of other investigators. The occurrence of flagellated swarm-spores and gametes in other groups, as the Amoebina, Volvocidae, Coccidia, etc., has never been used as an argument for placing these widely diverse groups in one order, and yet this is presented in good faith by Hartmann as one of the reasons for including the Protosoma with the trypanosomes.

The work of Gonder (1910) on Theileria and Babesia, of Neumann (1908) on Plasmodium, of Yakimoff, etc., (1911), on Achromaticus, of Nuttall and Graham-Smith (1906, 1907), and others, in spite of the fact that they find “blepharoplasts” or even traces of flagella, all afford the strongest proofs of the non-haemoflagellate affinities of these forms, as well as the undoubted resemblances in their developmental cycle to that of the Coccidia, in so far at least, as the course of development has been figured by these investigators.

In fact, we might go a step further and point out the similarities existing between the life-cycles of the Haemosporidia and many of the Rhizopoda, showing a well-marked differentiation into sexual and non-sexual cycles, with a distinct alternation of generations. Trichosphaerium sieboldi from the Rhizopoda and Plasmodium vivax from the Haemosporidia may be taken as typical examples. The life-cycle in each consists of the following phases; trophic phase, or growth-period, followed by schizogony, which may be repeated a number of
times before the next phase, gametogenesis. The gametes unite to form a zygote which grows to a larger organism and divides again. The Haemosporidia, to meet the exigencies of parasitic life and provide for possible destruction of the host, have evolved another phase of the life-cycle which is not found in the free-living forms, namely, sporogony, the production of resistant spores. The life-cycle of the Coccidia shows the same resemblances, differing only in minor details.

One group only among the Mastigophora offers a life-cycle of this type, the Rhizomastigina. On account of its morphology as well as its life-cycle, which bears a strong resemblance to that of some of the rhizopods, some authors have placed this group as a connecting link between the Rhizopoda and the Mastigophora. The life-cycle of Mastigella vitrea differs from that of the Sporozoa in that schizogony does not occur, and at the same time it cannot be said that it is typical of the process taking place in the Mastigophora.

The general lack of life-cycle of the sporozoan type in the Mastigophora, while it may yet be brought forth by further investigations, does not, at the present time at any rate, suggest a close relationship between them and the Sporozoa. The occurrence of life-cycles of similar types, together with the entire absence of cilia or flagella and the fact that very many genera of the Sporozoa are typically amoeboid in certain phases of the life-cycle, frequently in the trophozoite stage, would suggest closer affinities between the Sporozoa and the Rhizopoda than may be looked for in the flagellate line. Of the two characteristics of the Telosporidia which are said (Minchin, 1912) to ally them to the Mastigophora, the first, the possession of flagellated swarmspores, must be thrown out entirely as these also occur in the Rhizopoda; the second, the gregarine-like form of the body, is typical only of the gametocyte generation in some genera, as Plasmodium, and its formation may be compared to the encysted stages which occur in some amoebas at the same period. The whole evidence, as shown in the general outline of the life-cycle and in the morphology, points away from the Mastigophora and toward the Rhizopoda as the group with which the Sporozoa are allied.

The order "Binucleata," as it has been thus presented, is composed of the haemoflagellates, which possess a structure related to the motor apparatus, the parabasal body, for which nuclear value is postulated, and the Haemosporidia, which do not possess a motor apparatus of the flagellate type, but for which the presence of an accessory motor structure is claimed, having the value of the parabasal body.
of the haemoflagellates. These claims recognize a polyphyletic origin for the present class of Sporozoa, and give to the Haemosporidia a flagellate ancestry, which is as yet capable of no proof. This assumption has resulted in the attempt to find in the morphology and life-history of these forms flagellate stages and organelles.

From the facts presented here as well as from the figures cited, it is evident that the constant presence of a permanent cell organ in the Haemosporidia comparable to the parabasal body of the trypanosomes has not thus far been demonstrated, neither have undoubted flagellate stages in their life-history been shown to occur in a single authentic instance. With no recognizable data by which to relate them to the flagellates, and lacking a structure comparable to the parabasal body, this group cannot logically be placed among the "Binucleata."

With this review of the order Binucleata an estimate has been given of the validity of the claims for affinities between two widely diverse groups, and the grounds for the binuclear theory upon which these claims are based. It has been shown that these claims rest upon an insecure foundation of facts which are susceptible of other interpretations. Some of these interpretations have already been pointed out and in the following pages will be expanded more fully.

In the evidence thus presented, emphasis has been laid on those points in the structure of both the Haflagellata and Haemosporidia which have been used by the authors of the Binucleata as constituting the basis of their claims for a binuclear condition in these forms, as well as for flagellate affinities of the Haemosporidia.

D. THE PARABASAL BODY AS A SPECIALIZED STRUCTURE

I. PHYLOGENETIC DEVELOPMENT

The characteristic motor apparatus of the Mastigophora, as well as of the flagellated stages of other organisms, consists of one or more flagella attached either to the nucleus, or to a single granule or granule complex, the blepharoplast, which may or may not be connected with the nucleus. This type of organization is found generally throughout the free-living flagellates as well as in the parasitic forms and is evidently the primary condition.

The terminology for the neuromotor apparatus of the flagellates,
especially among the haemoflagellates, has become a matter of some confusion, and a word concerning the application of these terms in the present discussion will not be out of place. The basal granule complex has two distinct functions in many if not most of the flagellates, that is, as the definitive basal granule for the flagellum, and also as the division center for the cell. The latter function may be displayed either as the actual centrosome on the spindle, or as the organelle whose division begins the process in the entire cell. It may occasionally happen, as in one or two figures in the division cycle of *Trichomonas augusta* (Kofoid and Swezy, 1915b), that the blepharoplast becomes separated into two granules, one of which functions as the centrosome and the other as the basal granule. This is an exceptional occurrence, however, and in all cases in which it does not occur the term blepharoplast is applied to the granule complex. A further discussion of the term blepharoplast as applied to the parabasal body is given below. With the casting aside of the word *kinetonucleus*, the term *trophonucleus* becomes superfluous in the terminology of the trypanosomes, that structure then becoming the nucleus as in all other flagellates. The centrosome is the granule which by its own division initiates division in the cell, and, in the metazoan cell is surrounded by achromatic material which becomes the centrodesmose and central spindle. This latter structure has no relation whatever to the darkly staining fibril frequently found connecting the blepharoplasts in division in many of the flagellates. To this fibril the term *paradesmose* has been applied (Kofoid and Swezy, 1915a, b) as more correctly interpreting its function and origin. *Centrodesmose* is limited to the achromatic material of the central spindle.

The effects of parasitism on the protozoan cell are as yet but little understood, but it is certain that profound changes result from the attempts of the organism to adapt itself to its new environment in the various stages of its life as a parasite. In changing from a fluid to a semi-fluid medium, or to one filled with food or with blood cells, the greatest stress falls naturally upon the motor apparatus. This has been met in two ways, first, by the formation of an undulating membrane and second, by the growth of additional structures related to the basal granule of the flagella but intracytoplasmic. That these are adaptations conditioned on a parasitic mode of life is shown by the general lack of them in the free-living flagellates and the almost universal occurrence of one or the other, or of both, among parasitic species.
Hypotheses concerning the phylogenetic origin of parasitic forms must of necessity be mere guess-work, at the present time at any rate. It is quite certain that they arose from organisms not very unlike the present species of free-living Protozoa. Adaptation to a parasitic mode of life, taking place in a number of genera, might well be supposed to lead to the present conditions, where a number of parasitic species exhibit organelles which are homologous in structure and analogous in apparent function, or are at least convergent. This is analogous to what takes place in specialization throughout the animal kingdom and is a condition which would naturally be expected to occur here also. That such a condition does occur in this group, I hope to be able to show in the following pages, in evidence pointing to the homology of the parabasal body of the trypanosomes with the so-

Fig. 7. Schizotrypanum cruzi Chagas, after Chagas (1909, pl. 9, fig. 13). Trophozoite showing nucleus, parabasal body, blepharoplast, undulating membrane and flagellum; note fibrils connecting blepharoplast with parabasal body.

Fig. 8. Herpetomonas muscae domesticae (Burnett), × 2500. Trophozoite showing nucleus, parabasal body, blepharoplast and flagellum.

Figs. 9-10. Trypanoplasma carassii sp. nov. Fig. 9. Trophozoite, showing nucleus, parabasal body, blepharoplast, undulating membrane and flagellum. Fig. 10. The same.

called chromidial body of Prowazekia and Polymastix, the chromatic basal rod of Trichomonas and the parabasal bodies of the Trichonymphidae, structures which are correlated with an endoparasitic mode of life and are intimately related to the motor apparatus.

Two lines of development of the parabasal body may be noted here, the first springing from a uniflagellate ancestor and finally resulting
in the trypanosome type of structure, and the other, starting from a biflagellated ancestor, developing along the line of the Polymastigina and the Trichonymphida. These will be traced out separately, followed by a final summing up of the evidence therein given, and its application to the binuclear theory.

1. FIRST LINE—HAEMOFLAGELLATA

It has been claimed generally that the basal granule or blepharoplast of the flagellates has originated from the centriole of the nucleus (Minchin, 1912), which moves out, either remaining in close connection with the nucleus or becoming quite independent of it. The original type of flagellate would, in that case, be one in which the flagellum was connected directly with the centriole in the nucleus, assuming that the primitive position of the centriole is intranuclear. This primitive condition without blepharoplast or a basal granule is unknown among the flagellates, and is an entirely hypothetical one.

One place where the origin or the process of formation of the neuromotor apparatus in the individual, with its accompanying blepharoplast, may be actually observed is among some of the amoebae of the Limax group, as in Nāgleria gruberi (Schardinger). This is not the production of minute swarm-spores by the breaking up of the parent body, but is, instead, the actual change of an adult amoeboid organism into a flagellated one with blepharoplast and flagella complete (fig. 14). The actual transformation may be watched in the living forms and its stages analyzed in fixed material. This change from an adult amoeba to a flagellate has been described by Whitmore (1911), Alexeiff (1912), and more fully by Wilson (1915). As shown by the last-named investigator various factors may bring about this change, as a variation in temperature, medium, etc.

The first outgrowth of the flagellum appears as a minute bud on the edge of the karyosome within the nucleus. This granule moves out toward the nuclear membrane, drawing after it a slender fibril of presumably achromatic material from the karyosome (figs. 12, 13). When this granule reaches the periphery of the cell or very near it, the flagella make their appearance, and push out beyond the periplast. So far as direct observation goes, the material for the blepharoplast comes from the outer border of the karyosome and the centriole can still be seen at the center of the karyosome during all stages of the development of the flagella. Its exact origin is thus somewhat
uncertain, but the fact that the centriole does not move out to become the blepharoplast (Minehin, 1912) seems assured. It may, however, arise by a division of that body, a supposition that would explain its function as the division center for the cell, and also the occurrence of another division center in the nucleus itself, both of which conditions are common among the flagellates. This would, in reality, provide for two division centers in the cell, one of which may remain in the nucleus and the other functions as the blepharoplast. This condition is found in Polymastix bufonis. Both division centers may also be contained in one structure, as in the blepharoplast of Trichomonas (Kofoid and Swezy, 1915a, b), where there may be a temporary separation of the blepharoplast into the definitive centrosome and the basal granule.

The claim is not made here that the amoeba is a more primitive form than the flagellates. It is possible, however, that this change to a flagellate stage occurs only in the more primitive amoebas which may be on that account more unstable in their organization. Being nearly allied to the original monad from which both the Rhizopoda and the Flagellata may have sprung, the characteristics of both lines may thus appear in the individuals of one line, owing to adverse, or at least to some slight changes, in their environment. The tendency of the simpler flagellates to become amoeboid under like conditions, and also preceding degeneration, is probably another manifestation of this primitive relationship.
Flagellates are frequently found in which the flagellum with its blepharoplast is connected with the karyosome by a rhizoplast. It is found among the simpler as well as the more specialized flagellates and is probably the earliest form of flagellar insertion, a supposition towards which the conditions in the amoeba also point, as it is here a new formation in an amoeboid organism.

The origin of the blepharoplast in the centriole or karyosome of the amoeba may throw some light upon its function as the centrosome in the dividing flagellate. In many species of amoeba the division of the centriole to form the karyosome of the dividing nucleus is followed by division or elongation of the karyosome or a part of it to form the large "poleplates" of the spindle (Aragão, 1909, pl. 2, figs. 3-7, 13-18), the whole structure, apparently, performing the functions of the centrosome.

In all cases in which division of flagellates has been carefully worked out, it has been found generally that the process is initiated by a splitting of the blepharoplast. The blepharoplast thus forms a division center for the cell, even where an additional definitive centrosome for the nucleus is present. This function, then, combined with its position as the basal granule of the flagellum, constitutes it a blepharoplast as that organelle has been defined by Webber (1897), and this name will be used for it in the following discussion.

In the evolutionary development of the parabasal body, its more primitive form may be looked for among the simpler parasitic flagellates, the herpetomonads, as in these forms the change from the ordinary cercomonad type is but slight. In Herpetomonas (fig. 8) the primitively single flagellum arises at the anterior end of the body from a blepharoplast which is in turn connected with the parabasal body (Wenyon, 1913). The parabasal body here consists of a mass of deeply staining material which, in the majority of forms, shows but slight, if any, structural differentiation. The simple blepharoplast attached to the nuclear membrane in Cercomonas (Wenyon, 1910), has presumably migrated some distance from it in Herpetomonas, and formed the parabasal body by a secondary outgrowth from itself, between itself and the nucleus. Actual evidence in support of this last proposition has not been found among the herpetomonads, but it will be shown below that this process actually takes place in Prowazekia, and, by analogy, we would expect the same conditions to obtain here.
It has been claimed by many investigators on these forms that the flagellum arises directly from the parabasal body, or at least that no other granule is connected with it (Prowazek, 1904, Patton, 1908, and others), and many of their figures show only an undifferentiated chromatin mass at the base of the flagellum. This condition would be explained by the probability that the parabasal body was formed as an outgrowth of darkly staining granules from the blepharoplast which was not entirely separated from it in the earlier stages of its formation.

This view seems more in accord with the facts as shown in the intimate relation of these two structures, in the similarity in their methods of division and in the direct outgrowth of comparable structures in Trichomonas and in some of the Trichonymphida than does the theory which would make the blepharoplast a secondary derivative of the parabasal body, for two reasons. The first reason is that the simple basal granule, or blepharoplast, is the more primitive condition, and second, to have the blepharoplast of the trypanosomes take its origin from the parabasal body it would be necessary to suppose that the parabasal body was the original definitive nucleus, or part of it, or else that the original basal granule or blepharoplast was lost and a new one had to be secondarily formed. Neither of these suppositions is supported by the actual facts of mitosis as known at the present time.

The next stage in the evolution of this structure shows a further development resulting in a parabasal body more or less separated from the blepharoplast, as found in Crithidia. This flagellate possesses a short, often rudimentary, undulating membrane attached to the single flagellum, which arises from a blepharoplast near the posterior part of the proximal third of the body (fig. 16). The blepharoplast, as shown in the recent work of McCulloch (1915), is connected with the parabasal body by numerous fine unstained fibers, forming a cone-shaped structure between the two granules (fig. 15). The blepharoplast in these forms is minute and often difficult to demonstrate, as is also the case with the connecting fibrils between it and the parabasal body. In many figures given for Crithidia as well as for the trypanosomes by most investigators, the parabasal body is shown with a clear area between it and the end of the flagellum, evidence that the flagellum does not take its origin from that structure.

The rhizoplast connecting the motor organelles with the nucleus in Crithidia seems, in most cases, to pass through the parabasal body,
and to extend to and around the nuclear membrane (McCulloch, 1915, pl. 2, fig. 56). In some cases the rhizoplast extends directly to the karyosome of the nucleus. The connection between the rhizoplast and the blepharoplast in these cases is not clear. Another method of attachment of the rhizoplast is figured, however, which has important bearings on the question of the function of the parabasal body. This

Figs. 15-19. *Crithidia leptocoridis* McCulloch, after McCulloch (1915, pl. 3, figs. 57, 58, 60; pl. 4, fig. 74), × 2880. Fig. 15. Trophozoite, showing fibril arising from the blepharoplast. Fig. 16. The same. Fig. 17. The same, with a second fibril extending to the nuclear membrane. Fig. 18. Long, slender trophozoite, showing same method of attachment of the rhizoplast. Fig. 19. Trophozoite, showing the so-called "axostyle" arising from the blepharoplast.

method of attachment is shown in McCulloch’s plate 3, figures 57, 58, 60, and plate 4, figure 70, reproduced in figures 15 to 19 of the present paper. These show the rhizoplast arising from the blepharoplast and passing around the parabasal body on its way to the nucleus. A slight rotation of these figures would completely obscure its real origin, making it appear to arise from the parabasal body as shown in other figures. The relations of the entire neuromotor apparatus, consisting
of the flagellum, blepharoplast, rhizoplast, parabasal body, and nucleus, in these figures become exactly comparable with those shown for *Polymastix bufonis* (fig. 47) and *Trichomonas augusta* (fig. 58), in that a direct connection is established between the blepharoplast and nucleus, while the parabasal body occupies the position of an accessory organelle connected with the blepharoplast at one side, instead of in the direct line of structure from flagellum to nucleus; that is, it is in precisely the relation of the parabasal body. This relationship would suggest a function as an accessory organelle for the parabasal body, in opposition to the view which would give to it the position of the kinetic center, or kinetenucleus, of the cell. This relationship would also militate against the idea of its origin by a heteropole division of the nucleus.

The transition from *Herpetomonas* to *Crithidia* has been made by a backward migration of the blepharoplast with its attendant parabasal body and flagellum to the middle regions of the cell. This backward migration is followed, in the evolution of the trypanosomes, by a still further backward migration of this structure until the posterior region of the body is reached, with a corresponding development of an undulating membrane.

The relations of the neuromotor apparatus of the trypanosomes, consisting of the same organelles as found in *Crithidia*, are, at least in some stages of its life-cycle (fig. 7), the same as has been pointed out above for that genus (cf. Kofoid, 1916).

This condition found in the trypanosomes represents the highest stage of development reached by the *Crithidia* line of evolution of extra-nuclear organelles. That this is the probable course of evolution is shown by the fact that these stages, herpetomonad and crithidial, both occur in the life-cycle of the highest member of that group, *Trypanosoma* (Minchin and Thompson, 1915).

To conclude: It has been shown in the preceding paragraphs, that the only actual occurrence of the formation *de novo* of the blepharoplast is found in some of the primitive amoebas, in which a granule is budded off from the karyosome of the nucleus and migrates to the periphery of the cell where it gives rise to the flagella. This granule is not the centriole but may possibly be formed by a division of that body. The development of the neuromotor apparatus of the trypanosomes is found in two of its earlier stages in *Herpetomonas* and *Crithidia*. The relations of the parabasal body in *Crithidia* suggest that found in *Polymastix bufonis* and *Trichomonas augusta*, and also sug-
gest its function as that of an accessory organelle and not that of a kinetic center or nucleus.

2. SECOND LINE-BODONIDAE-TRICHONYMPHIDA

Starting from a simple, free-living *Bodo*, the stages of evolution of the parabasal body in another line are equally apparent in an almost orthogenetic series culminating in the elaborate differentiations of the Trichonymphida. Two forms of this structure are found in the Bodonidae, one of which is illustrated by *Prowazekia cruZi* (fig. 20) with the characteristic round parabasal body situated below the blepharoplast and connected with it by a short rhizoplast.

The parabasal body of *P. cruZi* is the only structure in this group which has been recognized by Hartmann as a "kinetomucleus." A careful study and comparison of the other forms with *Prowazekia cruZi* will, however, show that no line of distinction can be drawn between them. So far as their morphology, mode of division, apparent origin, and function are concerned, they present similarities too great to admit of any separation being made.

The second type is that shown in *Prowazekia lacertae* (Grassi) (figs. 26, 31), a flagellate which differs from the structure in the typical *Bodo* mainly in the presence of the organelle which I shall here term the parabasal body, regarding it as the homologue of the parabasal body of *Prowazekia cruZi* and of that of the trypanosomes.

This small protozoan I have found to be quite common in the amphibians Diemyctylus torosus, *Plethodon oregonensis*, and *Batracchoseps attenuatus* from California. It was described by Prowazek (1904) as *Bodo lacertae*. This name, however, has been reserved for those flagellates which do not possess a chromidial body or parabasal body in relation to the nucleomotor apparatus, hence it properly belongs with the genus *Prowazekia*. The proposal of Alexeieff (1912) to lay aside the generic term and substitute for it *Prowazekella* Alex. lacks adequate foundation. In his figures 1a and b he shows *Bodo caudatus*, neither figures of which are like the typical *Bodo* in structure. In figure 1c he portrays *Prowazekella* (nom. nov.) *lacertae*, which possesses the structure neither of the typical *Bodo* nor of *Prowazekia*. Both of these forms, however, have a parabasal body connected with the blepharoplast, a condition which has not been figured in the common free-living *Bodo*. 
Prowazekia lacertae is a small flagellate, ten to fifteen microns in length. It is pyriform in shape, with the nucleus situated in the broader, anterior half of the body. The blepharoplast, from which

Figs. 20-22. Prowazekia cruzi Hartmann and Chagas, X 2066. Fig. 20. Trophozoite, with nucleus, blepharoplast, parabasal body and flagella. Fig. 21. Trophozoite, showing variation in parabasal body. Fig. 22. The same.

Figs. 23-32. P. lacertae (Grassi), X 2066. Fig. 23. Trophozoite showing no evidence of parabasal body; rhizoplast connecting blepharoplast and nucleus. Fig. 24. Trophozoite showing enlarged blepharoplast. Fig. 25. Trophozoite with still further enlarged blepharoplast. Fig. 26. Trophozoite showing backward migration of the parabasal body; note connecting fibrils. Figs. 27-31. Trophozoites with variously shaped parabasal bodies. Fig. 32. Telophase of division; nuclei reorganized; parabasal body not divided. Fig. 33. Division of the parabasal body completed.
spring the two unequal flagella, is situated at the anterior end and is connected with the nucleus by a slender rhizoplast (fig. 30). Thus far it agrees in every essential detail with the typical *Bodo*. It will be seen in the following discussion of the various forms found in these amphibians that the propriety of separating them from *Bodo* may well be doubted, as all stages in the development of an elaborate parabasal structure from a simple blepharoplast, as in *Bodo*, will be shown.

Janicki (1915) also questions the propriety of separating *Prowazekia* and *Bodo*, on the ground that the nuclear nature of the parabasal body of *Prowazekia* is doubtful, and that this structure is susceptible of another interpretation. The chromidial body of *Bodo*, "corps sidérophile" of Alexeieff, he homologizes with the parabasal body of the Triehonymphida, a conclusion which agrees with my own reached before the appearance of his paper. The form which he figures as *Bodo lacertae* is apparently identical with the species designated here as *Prowazekia lacertae*.

The simplest form of the parabasal body is that shown in figures 24, 25, consisting of a number of granules surrounding the actual blepharoplast, or it might be described merely as an enlarged blepharoplast. No distinction in these figures can be drawn between the blepharoplast and the surrounding darkly staining material. A slight modification of this is shown in figure 25, where the parabasal body is becoming distinctly separated from the blepharoplast. A still further separation, with a rounding up of the chromidial mass, produces the type of parabasal body characteristic of *Prowazekia cruzi* (fig. 20). The figures 26 to 31 illustrate the backward migration of the parabasal body until it reaches a position close beside, or immediately behind the nucleus, a condition comparable to that found in *Critidia*, but differing from it in that the blepharoplast does not take part in this migration. It presents, however, some interesting modifications.

In figure 27 the parabasal body is still slightly in front of the nucleus. It has become greatly enlarged, exceeding the nucleus in size. The most interesting point here is its connection with the blepharoplast. This granule is slightly elongated, and starting out from the posterior portion of it, are a number of fibrils which spread out in a fan-shaped figure as they reach the parabasal body to which they are attached as a sort of a suspensory apparatus. At nearly equal distances between the points of attachment are two granules, unequal in size and spreading across the entire width of the fibrillar structure.
A comparison of this figure with that of *Schizotrypanum cruzi* (fig. 7) reveals an essentially similar structure, barring only the extra chromatin granules in *Prowazekia lacertae*. This denotes a relationship between these two bodies as intimate as that ascribed to the same organelles in the trypanosomes.

Similar structures have been figured for *Trypanosoma noctuae* by Rosenbusch (1909, pl. 25, fig. 10), for *T. rotatorium* by Machado (1911, pl. 7, fig. 3; pl. 8, fig. 50), in *T. lewisi* by Carini (Mayer, 1911, pl. 6, fig. 8), and in *Crithidia leptocoridis* by McCulloch (1915). This does not appear in all the figures given by these investigators, but much of the work has been done with Giemsa preparations and this is a difficult point to establish by that method.

Neither is its occurrence constant in *Prowazekia lacertae*. Various modifications of this suspensory apparatus may be frequently met with (figs. 26, 28, 30), or even forms in which it seems to have totally disappeared (fig. 29). The parabasal body itself is also modified in several ways. Figure 27 shows a well-developed fibrillar connection with the parabasal body which is here elongated into a long, band-like structure looped up around the nucleus. This band-like form is of frequent occurrence (figs. 28–30) and may assume various positions, sometimes occupying the whole central part of the cell. The amount of the chromidia in this organelle also varies greatly in different individuals, the entire structure sometimes appearing as a densely staining mass (fig. 31), and again showing more or less of a definite organization with very little chromidia.

The compact form shown in figures 31 and 32 is not far removed from that of *Prowazekia cruzi* (fig. 20), differing from it mainly in that its position is posterior to the nucleus, and farther removed from the blepharoplast. Division in the two species is essentially the same, consisting of an elongation usually in a tranverse direction in the cell and a simple constriction in the middle (figs. 32, 33).

The connection of this body with the blepharoplast and the different stages shown of its backward migration and change into an elongated, band-like form, afford very strong evidence for the view that we are here dealing with the same structure found in *Prowazekia cruzi* and in the trypanosomes, namely the parabasal body.

It is: (1) a body originating from the blepharoplast and intimately connected with it as, presumably, an accessory kinetic structure; (2) the steps in its backward migration may be followed, showing that this process is comparable with the backward migration of the para-
basal body in Crithidia and the trypanosomes; and (3) its division is by a simple constriction, as is the case also of the parabasal body of the haemoflagellates.

The next step in the evolutionary series has resulted in the production of two forms on divergent lines, which show striking similarities.

Figs. 34–36. *Trypanoplasma congri*, after Martin (1913, pl. 9, figs. 3, 7; 1910, pl. 21, fig. 1). Fig. 34. Normal active trophozoite. Fig. 35. Trophozoite showing elongated parabasal body. Fig. 36. Individual showing rounding-up stage.

Figs. 37–42. *T. dendrocoeli* Fantham and Porter, after Gelei (1913, pl. 7, figs. 9, 14, 23). Fig. 37. Individual in the beginning of division: note elongated parabasal body. Fig. 38. Later stage of division. Fig. 39. Dividing form with greatly elongated parabasal body. Fig. 40. *T. cypriini* Pilhn, after Martin (1913, pl. 10, fig. 26). Late stage of division, with two daughter nuclei still connected and the parabasal body undivided. Fig. 41. *T. congri*, after Martin (1910, pl. 21, fig. 8). Late stage of division. Fig. 42. *T. intestinalis* Léger, after Martin (1913, pl. 9, fig. 20). Late stage of division: organelles completely divided.
in the structure of their parabasal bodies as well as in the behavior of these organelles in division. These forms are *Trypanoplasma* and *Polymastix bufonis* (Dobell).

In *Trypanoplasma* an increase in the complexity of the motor apparatus is shown in the attachment of the trailing flagellum to the body by a membrane, which is more or less well developed (fig. 34).

Figs. 43-50. *Polymastix bufonis* (Dobell), × 2066. Fig. 43. Trophozoite; end view showing horseshoe-shaped parabasal body. Fig. 44. Normal active trophozoite showing the parabasal body with some evidences of organization. Fig. 45. Early prophase of division. Fig. 46. The same stage. Fig. 47. Ordinary trophozoite. Fig. 48. Prophase of division. Fig. 49. Late prophase. Fig. 50. Late anaphase.

The parabasal body presents many aspects in different species. In *T. carassii* sp. nov. (fig. 10) it is a rounded, compact body, connected with the nucleus on one side by a rhizoplast and on the other with the blepharoplast. In *T. congri* (Martin, 1913, fig. 35) it is stretched out lengthwise of the cell, sometimes lobed or constricted. Gelei (1913) shows how widely this structure may vary in *T. dendrocoeli*, in form as well as in its staining reactions, in the same species. At the time of division it elongates and becomes separated in the middle by a simple
constriction (Martin, 1913; Neresheimer, 1912; Gelei, 1913). It is to be particularly emphasized that it does not divide by a mitosis of its own.

*Polymastix bufonis* (Dobell) is a common flagellate of several species of Amphibia in California including *Dinemictylus torosus, Batrachosps attenuatus* and *Rana pipiens*. The shape of the body is pyriform and is fairly regular in outline, being sustained by a somewhat thickened cuticle, or periplast (fig. 44). This is marked with striations extending obliquely across the body in uniform, parallel lines. Four equal flagella arise from a blepharoplast at the anterior end, near which the nucleus is also situated. Connected with the blepharoplast by a more or less thickened fibril is a deeply staining, elongated body which forms an arch around the nucleus, as shown in the end view in figure 43. This structure presents various degrees of intensity in its staining reactions, usually appearing as dense, compact material, but occasionally staining deeply with iron haematoxylin in small areas only (fig. 44). In this peculiarity it agrees with the conditions figured for the parabasal bodies of *Trypanoplasma dendrococli* by Gelei (1913), and in *T. congri* and *T. cyprini* as given by Martin (1913), (figs. 34–42).

A careful comparison of the figures of these three species of *Trypanoplasma* and *Trypanoplasma borrelli* (Keysselitz, 1906) with figures of *Polymastix bufonis* brings to light some interesting points of resemblance. *T. congri* (fig. 36), with a closer approximation of the nucleus and blepharoplast, and a stronger curve to the parabasal body, will present the same structure shown in figure 43 of *Polymastix bufonis*. In no essential detail does the ordinary vegetative individual of *T. congri* (fig. 35) differ from the same stage of *P. bufonis* (fig. 47).

The parabasal body of *Trypanoplasma* elongates and divides by a simple constriction (Martin, 1913, Gelei, 1913), as does also the same structure in *Polymastix*. The various steps in this process figured in the two forms show a close resemblance in both cases. The orientation of the body of *T. borrelli* in the figures given by Keysselitz (1906) is somewhat difficult to interpret, but a comparison of them with Gelei’s figures seems to indicate that in the beginning of the process the parabasal body elongates in a direction parallel to the long axis of the body (fig. 37). In the later, more amoeboid stage of division, this direction becomes transverse (figs. 38, 40), owing, probably, to the tension exerted by the constantly moving flagella. In *Polymastix* the direction of elongation is transverse or slightly oblique (figs. 50–57).
and amoeboid changes in the body-form are entirely lacking. Gelei (1913, pl. 7, figs. 28, 29, 31, 32) has also described a process of longitudinal division in T. borreli as well as the transverse one shown here, but his figures do not bear out his interpretation.

The chief point of difference in the two structures here compared is found in their relative positions. In Polymastix buonis the parabasal body has migrated posteriorly to a position immediately sur-

![Figs. 51-57. Polymastix buonis (Dobell). X 2066. • Fig. 51. The same stage as in Fig. 50. • Fig. 52. Early telophase; parabasal body still undivided. Figs. 53, 54. Telophase. • Fig. 55. Telophase; constriction of parabasal body. • Fig. 56. The same stage. • Fig. 57. Telophase; organelles completely divided.]

rounding the nucleus, and remains connected with the blepharoplast by a fibril, while in Trypanoplasma it is connected directly with the blepharoplast, or by a very short fibril only, and its long axis coincides with the long axis of the cell, the reverse of which is true in Polymastix. The relative positions of this organelle with reference to the nucleus and blepharoplast are by no means constant in Trypanoplasma, as the figures of Gelei (1913) abundantly prove. His plate 7, figure 7, shows the blepharoplast and parabasal body occupying opposite ends of the cell with the nucleus between them. This is an extreme ease, but various intermediate stages are also figured.
With these comparisons in mind the conclusion seems inevitable that the parabasal body of Trypanoplasma has its homologue in the "chromidial body" of Polymastix bufonis, morphologically and functionally equivalent to it. This must therefore be considered the parabasal body of that species. That the backward migration of the parabasal body carries the blepharoplast with it in the haemoflagellates does not invalidate the homology here, as these structures are still connected by a rhizoplast, and hence the parabasal body still preserves its function, as an accessory kinetic reservoir.

Fig. 58. Trichomonas augusta Alexieff. × 2000. Normal active trophozoite, showing nucleus, axostyle, blepharoplast, parabasal body, undulating membrane and flagella.

A further step in the evolution of this structure is that exhibited in some of the trichomonads, in the presence of the chromatic basal rod. A full discussion of the morphology and relations of this organelle has been given elsewhere (Kofoid and Swezy, 1915b) and need not be repeated here.

The difficulties of homologizing a structure like the chromatic basal rod of Trichomonas augusta (fig. 58) with the parabasal body of the trypanosomes are considerably lessened when we regard the intermediate form, Prowazekia lacertae, as the connecting link. The long, ribbon-like parabasal body of the latter species, with its deep intra-cytoplasmic position and rhizoplast connecting it with the
blepharoplast, requires but few changes to transform it into the chromatic basal rod of *Trichomonas augusta*. This transformation may happen by a shortening of the rhizoplast until that structure disappears, leaving the parabasal body connected directly with the blepharoplast, and stretched out along the margin of the body. Its connection with the undulating membrane is a secondary modification which further emphasizes its function as a kinetic reservoir.

The only striking point of dissimilarity is found in the behavior of this structure during division of the cell. In *Trichomonas* this does not divide, but the old rod is retained by one daughter cell, while the new one is formed by a new outgrowth from the blepharoplast in the other daughter cell. Considering the original condition of the parabasal body as an outgrowth from the blepharoplast, this in no way hinders us from drawing the conclusion that in the chromatic basal rod of *Trichomonas* we are dealing with a modified form of parabasal body comparable, in probable origin and function, with the parabasal body of the trypanosomes. That this structure differs widely in these flagellates in form and apparent organization in no way invalidates this conclusion, a view which is supported by the great variety of form and structure exhibited by other organelles of the protozoan body. For example, the nucleus may consist of but little more than several masses of chromatin packed together without a membrane as in some of the simpler Sarcodina, or it may be built up of chromatin and achromatin until a complicated structure is produced, such as is found among the higher Sarcodina which bears but little resemblance to the simpler form. A third type, differing widely from either of these, is found in *Hexamitus intestinalis* (Swezy, 1915), with its club-shaped mass of chromatin, lacking apparent structure or membrane. If the most important and essential organelle of the cell varies so widely as these examples show, it would be expected that one developed under originally pathogenic conditions of parasitic life would also exhibit great modification in its structure. The results of evolution among the Metazoa point to the same conclusion, namely, that the effects of such evolutionary development are, more frequently than otherwise, quite diverse in the ultimate forms they reach, but remain comparable in origin and function.

The greatest advance in complexity of the parabasal apparatus is found in the parabasal bodies among the Trichonymphida, where they are correlated with a corresponding complexity of cell structure. This reaches its greatest development in *Lophomonas blattarum*. 
In its simpler form as found in *Devescovina striata* the parabasal apparatus is comparable to the "kinetonucleus" of *Prowazekia* and *Polymastix bufonis*. Here it consists of a long, narrow band or tube, connected with the blepharoplast at the anterior end by a delicate fibril, and passing backward around the nucleus, coiling about the axostyle with from two to eight spiral turns. At the time of division of the cell this body also divides.

In *Parajovonia grassii* the parabasal body consists of two distinct but similar parts, a condition which is associated with two lines of over twenty flagella each. These bodies have a more or less curved or horseshoe shape, lying in the immediate neighborhood of the nucleus, and are each connected with the blepharoplast by a slender fibril or rhizoplast. These structures divide in the formation of the new daughter cells as in *Devescovina*. In *Calonympha grassii* each group of flagella springs from its own blepharoplast, each of which is related to a single, small, rounded parabasal body, situated in the neighborhood of the nucleus.

A further complication is found in *Stephanonympha silvestrii*, with its several rows of nuclei, blepharoplasts, each associated with its parabasal body, and groups of several flagella. The blepharoplast and single parabasal body is connected by a slender rhizoplast. At the time of division the old structure is retained by one daughter cell and the other daughter cell is provided with one by a new outgrowth from the blepharoplast, as in the case of *Trichomonas*.

In *Lophomonas blattarum* the complexity of the parabasal apparatus is also related to a great development of flagella. The individual fibrils which in the other forms serve to connect the blepharoplast with its related organelle are here merged into a protoplasmic collar, or calyx, which serves to connect the different parts of the kinetic apparatus (Janicki, 1911). Its behavior during division is unlike that of the structures in the preceding species, as it appears to degenerate and to be formed anew in each daughter cell.

The possibility has been suggested by Janicki (1915) of placing the parabasal bodies of these flagellates, and especially the structure in *Devescovina*, in the same category as mitochondria. Only scant information exists at the present time on the subject of mitochondria in the Protozoa. The recent work of Lewis and Lewis (1915) on mitochondria in the cells of chick tissues would seem to point to the fact that in the metazoon cells these structures are probably connected with the metabolic activity of the cell, and vary in size and quantity
from one period to another, shown by their constantly changing shape, size and position. The chemical constitution of these bodies apparently varies greatly at different periods and in different cells. Further work on the subject from both the metazoan and protozoan standpoint is needed before any adequate generalization can be made, and yet certain resemblances can be traced which seem to be of some significance.

The parabasal bodies shown in figures 20 to 33, exhibit a great variety of sizes, shapes, and positions, as do also those grouped in figures 34 to 41. The same thing is found in any group of trypanosomes, as may be seen in the plates of Chagas (1909), Minehin and Thompson (1915) and others, though the variation is less in these forms than in the flagellates Trypanoplasma and Prowazckia. This might be expected from a comparison of the media in which these flagellates live, the blood forming a more nearly constant medium than does the intestinal contents with its constant fluctuations in both quantity and quality.

Considering the parabasal bodies as related in their origin and development to the processes of metabolism it seems not improbable that these structures may be homologous to the mitochondria in the metazoan cell.

The parabasal bodies of the Trichonymphida are structures related to the blepharoplast, the kinetic center of the cell, and, in two cases at least, are formed by direct outgrowth from it. They are permanent cell organelles, persisting from one division cycle to the next. Janicki (1911) has described their composition as "an fixierten und gefärbten Preparaten, besonders an osmierten, als aus dichten, durchaus homogenen Plasma zusammengesetzt; seine Konturen sind nicht sehr scharf ausgedrückt; eine Membran, welche das Plasma umschlüssle wird nicht beobachtet." With these, as is also the case with the chromidial bodies of Prowazckia, all stains, as, for example, Delafield's haematoxylin, do not produce nuclear reactions. This proves that it is not composed of nuclear chromatin, or at least not of unmodified nuclear chromatin. It will be pointed out in greater detail below that the parabasal body of trypanosomes is also not composed of nuclear chromatin in the sense that it is chemically identical with the intranuclear material.

In his latest communication, which appeared as this article was being prepared for the press, Janicki (1915) has extended the application of the term "parabasal body," including under that heading
the kinetonucleus of *Trypanoplasma*, the chromidial body of *Bodo*, both of which have already been discussed here, and the chromidial body of *Monocercomonas bufonis* Dobell. In regard to the latter species there is a strong probability that it and the form described above as *Polymastix bufonis* are one and the same form. A further discussion of this will, however, be given in a later communication.

These facts show that a comparison of the parabasal bodies of the Trichonymphida, the chromatic basal rod of *Trichomonas*, the chromidial bodies of *Polymastix* and *Prowazekia*, and the "kinetonuclei" of the haemoflagellates reveals similarities in origin, morphology and function sufficient to justify classifying them as homologous structures.

II. Function and Behavior

In the preceding discussion of the different members of the "Binucleata," as well as the other flagellates, the function and behavior of the parabasal body have already been indicated. It is necessary, therefore, only to sum up the evidence on these two points, with a consideration of some of the earlier views on these questions.

The majority of protozoologists who have dealt with this subject have agreed in calling the parabasal body a second nucleus, that is, the "kinetonucleus," and in giving it a rank equal to that of the nucleus, which is then designated the trophonucleus. The one notable exception to this is Doflein. He objects (1911) to the order Binucleata on the ground that the nuclear nature of this structure in trypanosomes has not been proven, and also that trypanosomes are not the only flagellates which possess "kinetonuclei." These objections have been amply confirmed in all work on these forms up to the present time, including even the investigations carried on by the supporters of the binuclear theory.

The views in regard to the function of the parabasal body are denoted by the different names which have been given to it. The French school of protozoologists, following the lead of Laveran and Mesnil (1902), generally regard it as the "centosome," a supposition for which but little, if any, adequate evidence can be found. The same views on the subject of its function were put forth by Moore and Breinl (1907), who thought it originated by a division of the intranuclear centrosome.

The use of the term blepharoplast for this structure among the Germans began with the adoption of it by Sehaudinn (1904). As
has already been pointed out, he considered it nuclear in nature and not a centrosome. Hartmann and Prowazek (1907) employed the same name, but claimed for the structure both centrosomie and nuclear value.

The term *kinetonucleus* was first employed by Woodcock. As the word indicates, he is convinced of its nuclear value, terming it the nucleus which controls the kinetic functions (1906). In this opinion Minchin agrees, as do also the majority of English investigators. Without elaborating his reasons for his conclusions, Minchin (1908) states that this structure "is a distinct kinetic nucleus, a specialization of the nuclear apparatus for a particular function." He later (1912) gives his views on the origin of the kinetonucleus as a division of the original nucleus into two nuclei of unequal size, and emphasizes its essential nuclear nature and function.

In a more recent memoir (Minchin and Thomson, 1915), giving what is, by far, the most complete account of the trypanosome life-cycle to date, no evidence is given to show that the parabasal body ever arises by a division of the nucleus, since, indeed, it is figured as a permanent structure in all stages of the life-history. Nor has any other investigator been more successful in bringing forward proofs for these claims, which rest solely upon the work of Schaudinn (1904). As has already been pointed out, these conclusions of Schaudinn's are based on erroneous interpretations of accidental appearances in individuals of two different species. The so-called mitotic figures of the parabasal bodies in division, have, in nearly every case, been shown to be accidental appearances in a cell which otherwise shows no evidence of division. Wherever other evidences of cell-division are present, the division of the parabasal body is almost invariably figured as a simple constriction.

The origin of the parabasal body by an unequal heteropole division of the nucleus is another claim for which the evidence is very slight, if indeed there is any beyond serious criticism, in spite of the attempts of Neumann (1909) and Berliner (1909) to figure this process in the Haemosporidia, where the entire absence of a motor apparatus is one of the characteristic features of its morphology.

Alexeieff (1910) has pointed out the lack of nuclear and centrosomie value of the parabasal body of *Trypanoplasma*, giving as his reasons the fact that it is often composed of several pieces, presents no definite structure, and does not divide mitotically. For its probable function he suggests that it may be composed of reserve material for the motor
apparatus. Its homology with the "kinetosome" of the trypanosomes he rejects on the ground that the latter is a second nucleus. His conclusions in regard to the function of the parabasal body of Trypanoplasma agrees with the ideas that have been brought out here. His error lies in ascribing to that structure in the trypanosomes a value which it does not possess, and in failing to note its homology with the parabasal body of Trypanoplasma.

That this organelle is not the main center of kinetic activity of the cell is shown in the "blepharoplastose" trypanosomes of Werbitzki (1910), where its loss was followed by no diminution of kinetic activity. Its intimate connection with the motor apparatus suggests that its function is that of an accessory kinetic structure, or kinetic reservoir, secondary to the blepharoplast, which is the chief kinetic center or nucleus, and is related to the metabolic processes incident to increased motor activity resulting from a parasitic mode of life, and may possibly be, as Alexeieff (1910) has suggested, a mass of reserve material. On this account the term coined by Janicki (1911), "parabasal body," is a more appropriate name for this structure than any of the others which have been commonly applied to it, and its general adoption for these structures is hereby proposed.

All of the facts thus far brought out point to the same conclusion in regard to this structure in Prowazekia, Polymastix, Trichomonas, and the Trichonymphida, that is, that it is an accessory part of the motor apparatus, correlated with an endoparasitic mode of life.

It has already been pointed out in the discussion of the various flagellates which possess "kinetosome" that the division of these structures is in no sense mitotic. The idea, however, has been advanced by those (Schaudinn, 1904, Minchin, 1912) who do not hold the centrosomic view of its function above sketched, but who do regard it as a secondary nucleus, that it contains a centrosome by which its own division is controlled. Minchin (1912) terms this centrosome the "centrosome blepharoplast" and claims it as the basal granule of the flagellum. Proof on this point, however, is entirely based on pathogenic or abnormal figures and erroneous interpretation. The true blepharoplast, as we have used the term, initiates division in the cell by dividing first (Wenyon, 1913, Minchin and Thomson, 1915) but no evidence has yet been produced by any investigator to show that it actually functions as a centrosome on a spindle for the division of the "kinetosome" by mitosis.
III. Nuclear Value Not Established by Evidence

Undoubtedly the most important piece of work bearing on the question of the nuclear value of the parabasal body is that of Werbitzki (1910), who was able to produce a strain of trypanosomes lacking these organelles. He treated infected animals with orthochinoid substances and found that under their influence the parabasal bodies of the trypanosomes disappeared. In this way, by successive passages six to ten times through mice treated with oxazin, a strain of trypanosomes was obtained in which every individual was devoid of a parabasal body. This strain remained constant in its subsequent passages through untreated mice.

Kudieke (1911a) confirmed this work of Werbitzki. He did not find it possible, however, to obtain a strain (1911b) which would regenerate the parabasal body, either by treatment with a drug, or by transplantation into other animals as Werbitzki claims to have done.

Laveran and Roudsky (1911) found that when oxazin and aeridine were injected into an infected animal the parabasal body of the trypanosomes were stained pink or violet with the dye. This could be observed in the living forms in a drop of blood from a treated mouse. In the actively motile organisms the parabasal body began to dwindle in size and finally disappeared. Various experiments showed that these substances have a specific action on the parabasal body. The suggestion has been made that its disappearance is brought about by auto-oxidation in the living animal, and the results seem to support this view.

This change has been found to occur in eight species of trypanosomes: Trypanosoma brucei, evansi, soudanense, gambiense, dimorphon, pecorum, congolense, and lewisi.

The total lack of action of the chemical on other parts of the cell, particularly on the nucleus and blepharoplast, affords the strongest possible evidence for the view that the component chemical substances of the parabasal body differ from those of the nucleus and blepharoplast. It follows that the nuclear value of the parabasal body, in thus lacking what has always been considered the essential part of the nucleus, the nuclear chromatin, is absolutely disproved, so far as its composition is concerned.

Curiously enough Werbitzki (1910) begins his account of the results of his work along this line with the statement that the nuclear character of the blepharoplast, as he calls the parabasal body, is fully
established. His own work affords the strongest evidence yet brought forth to disprove this theory.

These results also strike at the root of Hartmann's whole binuclear theory, namely, at the idea that the parabasal body is composed of "lokomotorisch-generative" und "idio-generative" chromatin. It is conceivable that we may be here dealing with chromatin in a different physiological condition, which may, therefore, be acted upon by substances which do not affect the nuclear chromatin. This, however, will not agree with Hartmann's contention that these bodies are composed of the same kinds of material. The same may be said of any other structure of the cell body, and proves nothing in regard to its nuclear value.

The effects of the loss of this structure on the trypanosomes thus treated is shown only by a slightly diminished virulence. The structure, division, activity, etc., all appear to be absolutely normal. It cannot be maintained that this disproves the idea of the function of the parabasal body as part of the motor apparatus. The motor functions were undoubtedly possessed wholly by the blepharoplast originally, as they still are in all free-living flagellates and a few parasitic forms. It is therefore quite probable that with the secondary loss of this organelle, the parabasal body, the blepharoplast assumes all of its original functions again. That the loss of one organ or part of it may be compensated by the remaining part and its functions carried on, is of such frequent occurrence among the Metazoa that we would naturally expect to find some evidence of the same ability among the Protozoa.

Kudicke (1911) has pointed out the fact that in normal trypanosomes in laboratory cultures individuals are frequently observed without parabasal bodies. The loss of the nucleus under the same conditions has been observed by Hartmann and Prowazek (1907) in Leishmania. Fln (1908) and Berliner (1909) described the same abnormality in Crithidia and Herpetomonas. It cannot be emphasized too freely in this connection that laboratory cultures, under the best possible conditions, present an abnormal, or it may be even pathogenic, environment to the organisms which normally live in the body of another animal. Under such circumstances the production of abnormal and degenerating forms are of frequent occurrence. These abnormalities disappear when the flagellates are replaced in their normal habitat; hence their development proves nothing except the presence of abnormal or pathological conditions for the protozoans.
So far as the actual facts which bear on the nuclear value of the parabasal body are concerned, a few definite statements may be made. In all the results of investigations in this field which it has been possible to examine, not the slightest evidence has been found to justify the current practice of attributing to the parabasal body nuclear homology, function, or behavior. It is a structure which is not composed of nuclear chromatin, and hence cannot carry on the functions of that organelle. It is not a kinetic center, for all kinetic activities are carried on, for a time at least, with undiminished vitality after its total disappearance from the cell. As Janicki (1911) has suggested in the case of the parabasal bodies of the Trichonymphida, these structures are probably reserve material connected with the very great kinetic activity of the cell. Its relation to kinetic activities is thus wholly secondary.

IV. USE AS A BASIS FOR CLASSIFICATION NOT CRITICALLY DEFENDIBLE

The classification of the Protozoa must be based upon well-defined characteristics, as to great permanency as possible, correlated with full consideration of the life-cycle as a whole. This is especially necessary in those forms in which an alternation of generations occur.

An examination of the order Binucleata shows that, in the first place, it is founded upon an hypothesis, the binuclear theory, which, in its essential point, has been contradicted by the results of Werbitzki (1910), Kudieke (1911) and others, in proving that the parabasal body is not composed of nuclear chromatin. This is positive evidence and must stand until some further test shall show that the two structures are identical in composition.

In regard to the claim that the parabasal body ever arises de novo by a heteropole division of the nucleus, not a single instance, critically proven, have I been able to find of its actual occurrence, either in the literature, or in working over the organisms themselves. And likewise it has been impossible for any workers subsequent to Schaudinn (1904) to detect its de novo origin among the trypanosomes in a single instance. It seems to be a constant, permanent cell organ in these flagellates, passed on by division in each mitosis. Its fate in gamete formation and its subsequent history in the zygote awaits the critical analysis of those stages, if they occur, in these flagellates.

These two points cover the only reason for forming the order Binucleata in the first place, namely, that these organisms possess two distinct nuclei, both composed of "lokomotorische-generative" and
"idio-generative" chromatin and both arising by an unequal division of the original nucleus.

With the refutation of these points the only reason that might be advanced for retaining this classification would be the permanency of the parabasal body as a cell organ and its specificity for these forms. Among the Trypanoplasmidae and the Trypanosomidae, the parabasal body has been figured, by almost every investigator in this field within recent years, as a permanent cell organ, present throughout all the known stages of the life-cycle. The earlier observations have too much of doubt attached to them to withstand later results. On the other hand, among the Haemosporidia included in the order Binucleata no single undoubted instance can be found which shows a structure comparable in its permanency and function to the parabasal body of the trypanosomes. The granules which occur occasionally in these organisms, and which have been claimed as the homologue of the parabasal body, have no resemblance, either in morphology or in function, to that organelle in the flagellates, and require a far-fetched interpretation so to designate them.

As has already been indicated, the life-cycle of the Haemosporidia widens instead of bridges the gap between them and the flagellates, and also indicates their relations to the Coccidia. On the other hand, going outside the order Binucleata, other flagellates are found which possess structures identical morphologically and functionally with that found among the Haemoflagellata about which Hartmann built up his order. These occur in at least three distinct orders, the Protomonadina, the Polymastigina and the Trichonymphida. These groups comprise the great majority of the parasitic flagellates, and the occurrence of an accessory motor structure in them is significant when the probable origin and function of that structure is in question. Its formation in three distinct orders by a process of parallel evolution, correlated with an endoparasitic mode of life, reduces the value of the claims made for it as a basis of classification.

From a consideration of these facts there seems to be no good reason for retaining the Binucleata as an order of the Mastigophora (Kofoid, 1916), and for uniting the Haemoflagellata with the Haemosporidia. Far simpler is it to consider Trypanosoma, Trypanoplasma, Leishmania, and Prowazekia as part of the order Protomonadina, and the Haemosporidia in their present position in the Sporozoa. A more natural system of classification can be attempted only with fuller knowledge of developmental data than is now available.
E. SUMMARY

1. The "kinetonucleus" is a structure which, in the trypanosomes at least, is not composed of nuclear chromatin, nor can nuclear behavior, shown in mitosis, be claimed for it. It is a structure correlated with an endoparasitic mode of life, and is part of the extranuclear motor apparatus, which, in those cases where its origin can be traced, arises from the blepharoplast, and not by a division of the nucleus.

2. The "kinetonucleus" has been developed phylogenetically along two lines: first, from a uniflagellate ancestor possessing a simple basal granule, producing Trypanosoma, Crithidia and Herpetomonas; second, from a heteromastigote ancestor, also exhibiting only a simple basal granule, along the line of Prowazekia, Polymastix, Trichomonas, and the Trichonymphida.

3. The chromidial body of Prowazekia arises as an outgrowth from a simple basal granule. Different stages in its development can be traced from this condition to the "kinetonucleus" of P. cruzi, followed by its backward migration to a position posterior to the nucleus. The various forms which it assumes are all different aspects of the same structure, morphologically and functionally equivalent to the "kinetonucleus" of the trypanosomes.

4. The next stage in the development of the "kinetonucleus" has produced, by a course of parallel evolution along two different lines, organelles in Polymastix and Trypanoplasma similar in their morphology and behavior in division. These are accessory kinetic structures comparable to the "kinetonuclei" of the haemoflagellates.

5. A slight modification of the long, band-like "kinetonucleus" of Prowazekia lacertae is found in the chromatic basal rod of Trichomonas. This is an accessory structure intimately related to the blepharoplast and the entire neuro-motor apparatus and probably kinetic in its function, and hence an organelle homologous with the "kinetonucleus" of the trypanosomes.

6. A further development of the "kinetonucleus" is found in the parabasal bodies of the Trichonymphida, a group phylogenetically close to Trichomonas. These bodies are accessory kinetic structures and in every respect comparable with the same organelles found in the other flagellates.
7. This structure is not the kinetic center of the cell, but is an accessory part of the motor apparatus, a kinetic reservoir, hence the term "kinetonucleus" denotes a wrong interpretation of its function, as does also the name blepharoplast, which is reserved for the definitive basal granule of the flagella plus the centrosome in some cases at least. As a more appropriate substitute for these names, and one which better indicates its probable relations in the cell, is suggested the term "para-basal body," first proposed by Janicki for these organelles in the Trichonymphida. Its application is here considerably extended.

8. The binuclear theory of Hartmann and the foundation of the order Binucleata, rest upon three main propositions, namely, that the "kinetonucleus" is composed of nuclear chromatin, that it originates by division of the trophonucleus, and that it divides by mitosis. These facts are contradicted by the results of careful investigations on both the Haemoflagellata and Haemosporidia, where these conditions are claimed to occur, which show, (1) by actual experimental proof, that the "kinetonucleus" is not composed of nuclear chromatin, (2) that in no single instance has it been found to arise by division of the nucleus, and (3) that in no instance has a process of mitosis been found which could be correlated with division of the other organelles and with the cell.

9. The Haemosporidia are affiliated with the Haemoflagellata neither morphologically nor by a comparison of the developmental data of the two groups. On the contrary, they are more nearly allied to the Coccidia and should be retained therewith in the Sporozoa.

10. The order Binucleata, being founded on false premises and composed of families totally unrelated either morphologically or phylogenetically, should not be retained as a valid order of the Mastigophora.

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LITERATURE CITED

ALEXEIEFF, A.
1912b. Sur la revision du genre Bodo Ehrbg. Arch. Prot., 26, 413–419, 1 fig. in text.

ARAGÃO, H. B.

BELAÉ, K. WEIN.
1914. Bau und Vermehrung von Prowazekia josephi n. sp. Arch. Prot., 35, 103–116, pl. 9, 8 figs. in text.

BERENBERG-GOSSLER.

BERLINER, E.

BOTT, M.
1907. Fortpflanzung von Pelomyxa. Ibid., 8, 120–156, pls. 3, 4, 1 fig. in text.

BREINL, A. AND HINDE, E.

BÜTSCHLI, O.

CALKINS, G. N.
1909. Protozoology (New York, Lea & Febiger), ix + 349, 4 pls., 125 figs. in text.

CARINI, A.

CHAGAS, C.

DOBELL, C. C.

DOLFLIN, F.
1911. Lehrbuch der Protozoenkunde, 3rd ed. (Jena, Gustav Fischer), xii + 1043, 951 figs. in text.

FLU, P. C.

FRANCHINI, G.

Goldschmidt, R.
1904. Die Chromidien der Protozoen. Ibid., 5, 126–143, 1 fig. in text.

Gonser, R.
1910. Die Entwicklung von Theileria parva, dem Erreger des Küstenfiebers der Rinder in Afrika. Ibid., 21, 143–163, pls. 9–13, 1 fig. in text.

Häcker, V.

Hartmann, M.
1911. Die Konstitution der Protistenkerne (Jena, Gustav Fischer), 1–54, 13 figs in text.

Hartmann, M. and Chagas, C.

Hartmann, M. and Jollos, V.

Hartmann, M. and Prowazek, S.

Heidenhain, M.

Hertwig, R.

Janicki, C.

Kellicott, W. E.
1913. A textbook of general embryology (New York, Henry Holt & Co.), iv + 376, 168 figs. in text.

Keysselitz, G.

Kofoid, C. A.
KOFIOD, C. A. AND CHRISTIANSEN, E. B.
KOFIOD, C. A. AND SWEZY, O.

KUDICKE, R.
1911b. Beiträge zur Biologie der Trypanosomen. Ibid., 61, 113–128.

LAUTERBORN, R.

LAVERAN, A. AND MESNIL, P.

LAVERAN, A. AND ROUSDKEY, D.

LEWIS, M. R. AND LEWIS, W. H.

McCulloch, I.

MACHADO, A.

MARTIN, C. H.
1913. Further observations on the intestinal trypanoplasma of fishes. Ibid., 59, 175–193, pls. 9, 10, 2 figs. in text.

MAYEE, M.
1911. Pathogenen Trypanosomen, in Prowazek, Handbuch der Pathogenen Protozoen, 249–323, pl. 6, 18 figs. in text.

MINCHIN, E. A.
1912. An introduction to the study of the Protozoa (London, Edward Arnold), xi + 517, 194 figs. in text.

MINCHIN, E. A. AND THOMSON, J. D.
MINCHIN, E. A. AND WOODCOCK, H. M.


NERESHEIMER, E.

1912. Die Gattung Trypanoplasma, Prowazek, in Handbuch der Pathogenen Protozoen, 101-117, 22 figs. in text.

NEUMANN, R. O.


NOVY, F. G. AND McNEAL, W. J.


NUTTALL, G. AND GRAHAM-SMITH, G. S.


1908a. Multiplication of Piroplasma bovis, P. pitheci, in the circulating blood compared with that of *P. canis*. Parasit., 1, 134-143, pl. 11, 4 figs. in text.

1908b. Development of Piroplasma canis in cultures. *Ibid.*, 1, 243-260, pl. 19, 1 fig. in text.

PATTEN, W. S.

1909. Herpetomonas lygaei. Arch Prot., 13, 1-16, pl. 1, 2 figs. in text.

PORTER, A.


PROWAZEK, S.


REICHENOW, E.


ROBERTSON, M.


ROSENBUSCH, F.


SALVIN-MOORE, J. E. AND BREINL, A.


SCHAUDINN, F.

1896b. Ueber die Copulation von Actinophrys sol Ehrbg. Ibid., 1, 83-89, 1 fig. in text.
SERGENT, E. AND E.
SWARCZEWSKY, B.
SWEZY, O.
WEBBER, H. J.
WENYON, C. M.
WIRBITZKI, P. W.
WITMORE, E. R.
WILSON, E. B.
1911. The Cell (New York, Columbia Univ. Press), xx + 483, 193 figs. in text.
WILSON, C. W.
WOODCOCK, H. M.
1909. On the occurrence of nuclear dimorphism in Halteridiwm parasite in the chaffinch. Ibid., 53, 339-349, 14 figs. in text.
1912. Notes on Sporozoa. Ibid., 58, 171-238, pls. 9, 10.
1914. Studies on avian Haemoproteinza. III. Ibid., 60, 399-432, pls. 29-31, 1 fig. in text.
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ON THE LIFE-HISTORY OF A SOIL AMOEBA

BY

CHARLIE WOODRUFF WILSON

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A. INTRODUCTION

I. HISTORICAL

Since amoebas may be obtained easily, cultured without difficulty in the case of many species, and experimented upon readily owing to their size and numbers, they have been the objects of extensive observation and investigation. Most of the early work was necessarily observational on the living vegetative stages. According to Dujardin (1841), an amoeba was first seen by Rösel (1746–1761), then cited by Linnaeus (1758) and Pallas (1766) under the name of Volvox chaos, Chaos proteus, and Volvox proteus. Müller (1786) later saw what he named Proteus difflicus. Many others are recorded from this time on. Gleichen (1778) found a small form in some infusions. Bory (1826) created the genus Amiba, the spelling of which was changed to Amoeba later by Ehrenberg (1839), in which he put three true amoebas and some other forms entirely different. In his enthusiasm Losana de Turin (1827) described no less than sixty-nine species which were for the most part modifications of the form of A. difflicus. Dujardin (1841) described the first limax. He found it in some water from the Seine and named it Amoeba limax. Its size he gave as 0.10 mm. by 0.03 mm. He characterizes it as “Diaphone arrondié aux deux bouts, très peu lobée; contenant des granules très distincts et une vacuole très prononcée.” There were numerous other species described after this date, but they were characterized principally by pseudopodia and size. The most complete and critical attempt to systematize our knowledge of these organisms is that of Penard (1902). The first cyst, probably, was described by Auerbach (1856) in A. bilimbosa. Later Wallich (1863) described one and says that Schneider had done so before he did.

The binary fission of amoebas was described in the early work as amitotic. Schulze (1874) from his observation on A. polypodia came to this conclusion and his figures of this are found in many textbooks of zoology. With the advent of the staining method it was found that division took place mitotically. Then arose discussions as to whether or not amitosis was degenerate and mitosis the normal process. Gruber (1894) held that mitosis was normal. Promitosis was first described in limax amoebas by Vahlkampf (1904), then by Nägler (1909) who gave it the name. Both of these
found that the equatorial plate came from the karyosome, while Chatton (1910) and Wasielewsky and Herschfeld (1910) described it as coming from the peripheral chromatin. Alexeieff (1911) says it comes from both.

A flagellate stage in the life-cycle of an amoeba has been found by a number of workers. Among these Schaudinn (1896), in Paramoeba cilhardii, described this stage and found division occurring in it, as does Craig (1910, 1911) in his Paramoeba hominis. Schardinger (1899) in his paper on "Entwicklungskreis einer Amoeba lobosa" describes various experiments on the flagellated stage which he calls Amoeba gruberi. His material came from diarrhea stools and the medium used was hay- or straw-agar. He found that amoebas in a hanging drop at 25°-30° C began turning into flagellates after seventy minutes, at which time the pseudopodia were shorter. In one hour and fifteen or twenty minutes the flagella were formed on about ninety per cent of the individuals. He found that he could turn the flagellates thus obtained back into amoebas when he chose. There were four methods which he found would accomplish this: (1) increase of temperature to about 34° C, (2) intense lighting, (3) hindrance of free movement, and (4) the addition of fresh food.

Wasielewsky and Herschfeld (1910) described a biflagellate stage in an amoeba from hay infusion and one from an infusion from tanbark. Whitmore (1911) in Trimastigamoeba philippinensis found individuals with three and sometimes two or four flagella, some of which he showed with basal granules and rhizoplast. Alexeieff (1912a) described the flagellate stage of Amoeba punctata Dangeard. Wherry (1913) working on the same species as that of this paper found this stage. He produced it by diluting the medium and exposing it to atmospheric oxygen.

There are numerous other accounts of Amoeba, but in no case has the life-history of any one species been described. This discrepancy was pointed out by Professor C. A. Kofoid, to whom I am indebted for direction and much kindly assistance.

The purpose, then, of this work is to contribute as much as possible to our knowledge of the life-history of a single species. In pursuance of this, daily observations have been made for most of the time for the past two years.

II. MATERIAL AND METHOD

The material was obtained from undisturbed alluvial soil along Strawberry Creek in the glade by the Faculty Club, on the campus of
the University of California. Small amounts of soil, about ten cubic centimeters, were placed in previously sterilized, covered glass dishes of creek water or culture medium, the latter giving a greater abundance in a shorter time. In a week amoebas were found in the scum which formed on the surface. From these cultures were started, each being made from a single individual amoeba with bacteria of the culture.

At first a mechanical pipette was used for isolation, but the amoeba stuck to the sides so that an ordinary pipette which has been drawn out very small was used in all subsequent work. With this the amoeba can be ejected before it has had time to adhere to the glass. The flagellate stage is much more easily handled and so it has been used a great deal for inoculation of cultures. Subcultures were also made from cultures which were pure by the transfer to the new medium of a cover-glass which had been floated on the given culture. In this way numerous amoebas may be obtained more quickly than by the use of a single individual. It has been possible by this method to keep "pure mixed" cultures of amoebas and of the bacteria which form their food in great numbers continuously in the laboratory for the past two years.

The medium used was boiled, filtered creek water to which one per cent of a mixture in water of ground cabbage and cracker in equal proportions was added. The mixture was then sterilized in the dishes used for cultures for from forty-five minutes to one and a half hours, usually the latter, at eighteen pounds pressure in a steam autoclave. Enough water is always used in the cabbage-cracker mixture to make it thin enough to pour; and it is kept indefinitely in a flask plugged with cotton, being sterilized each time after being opened. Agar plates have also been employed, but the films for study and fixation are much more easily made from the liquid medium and the amoebas are more abundant and better distributed here, so that the culture fluid has been used for the most part rather than the agar.

The method has been the moist-film one. A cover is floated on the bacterial film on the surface of the culture, left for from a few seconds to a minute or two, removed, drained of excess water by standing on edge on a blotter or by merely shaking, then dropped film-side down on the fixing fluid. It is not necessary to use any fixation to make the film adhere to the glass. After it has been on the surface of the fixative for about a half minute or longer, the cover is turned over and allowed to sink.

The following fixing fluids were used: Bouin's, Carnoy's, picromercuric, carbo-formalin-aceto-methyl-alcohol, and alcoholic sublimate
with five per cent acetic acid. The last is entirely satisfactory. The sublimate is always removed by washing in iodine alcohol.

The stains used were blood stains such as Leishman’s, Wright’s, and Giemsa’s; Rosenbusch’s haematoxylin; saffranin lichtgrün; Mayer’s paracarmine; Delafield’s haematoxylin; Haidenhain’s iron haematoxylin; Dobell’s (1914) alcoholic iron haematin; and Alexeieff’s (1912 a) triple stain. Of these the last three have proved most satisfactory. Dobell’s stain is best for the cysts and for the division stages. In addition to its efficiency it has the decided advantage of taking but a short time for the whole process. Iron haematoxylin is good for the flagellate stage, because the differentiation may be carried further with this than with Dobell’s method without removing the stain from the basal granule, though excellent preparations have been obtained with the latter. Alexeieff’s triple stain is good for staining the rhizoplast. In this method haematin was also used instead of haematoxylin and the preparations came out clearly.

The counter-stains employed were erythrosin, eosin, and orange G. The intra-vitam stains tried were new methylene blue X, Janus green, neutral red, Bismark brown, niblau, and niblau chlorohydrate, but they were not successful in differentiating anything but cytoplasmic granules.

B. OBSERVATIONS

I. THE TROPHOZOITE

1. MOVEMENT

In the amoebas under observation here the body has one constant character, namely that it progresses by means of one blunt, broadly rounded or lobose, anterior pseudopodium. While the general direction of movement is in a straight line, there is not a constant flowing direction forward, but rather outward from very near the anterior end and alternating from one side to the other. When not in locomotion the amoeba often alternately draws in and thrusts out a pseudopodium on either side, advancing the one while withdrawing the other (pl. 18, fig. 6). This may take place at any point and may be repeated indefinitely. The floating forms have from three to six blunt pseudopodia which may be long or short.
2. Morphology

In this stage of the amoeba the general form is elongated with the length 1.5 to 2.5, rarely equalling 5 times the diameter. The large diameter varies from 6 to 46μ (pl. 19, figs. 19, 27); the transverse diameter in the different individuals varies from 4 to about 11.5μ. In a young culture, one in which the amoebas have not divided very many times, the average size is larger (pl. 18), but after numerous divisions have taken place there is a decrease in size of the individual and likewise of the nucleus. Therefore a young culture is much more satisfactory for the study of mitosis.

In form the trophozoite is characteristically of the limax type. It has blunt, rounded ends, but its proportions vary in different individuals, in the same individual from time to time, and so on. It may have its outline uneven; or it may be long and slender, the length being more than twenty times as great as the smallest diameter. Or again it may be shorter and thicker, having the greatest diameter only about three times that of the smallest, which, in a given individual, varies very little.

When the animal is at rest, the ectoplasm is not differentiated from the endoplasm (pl. 18, fig. 10) but when it is moving, the pseudopodium is largely of ectoplasm which is clear and homogeneous, while the endoplasm is granular (pl. 19, fig. 19). With neutral red there are rather numerous large granules in the endoplasm which take the stain.

A contractile vacuole is present. It is formed by the fusion of three or four small vacuoles. Its position is posterior to the nucleus in the moving form. The time from one contraction to the next is usually from forty to sixty seconds. Food vacuoles lie in the endoplasm and are filled with bacteria in the process of digestion. The bacteria found in the cultures come partly from air contamination, but some come from the soil being inoculated into the culture with the amoeba. These serve as food. Some amoebas may be found very full of food vacuoles, but ordinarily there is only one or few (pl. 18, fig. 13). Quite often none are found.

The resting nucleus, which is spherical except in very much elongated individuals (pl. 19, fig. 19), will average two to four microns in diameter. There is a distinct, not a double-contoured, nuclear membrane, but at the periphery of the nucleus is "'cosinophile'" chromatin (Chatton and Lalung-Bonnaire, 1912). This chromatin may be barely observable (pl. 19, fig. 19) or very evident (pl. 20, fig. 40), being in the
first case only slightly granular and close to the membrane, giving it a thickened appearance, and in the second case very granular and in a looser layer. There is a clear zone between this and the single large centrally located, spherical, compact karyosome, which may have stainable lines extending outward from it to the peripheral chromatin in a delicate network (pl. 20, figs. 39, 40, 44). Sometimes its central region stains very much less than the peripheral portion, in which case a dark granule, the centriole, may sometimes be distinguished in or near its center (pl. 20, fig. 59).

3. Binary Fission

Division is promitotic, that is, with large chromatic polar masses coming from the karyosome, and spindle within the nuclear membrane between these masses. The amoeba does not round up, but may move about during most of the process.

(a) Prophase

The prophase is characterized by an increase in the size of the nucleus, the diameter sometimes reaching seven microns (pl. 20, fig. 39). The membrane is distinct, and the peripheral chromatin is more evident than it is in the resting condition. The central karyosome, within which a central granule or centriole may sometimes be distinguished, increases in size, and then elongates. The peripheral chromatin migrates in from the nuclear membrane towards the karyosome, which at this stage becomes "bent dumb-bell" in shape (pl. 20, fig. 47) and the peripheral chromatin comes to lie in the angle.

Spindle fibers are formed between the chromatic polar masses (pl. 18, figs. 2, 3), and an equatorial plate of chromosomes appears on them (pl. 18, fig. 9), at which time the connection between the polar masses or central spindle is within the spindle, that is, the chromosomes are largely peripheral. Polar caps, which take the plastin stain, may be detected in the late stages of the prophase on the outer ends of the chromatic masses (pl. 18, figs. 6, 7, 9, 10). They are conical in shape with the broad part of the cone resting on the chromatic polar mass.

(b) Metaphase

Eight subequal chromosomes organized out of the chromatin granules in the angle between the parting polar chromatic masses appear in the equatorial plate. They are ellipsoidal, about twice as long as wide, and show no marked differentiation among themselves. When
formed, the plate is very regular in the arrangement of the equidistant chromosomes, which are in some cases all in one plane.

There is no evidence of a longitudinal splitting of the chromosomes in the metaphase, but each is constricted at its middle into two equal parts. The polar caps may (pl. 20, fig. 48) or may not (pl. 18, fig. 11) be visible at the poles of the spindle beyond the polar masses.

(c) Anaphase

Eight daughter chromosomes are then drawn to each pole (pl. 18, figs. 11, 13). The polar caps are evident in the first stages (pl. 18, figs. 6, 10), but are covered by the chromatin in the later ones (pl. 18, figs. 8, 13, 14).

The shape of the nuclear membrane conforms to that of the karyosome until the spindle fibers appear, and then to that of the general shape of the whole mitotic figure. It becomes constricted in the equatorial region in the early anaphase (pl. 18, fig. 10) and the two parts become more and more separated (pl. 18, figs. 13, 14). The membrane finally closes around the daughter nuclei in the later anaphase (pl. 18, fig. 8).

(d) Telophase

The polar masses become elongated at right angles to the long axis of the spindle, the chromosomes are fused, and the masses become more closely connected with the polar clumps; then the peripheral chromatin is extruded in each nucleus from the region between the two masses, and migrates towards the nuclear membrane. There is a connection between the two daughter nuclei which stains like plastin (pl. 18, fig. 15) and which persists until immediately before cytoplasmic division is complete (pl. 18, fig. 12).

(e) Nuclear Reorganization

In the reorganization of the nucleus, the nuclear "membrane" becomes spherical, the peripheral chromatin and plastin come out from the region between the polar and chromosome masses (pl. 20, figs. 50, 53, 57) and take their position at the nuclear "membrane." The polar and chromosome masses fuse, forming a karyosome with a lighter-staining mass in the center (pl. 20, figs. 43, 61) which is the remains of the plastin of the spindle. Most of this process takes place after the daughter amoebas are separated.
(f) Cytoplasmic Division

The amoeba may become elongated in the direction of the long axis of the spindle in the early anaphase (pl. 18, figs. 10, 13) and always does so in the late anaphase (pl. 18, figs. 8, 14). It begins to constrict before the connection between the two nuclei is lost (pl. 18, fig. 15). When it has become very slender, the daughter amoebas put out pseudopodia (pl. 18, figs. 12, 15) and pull apart. The separation is evidently accomplished with no little force from the contraction of the released ends which may be observed in the living material. After separation the cytoplasm immediately rounds off so that there is no evidence of the point at which the separation finally took place (pl. 19, fig. 25). However, the remains of the nuclear connection, comparable to the achromatic strands or interzonal fibers leading to a cell plate, persist for some time (pl. 19, fig. 25; pl. 20, fig. 43).

(g) The Behavior of Organelles in Mitosis

The organelles do not behave in exactly the same manner in every dividing individual, and while the brief account of mitosis as given above describes the process in general, it will be well to add a discussion of the more minute structure and variable behavior of the organelles during mitosis.

The karyosome, averaging 1.5 μ in diameter, often increases to 2.5 and even 4 μ prior to division (pl. 20, figs. 38, 39). It is very dark at the periphery and light in the plastin center, which may be the equivalent of the centrodesmose in the restricted sense of Heidenhain (1907), appearing almost vacuolated in both the living and stained material. There may be one or several of these "vacuoles" (pl. 18, figs. 1, 4; pl. 20, figs. 39, 44). This vacuolated condition may persist to late prophase (pl. 18, fig. 3), but not in all individuals (pl. 20, figs. 47, 60).

The karyosome is not granular until in the late prophase (pl. 18, figs. 1-5, 9; pl. 20, figs. 37-39, 41, 55, 56), and even then it is not invariably so (pl. 20, figs. 47, 60). Also in all the later stages of division it may be more or less granular, though often homogeneous in appearance. When a granular condition is found, the slender connection between the two migrating chromatic polar masses may also be somewhat granular (pl. 20, fig. 55). This is not true when the connection has become very slender (pl. 18, fig. 9).

The karyosome elongates, bends in on one side (pl. 18, fig. 1; pl. 20, figs. 39, 45), and the ends round up so that it is shaped like a bent dumb-bell. The connection between the two ends gradually diminishes
in size and continues to diminish until only the slender central spindle is left (pl. 20, fig. 52). In the dumb-bell stage this connection between the polar masses is quite evident on the outside of the spindle, but in the later stages it is within the spindle in an axial position. The stage at which this change of position takes place seems to be that in which there is a separation of the two chromatic polar masses (pl. 18, fig. 9; pl. 20, fig. 52) with only the central spindle connecting them.

Part of the plastin of the karyosome may project out beyond the chromatic portion to the nuclear membrane as a polar cap in the metaphase. This projection, conical in shape, with the base resting on the chromatic polar mass and the point to the membrane, is variable in its appearance and relative size. It is sometimes very evident (pl. 18, figs. 6, 7, 10), sometimes only barely visible (pl. 18, fig. 9; pl. 20, fig. 48) and sometimes completely obscured, being entirely covered by the chromatin of the polar mass (pl. 18, fig. 11). While this last condition may be found occasionally in the early stages of division beginning with late prophase, in which case the polar mass is conical in shape, it is always found in the late anaphase and the telophase (pl. 18, figs. 8, 12–15; pl. 20, figs. 37, 51). Here the polar mass is flattened at the end instead of coming to a point as it does in the earlier stages. Likewise, there are no polar caps in the reorganization stages of the nucleus (pl. 19, figs. 17, 25).

The chromosomes, the formation of which will be discussed later, behave in various ways after the metaphase. They may be pulled to the pole as separate entities (pl. 18, fig. 13) gradually fusing in the later stages, they may become separated into granules as they are pulled apart (pl. 18, fig. 10), or they may early become more or less fused (pl. 18, fig. 6). However, they finally fuse into a mass which is more or less closely connected with the chromatic polar mass (pl. 18, fig. 15). This connection becomes closer until fusion is finally complete in the resting nucleus (pl. 19, fig. 19; pl. 20, fig. 43). The region between the chromosome mass and the polar mass, made up of the remains of the spindle fibers, becomes the plastin-like center of the new karyosome (pl. 20, figs. 43, 53).

In the karyosomes of preparations which have been nearly completely destained, a granule much darker than its surroundings may be distinguished (pl. 20, fig. 59). Also, in some elongated karyosomes, which are not very deeply stained, may be found two granules, the centrioles, with a slender connection, the central spindle (pl. 20, fig. 62). This last condition may also be distinguished in late prophase.
stages (pl. 20, fig. 52), in a few stages of the metaphase (pl. 20, fig. 48), and usually may be seen in the earlier stages of the anaphase (pl. 18, fig. 10; pl. 20, figs. 37, 42). In the later anaphase it is not usually easily distinguished, because in most individuals the chromatic polar masses are very dark. However both centrioles and a portion of the central spindle may be clearly made out in some instances (pl. 20, fig. 37).

The granules or centrioles are generally within the chromatic polar masses (pl. 20, figs. 37, 42, 48, 52), not at the tips of the caps nor in the metaphase even within the chromatic polar masses, but are, however, to be found on focusing down even in the region between the polar mass and the chromosomes, but nearer the former (pl. 20, fig. 42). The connection between them, the central spindle, stains a little deeper than a spindle fiber, is slender, and during the stages in which the karyosome is dumb-bell shaped is in the narrowed region of the dividing karyosome. When the two polar masses are separated, it remains as a connection between them (pl. 20, fig. 52), having, as previously stated, changed its position from the periphery to the center of the spindle.

Some of the workers on amoebas hold that there is no centriole, others that there is. Among these, Hogue (1914) figures "a structure closely resembling a centriole in a few amoebas." Gläser (1912 a) says he occasionally finds a granule which is a part of the karyosome and retains the stain longer than the remaining part, but that it is not a centriole. Hartmann (1913) figures a centriole in Amoeba hyalina, but in this species there are no polar caps. He (1914) also figures centrioles in A. lacertae, though Dobell (1914) says that they do not occur in that species. Likewise Alexeieff (1913) says that there is no centriole in the amoebas which he has studied.

The preparations from which the drawings of the centriole were made were stained by Dobell's alcoholic haematin method (1914) and so the structures figured are certainly not the "fortuitous appearances which may occasionally be met with in Heidenhain preparations," as he says in speaking of those described by Xägler (1909). They are definite structures which behave as fixedly as the karyosome does.

The peripheral chromatin or chromatin net is not very evident in resting nuclei, the only indication of its presence being that the nuclear membrane has a thick appearance (pl. 19, fig. 19). Chatton and Lahnung-Bonnaire (1912), in characterizing Amoeba limax Dujardin, say that there is "dans l'état périphérique compris entre la parai de
la vesicule et la caryosome, un léger coagulum eosinophile de chromatine périphérique." In the early prophase, however, there is a very granular layer next to the membrane (pl. 18, fig. 4; pl. 20, figs. 40, 44). The granules may be large and deeply stained, with stainable lines connecting some of them with the karyosome, or they may be finely granular, with or without visible lines (pl. 20, figs. 40, 44). Only rarely are the granules to be found which take the chromatin stain or retain it after destaining. However, this may be due to the fact that in general the chromatin is so diffuse that all the stain comes out by the time the cytoplasm and karyosome are sufficiently destained.

 Coincident with the elongation of the karyosome in division, the peripheral net becomes loose in structure, collects at one side between the membrane and karyosome (pl. 20, figs. 39, 44), and finally takes up its position on one side of the karyosome, becoming more fibrillar in appearance as the elongation of the karyosome progresses (pl. 19, figs. 38, 45). The connection with the karyosome may take place after the latter has become dumb-bell shaped (pl. 18, figs. 2, 5), but its position is always on one side until the late prophase, at which time it is midway between the two chromatic polar masses, which are now connected only by the central spindle (pl. 20, fig. 52).

 In the process of reorganization of the nucleus, which may begin in the late anaphase (pl. 20, fig. 37), there is an extrusion of chromatin network from the region between the chromatic polar mass and the chromosome mass to a position around the nuclear membrane (pl. 18, figs. 12, 15; pl. 20, figs. 51, 53, 57).

 The spindle as it appears in the metaphase, with its large polar masses made up of plastin, chromatin, and centrioles, its eight distinct spindle fibers upon each of which is a chromosome, all of which are in a regular equatorial plate, and its central spindle, is characteristic in its formation. In the early stages there is an elongation and bending of the karyosome, with a gradual decrease in size of the part connecting the two separating karyosome ends or masses, and an entrance into the angle between the polar masses of the peripheral net. The centriole divides in the early elongation stage of the karyosome (pl 20, fig. 62) and, as elongation continues, one centriole is to be found in each polar mass with the central spindle in the gradually decreasing region between the two chromatic polar masses. From the plastin of the chromidial net, which is evidently from both the peripheral chromatin and the karyosome (cf. pl. 20, figs. 38, 45, 60), the eight spindle fibers are formed.
After the separation of the chromosomes is completed, the parts of the spindle gradually resume their original place in the resting nucleus. In each daughter nucleus fused chromosome and chromatid masses become the chromatin of the new karyosome. Remains of the central spindle and of the spindle fibers, centrioles, and perhaps some of the plastin of the chromatid polar mass, become the centrodesmose, or central "vacuole," of the new karyosome. While the chromatin net is extruded from the region between the chromosome and chromatid polar masses to become the peripheral chromatin (pl. 18, figs. 12, 15; pl. 19, figs. 17, 25; pl. 20, figs. 43, 46, 50, 51, 53, 57, 61).

Upon each of the eight spindle fibers one chromosome is formed, the source of the chromatin of which has been a disputed matter. Some describe it as coming entirely from the karyosome, some as coming entirely from the peripheral chromatin, while Alexieff (1911) says that it comes from both. The evidence of the material used in this work is in support of the last view, and it falls into four heads, as follows: (1) the character and behavior of the peripheral chromatin; (2) character of the spindle fibers and staining of the equatorial plate; (3) the occasional occurrence of large granules with variable staining capacity continuous with those of the karyosome; and (4) the behavior of the chromosome mass and extrusion of chromatin net in reorganizing nuclei.

Part of this evidence has already been discussed, and for that reason will be given only briefly here. In some early prophase figures there are to be found, between the nuclear membrane and karyosome, granules which take the chromatin stain (pl. 20, figs. 39, 40). This indicates that chromatin is present in the peripheral mass, though generally it is too diffuse to hold the stain. All the peripheral mass comes onto the karyosome, becoming more fibrillar as it does so. The fibers are somewhat granular in appearance, but do not take the stain of chromatin at any point. The unstaining thickenings of these take up their position in a regular equatorial plate, each fiber having at its middle a mass evidently made up of several granules (pl. 18, fig. 9) which later fuse into a deeply staining chromosome, all of the chromosomes of a spindle being regular and subequal (pl. 18, fig. 7). These chromosomes could have come from the diffusion of chromatin from the karyosome, except that this view leaves the peripheral granules out of account.

On the other hand, a large part of the chromosome material may come from the karyosome, since the total quantity in each is in-
adequately provided for in the peripheral material. Often there are found prophase stages in which the karyosome, both polar masses and their connecting portion, are granular (pl. 20, fig. 55). The chromatin masses of the karyosome are large and loose and some of the chromatin granules in the chromatic polar masses are in an unbroken line with those on the fibers (pl. 20, figs. 41, 55, 56). Those nearest the polar mass stain dark and there is a slight decrease in staining capacity toward the equator. This seems to indicate that part of a chromosome, and a large part at that, comes from the karyosome. Also the late division stages and nuclear reorganization indicate the same thing. During the anaphase the chromosomes become fused (pl. 18, figs. 8, 14) and a chromosome mass is clearly distinguishable in every stage after that until the nucleus is reorganized (pl. 20, figs. 43, 46, 50, 51) and the chromosome mass and chromatic polar masses are fused in the central karyosome.

Extrusion of chromatin from the region between the polar mass and the chromatin mass, begins in the telophase (pl. 18, fig. 15). Certainly all of the chromosome chromatin is not given out, from the later reorganization stages (pl. 20, figs. 43, 46, 58, 61). For that matter none of it may be given off, but some from the polar mass, or from both, or none at all, may be given off. The last assumption, however, would leave out of account the stainable peripheral granules unless these were later given off by the karyosome. From the slight decrease in the relative sizes of the chromatic masses, polar and chromosome, in reorganization, it seems probable that some must be given off from the chromosome mass (pl. 20, figs. 50, 51, 53, 57) to the peripheral chromatin.

(h) MITOSIS AND AMITOSIS

Stages have been found which correspond to some of those figured by Hogue (1914) as amitosis, but on careful analysis have proved to be stages in mitosis. As described by Hogue (1914, pl. 16, figs. 5–7) amitosis is most often unequal. "The two parts of the karyosome always bend up into a u-shape." In the "unequal division" the small part is the chromosome mass, the large part the chromatic polar mass, the narrowed portion the connection between the two, and the appearance of a pinched-in nuclear membrane is given by the extruding peripheral chromatin (pl. 20, fig. 57) as found in a nucleus reorganizing after division. The case in which Miss Hogue's amitotic division is "equal" corresponds to the prophase in which the karyosome has become dumb-bell shaped before the
peripheral chromatin came in (pl. 20, fig. 54). The case she mentions in which the unequal nuclei are close together with pointed karyosomes is analogous to the nuclei in plasmotomy (pl. 20, fig. 58), occurring after multiple mitosis, which are exactly comparable to some produced in binary fission in which there may also be found the remains of the plastin connection of the daughter nuclei (pl. 20, fig. 43). This still agrees with her material, for she says that where there was much amitotic division there were many multinucleated forms. Of course it is possible that the laboratory conditions on agar plates were in some instances pathological for the form she worked with, and so some of the stages she figures are amitotic, but the evidence indicates that amitosis does not occur.

The comparison made above between the prophase stage and Miss Hogue's "equal amitosis" cannot hold if she has correctly interpreted mitosis which she has characterized as promitotic. However her series is incomplete, and there appear to be discrepancies in her interpretation of the stages she has figured (see her plate 16, figures 10-13), but using her figures and changing the interpretation somewhat the analogy drawn seems correct.

4. Multiple Fission

Multiple fission has not been observed in full, but there is evidence that it occurs. The only cases of division observed in individuals in which there had been multiple mitosis was plasmotomic. Multiple mitosis is very common in our multinucleate forms, which have been found with two (pl. 19, figs. 22, 26), three (pl. 19, figs. 16, 20, 23), and rarely four nuclei (pl. 19, fig. 18), but not more than three spindles have been found. Wherry (1913), however, in the same species figures individuals with two and four nuclei all in coincident mitosis, and individuals with ten and more nuclei. Usually all nuclei are in the same stage of division, but in a binucleate form one nucleus may divide and the other not (pl. 19, fig. 16). Cytoplasmic division does not follow, in some cases at least, because in the resting condition individuals are often to be found with one large and two small nuclei.

The spindles are ordinarily more or less parallel (pl. 19, figs. 20, 26), but they may be at right angles in the binucleate forms. In the case of the parallel or nearly parallel spindles the division would probably be plasmotomic (pl. 19, fig. 26), which has been found in the case of forms with two spindles. When at right angles, the division might be by coincident multiple fission, but this has not been observed.
However, the position of the spindles and the outline of the cytoplasm indicate that that would be the case. That some kind of cytoplasmic division must sometimes take place is evident from the fact that the amoebas found in my cultures rarely had more than three nuclei, at most only four, while two and three spindles are of most common occurrence. The multinucleate condition evidently comes from cases in which the cytoplasm failed to divide after mitosis.

Figure 21, plate 19, shows an amoeba with two reorganizing nuclei. From the position of the upper nucleus it does not seem probable that the two are daughter nuclei, but that they came from nuclei which formerly had their spindles nearly at right angles to each other but the upper one of which had been shifted by movement. In this case there may have been a recent plasmatomonic division such as might part the upper from the lower half of the four-nucleate plasmodium shown in plate 19, figure 26.

5. Budding

Two types of budding, exogenous and endogenous, occur as a method of asexual multiplication in the life-history of this species of amoeba. In the first the bud is constricted off at the periphery of the cytoplasm and has chromidia but no definite nucleus at the time it is separated from the parent individual. In the second, the bud is constricted off within a vacuole in the cytoplasm and contains a typical nucleus.

In the process of multiplication by exogenous budding, the peripheral lobes of cytoplasm are constricted off, each containing chromidia given off by the nucleus. These chromidia then reorganize to form nuclei of the new small individuals in which later coincident growth of nucleus and cytoplasm ensues.

Exogenous budding has been found in a few cultures, whose conditions are not noticeably different from those of other cultures. In the individuals in which this form of multiplication occurs there are noted small karyosomes (1.5μ), heavy peripheral chromatin and chromatin granules or chromidia in the cytoplasm. The karyosome is either less deeply stained than that of the usual resting nucleus, or it has its chromatin all at the periphery (pl. 19, figs. 33, 34). In the living forms a tendency to round off portions of the cytoplasm at the periphery may be observed. In the buds there is not a distinct nucleus, but several small chromatin granules or chromidia. Later, judging from the decrease of the chromatin of the karyosome and its appearance at the periphery, the chromidia are formed at the expense of
nuclear chromatin (pl. 19, figs. 33, 34). Sometimes the buds have few granules (pl. 19, figs. 32, 36), but they may have many. Later these go to form the characteristic nucleus, which is not organized until after separation takes place (pl. 19, figs. 27, 28, 31). The cutting off of these buds has not been observed, though they have been watched for more than an hour at a time. But the evidence indicates that this process does occur in the multiplication of this species of amoeba. Since no bud in situ has ever been found with a small nucleus, but small amoebas are found without nuclei and with chromidia, slightly larger amoebas with small nuclei and few chromidia, and small amoebas with large nuclei and no chromidia, it is evident that the nuclei are formed from these chromidia after separation takes place and that as nuclear formation takes place there is an increase in size of both nucleus and cytoplasm and a progressive disappearance of the chromatin.

The other form of budding, which is endogenous, is occasionally found in this species. It has been noted by me only in binucleate forms and occurs by the internal constriction of the cytoplasmic mass around one of the two nuclei. The fluid-filled space gradually enlarges until it severs the internal bud from the parent mass.

It is evident that endogenous budding does not often occur, at least in cultures, since it has been observed in but three preparations, and even in these in only a very few individuals. There was in no case more than one bud, though Wherry (1913) figures for this species one or two, and Miss Hogue (1914) in Vahlkampfia calcensi from one to three. Liston and Martin (1911) also figure endogenous buds in "large amoebas from liver-abscesses," in which in the early stage of bud-formation there are chromidial strands. These later condense to form the nucleus of the bud. This same process is suggested by my figures 29 and 35, plate 19. However, a complete series of the stages in this process has not been found in my material, but so far as observed it does not seem to differ from that which the above investigators have described. A portion of the cytoplasm (pl. 19, figs. 29, 30, 35) containing a nucleus is separated from the rest of the cytoplasm within the amoeba and finally lies entirely free within a vacuole. After this process it is probable that it is ejected from the amoeba, though there has been no absolute evidence that this occurs, except that small amoebas are found in the preparation. There is no direct evidence of the origin of the endogenous buds by chromidial formation nor of their separation as a phase of mitosis.
6. CHROMATIN EXTRUSION

Chromidia have been found in trophozoites, in flagellates, and in individuals during the period prior to, and during the early phases of encystment. In all cases chromidia formation is at the expense of the karyosome, and there is always heavy peripheral chromatin on the nuclear membrane during chromidial formation. The formation in the trophozoite, which has already been described, has to do with the formation of exogenous buds. That found in the flagellate stage would probably have been cut off in exogenous buds had the individuals not been transformed into flagellates. Chromidia found coincidently with cyst formation, the details of which are given below, differ from the above in early process of production, in size and in fate.

(a) CHROMIDIAL FORMATION IN CYSTS

The process of the formation of chromidia in cysts seems to be a normal one, because it has been found in every encysting culture regardless of whether or not the culture came from an individual recently isolated from the soil, or from one from a culture which had been kept under laboratory conditions for more than a year. It does not appear to be a pathological phenomenon. It is begun in an amoeba while it is still moving about or after it has rounded up to encyst. It is initiated in the karyosome and ends in the formation of large spheroidal, sometimes irregular, chromidia which later disappear. Throughout the process stainable lines may or may not be found extending from the nuclear membrane into the karyosome.

The first indication of the process is an enlargement of the karyosome as well as an increase in the entire nuclear volume (pl. 21, fig. 67). The karyosome has darkly stained granules at the periphery, then a lightly stained region, in the center of which is a darkly stained spherical mass, a condition which differs from that of the enlarged karyosome of mitosis as well as from that prior to chromidial formation as seen in budding individuals. Fusion takes place among the granules at the surface of the karyosome so that they become larger and more deeply stained (pl. 21, fig. 68). After this stage is reached the central sphere, the expurged karyosome, begins to move out of its globe of peripheral chromatin (pl. 21, fig. 70). It breaks through one side (pl. 21, fig. 71) and the peripheral globe takes on the appearance of a cup (pl. 21, fig. 76). The process of separation continues (pl. 21, figs. 72, 73) and is evidently completed with force, for the large dark karyosome is found pressed against the nuclear membrane, while the
extruded chromatin lies at the opposite side (pl. 21, figs. 69, 74). The karyosome resumes its central position and the peripheral chromatin flattens out on the nuclear membrane.

The chromatin from the periphery of the nucleus is gradually given off into the cytoplasm, for soon after its flattening out chromidia begin to appear (pl. 21, fig. 75) and these increase in size and number as the amount and staining capacity of the peripheral chromatin decreases (pl. 21, fig. 78). The latter finally all disappears and mononucleate cysts are found with numerous darkly stained spheroidal chromidia of variable size and no peripheral chromatin in the nucleus (pl. 21, fig. 81).

The chromatin is evidently diffused out into the cytoplasm, for the stain of the cyst is much deeper in the region of the nucleus than it is at the periphery, a condition which is not found in old cysts. This diffused chromatin collects in clumps at first near the nucleus (pl. 21, figs. 75, 78) and later extends to the region further out in the cytoplasm (pl. 21, fig. 81), forming chromidia in slightly irregular spheres of different sizes. Though at first small, these spheres are later nearly equal in size to the karyosome.

In the older cysts the chromidia are large and few in number, sometimes as few as six, and they finally all disappear in about three days after encystment. None have ever been found ejected into the region between the amoeba and the cyst wall, as Dobell (1914) has figured for some in Amoeba lacertae, though the fact that in the older cysts they are found nearer the periphery of the cytoplasm than in the younger ones, might be considered to indicate that fate (pl. 21, figs. 78, 81). The evidence, however, seems to me to indicate that the chromidia are digested or absorbed.

A complete series has not been found in binucleate forms, but from the stages found the process of chromatin extrusion in these is like that in the mononucleate cyst (pl. 21, figs. 77, 80).

Another point which should be mentioned here is that while amoebas may ordinarily be induced to come out of a cyst, never has an amoeba been induced to do so in which there were chromidia which can be seen in the living cysts, but these same amoebas will come out after the chromidia have disappeared.

(b) CHROMIDIA IN OTHER AMOEBA

Process of chromidial formation somewhat similar to that given above have been described by investigators in other species of Amoeba.
Hogue (1914) finds, in Vahlkampfia calcensi, that chromidia are produced in the trophozoite at the expense of the karyosome and are later reorganized to form nuclei in amoebas produced by exogenous budding. Dangeard (1910) finds chromidia in cysts of Amoeba limax and A. punctata which appear coincidently with the process of encystment and disappear little by little as that process advances. In his discussion of extranuclear chromatin he states for amoebas that there is a complete independence between the chromidia and the nucleus, by which he means that nuclei cannot arise from these, but the chromidia represent a simple transportation of substance of the nucleus to the cytoplasm or the reverse, and these are elements which are often transitory.

In the process described by Dobell (1914) for A. lacertae, the karyosome increases in size and radiating lines extend from it to the nuclear membrane. There is a decrease in the size of the karyosome and the appearance in the cytoplasm of chromidia irregular in size and shape. Most of these later disappear and the remaining ones are cast out into the region between the cyst wall and the amoeba.

(c) Significance of the Chromidia

Briefly the facts when completely worked out in regard to chromidia in cysts are these: (1) there is an enlarged karyosome, (2) an extrusion of chromatin, (3) the appearance of chromidia in the cytoplasm, (4) a decrease in number and final disappearance of the chromidia, (5) failure to induce an amoeba in this condition to come out of the cyst. All of these facts suggest strongly one explanation, namely, that it is a process for the purpose of re-establishing the normal nucleo- cytoplasmic ratio as conceived by Hertwig (1903, 1908), the gist of which it will be well to recall here.

Hertwig considers that there is a certain quantitative relation of nucleus and cytoplasm which is necessary in any cell for its vital functions to continue normally. This ratio is subject to variations at different periods of the life-history, but is the same for corresponding phases in the life of the cell. The depression periods are characterized by increase of the nuclear substance in proportion to the amount of cytoplasm and unless part of the nuclear substance is eliminated and absorbed, death of the individual will follow.

The large karyosome and nucleus indicate lack of balance or disturbance of this normal ratio as a result possibly of prolonged vegetative activity. The chromatin extrusion, chromidial formation and
final absorption, suggest the restoration of this balance; and the
failure of inducements for the amoeba to come out suggest that it is a
depression period, and one of internal readjustment, since, when the
chromidia disappear, the amoeba comes out and goes through another
vegetative cycle.

It is the mere elimination of nuclear substance and is not sufficient,
as Hartmann (1913) says, to be interpreted as chromatin reduction in
gametogenesis. If it were, there would be fertilization after the
amoebas came out of the cysts, not a single case of which has been
found, though preparations have been fixed at short intervals during
ecystment extending over a period of more than twelve hours.

The differences in structure and fate of the chromidia of tropho-
zoites and those of cysts, though chemically alike in so far at least as
their reaction to stains is concerned, suggest that they are different
in kind, the former being generative chromatin and the latter vegetative
chromatin. However, the proof is not conclusive. All chromidia of
the trophozoite may not produce new nuclei. Those that do may be cut
off by mere chance and then may or may not reorganize into nuclei of
new individuals. The chromidia of cysts may not always be absorbed.
However, they sometimes behave as described above and as far as the
evidence goes, they are of the two kinds.

II. THE CYST

1. Characters

The cysts are spheroidal, 7–14 μ in diameter, and have transparent
hyaline walls with definite "marks" or openings which are variable
in number (pl. 23, fig. 114). The amoeba within a mature cyst is free
from food and contractile vacuoles. It is so translucent that it may
be studied without difficulty in both living and stained material. The
nucleus with its large karyosome is clearly discerned.

The mature cyst is double-walled. Two walls may not always be
distinguished in that stage, but they are clearly defined in its develop-
ment, in which there is first formed a very thin outer wall in which
no openings have been observed (pl. 21, fig. 79). Within this is later
formed a thick wall in which from three to eight openings have been
found, each in a local thickening.

In a newly formed cyst the cytoplasm may fill the entire pace
within the cyst wall, but later withdraw more or less from the wall
(pl. 21, fig. 81), apparently by condensation. It is dense and slightly
granular in both living and stained material (pl. 20, figs. 63, 66). As stated above, there are neither food nor contractile vacuoles present, though the latter are found during early encystment and during excystment. There are many chromidia in a newly formed cyst, but only an occasional one during later stages.

The nucleus of the cyst is from one-fourth to one-third the diameter of the entire cyst. It has two characteristic conditions. One of these is that found in a cyst immediately after encystment and prior to excystment, in which the karyosome is spherical and compact (pl. 21, figs. 79, 83). The other is found in old cysts and in it the chromatin is broken up, but lies within the nuclear membrane (pl. 20, figs. 64–66; pl. 21, figs. 84–86). No cyst has ever been found without a nucleus and in which the chromatin is all in the form of chromidia as Brodsky (1910) describes for Amoeba hyalina Dangeard, and Arndt (1914) for A. chondrophora.

In the compact condition the karyosome has a diameter from a little more than 0.5 to 0.8 that of the nucleus. It does not take the stain deeply in the recently encysted individuals.

There are various modifications of the broken-up condition to be found in old cysts. There is usually a single rounded mass, though there may be two or sometimes more, which are probably karyosome remnants. It has around it, rather closely connected with it, other chromatin. This condition looks not unlike a short spireme which has a nucleolus associated with it (pl. 21, fig. 86). The outer chromatin is never in a sphere and is not on the nuclear membrane as is the case with the peripheral chromatin of the trophozoite (pl. 20, figs. 64–66; pl. 21, figs. 84–86).

The condition seems to be normal and is rather general in cultures five days after encystment. In our culture from which preparations were fixed at intervals during the day every day for seventy days it was found in most of the cysts, not in all, for excystment occurred continually during that period. The same thing was done in another culture for twenty days and in others for shorter lengths of time with the same results.

Then to determine the effect of excystment and to find out whether the condition was one which had anything to do with fertilization or not, the amoebas were stimulated to come out of the cysts. A large culture was chosen in which the amoebas had been encysted for forty days and in which the chromatin was generally broken up. Numerous cover-slips taken from this, with fresh medium added, were put in a
moist chamber film side up. After seventeen hours they were fixed at intervals of fifteen minutes for more than five hours. The amoebas had all come out very near the beginning of the experiment. Another series was started in the same way, but fixed at the same intervals beginning at the time the covers were put into the moist chamber and extending over a period of five and a half hours. Here the amoebas began coming out in two and a half hours. The karyosomes of the emerging and emerged amoebas were compact. There was no evidence of fertilization, but division stages were common soon after excystment.

2. Encystment

Encystment follows a period of rapid division. The amoebas are always very numerous before this takes place and only an occasional division stage may be found in a preparation in which most of the individuals are encysting. Encystment occurs on the fourth day or later, depending on the heaviness of the inoculation or the relative numbers of amoebas in the culture. Encystment is delayed when numbers are small.

Amoebas going into this condition have no food vacuoles. They round up with a very large contractile vacuole pulsating (pl. 21, fig. 82). The protoplasm becomes dense and a wall is formed around the amoeba. First a thin outer wall is formed in which no openings have been observed. A thick wall is formed within the outer thin one, and in it round openings are left (pl. 21, fig. 83). Around these the wall in some instances seems slightly thickened.

3. Excystment

Excystment has been found in cultures which have been depopulated by removal of individuals for study and fixation. It may also occur when none have been removed, but no investigation has been made to prove this because when once a cover is removed it is not put back for fear of contamination. It may also be induced by the addition of fresh medium and is apparently a reaction to a favorable change in the medium.

An amoeba that is coming out may be easily recognized by the appearance of contractile vacuoles, which are from one to four or more in number. These contract frequently and their activity may extend over a period of twenty minutes or longer before a pseudopodium is put out. The first appearance of the latter is a little rounded mass protruding beyond the cyst wall through one of the micropyles (pl. 21, fig. 88).
The part of the protoplasm protruded is very much lighter stained than that within, which may be partly due to the fact that it is exposed and hence destains easily. It is much less dense than that within the cyst (pl. 21, fig. 89). Vacuoles may be found in the cytoplasm within or without the cyst or in both places (pl. 21, figs. 89, 90; pl. 23, fig. 111). The karyosome is always compact and stains very dark.

The flowing out may continue after the pseudopodia are protruded or it may not proceed further for thirty-five minutes or longer. After it does begin again it is nearly continuous until the amoeba is out. An amoeba has been observed to flow back part of the way, then out (cf. pl. 23, figs. 110–112), and this may be repeated several times before it emerges. The process may require from seven to thirty-five minutes and even longer. The series figured (pl. 23, figs. 107–113) was completed in seven minutes, but a large amount of protoplasm was extruded before the drawings were begun (pl. 23, fig. 107).

Often amoebas are found emerging at two or three exits. It is very common to find them protruding from two (pl. 21, fig. 88). Usually the protoplasm extends a little way out as in figure 88, then flows out at one, having flowed back in from the others. Occasionally, however, individual conditions are found in which a large mass has flowed out at two openings (pl. 21, fig. 90).

That there are two layers in the wall of the cyst is evident from development stages, as before mentioned, and the outer wall serves as a covering for the "pores" (Dangeard, 1910), or "cyst markings" (Wherry, 1913) which are found in the inner thick wall. This outer thin wall is probably easily broken or dissolved. This may be accomplished by the force of the products from the vacuoles which are pulsating frequently prior to excystment or the protruding pseudopodia may break through or may dissolve the outer wall locally.

The number of pores may be readily counted in the abandoned cyst. In those counted there have been from three to eight of these. What the fate of the old wall is has not been ascertained, but they have been observed intact two and three weeks after they were abandoned.

III. THE FLAGELLATE

A flagellate stage was first found in this species, Naegleria gruberi, by Schardinger (1899), who called the organism Amoeba gruberi, as has been stated previously. Wherry (1913) also experimented with the flagellate and other stages of what appears to be this species, from the city water-supply of Oakland, California.
The flagellate occurs regularly in the cultures, but there are few in comparison with the number of amoebas. For example, on a cover-glass which has so many amoebas on it that there is very little space not covered by them, there will be from one to five, occasionally more, flagellates in a field of a \(\frac{2}{3}\)-inch objective. If the medium next to the upper and lower surfaces of the culture is examined about the same number will be found.

The first flagellates found in the pure mixed cultures of amoeba and bacteria with which this work is concerned were found in a hollow-slide preparation which had been made by sealing an amoeba film with vaseline over the cell filled with distilled water. It was examined after sixteen hours and there were a few flagellates among the amoebas, showing that flagellates may be produced entirely cut off from the air. Since then they have been produced in material from old cultures and in those from individuals recently obtained from the soil. Once they were found on an agar plate, but this medium was very little used and so their behavior on it cannot be discussed.

To turn a whole film of amoebas into flagellates, it is only necessary to add a few drops of distilled water to the cover-slip containing them. It has been found by experiment that more of the amoebas enflagellate in this than when sterile medium is used. The cover-slip is then left film side up exposed to the air of the room, which is better than that of a moist chamber. A preparation which has turned into amoebas overnight may be stimulated to enflagellate in the morning by the addition of more distilled water. Most of the amoebas of a preparation will be transformed to flagellates in three hours.

A little over an hour after the preparation is made (the time varies from one hour to one hour and fifteen minutes), contracting amoebas will be found at different places over the cover. These will be found to be more or less free from the substratum and to be moving the free portion through a rather large extent of space by this jerky movement. Short pseudopodia are sent out and drawn in almost continuously. The amoebas twist and turn, getting nearer all of the time to the rounded-up condition. When they reach a thick, short, pyriform shape, they swim away with the narrow flagellated end anterior (pl. 22, fig. 97).

The flagellate stage is not of long duration. If undisturbed, a preparation in which the first flagellates appeared at eleven o'clock in the morning, was practically free of all flagellates by nine o'clock in the evening, and only two or three, even fewer, could be found in a low-power field the next morning. These may have been some that
were late in transforming, they may have enflagellated again, they may
have been flagellates all of the time, or they may have come from some
of the amoebas transferred to the flagellate cover, for it is impossible
to get a preparation entirely free from them because there are always
a few left at the two surfaces of the original preparation and the
poured-off liquid must necessarily have a few in it. From which it will
be seen that the exact duration cannot be ascertained, but it is between
ten and twenty-four hours.

Not only does a flagellate turn back to an amoeba in the ordinary
course of its life, but it may be stimulated to do so at any time during
its flagellated state. This has been done by raising or by lowering the
temperature slightly, and by mechanical disturbance.

After a stimulus has been applied, the flagellate immediately begins
to whirl about until it seems exhausted, when it settles to the bottom
and assumes its amoeboid motions. The flagella are present for a time
and remain in motion, but they are found to extend from any part of
the body, front, back or side (pl. 22, figs. 100-103; pl. 23, figs. 120,
122), but still with the nucleus near their base. If a temperature
stimulus is used and is removed in a few minutes, normal swimming is
resumed, but if it is left during the whole time, exflagellation continues.
After two hours most of the individuals may be amoebas.

After any mechanical stimulus, such as pouring the liquid contain-
ing the flagellates on to another cover (which may also be done without
causing a great disturbance), by touching the medium with the micro-
scope objective, by the addition of fresh medium added so that the
water of the preparation is stirred up, or after temperature change,
the flagellates become sticky. In whirling about they run into each
other and it is with difficulty that they become separated. They may
settle down together but they do not copulate or conjugate as might be
expected, for in the first place small clumps are soon found, and also
when followed up this sticky condition has no further appearance of
reproductive significance, but seems to be merely a reaction to un-
favorable conditions.

The sticky condition of the exflagellating individuals may last only
a short time or it may persist until clumps of from few to many indi-
viduals may be found (pl. 23, figs. 115-118, 121, 124-126) which are
in the amoeboid stage. These move over and over each other, seeming
to be unable "to get out of touch" with each other.

Material at room temperature does not agglomerate as often as it
does at a lower one. Flagellates put in a refrigerator were invariably
found in this condition the next morning, in spite of the fact that the
temperature was that of the room by that time. After the addition
of fresh sterile medium the clumps at room temperature broke up and
the amoebas spread out over the cover-glass, but if left at low tempera-
ture there was no change.

1. Morphology

The size of the flagellate, which is pyriform in shape with two equal
flagella slightly longer than the body and situated at the narrower
anterior blunt end (pl. 22, fig. 106), is less than that of the amoeba
because it is in a more rounded-up condition and it varies proportion-
ately as that of the trophozoite does. When first formed a flagellate
is ovoid in shape and has its greatest cross-diameter about two-thirds
that of its longitudinal diameter (pl. 22, fig. 97), but when the pyrim-
form shape, which is characteristic of older flagellates, is reached the
greatest cross-diameter is less than half that of the longitudinal
diameter (pl. 22, fig. 106). After several hours in the flagellate con-
dition it is not uncommon to find individuals with a knob of cytoplasm
projecting posteriorly (pl. 22, fig. 95). The size of this may increase
until only a neck of cytoplasm is left at the anterior end.

The cytoplasm and endoplasm are not differentiated. Occasionally
chromidia are formed in the cytoplasm. The contractile vacuole is
situated posterior to the nucleus, which is anterior near the base of the
flagella. The nucleus may (pl. 22, figs. 98, 102) or may not (pl. 22,
fig. 95) have heavy peripheral chromatin. There is a plastid connection
the rhizoplast, from the karyosome to a darkly staining granule, the
blepharooplast, which is situated anteriorly at the periphery of the
cytoplasm. Extending from this are the two flagella, which with
Alexeieff's triple stain are slightly rose-colored like the rhizoplast and
the nuclear plastin.

When heavy peripheral chromatin has been found in the flagellates
there has always been the same condition in the material prior to
enflagellation. As suggested before, the same thing is probably true of
the chromidia, that they would probably have formed nuclei of new
individuals had enflagellation not occurred.

The description as given above for mononucleate forms is also true
for the multinucleate ones which are found in flagellates produced from
multinucleate amoebas. In this case there may be only two flagella
and these are connected with one nucleus (pl. 22, fig. 97) or rarely
there are found flagellates with two nuclei with two flagella related to
each. In the last case all four flagella ordinarily project from the same end, but three cases have been found in which they come off from nearly opposite ends, making the flagellate somewhat triangular in shape. One of these was watched until it turned back into an amoeba and it proved not to be a division stage, for it was still binucleate as an amoeba.

(a) Process of enfagellation.—In enfagellation there is a progressive change in form of the individual which may be slightly modified. Differing from the characteristic limax form, the amoeba first has short pseudopodia which are constantly being withdrawn. It gradually rounds up either before (pl. 22, figs. 92, 93) or after the flagella are formed (pl. 22, fig. 94). The flagella appear in the first individuals in about an hour, depending on the temperature, and with their appearance a side-to-side motion at the anterior end begins and increases until the whole flagellate is involved. Finally an oval flagellate swims away with a movement that is at first uncertain in appearance, but soon becomes the characteristic side-to-side, forward spiral flagellate motion and its shape is then pyriform.

In a preparation fixed when flagellation is beginning the karyosome of some of the individuals has a small projection at one end and is slightly elongated (pl. 22, fig. 91), differing from a reorganization nucleus only in the absence of peripheral chromatin. Later it is rounded and at the nuclear membrane there is a dark-stained granule which is connected with the karyosome (pl. 22, fig. 92). This granule in others is found to be located at varying distances from the nucleus to the periphery but always with a plastin connection with the karyosome (pl. 22, fig. 93). No stage has been found in which it is at the periphery without flagella being present.

Individuals are found with short, blunt flagella and the nucleus at a distance from their bases (pl. 22, figs. 94, 99), individuals with nuclear membrane pulled out in the direction of the blepharoplast (pl. 22, fig. 99), and individuals with long flagella and nucleus close to the blepharoplast (pl. 5, figs. 95, 98). The increase of flagellar length and coincident movement of the nucleus to a position near the blepharoplast has been observed in living material.

Judging from these conditions the basal granule comes out from the karyosome and is always connected with it. It may be derived from the centriole or it may be budded off from the karyosome around the centriole. Our data do not enable us to determine this. It takes its position at the periphery of the cytoplasm and coincident with that
the flagella grow out. These increase in length as the nucleus gets
closer to the basal granule, evidently at the expense of the rhizoplast
and nuclear plastin.

(b) Process of exflagellation.—In material fixed one hour after
having been stimulated to exflagellate, amoeboid individuals with
shorter flagella and elongated karyosomes and those with blepharoplast
or with none distinguishable are to be found. In the latter case the
karyosome looks like that of the early stage of enflagellation (pl. 22,
fig. 101). However, this may be due to a pull on the karyosome.
Sometimes there is a stainable mass on the rhizoplast midway between
the periphery and the nucleus (pl. 22, fig. 100). The flagella have
been observed during the process of being pulled in in living material.
The indications are that the material returns to the nucleus. Early
exflagellation begins with a change in form (pl. 22, figs. 100-103).
Stages are found in the same material with granule connected with the
karyosome (pl. 22, fig. 104) and with an elongated pointed karyosome
and no flagella or peripheral chromatin.

These facts of themselves are incomplete and inadequate, but taken
together with those of enflagellation, it seems clear that the basal
granule resumes its position in the karyosome, and that the plastin,
both flagellar and that of the rhizoplast, is drawn in and takes up its
position with the basal granule within the nucleus.

C. SEXUAL STAGES IN CULTURES

Gametogenesis and syngamy have not been conclusively found in
the cultures. In the cysts, isolated instances of conditions simulating
maturation have been found, and binucleate trophozoites and flagellates
simulating syngamy occur. In old cultures it is very common to find
one large chromidium and the nucleus with its chromatin broken up
(pl. 21, fig. 86) superficially suggesting a maturation division. Some-
times the nucleus is large and somewhat elongated and the chromatin
has a form which closely resembles a spindle (pl. 21, fig. 85), but no
spindle fibers have been found and the chromatin is not in a regular
equatorial plate between the two small spherical granules, but it so
closely resembles an equatorial plate that it is with difficulty that its
banded nature is detected. Sometimes a cyst has been found with one
large chromidium in the cytoplasm and with two nuclei, in each of
which the chromatin was broken up so that it resembled a spireme with
its nucleolus. In some cultures, in which multinucleate forms were
common, after encystment cysts were found with from one to four nuclei. Occasionally the chromatin looks like a spireme and nucleolus (pl. 21, fig. 58); again, the spherical granule has divided and has a central connection with the remaining chromatin in a ring around the connection (pl. 21, fig. 85), resembling a late prophase except that no fibers were present.

These might be interpreted as an incomplete series of maturation divisions occurring in cysts, if it were not for other facts as follow. Many cysts from many cultures, fixed at intervals during the day, have been examined and no other stages have been found. Chromidia are formed at encystment and some might perhaps persist. A broken-up condition of chromatin is general and characteristic in old cysts. Where the amoebas of cysts in this condition are induced to come out, no copulation has ever been observed. The cysts with more than one nucleus are found in cultures in which the amoebas were multinucleate and so were derived from the encystment of multinucleate individuals (pl. 21, fig. 80) and the nuclei are not reduction nuclei. From which it follows that these are not maturation stages, but by mere accident resemble them.

Gläser (1912 b) figures maturation in *Amoeba mira*. In his plate 7, figures 4a, 4b, and 7–13, are amoebas immediately prior to encystment in which there is heavy peripheral chromatin. Of these, figures 8–11 are division stages in which the karyosome is more or less like a dumb-bell in shape and has the peripheral chromatin about its two extremities. Except for the last-mentioned figures, the stages figured are comparable to chromatin extrusion prior to encystment as shown in our plate 21, figures 67, 68, 70, 75 of *Naegleria gruberi*. The reduction of Gläser’s thirty-two double chromatin rods, which become sixteen on the spindle, is possibly the parallel of the chromosome formation in vegetative mitosis of *N. gruberi* in which numerous granules assemble to form the eight chromosomes (pl. 20, figs. 41, 56). His early synapsis looks very much like figure 86 of plate 21, which is an old cyst with the karyosome broken up. The fact that there are small spindles in some cysts and large in others, small nuclei in some and large in others, and large nuclei and small spindles in some, does not necessarily signify maturation. In *Naegleria gruberi* nuclear size varies decidedly in the vegetative condition (cf. pl. 18, figs. 10, 11) and also in cysts (pl. 20, fig. 64). The presence of five nuclei in one cyst does not necessarily mean that it is a stage in maturation. The unlike karyosomes (Gläser, 1912 b, pl. 8, fig. 34) may merely be a condition
common in old cysts, and his culture had plenty of time after encystment for the karyosome to break up.

Flagellates and trophozoites are found with two nuclei close together. No evidence, however, of reduction of chromosomes has been found, and so these must be regarded as merely binucleate, produced by failure of the cytoplasm to divide. The proximity of the nuclei must be purely accidental.

These facts regarding the nuclear conditions and the absence of observed sexual behavior in living material lead us to conclude that the evidence for sexual reproduction in this amoeba is as yet lacking.

No indication of flagellated gametes as described by Metcalf (1910), nor anything resembling fertilization like that described by Calkins (1904, 1907, 1909) has been found in Naegleria gruberi. Likewise copulation, as Hedges (1914) observed in an amoeba from a hay infusion has not been found, though possibly the agglomeration phenomenon is suggestive of this process. However, since there is apparently no chromosome reduction accompanying this process it seems that it is only a reaction to environmental changes, and has therefore no reproductive significance. It is possible that the process described by Hedges, also, is not of reproductive significance, but this can be determined only by nuclear behavior which he was unable to follow. From all of which it seems that, even if reduction does take place as shown in Gläser's figures 24–27, all his other figures are not unquestionably maturation stages.

D. COMPARISON WITH RELATED AMOEBAAS

Vahlkampfia calkensi (Hogue, 1914) is similar in some respects to the species investigated here. Endogenous buds are formed in multinucleate individuals as in Naegleria gruberi, except that in the former two nuclei may sometimes be cut off in one bud, a condition which has not been found in the latter. Exogenous buds are produced in individuals in which chromidia, formed at the expense of the karyosome, are found.

Miss Hogue says that division is amitotic and mitotic. In the former her figures resemble stages found in reorganizing nuclei of Naegleria gruberi, an interpretation which has been discussed under amitosis. The latter she says is by promitosis, but she does not give a complete series of this, and the ones she does give do not seem to be
correctly interpreted. She figures (her pl. 16, fig. 11) an equatorial plate without chromatic polar masses. Following this she gives a stage with two chromatic polar masses and two chromosome (?) masses, while the next stage has two chromatic polar masses. If division is promitotic it does not seem that an equatorial plate would be formed prior to the formation of the chromatic polar masses. When these are formed, from what do they arise? Again, she figures an individual with an elongated karyosome which is dumb-bell shaped with evident peripheral chromatin comparable to a prophase of promitosis (see her pl. 1, fig. 3, and Dobell, 1914), but she says it is elongated because the amoeba is elongated. A complete series is needed to clear up these discrepancies.

Gläser (1912 a) figures division for Amoeba tachypodia which in most respects is like that of Naegleria gruberi. He does not find centrioles, but the karyosome becomes a bent dumb-bell, and the peripheral chromatin comes into the angle of the karyosome. The equatorial plate comes from the peripheral chromatin and is probably added to from the karyosome. During reorganization there is an extrusion of peripheral chromatin. Polar caps are not shown, but notches in the chromatic polar masses suggest their occurrence, as pointed out by Ford (1914). Gläser did not find a flagellate stage, but since his division figures are so nearly like those of Naegleria, it is probable that they occur and that he may have been dealing with Naegleria.

Calkins (1909) figures an Amoeba limax which produces exogenous buds in the manner described for Naegleria. Chromatin extrusion prior to encystment is also very similar. The karyosome has a globe of peripheral chromatin which is given off into the cytoplasm, but it does not take up its position on the nuclear membrane.

E. SYSTEMATIC POSITION

Several new generic names have been proposed for amoebas of the limax group. Chatton and Lalung-Bonnaire (1912) proposed Vahlkampfia for all of these. Alexeieff (1912c) proposed Naegleria as the generic name of limax amoebas in which during division there are voluminous polar masses representing all of the karyosome, and Hartmannia for those in which there are no polar masses. Calkins (1913) recognizes the genus Vahlkampfia for the limax amoebas in which a
flagellate stage has not been found and limits Naegleria to those in which a biflagellate stage occurs. This takes a number of species out of the genus Vahlkampfia which will have to be put back in case all placed in Vahlkampfia prove to have a biflagellate stage, and the occurrence of a biflagellate stage will have to be made a generic character of the genus Vahlkampfia. As a matter of convenience it may be well, pending further investigation, to use the distinctions as Calkins proposed them.

Calkins gives the generic characters of Naegleria as follows:

Small limax-like forms with no essential morphological differences from Vahlkampfia, except for the fact that the adult amoebae acquire and lose flagella under conditions not fully recognized. They are viable, possess one contractile vacuole and a single nucleus of the limax type. The flagellated stage has a definite oval form. Nuclear division promitotic; division of the flagellate stage unknown in the majority of cases.

The genus, then, to which the species described in this paper belongs is Naegleria Alexeieff.

The species on which this work has been done resembles certain other described species of Amoeba very closely as far as the life-history has been worked out. The species A. gruberi described by Schardinger (1899) agrees with it in all of the characteristics which he has given. The habitat, however, is different. In this regard he says in one place "Ich vermochte, ohne sonderliche Mühe aus Wasser eine Amoeba spinosa, eine andre A. lobosa (oblonga) . . ." Later he says "Da die Amöbe aus einem diarrhöischen Stuhle gezüchtet ist . . .".

These are the only places where Schardinger mentions his sources. It is possible that he may have obtained a soil amoeba in his culture of diarrheal forms, but what the possibilities of contamination were in his cultures is not clear from his paper. On the other hand, however, the cyst may have passed through the intestinal tract uninjured with excystment taking place in the water from which the culture was made; or, since amoebas live almost everywhere that bacteria are found, it is possible that it may have endured or may have even become adapted temporarily to the habitat of the digestive tract.

Schardinger calls the species Amoeba lobosa evidently by way of allocating it in the system of Rhizopoda, but not as a generic and specific designation; in other words, it is a limax form. The size in locomotion is 32–40μ long and 16–24μ wide, with an occasional larger one. The pseudopodia are of ectoplasm only. There is one contractile vacuole. The nucleus has peripheral chromatin slightly evident or
very heavy. Multinucleate forms are found which may divide by plasmotomy. Small "young" forms are figured and these grow into the large ones. Schardinger has figured what looks like an endogenous bud but he says nothing about it.

The cyst, 14–16 $\mu$ in diameter, has a double, contoured wall in which there are from three to six thickenings which look like pores of a pollen grain. The cytoplasm is granular. The nucleus from Schardinger's figures may or may not have the chromatin broken up. He found one, two, and four nuclei; in the last case the cyst was 24 $\mu$ in diameter.

The flagellate behavior of Schardinger's species agrees with that of the species of this paper, in time required for enflagellation, in reaction to stimuli, in number and position of flagella, position of contractile vacuole, character of cytoplasm, and modification of form. Schardinger figures the nucleus of the flagellate as having rather evident peripheral chromatin in every case, while in my form it may or may not have it. Also he figures an occasional mononucleate flagellate with three and four flagella. The nucleus, he says, is always close to the base of the flagella but he was unable to demonstrate a connection between the two organelles.

In so far as the life-cycle in certain other forms is known it agrees very nearly with some of those just mentioned, as for example Gläser's (1912a) division stages of Amoeba tachypodia, obtained from pond water, but for which a flagellate stage has not been found.

However, Wherry (1913) described a form obtained from the water-supply of Oakland, California, which becomes a flagellate and which agrees, so far as he went, in every respect with the one upon which I have worked, except in the matter of amitosis which has not been found by me. The latter he says he has watched, but he does not describe or figure it. It may well have been mitosis which he saw. He describes the characters of the amoeba but does not figure division stages, except plasmotomic cytoplasmic division. He figures three and four coincident spindles, multinucleate forms, endogenous buds, a flagellate with a contractile vacuole and two equal flagella, but he does not figure these showing any rhizoplast or other connection with the nucleus. He figures a cyst with a double wall, in the inner one of which are five "markings" or pores. The size of the cyst is 9.2 $\mu$ to 39.6 $\mu$, generally uninucleate, but sometimes with from one to eight nuclei.

From these facts it follows that the species worked on by Schar-dinger (1899), that worked on by Wherry (1913) and the one described
in this paper are probably all the same species, Naegleria gruberi (Schardinger).

Attention should be called to Alexeieff’s (1912d) classification of this species. He calls it Dimastigamoeba gruberi (Schardinger, 1899).

**Synonyms:**

*Amoeba gruberi* Schardinger, 1899.
*A. punctata* Dangeard, 1910.
*A. punctata* Dangeard, Alexeieff, 1911.
*Vahlkampfia punctata* (Dangeard) Chatton et Lahumb-Bonnaire 1912.
*Naegleria punctata* (Dangeard) Alexeieff, 1912 c.

It should be noted in this connection that *Amoeba gruberi* agrees with *Naegleria punctata* (Alexeieff, 1912c) in every respect so far as described, except in the character of the cyst wall, which is of even thickness in the first-named species and not as Alexeieff (1912c) has figured it in *Naegleria punctata*. All the cysts examined by me in *Naegleria gruberi* have walls of even thickness. In this species also the rhizoplast is found connecting the karyosome and blepharoplast, and not nuclear membrane and blepharoplast as figured in *Naegleria punctata*.

Alexeieff says that there is a complete resemblance between *Vahlkampfia punctata* and *Dimastigamoeba radiata* Bloehmann (1895). If this resemblance proves valid, then *Dimastigamoeba* will have to be used instead of *Naegleria* for biflagellated amoebas.

Should *Dimastigamoeba* be used for the generic name of the biflagellate amoebas, they would be placed in a genus in which the other species are flagellated throughout their free state in so far as is now known, while in these forms a flagellate condition is occasional and temporary. The return to the flagellate state is perhaps not of long enough duration and frequent enough, so far as is apparent, to be considered an adult characteristic, the evidence seeming to indicate that it is for distributional purposes only, and for this reason that name should not be used for this species. The genus was created for two amoeboid flagellates, and unless these have a non-flagellated amoeba stage, *Naegleria* should be retained as the generic name for the biflagellated amoebas, which seem more closely related to the Rhizopoda than to the Mastigophora.
Should the two species of *Dimastigamoeba* prove to have a life-history comparable to that of *Naegleria*, then *Dimastigamoeba* would be applicable, and not until then.

Other forms which should be added to the above list are the "kulturamöben" of Wasielewsky and Hirschfeld (1910), the *limax* amoeba described by Wherry (1913), and possibly *Amoeba tachypodia* Gläser (1912a).

F. SUMMARY

1. Pure mixed cultures of a soil amoeba, *Naegleria gruberi*, have been maintained under laboratory conditions during two years. In these cultures encystment and excystment, enflagellation and exflagellation, exogenous and endogenous budding occur.

2. In division by binary fission, which is promitotic, the spindle is within the nuclear membrane. It has two large chromatic polar masses of karyosomic origin with polar caps of plastin more or less evident. The spindle fibers are formed in the angle of the dividing dumb-bell shaped chromatic karyosome. They come from the peripheral and karyosomic plastin. The eight subequal chromosomes come from the peripheral chromat in with an addition from that of the karyosome. They are formed in a regular equatorial plate and later are divided by constriction.

3. The nucleus is reconstructed as follows: the karyosome is formed from the chromatic polar mass containing the centriole and from part at least of the chromosomic mass, while its plastin center comes from the centrodesmosome and the remains of the spindle. The peripheral chromat in and plastin emerge from the network between the chromatic polar mass and the chromosome mass. Stages of nuclear reconstruction simulate amitosis and have been so interpreted by Hogue (1914) and others. No cases of true amitosis have been found.

4. Evidence of multiple fission, which is apparently rare, was found in amoebas containing two and three spindles.

5. Under conditions which have not been determined, exogenous budding occurs in which peripheral lobes of cytoplasm are constricted off, each containing chromidia given off from the nucleus. These chromidia then reorganize to form nuclei of the new small individuals in which later coincident growth of nucleus and cytoplasm ensues.
6. Endogenous budding is rare. It occurs by the internal constriction of the cytoplasmic mass around one of two nuclei. The fluid-filled space gradually enlarges until it severs the internal bud from the parent mass.

7. Chromidial formation occurs rarely in flagellate stages and in those trophozoite stages in culture in which exogenous budding is occurring. In such individuals the karyosome becomes granular and the quantity of its stainable material becomes reduced and is peripherally located. The peripheral chromatin on the nuclear membrane is temporarily increased in quantity and minute stainable granules appear and increase in number in the cytoplasm.

Chromidial formation accompanies encystment as a normal process. It is initiated on the periphery of the karyosome and results in the formation of large stainable spheres in the cytoplasm which later disappear entirely in old cysts. A sharp distinction exists in both process and form of the stainable material from which the nuclei of the buds are formed and that which is cast out from the nucleus in cysts.

8. Encystment regularly ensues after a period of binary fission, occurring more quickly in densely populated cultures on the fourth day after heavy inoculation. Encysting individuals are spheroidal, lack food vacuoles, and have an enlarged contractile vacuole. A thin membrane without openings is first formed and within this a heavy hyalin cyst wall containing three to eight minute eirenal micropyles. It is during the early stages of encystment that chromidial formation is intense.

9. Ex cystment occurs continuously in small numbers in standing cultures and may be generally induced by renewal of the culture medium. The protoplasm becomes active and starts to emerge at the various micropyles, finally leaving the cyst through a single one.

10. Enflagellation occurs sparingly in cultures and may be induced generally by the addition of distilled water and by the exposure of films to the air at room temperature. The flagellate has two equal flagella, plastin in nature, at the base of which is a darkly stainable granule, the blepharoplast. This granule is connected with the karyosome of the nucleus by a slender plastin line, the rhizoplast. The flagella arise by an outgrowth of the karyosome, presumably from the centriole which crosses the clear nuclear zone, emerges through the nuclear membrane into the cytoplasm and the flagella grow out from it.

11. The flagellate stage is of brief duration, rarely exceeding twenty-four hours, and exflagellation accompanies slight changes in
temperature or mechanical disturbance. The flagellates whirl about, settle down on the substrate, and become actively amoeboid. The flagella shorten and are withdrawn.

12. Gametogenesis and syngamy have not been conclusively found in the cultures, although isolated instances occur of chromatin extrusion simulating maturation, and of binucleate trophozoites and flagellates simulating stages in syngamy.

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LITERATURE CITED

ALEXEIEFF, A.
1912c. Sur les caractères cytologiques et la systématique des amibles du groupe limax (Naegleria nov. gen. et Hartmannia nov. gen.) et des amibles parasites du vertèbres (Proctamoeba nov. gen.). Ibid., 55-74, 7 figs. in text.
1912d. Quelques remarques à propos de la spécificité parasitaire. Sur le véritable nom de Cryptobia (Trypanoplasma) intestinalis et sur celui du trypanosome pathogène des mammifères; quelques autres questions de synonymie chez les protozoaires. Zool. Anz., 41, 17-37, 3 figs. in text.
1913. A propos de la question du centriole chez les amibles limax. Ibid., 42, 327-331.

ARNDT, ARTHUR.
1914. Über generative Vorgänge bei Amoeba chondrophora n. sp. Arch. Prot., 34, 39-60, pl. 3.

AUERBACH, L.

BLOCHMANN, F.

BRODESKY, A. L.
CALKINS, G. N.

CHATTON, E.

CHATTON E. ET LALUNG-BONNAIRE.

CRAIG, C. F.
1911. The parasitic Amoeba of man (J. B. Lippincott Company), x + 2.53, 30 figs. in text.

DANGEARD, P.

DOBELL, C.
1914. Cytological studies on three species of Amoeba—A. lacertae Hartmann, A. globae n. sp., A. flavialis n. sp. Arch. Prot., 34, 139-190, pls. 7-11.

DEJARDIN, P.

FORD, E.

GLÄSER, H.
1912a. Untersuchungen über die Teilung einiger Amoeben, zugleich ein Beitrag zur Phylogenie des Centrosomes. Ibid., 25, 211-152, pls. 3-8, 5 figs. in text.

GRUBER, A.

HARTMANN, M.
1913. Morphologie und Systematik der Amöben, in Kolle-Wassermann, Handbuch der Pathogenen Mikroorganismen, 7, 607-650, 64 figs. in text.

HODGES, R. E.

HEIDENHAIN, M.
1907. Plasma und Zelle (Jena, Gustav Fischer), I, (1), viii + 506, 276 figs. in text.
Hertwig, R.
1908. Neue probleme der Zellenlehre. Arch. Zellforsch., 1, 1–33, 6 figs. in text.

Hogue, M. J.
1914. Studies in the life-history of an amoeba of the limax group. Vulh-

Liston, W. G., and Martin, C. H.

Metcalp, M. M.

Nägler, K.

Pensad, E.

Schaudiner, F.

Schaudinx, F.

Vahlkammef, E.

Viereck, H.

Wallich, G.

Wasielewsky, T. V. und Hirschfeld, L.

Wherry, W. B.
1913. Studies on the biology on an amoeba of the limax group. Vahl-
kampff sp. no. 1. Arch. Prot., 31, 77-94, 2 pls., 8 figs. in text.

Whitmore, E. R.
1911a. Studien über Kulturamöben aus Manilla. Ibid., 23, 11-81, 3 figs. in text.
1911b. Studien über Kulturamöben aus Manilla. Ibid., 23, 81-95, pls. 3, 4.
EXPLANATION OF PLATES

All the figures of Naegleria gruberi (Schardinger) were drawn with a camera lucida. Most of the material drawn was fixed with acetic-sublimate-alcohol and stained by Dobell’s method, except the following: plate 20, figure 40, plate 21, figures 79 and 83, plate 22, figure 92, haematoxylin; plate 20, figure 49, plate 22, figures 93 and 94, Alexeieff’s triple stain. × 2000.

PLATE 18

Fig. 1. Prophase with elongated nucleus and karyosome, the latter vacuolated.

Fig. 2. Vacuolated, dumb-bell shaped karyosome with spindle fibers forming. Fibers at the end show, others are covered by the karyosome.

Fig. 3. Same as fig. 2. Spindle developed farther.

Fig. 4. Early prophase. Karyosome vacuolated. Peripheral chromatin more evident.

Fig. 5. Prophase with karyosome dumb-bell shaped. Peripheral chromatin still at nuclear membrane.

Fig. 6. Anaphase. Two polar caps visible. Chromosomes fused early.

Fig. 7. Spindle of late prophase with definite, deeply staining chromosomes.

Fig. 8. Late anaphase. Nuclear membrane closing around daughter nuclei.

Fig. 9. Late prophase showing polar caps, granular chromatic polar masses, eight spindle fibers with lightly stained chromosomes, and central spindle.

Fig. 10. Early anaphase. One distinct polar cap, all chromatin granular, central spindle visible at side.

Fig. 11. Very early anaphase. Polar caps not visible, chromosomes separating.

Fig. 12. Late telophase. Daughter amoebas almost separated.

Fig. 13. Nuclear membrane pinched in, chromosomes separate, no polar caps. Food vacuole in cytoplasm.

Fig. 14. Late anaphase. Further elongation and beginning of chromosome fusion.

Fig. 15. Telophase. Pseudopodia put out to pull daughter amoebas apart. Extrusion of peripheral chromatin net. Two chromatic masses fusing around centrodesmose of new nucleus of upper amoeba.
PLATE 19

Fig. 16. Amoeba with one large and two small probably daughter nuclei.

Fig. 17. Reorganization of nucleus of daughter amoeba in which the two chromatin masses are late fusing.

Fig. 18. Amoeba with four nuclei of same size.

Fig. 19. Amoeba elongated. Resting nucleus with little peripheral chromatin. Pseudopodium ectoplasmic as in those of figures 1-7 and 16-18.

Fig. 20. Amoeba with three nuclei in same stage of late prophase.

Fig. 21. Amoeba in which the cytoplasm failed to divide. Nuclei reorganizing.

Fig. 22. Amoeba with two nuclei, in very early prophase. Peripheral chromatin very evident.

Fig. 23. Amoeba with three nuclei, one of which is in resting condition, one is in very early prophase, and the other in late prophase.

Fig. 24. Amoeba with three nuclei in same stage.

Fig. 25. Daughter amoeba in which the two chromatin masses are nearly fused, but the nucleus is not rounded off. Remains of nuclear connection visible.

Fig. 26. Amoeba with two nuclei in early telophase, the position of which indicates that plasmotomy may occur.

Fig. 27. Amoeba with large nucleus and no chromidia.

Fig. 28. Small amoeba with small nucleus and few remaining chromidia.

Fig. 29. Endogenous bud in vacuole.

Fig. 30. An individual showing an endogenous bud nearly cut off.

Fig. 31. Nucleus larger than that in figure 28. Few chromidia.

Fig. 32. A budded individual in which the nucleus is not organized.

Fig. 33. Binucleate individual about to form an exogenous bud. Nuclei are very light. Chromidia present.

Fig. 34. An amoeba constricting off a cytoplasmic lobe at the periphery containing chromidia; vacuole and chromidia in cytoplasm, nucleus with heavy peripheral chromatin and karyosome with little chromatin.

Fig. 35. Endogenous bud in vacuole. Nucleus not well organized.

Fig. 36. Endogenous bud containing chromidia almost separated from parent individual.
Fig. 37. Late anaphase in which centrioles and part of the central spindle may be distinguished.

Fig. 38. Peripheral chromatin free from nuclear membrane and taking position on karyosome.

Fig. 39. Early prophase with peripheral chromatin moving into the karyosome which is elongated.

Fig. 40. Nucleus in dividing material showing darkly stained peripheral chromatin, some of which is connected by lines with the karyosome.

Fig. 41. Polar view at small angle showing relation of karyosome and spindle chromatin.

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Fig. 66. Encysted amoeba in which the karyosomes of the two nuclei are broken up.

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PLATE 23

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OF SOUTHEASTERN WASHINGTON

BY

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DISTRIBUTION OF THE LAND VERTEBRATES
OF SOUTHEASTERN WASHINGTON

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LEE RAYMOND DICE

CONTENTS

INTRODUCTION

In the following paper the local and geographical distribution of
the land vertebrates of Walla Walla and Columbia counties, south-
eastern Washington, is considered. The region selected is of consider-
able geographical extent. It is especially adapted to the present study
 because it possesses a wide range of climatic conditions, giving a
variety of habitats. In the presentation of the facts the endeavor is
made to show how the consideration of local distribution may be com-
bined with the consideration of geographical distribution.
The dependence of animals on their habitats does not seem to have been sufficiently taken into account in the study of distribution. Many species are closely restricted to certain kinds of habitats. If a particular kind of habitat does not occur in a region the species of animals restricted to it must also be absent. This applies to the habitats of great extent as well as to the habitats which always occur in limited areas. We should not expect to find typically desert animals except in a desert habitat. In order to explain distribution we should give much attention to the causes producing the different kinds of habitats.

Usually the fauna of each geographical region has been studied as a whole and whole faunas have been compared together without regard to differences in the habitats of the component species. It seems that such a method cannot lead to the most exact knowledge of the factors limiting the distribution of animals on the continent of North America or of the relation in origin of particular species or faunas.

In order to show the relation of the faunal areas of southeastern Washington to the faunal divisions of North America, the position of the region in the zoogeographical system of considering distribution and in the life-zone system is determined as closely as possible. Finally, a critical comparison is made of the several methods of studying animal distribution which are at present in use.

The latter part of the field-work and the preparation of the results have been supervised by Professor S. J. Holmes, who has given much valuable criticism. I am also indebted to Dr. Joseph Grinnell for advice and criticism. Professor J. C. Merriam furnished most of the notes on the geological history of the region.

TOPOGRAPHY

The topographical features of southeastern Washington have been described in detail by Russell (1897, pp. 14–28). The western part of Walla Walla County has in general a low relief and is largely formed of several "Flats." However, south of the Walla Walla River in this region there is a high range of hills.

East of Lamar the hills become somewhat abruptly higher and the prairie region of eastern Walla Walla County and western Columbia County is characterized by high, rolling hills. The hills are steepest on the northeast slopes and these northern hillsides are damper and colder and support a more luxuriant growth of vegetation than the southern slopes. Along the northern boundary of Walla Walla and Columbia
Fig. A. Map of Walla Walla and Columbia counties, southeastern Washington, showing the principal streams and localities.
counties the Snake River has cut a deep cañon, reaching in places to a depth of 1500 feet or more.

The Blue Mountains in southeastern Columbia County form a region of steep slopes and rather sharp ridges, with an elevation of 4000 to 6000 feet. The cañons are deep and the valleys narrow.

GEOLOGICAL HISTORY

In early Miocene time the greater portion of eastern Washington and also a portion of eastern Oregon and Idaho were flooded by a series of lava flows. These Columbia Lavas have a thickness in places of probably over 4000 feet. All the visible rocks of Walla Walla and Columbia counties are basalts probably formed at the time of these lava flows. The cañon walls of Snake River indicate at least ten successive flows, between some of which sufficient time elapsed for soil to form and forests to grow.

Volcanic outbursts continued during the Middle Miocene and much volcanic ash was thrown out. In eastern Oregon extensive lake deposits, the Mascall beds, were formed at that time.

The climate of the region in the period succeeding the lava flows must have been more moist than at present. Before the middle of the Pliocene, however, the Cascade Mountains had been elevated and these mountains robbed the winds from the ocean of their moisture long before they reached eastern Washington. A dry period necessarily ensued.

The time of the uplifting of the Blue Mountains is not certainly known, but their present elevation had probably been reached before the beginning of the Pliocene.

In Pleistocene time the presence of glaciers in northern Washington seems to have caused a return of moist conditions over the region. At that time also the gorge of the Columbia River through the Cascade Mountains seems to have been blocked, resulting in the formation of a large lake, Lake Lewis, which covered a large part of the Columbia Basin. In Walla Walla County this lake probably extended eastward as far as Eureka, but no evidences of it east of this point have been found.

METHODS OF STUDY EMPLOYED

The principal aim in the present study has been to determine the vertebrate associations of the region. The wide range of climatic and vegetational conditions which occurs gives a very good opportunity to
study the extent to which the different species are restricted to particular habitats. The habitats have been described in some detail in order to facilitate comparison with the habitats of other regions.

The attempt has been made to study and describe the habitats and associations in as nearly their native and normal conditions as possible, but for many habitats it has been difficult to find a sufficient area retaining anything like the original conditions.

While many of the associations here considered could be easily subdivided or several could perhaps be combined, yet it is believed that the present arrangement is the most useful one for the study of vertebrate distribution in the region. A region is divided into associations as a matter of convenience in studying and describing the environments and the habitat preferences of the animals. To increase the number of associations unduly would destroy the convenience of use for which the classification is made, while to lump the associations might obscure important facts. It cannot be hoped that the relative abundance of the species in the different associations as here given is fully accurate, for the observations on which the results are founded are known in many cases to be too few in number.

In order to show the relative abundance of each species in the different habitats it has been desirable to use a system of nomenclature modified from that used by Grinnell (1914, p. 67). As here considered, the relative abundance of each species in the different habitats of the same area is compared. No attempt is made to compare the relative abundance of a species in the different faunal areas. When a species occurs in only one habitat in an area, this is designated the exclusive habitat. If the species occurs in more than one habitat in the area, the habitat in which it occurs most abundantly is said to be its major habitat and all others are said to be minor habitats. If the data are insufficient to determine the major habitat, the term reported is used to refer to each habitat in which the species is known to occur. The relative abundance in the different habitats has been determined on the basis of the comparative number of individuals actually observed or trapped in each.

Active observation of the birds in the region near Prescott was begun by the author in December, 1904, and attention was later directed to the other vertebrates. The observations have been much interrupted by the conflicting claims of other duties and by prolonged absences from the region. In the summer of 1914 ten days were spent in the region near Wallula, a shorter excursion was made to Lyon’s
Ferry on the Snake River, and three weeks were spent in the Blue Mountains in very intensive study. Notes published by others bearing on the distribution of the vertebrates of the region have been used whenever possible. Mr. S. H. Lyman of Dayton has kindly allowed the use of his unpublished notes on the birds of the region. It is hoped that the full accounts of the distribution of each species of vertebrates in the region, which were prepared in working up the present paper, may soon be published.

Great care has been taken to obtain accuracy in the specific identifications. Specimens have been secured when possible, except of easily recognized forms. In a few cases the subspecific identification is based on the geographical distribution as given by recognized authorities. Dr. Joseph Grinnell has checked a large number of identifications of mammals and birds. The identification of the reptiles and amphibians is due to Mr. Charles L. Camp.

The botanical names used have been taken from Piper (1906) except in a few cases where other names seemed more desirable. Specimens of many of the more important species of plants were collected. Dr. H. M. Hall identified a number of specimens and Dr. H. S. Yates determined several grasses.

FAUNAS AND ASSOCIATIONS OF THE REGION

In southeastern Washington three prevailing types of vegetation may be recognized. Along the Columbia River there is an area where sagebrush is the dominant plant; further east is a region where bunchgrass forms the most prominent part of the natural vegetation; and the Blue Mountains are largely covered by conifer forests. Correlated with these differences in vegetation there are important differences in the species of vertebrates found in each of these districts. The assemblage of species found in each such region delimited by climatic features may be called a fauna, and the region itself may be called a faunal area, or, more simply, an area. The term “area” as here used refers to the whole of any geographical district where a particular type of vegetation is dominant and includes all of the habitats in such a district.

Each faunal area is made up of several different kinds of habitats and each habitat shelters a different association of vertebrates. Some habitats and associations in the different faunal areas are very similar,
but in the present paper an association is not considered to extend beyond the limits of a single faunal area.

The lines separating the different faunal areas are not sharp. Probably the best criterion for characterizing faunal areas is the dominance of particular habitats. It is evident that in passing from one area to another a situation will be met where the dominant habitat of one area will equal in extent the dominant habitat of the other area. It is at this point that the line separating the two must be drawn. Each area will usually show at its edges some development of the dominant habitats of the adjacent areas. In many cases a dominant habitat from one area may recur as a subdominant habitat throughout an adjacent area. However, it is best to consider each area in sections where it is typically developed and not along its edges.

Lists of the characteristic species of each of the faunas are given. Each list includes those native breeding species which in southeastern Washington are definitely known from only one faunal area. Lists are also given of the species making up the different associations of each fauna. As here given the lists record all the forms noted in each of the corresponding habitats. Trapping records are included in some instances to show the relative abundance of some of the smaller mammals. Unless otherwise noted, only the results of the first night’s trapping on any trap line are included. Traps are usually set from five to ten yards apart and in a continuous line.

Fig. B. Map of Walla Walla and Columbia counties, southeastern Washington, showing the extent of each of the three faunal areas, Columbia Basin sagebrush area, Columbia Basin prairie area, and Blue Mountain area.
COLUMBIA BASIN SAGEBRUSH FAUNAL AREA AND FAUNA

Habitats and Associations:

- Sagebrush
- Rocky-slope
- Willow
- Water-margin
- Aquatic
- Aerial

The Columbia Basin sagebrush area is characterized by the dominance of the sagebrush habitat. This is found well developed in the western end of Walla Walla County near the Columbia and Snake rivers. Sagebrush extends up the valley of the Walla Walla River to the neighborhood of the town of Touchet or a little above this. It also extends up Snake River for some distance, but the exact limits are not known. Sagebrush is not dominant south of the Snake River at Lyon’s Ferry, so the sagebrush area does not extend eastward that far. Between the Walla Walla and Snake rivers the sagebrush area extends eastward a number of miles, but as the land rises the sagebrush gradually gives place to the prairie. The change is very gradual and no abrupt line of demarcation can be drawn. The typical sagebrush area probably does not extend eastward from the Columbia River more than about ten miles.

There are some rocky slopes in all the higher hills of the sagebrush area and along the streams there are numerous basaltic bluffs. Along the Walla Walla River there is a narrow growth of willows, but along the Columbia and Snake rivers in western Walla Walla County there are almost no trees, and brush is developed in only a few places, so that along these streams the willow habitat appears only in isolated patches.

Characteristic Species of the Columbia Basin Sagebrush Fauna

- Scaphiopus hammondii hammondii
- Sceloporus graciosus
- Phrynosoma douglassii douglassii
- Centrocercus urophasianus
- Amphispiza nevadensis nevadensis
- Lanius ludovicianus exubitorides
- Onychomys leucogaster fuscofusca
- Perognathus parvus parvus
- Perognathus lordi columbianus
- Perodipus ordii columbianus

The Columbia Basin sagebrush fauna is characterized by the presence of a considerable number of species nearly all of which are specially adapted to semi-desert conditions and are inhabitants of the sagebrush habitat.
SAGEBRUSH HABITAT AND ASSOCIATION (SAGEBRUSH AREA)

Exclusive:
- Seeloj^orus gracioso nurs.
- Phrynosoma douglassii douglassii
- Speotyto cunicularia hypogaea — summer.
- Sturnella neglecta — summer.
- Chondestes grammacus strigatus — summer.
- Amphispiza nevadensis nevadensis — summer.
- Lanius ludovicianus exuberitorides — summer.
- Canis latrans lestes.
- Taxidea taxus neglecta.
- Perognathus parvus parvus.
- Perodipus ordin columbia m.
- Citellus townsendii.
- Lepus californicus wallawalla.

Major:
- Scaphiopus hammondi i hammondi.
- Chordeiles virginianus hesperis — summer.

Minor:
- Crotalus oregonus.
- Oxyechus vociferus vociferus — summer.
- Zenaidura macroura marginella — summer.
- Pica pica hudsonia — resident.
- Icterus bullocki — summer.
- Euphagus cyanoccephalus — summer.
- Thomomys columbianus.

Reported:
- Pedioecetes phasianellus columbiana — resident.
- Centrocercus urophasianus — resident.
- Falco sparverius sparverius — summer.
- Asio flammeus — summer.
- Astragalus tristis pallidus — summer.
- Onychomys leucogaster fuscogriseus.

The sagebrush habitat, where it was studied three miles east of Wallula, is not entirely homogeneous. The dominant plant is the common sagebrush (Artemisia tridentata). Commonly mixed with this are two species of rabbit brush (Chrysothamnus viscidiflorus and Chrysothamnus nauseosus graveolens). In places one or other of these shrubs may be more abundant than the sagebrush. The hop sage (Grayia spinosa) and the antelope brush (Kunzia tridentata) occur in lesser abundance. In sandy areas a cactus (Opuntia polyacantha) is often found. The wheat bunchgrass (Agropyron spicatum) is found very sparingly, but under native conditions was evidently much more abundant than at present. Where there has been extensive pasturage and tramping by stock the yarrow (Achillea millefolium lanulosa) is common.
The soil in the sagebrush habitat is light and sandy and being subjected to high winds often drifts, and areas of drifting sand are common. The sand heaps up about the various shrubs, forming small dunes. Being continually shifting it would not seem to be a good place for ground-dwelling animals to make their homes. There are small areas where there are no shrubs or plants but only drifting sand, which in some places near the larger rivers forms good-sized dunes. Over large areas covered by sagebrush the sand is packed and is being eroded by the wind. In these places the sand is removed as soon as it is loosened so that little loose sand is present. By the erosion small sand bluffs are sometimes exposed.

The sagebrush association is represented by a considerable number of species, most of which are characteristic of semi-desert conditions. A few birds, which breed along the streams, forage out a considerable distance into the sagebrush.

Trapping in sagebrush three miles east of Wallula on the nights of June 10, June 12, and June 17, 1914, produced a total of 1 Onychomys leucogaster fuscogriseus, 6 Perognathus parvus parvus, and 6 Perodipus ordii columbianus. On these nights there were 61, 66, and 61 traps used respectively. This gives a total of 188 "trap-nights" (Grinnell, 1914, p. 92). Most of the traps were "out-o-sight" mouse traps, but 5 or 6 were rat traps.

ROCKY-SLOPE HABITAT AND ASSOCIATION (SAGEBRUSH AREA)

Exclusive:
Salpinctes obsoletus obsoletus—summer.

Major:
Crotalus oreganus.

Minor:
Peromyscus maniculatus gambeli. Sylvilagus nuttallii nuttallii.

Reported:
Buteo borealis calurus—summer Neotoma cinerea occidentalis.

The rocky-slope habitat is made up of the slopes covered by broken rock and of the exposures of solid basalt and their talus slopes. Some vegetation is usually found in the soil among the rocks and, because the basalt rapidly decomposes, there is a tendency for plants to increase rapidly in numbers. The vegetation usually agrees in character with that of the surrounding country. Sagebrush (Artemisia tridentata) and wheat bunchgrass (Agropyron spicatum) usually both
occur and one or the other is dominant, depending on whether the
region is dominated by sagebrush or bunchgrass. In general the
habitat is strikingly arid.

The species of the rocky-slope association are few in number and
represent species which in general show a fondness for the neighbor-
hood of rocks.

In ten traps set among rocks on a steep hillside three miles south-
east of Wallula one _Peromyscus maniculatus gambelii_ was taken on
June 16, 1914.

**WILLOW HABITAT AND ASSOCIATION (SAGEBRUSH AREA)**

*Exclusive:*
- *Colinus virginianus* virginianus—resident.
- *Asio wilsonianus*—resident.
- *Otus asio macfarlanei*—summer.
- *Colaptes cafer collaris*—summer.
- *Corvus brachyrhynchos hesperis*—summer.
- *Molothrus ater artemisiae*—summer.
- *Melospiza melodia merrilli*—resident.
- *Zamelodia melanochroa*—summer.
- *Passerina amoena*—summer.
- *Dendroica aestiva aestiva*—summer.
- *Icteria virens longicauda*—summer.
- *Dumetella carolinensis*—summer.
- *Penthestes atricapillus septentrionalis*—resident.
- *Planestes migratorius propinquus*—summer.
- *Sorex vagrans dobsoni.*
- *Reithrodontomys megalotis nigrescens.*
- *Mus musculus musculus.*

*Major:*
- *Zenaidura macroura marginella*—summer.
- *Tyrannus tyrannus*—summer.
- *Tyrannus verticalis*—summer.
- *Pica pica hudsonia*—resident.
- *Icterus bullocki*—summer.
- *Euphagus cyanoccephalus*—summer.
- *Mephitis occidentalis major.*
- *Peromyscus maniculatus gambelii.*
- *Thomomys columbianus.*

*Minor:*
- *Thamnophis elegans.*

*Reported:*
- *Pituophis catenifer catenifer.*
- *Sylvilagus nuttallii nuttallii.*
- *Erethizon epixanthum epixanthum.*

The timber found along the Walla Walla River near Wallula is
made up largely of willows (_Salix_) of several species. Cottonwoods
(_Populus trichocarpa_) are rare and so are many of the shrubs which
grow along the streams nearer the Blue Mountains. The habitat is
limited in extent and does not usually extend more than a few rods
from the banks of the river, when it ceases abruptly and gives place to
the sagebrush. The willows are mostly small in size and grow very
thickly together, forming a dense thicket. On some of the very low land near the river there are small meadows where the willows have not been able to establish themselves or from which they have been cleared by man.

The majority of the species of the willow association are not found in the adjacent sagebrush association and these two associations are very distinct. The species of the willow association in the sagebrush area are all found also in the cottonwood-willow association of the prairie area.

Twenty-nine traps set at the edge of the willows along the Walla Walla River three miles east of Wallula took, on June 13, 1914, 7 Reithrodonotus megalotis nigrescens, 8 Peromyscus maniculatus gambelii, and 6 Mus musculus musculus. On June 15, the third day's trapping in this trap-line, one Sorex vagrans dobseni was taken, and also several mice. It is evident that small mammals are much more numerous in these willows than in other habitats of the area. This is true of birds also, for more were seen here than elsewhere.

WATER-MARGIN HABITAT AND ASSOCIATION (SAGEBRUSH AREA)

Major:

Thamnophis elegans. Oxyechus vociferus vociferus—summer.

Minor:

Scaphiopus hammondii hammondii.

Reported:

Rana pipiens brachycephala. Agelaius phoeniceus neutralis—summer.

The water-margin habitat comprises the shores of streams, irrigating ditches, and lakes. Only a few seepage lakes occur in western Walla Walla County and streams and irrigating ditches are not numerous. There is a considerable extent of barren sandy and gravelly shore along the Columbia and Snake rivers. Little study was made of the life of those places. Along the Walla Walla River the water-margin habitat is a very narrow strip between the river and the growth of willows. A few bars of mud and gravel occur, but these are not extensive.

In the sagebrush area the water-margin association is not very important and is made up of only a few characteristic species.
AQUATIC HABITAT AND ASSOCIATION (SAGEBRUSH AREA)

Reported:

Osalatra zibethicus osoyoosensis.

The aquatic association as here considered includes the animals which inhabit the open water of the streams away from the proximity of the shores. Vertebrate members of the association other than the fishes are few in the region. The Columbia and Snake rivers are both swift and during most of the year carry much mud and sand. The lower part of the Walla Walla River is much more sluggish and there are a number of quiet pools. However, there is little aquatic vegetation and few aquatic insects, and the habitat does not appear very suitable for the higher vertebrates.

AERIAL HABITAT AND ASSOCIATION (SAGEBRUSH AREA)

Minor:

Chordeiles virginianus hesperis—summer.

Tyrannus tyrannus—summer.

Tyrannus verticalis—summer.

Reported:

Tachycineta thalassina lepida—summer.

The aerial association is considered to be made up of those animals which feed in the air. Most birds fly about in the air more or less, but the association should be limited to those species which carry on a vital activity in the habitat.

COLUMBIA BASIN PRAIRIE FAUNAL AREA AND FAUNA

Habitats and Associations:

Bunchgrass. Water-margin.

Rocky-slope. Aquatic.

Cottonwood-willow. Aerial.

The bunchgrass habitat is dominant over most of Walla Walla County and the western part of Columbia County. In typical parts of the area it covers the entire region with the exception of small areas of rocky slopes and the small amount of surface occupied by the streams and their adjacent habitats. Sagebrush is well developed in local areas on the flats to the west of Lamar and in a few places in the canions near Snake River, but in the typical part of the prairie area sagebrush is clearly subordinate to the bunchgrass and does not form
a distinct habitat. Near Nine-mile, on the Walla Walla River, the valley is definitely semi-desert, and sagebrush extends up the side canons, but the vegetation of the higher land is dominated by bunchgrass. Bunchgrass is also dominant on the upper parts of the range of hills south of the Walla Walla River. Yellow pines invade the prairie from the Blue Mountains, coming down along the north and northeastern hillsides and appearing in the bottoms of the canons in the foothills.

Along Snake River the region is drier than at Prescott and the rocks lie closer to the surface. Along the canons in that region there are high basaltic bluffs and many rocky slopes. Toward the Blue Mountains also there are numerous outcroppings of rocks, but over most of the prairie area rocks are rarely found at the surface of the ground.

Streams are not numerous in the prairie area. There is a growth of willows and other deciduous trees and shrubs along the smaller streams, but along Snake River there are few native trees or shrubs, so that no cottonwood-willow habitat is formed along this stream.

Characteristic Species of the Prairie Fauna

Aneides iœcanus.  
Hyla regilla.  
Chrysemys bellii.  
Actitis macularius.  
Numenius americanus.  
Aecipiter velox.  
Buteo swainsoni.  
Bubo virginianus occidentalis.  
Dryobates pubescens homorus.  
Asyndesmus lewisi.  
Archilochus alexandri.  
Sialia rufus.  
Myiarches richardsoni richardsoni.

Empidonax trailli trailli.  
Ammodramus savannarum bimaculatus.  
Riparia riparia.  
Stelgidopteryx serripennis.  
Vireosylva gilva swainsoni.  
Geothlypis trichas occidentalis.  
Sectophaga ruticilla.  
Troglodytes aëdon parkmani.  
Scapanus orarius schefferi.  
Mustela arizonensis.  
Microtus nanus canescens.  
Perognathus lordi lordi.

The majority of species known from the Columbia Basin prairie area are known also from the sagebrush area or from the Blue Mountains. Of the species here given as unique most will probably later be found to occur in the adjacent faunas.
BUCHGRASS HABITAT AID ASSOCIATION (PRAIRIE AREA)

Exclusive:
- Numenius americanus—summer.
- Archibuteo ferrugineus—summer.
- Asio flammeus—summer.
- Speotyto cucullaria hypogaena—summer.
- Otocoris alpestris arcticola—winter.
- Otocoris alpestris merrilli—resident.

Major:
- Pituophis catenifer catenifer.
- Pedioecetes phasianellus colubrionus—resident.
- Buteo borealis calurus—resident.
- Buteo swainsoni—summer.
- Falco mexicanus—resident.

Minor:
- Crotalus oregonus.
- Ana platyrhynchos—winter.
- Ardea herodias treganzai—resident.
- Oxyechus vociferus vociferus—resident.
- Phasianus torquatus—resident.
- Zenaidura macoura marginella—resident.
- Aecipiter velox—resident.
- Falco sparverius sparverius—resident.
- Asyndesmus lewisi—summer.
- Colaptes cafer collaris—resident.
- Tyrannus tyrannus—summer.
- Tyrannus verticalis—summer.
- Sayornis saya—summer.
- Pica pica hudsonia—resident.
- Molothrus ater artemisiae—summer.

Reported:
- Pisobia hairdi—migrant.
- Ammodramus savannarum bimaculatus—summer.
- Chondrostes grammacus strigatus—summer.
- Spizella breweri—summer.
- Anthus rubescens—migrant.
- Perognathus lorida lorida.
- Lepus campestris townsendii.
- Lepus californicus wallawalla.

- Passer eulophus—migrant.
- Siala currucoides—summer.
- Canis latrans lestes.
- Taxidea taxus neglecta.
- Citellus townsendii.

- Icterus bullocki—summer.
- Euphagus cyanocephalus—resident.
- Astragalinus tristis palidus—resident.
- Spizella passerina arizonae—summer.
- Salpinctes obsolitus obsolitus—summer.
- Planesticus migratorius propinquus—resident.
- Sialia mexicana occidentalis—migrant.
- Mustela arizonensis.
- Mephitis occidentalis major.
- Reithrodontomysegs megalotis nigrescens.
- Peromyscus maniculatus gambelli.
- Thomomys columbianus.
- Citellus colombianus colombianus.

The bunchgrass habitat is characterized by the wheat bunchgrass (Agropyron spicatum). With this are associated the balsam root (Balsamorhiza sagittata), elarkia (Clarkia pulchella), Indian bullet (Lithospermum ruderale), phlox (Phlox sp.) and several lupines.
(Lupinus). Common sagebrush (Artemisia tridentata) and rabbit brush (Chrysothamnus viscidiflorus and Chrysothamnus nauseosus graveolens) occur sometimes in the bottoms of the drier ravines and on exposed hillsides. In damp situations, in the bottoms of ravines or on north hillsides, the rye grass (Elymus condensatus) forms large clumps. Where the land has been much pastured the yarrow (Achillea millefolium lanulosa) grows abundantly. There are also a number of kinds of mustards and many other less important plants. In damp places on the north hillsides a few woody shrubs may be found and these are more numerous the more closely the mountains are approached. The more important of these are the rose (Rosa) and service-berry (Amelanchier florada).

Near Prescott almost all of the bunchgrass hills have been plowed and are planted to wheat and barley. On alternate years the land is allowed to lie fallow. The bunchgrass which remains unplowed has been heavily pastured, so that the grass has been partially killed out and yarrow, lupine, and other weeds have greatly increased.

The bunchgrass association includes a considerable number of plains-loving species.

Traps set in bunchgrass habitat in the hills two miles southeast of Prescott produced, for a total of 148 "trap-nights," on June 27, July 1, and July 7, 1914, five Perognathus lordi lordi. One of these trap lines, of 33 traps, produced on the second day's trapping, July 8, one Peromyscus maniculatus gambelii.

ROCKY-SLOPE HABITAT AND ASSOCIATION (PRAIRIE AREA)

Major:
Crotalus oregonus.
Salpinetes obsoletus obsoletus—summer.

Minor:
Pituophis catenifer catenifer.
Zenaidura macoura marginella—resident.
Tyrannus verticalis—summer.
Petrochelidon numifrons numifrons—summer.
Buteo borealis calurus—resident.
Peromyscus maniculatus gambelii.
Falco sparverius sparverius—resident.
Sylvilagus nuttallii nuttallii.

There is usually very little sagebrush growing among the rocks in the rocky-slope habitat of the prairie area. The wheat bunchgrass (Agropyron spicatum) grows abundantly among the rocks wherever soil is present. Along Snake River a few shrubs grow among the
One of these are a serviceberry (*Amelanchier* sp.) and a rose (*Rosa* sp.).

The characteristic species of the rocky-slope association are few in number. Several species of birds find suitable nesting sites about rock cliffs.

About the rocks and rock cliffs near Lyon's Ferry 7 *Pomyscus maniculatus gambelii* were taken on June 23 and 24, 1914, from 115 trap-nights.

**COTTONWOOD-WILLOW HABITAT AND ASSOCIATION (PRAIRIE AREA)**

*Exclusive:*

- *Aneides iecanus.*
- *Bufo colombiensis.*
- *Hyla regilla.*
- *Perdix perdix*—resident.
- *Colinus virginianus virginianus*—resident.
- *Bonasa umbellus togata*—resident.
- *Accipiter cooperi*—summer.
- *Astur atricapillus striatulus*—winter.
- *Asio wilsonianus*—resident.
- *Bufo virginianus lagophonus*—winter.
- *Bufo virginianus occidentalis*—resident.
- *Dryobates villosus orius*—winter.
- *Dryobates pubescens homorus*—resident.
- *Phloctomus pileatus picinus*—migrant.
- *Archilochus alexandri*—summer.
- *Selasphorus rufus*—summer.
- *Cyanocitta stelleri annectens*—winter.
- *Corvus brachyrhynchos hesperis*—resident.
- *Hesperiphona vespertina montana*—winter.
- *Spinus pinus pinus*—winter.
- *Passer domesticus*—resident.
- *Zonotrichia leucophrys gambeli*—winter.
- *Spizella monticola ochracea*—winter.
- *Junco hyemalis shufeldti*—winter.
- *Melospiza melodia merrilli*—resident.
- *Passerella iliaca sechistacea*—summer.
- *Pipilo maculatus curtatus*—winter.
- *Zamia melanecephala*—summer.
- *Passerina amoenus*—summer.
- *Piranga ludoviciana*—summer.
- *Bombus pallida garrula*—winter.
- *Viresolva olivacea*—summer.
- *Viresolva gibla swainsoni*—summer.
- *Dendroica aestiva aestiva*—summer.
- *Dendroica auduboni auduboni*—migrant.
- *Dendroica townsendi*—migrant.
- *Oporornis tolmiei*—summer.
- *Geothlypis trichas occidentalis*—summer.
- *Icteria virens longicauda*—summer.
- *Wilsonia pusilla pileolata*—migrant.
- *Dumetella carolinensis*—summer.
- *Troglydytes aedon parkmani*—summer.
- *Xanthus hiealis pacificus*—migrant.
- *Chidemia familiaris montana*—winter.
- *Sitta carolinensis aculeata*—summer.
- *Sitta canadensis*—winter.
- *Penthestes atricapillus septentrionalis*—resident.
- *Penthestes gambeli gambeli*—winter.
- *Penthestes rufescens rufescens*—winter.
- *Regulus satrapa olivaceus*—winter.
Regulus calendula—calendula—winter.
Myadestes townsendi—winter.
Hylocichla guttata sequoensis—summer.

Major:
Phasianus torquatus—resident.
Zenaida macourea marginella—resident.
Accipiter velox—resident.
Falco sparverius—resident.
Pandion haliaetus carolinensis—summer.
Asyndesmus lewisi—summer.
Colaptes cafer collaris—resident.
Tyranthus tyrannus—summer.
Tyranthus verticalis—summer.
Sayornis saya—summer.
Nuttallornis borealis—migrant.
Myiouchus richardsoni richardsoni—summer.
Empidonax difficilis—summer.
Empidonax trailli—summer.
Pica pica hudsonia—resident.

Minor:
Rana pipiens brachycephala.
Pituophis catenifer catenifer.
Thamnophis elegans.
Ardon berolius tregannzi—resident.
Pedioecetes phasianellus columbianus—resident.
Buteo borealis calurus—resident.
Buteo swainsoni—summer.
Falco mexicanus—resident.
Streptoceryle aleyon caurina—resident.
Agelaux phoeniceus neutralis—summer.
Sturnella neglecta—resident.

Reported:
Basecanion constrictor vetustum.
Cryptoglaux acadica acadica—winter.
Lanius borealis—winter.

Passerculus sandwichensis alaudinus—migrant.
Stelgidopteryx serripennis—summer.
Sialia currucoides—summer.
Myotis yumanensis (?).
Myotis californicus californicus.
Lasius cinereus.
Canis latrans lestes.
Procyon psora pacifica.
Mustela vison engerumenes.
Taxidea taxus neglecta.
Citellus townsendii.
Castor canadensis canadensis.

Seiurus noveboracensis notabilis—migrant.
Lynx sp.
The growth of timber along the smaller streams of the prairie area does not extend far from the banks of the streams. In most places along the Touchet River trees do not naturally grow more than a quarter of a mile from the stream, and often the width of the habitat is much less than this. As the valley of the Touchet near Prescott is nearly a mile broad on the average, it is evident that the growth of trees and brush covers only a portion of the nearly level floor of the valley.

The most conspicuous plants of the habitat are the cottonwood (*Populus trichocarpa*) and willows (*Salix*) of several species. Other trees and shrubs which are common along the banks of the Touchet River near Prescott are the birch (*Betula microphylla*), alder (*Alnus rhombifolia*), chokecherry (*Prunus demissa*), thorn (*Crataegus brevispina*), service-berry (*Amelanchier florinda*), red osier (*Cornus stolonifera*), and syringa (*Philadelphus lewisii*). Less important species are the cascara sagrada (*Rhamnus purshiana*), ninebark (*Opulaster pauciflorus*), elder (*Sambucus glauca*), wild cherry (*Prunus camarginata*), snowberry (*Symphoricarpus sp.*), and clematis (*Clematis ligusticifolia*). Roses (*Rosa sp.*) occur commonly, especially along the outer margins of the timber. The cottonwood often makes very large trees with a height of 80 to 100 feet and with trunks three to four feet in diameter, but the other trees are much smaller. Under the trees there is nearly always a heavy growth of shrubby underbrush. A growth of shrubs also covers many small areas over which trees have not become dominant. Where the habitat has not been disturbed by man the thick tangle of smaller shrubs, thorns, and vines makes excellent refuges for birds and mammals.

The cottonwood-willow association is made up of a great number of species. A large number of these are closely restricted to the cottonwood-willow habitat. A few species which reach their greatest abundance in the cottonwood-willow association are found in lesser abundance in the bunchgrass association. Other species of greatest abundance in the bunchgrass association are sparingly represented in the cottonwood-willow association. Several species of birds nest or obtain shelter in the cottonwood-willow habitat but forage out into the adjacent bunchgrass.

Sixty traps set in the timber and brush along the Touchet River two miles east of Prescott caught on July 2, 1914, 2 *Reithrodontomys megalotis nigrescens*, 8 *Peromyscus maniculatus gambelii*, and 5 *Microtus nanus canescens*. 
WATER-MARGIN HABITAT AND ASSOCIATION (PRAIRIE AREA)

Exclusive:
Chrysemys bellii.
Grus mexicana—summer.

Major:
Rana pipiens brachycephala.
Thamnophis elegans.
Anas platyrhynchos—winter.
Nettion carolinense—winter.
Ardea herodias treganzai—resident.
Oxyecheus vociferus vociferus—resident.

Minor:
Spatula clypeata—migrant.
Streptoceryle aleyon caurina—resident.

Reported:
Baseanion constrictor vetustum.

Along the smaller streams of the prairie are numerous small gravel and dirt bars. These usually become very dry in summer and the grasses and herbs growing on them dry up, except in a few places at the level of the water or along the rare sloughs.

Along Snake River there is a considerable width of water-margin habitat, which is annually covered during the spring high water. There are few willows or shrubs along this stream and the water-margin habitat is broad except where cliffs reach the edge of the water. Near the edge of the water plants are almost absent, only a few herbs being found. On the higher level of the beach there is considerable driftwood and the ground is quite sandy. In among the logs the plants of the bunchgrass habitat appear and so also do a number of weeds.

The water-margin association of the prairie is chiefly made up of species which feed along the shores of the streams. Kingfishers and bank swallows are known to nest in holes in the soft dirt banks. Robins gather mud for plastering their nests from along the shore. A number of species from both cottonwood-willow and bunchgrass associations probably come to the water’s edge to drink.

Twenty traps set among rocks and driftwood on the shore of Snake River near Lyon’s Ferry caught two Peromyscus maniculatus gambelii on June 25, 1914.
AQUATIC HABITAT AND ASSOCIATION (PRAIRIE AREA)

Exclusive:
Charitonetta albeola—migrant.

Major:
Spatula alceata—migrant.
Streptoceryle aleyon caurina—resident.

Minor:
Anas platyrhynchos—winter.
Nettion carolinense—winter.
Pandion haliaetus carolinensis—summer.

Reported:
Mergus americanus—winter.
Mareca americana—migrant.
Fulica americana—migrant.

The Snake River is a large stream with a rapid current, but the other streams of the prairie area are small. These smaller streams have usually a rapid current, and quiet pools are rare. Lakes are entirely absent.

The kingfisher, osprey, and mink feed in the river habitat. Of the other forms observed on the rivers some probably feed in the habitat while others rest or take refuge there.

AERIAL HABITAT AND ASSOCIATION (PRAIRIE AREA)

Major:
Petrochelidon luniferous luniferous—summer.
Riparia riparia—summer.
Stelgidopteryx serripennis—summer.
Myotis yumanensis (?).
Myotis californicus californicus.
Lasiurus cinereus.

Minor:
Tyrannus tyrannus—summer.
Tyrannus verticalis—summer.
Sayornis saya—summer.
Myiochanes richardi richardi—summer.
Stetophaga ruticilla—summer.

Reported:
Chordeiles virginianus hesperis—summer.
Tachycineta thalassina lepida—summer.

The aerial association is represented in the prairie area by night-hawks, flycatchers, swallows, and bats. Some of the swallows are present at certain localities in immense numbers.
Blue Mountain Faunal Area and Fauna

Habits and Associations:

- Rocky-slope.
- Yellow-pine.
- Buckbrush.
- Alpine-fir.
- Lowland-fir.
- Water-margin.
- Aquatic.
- Aerial.

The Blue Mountain area is characterized by the dominance of conifer forests of several kinds. In the bottoms of the canons the forest is often very heavy and is dominated by the lowland fir (*Abies grandis*). On the tops of the higher ridges the alpine fir (*Abies lasiocarpa*) is the dominant type of tree. On the lower ridges and slopes and in the valleys of the more arid parts of the mountains the open yellow-pine type of forest prevails. It has been very difficult to determine the relation of the species of vertebrates to the different kinds of conifer forest. There seems to be a restriction of certain species to the higher ridges and of others to the canons, but no species seems to be clearly limited to any particular type of forest. It will require a very considerable amount of further study before the distribution of the vertebrates in the area is at all satisfactorily known.

The Blue Mountains are at the present time only partially covered by forests. Many of the steep, rocky slopes are nearly or quite bare of timber. Also, much of the region has been burned over, destroying the forests. Following the fires, or in some cases starting on bare slopes where probably no fire has been, there have sprung up extensive growths of deciduous brush. This brush is best developed near the summits of the ridges, but extends down the slopes for considerable distances, reaching the bottoms of the canons at the heads of a number of streams.

Species Characteristic of the Blue Mountain Fauna

- *Rana pretiosa.*
- *Charina bottae.*
- *Dendrapagmus obscurus richardsoni.*
- *Piceoides americana dorsalis.*
- *Sphyrapicus thyroideus.*
- *Philecotomus pileatus picinus.*
- *Stellula calliope.*
- *Empidonax hammondii.*
- *Perisoereus canadensis capitalis.*
- *Nyctrograga columbiana.*
- *Junco hyemalis shufeldti.*
- *Dendroica townsendi.*
- *Cinclus mexicana unicolor.*
- *Namus hyemalis pacificus.*
- *Sitta pygmaea pygmaea.*
- *Peniestes gambeli gambeli.*
- *Regulus satrapa olivaceus.*
- *Myalestes townsendi.*
- *Neosorex navigator navigator.*
- *Myotis longicrus.*
- *Ursus altifrons.*
- *Vulpes macrourus.*
- *Martes sp.*
- *Mustela cicognani lepta.*
Evotomys gapperi saturatus.  
Microtus mordax mordax.  
Thomomys fuscus fuscus.  
Zapus princeps oregonus.  
Callospermophilus chrysodeirus

The Blue Mountain fauna contains a number of species which in southeastern Washington are not found in the other faunal areas. Many of these species are characteristic of conifer forests elsewhere.

ROCKY-SLOPE HABITAT AND ASSOCIATIONS (BLUE MOUNTAIN AREA)

Exclusive:
Salpinctes obsoletus obsoletus—summer.

Major:
Peromyscus maniculatus gambelii.  
Callospermophilus chrysodeirus chrysodeirus.

Minor:
Fledo sparverius sparverius—summer.  
Citellus columbianus columbianus.  
Eutamias amoenus amoenus.

Thomomys fuscus fuscus.

Outcroppings of basaltic rock are common in the Blue Mountains. Besides the numerous small rock bluffs there are many rocky slopes covered by broken pieces of rock of various sizes. These rocky slopes often cover large areas. On exposed slopes grasses and small shrubs such as ninebark (*Opulaster pauciflorus*) grow among the rocks and in many places there are scattered yellow-pine trees (*Pinus ponderosa*). The habitat in such places often gradually gives way to the yellow-pine forest habitat.

Seventy-eight traps set on a rocky slope near Hompeg Falls captured 23 *Peromyscus maniculatus gambelii* and 2 *Eutamias amoenus amoenus* on July 24, 1914.

YELLOW-PINE HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)

Minor:
Dendrapagus obscurus richardsoni—resident.  
Fledo sparverius sparverius—summer.  
Sphyrapicus thyroideus—summer.  
Empidonax wrighti—summer.  
Corvus brachyrhynchos hesperis—summer.  
Spizella passerina arizonae—summer.  
Junco hyemalis shufeldti—summer.

Reported:
Sialia mexicana occidentalis—summer.
Over the foothills and exposed, lower slopes of the Blue Mountains the yellow pine (Pinus ponderosa) forms the dominant forest. This species seems to be able to endure much drier conditions than any of the other conifers. It is limited in vertical range and is not found on the higher parts of the Blue Mountains. As found near Hompeg Falls yellow pine is in many places associated with Douglas spruce (Pseudotsuga taxifolia). The trees in this forest usually grow rather far apart. On the exposed slopes there is little underbrush, but the ground is stony or covered by grasses and prairie plants. On somewhat sheltered slopes a considerable amount of underbrush may be developed. In this the ninebark (Opulaster pauciflorus) is most abundant and in places on eastern slopes forms a thick covering to the ground. Other shrubs such as service-berry (Amelanchier), currants (Rubus), spirea (Spirea), willows (Salix), and alders (Alnus) occur also. On the higher slopes the forest is heavier and Douglas spruce tends to become dominant.

No trapping was done in the yellow-pine habitat and only incidental observations were made in this type of forest. The animal inhabitants are surely much more numerous than indicated in the above list.

BUCKBRUSH HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)

Exclusive:
Taxidea taxus neglecta.

Major:
Buteo borealis calurus—summer.  Zapus princeps oregonus.
Spizella passerina arizonae—
summer.
Junco hyemalis shufeldti—summer.
Thomomys fusicus fusicus.

Minor:
Colaptes cafer collaris—summer.  Citellus columbianus columbianus.
Peromyscus maniculatus gambeli.  Callospermnophilus chrysodeirus
Evotomys gapperi saturatus.  chrysodeirus.
Microtus mordax mordax.

Reported:
Vulpes macrourus.  Lya sp.

The principal plant of the buckbrush habitat is the buckbrush (Ceanothus velutinus). Associated with this are often willows (Salix), alders (Alnus), and a number of other shrubs. Near the tops of the ridges stunted aspens (Populus tremuloides) sometimes appear.
The brush often grows so thickly that it is very difficult to force one's way through it, but it is likely to be in clumps and there are many open places. Rock outcroppings are numerous. The height of the brush is usually from four to eight feet, but around springs or damp places it may grow much higher. There is much down and partly burned timber in some parts of the habitat, and an occasional stump or tree has survived the fires. Young conifers are springing up in places and of these the lodgepole pine (Pinus murrayana) is most numerous on the ridges. On the lower slopes the buckbrush habitat overlaps in some places the range of the yellow pine (Pinus ponderosa) and isolated old yellow pine trees may often be found growing in among the shrubs.

Trapping on August 3 and August 9, 1914, in buckbrush on the ridge near Twin Buttes R.S. produced 1 Peromyscus maniculatus gambelii, 1 Evotomys gapperi saturatus, 6 Zapus princeps oregonus, and 2 Eutamias amoenus amoenus from a total of 50 trap-nights.

ALPINE-FIR HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)

**Exclusive:**

- Dryobates villosus oriuss—summer.
- Nueifruga columbiana—resident.

**Major:**

- Colaptes cafer collaris—summer.
- Spinus pinus pinus—summer.
- Penthestes gambeli gambeli—summer.

**Minor:**

- Dendrapagus obscurus richardsonii—resident.
- Buteo borealis eulurus—summer.
- Falco sparverius sparverius—summer.
- Sphyrapicus thyroideus—summer.
- Cyanocitta stelleri annectens—resident.
- Spizella passerina arizonae—summer.
- Junco hyemalis shufeldti—summer.
- Narrus hiemalis pacificus—summer.
- Regulus satrapa olivaceus—summer.

**Reported:**

- Picoides arcticus.
- Picoides americanus dorsalis—summer.
- Picoides villosus oriuss—summer.
- Sitta canadensis—summer.
- Sialia currucoides—summer.
- Evotomys gapperi saturatus.
- Myadestes townsendsii—summer.
- Planesticus migratorius propinquus—summer.
- Peromyscus maniculatus gambelii.
- Microtus morax mordax.
- Thomomys fuscus fuscus.
- Zapus princeps oregonus.
- Eutamias amoenus amoenus.
- Callospermophilus chrysodeirus chrysodeirus.
- Scirius hudsonicus richardsonii.
- Lepus bairdii bairdii.
- Odocoileus hemionus hemionus.
- Passerella iliaca schistacea—summer.
- Ursus altifrontalis.
- Lynx sp.
The alpine fir (Abies lasiocarpa) forms extensive forests on the higher ridges of the Blue Mountains. It does not grow in the canyons or on the lower slopes, so it may be considered the characteristic tree of the ridge forest. Alpine-fir forest is abundantly developed in many of the sheltered coves near the tops of the ridges. In these coves the ground is fairly moist. The forest developed is not dense, and there is plenty of room to walk between the trees. The size reached by the alpine firs is not very large and few of the trunks would measure over fifteen inches in diameter. Some lodgepole pines (Pinus murrayana) are often mixed with the alpine firs and in places where new growth is springing up lodgepole may be the dominant tree. There is usually very little undergrowth under the alpine-fir forest, there being commonly only a few very low shrubs. On the ridges alpine fir is less common and the forest is more open and commonly includes many Douglas spruces (Pseudotsuga taxifolia). The ground here is drier than in the coves and there is much exposure to sun and wind. The trees occur singly or in small groups. Douglas spruce is the dominant tree in the most exposed places.

In many of the coves and on protected slopes near the tops of the ridges forests of western larch (Larix occidentalis) are developed. This may be developed as a pure forest or may be mixed with alpine firs or Douglas spruces. The pure larch forest is very open and underbrush is scanty and the forest floor may be covered entirely by grasses. The trees reach a good size with trunks several feet in diameter. On the damper slopes Douglas spruce usually dominates over the larch and a rather dense forest is developed. This contains much more underbrush and the ground is commonly quite damp. In such places Engelmann spruces (Picea Engelmannii) may occur commonly.

Although the alpine-fir association is made up of a considerable number of forms very few are restricted to the association. Neither do very many reach their greatest abundance in this habitat.

Trapping in forest habitats on the ridges near Twin Buttes R.S. on July 28, July 29, and August 3, 1914, produced 8 Peromyscus maniculatus gambelii, 1 Microtus mordax mordax, 4 Eutomys gapperi saturatus, and 1 Eutamias amoenus amoenus from a total of 181 trap-nights.
LOWLAND-FIR HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)

Exclusive:
- Phloeotoimis pileatus picinus—summer.
- Melospiza melodia merrilli—summer.
- Penthestes atricapillus septentrionalis—summer.

Major:
- Dendrapagrus obscurus richardsoni—resident.
- Falco sparverius sparverius—summer.
- Sphyrapicus thyroideus—summer.
- Empidonax difficilis difficilis—summer.
- Empidonax hammondii—summer.
- Empidonax wrightii—summer.
- Nannus hiemalis pacificus—summer.
- Cyanocitta stelleri annectens—resident.
- Corvus brachyrhynchos hesperis—summer.
- Piranga ludoviciana—summer.
- Regulus satrapa olivaceus—summer.
- Myadesus townsendi—summer.
- Planesticus migratorius propinquus—summer.
- Citellus columbianus columbianus.
- Sciurus hudsonicus richardsonii.

Minor:
- Thamnophis elegans.
- Colaptes cafer collaris—summer.
- Spinus pinus pinus—summer.
- Spizella passerella arizonae—summer.
- Junco hyemalis shufeldti—summer.
- Peromyscus maniculatus gambeli.
- Evotomys gapperi saturatus.
- Microtus morlas morlas.
- Thomomys fuscus fuscus.
- Eutamias amoeneus amoeneus.
- Castor canadensis canadensis.
- Lepus bairdii bairdii.
- Odocoileus hemionus hemionus.

Reported:
- Bufo columbiensis.
- Charina bottae.
- Bonasa umbellus togata—resident.
- Otus asio macfarlanei—summer.
- Stellula calliope—summer.
- Passerella iliaca schistacea—summer.
- Dendroica townsendi—summer.
- Oporornis tolmiei—summer.
- Certhia familiaris montana—summer.
- Penthestes rufescens rufescens—summer.
- Hylocichla ustulata swainsoni—summer.
- Sorex vagrans dobsoni.
- Martes sp.
- Mustela eicognanii lepta.

In the deeper canyons of the Blue Mountains the lowland fir (Abies grandis) is the dominant tree. These trees reach quite a large size, trunks estimated at over four feet in diameter being seen. Near Hompeg Falls this type of forest is well developed. However, there are many open places washed out by the stream or due to the action of former fires. Associated with the lowland fir are Douglas spruce (Pseudotsuga taxifolia), yellow pine (Pinus ponderosa), western yew
(Taxus brevifolia), western larch (Larix occidentalis), cottonwood (Populus trichocarpa), and birches (Betula microphylla). A few Englemann spruces (Picea Englemanni) occur and one silver pine (Pinus monticola) was seen. There is a small amount of underbrush, composed chiefly of dwarf maples (Acer glabrum) and alders (Alnus sp.). The lowland-fir type of forest occurs only in the bottoms of deep canons and in very damp places, and does not extend up on the mountain slopes.

On sheltered lower slopes the western larch and Douglas spruce make up the larger part of the forest. Sometimes one and sometimes the other is dominant. The larch is best developed in damp situations, while the Douglas spruce covers drier slopes. Near Hompeg Falls the larch-Douglas spruce forest occupies the north slopes of the side ravines which branch from the main canyon. The larch largely occupies the bottoms of the ravines, while the Douglas spruce extends further up the sides, and towards the tops of the ridges spreads out to form a more extended forest. The Douglas spruce forest is usually fairly dense in this situation and many of the slopes which it covers are very steep. Under the heaviest forest of this kind there is no underbrush, but the ground is entirely covered by dead needles. In other places the forest is more open and more or less brush occurs, in which the alder (Alnus sp.) is the most abundant type. The larch forest is more open and usually does not have a heavy growth of underbrush.

Along Butte Creek, where the bottom of the narrow canyon has been much washed over by the stream, much of the lowland-fir forest has been washed out and is replaced in patches by a deciduous forest which is notable for the thickness of the underbrush. The dominant trees are cottonwood (Populus trichocarpa) and the willows (Salix). The brush was composed principally of alder (Alnus sp.), thorn (Crataegus brevispina), service-berry (Amelanchier floridana), wild cherry (Prunus demissa), red osier (Cornus stolonifera), dwarf maple (Acer glabrum), and snowberry (Symphoricarpus).

Traps set in the lowland-fir habitat near Hompeg Falls on July 23, 25, and 26, 1914, caught 1 Sorex vagrans dobsoni, 23 Peromyscus maniculatus gambelii, 1 Evotomys gapperi saturatus, and 2 Eutamias amoenus amoenus from a total of 201 trap-nights.
WATER-MARGIN HABITAT AND ASSOCIATION  
(BLUE MOUNTAIN AREA)  

Exclusive:  
Rana pretiosa.  

Major:  
Thamnophis elegans.  
Mierotus mordax mordax.  

Minor:  
Cinelus mexicana unicolor—summer.  
Evotomys gapperi saturatus.  

Reported:  
Telmatodytes palnstris plesius—summer.  

Along the streams of the Blue Mountain gravel or mud bars are rare and the forest often reaches the edge of the stream and partially overhangs the water. Springs are quite numerous in the bottoms of the canons and these often produce moist areas which make little swamps. Such swamps are often shaded by the heavy lowland fir trees and have only a low growth of vegetation, but in more open spots grasses and horsetails as well as smaller herbs make a luxuriant growth. Swamps also occur along small sloughs diverted from the main streams or about ponds caused by the damming of some stream by a beaver dam or by the natural accumulation of drift.  

AQUATIC HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)  

Major:  
Streptoceryle alecyon caurina—Cinelus mexicana unicolor—summer.  

Minor:  
Castor canadensis canadensis.  

The streams in the Blue Mountains are all small and have swift currents. There are many rapids and low falls and the pools are small. There is very little extent of open water free from the margins of the streams.
AERIAL HABITAT AND ASSOCIATION (BLUE MOUNTAIN AREA)

Minor:
Empidonax difficilis difficilis—Empidonax hammondii—summer.
Empidonax wrighti—summer.

Reported:
Chordeiles virginianus hesperis—Myotis longicornis.

Flycatchers, nighthawks, and bats make up the members of the aerial association in the Blue Mountains. Swallows were seen flying over some of the ridges but the species was not determined.

CLIMATE

Climatological records have been taken for a number of years in southeastern Washington by Weather Bureau Stations. The accumulated data have been kindly furnished by the United States Weather Bureau. A summary of this is presented in Table 1. The data is most complete for the prairie area and least so for the Blue Mountains. The stations are usually located in towns and so the records do not indicate the conditions in any particular habitat, but they do give a basis for comparing the climatic conditions in different faunal areas.

Table 2, which gives the climatological data for each month at Walla Walla, is presented to illustrate the weather conditions in the region at the different seasons. In southeastern Washington the precipitation is unequally distributed throughout the year, being greatest in winter, while in summer very little rain falls. The summers are very hot, and the winters moderately cool with occasional very cold periods of short duration. There is a considerable daily range of temperature and even in the hottest weather the nights are cool. The humidity of the air is very low in summer, but is higher in winter. There is an abundance of sunlight in summer, while in winter the light is much weaker. Winds are quite common and especially in spring may be very strong. Their usual direction is from the southwest. A peculiar wind which deserves notice is the "chinook." This is a dry, warm wind from the southwest which may start at any time of the day or night in winter. It rapidly melts the snow and dries the surface of the ground. In consequence, snow seldom lies for any length of time upon the ground, except in the Blue Mountains.
<table>
<thead>
<tr>
<th>Year</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- July, August, September, October, November, and December are the months of the year.
- 1916 is the year mentioned in the table.

**Table 1:**

<table>
<thead>
<tr>
<th>Station</th>
<th>Rainfall</th>
<th>Temperature</th>
<th>Evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dixie (near Tumwater)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dishman's Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumwater</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Comparison of Climatological Data from Southeastern Washington**

**Rainfall:**
- Dixie (near Tumwater): 1916
- Dishman's Point: 1916
- Tumwater: 1916

**Temperature:**
- Dixie (near Tumwater): 1916
- Dishman's Point: 1916
- Tumwater: 1916

**Evaporation:**
- Dixie (near Tumwater): 1916
- Dishman's Point: 1916
- Tumwater: 1916
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>33.2</td>
<td>36.4</td>
<td>44.0</td>
<td>52.8</td>
<td>60.7</td>
<td>68.2</td>
<td>74.1</td>
<td>73.8</td>
<td>65.4</td>
<td>53.7</td>
<td>42.9</td>
<td>36.0</td>
<td>53.4</td>
</tr>
<tr>
<td>Mean maximum</td>
<td>38.0</td>
<td>44.0</td>
<td>54.0</td>
<td>63.0</td>
<td>72.0</td>
<td>78.0</td>
<td>87.0</td>
<td>87.0</td>
<td>75.0</td>
<td>64.0</td>
<td>50.0</td>
<td>43.0</td>
<td>63.0</td>
</tr>
<tr>
<td>Absolute maximum</td>
<td>70.0</td>
<td>69.0</td>
<td>79.0</td>
<td>92.0</td>
<td>100.0</td>
<td>105.0</td>
<td>111.0</td>
<td>113.0</td>
<td>100.0</td>
<td>87.0</td>
<td>78.0</td>
<td>64.0</td>
<td>113.0</td>
</tr>
<tr>
<td>Mean minimum</td>
<td>27.0</td>
<td>30.0</td>
<td>36.0</td>
<td>42.0</td>
<td>49.0</td>
<td>54.0</td>
<td>60.0</td>
<td>60.0</td>
<td>52.0</td>
<td>44.0</td>
<td>36.0</td>
<td>32.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Absolute minimum</td>
<td>-17.0</td>
<td>-15.0</td>
<td>2.0</td>
<td>29.0</td>
<td>34.0</td>
<td>40.0</td>
<td>45.0</td>
<td>41.0</td>
<td>36.0</td>
<td>24.0</td>
<td>-9.0</td>
<td>-2.0</td>
<td>-17.0</td>
</tr>
<tr>
<td>Mean daily range</td>
<td>12.0</td>
<td>14.0</td>
<td>17.6</td>
<td>21.2</td>
<td>22.8</td>
<td>24.4</td>
<td>27.4</td>
<td>26.8</td>
<td>23.6</td>
<td>19.8</td>
<td>14.4</td>
<td>11.0</td>
<td>19.6</td>
</tr>
</tbody>
</table>

**Precipitation, inches**

<table>
<thead>
<tr>
<th>Mean</th>
<th>2.92</th>
<th>1.62</th>
<th>1.85</th>
<th>1.57</th>
<th>1.80</th>
<th>1.17</th>
<th>0.41</th>
<th>0.44</th>
<th>0.93</th>
<th>1.46</th>
<th>2.01</th>
<th>2.06</th>
<th>17.34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>4.99</td>
<td>2.75</td>
<td>4.17</td>
<td>3.88</td>
<td>4.81</td>
<td>3.61</td>
<td>1.47</td>
<td>2.16</td>
<td>2.60</td>
<td>4.02</td>
<td>5.15</td>
<td>4.41</td>
<td>23.07</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.17</td>
<td>0.11</td>
<td>0.69</td>
<td>0.04</td>
<td>0.21</td>
<td>0.04</td>
<td>T.</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
<td>0.01</td>
<td>0.50</td>
<td>11.66</td>
</tr>
<tr>
<td>Average snowfall</td>
<td>9.5</td>
<td>6.2</td>
<td>1.5</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>T.</td>
<td>1.9</td>
<td>5.1</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td>Rainy days</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>14</td>
<td>112</td>
</tr>
<tr>
<td>Sunshine, %</td>
<td>26</td>
<td>38</td>
<td>62</td>
<td>70</td>
<td>69</td>
<td>75</td>
<td>57</td>
<td>81</td>
<td>69</td>
<td>62</td>
<td>37</td>
<td>22</td>
<td>58</td>
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</table>

**Relative humidity—**

<table>
<thead>
<tr>
<th>5 A.M., %</th>
<th>86</th>
<th>84</th>
<th>80</th>
<th>72</th>
<th>72</th>
<th>69</th>
<th>58</th>
<th>56</th>
<th>67</th>
<th>75</th>
<th>79</th>
<th>85</th>
<th>74</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 P.M., %</td>
<td>83</td>
<td>75</td>
<td>62</td>
<td>48</td>
<td>44</td>
<td>39</td>
<td>27</td>
<td>29</td>
<td>44</td>
<td>59</td>
<td>73</td>
<td>83</td>
<td>56</td>
</tr>
<tr>
<td>Mean daily range, %</td>
<td>3</td>
<td>9</td>
<td>18</td>
<td>24</td>
<td>28</td>
<td>30</td>
<td>31</td>
<td>27</td>
<td>23</td>
<td>16</td>
<td>6</td>
<td>2</td>
<td>18</td>
</tr>
</tbody>
</table>

**Wind—**

<table>
<thead>
<tr>
<th>Average hourly velocity</th>
<th>7</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>7</th>
<th>7</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Highest velocity</td>
<td>45</td>
<td>50</td>
<td>45</td>
<td>50</td>
<td>40</td>
<td>65</td>
<td>52</td>
<td>50</td>
<td>38</td>
<td>44</td>
<td>46</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Prevailing direction</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
<td>s.</td>
</tr>
</tbody>
</table>
The growing season of southeastern Washington is comparatively long. At Prescott it is often possible to plant the seeds of hardy vegetables in the open ground in the first week of March or earlier. The frostless season is also comparatively long (Table 3), although irregular frosts late in spring often do considerable damage to fruit and garden crops.

**TABLE III**

<table>
<thead>
<tr>
<th>Station—</th>
<th>Columbia Basin sagebrush area</th>
<th>Columbia Basin prairie area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kennewick</td>
<td>Touchet</td>
</tr>
<tr>
<td>Length of record</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Autumn first frost in</td>
<td>Oct. 15</td>
<td>Sept. 13</td>
</tr>
<tr>
<td>Average date last killing frost in Spring</td>
<td>Apr. 28</td>
<td>Apr. 23</td>
</tr>
<tr>
<td>Earliest date of killing frost in Autumn</td>
<td>Sept. 25</td>
<td>Aug. 25</td>
</tr>
<tr>
<td>Latest date of killing frost in Spring</td>
<td>May 25</td>
<td>May 8</td>
</tr>
<tr>
<td>Average season between frosts—days</td>
<td>170</td>
<td>143</td>
</tr>
<tr>
<td>Frostless season—days</td>
<td>123</td>
<td>109</td>
</tr>
</tbody>
</table>

No records of humidity are available from the various habitats of Walla Walla and Columbia counties, but in Whitman County, Washington, and in the Thatuna Hills of Idaho, Weaver (1914) has obtained records of the rate of evaporation as determined by a porous-cup atmometer during the summer. These records show that the rate of evaporation is highest in the rocky-slope habitat and that in the other habitats it decreases in the following order: prairie, S.W. exposure; prairie, N.E. exposure; yellow pine; fir-tamarack; cedar. The sagebrush habitat was not included in these observations.

The prominent features of the climate of the sagebrush area is the small annual precipitation and the high temperature of summer. No records of wind velocities are available from these stations, but it is known that the area is subject to strong winds which act powerfully to drift the sandy soil.

The prairie area as a whole shows a lower average temperature and particularly a lower temperature in the summer months than is found in the typical part of the sagebrush area as recorded at Kennewick. Also the prairie area shows a greater rainfall and this rainfall is greater the more closely the Blue Mountains are approached. It may
be said also that the winds of the prairie area are probably less strong than those of the sagebrush area.

Climatological data from the Blue Mountains is very scanty and consists only of records of precipitation at two stations on the lower ridges. It is evident that the precipitation is very much higher on these ridges than in the prairie area. The temperature of the area is considerably lower than in the adjacent areas, but no definite records could be obtained. There is a much greater snowfall in the mountains than in the lower country and the snow lies on the mountains all winter and often until late in the spring.

INFLUENCE OF ARTIFICIAL CONDITIONS

The animal habitats of southeastern Washington have been greatly altered by the work of man. Farming is extensively carried on and in the prairie area a very large percentage of the land is under cultivation. Irrigation is also practiced in the valleys of both the prairie and sagebrush areas. All of the land not under direct cultivation has been heavily grazed by cattle and stock. Part of the timber along the streams has been cut down and much of the brush has been cleared away. Houses have been built and shade trees planted in places where formerly no trees grew. In the Blue Mountains there have been many destructive forest fires and much timber has been cut. In the region it is now difficult to find an area of any size which shows the primitive conditions in completeness.

These changes in the environment have caused great changes in the abundance of the different species of vertebrates. Some species are greatly reduced in numbers or have been exterminated in the region; others have held their own or have increased to some extent. The species of the open fields have probably suffered most by the occupation of the region by man. Extensive hunting has operated to reduce in number or exterminate some of the game animals. On the other hand, a few game species have been intentionally introduced by man, and a few obnoxious species have been unintentionally introduced.
COMPARISON WITH OTHER SCHEMES OF ECOLOGICAL DISTRIBUTION

The vertebrate associations as here recognized cannot be compared directly with other schemes of vertebrate associations, because the local distribution of no other region of similar climatic conditions has been studied by the associational method. However, a comparison can be made with several schemes of ecologic distribution used in other regions.

Weaver (1914) has studied the plant associations found in Whitman County, Washington, and the Thatuna Hills of adjacent Idaho, where the vegetational features are somewhat similar to those of the region we are studying. He recognizes the following plant associations: bunchgrass-rimrock association, prairie association, yellow pine association, fir-tamarack association, and cedar association. The bunchgrass-rimrock association corresponds to the rocky-slope association of the prairie area as used in this paper. The prairie association is the same as our bunchgrass association.

Gates (1911, pp. 9-11) has included flycatchers and swallows in the aquatic association because they capture insects in the air over the water. However, such forms cannot be considered to be aquatic in any sense of the term and we have therefore placed them in the aerial association.

Shelford (1913, p. 262) in the Chicago area has recognized a distinct animal community in the narrow border of shrubs and weeds occurring between the prairie and the forest proper. This forest-margin community is very distinct in many regions, but it has been thought undesirable to recognize it as an association between the willow associations and the bunchgrass or sagebrush associations of southeastern Washington. The willow habitat in the region is usually narrow and is often rather open. It resembles in these respects the forest margin rather than a true forest habitat. In the Blue Mountains the yellow pine forests pass over into the prairie usually without any indication of a marginal habitat. The other conifer forests of the area are sometimes bordered by an extensive growth of brush and this has been called the buckbrush habitat.

Kennedy (1914) in a study of the birds of the Yakima Valley, Washington, gives separate lists of the birds of the sagebrush and of those found along the streams. No attempt is made to distinguish
those of the timber and brush from those of the stream shore. The Yakima Valley belongs in the Columbia Basin sagebrush faunal area and the environmental conditions of the sagebrush in that valley seem to be very similar to those of the sagebrush in western Walla Walla County. Of the species of birds stated to be characteristic of the sagebrush of the Yakima Valley all except five, Sayornis sayus, Otocoris alpestris merrilli, Poecetes gramineus confinis, Spizella breweri, and Oreoscoptes montanus, have been reported from the sagebrush of western Walla Walla County.

The roadside association recognized by Jackson (1914, pp. 23, 24) in the conifer forests of Wisconsin belongs to a habitat at the edge of a clearing in a heavy forest and seems to have many features in common with the forest-margin communities recognized in other regions. Such an association might be recognizable in the Blue Mountains, but roads are few in that area and it is impossible to define such an association without more data than is at present at hand.

Animal habitats are sometimes divided into strata. Shelford (1913, p. 165) recognizes five strata in some terrestrial habitats, extending from the subterranean stratum to the tree stratum. No attempt has been made to divide the habitats of southeastern Washington into strata, although various strata could undoubtedly be distinguished.

Much has been made of the succession of animal species due to the change in habitats induced or correlated with plant succession (Adams, 1908). In southeastern Washington many of the associations and habitats seem to have reached an equilibrium and succession is not very prominent. In the sagebrush and prairie areas the rocky-slope habitat tends to change to the sagebrush or bunchgrass habitat. Modifications which occur by the shifting of the stream channels produce changes in the riparian associations. Floods sometimes wash out part of the willow habitat and even at times part of the bunchgrass or sagebrush habitat. Also, the willow habitat tends to invade the river beds. At every shifting of the stream channel there are changes in the extent and position of the water-margin habitat. In the Blue Mountains the conditions are probably less stable and changes in habitats are probably in more active progress. Weaver (1914) has suggested that in Whitman County, Washington, and in the adjacent parts of Idaho the succession is the following direction: (1) bunchgrass; (2) yellow pine; (3) Douglas spruce and western larch; (4) cedar. Cedar does not occur on the Blue Mountains as a distinct habitat, but its place is probably taken by the alpine fir.
Associations may, for ease in comparison, be grouped in either of several different manners. Grinnell and Swarth (1913, pp. 218-220) have considered two kinds of associations, major and minor. Each major association is made up of one or more minor associations. Major associations recognized in the San Jacinto area of California are: chaparrel, forest, riparian, rupestrine, meadow and sand-flat. It is considered that a given major association may occur in several faunal areas and life-zones, but its minor divisions are much more restricted. The associations of southeastern Washington recognized in this paper would belong in general to the class of major associations according to this classification, for no attempt has been made in most cases to work out the finer divisions of the associations.

Another method of comparing associations is to group them into formations. A formation is stated to be a group of physiologically similar associations (Shelford, 1913, p. 38). Formations may themselves be combined into still larger groups. The classification of the formations of the world is still in its preliminary stages. Shelford (1911, pp. 604, 605) has proposed a classification of formations with which it will be illuminating to compare the associations of southeastern Washington. The conifer forest associations of the Blue Mountains must be referred to his second division, formations of forests with narrow, thick leaves. The bunchgrass associations belong to the third division, formations of savannas and grasslands, and to the subdivision c. cool steppe formations. The associations of the rivers belong to division seven, formations of fresh water. The other associations are harder to place in the system.

ZOOGEOGRAPHIC POSITION OF SOUTHEASTERN WASHINGTON

The accompanying table (Table 4) shows the general relations of the vertebrate faunas of southeastern Washington to the faunas of adjacent regions. In this table the occurrences in the adjacent regions are given of those species whose ranges are well known and which have been definitely identified from Walla Walla County or Columbia County. Of the birds only those species occurring in the regions in summer and which are presumably breeding are included.

The Columbia Basin sagebrush fauna shows in this comparison much greater affinity to the fauna of the Great Basin than to the
faunas of other adjacent regions. It must be placed in the Great Basin district of Allen (1892, p. 237). As the Great Basin seems to have had a rather stable climate and a continuous sequence of forms for a long period of geologic time it is allowable to suppose that many of the forms of the Columbia Basin sagebrush fauna originated in the Great Basin and migrated into southeastern Washington at some period later than the Middle Miocene.

The fauna of the Blue Mountains is most closely related to the fauna of the Rocky Mountains. The Blue Mountain area must be placed in a subdivision of the Canadian subregion of Holarctica (Lydekker, 1896, p. 360). It seems logical to suppose that the fauna of this area has been derived largely from the North.

The fauna of the Columbia Basin prairie area is related to the faunas of both the Rocky Mountains and the Great Basin. It has seemingly been produced largely by an admixture of elements from these two places.

The maintenance of the distinctness of the fauna of the Columbia Basin prairie area must be due to the climatic barriers which separate it from the Columbia Basin sagebrush fauna and from the Blue Mountain fauna. Differences in temperature and rainfall and perhaps other factors are effective in separating the fauna of the prairie from that of the Blue Mountains. The difference in temperature between the prairie area and the sagebrush area in southeastern Washington is not marked and the difference in rainfall is probably the chief factor separating the faunas of the two places.
Life-Zones of Southeastern Washington

The sagebrush region about Wallula belongs certainly to the Upper Austral life-zone. It is placed in this zone on the basis of the flora by Piper (1906, p. 35). Merriam (1898, p. 30) states that a part of the Upper Austral zone in Washington, in the valleys of the Snake and Columbia rivers, has so hot a climate that it might almost be placed in the Lower Austral zone.

The Columbia Basin prairie area must be placed in the Transition life-zone although it contains a strong Upper Sonoran element. In the area there are no species which have not elsewhere been reported to occur in zones above the Upper Sonoran. Four breeding species, Mustela arizonensis, Citellus columbianus columbianus, Passerella iliaca schistacea, and Oporornis tolmiei, are characteristic of the Transition or higher life-zones. Further, the area has been placed in the Transition life-zone by Piper (1906, p. 48) on the basis of the flora.

In the Transition life-zone must be included the bunchgrass hills south of Wallula and also those north of the Walla Walla River east of Nine-mile. Sagebrush as a dominant habitat extends up the Walla Walla Valley as far as Touchet, and this would seem to mark the eastern limit of the Upper Austral life-zone in the region. Piper (1906, map) extends a tongue of Upper Austral as far east as Walla Walla, but there seems no justification for this, for the plant and animal associations at Walla Walla, so far as can be judged under the present altered conditions, are essentially the same as in the bunchgrass region to the north and east. Piper also places the cañon of Snake River, for the whole of the distance that this extends through Washington, in the Upper Austral life-zone. However, in the cañon of Snake River at Lyon’s Ferry sagebrush was not the dominant vegetation and the characteristic vertebrates of the Upper Austral life-zone found at Wallula were not present.

Temperature records of the kind used by Merriam (1894) in defining the limits of the life-zones are available only for Walla Walla (Bigelow, 1908, p. 90). At Walla Walla daily normal temperatures of 43° F. and above occur throughout the period between March 12 and November 16, giving an average growing season of 249 days. The sum of the daily normal temperatures for this season is 15352° F. The hottest six weeks of summer at Walla Walla are the last three weeks of July and the first three weeks of August. The average tem-
perature of this period is 74.8° F. On the basis of temperature it would be necessary to place Walla Walla and the Columbia Basin prairie area about midway in the Upper Austral life-zone (Merriam, 1894, p. 236), but the fauna indicates closer affinity to the Transition life-zone. In this matter the fauna is probably a better criterion than the temperature, because the life-zones are founded primarily on faunal relationships.

The yellow-pine areas of the lower parts of the Blue Mountains in Columbia County make up the timbered division of the Transition life-zone as recognized by Piper (1906, p. 35).

The part of the Blue Mountain area above the Transition life-zone belongs to the Boreal region of Merriam. Piper (1906, pp. 58, 60, 62) recognizes Canadian, Hudsonian, and Arctic life-zones in the flora of these mountains. In the vertebrate fauna the Arctic life-zone cannot be distinguished and the Canadian and Hudsonian life-zones are very difficult to separate. If the Hudsonian life-zone be recognized as distinct it must be restricted to the summits of the ridges. Here is found the alpine fir (Abies lasiocarpa), a characteristic Hudsonian tree (Piper, 1906, p. 60). The vertebrate species found on these higher ridges, and not reported from lower altitudes, are Zapus princeps oregonus, Picoides americanus dorsalis, and Nucifraga columbiana. None of these species can be considered strictly Hudsonian. It seems best to place the part of the Blue Mountain area above the Transition life-zone in a single life-zone, the Boreal.

**COMPARISON OF THE DIFFERENT SYSTEMS OF CONSIDERING DISTRIBUTION**

The facts of animal and plant distribution are very complex and it is convenient to have some system or systems of arranging these facts so that they can be considered in groups rather than as isolated instances. Several systems are now in use. Each of these emphasizes different features of the facts of distribution.

**The Zoogeographical System**

The system of zoogeography points out the barriers to distribution, and indicates something as to the origin of the faunas of different regions. Because different species of animals are not limited by the same barriers, they do not all fall evenly into zoogeographical divisions.
Routes of migration have been opened and closed at irregular times, and many groups have become differentiated only to become exterminated. Climatic barriers are hard to determine and different species show various degrees of limitation by such barriers. It is a difficult matter to divide any region into satisfactory zoogeographical areas. It has been pointed out that the zoogeographical divisions of the globe are different for each group of animals and that in any one group these divisions indicate roughly the length of time the different sections of the group have been separated (Gadow, 1913, p. 13-15).

The zoogeographical method has many limitations and it is unwise to attempt to apply it too closely. Species are limited in distribution by various factors or complexes of factors. The zoogeographical divisions are founded on comparative statistics and there will always be exceptions. In some cases the exceptions will almost equal the number following the rule. The zoogeographical divisions are more or less arbitrary and there are sure to be many places of uncertain position. Still, the system greatly simplifies the consideration of the facts of distribution.

The Life-Zone System

The significance of the life-zone method lies in its indication of climatic barriers on the continent of North America, and the origin of the faunas of the several life-zones. Although this method of considering distribution has come into rather general use, it has a number of disadvantages and difficulties, and a considerable amount of criticism has been directed at the system. It seems advisable therefore to consider its history and some of the objections which have been raised against it.

On the high mountains of the western United States there are different zones of vegetation at different levels, and with each of these vegetational zones there are associated particular species of animals. Merriam (1890, pp. 7-11) found seven such zones of life on San Francisco Mountain, Arizona. Beginning at the top he gave these the names of Alpine zone, Subalpine or Timberline zone, (Central) Hudsonian or Spruce zone, (Central) Canadian or Balsam Fir zone, Neutral or Pine zone, Piñon zone, and the Desert Area. He showed that some species in the fauna and flora of the uppermost four of these zones were characteristic of much more northern regions. On the mountains of central Idaho, Merriam (1891, pp. 21–25) distin-
guished six life-zones which he called respectively the Arctic-alpine zone, the Subalpine or Timberline zone, the (Central) Hudsonian or Spruce zone, the (Central) Canadian or Douglas Fir zone, the Neutral or Transition zone, and the Upper Sonoran zone.

The faunal divisions of eastern North America generally recognized by students of distribution, particularly by ornithologists, at the time Merriam began his work on correlation, were eight in number. Passing from north to south these divisions were (1) Arctic, (2) Hudsonian, (3) Canadian, (4) Alleghanian, (5) Carolinian, (6) Louisiana, (7) Floridian, and (8) Antillean (Merriam, 1890, p. 18).

Merriam (1890, p. 18) was much impressed with the similarities between the zones of the higher parts of San Francisco Mountain and the faunal areas of northeastern North America and states that

in many instances, the zones of the mountain may be recognized by the identical species which characterize them in New England and Canada. In short it was found that the faunal and floral zones which go to make up the Boreal province in the East may be traced in a northwesterly direction around the northern end of the Plains of the Saskatchewan, and then south along the sides of the Rocky Mountains even to this isolated peak in Arizona.

Merriam (1892, p. 22) later extended the correlation of the zones of the eastern and western United States and stated "with some confidence" that the Transition zone of the mountains of the West is the equivalent of the Alleghenian of the East and also that the Upper Sonoran is the equivalent of the Carolinian, and the Lower Sonoran of the Austroriparian. He thought that these life-zones followed "the lines of equal temperature during the season of reproduction," and based the correlation mainly on that factor.

Since that time members of the United States Bureau of Biological Survey and others have extensively followed the life-zone method in describing distribution in North America. A brief statement of the birds and mammals characteristic of each life-zone was published by Merriam in 1898. As used as present, the Timberline zone originally recognized by Merriam has been merged into the Hudsonian zone, but no other important modification has been made.

Each of the life-zones of the Sonoran region is divisible into two or more faunal areas (Merriam, 1898, pp. 20–49). These faunal divisions are based upon differences in the atmospheric humidity in different parts of the same life-zone (Grinnell and Swarth, 1913, p. 217). In California a considerable number of these faunas have been distinguished by Grinnell (1902, p. 7).
The idea that temperature is the fundamental factor in limiting the distribution of species is dominant in the conception of life-zones, and in 1894 Merriam made an attempt to determine the temperature limits of each life-zone. By running various isothermal lines he determined that the northern limits of the life-zones agreed fairly well in having the same total quantity of heat. The total quantity of heat is the sum for the year of the daily mean temperatures above 6° C. This temperature is assumed to be the point at which life begins activity. The southern limits of the life-zones, however, did not agree with the isotherms thus determined, but did approximately agree with isotherms of the hottest period of the year.

One criticism which should be made of this correlation of life-zones and isotherms is that the northern and southern limits of the life-zones are not determined by the same temperature criteria and that therefore in some places the life-zones may not meet each other. If dependence be placed on these temperature criteria alone, some regions must be placed in two life-zones and theoretically some in none at all. Along the Pacific Coast in particular there is much overlapping of the life-zones. Merriam (1894, p. 233–235) considers that in that region the northern forms are able to come far south on account of the low temperature of the summers, while the southern forms are able to extend their ranges far to the north on account of the long growing season. Thus is explained the great overlapping of northern and southern forms in the "Pacific Coast strip." However, it cannot be considered proved that the temperature relations established by Merriam are the particular ones which determine the limits of distribution of any species of animal.

Another criticism of Merriam's determination of life-zone temperatures is that no thorough attempt has been made to determine if these temperatures actually do apply to all parts of the life-zones as they have been plotted in North America. Indeed, the temperatures of some parts of the life-zones in the West were obtained by applying temperature data obtained in the corresponding faunal areas of the eastern United States (Merriam, 1890, pp. 31, 32). There are some facts which seem to indicate that the temperatures determined by Merriam do not apply in parts of some life-zones. For instance, according to temperature Walla Walla and the Columbia Basin prairie area of southeastern Washington would be placed well within the Upper Austral zone, but the faunal relationships are with the Transition zone, or at least are not definitely Upper Austral.
In the life-zone system, humidity is recognized to have considerable influence on distribution, but is held to be always subordinate to the influence of temperature. However, it seems that either of several climatic factors may be of importance in limiting organic distribution. An extreme variation of humidity, or probably of other climatic factors besides temperature, may form a positive barrier to the distribution of species. All the climatic factors are complexly interrelated and a variation of any factor has an influence on the effect of the others. Different organisms are adapted to different climatic complexes and react in different manners to different factors and to varying degrees of the same factor. Temperature perhaps often is the most important factor in limiting distribution, but it would seem to be impossible to base a system of distribution on variations in any one climatic factor without obscuring many facts of prime importance. It has not yet been established that small differences of temperature of the degree supposedly separating some of the life-zones are as important barriers to distribution as are some of the more marked differences due to variations in rainfall and humidity.

The zones of life which occur in any given locality may be dependent in part on temperature, yet there are other factors which evidently have a very strong modifying influence. Differences in rainfall, in the humidity of the air, in slope exposure, or in other factors may greatly modify the position of zones. It may be that differences in some of those factors, other than temperature, might even be the principal cause in the production of certain zones. In each given case it is probably the complex of climatic factors which determines the occurrence of the zone rather than the action of one factor alone.

Three distinct regions of life may be recognized in North America, the Holarctic (Boreal) region, the Sonoran (Austral) region, and the Neotropical region (Tropical zone) (Lydekker, 1896, frontispiece). The limitation of many characteristic species and genera to each of these regions is probably due principally to the action of temperature as a barrier. In the Holarctic region of North America three transcontinental belts of life, Arctic, Hudsonian, and Canadian, have been recognized by nearly all students of geographical distribution. These belts of life are probably also determined largely by the effect of temperature. However, there is much more difficulty in recognizing transcontinental life belts within the Sonoran region, and in the truly tropical regions zones of distribution corresponding to isotherms have not been recognized.
Zones of life are clearly evident upon many mountains and in many regions which are not mountainous. The life of the uppermost of some of these mountain zones is evidently related to the life of more northern regions. However, the life of the mountain zones is never identical with the life of any particular northern transcontinental belt. Neither do the zones found on mountains in different parts of the United States exactly correspond. Grinnell finds the Canadian and Hudsonian life-zones in California to be far less distinct than the other life-zones in the state. Also in California those two life-zones are much less distinct than they are in the northern part of the continent. In the Blue Mountains of Washington it is almost impossible to separate the Hudsonian life-zone from the Canadian. In the Pine Forest Mountains of Nevada, Taylor (1912, p. 339) recognizes an area which is referred to the Transition life-zone, but which has a "Boreal infusion." It seems that the zones of life found on the upper parts of southern mountains show less affinity to particular northern transcontinental life belts than they do to an alpine or Arctic type of life in general.

The life-zones of the various parts of the Sonoran region present still greater difficulties in homologizing. In the first place it may be doubted if transcontinental life-zones really show in the best manner the similarities and differences of the faunas in the various parts of the region. Allen (1892, pp. 217-218) has demonstrated that the genera and subgenera of mammals of the arid division of the Sonoran region are more different from those of the humid division, than are those of a northern transcontinental division of the region from those of a southern division. It seems, then, that the first division of the Sonoran region should be into eastern and western sections.

The zones of the Sonoran region found in the various parts of the western United States are very difficult to correlate. The number of zones to be distinguished is variable and those of different regions do not seem to be exactly homologous. Following Merriam's classification the three life-zones, Transition, Upper Sonoran, and Lower Sonoran have usually been recognized. However, other zones are sometimes apparent. Grinnell and Swarth (1913, p. 217) have split the Transition zone in the San Jacinto area of southern California into an upper and a lower division. In eastern Washington a division of the Transition zone has also been made and these divisions are as distinct as are any other two zones. As an instance of the difficulty of homologizing zones in different regions, we may mention the Columbia Basin prairie
area. This area seems to show homologies to both the upper Sonoran and to the Transition zones as found in other parts of the West.

Certain species seem to have a different "zonal" position in different regions. Those which in one place are restricted to a certain life-zone range elsewhere into areas which must be placed in other life-zones. Many of the species and several of the genera given by Merriam (1892 and 1898) as characteristic of the various life-zones are now known to range beyond the limits stated by him. Grinnell and Swarth (1913, p. 217) mention the case of a "Transition infiltration into a prevailing Upper Sonoran area" in the San Jacinto region of southern California. Cases like this indicate very strongly that there is often a lack of homology between the zones of life found in different regions.

In a restricted region of general climatic similarity the zones of life may usually be easily homologized. In California Grinnell (1902, p. 6) has recognized several zones which are evidently natural divisions of the fauna, and each of which is seemingly homologous throughout its extent in the state. However, the zones of life found in different regions, particularly in regions under different climatic conditions, show much less similarity and in many cases are certainly not directly homologous.

In some cases the life-zone system seems to be largely dependent upon the distribution of particular associations of plants and animals. The life-zones are based on temperature differences, yet "it is obvious that, throughout considerable portions of the continent, the details of temperature distribution are not known with any approach to precision. Thus, the actual criterion which the field zoologist falls back upon in any given case is the character of the fauna and flora which he finds associated together. The presence of certain species shows him that he chances to be in this or that "life-zone" " (Sumner, 1915, p. 67). On Alder Creek in northern Nevada, Taylor (1912, p. 331) has placed the vegetation along the stream in the Transition zone, while the treeless slopes away from the narrow strip of vegetation are placed in the Upper Austral zone. There may be a temperature difference between the strip along the stream and the immediately adjoining timberless slopes sufficiently great to maintain different life-zones in the two places, but there is no proof that such is the case. On the contrary, it seems that the differences are those that would naturally be produced by habitat differences. There is no justification for assuming that the differences in this and many other similar cases are
due to differences in temperature until the effect of difference in habitat has been eliminated.

We believe that the true significance of the facts considered in the life-zone method of studying distribution in North America would be better presented by an extension of the zoogeographical method of Lydekker and Allen. This method would recognize the zones of life found in the various parts of the continent. It would recognize the relation of the zones on the higher parts of the mountains to the belts of life in the North by placing these higher zones in subdivisions of the Holarctic region. Under this system there would be no compulsion to recognize a certain number of life-zones in each region, but the number of divisions could be varied to fit the circumstances. The effect of temperature as a barrier to distribution in places where that is important would be shown, and the effects of other climatic barriers could also be emphasized.

The Ecological Method

The ecological method of studying the distribution of animals and plants brings out chiefly the relations of the organisms to their environments. It makes as simple as possible the comparison of environments and of adaptational structures and habits in different species and in different localities. The different associations and formations of any region can be compared with associations and formations in any other part of the world. At present our knowledge of associations and formations in general is too slight to point out the significance of each ecological division in southeastern Washington, but we feel certain that the study of the distribution of the species of animals in relation to the distribution of different kinds of environments will lead to results of the highest value.

The classification of the habitats of a region and the placing of the species in associations, which to some extent at least are arbitrary divisions, may be objected to on the ground that such a system apparently indicates a discontinuity in nature which does not exist. However, it is thought that the use of terms showing the relative abundance of each species in the different habitats, prevents the associations from assuming more of a definite character than they actually possess.

The different systems used for describing animal distribution are used for convenience in classifying the complexly related facts in-
It seems impossible to organize all the facts into a perfect system, but it is desirable to have as great uniformity as possible and not unduly to increase systems or complexity of nomenclature. As the facts become better known systems will have to be changed to agree with the increased knowledge. At present it seems desirable to use two systems, both starting with the same unit, the species. By the first method, zoogeography, species and taxonomic groups are considered in relation to geographical divisions. The second method, the ecological method, groups species according to similarity of adaptational features and of environmental conditions.

A combination of these two methods of studying distribution should lead to excellent results. Usually the study of zoogeography has been carried on without reference to the particular habitats in which the organisms live. A comparison of the animals in similar habitats in different faunal areas is sure to bring to light many important facts about the evolution of the different groups and of topographical and climatic changes in general. Further, the relation between an organism and its environment cannot be fully understood without reference to the mode of origin both of the organism and of the environment.

SUMMARY

In southeastern Washington we may distinguish three faunal areas, each containing a number of distinct habitats. Each habitat is occupied by a different vertebrate association.

The Columbia Basin sagebrush fauna belongs to the Great Basin division of the Sonoran region. The Blue Mountain fauna belongs to the Canadian subregion of Holarctica. The Columbia Basin prairie fauna shows affinities to the life both of the Rocky Mountains and of the Great Basin.

An Upper Austral life-zone, a timberless and a timbered division of the Transition life-zone, and a Boreal life-zone may be recognized in the region.

Although temperature seems to be the climatic barrier which is most important in separating the faunas of the zoogeographical regions (Holarctic, Sonoran, and Neotropical) represented in America, yet within the limits of the Sonoran region it has not been proved that temperature is as important a barrier to distribution as are the factors connected with differences in rainfall and humidity.
Zones or belts of life may be recognized in many regions. However, it is very difficult to homologize the zones of life which occur in widely separated parts of North America. The division of the continent into a definite number of transcontinental life-zones seems to be contrary to a number of the facts of distribution.

The ecological method of studying distribution furnishes valuable information about the relation between organisms and their environments. The use of this method in conjunction with the zoogeographical method should lead to results of great value.

LITERATURE CITED


GADOW, HANS. 1913. The wanderings of animals (University Press, Cambridge), viii + 150 pp., 17 maps.


GRINNELL, J., AND SWARTH, H. S. 1913. An account of the birds and mammals of the San Jacinto area of southern California, with remarks upon the behavior of geographic races on the margins of their habitats. Univ. Calif. Publ. Zool., 10, 197-406, pls. 6-10, 3 figs.


MERRIAM, C. HART.


Piper, C. V.

RUSSELL, I. C.

Shelford, V. E.


Sumner, F. B

Taylor, W. P.
1912. Field notes on amphibians, reptiles, and birds of northern Humboldt County, Nevada, with a discussion of some of the faunal features of the region. Univ. Calif. Publ. Zool., 7, 319-436, pls. 7-12.

Weaver, John E.
PLATE 24

Fig. 1. Packed sand situation in sagebrush habitat, three miles east of Wallula, June 13, 1914. The plants are common sagebrush (*Artemisia tridentata*) and rabbit brush (*Chrysothamnus viscidiflorus* and *Chrysothamnus nauseosus graveolens*).

Fig. 2. Drifting sand situation in sagebrush habitat, three miles east of Wallula, June 13, 1914. The plants are common sagebrush and rabbit brush and a few individuals of the hop sage (*Grayia spinosa*). In the distance are the bunchgrass-covered hills south of the Walla Walla River.
Fig. 3. Bunchgrass habitat in the prairie area one mile south of Lyon's Ferry, June 24, 1914. The principal plant is the wheat bunchgrass (*Agropyron spicatum*).

Fig. 4. The Touchet Valley, two miles east of Prescott, July 9, 1915. The cottonwood-willow habitat is shown in typical development along the river. The conspicuous trees are cottonwoods (*Populus trichocarpa*). The hills and the greater part of the valley were natively covered by bunchgrass, but now are nearly entirely in cultivated fields.
PLATE 26

Fig. 5. The eastern side of the cañon at Hompeg Falls, Blue Mountain area. The considerable extent of the rocky-slope habitat at this point is shown. The trees on the slopes are mostly yellow pines (*Pinus ponderosa*). Toward the upper part of the ridge a yellow-pine habitat occurs. In the bottom of the cañon is a lowland-fir habitat.

Fig. 6. Forests in the Blue Mountains near the head of the South Fork of the Touchet River, Aug 1, 1915. The forest in this section is chiefly made up of young trees. Douglas spruce (*Pseudotsuga taxifolia*) and western larch (*Larix occidentalis*) are the dominant species.
Figure 5

Figure 6
THE ANATOMY OF *HEPTANCHUS MACULATUS*

THE ENDOSKELETON

BY

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3. The Reptiles of the San Jacinto Area of Southern California, by Sarah Rogers Atsatt. Pp. 31-50. November, 1913 .20


5. Aplodonta chrysea, a New Mountain Beaver from the Trinity Region of Northern California, by Louise Kellogg. Pp. 295-396 .10

6. A Previously Undescribed Aplodonta from the Middle North Coast of California, by Walter P. Taylor. Pp. 297-300. Nov. 5 and 6 in one cover. April, 1914 .05


THE ANATOMY OF HEPTANCHUS MACULATUS

THE ENDOSKELETON

BY

J. FRANK DANIEL

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INTRODUCTION

Notwithstanding a late geological appearance, the notidanid sharks are usually taken to represent a more generalized plan than do pentanchid elasmobranchs. The presence of a greater number of branchial clefts alone strongly enforced such a conclusion. In addition to this it is known that many of the internal structures are built on a simple plan.
In order to determine in how far the plan is a generalized one I have set myself the task of studying in detail the anatomy of *Heptanchus maculatus*, one of the species of notidanids found along the Pacific coast, and I may say summarily that most of the systems compared with those, say, of *Heterodontus francisci*, a type geologically old, indicate so great a generalization that *Heptanchus* may be looked upon as an elasmobranch inheriting to a great extent the features of its remote ancestors.

It is my purpose within a short time to publish all of these studies in a book dealing with the anatomy of the elasmobranch fishes, but since it is desirable to give some of them at this time, these will appear as a series in the publications of the University of California.

The Endoskeleton

The first system which I wish to consider is the endoskeleton. This in *Heptanchus* as in other elasmobranches is composed of cartilage; but in the notidanids, as Roth (1911) has shown, the cartilage is of a simple type. In all of those specimens of relatively immature age which I have examined of *Heptanchus maculatus* the cartilage is exceedingly soft, and over large areas it is often devoid of calcification. In the more mature specimens, however, some of which have measured seven to nine feet in length, a considerable amount of calcification is present; but even in these it cannot be said to be a marked feature of the skeleton.

I. AXIAL SKELETON

The Skull

The skull in *Heptanchus maculatus* is like that of other elasmobranchs in that the capsules for the nose and ear are fused to the cranium; but this shark is unlike others in having the greatest number of visceral arches, supporting buccal and branchial areas, known among the elasmobranchs.

The cranium in general shape is unlike that of *Heptanchus cinereus*, figured by Gegenbaur (1872) or more recently by Reynolds (1913), but it is remarkably like that of *Hexanchus griseus* (Gegenbaur, 1872, pl. 1, fig. 2). From dorsal view (pl. 27, fig. 1) the cranium is shaped like a violin box, broadly pointed in front and squarish at the posterior end. Anterior to the middle segment it is constricted and back of this constriction are the heavy postorbital processes (po.o.).
In the mid-dorsal line and at the posterior part of the cranium is the occipital crest \((o.\, cr.)\) which extends upward from the foramen magnum \((f.\, m.)\) to the parietal fossa \((p.\, f.)\). At the bottom of this fossa, as was described for *Heterodontus francisci* (Daniel, 1915), are the small openings for the endolymphatic ducts \((e.\, d.)\), and nearer the middle line the much larger foramina or fenestrae \((f.n.)\). Behind the parietal fossa, the posterior oblique semicircular canals radiate outward and backward to the angles of the skull. Forward from the fossa the roof is slightly convex and leads to the large anterior fontanelle \((F.)\).

To the side of the anterior fontanelle there is a small perforation \((f.\, o.\, p.\,')\) for the ophthalmic division of the fifth nerve, and back of this a large opening \((f.\, o.\, VII\,')\) for the superficial division of the seventh nerve. Continuing on a line posteriorly from this there is a row of small foramina which mark the boundary of the supraorbital crest. Through these, branches of the superficial ophthalmic nerve pass to the supraorbital canal.

In side view \((p l.\, 28,\, f i g s.\, 2\, a n d\, 3)\) the cartilaginous rostral supports for the nose project slightly forward; at the base and sides of these are the prominent olfactory capsules \((o.\, c.)\) for the nasal apparatus. The cartilages for the capsules are exceedingly thin-walled and open to the exterior by the nasal apertures. Surrounding the aperture is the arch-like nasal cartilage \((n.\, c.)\) which in *Heterodontus* is like the letter A, the cross-bar of which is formed by a posterior and an anterior projection. These projections serve to divide the aperture into a smaller upper and a larger lower opening.

At the posterior third of the cranium is the auditory capsule \((a.\, c.,\, f i g.\, 2)\), in which the semicircular canals and the organs of hearing are located. In dorsal view these capsules are strongly marked by the anterior and posterior oblique \((p.\, o.\, s.,\, f i g.\, 1)\) semicircular canals; while laterally \((f i g.\, 2)\) each capsule is convex and but slightly affected by the attachment of the hyoid arch. In side view, furthermore, the foramina for the ninth \((f.\, IX)\) and tenth cranial nerves are in close relation to the capsules.

Between the auditory and the nasal capsule is the large orbit for the eye. Overhanging this is the supraorbital crest \((s.\, o.,\, f i g.\, 3)\), the pre- \((p r.\, o.)\) and the postorbital \((p o.\, o.)\) processes from which are well developed. The preorbital process extends far ventralward and the postorbital serves as an important attachment for the first visceral arch. Ventral to the posterior part of the orbit the cranium bends
sharply downward, forming the strong basal angle (b.a., fig. 2). On the anterior face of the basal angle there is a flattened articular surface against which the orbital process of the upper jaw fits. Extending from the margin of the cranium and below the preorbital process is an antorbital process (a.pr.). This in *Heptanchus maculatus* serves for the attachment of a slip of muscle which runs to the adductor mandibulae. In the rays the antorbital process is connected with the propterygium.

The foramina perforating the walls of the brain-case differ considerably from those figured by Gegenbaur (1872) for *Heptanchus*; but they occupy essentially the same locations as those which he has given for *Hexanchus* (see his pl. 1, fig. 2). The first of these, between the nasal capsules and the orbit, is the anterior opening of the orbitonasal canal (o.-n., my fig. 2). The posterior opening of this canal (o.-n') lies in the anterior part of the orbit. Above the latter is a smaller foramen for the anterior cerebral vein (f.a.c.). Ventrally and at the middle of the orbit is the large optic foramen (f. II) through which the second cranial nerve reaches the brain. Directly above the optic is the ophthalmic for the superficial branch of the seventh nerve (f.o.VII), while just posterior to the ophthalmic is the smaller trochlear foramen (f.IV) through which the fourth cranial nerve passes to the superior oblique muscle of the eye. Considerably behind the optic and below and slightly back of the tip of the postorbital process is the enlarged orbital fissure (o.f.), through which pass the fifth, sixth, and a part of the seventh cranial nerves. Below and slightly posterior to the orbital fissure is the facial foramen (f.VII) for the hyomandibular branch of the seventh or facial nerve. On a line between the facial and the optic foramina are two perforations, the nearer and more crescentic of which is for the interorbital canal (i.o.); by means of this the orbital sinuses mediad of the two eyes communicate. The other of these perforations (f.r.a.) is for the entrance of the ramus anastomoticus artery. Above this is the small opening (f.III) for the exit of the third cranial nerve to muscles of the eye.

The visceral skeleton in *Heptanchus* is composed of a series of nine pairs of cartilaginous arches which more or less completely surround the buecal cavity and the pharynx. The first and largest of these arches, the mandibular, is formed of the upper and lower jaws (pl. 28, fig. 3). This arch bears the teeth and has become the most highly specialized of all the visceral arches. Its massive upper segment, the
palato-quadrate \((p-q,.)\) suspends the mandible \((imd,.)\) or lower segment and is itself attached to the postorbital process of the cranium by the strong quadrate process \((ql, p.)\). Anteriorly the upper segment comes in contact with the cranium at the orbital process \((or, p.)\), which, as we have said, fits against the basal angle. The upper and the lower segments of the first arch on the left side are connected loosely in front to similar segments on the right side; but the articulations in *Heptanchus maculatus*, in so far as I can make out, are devoid of extra cartilages like those given by White (1896, p. 58, fig. 2) for *Heronichus*. Gadow (1888) has called attention to the simple condition of the joint between the palato-quadrate and mandible in *Heptanchus cinereus*. In the species under observation I find that the joint is essentially identical with that of *Heptanchus cinereus*, and further that the ligaments binding this joint differ so slightly in the two species as to need no additional description.

The slender segments making up the hyoidean or second visceral arch are entirely hidden in lateral view by the first or mandibular arch. The upper segment, the epihyal, unlike that in a more highly specialized form as, for example *Heterodontus* (Daniel, 1915), is not a suspensorium for the mandibular arch. The lower and much longer ceratohyal segment of the hyoid arch projects forward and inward and is connected with the ceratohyoid of the opposite side by a broad basihyoid \((bh,\) text-fig. B.\)

Both the epihyal and the ceratohyal segments are provided with numerous cartilaginous rays \((b.r., pl. 28, fig. 3)\) which support the main respiratory structures. The cartilaginous rays in this arch, due to the fusion of numerous rays into a common stem, are more complex than are similar rays found on the branchial arches.

The branchial arches of right and left side form a complete ring around the pharynx only with the most anterior segment. Back of this, the arches unite ventrally but do not join dorsally. The first branchial arch (text-fig. A) consists of: (1) a long slender pharyngo-branchial \((pb,)\) the tip of which is joined to the arch from the opposite side; (2) a short epibranchial \((eb,)\); (3) a long ceratobranchial \((eb,)\); and (4) a relatively insignificant hypobranchial \((hb,)\) segment. Like the branchial arches in pentanchid sharks, these segments slant obliquely forward and downward, roughly forming the letter S.

In the arches following the first branchial the segments are similar to those of the first, back to and including the sixth arch, with the exception of the hypobranchial segments. The hypobranchials \((hb,\)
text-fig. B) back to the fifth are of large size and the fifth is divided into two segments, the upper of which is similar to the sixth hypobranchial. The seventh or last arch, like the fifth in pentanchid forms, has its pharyngobranchial fused to that of the sixth arch. Like pentanchid forms also, the ceratobranchial of this arch is hypertrophied, but the most interesting departure seen in the seventh arch is a clearly marked rudimentary hypobranchial (see $hb'$, left side, text-fig. B). To a further discussion of the region we shall return.

In the mid-ventral line (text-fig. B) right and left arches are united by basal pieces, or copulae. These pieces in *Heptanchus maculatus* differ greatly from those described for *Heptanchus cinereus* (Gegenbaur, 1872, pl. 18, fig. 1), but they are similar in general plan to those figured by him for *Hexanchus*. The first of these is the broad basihyal piece ($bb\)$. This cartilage, unlike that described in *Chlamydoselachus* (Goodey, 1910, pl. 43, fig. 6), is not perforated by
the duct from the thyroid gland. It is further without a glossal projection like that of *Heterodon* (Daniel, 1915).

A first basibranchial piece is lacking in *Heptanchus maculatus* unless, as seems improbable, it be represented by the point at the

posterior part of the basihyoid as suggested by Goodey (1910) for *Chlamydoselachus*. At the union of the second to the fourth pairs of hypobranchials basibranchials (*bb.2-4*) are present. A large median piece serves as an attachment for the fifth and the sixth hypobranchials, and the seventh ceratobranchials.
Our attention has been called by Braus (1906) to an additional rudimentary arch in the embryo of *Heptanchus cinereus*. Such an arch is represented in text-figure B by the piece split off on the left side from the ceratobranchial (*cb*). But a more interesting condition obtains on the right side. It will be observed that a slight asymmetry is shown in text-figure B, which gives a somewhat greater development in the mid-ventral region on the right than on the left side. Through this asymmetry the rudimentary arch and adjoining area on the right side are more highly developed than on the left.

Upon examination of the ventral side of the rudimentary arch of the right side I found certain rays (*r.*, text-fig. C) arising from the

![Fig. C. Area of rudimentary arches, *Heptanchus maculatus*. Camera lucida outline, ventral view.](image)

*ar.9*, evidence of a ninth arch; *r.*, rudimentary rays; *x.*, part of eighth arch.

seventh ceratobranchial practically at right angles to its long axis. These extended posteriorly between the piece X and the median piece. These are round and pointed and are of clear hyalin cartilage. I am not certain whether they represent rudimentary branchial rays on the seventh arch like those described by Gegenbaur (1872, pl. 12, fig. 5) on the anterior margin of the fifth arch for *Scyllium*, or whether they have to do with the rudimentary arch following.

On the middle piece a similar arrangement is found. Here there are three pieces which are successively longer toward the middle line. They are essentially identical in appearance with those above described on the seventh ceratobranchial segment, but they are attached along their whole dorsal length as flattened lamellae. Terminally the
median two of them look very much like the rays above described and they are much like them also in that they are of clear hyalin cartilage. Further toward the median line on the middle piece there is clear evidence of another group similar to these, excepting that it is not separated into rays or lamellae. This group is also of clear hyalin cartilage differing distinctly from the median piece, which is a dark color in the specimen. I have interpreted this as a remnant probably having to do with a ninth arch (ar. 3), although I am not certain what part it represents.

The condition found in this specimen is suggestive as to the method of formation of the enlarged median piece so characteristic of the clasmobranchs. It would appear that in this region the rudimentary arches are forced more and more to take a longitudinal direction nearer the middle line, and that the median piece represents in its most posterior part the fusion of these arches from side to side.

Well-developed cartilaginous branchial rays are present on the epibranchial and ceratobranchial segments of all of the branchial arches (see b.r., text-fig. A) excepting the last. These rays, although much simpler than are those of the hyoid arch (cf. pl. 28, fig. 3), have the same function to support the gill-septa.

The extravisceral cartilages in Heptanchus maculatus, although well developed, are not so pronounced as those of Heterodontus (Daniel, loc. cit.). The labial cartilages in the notidanids are of great interest since here they are found in a comparatively simple condition and may be traced in series. In Heptanchus cinereus they were thought to be absent until Fürbringer (1903) discovered a single small cartilage on each side lying against the upper jaw. In Heptanchus maculatus each labial (l., fig. 3) appears in a slightly higher stage of development. It is an irregular cartilage of considerable size, shaped in general like a tuning-fork, the two prongs of which lie against the upper jaw, and the single stem extends backward and downward against the lower jaw. In this, so far as I can find, there is no indication of a separation into parts. Only a little more complex than this is that figured for Hexanchus by Gegenbaur (1872, pl. 10, fig. 1), in which the labial is perforated by a foramen and separated longitudinally into two parts. From some such separation the two dorsal and single ventral labials characteristic of pentanchid sharks evidently have arisen.

An extravisceral cartilage is present dorsally over the hyoid arch (ex.h., pl. 28, fig. 3), but ventrally such a segment appears to be absent.
Over all of the branchial arches excepting the last these cartilages are found both dorsally and ventrally. On the anterior branchial arch (ex.b., text-fig. A) they attain a large size, yet even here they do not overlap as in *Heterodontus*. They curve laterally around the tips of the branchial rays and serve to protect the underlying structure and to support the septa.

**The Spinal Column**

The spinal column in *Heptanchus*, because of its generalized character, is especially interesting. It consists of a long central column (c.) which anteriorly is more or less indistinguishable from the occipital region of the cranium (see pl. 28, fig. 3) and which in the body region is undivided into separate centra (text-fig. F). If the column is allowed to dry slightly, differentiation of this central column into segments may be made out. The septa which produce this segmentation of the column, however, run through what would be the middle of a centrum in a more specialized form. A series of dorsal arches protecting the spinal cord extends practically the whole length above this central column, and a series of haemal arches is present ventral to it in the region of the tail (h.a., text-fig. F).

For convenience of study we may divide the column into regions: a section in the so-called cervical region shows the segments (c., text-fig. D, and pl. 28, fig. 3) relatively well developed. Above these are the plates making up the neural arch, each arch being composed of a basidorsal (bd.) and an interdorsal (id.) plate. Both of these cartilages are more or less triangular in shape, the former having its base on the centrum, the latter with apex pointing toward the centrum. Above the basidorsal there is a piece segmented off as the suprabasidorsal (s.bd.) and in the anteriormost part of the column two such pieces are formed, one above the other (fig. 2). Each basidorsal is further perforated by the ventral root (v.) of a spinal nerve, and each interdorsal is pierced by the dorsal ramus (d.) of a spinal nerve. Ventral to the central column are also basiventral pieces (bv.) between which may be interpolated small interventrals (iv.). From the third and succeeding basiventrales back to about the forty-fourth, ribs (r.) are formed. Beginning with the eighth vertebra and continuing back in cases to the twenty-fourth the ribs in *Heptanchus maculatus*, like those in *Laemargus*, are divided into an anterior and a posterior part, the former of which is a curious plate-like process projecting forward and downward.
A sagittal section through the column of this region shows the finer internal structures of the segments (text-fig. E). In this the plates of the neural arch are regularly arranged and need no further description. The central part of the column offers great simplicity. It consists largely of the notochordal sheath \((sh.)\), around which is a thin layer of cartilage. The sheath is slightly thicker dorsally than ventrally and has the appearance of being traversed by multitudes of fibers. A tissue much lighter in color \((m.)\) lies within this and passes inward, causing a constriction of the central notochord, the constrictions being much more pronounced ventrally than dorsally. In a specimen nine feet in length these constrictions of the notochord formed a series of septa \((s.)\) like the nodes of a plant. In this, however, the septa had passed from the ventral wall almost completely to the dorsal and were so thin as to be transparent.

In the ventral part of these septa (Hasse, 1882) described for \(H.\ cinereus\) a considerable amount of calcification. It has long been recognized that in this regard \(H.\ cinereus\) is even more
specialized than *Heranchus*. Such certainly cannot be said of *Heptanchus maculatus*, for in this practically all traces of calcification are absent from the central column, which represents only a slight improvement over a notochord of similar size.

In the mid-body the central column assumes its simplest character. Although there is considerable variation in the different specimens dissected, our description will refer to what may be characterized as the ordinary plan. In an immature specimen the mid-column (text-fig. F) consists essentially of a heavy tube containing the constricted notochord; upon this cartilaginous tube clearly defined arches rest. Immediately posterior to the rib-bearing segments the basiventrals bend downward and on the forty-seventh join below to form the first haemal arch *(h.a.)*. From about the fiftieth to the fifty-fifth segment of this region the basidorsal pieces extend entirely to the top of the arch, no suprabasidorsals being present. Back of the region where the high basidorsals occur *(56, text-fig. F)* it is observed that the septa, constricting the notochord, are farther apart. Since each septum strikes the middle of a segment of the central column it is clear that the segments of the column in this area are much longer than in the anterior regions. It will be observed that to each of these longer segments two neural arches occur. This is the beginning of diplospondylous, although the segments of the central column are not themselves divided. Following this there is considerable irregularity in the arches, but in general two types of basidorsals obtain, one of which is high, the other is much lower. The higher of the two in text-figure F is perforated by the ventral root of the nerve *(f.v.)*; the lower is imperforate. The higher is followed by an interdorsal which is perforated by the foramen of the dorsal root-nerve *(f.d.)*. In the haemal or ventral arches the doubling of the plates in this specimen begins unusually far forward. The first evidence of this is in the forty-eighth segment. A regular doubling begins on the fiftieth and intraventrals are added on the fifty-second segment.

A segment of the column through the tail shows a typical diplospondylous condition. In this region both the perforate and imperforate basidorsals are practically uniform in size as well as are the interdorsals. Above the interdorsals are the cartilages which support the dorsal lobe of the caudal fin. These are much more numerous than the basidorsal and basiventral pieces, more than one hundred being present back of the seventy-first vertebra. Below the seventy-third segment in this region the haemal arches are well formed and
The Aiialiituij of Ilepleuielnis intaeual us have projecting from them ventralward haemal spines which serve as a framework for the ventral lobe of the caudal fin.

II. APPENDICULAR SKELETON

The part of the skeleton known as appendicular is a framework for the fins and the girdles to which these, if paired, are attached.

The Skeleton of the Paired Fins and their Girdles

The Pectoral Fin.—The skeleton of the pectoral fin (pl. 29, fig. 4) is fan-shaped, the proximal part of which consists of three basal cartilages, pro-, meso-, and metapterygium; from the last two of these radiate numerous rows of radials.

The propterygium (pr.p) is a small nodule located against the mesopterygium. The mesopterygium (ms.p.) is a stout cartilage, from the enlarged distal end of which extend ten or twelve rows of radials (ra.) depending upon the amount of fusion which has taken place proximally. The first or most anterior of these is made up of large and irregular plates. The remaining rows are broken up into small segments. The metapterygium (mt.p.) is a triangularly shaped cartilage, the base of which points posteriorly. It is segmented both proximally and distally and is then continued into the most distal radial. From the metapterygium diverge numerous rows of preaxial radials, in addition to which are several clearly marked postaxial radials (po.r.).

The pectoral girdle in Heptanchus is a slender arch unclosed dorsally, to which the framework of the pectoral fin is attached. It is composed of a right and a left cartilaginous half which in Heptanchus maculatus are united in the middle line below by means of an unpaired median piece. The point of the girdle which extends the more dorsalward is known as the scapular portion (sc.). That which by means of the median piece joins a similar part from the opposite side below is the coracoid portion (ca.). At the middle and postero-lateral part of each half of the girdle there is an irregular surface for articulation with the pectoral fin (a.pt.), and in front of and below this projection there is a broad surface for the attachment of the ventral pectoral muscles. Perforating the girdles in this surface is a large foramen (f.plt.) through which the blood and nerve supplies of the fin pass.

The Pelvic Fin.—The framework of the pelvic fin proper consists of a longer posteriorly projecting basal cartilage, the basipterygium
(ba.p., text-fig. G) which bears one or two small terminal segments. From this in the female proceed twenty-one or twenty-two radials, all of which excepting the last five are segmented. Anteriorly a much enlarged plate strikes the basal piece, forming an obtuse angle. From this also run three rows of radials. At the proximal end of this enlarged plate and the basal piece are the two fossae with which the protuberances from the pelvic girdle above described articulate.


β, the beta cartilage; β1-2, connecting segments; ba., basal piece; ba.p., basipterygium; pl., pelvic girdle.

In the male (text-fig. H) the long basal piece is continued by the axial cartilage of the claspers (ba.). Where the two join there are two segments β1-2 and dorsal to these is the so-called β cartilage. Distally on the main axis I was unable to find the blade-like terminal cartilages described by Krall (1908) for *Hexanchus*, but this is probably due to the fact that the specimen from which text-figure H was taken was small.

The pelvic girdle (pl., text-figs. G and H) consists of a flattened band of cartilage slightly concave dorsally and enlarged at the ends.
Perforating the terminal parts of the girdle is a foramen through which the nerve passes to the pelvic fin. At the termini of the girdle are the articular processes, each consisting of two protuberances which fit into depressions (fossae) of the pelvic fin skeleton. These are not well seen in the figure.

**Skeleton of the Unpaired Fins**

*The Dorsal Fin.*—The unpaired fins are essentially like those of *Heptanchus cinereus* figured by Mivart (1879). In *Heptanchus maculatus* the thin basal cartilage of the dorsal fin extends from about the forty-ninth to the fifty-fifth segment of the vertebral column. From this plate in *Heptanchus maculatus* arise seventeen or eighteen radial cartilages, the anterior of which is unsegmented and the posterior of which is apparently a fusion of several pieces.

*The Caudal Fin.*—The essential parts of the caudal fin have been described (p. 360). But it may be repeated that its ventral rays are an integral part of the axial skeleton, being the prolongations of the haemal spines. These consist of a series of rays two of which correspond to a segment. Toward the end, however, the column is more or less undifferentiated. The dorsal lobe of the fin is also supported by rays which, unlike those supporting the ventral lobe, greatly exceed in number the segments of the central column.

*The Anal Fin.*—The base of the anal fin abuts against the fifty-third segment of the spinal column. The basal piece, barring the fact that it is segmented in front, is remarkably similar to that of the dorsal. From it, however, the radials proceed in a less definite fashion.

*Transmitted June 30, 1916.*
LITERATURE CITED

BRAUS, H.

DANIEL, J. Frank.

FÜRBRINGER, Karl.

GADOW, Hans.

GEGENBAUR, C.

GOODEY, T.

HASSE, C.

KRAAL, A.

MAYER, Paul.

MIVART, St. George.

REYNOLDS, S. H.
1913. The vertebrate skeleton. Cambridge, Univ. Press.

ROTH, Wilhelm.

WHITE, Ph. J.
1896. The existence of skeletal elements between the mandibular and hyoid arches in Hexanchus and Laemargus. Anat. Anz., 11, 57-60, 3 figs. in text.
EXPLANATION OF PLATE 27

Fig. 1. Dorsal view cranium *Heptanuchus maculatus* (3/4 natural size). *e.d.*, endolymphatic duct; *F.*, anterior fontanelle; *f.m.*, foramen magnum; *fn.*, fenestra; *f.o.p.*, foramen for opthalmicus profundus nerve; *f.o. VII.*, foramen for opthalmicus superficialis nerve; *o.cr.*, occipital crest; *p.f.*, parietal fossa; *po.o.*, postorbital process; *p.o.s.*, posterior semicircular canal.
Fig. 1
EXPLANATION OF PLATE 28

Fig. 2. Lateral view of cranium, Heptanchus maculatus (½ natural size).

Fig. 3. Lateral view of cranium with first and second arches attached.

a.c., auditory capsule; a.pr., antorbital process; b.a., basal angle; b.r., branchial cartilaginous rays; l., labial cartilage; c.x.-h., extra-hyoid cartilage; f.a.c., foramen of anterior cerebral vein; f. II., optic foramen; f. III., oculomotor foramen; f. IV., trochlear foramen; f. VII., facial foramen; f. IX., foramen for ninth cranial nerve; f.o. VII., ophthalmic foramen for seventh nerve (leaving orbit); f.r.a., foramen for ramus anastomoticus artery; i.o., interorbital canal; m.d., mandible; n.c., nasal cartilage; o.f., orbital fissure; o.-n., orbito-nasal canal; o.-n', orbito-nasal canal (entrance to orbit); o.l.c., olfactory capsule; o.r.p., orbital process; p.o.o., postorbital process; p.r.o., preorbital process; p.-q., palato-quadrate cartilage; q.d.q., quadrate process; s.o., supraorbital crest.

[368]
EXPLANATION OF PLATE 29

Fig. 4. Lateral view of pectoral girdle (x1) and fin (½ natural size). a.pt., articular process for pectoral fin; co., coracoid; f.pt., foramen through the pectoral girdle; ms.p., mesopterygium; mt.p., metapterygium; po.r., postaxial radials; pr.p., propterygium; ra., radial cartilage; sc., scapula.
SOME PHASES OF SPERMATOGENESIS IN THE MOUSE

BY

HARRY B. YOCOM

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IN THE MOUSE

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HARRY B. YOCOM

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INTRODUCTION
The problems of the number, behavior, and peculiarities of the chromosomes during the process of spermatogenesis have been of absorbing interest for many years. Until recently they have been studied largely in insects, perhaps because many species of these animals have relatively few chromosomes, a fact which enables the investigator to work with greater precision and certainty. The chromosomes of mammals have been less studied, and only within recent years has there been much intensive work done on them. Many of the less difficult problems have even been overlooked. The number of chromosomes in many of our more common mammals has not been definitely established; and as to their behavior during the process of maturation of the germ cells, little has been proved beyond question.

The common house mouse has been the subject of considerable cytological investigation. Tafani (1889) claimed that it had twenty chromosomes. Sobotta (1895) fixed the number at twelve, but later
(1907) changed his opinion and stated that there were sixteen. Kirkham (1907a, b) counted twelve. Long and Mark (1911) determined the number to be twenty, thus agreeing with Tafani. The above numbers were all obtained in the study of the egg in maturation, and thus represent the haploid number of chromosomes in the female. No one has counted the chromosomes in the male, with the exception of Loukianow (1898), who stated the number to be twelve in the primary spermatocyte, with six double chromosomes in the secondary spermatocyte. His figures, however, are small and poorly represent the shape of the chromosomes, so that they would lead one to think that his technique was deficient and his results inaccurate.

The primary object in beginning this study of spermatogenesis in the mouse was the determination of the number of chromosomes in the spermatocytes and their correlation with those found in the egg. This correlation in size, shape, and number of chromosomes, although found in insects, has not been heretofore described for mammals. Such a relation is very important, since our ideas of the mechanism of heredity and the determination of sex are based largely upon evidence obtained from studies of the chromosomes of germ cells. This paper covers only a small part of the process of spermatogenesis, being confined mainly to the chromosomes of the primary and secondary spermatocytes.

This work was undertaken under the direction of Dr. J. A. Long, to whom my thanks are due for his many helpful suggestions and criticisms.

METHODS

The mice used were active, healthy individuals. They were of different ages, some being adult and some three, four, and five weeks old. The testes were taken from freshly chloroformed animals, cut into two or three pieces and put into the killing fluids. Several killing agents were employed: namely, Zenker's, Carnoy's, and Fleming's strong solution, and Benda's modification of the latter. While any of the above gave fairly good results, Carnoy's and Flemming's were found most satisfactory.

Sections six to twelve microns thick were stained in various ways. A few were treated in Heidenhain's iron-alum haematoxylin, others with Mallory's phosphotungstic-acid haematoxylin, while the majority were stained with an alcoholic iron-alum haematin with or without a counter-stain.
The latter two stains were used on the slides upon which most of the studies were carried on. Each has its own advantages. The phosphotungstic-acid haematoxylin is a much more satisfactory stain for a study of the spindle fibres and centrosomes, and has the advantage of being a much less opaque stain than the others. It permits a study of the shapes of the chromosomes and aids in the study of the equatorial plate stages as seen in side view. It also has the advantage of staining the cytoplasm, thus doing away with the need of a counter-stain. The haematin is a better stain for the study of the prophases, since it is opaque and causes less confusion of the spireme.

The optical equipment employed consisted of a Zeiss 2 mm. oil immersion objective and a Zeiss No. 6 compensating ocular; and for higher magnifications, a Holos No. 20 ocular manufactured by W. Watson & Sons, London.

STRUCTURE OF THE TESTES

The structure of the mammalian testis is so well known that it needs little description. In the mouse there is little connective tissue binding the tubules together, and when the tough outer membrane is cut the tubules are pushed out as if they had been held under pressure. In a given section of a testis the tubules are cut at all angles. In those cut longitudinally it is easily seen that the cells along the entire length of the tubule are not in the same condition.

A cross-section of any tubule shows several of the stages of spermatogenesis. Next to the basement membrane lie the spermatogonia and the Sertoli cells, the former often scarce and quite indistinct, apparently crowded out by the foot cells. Sometimes spermatocytes are next to the membrane, but usually they are separated from the outer edge of the tubule by the spermatogonia. Still farther towards the lumen of the tubule come the spermatids and finally the spermatozoa in various stages of development. In the testis of a mouse three weeks old there are no spermatids. The majority of the cells are the primary spermatocytes, and none is older than the young secondary spermatocyte. In most cases the lumen of the tubule has not formed, the cells making a solid cord.

In a mouse aged five weeks, development has continued so far that many spermatids may be found and even a few spermatozoa; but apparently the latter are not fully matured, being well imbedded in the Sertoli cells.
It would seem from the examination of the testes of mice of different ages that the whole process of spermatogenesis might be worked out in the mature testis and on a few slides, since all stages from the spermatogonia to the mature spermatozoa are found in sections of the tubules of the adult mouse. Such a condition offers a great advantage over the study of mammalian eggs, because in this work a great number of specimens is needed and in many cases specimens of different ages.

THE PRIMARY SPERMATOCYTES

The spermatogonium in becoming a mature spermatoocyte increases greatly in size and undergoes a series of important changes in which several stages may be recognized. If the duration of one of these stages is measured by the number of cells in that state, it would seem that a greater part of the developmental period of a primary spermatoocyte is passed in the spireme condition, for most of the primary spermatocytes have the chromatin in the form of a spireme in various stages of development. The behavior of the spireme during synopsis, growth, and the formation of the chromosomes will be left for a more complete description of the whole process of spermatogenesis. Suffice it to say that the spireme segments and the chromosomes form against the nuclear membrane.

Soon after their formation, the chromosomes become arranged at the equator of the spindle with their long axes parallel to the long axis of the spindle. When examined in a polar view they are seen to be connected by threads taking the stain very similarly to the chromatin itself (pl. 30, fig. 1). This condition has not been heretofore described in mammals. The chromosomes vary somewhat in size, but usually there is one considerably larger than the others. Whether this is the accessory chromosome or not has not yet been determined, but one is led to that belief since often a large, round chromosome may be seen when the spindle is examined in side view.

The chromosomes vary in shape as well as in size, as shown in side views of the primary spermatoocyte spindles (pl. 30, figs. 3 and 5). Moreover, they have a fairly constant structure resembling very closely the chromosomes of the egg as described by Long and Mark (1911), and by Kirkham (1907a, b). Those that can be seen clearly, exhibit a lighter area running longitudinally, and at the middle of the sides darker, knob-like projections (pl. 30, fig. 5). These knobs are quite prominent on the large chromosomes in figure 3.
Polar views of spindles of the division of the primary spermatocytes show twenty chromosomes. This number corresponds to that found by Long and Mark (1911) in the eggs, as is to be expected since in each case it is the haploid number. As further evidence that twenty is the number, figure 2 (pl. 30,) shows an anaphase of the first division with twenty chromosomes migrating to each pole. The scarcity of such cases indicates that the period of migration is rapid compared with the other stages. Good polar views of the spindles of more than fifty primary spermatocytes were examined, and the number of chromosomes was found not to vary from twenty.

**SECONDARY SPERMATOCYTE**

There is no period of rest between the first and second divisions, but the chromosomes become arranged on the spindle in an equatorial plate for the second division. What the nature of the division is cannot be stated at present, for it has not been possible to determine the exact method of the formation of the chromosomes from the spireme, but there is some indication that they form so that the first division, which is transverse, results in the separation of the homologous elements. If this is true, the second division follows a splitting of the autosomes. There is one chromosome that does not divide in the second division, and that will be described later.

When viewed from the pole, the chromosomes of the secondary spermatocyte appear so similar to those of the first spindle that it is sometimes difficult to distinguish between them. There are two characteristics, however, which aid in their recognition. First, the secondary spermatocytes lie in a region of the tubule in which there is a great number of spermatids; and, secondly, there are few late spiremes to be seen in the region of the numerous secondary spermatocytes. After a little study the different kinds of cells can be distinguished beyond doubt. The points brought out in this paper are based only upon the study of the cells about which there is no doubt as to the stage of development.

In polar views of spermatocytes of the second order it was noticed that some had nineteen chromosomes, and others had nineteen in the equatorial plate and one at a higher or lower level. Figure 4 (pl. 30) represents a cell of the former type, and figure 6 shows one of the latter. An examination of figure 7 will indicate how such a condition may be brought about. If the advancing chromosome has migrated
toward one of the poles to a considerable extent ahead of the others, the plane of sectioning might come between it and the equatorial plate. This would leave nineteen chromosomes in one section as seen in polar view. If, on the other hand, the plane of sectioning did not come between the equatorial plate and the advancing chromosome, a polar view would show nineteen chromosomes in one plane and a single one at another level.

In making counts of the number of secondary spermatocytes having twenty or nineteen chromosomes, it was found that more had twenty than had nineteen. This is to be expected, since it is not at all likely that one-half of the sections would be made separating the advancing chromosomes from the equatorial plate.

A side view of a secondary spindle shows that the chromosomes are very different in shape from those of the first spindle. Instead of having the quadripartite form, they have assumed a dumbbell shape, with their long axes lying parallel to the long axis of the spindle (pl. 30, fig. 7).

With the exception of one, all of the chromosomes divide at about the same time. As they migrate they become so massed together that it is extremely difficult to count them, and by the time they have reached the poles few of the chromosomes can be distinguished in the mass of chromatin. Here too it seems that the period of migration is rapid, for anaphases are very rare.

Guyer (1910), Wodsedalek (1913), Jordan (1911), and others working on various vertebrates have claimed a second pairing of the chromosomes previous to the secondary division. This fusion I have failed to find in the mouse. Many metaphase plate stages of the secondary spermatocytes have been studied and all showed the number of chromosomes to be nineteen or twenty, and in figure 7 (pl. 30) seventeen chromosomes may be counted. Wodsedalek (1913) finds that in this secondary pairing the accessory chromosome does not pair. This process of pairing could not take place in the mouse unless one of the autosomes should pair with the allosome, since there is an uneven number of autosomes.

A chromosome has been mentioned which behaves differently from the others and which in several ways resembles an accessory chromosome. Throughout the development of the early spermatocytes, a mass of chromatin is to be seen lying against the nuclear membrane, and it is particularly conspicuous during the late spireme stages. This appears similar to a chromatin nucleolus found in some insects.
In polar views of the primary spermatocyte spindle, one chromosome appears larger than the others; and in side views of similar spindles, one chromosome does not have the distinct quadripartite form, but is more rounded. In the first division there is no indication that any chromosome does not divide and but a slight indication of any preecious splitting. Figure 3 (pl. 30) shows one chromosome marked $x$ which does begin its migration slightly ahead of the others, but not to a very marked extent. In the second division there is a chromosome with a somewhat different behavior. Without division it begins its migration ahead of the others and may have advanced half of the way to the pole before the allosomes begin their separation. This is different from the behavior of the accessory chromosomes, as described by Wodsedalek (1913) in the pig and horse, and by Jordan (1911) in the opossum. In these forms the accessory chromosome divides, not in the primary spermatocyte, but in the secondary. This would make the reduction division in the first spermatocyte. Secondary spermatocytes are therefore dimorphic. In the mouse, dimorphism is not brought about until the formation of the spermatids, half of which have nineteen chromosomes and half twenty.

**SPINDLE FIBRES AND CENTROSUME**

In sections stained with phosphotungstic-acid haematoxylin, the spindle fibres show with remarkable clearness, while with the alcoholic haematin stain they can be seen only faintly. In the second division the Zwischenkorper is very conspicuous (fig. 9), but in the earlier divisions it has not been noticed.

A structure which is ever interesting is the centrosome. When stained by the phosphotungstic-acid haematoxylin, this structure appears as a small, bright red granule, toward which the spindle fibres converge. The granule seems to be single in the primary spermatocytes, but in the secondary cells there is some indication that it divides before the division of the chromosomes (pl. 30, fig. 8). If this is true, it enters the spermatid as a double granule.
SUMMARY

The following seem to be the cardinal points in the division of the spermatocytes:

1. In the primary spermatocyte there are twenty chromosomes, which correspond in number, shape, and seemingly in size variation, to those found in the mouse egg.

2. In the first division all of the chromosomes divide.

3. In the secondary spermatocytes some sections show only nineteen chromosomes, while others show nineteen in one plane or focus and one in another.

4. There is one chromosome which does not divide in the second division, but passes in advance toward one pole of the spindle.

5. There is no secondary pairing of the chromosomes such as has been described in the horse, pig, guinea-pig, opossum, and in man.

6. The spermatids are dimorphic, half having nineteen, and half having twenty chromosomes.

Transmitted October 30, 1916.

Zoological Laboratory,
University of California.
LITERATURE CITED

GYVER, M. F.

JORDAN, H. E.
1911. The spermatogenesis of the opossum (Didelphys virginiana) with special reference to the accessory chromosome and the chondrio- somes. Arch. Zellforsch., 7, 41-86, 3 pls., 3 figs. in text.

KIRKHAM, W. B.
1907b. Maturation of the egg of the white mouse. Trans. Conn. Acad. Arts and Sci., 13, 65-87, pls. 1-8, 10 figs. in text.

LONG, J. A., and MARK, E. L.
1911. The maturation of the egg of the mouse. Carnegie Inst. Publ., 142, 1-72, pls. 1-6, 10 figs. in text.

LOUKIANOW, S. M.

Sobotta, J.

TAFANI, A.
1889. I primi momenti dello sviluppo dei mammiferi. Studi di morfologia normale e patologica eseguiti sulle uova dei topi. Arch. anat. norm. e patolog., 5, 1-59, pl. 1.

Van Hoof, L.

WoosEdalek, J. E.
1913. Spermatogenesis of the pig, with special reference to the accessory chromosomes. Biol. Bull., 25, 8-32, pls. 1-6, 1 fig. in text.
EXPLANATION OF PLATE 30

All drawings made with camera lucida from cells of the testis of the mouse fixed in Carnoy's solution. Magnification, × 2640.

Fig. 1. Primary spermatocyte in the equatorial plate stage, showing twenty chromosomes, some of which are connected by chromatin-like threads. Alcoholic haematin stain.

Fig. 2. Anaphase of primary spermatocyte division, showing twenty chromosomes migrating to each pole. The chromosome marked b shows indication of early splitting of chromosomes of second order. Alcoholic haematin stain.

Fig. 3. Side view of spindle of primary spermatocyte in early anaphase. Phosphotungstic-acid haematoxylin stain.

Fig. 4. Polar view of secondary spermatocyte in equatorial plate stage, showing nineteen chromosomes. Phosphotungstic-acid haematoxylin stain.

Fig. 5. Side view of spindle of primary spermatocyte. Alcoholic haematin stain.

Fig. 6. Secondary spermatocyte in equatorial plate stage, showing twenty chromosomes: nineteen in one plane, and one marked x at another level. Alcoholic haematin stain.

Fig. 7. Side view of spindle of secondary spermatocyte, showing one undivided chromosome marked x beginning to migrate ahead of the others. Phosphotungstic-acid haematoxylin stain.

Fig. 8. Similar to figure 6, but showing centrosome divided. The chromosome marked x is the undivided chromosome. Phosphotungstic-acid haematoxylin stain.

Fig. 9. Telophase of secondary spermatocyte, showing Zwischenkörper and massing of chromosomes at each pole. Phosphotungstic-acid haematoxylin stain.
SPECIFICITY IN BEHAVIOR AND THE RELATION BETWEEN HABITS IN NATURE AND REACTIONS IN THE LABORATORY

BY

CALVIN O. ESTERLY
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SPECIFICITY IN BEHAVIOR AND THE RELATION BETWEEN HABITS IN NATURE AND REACTIONS IN THE LABORATORY

BY

CALVIN O. ESTERLY

(Contribution from the Scripps Institution for Biological Research)

One way of studying behavior consists in subjecting organisms to physical or chemical agents and ascertaining the effect on the living material. In this way it is possible to learn what organisms can do under diverse experimental conditions; that they are capable of responses that would never be evoked in nature. This method emphasizes the agent rather than the organism; the aim is to "work out the physics and chemistry of biological phenomena" (Jennings, 1910, p. 353). The brilliant results of this way of working are well known, and the results abundantly justify it. Its bearing on the study of adaptation, if nothing else, renders it unnecessary to defend the method. This synthetic mode of procedure, as it is called by Jennings, is the one to use in studying organisms from the strictly physiological point of view.

There is, however, another point of view, in which the principal interest is to discover, if possible, what organisms do in nature, and why they do it. This gives rise to another method of studying behavior. "It is based upon interest in the organism rather than on interest in physics and chemistry, and it makes the organism the unit of work, the object of investigation" (Jennings, 1910, p. 353). The actual mode of work is to learn what the particular organism does in nature and to discover by means of the laboratory the reasons for its behavior. In this way of working the laboratory studies, as such, are at least of no more consequence than those made in the field. Such a standpoint is fundamental and important, if it is granted
that the primary object of all study of living things is to further our knowledge of them as they exist in nature. An organism is first of all something in nature, and its natural history must be known if we are studying nature and not merely objects in a laboratory. The far-reaching importance of natural history is well presented by Jordan (1916), and the "natural history" method is evidently the one Jennings has called analytic.

Both the synthetic and analytic methods are necessary and valuable means of getting information, and the method to be used depends on what the investigator wants to do. My work for the last half-year has been the study of the responses of as many different plankton organisms as possible, with a view to applying the results to the habit of diurnal depth migration. As I regard it, this requires the analytic method, and my position as to the relation between experiment and field observation coincides with that more fully presented by Michael (1916, p. xi), and so well emphasized by Hargitt (1912, p. 51).

Certain considerations have appeared with special bearing on this particular problem; but they also have important significance in any investigation intended to furnish some of the reasons for habits in nature. It is the more general application of my results that I wish to point out, rather than their meaning for the immediate problem.

The relation between the reactions of plankton organisms and the habit of periodic vertical migration has received much attention (Groom and Loeb, 1890; Parker, 1902; Loeb, 1908, 1913; Dice, 1914). Such migration in nature is abundantly established by field observations. The reasons for the movement, however, will apparently come from studying reactions. But, whatever the explanation of the movement may be, there are certain matters involved in the experimental side that will inevitably affect the results of the tests. Habit will therefore be wrongly interpreted in the light of experiment, unless these modifying factors are considered.

The discussion that follows deals with three such factors.

1. Generic and Specific Differences in Behavior

The most general result, probably, of a prolonged study of the collecting records of the Scripps Institution is the outstanding fact that each kind of organism has its own habit, particularly as regards the vertical migration. Michael (1916, p. xiv) has referred to this, and his paper dealing with the chaetognaths (1916) gives further
Esterly: Specificity in Behavior

Evidence, as does mine (Esterly, 1912) on the copepods. Specificity appears even more strikingly in the laboratory responses of different forms. It is outside the purpose of this paper to enter into generic differences, but it may be said that the representatives of each of five genera of copepods are unique in their behavior as compared with the others; the chaetognath, Sagitta bipunctata, and a schizopod have also their peculiarities.

The existence of specificity in behavior as well as in structure has not received the attention it deserves. Walter (1907) has ably presented the matter and the papers of Shelford (1911, 1912, 1914, 1915) deal with the same topic, at least incidentally. Allee (1912, 1913) also has had this point in mind, apparently. Though these writers have not dealt with plankton organisms, they have shown that the general principle holds in other forms.

As regards the plankton group in particular, I know of no work that has dealt with the reactions of various forms with a view to ascertaining specific or generic modes of response. One gets the impression from Loeb's papers (for example, Loeb, 1908, p. 732; 1913, p. 480) that the results from a few forms are applicable in general to the plankton. It is doubtful if we can rightly conclude that the reactions of any species of a genus are typical of the others until the comparison has really been made. Bauer (1909, p. 80) was of this opinion to a certain degree when he wrote that through experiment

Ich hoffe ... zu zeigen, dass die Mechanismen der Tiefenregulierung bei den verschiedenen Formen recht verschiedene sind und dass die beispielsweise für eine Daphnidenform festgestellte Reaktionsweise nicht einmal für andere Art derselben Familie, geschweige denn für alle Planktonformen, verallgemeinert werden kann.

My results lead me to extend Bauer's statement to species of the same genus. The basis for comparing the reactions of two species in the same genus is afforded in the copepods Acartia tonsa Dana and A. clausi Giesbrecht. There can be no doubt of the generic identity of these forms nor of their specific distinctness. Tonsa belongs to one general division of the genus, clausi to the other (Steuer, 1915).

The two occur at La Jolla together during some seasons of the year, and they are obtained in large numbers even in the same collections. Such conditions offer unusually favorable opportunities for comparisons. No amount of experiment, however, would tell us that tonsa occurs both summer and winter, while clausi appears off the station pier about the first of November. This in itself shows a specific difference that knowledge of the animals in nature alone can supply. It is
also something that must be known if habits are to be accounted for on the basis of reactions.

The animals were always taken in an open plankton net, with a wide-mouthed four-ounce bottle tied into the peak of the net. Thus they were never out of water and were not subjected to rough treatment. Collecting was done at the end of the pier (about 1000 feet from shore) or further out, in deeper water, from a boat. In the laboratory the animals were kept in finger-bowls. No attempt was made to furnish running water, but that in the stock vessels was changed at intervals. The animals used in experiment were handled by means of a wide-mouthed pipette and they were never forcibly drawn into or expelled from the tube. Mechanical stimulation of any sort was avoided in all possible ways. The lighting conditions in the general laboratory are those of ordinary diffuse light, chiefly from the north. The animals were never in direct sunlight.

All of the tests here referred to consisted in releasing single animals at the center of a circle 16 cm. in diameter, in a shallow dish. The nature of the response and the time taken to travel 8 cm. were noted at the circumference of the circle. The work on reactions was done in a room with black walls to which north light could be admitted through a window 50 by 40 cm. The inside of the wall of the dish was covered on the half away from the light with a mixture of lampblack and paraffine, and reflections from other sources were cut off as far as practicable by black screens. In the tables that follow the number of animals is not set down, but on the average there are about half as many animals as trials. Only adult males or females were used.

Table 1 summarizes the results obtained with females of the two species that came in the same hauls at the surface.

**TABLE 1**

**FEMALES OF ACARTIA TONSA AND A. CLAUSI FROM THE SURFACE**

Comparison of responses of the two species to light from window 1 metre away; distance and time taken from center to circumference of 16-cm. circle. The effect of low temperature is shown.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>No. of hours after capture</th>
<th>No. of trials</th>
<th>Per cent of whole no. of trials</th>
<th>Av. time in secs. to go 8 cm.</th>
<th>Av. no. of cm. per sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tonsa Over 15° C 1½—37</td>
<td>34 34 0 100 0</td>
<td>0 0</td>
<td>30.7 0 .263 0</td>
<td>31.1 42.5 .25 .18</td>
<td></td>
</tr>
<tr>
<td>2. Clausi Over 15° C 1½—37</td>
<td>37 37 0 100 0</td>
<td>0 0</td>
<td>23.3 0 .19 .0</td>
<td>31.1 42.5 .25 .18</td>
<td></td>
</tr>
<tr>
<td>3. Tonsa 15° C or less ½—7</td>
<td>57 44 13 77 23</td>
<td>14.7 9.0 .56 .34</td>
<td>31.1 42.5 .25 .18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Clausi 15° C or less ½—7</td>
<td>81 21 57 26 70</td>
<td>31.1 42.5 .25 .18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note.—The sum of the numbers in the fourth and fifth columns, fourth line, does not equal the total, because in some trials the animals did not cross the circle.
The table gives reasonable ground for the following conclusions: (1) Both species are always positive to daylight if the temperature is above 15° C. (2) At lower temperatures, most individuals of *tonsa* are positive, while most of *clausi* are negative. (3) Laboratory conditions do not have any ascertainable effects at the higher temperatures. (4) The locomotion of *clausi* is always slower than that of *tonsa*.

The second item reveals the most striking difference between the two species. I have made several different tests of sets of *tonsa* and *clausi* from the same hauls, first at temperatures of 11° C to 13° C, and then with the same animals at 19°-21° C. In the cold water *clausi* is practically always negative and *tonsa* practically always positive. In the warmer water both are always positive. These results are incorporated in table 1, but the conditions just mentioned do not appear.

It is worth emphasizing that here are two species of one genus, neither of which is made positive by cooling, while both become positive by warming. On the contrary, cooling has the effect of causing negative responses in representatives of both the species, but to a greater extent in *clausi*. One of the generalizations given by Loeb (for example, 1913, p. 480) is that cooling induces positive heliotropism which is displaced by raising the temperature; yet our commonest forms do not come under this general rule. This lack of conformity between the results obtained from many forms studied by Loeb and the results so evident here should make plain the need of ascertaining the differences between species before drawing general conclusions.

2. Effect of Laboratory Conditions

I believe that this effect must be considered in any study of behavior the object of which is to furnish the explanations of habits in nature. If the experimenter wishes only to study the general physiology of any organisms, it is well to "accustom them" to laboratory conditions, for in that way he will get rid of some troublesome variations. It is necessary, however, if one works with the former end in view, first to determine whether animals just taken from their natural surroundings react as they do several hours later.

For example, specimens of *tonsa* that have come from the surface are apparently not affected by the change from the sea to the laboratory. In 126 trials, that do not enter into table 1, made after the animals had been in the laboratory from twelve to eighteen hours, all
the responses were positive. Isolated animals have been kept during
the summer for as long as three days and tested twice a day; yet
they are always positive. I have not tried to keep animals so long
at the lower temperatures, but there is no change in the responses in
seven hours.

*Clausi* reacts similarly to *tonsa* at the higher temperatures; this
is shown in table 1, and other data agree. But at temperatures below
15° C the responses of *clausi* are changed noticeably if the animals
are kept too long in the laboratory. There is a summary in table 2
that will bring this out.

### TABLE 2

**Females of A. clausi from the Surface**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>No. of trials</th>
<th>Per cent of</th>
<th>Av. time</th>
<th>Av. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>capture</td>
<td>whole no.</td>
<td>in secs. to go 8 cm.</td>
<td>of cm. per sec.</td>
</tr>
<tr>
<td>1°-6°</td>
<td>70</td>
<td>61</td>
<td>16.5</td>
<td>.48</td>
</tr>
<tr>
<td>16-21</td>
<td>44</td>
<td>18</td>
<td>27.0</td>
<td>.29</td>
</tr>
</tbody>
</table>

The table may be said to be self-explanatory and the effect of
laboratory conditions, whatever they are, is evident. No records that
enter into table 2 were used in table 1, yet the two tables show similar
results so far as *clausi* is concerned.

The effect of the laboratory is correlative with the third of the
factors under discussion. These two can hardly be separated in
practice, but they may be for the sake of discussion.

### 3. Influence of Habitat (Environment) from Which Animals are Taken

This influence has been noted by some observers, but not in the case
of plankton animals, so far as I know. A recent note by Kepner and
talianferro (1915) is along this line, as are the interesting observations
of Hargitt (1909) on tubicidous annelids. Allee (1912, 1913) has
shown that individuals of the same species of isopod, some from a
pond and some from a stream, have different sorts of behavior. And
Shelford (1914) has found that, in comparing the behavior of different
crinds of stream and pond animals, as *groups* they are contrasted
with each other; but within a group there are certain characteristics
in common.

These instances, however, all relate to animals that are permanent
residents of any particular habitat. Yet, under certain circumstances,
a form that moves periodically from one region to another can be shown to be in different states or conditions that depend, apparently, upon the environment from which it comes. It is true, as Michael (1916, p. xiii) has said, that "Reactions in a laboratory ... may be largely due to the particular individuals collected."

If specimens of *tonsas* or *clausi* are used that have been collected from depths of 10–20 fathoms during the middle of the day (at these times it is practically impossible to get them at the surface), the number of negative responses to light increases while the positive responses decrease. Evidence to this effect appears in table 3.

**TABLE 3**

**FEMALES OF A. TONSA AND A. CLAUSI, OBTAINED FROM 10–20 FATHOMS DURING THE DAY, IN THE SAME HAULS**

Responses to light from window 1 metre away; time and distance taken from center to circumference of 16-cm. circle; animals kept in laboratory 3/4 to 2 1/2 hours; temperatures 11°–14° C.

<table>
<thead>
<tr>
<th></th>
<th>No. of trials</th>
<th>Per cent of whole no. of trials</th>
<th>Av. time in secs. to go 8 cm.</th>
<th>Av. no. of cm. per sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonsa</td>
<td>29</td>
<td>13</td>
<td>45</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>16</td>
<td>55</td>
<td>31.5</td>
</tr>
<tr>
<td>Clausi</td>
<td>12</td>
<td>0</td>
<td>100</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
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</tbody>
</table>

A comparison of the results set forth in table 3 with those in tables 1 and 2 strongly indicates that animals obtained where the light is of relatively low intensity are negative to higher intensities. That is, there is some relation between habitat and response. It can not yet be established whether the animals are negative because of residence in that habitat or whether they reach the habitat because they previously became negatively heliotropic. But it is evident that the kind of response is in some way related to environment. This conclusion is reinforced by some other data which also show the change produced in the animals by laboratory conditions.

**TABLE 4**

**MALES AND FEMALES OF A. TONSA, OBTAINED FROM 10–12 FATHOMS DURING THE DAY**

Responses to light from window 1 metre away; time and distance taken from center to circumference of 16-cm. circle; temperatures 12°–17° C. Sign of response effected by laboratory conditions.

<table>
<thead>
<tr>
<th>No. of hours after capture</th>
<th>No. of trials</th>
<th>Per cent of whole no. of trials</th>
<th>Av. time in secs. to go 8 cm.</th>
<th>Av. no. of cm. per sec.</th>
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<tr>
<td>1/4–1 1/4</td>
<td>51</td>
<td>9</td>
<td>82</td>
<td>28.2</td>
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<td></td>
<td>42</td>
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</table>

Note 1.—Line 1 contains: 14 trials at 16°–17° C, 14 negative; 23 trials at 14°–15° C, 19 negative; 14 trials at 12°–13° C, 9 negative.

Note 2.—The temperature during records of line 2 was 14°–15° C.
In table 4 is brought out the fact that the animals from below the surface are overwhelmingly negative to light if they are tested soon after collection. This is in sharp contrast to the behavior of surface animals at any time. On the other hand, if the subsurface forms remain in the laboratory too long, the reaction is reversed. The second line of table 4 contains the records of five males and five females from a collection in which every animal tested was negative on the preceding day. On that day the animals moved away from the light when released one by one, and they remained on the room side of the stock dish. It is worth noting that the proportion of negative trips is greater at higher temperatures (see note 1), though it is at higher temperatures that surface animals are all positive.

It is evident that animals kept beyond a certain length of time in the laboratory do not give responses that will help in reaching the true explanation of their behavior under natural conditions. Experiments with such material are more likely to mislead us than to further our knowledge of habits. In addition to determining what effect confinement in the laboratory has, it is also necessary that experimental animals be obtained from as many different habitats as possible. It can not be said how general the influence of environment on reaction is but this influence should always be ascertained if possible.

The importance of negative phototropism in Acartia tonsa may be more appreciated if a summary of still other data is given. When the source of light is a Mazda lamp, surface animals are always positive, whether it is a 100-watt 19 cm. from the center of the circle, or a 15-watt at a distance of 32 cm. The response is positive no matter what the preceding intensity of illumination may have been. At temperatures of 15°-16° there were no negative trips in 202 trials, whether intense light preceded weaker or vice versa, or whether the animals were in the dark previous to testing. It is not fully established whether freshly caught animals from below the surface would be negative to all intensities. A few, however, were negative to the 15-watt lamp under conditions when all others have been positive.

The preponderance of positive reactions under most circumstances emphasizes the conditions under which negative responses appear, and it is important to note that, so far as the experimenter is concerned, these conditions are resident in the animals.

Another indication of the influence of habitat on reaction may
be given here, though it is outside the field of this particular paper. It has been found from many trials that A. tonsa, if taken from the surface, is predominatingly negative to gravity in diffuse light, and positive, for the most part, in the dark; and that an animal will swim up toward a light at the top and down toward a light at the bottom. But animals from below the surface are positive to gravity in diffuse light, and swim down from a light at the top.

It has been shown that Acartiæ taken from below the surface do not react to light, if tested at once, as they do after several hours, or as surface animals always do. For the sake of discussion we may say that, so far as collecting shows, the copepods caught at ten fathoms are negative to light and positive to gravity, and that surface animals are positive to such intensity of light as exists at the time, and negative to gravity. Such, in fact, appears to be the case from experimental evidence, but we should not discover it to be so if we use only surface animals or those from below the surface that have been in the laboratory too long. Even admitting, furthermore, that the essential features of responses as related to habits can be satisfactorily worked out in the laboratory alone, it is necessary to use animals from different habitats and before the corresponding physiological state has changed. In addition, as Ritter (1916, especially p. 461) has set forth in a more general way, different species show diversities in other regards than structure, and this must be taken into account in general explanations of habits based on experiment.
SUMMARY

1. A comparison of the heliotropic reactions of two species of copepods, *Acartia tonsa* and *Acartia clausi*, shows that while they are alike in some respects there are also significant differences.

2. Both species, if collected from the surface, are always positive to light at temperatures above 15° C, but the majority of *clausi* are negative at lower temperatures, while most (but not all) specimens of *tonsia* are positive (table 1).

3. *Clausi* is the slower of locomotion.

4. Laboratory conditions do not affect the light reactions of *tonsia* if the animals come from the surface, but those of *clausi* are reversed by retention in the laboratory (table 2).

5. The effect of laboratory conditions is also shown in animals that come from ten fathoms or below, in that the responses of most of them to light are changed from negative to positive (table 4).

6. "Physiological states" connected with habitat are evident, and plainly have an influence upon the reactions (tables 3 and 4). The number of negative responses increases and the number of positive responses decreases significantly if animals that have come from ten or twenty fathoms are used within a short time, as compared with the reactions of surface animals.

7. It is suggested by these facts that the results of experiment can not be fairly used in accounting for habits unless animals from different regions are tested. In any case the effects of laboratory conditions should be ascertained before it is assumed that "accustoming to laboratory conditions" is advisable and will not lead to erroneous interpretations.

8. In generalizing about the reasons for the habits of such a group of organisms as those in the plankton, from experiments on a few representative kinds, false conclusions are practically certain. It is necessary that at least all the genera be used, and desirable that different species of the same genus be tested.

9. The mutual support of laboratory and field study is necessary to determine fully the relations between responses and habits.

*Transmitted January 26, 1917.*

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KEPNER, W. A., AND TALLAFERRO, W. H.

LOEB, J.

MICHAEL, ELLIS L.
Parker, G. H.

Ritter, Wm. E.

Shelford, Victor E.
1914. An experimental study of the behavior agreement among the animals of an animal community. Ibid., 26, 294–315.

Steuer, Adolf.

Walter, Herbert Eugene.
THE OCCURRENCE OF A RHYTHM IN THE GEOTROPISM OF TWO SPECIES OF PLANKTON COPEPODS WHEN CERTAIN RECURRING EXTERNAL CONDITIONS ARE ABSENT

BY

CALVIN O. ESTERLY

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THE OCCURRENCE OF A RHYTHM IN THE GEO-TROPISM OF TWO SPECIES OF PLANKTON COPEPODS WHEN CERTAIN RECURRING EXTERNAL CONDITIONS ARE ABSENT

BY

CALVIN O. ESTERLY

(Contribution from the Scripps Institution for Biological Research)

It is known that rhythmic activity may occur in animals even when periodic changes in external conditions, that may have caused the rhythm in the first place, can no longer affect the organisms directly. Examples of such behavior have been given by Holmes (1911, pp. 16, 79, 80, 155-158) and other instances may be found in the paper by Menke (1911). Activities of this character in strictly pelagic animals of the plankton have not been observed, so far as I know, under experimental conditions.

The marine copepods Acartia tonsa Dana and A. clausi Giesbrecht show a periodic change in behavior under uniform external conditions. Temperature is a modifying factor, however, for tonsa. Both species are common and easily obtained by towing from the pier of the Scripps Institution. Tonsa has been taken all the time from August, 1916, to the present date, while clausi appeared about November 1.

Acartia tonsa, in the ordinary diffuse light of the laboratory, is for the most part negatively geotropic, providing the animals have been obtained at the surface. As might be expected, the individuals do not all show the same response, but most animals of most sets stay at the surface or near it when in a vertical container. The animals were released at the top of a flat-bottomed tube 50 cm. high and 30 mm. in diameter, and the tube was left standing about 75 cm. from the north windows. The column of water was considered to be
divided into five sections, each 10 cm. in height; section V is at the top and section I at the bottom.

If such preparations are kept in the dark all the time, a smaller proportion of animals is found in the upper parts of the tube, if all records are considered together, than when the illumination is constant.

All of the tables in this paper are constructed on the same plan, and the following explanation applies to all. The "whole number of animals observed" is the sum of all records of distribution in all experiments, several records having been made in each one. Each animal (if alive) was counted as many times as there were observations made during that experiment. "Percentage distribution" is the proportion of the total number of animals observed in each section, for all experiments, to the whole number observed. "Number of observations" means the number of times the distribution was recorded. The "center of distribution" is the average position (as between the top and bottom) of the whole number of animals observed. It is obtained by multiplying the total number of animals recorded in each section of the tube by the number of the section, and dividing the sum of the products by the "whole number of animals observed." The centers are set down in units and tenths and the unit is considered as the middle point of the section having the corresponding Roman numeral. If one center is compared with another, the difference between them, if any, is the magnitude of the shift of the whole population. For example, if the centers in the third and fourth lines of table 2 are compared, the difference is 1.5; and since the center changes from 2.5 to 4 this signifies that the population as a whole shifted a section and a half (15 cm.) upwards. The method of determining the average position is that of Banta (1910, p. 253).

A comparison of a number of records of the distribution of Acartia tonsa in light and in darkness is found in table 1.

**TABLE 1**

**Acartia tonsa, Adults of Both Sexes: Summary of Records of Distribution in a Column of Water in Diffuse Light and in Darkness**

<table>
<thead>
<tr>
<th>Whole no. of animals observed</th>
<th>Percentage distribution in the sections</th>
<th>No. of observations</th>
<th>Average % showing interval Cen- more ans., in between 1/2 upper lower obs., of Total 2 1/2 2 1/2 (hours) distr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>In the light</td>
<td>380</td>
<td>56.5</td>
<td>6.0</td>
</tr>
<tr>
<td>In the dark</td>
<td>738</td>
<td>52.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>
It is shown in the table that a good many more animals are found in the upper than in the lower two-fifths if the tubes are kept in the light. Temperature seems to have no effect on the distribution, since experiments were made at intervals from July through December, during which time there has been a reduction in average room temperature of 8° C.

The second line of table 1 contains a summary of all the records of distribution in darkness without regard to any modifying factor, and it is shown that the number of animals decreases in the upper and increases in the lower sections in comparison with the proportions in the light; the center of distribution is also at a lower level in the dark. This general result as regards darkness is affected, however, by two conditions—temperature and time of day. The effects of these factors can be seen in such a tabular summary as that in table 2, wherein the records in the second line of table 1 are arranged according to the time of day at which the observations were made, as well as according to temperature. It should not be forgotten that the experiments were set up during the forenoon and the animals kept in the dark except for the brief time necessary to note their distribution.

**TABLE 2**

| ACARTIA TONSA, ADULTS OF BOTH SEXES: SUMMARY OF RECORDS OF DISTRIBUTION IN A COLUMN OF WATER IN DARKNESS, TO SHOW EFFECT OF TEMPERATURE AND TIME OF DAY |

<table>
<thead>
<tr>
<th>Whole no. of animals observed</th>
<th>V</th>
<th>IV</th>
<th>III</th>
<th>II</th>
<th>16° C or MORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-12 A.M.</td>
<td>135</td>
<td>22</td>
<td>1.5</td>
<td>0.74</td>
<td>2.2</td>
</tr>
<tr>
<td>12:01-3:59 P.M.</td>
<td>141</td>
<td>25</td>
<td>1.4</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>4-5:59 P.M.</td>
<td>65</td>
<td>37</td>
<td>1.5</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>6-8 P.M.</td>
<td>70</td>
<td>72</td>
<td>0.43</td>
<td>4.3</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Average interval between observations (hours) Center of distr.

| Below 16° C                  | 68  | 69 | 4.4 | 0  | 3.9 | 22.3 | 12 | 66 | 34 | 1.08 | 3.9 |
| 12:01-3:59 P.M.              | 128 | 78 | 3.9 | 2.3 | 2.3 | 14 | 20 | 90 | 8  | 1.0 | 4.2 |
| 4-5:59 P.M.                  | 53  | 81 | 3.8 | 0  | 3.8 | 11.3| 9  | 100| 0  | 1.5 | 4.6 |
| 6-8 P.M.                     | 78  | 74 | 5.1 | 5.1| 0.1 | 14 | 16 | 81 | 19 | 0.55 | 4.2 |

Note.—The reason for the proportional reduction in numbers from late afternoon on, as compared with the rest of the day, is that some individuals die in the course of the day, so that as the tube is left standing the whole number becomes less. This, however, does not affect the percentages.

It is obvious that at the higher temperatures there is a marked upward movement of the animals from 6 to 8 P.M. This is shown by
the percentage distribution, by the number of times an excess of animals was observed in the upper or lower portions of the tubes, and by the location of the center of distribution. Every experiment was continued as long as enough animals were alive to make it worth while—in some cases to the next or even to the second day. So few observations were made after 8 p.m. that nothing need be said about them except that they indicate that the descent begins before 9 p.m.

At the lower temperatures, on the other hand, the ascent is much less marked, although it can be said to occur. It is probably significant that, in the lower half of the table, there is a gradual increase in the percentages in the upper fifth of the tube accompanied by corresponding decreases in the lower fifth, from 8 a.m. to 6 p.m. Furthermore, the proportion of observations showing the excess in the upper two-fifths increases during the time that the proportion in the lower two-fifths steadily decreases. During these hours, also, the center of distribution is gradually rising. All of these indications, taken together, lead one to conclude that there is a certain amount of upward movement. This conclusion is strengthened when it is noted that from 6 to 8 p.m. the percentage distribution in section V decreases, while that in sections IV, III, and I increases; that the proportion of observations showing an excess of animals in the upper two-fifths has decreased as compared with the preceding period, while the proportion in the lower two-fifths has increased; and that the center of distribution has been shifted to a lower level. Nevertheless, the effect of lower temperatures is striking, on the whole.

The upward movement in the dark is shown even more clearly when the animals have been obtained from ten or twenty fathoms. Temperature does not seem to affect such animals, but I have used them only once when the temperature was 16° C or over. All records, therefore, of the distribution of deep-water animals in the dark are

**TABLE 3**

| Acartia tonsa, Adults of Both Sexes, from 10-20 Fathoms: Summary of Records of Distribution in a Column of Water in Darkness, to Show Effect of Time of Day |
|---|---|---|---|---|---|---|
| | Whole | Percentage | No. of | Average | |
| | no. of | distribution | observations | interval | |
| | animals | in the sections | showing | between | between |
| | observed | | | more ans. in | ter obsrvns. |
| | | | | upper | of |
| | | | | lower | (hours) |
| | | | | | distr. |
| | | | | | |
| | | | | | |
| 8-12 A.M. | 50 | 0 | 4.0 | 2.0 | 2.0 | 62 | 10 | 0 | 100 | 0.88 | 1.3 |
| 12:01-3:59 P.M. | 156 | 13 | 0.65 | 2.5 | 2.5 | 81 | 24 | 0 | 100 | 0.92 | 1.6 |
| 4-5:59 P.M. | 57 | 37 | 0 | 0 | 0.7 | 62 | 10 | 36 | 70 | 1.2 | 2.5 |
| 6-8 P.M. | 61 | 62 | 7.8 | 4.7 | 3.1 | 22 | 11 | 82 | 0.91 | 1.0 | 3.8 |
included in table 3, regardless of temperature. It should be stated that animals from such depths are positively geotropic in the light if not left too long in the laboratory before being tested, and their positive reaction is more strongly marked than the negative response of surface animals.

Comments on table 3 are needless, since the results are in all respects similar to those in table 2.

It seems reasonable to conclude that the stimulus for the upward movement shown in tables 2 and 3 is an inner unknown factor, since the periodic behavior takes place in the absence of known recurrent changes in the environment. This statement may pass as a matter of observation, aside from the question of how the rhythm may have been impressed on the organisms in the first place. On account of the mortality among the animals, there is not much that can be said about the duration of the tendency to ascend at certain times. In two separate experiments on deep-water animals the upward movement was manifested on two successive days. In each case the animals were in constant darkness (except when the distribution was recorded) from twenty-eight to twenty-nine hours. In another, using surface animals, the ascent took place on two days, the animals having been in darkness for fifty-three hours and under intermittent observation for twenty-nine hours.

*Acartia clausi*, as well as *A. tonsa*, exhibits this sort of behavior, but the former is not affected by colder water as is the latter. All experiments using *clausi* were at temperatures below 16° C. Table 4 is for the general comparison of the distribution of *clausi* in diffuse light and in darkness.

**TABLE 4**

**ACARTIA CLAUSI, ADULTS OF BOTH SEXES: SUMMARY OF RECORDS OF DISTRIBUTION IN A COLUMN OF WATER IN DIFFUSE LIGHT AND IN DARKNESS**

<table>
<thead>
<tr>
<th></th>
<th>Whole no. of animals observed</th>
<th>Top section</th>
<th>Percentage distribution in the sections</th>
<th>No. of observations</th>
<th>% showing more animals in upper section</th>
<th>Average interval between observations (hours) distrib.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>III</td>
<td>II</td>
<td>I</td>
<td>Total</td>
</tr>
<tr>
<td>In the light</td>
<td>392</td>
<td>55</td>
<td>4.6</td>
<td>1.3</td>
<td>1.6</td>
<td>38</td>
</tr>
<tr>
<td>In the dark</td>
<td>382</td>
<td>27</td>
<td>8.7</td>
<td>3.7</td>
<td>2.4</td>
<td>58</td>
</tr>
</tbody>
</table>

The table shows that there is a strong tendency for the animals to gather in the upper portions of the column of water when in the light, and to congregate at lower levels in the dark if the time is considered as a whole.
But if we put together the records of distribution in the dark according to the time of day, we shall find that at one period of the day the animals are at or toward the top. A summary of this kind appears in table 5.

**TABLE 5**

**ACARTIA CLAUSI, ADULTS OF BOTH SEXES, FROM THE SURFACE: SUMMARY OF RECORDS OF DISTRIBUTION IN A COLUMN OF WATER IN DARKNESS TO SHOW EFFECT OF TIME OF DAY**

<table>
<thead>
<tr>
<th></th>
<th>Whole no. of animals observed</th>
<th>Percentage distribution in the sections</th>
<th>No. of observations showing more anim. in upper lower</th>
<th>Average interval between observ. (hours)</th>
<th>Center of distr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>IV</td>
<td>III</td>
<td>II</td>
</tr>
<tr>
<td>8-12 A.M.</td>
<td>113</td>
<td>31</td>
<td>8.9</td>
<td>4.4</td>
<td>0.89</td>
</tr>
<tr>
<td>12:01-3:59 P.M.</td>
<td>133</td>
<td>20</td>
<td>9.0</td>
<td>3.7</td>
<td>1.5</td>
</tr>
<tr>
<td>4-5:59 P.M.</td>
<td>67</td>
<td>16</td>
<td>6.1</td>
<td>6.1</td>
<td>1.5</td>
</tr>
<tr>
<td>6-8 P.M.</td>
<td>69</td>
<td>43</td>
<td>10.1</td>
<td>7.5</td>
<td>0</td>
</tr>
</tbody>
</table>

The results set forth for *clausi* in table 5 are evident, and along the same lines as those for *tonsa*. One experiment with *clausi* ran through three successive days, with the ascent at about the same time on each day; during the rest of the time most of the animals were in the bottom section. The total time in darkness was 58 3/4 hours, with observations made at intervals for 32 3/4 hours.

It is not desired to discuss at this time the question of what effects, varying periodically previous to constant darkness, may have been responsible for the rhythm under practically uniform conditions, or whether the rhythm is to be accounted for at all by the action of antecedent recurring stimuli.

As yet it is unestablished whether the upward movement would take place in constant illumination, but it is doubtful if it would, because of the strong tendency of the animals to stay at the top in the light. Loeb (1913, p. 480) states that he has directly observed the vertical movement of certain forms under the influence of light, and Groom and Loeb (1890, pp. 172-173) say that the daily depth migration of barnacle larvae goes on in a glass of water as on the high sea. If these observations relate to animals that are kept in the light, the behavior may be due to changes in the light-intensity. *Acartia*, however, does not descend during the day from 8 A.M. to 4 P.M. if in the light. I have watched a good many sets and have recorded their distribution at intervals of from five to fifteen minutes, and the percentages of animals observed in the upper and lower sections does not vary from morning to late afternoon. Some animals
are in the lowest section at any time, but tubes standing before the windows show most animals in the upper sections.

The bearing of the experimental data given here on an explanation of the diurnal depth migrations in the sea will be considered when the field collections and observations, that are being made now, can be presented.

**Summary**

1. The marine copepods *Acartia tonsa* and *A. clausi* both show a marked tendency to stay toward the top of a column of water in diffuse light.

2. Both species, if kept in the dark, show larger numbers on the whole toward the bottom.

3. But there is a marked increase in relative numbers in the upper parts of the column, as compared with the lower portions, from 6 to 8 p.m., and not at other times of the day. This may be repeated on the second day although the animals have been in darkness all the time.

4. The descent begins, apparently, between 8 and 9 p.m. At any rate, in experiments that extend into a second day, the animals are in the lower section for the most part, in the morning; they ascend again in the evening.

5. Temperatures below 16° C have the effect in the case of *tons a* of causing more animals, relatively, to remain toward the top, though the periodic ascent is still evident.

*Transmitted January 29, 1917.*

**Scripps Institution,**

**La Jolla, Calif.**
LITERATURE CITED

BANTA, A. M.

GROOM, T. T., and LOEB, J.

HOLMES, S. J.

LOEB, J.

MENKE, HEINRICH.
ON SOME NEW SPECIES OF APHRODITIDAE
FROM THE COAST OF CALIFORNIA

BY

CHRISTINE ESSENBERG
UNIVERSITY OF CALIFORNIA PUBLICATIONS

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On some new species of Aphroditidae from the coast of California

By

Christine Essenberg

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A. INTRODUCTION

While examining the abundant material in the Zoological Museum at the University of California, my attention was first drawn to the Aphroditidae. This family having received little attention, some species were found which evidently were new, and it is the aim of the present paper to describe them.

B. ACKNOWLEDGMENTS

I gladly avail myself of this opportunity to express my sincerest thanks to Professor Charles A. Kofoid for his encouragement, his valuable suggestions, and his criticism in this work. I further wish to extend hearty thanks to Dr. Olive Swezy for her kind criticism and her interest in the work.
C. GENERAL DESCRIPTION OF THE APHRODITIDAE

The Aphroditidae are exclusively deep-water annelids, although occasionally, after severe storms, specimens are found on the shores, where they have been driven by the waves. The distribution of the Aphroditidae is known over the various parts of the Atlantic, Pacific, and Indian oceans. They occur in all zones from the boreal to the subtropic regions.

Some Aphroditidae are noted for their beautiful, iridescent fibers. Especially *Aphrodita aculeata*, the well-known seamouse, has been the object of the great admiration of many observers. Cuvier (1834) says of *Aphrodita aculeata*: "From its sides spring bundles of flexible bristles, shining brilliantly with all the splendor of gold, and changing into all the hues of the rainbow. They do not yield in beauty either to the plumage of the hummingbird or to the most brilliant of the precious stones." Linnaeus compares its vivid colors with those of the peacock. On the Pacific Coast *Aphrodita refulgida*, with its brilliant iridescent lateral fibers, takes the place of the *Aphrodita aculeata* of the Atlantic Coast.

The species of *Aphrodita* are less numerous than are those of the nearest related families. Dr. J. P. Moore, who has done the most work on the Pacific Coast annelids, reports six species of *Aphrodita* from this coast: *Aphrodita castanea*, *A. refulgida*, *A. negligens*, *A. parva*, *A. armifera*, and *A. japonica*. Treadwell (1914, p. 177) in his report on the polychaetous annelids from the collections of the University of California, enumerates four species of that genus: *Aphrodita castanea*, *A. refulgida*, *A. parva*, and *A. negligens*. The first two species are in abundance in the collection. However, I failed to find any representatives of *Aphrodita negligens*, although some specimens were labeled as *Aphrodita negligens* which belonged to other species. The same may be said in regard to *Aphrodita parva*. I have not found any specimens that agree with Dr. Moore's *Aphrodita parva*.

The Aphroditidae may be characterized as follows: They are ovate or oblong in shape, convex dorsally, with a distinct, lobed head or prostomium on the anterior end of which are a pair of ocular hemispheres, each usually bearing a pair of eye-spots. From the center of the anterior margin of the prostomium springs a median tentacle. Ventrad to this is a papillose facial tubercle. Two palpi arise from the base of the prostomium. The mouth is ventral, bordered by a
broad lip. There are usually fifteen pairs of elytra, occurring on segments 2, 4, 5, 7, and on all alternate segments to 25; then on segments 28 and 32. Beginning with the sixth segment are the fimbriated organs occurring on all eirriferous segments. Over the elytra is a thick coat of a felty layer, formed by the tufts of dorsal fibers arising from the notopodia. The parapodia are biramous, each ramus being supported by a strong bristle or aciculum. The neuropodium terminates in a peculiar, three-step-like fashion. There are no lateral prostomial tentacles. The notocirri are long and occur on all alternate segments; the neurocirri are short, occurring on all segments.

The presence of the lateral and of the felt fibers, of ocular hemispheres, of the facial tuberele, of the fimbriated organs, and neuropodia terminating in the three-step-like fashion, and the absence of the lateral prostomial tentacles distinguish the Aphroditidae from the Polynoidae and from any other family of annelids. The genus Aphrodita differs from the genus Lactmatonice in the sessile eyes, in the simple ventral setae, and in a thicker dorsal felt. In Lactmatonice the eyes are pedunculate, the dorsum is covered with thin felt, or the latter may be absent from the dorsum, and the ventral bristles are semipinnate.

The differentiation of the species is based partly on the size and the shape of the body. Further characteristics concerned in diagnosis are: the relative length of the neuropodia; the shape of the prostomium; the shape and the size of the ocular peduncles, and the size of the eyes; the length of the median tentacle; the relative length of the palpi; the arrangement of the notosetae; the form and the structure of the setae; the shape of the fimbriated organs and of the elytra; and, to some extent, the shape and the size of the papillae.

1. Aphrodita longipalpa, sp. nov.

Pl. 31, figs. 1-14; pl. 37, figs. 77-78

Comparison.—The description of this species is based on two specimens which are in the collection of the University of California. They have a slight resemblance to Aphrodita castanea (Moore, 1910). The chief resemblance lies in the arrangement of the dorsal setae, which are recumbent, pointing posteriorly, and covering the dorso-lateral surface of the worm, as in Aphrodita castanea. Probably the latter characteristic led Treadwell to the conclusion that the speci-
mens belonged to the species castanea, as he so classified these specimens (1914). Closer observation and comparison of the specimens with Aphrodita castanea, which Dr. Moore had kindly sent to us, as also with the numerous examples of A. castanea in the collections of the University, showed some essential characteristics distinguishing these specimens from Aphrodita castanea. We have therefore assigned them to a new species, A. longipalpa, distinguished from A. castanea as follows: (1) the shape of the body of Aphrodita longipalpa is narrower than that of A. castanea; (2) the dorsum is more convex; (3) the notosetae, although arranged in the same manner as those of A. castanea, lack the golden-brown, lustrous appearance of the latter, and are rather dull and inconspicuous; (4) the parapodia are relatively longer than in A. castanea; (5) the neurosetae are very long, of a dark-brown, almost black, color; (6) the palpi are the longest known in any species of the genus; (7) the shape of the prostomium differs also from that of A. castanea; (8) the ocular hemispheres are less prominent, without any trace of pigmented eye-spots, while in A. castanea the two pairs of eye-spots show very distinctly; and (9) the fimbriated organs in A. longipalpa are less deeply lobed, and the lobes are less numerous, than they are in castanea, being obtusely rounded instead of long, narrow, and pointed.

Description.—The body (pl. 37, figs. 77, 78) is narrow and the dorsum is convex. The length of body in the two specimens is 26 and 34 mm. respectively, and the greatest width is 19 and 22 mm. respectively, measured from tip to tip of the setae. The width of body between the parapodia is 6 and 8 mm. respectively. The number of segments is 33.

The width of the prostomium (pl. 31, fig. 1) is slightly greater than its length. The two ocular prominences are hemispherical, slightly depressed from above. No eye-spots are visible on either of the specimens. The palpi are 11.5 times the length of the prostomium, having a definite short basal part. They are stout at the base, tapering very gradually toward the distal end, covered with very fine sensory papillae, which are visible only under high magnification. The median tentacle consists of a short cirratophore and a style of equal length. It is so far ventrad that it is invisible when viewed from the dorsal surface. The facial tubercle is large, compressed between the palpi. It ends in a long, finger-like projection extending ventrally over the mouth, and is covered with prominent papillae. The mouth is ventral. The ventral lip is definitely marked off by
deep furrows on each side and extends to the third segment. The dorsum is covered with a thick, felty layer; beneath this are fifteen pairs of tough, widely overlapping elytra (pl. 31, fig. 10) covering the entire dorsum. They are arranged in the usual order characteristic of the genus and are partly covered with brown pigment. The fimbriated organs (pl. 31, fig. 12) are hatchet-shaped, consisting of three or more lobes, each lobe having two or three short projections.

The ventral and dorsal surfaces are studded with papillae. The latter are simple, without the caps (pl. 31, figs. 13, 14). The parapodia are biramous (pl. 31, fig. 11). The notopodium is a mere protrusion. The neuropodium is very long and slender. The neurosetae, which are arranged in the usual three series, are very dark brown, almost black, and are very long. The dorsal row consists of two stout setae, the ends of which are tapered, slightly curved, and pillose (pl. 31, figs. 2, 2'). The median row has four setae, equal in structure to the former, but smaller in diameter (pl. 31, fig. 4). The six setae from the ventral series are lighter brown in color, smaller in diameter, with a subterminal enlargement ending in an attenuated, curved, and richly pillose extremity (pl. 31, fig. 3). The neurosetae of the second parapodium are specially modified with long, dentate projections (pl. 31, fig. 6). The caudal neurosetae are long, ending bluntly, with the distal portion covered with conspicuous protuberances (pl. 31, figs. 5, 5', 5''). The notosetae are in two rows, the lateral ones forming a fan-like arrangement pointing dorsally. The dorsalmost are very long, with colorless, slightly hooked tips (pl. 31, fig. 9), meeting in the dorsum or overlapping. The notosetae are covered with asperities, which are visible only under higher magnification (pl. 31, fig. 7). Some of the dorsal setae near the posterior extremity end more bluntly and are surrounded by a gelatinous envelope (pl. 31, fig. 8).

The lateral fibers are short, coarse, and colorless. They are sparse and do not conceal the neuropodia. The long fibers form a heavy, felt layer over the dorsum.

The neurocirrus arises from the parapodium at a point about two-thirds of its length from the body; it is only about one-fourth of the length of the notocirrus (pl. 31, fig. 11).

Occurrence.—The two specimens, type and cotype, were found at Station 1124 at 32° 55' 1 N, 117° 18' 5 W, off La Jolla, California, at a depth of 292 metres, on green mud bottom, June 25, 1906.
2. Aphrodita californica, sp. nov.

Pl. 32, figs. 15-26; pl. 37, figs. 79-80

Comparisons.—Only one specimen of this interesting species is in the collection. It had been included with those annelids identified by Treadwell (1914) as Aphrodita castanea. However, it differs from the latter in the following characteristics: the shape of the body is shorter and broader than that of A. castanea; the golden-brown dorsal setae, which are conspicuous in A. castanea, are covered by the colorless lateral fibers and are of a dull, pale color; the shape of the prostomium is somewhat squarish; the eyes are very large; the median tentacle is unusually long in A. californica, while in A. castanea it is very short; the fimbriated organs have short projections ending bluntly; the neurosetae in A. californica are perfectly smooth, without any trace of hairiness, while those of A. castanea have pillose tips.

Description.—The body (pl. 37, figs. 79, 80) is oval, broadly rounded anteriorly, tapering abruptly posteriorly to a narrow caudal region. It is dark gray in color. The dorsal surface is covered with a heavy feltly covering and with debris, so that the animal at first sight looks more like a piece of inorganic matter than like an annelid. The length of the body is 18 mm.; the width, 8 mm., from tip to tip of setae; distance between the parapodia, 4 mm. There are thirty-three segments, very definitely marked off. The parapodia are marked off from the main body by a deep groove on each side of the ventral surface. The ventral surface is thickly covered with large papillae (pl. 32, fig. 22) which have capped tips.

The prostomium (pl. 32, fig. 15) is somewhat squarish in shape, subglobular, the width slightly exceeding the length. The narrow isthmus by which it is attached to the peristomium is about one-third of the width of the prostomium. The ocellar prominences are large, each bearing a pair of comparatively large eyes of which only one is visible from the dorsal surface, while the other is located at the extreme anterior end of the ocellar hemisphere. The palpi arise from short basal portions. They are stout, somewhat uniform in diameter except the distal ends, which slope gradually to attenuated tips. The length of the palpi is four times that of the prostomium, and their surface is covered with fine sensory papillae. The median tentacle arises from a prominent cirrophore, which is one-fifth of the style and is covered with papillae. A few papillae are also scattered on
the dorsal surface of the anterior portion of the prostomium. The median tentacle is unusually long in this species. In this respect it resembles Kinberg's (1855) *Aphrodita longicornis*, but differs from it in the absence of the iridescent lateral fibers and in the shape and the size of the body.

The ventral lip extends to the third segment and is well marked off laterally by deep grooves on each side. The facial tubercle reaches ventrally to the mouth.

There are fifteen pairs of elytra, arranged in the usual order characteristic of the genus. The elytra (pl. 32, fig. 18) are large, thin, and transparent, covering the entire dorsum. The branchiae (pl. 32, fig. 23) are hatchet-shaped, having three or four main lobes, which are more or less deeply indented.

The parapodia (pl. 32, fig. 24) are biramous, supported by two long, dark-brown acicula, piercing the tips of parapodia. The neuropodium is of median length, bearing the three rows of setae. The dorsal series consists of two very stout, bluish-brown setae (pl. 32, fig. 16), arising one on each side of the aciculum. They are perfectly smooth, with slightly curved and attenuating, bluntly ending tips. The second row is made up of three or more setae (pl. 32, fig. 20), similar to the former, but differing from them in the smaller size and the straighter, more bluntly-ending tips. The ventral series consists of five or six setae (pl. 32, fig. 17) similar to the others in shape, but smaller. The neurosetae of the second parapodium (pl. 32, fig. 19) are specially modified, bearing spinous projections with the distal ends spirally twisted. The posterior or caudal neurosetae (pl. 32, fig. 26) are similar to the neurosetae of the second parapodium except that they end more bluntly and that the large protuberances are distributed more evenly on the entire surface of the distal portion.

The notopodium is a broad, low protuberance. It bears two rows of setae. Each row is made up of ten or eleven pale-brown setae, which are completely covered by debris. The tips of the notosetae are almost colorless, very fragile, fine, ending in an abrupt curve or hook (pl. 32, fig. 25). The fibers of the ventral tuft are very coarse. They are abundant, partly concealing the neuropodia, and are white or colorless. Those of the dorsal tufts are long and form a very thick, feltly covering over the dorsum.

The neurocirrus is about one-fifth of the length of the notocirrus (pl. 32, figs. 21, 24). The notocirrus arises from a prominent cirrato-phore and extends posteriorly and laterally, lying on the surface of
the felty layer. It has a subterminal enlargement and ends in a blunt tip.

Occurrence.—The only specimen, the type, was taken at Station LXV, 32° 42'7 N, 117° 13':4 W, off Coronado, California, from sandy bottom at a depth of 6-5 metres, on July 20, 1901.

3. Aphrodita solitaria, sp. nov.

Pl. 37, figs. 81, 82; pl. 33, figs. 27-38

Comparisons.—The description of Aphrodita solitaria is based on a single specimen that had been previously identified by Treadwell (1914) as Aphrodita refulgida. It differs from the latter in some essential characteristics. The shape of the body is narrower and more attenuated at the anterior and the posterior extremities. The brilliant, iridescent lateral fibers, which are conspicuous in A. refulgida, are absent in this species, and their place is taken by short, colorless fibers. The neurosetae of A. solitaria have strongly pillose tips, while those of A. refulgida are perfectly smooth. Furthermore, the shape of the prostomium and of the fimbriated organs differs from that in A. refulgida.

Description.—The shape of the body is narrowly ovate. The length of the body is 34 mm. The greatest width of the body, between segments 13 and 15, is 23 mm, from tip to tip of the setae, and 10 mm. between parapodia. The body tapers very gradually towards both ends. The posterior segments decrease abruptly in width, and the last 12-13 caudal segments form a narrow portion about 0.5 mm. in width. The specimen has forty segments, which are well marked ventrally. The ventral surface is gray (in alcohol), densely studded with papillae. The latter are prominent, with capped tips (pl. 33, fig. 22). The dorsum is arched. The dorsal setae are partly concealed by the felty layer and by the adherent debris.

The prostomium (pl. 33, fig. 27) is slightly wider than long, and is attached to the peristomium by the narrow isthmus, which is about one-sixth of the width of the prostomium. The ocular hemispheres are inconspicuous, each having a pair of minute eye-spots. The median tentacle consists of a prominent cirrophore and a style three times the former. The length of the palpi is five times that of the prostomium. The palpi are comparatively stout, gradually decreasing in diameter towards their distal ends, grooved longitudinally, and are covered with fine sensory cilia, visible only under high magnification.
The ventral lip extends to the third segment. The facial tubercle is prominent, covered with papillae, and ends in a finger-like projection hanging over the mouth ventrally.

The fifteen pairs of clytra (pl. 33, fig. 35) are thin, tough, and transparent, and are attached to the notopodia by strong elytraphores. Slight venations radiate from the place of attachment in all directions.

The branchiae begin on the sixth segment, occurring thence posteriorly on all cirriferous segments except the last few caudal ones. They are hatchet-shaped (pl. 33, fig. 36), with from seven to nine irregular, prominent projections.

The parapodia are biramous (pl. 33, fig. 28). The neuropodia are subirnuneate, ending in the usual three-step-like fashion. The tips of all the neurosetae are strongly pillose. Their color is dark brown, with a bluish reflection in light. The neurosetae from the dorsal series are the largest (pl. 33, figs. 29, 30). The four setae of the second row are finer (pl. 33, fig. 33). The six setae from the ventral series are the finest and have a subterminal enlargement (pl. 33, fig. 31). The neurosetae from the second parapodium (pl. 33, fig. 34) are specially modified, ending in a fine point, and are covered with prominent spines. The caudal setae have the same shape as the setae of other parapodia except that their ends are perfectly smooth. The dorsal setae are arranged in two rows. The dorsal row consists of four setae and the ventral of from eight to ten. The notosetae are long and brown, with pale, colorless ends terminating in a fine, strongly curved hook (pl. 33, fig. 32).

The dorsal cirrus is long, smooth, tapering gradually towards the distal end, and terminating in a bulbous tip. Its length is about eight times that of the neurocirrus (pl. 33, fig. 28).

Occurrence.—No data as to its habitat are available, but it is probable that the specimen came from collections made off the coast of southern California in 1901–1904.

4. Aphrodita cryptommata, sp. nov.

Pl. 34, figs. 39–50; pl. 37, fig. 83

Comparisons.—This species was labeled as Aphrodita parva and was evidently identified as such by Treadwell (1914). Comparing the specimen here described with the one sent to us by Dr. P. J. Moore, some essential differences were discovered.
The shape of the body in Aphrodita cryptommata is more slender, ending in a narrow caudal region; in A. parva the body ends posteriorly more obtusely. The neuropodia are more slender in A. parva. The shape of the prostomium is also different in the two species. The median tentacle is very short in A. cryptommata; it is very long in A. parva. The neurosetae of A. cryptommata are perfectly smooth, without any hairiness; they are pilose with a prominent spur in A. parva. The fimbriated organs in A. cryptommata differ from those of A. parva and from any other known species by having four to five conspicuously long, finger-like lobes. The elytra are somewhat squarish in A. cryptommata; they are more rounded in A. parva.

Description.—The body is ovately elongated, tapering towards both extremities, more toward the posterior end (pl. 37, fig. 83). The dorsum is arched, and the segmentation is well marked on the ventral surface. The two specimens in the collection of the University of California are 28 and 29 mm. long respectively. The width of the body is 16 mm. from tip to tip of the setae, and 10 mm. between parapodia. The greatest width of the body is between segments 10 and 11. The width decreases thence towards both ends. The ventral surface is thickly covered with prominent, capped papillae. There are thirty-eight segments.

The prostomium (pl. 34, fig. 39) is semiglobular, having the width equal to the length. The ocular protuberances are prominent, each bearing a pair of minute eyes, of which the dorsal pair only is visible from the dorsal surface. The median tentacle is very short, consisting of a short cirrophore and a short style. The palpi are white, stout, slightly grooved longitudinally, tapering gradually toward the distal end, and are covered with fine cilia. Their length is about five and one-half times the length of the prostomium. The fifteen pairs of elytra are thin, squarish in shape (pl. 34, fig. 40), strongly overlapping, and completely covering the dorsum. They are sparsely covered with papillae and fine venations radiating from the point of attachment in all directions.

The fimbriated organs have from four to six long, smooth, finger-like projections (pl. 34, fig. 47).

The parapodia are of the usual shape characteristic of the genus (pl. 34, fig. 49). The neurosetae are smooth, with abruptly narrowing ends terminating bluntly. There are two dark-brown, stout neurosetae in the dorsal series (pl. 34, fig. 43); five finer setae from the middle series (pl. 34, fig. 42) equal in structure to the former; eight
setae from the ventral series (pl. 34, fig. 41) lighter in color and finer than either of the former, with a slight subterminal enlargement. The notosetae pierce the felty covering. They are long and fine, and are arranged in two rows. The tips are strongly hooked, forming almost a ring (pl. 34, fig. 44). The lateral and the dorsal fibers also have hooked tips. The neurosetae of the second parapodium (pl. 34, figs., 45 and 46) have spinous protuberances and end in a fine point. The caudal neurosetae (pl. 34, fig. 50) are long, ending bluntly. They are covered with spiny hooks.

The notocirri occur on all non-elytroferous segments. They are four times the length of the neurocirri, terminating bluntly.

Occurrence.—One of the specimens, the type, was taken on June 14, 1901, at Station XIX, Haul 1, at 33° 34.6' N, 117° 55.6' W, off Newport, California, in a haul of the trawl at a depth of from 185 to 55 metres, on a bottom of soft mud, sand, and pebbles. The other one, the paratype, has no data.

5. Aphrodita brevitentaculata
Pl. 35, figs. 51–63; pl. 37, fig. 84

Comparisons.—A single specimen, the type, is in the collection of the Zoological Museum of the University of California. It was previously identified by Treadwell (1914) as Aphrodita negligens. Comparing the specimen with a cotype of A. negligens which Dr. J. P. Moore kindly sent to us, some essential differences were discovered.

The size of the body differs considerably. Of the two specimens from Dr. Moore, one, the type, is 40 mm. long and 17 mm. wide, excluding the setae; the other one is 60 mm. long and 40 mm. wide. Aphrodita brevitentaculata is only about one-third of that size, its length being 23 mm. and its width 9 mm. Further differences are found in the shape of the body, which is more obtusely rounded anteriorly in A. brevitentaculata, with the dorsum less arched. The prostomium differs in shape in the two species. The eyes are very small in A. negligens, but are unusually large in A. brevitentaculata. The palpi are relatively shorter in A. brevitentaculata, being two and a half times the length of the prostomium, while those of A. negligens are four and a half times the length of the prostomium. The lateral and the felt fibers of A. negligens are iridescent, of a dull green or bluish color; those of A. brevitentaculata are colorless. In A. negligens
the neurosetae are curved, with the tips slightly covered with hairs; in *A. brevitentaculata* the neurosetae are smooth, with the tips less curved—almost straight. The elytra are granular in *A. brevitentaculata*; they are thin and smooth in *A. negligens*.

*Description.*—The body (pl. 37, fig. 84) is ovate, slightly arched on the dorsum and slightly convex ventrally, i.e., the rest of the body from the base of the parapodia is slightly elevated. The length of the body is 23 mm. The width at the widest part of the body, between segments 9 and 17, is 14 mm. from tip to tip of setae, 9 mm. between parapodia. The anterior end of the body is broadly rounded. Towards the posterior portion the body width decreases very gradually, then attenuates abruptly before the last seven or eight segments in a narrow caudal portion about 0.5 mm. in width. There are thirty-five well-defined segments. The ventral surface is gray, thickly covered with fine papillae varying in size. The dorsal papillae (pl. 35, fig. 58) are still smaller than the ventral, with capped tips.

The prostomium (pl. 35, fig. 51) is slightly broader than it is long and is attached to the peristomium by a long, narrow isthmus about one-fourth of the width of the prostomium. The median tentacle is short, bends upwards and ends in a bulbous tip. Each ocellar prominence bears a pair of large eyes which are slightly fused. The palpi are stout, decreasing in diameter very gradually towards the distal ends. They have a short basal portion, and are two and a half times the length of the prostomium. They are smooth without any grooves and are sparsely covered with minute sensory cilia, visible only under high magnification. The facial caruncle is compressed between the palpi, ending in a long finger-like projection that hangs over the mouth ventrally. The mouth is situated ventrally, bounded by the third segment, and the broad ventral lip is well marked off from the rest of the body by deep grooves on each side of it.

There are fifteen pairs of elytra (pl. 35, fig. 55) arranged in the usual order. They are attached to the body by long elytraphores, and are covered with fine papillae and brown incrustations.

The branchiae (pl. 35, fig. 59) begin at the sixth segment, occurring thence on all cirriferous segments to the thirtieth. As usual, the anterior and posterior ones are smaller and less developed, those toward the center of the body are broad, each having 8 to 11 simple, finger-like projections.

The parapodia (pl. 35, fig. 61) are comparatively short, biramous, and supported by two aciculi. The neuropodium is of the usual shape.
characteristic to the genus. The three series of neurosetae consist of two setae in the dorsal series, three in the middle series, and five in the ventral series. They are similar in shape and structure, except for the difference in size (pl. 35, fig. 57, 60, 63). The neurosetae of the second parapodium (pl. 35, figs. 52, 53) are decorated with spinous projections ending bluntly or in a spiral twist. The neurosetae of the caudal parapodia (pl. 35, fig. 54) are covered with spiny hooks.

The dorsal setae are arranged in two rows inserted at different angles. Each row consists of ten or eleven setae, which pierce the layer of felt fibres, extending dorsad. The tips of the notosetae are fine and brittle, ending in an abruptly bent hook (pl. 35, fig. 56). The fibers arise as usual in three tufts, the dorsal, intermediate, and ventral. The felt fibers arise in tufts immediately above the dorsal notopodial setae on eleytroferous segments only. A smaller tuft arises between the two facicles of setae on all segments. The lateral tufts of fibers arise below the notopodial setae. On the eleytroferous segments the dorsal bundle of fibers, which furnishes the greater part of the dorsal felt, is lacking. The lateral fibers are covered with fine hairs and terminate in a hook (pl. 35, fig. 62).

The notocirri are about four times the length of the neurocirri. The latter are fusiform and reach to the base of the second row of the neuropodial setae.

Occurrence.—There is only one specimen, the type. It was taken off San Diego, in September, 1898, by Professor S. J. Holmes, from the holdfasts of kelp.

6. Aphrodita raripillata, sp. nov.
Pl. 36, figs. 64-76; pl. 37, figs. 85-86

Comparisons.—The description of this species is based on three examples which are in the Zoological Museum of the University of California. Two of these were labeled, probably by Treadwell (1914), Aphrodita parva, while the third specimen was labeled Aphrodita negligenus. The characteristics of the latter species have been previously discussed in this paper in comparison with other species. It is unnecessary, therefore, to repeat the description here. It may be said, however, that observation shows at once that the specimens here described are neither A. parva nor A. negligenus, as they differ from both in the shape of the body, which is more slender, with the dorsum
apparently more arched. This appearance, however, is due to the prominent dorsal setae, which stand out arcing over the dorsum, thus making the animal appear deeper dorso-ventrally. A further distinguishing characteristic is the very thin, clear, felty covering over the dorsum. The dorsal setae are very stout dark brown, covered with asperities, and ending more bluntly than they do in any other species of Aphrodita except A. armifera.

Aphrodita raripillata has a great resemblance to Aphrodita armifera (Moore, 1910), and is undoubtedly closely related to it. The chief difference is in the structure of the neurosetae. In Aphrodita armifera the neurosetae of the ventral series are covered with asperities and have a subterminal spur, while in A. raripillata no spur or asperities are seen even under the highest magnification. Moreover, Dr. Moore (1910) in his description of A. armifera states that the lateral and the felt fibers of this species are almost colorless, while in A. raripillata they are colorless.

In a specimen of A. armifera in the collections of the Marine Biological Laboratory of the Scripps Institution at La Jolla, the neurosetae are covered with the asperities and possess a subterminal spur. The lateral and the dorsal fibers, although not conspicuously colored, show a slight tinge of dull green color.

I feel justified in separating this species on the difference in the setae, since Dr. Moore (1910), in a similar case, based the distinction of Aphrodita parva from A. intermedia McIntosh on the structure of the setae, the only difference between them. Bourne (1883), in his discussion of variable and constant characters in the Polynoidae, states that the characteristics of the setae of corresponding segments are constant. Comparing a large number of A. refulgida and A. castanea, I have found that the structure of the setae is a reliable distinguishing characteristic in these species of Aphrodita.

Description.—The shape of the body is ovate (pl. 37, figs. 85, 86), obtusely rounded at the anterior end, reaching its maximum width about the tenth segment, decreasing thence towards the posterior end until the last five or six segments form a very narrow caudal end (pl. 37, fig. 85). The dorsum is arched and is covered with a very thin, felty layer over which the golden brown setae arch with their ends nearly meeting over the dorsum near the anterior end. At the posterior end of the body the ends of the notosetae meet or overlap. On the ventral surface (pl. 37, fig. 86) the segmentation is well indicated by transverse ridges. There are thirty-three segments. The
respective length of the three specimens at hand is 12, 15, and 28 mm. The respective width, from tip to tip of the setae, is 8, 12 and 15 mm.; between the parapodia, 5, 8, and 9 mm. respectively.

The prostomium (pl. 36, fig. 64) is subglobate, slightly wider than long. The ocular hemispheres are prominent, each bearing a pair of median-sized eyespots located on the dorsal and the antero-ventral surface of the hemispheres. The median tentacle consists of a short cirrophore, bearing a style almost equal in length to the cirrophore, ending in a bulbous tip. The white, stout palpi are almost uniform in width, tapering very slightly towards the distal ends, and are covered with sensory cilia. Their length is four times that of the prostomium.

The fifteen pairs of round, transparent elytra (pl. 36, fig. 66) are arranged in the usual order. The fimbriated organs (pl. 36, fig. 67) begin at the sixth segment, occurring thence on all cirriferous segments. They consist of six to eight prominent lobes. Each is subdivided into two or more smaller lobes, although undivided lobes occur.

The parapodia (pl. 36, fig. 73) are of median size, biramous, and are supported by two strong, dark-brown aciculi. The neuropodial setae from the three series (pl. 36, figs. 74, 75, and 76) are very much alike except for the difference in size. They are smooth, with slightly bent tips ending bluntly. The tips of the setae of the middle series are less curved than those of the other two series. The neurosetae of the second parapodium (pl. 36, fig. 71) are covered with prominent spines, except the extreme distal tip, which is smooth, and terminates in a pointed end. The caudal neurosetae (pl. 36, fig. 65) bear prominent spinous projections which are evenly distributed on the distal portion of the setae, except on the extreme distal end, which is smooth.

The notopodium is an inconspicuous tuberosity bearing two rows of dark brown setae and the lateral and the dorsal fibers. The dorsal setae (pl. 36, fig. 72) are almost straight, ending bluntly, and are covered with asperities. The latter are less conspicuous near the distal end, increasing in size toward the proximal end.

The neurocirrus arises from a strong cirrophore and is about one-sixth of the length of the notocirrus.

The body of the worm is covered with papillae, of which the dorsal papillae are less prominent, and with papillae of the simple type without the caps (pl. 36, figs. 68, 69, 70).
Occurrences.—The three specimens of *Aphrodita raripillata* in the collection of the University of California are of different sizes. One of them has the egg-sacs attached to the ventral surface. Another specimen, judging by the size, is young. These three specimens, the type and two paratypes, were taken off Southern California at different depths, from 27 to 55 metres. The type is from Station LV, Haul 2, at $32^\circ$ 32' N, $117^\circ$ 14' W, off San Diego, where it was taken July 18, 1901, at a depth of 46 to 41 metres, on bottom of soft mud, sand, and rocks. One of the paratypes is from Station LVIII, at $32^\circ$ 26' N, $117^\circ$ 15' 2" W, off San Diego, at a depth of 33-27 metres on a bottom of sand and broken shells, taken July 19, 1901. The other paratype was taken at Station XXIII, Haul 2, at $33^\circ$ 19' 3" N, $118^\circ$ 18' 2" W, off Avalon, Catalina Island, at a depth of 55-42 metres, on a bottom of fine gray sand and broken shells, on June 22, 1901.

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E. LITERATURE CITED

BAIRD, W.

BOURNE, A. G.

Cuvier, G.

KINBERG, J. G. H.

MOORE, J. P.


TREADWELL, A. L.
1914. Polychaetous annelids of the Pacific coast in the collections of the Zoological Museum of the University of California. *Univ. of Calif. Publ. Zool.,* 13, 175-238, pls. 11, 12, 7 figs. in text.
F. EXPLANATION OF PLATES
All figures drawn with camera lucida

PLATE 31
All figures of Aphrodita longipalpa, sp. nov.

Fig. 1. Prostomium, × 10.
Figs. 2 and 2'. Tips of neurosetae from dorsal series. × 75.
Fig. 3. Tips of neurosetae from ventral series. × 75.
Fig. 4. Tip of median neuroseta. × 75.
Figs. 5, 5', 5''. Tips of caudal setae. × 75.
Fig. 6. Tip of neuroseta from the second parapodium. × 75.
Fig. 7. Tip of dorsal seta. × 320.
Fig. 8. Tip of dorsal seta, showing the gelatinous envelope. × 75.
Fig. 9. Tip of dorsal seta. × 160.
Fig. 10. Fourth elytron. × 10.
Fig. 11. Twelfth parapodium. × 10.
Fig. 12. Fimbriated organ. × 75.
Fig. 13. Dorsal papillae. × 320.
PLATE 32

All figures of *Aphrodita californica*, sp. nov.

Fig. 15. Prostomium. $\times 20$.
Figs. 16, 17, 20. Tips of neurosetae. $\times 320$.
Fig. 18. Fifth elytron. $\times 20$.
Fig. 19. Tip of neuroseta of second parapodium. $\times 320$.
Fig. 21. Dorsal cirrus. $\times 20$.
Fig. 22. Dorsal papillae. $\times 20$.
Fig. 23. Branchiae. $\times 75$.
Fig. 24. Tenth parapodium. $\times 10$.
Fig. 25. Tip of dorsal seta. $\times 320$.
Fig. 26. Tip of caudal neuroseta. $\times 320$. 
PLATE 33

All figures of *Aphrodita solitaria*, sp. nov.

Fig. 27. Prostomium. × 10.
Fig. 28. Twelfth parapodium. × 10.
Fig. 29. Tip of dorsal neuroseta. × 160.
Fig. 30. Dorsal papillae. × 160.
Fig. 31. Tip of ventral neuroseta. × 160.
Fig. 32. Tip of dorsal seta. × 75.
Fig. 33. Tip of median neuroseta. × 160.
Fig. 34. Tip of neurosetae from second parapodium. × 320.
Fig. 35. Fifth elytron. × 10.
Fig. 36. Branchiae. × 45.
Fig. 37, 38. Tips of median neurosetae. × 160.
PLATE 34

All figures of *Aphrodita cryptomata*, sp. nov.

Fig. 39. Prostomium.  $\times$ 10.
Fig. 40. Fifth eleytron.  $\times$ 10.
Figs. 41, 42, 43. Tips of neurosetae.  $\times$ 160.
Fig. 44. Tip of notoseta.  $\times$ 320.
Fig. 45. Tip of neuroseta from second parapodium.  $\times$ 360.
Fig. 46. Neuroseta from second parapodium.  $\times$ 160.
Fig. 47. Branchiae.  $\times$ 45.
Fig. 48. Dorsal papillae.  $\times$ 160.
Fig. 49. Twelfth parapodium.  $\times$ 10.
Fig. 50. Portion of caudal seta.  $\times$ 320.

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PLATE 35

All figures of *Aphrodita brevitentaculata*, sp. nov.

Fig. 51. Prostomium. $\times 20$

Figs. 52, 53. Neurosetae from second parapodium. $\times 160$

Fig. 54. Caudal neuroseta. $\times 75$

Fig. 55. Third elytron. $\times 10$

Fig. 56. Tip of notoseta. $\times 160$

Fig. 57. Tip of median neuroseta. $\times 160$

Fig. 58. Dorsal papillae. $\times 160$

Fig. 59. Branchiae. $\times 45$

Fig. 60. Tip of neuroseta from dorsal series. $\times 160$

Fig. 61. Twelfth parapodium. $\times 10$

Fig. 62. Tip of felt fiber. $\times 320$

Fig. 63. Tip of neuroseta from the ventral series. $\times 160$

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PLATE 36

All figures of *Aphroditina raripillata*

Fig. 64. Prostomium. $\times 20$.
Fig. 65. Caudal neuroseta. $\times 75$.
Fig. 66. Fourth elytron. $\times 10$.
Fig. 67. Branchiae. $\times 75$.
Figs. 68, 69, 70. Dorsal papillae. $\times 160$.
Fig. 71. Neuroseta from second parapodium. $\times 310$.
Fig. 72. Tip of dorsal seta. $\times 160$.
Fig. 73. Thirteenth parapodium. $\times 10$.
Figs. 74, 75, 76. Neurosetae. $\times 160$. 

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Fig. 77. *Aphrodita longipalpa*, dorsal surface. × 2.
Fig. 78. The same, ventral view. × 2.
Fig. 79. *Aphrodita californica*, dorsal surface. × 2.
Fig. 80. Ventral view of the same. × 2.
Fig. 81. *Aphrodita solitaria*, dorsal view. × 2.
Fig. 82. The same, ventral view. × 2.
Fig. 83. Ventral view of *Aphrodita cryptommata*. × 2.
Fig. 84. Ventral view of *Aphrodita brevitentaculata*. × 2.
Fig. 85. Dorsal view of *Aphrodita raripilata*. × 2.
Fig. 86. Ventral view of the same. × 2.

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NOTES ON THE NATURAL HISTORY AND BEHAVIOR OF *EMERITA ANALOGA* (STIMPSON)

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HAROLD TUPPER MEAD
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Nos. 5 and 6 in one cover. April, 1914 .05


NOTES ON THE NATURAL HISTORY AND BEHAVIOR OF **EMERITA ANALOGA** (STIMPSON)

BY

HAROLD TUPPER MEAD

(Contribution from the Scripps Institution for Biological Research, La Jolla, California)

Along sandy beaches in the vicinity of San Diego are countless numbers of the burrowing crustacean, *Emerita analoga* (Stimpson) commonly called the sand-crab. They are roughly oval creatures having an elongate, transversely rounded carapace which, in adult females, has an average length of about 22.4 millimeters and an average width of about 17 millimeters, computed from measurements of twenty-three individuals. Adult males have a carapace length of from 12 to 14 millimeters (Barnhart). The abdomen and telson resemble somewhat those structures in the true crabs. The first pair of pereopods are simple, flattened and extended forward a little in advance of the head. The other pereopods are less conspicuous, being shorter and not protruded so far from underneath the body. The eyes are mounted on comparatively long, slender stalks. These crabs always move backwards, whether swimming, crawling or burrowing. Swimming is accomplished almost entirely by the backward beating of the uropods above the posterior margin of the carapace, thereby drawing the animal through the water. Burrowing is accomplished by the combined action of the uropods and pereopods, the latter being the more serviceable. Animals from which the uropods were clipped could not swim, while apparently making frantic efforts, but they could burrow, although more slowly than normal animals. When the pereopods were clipped and the uropods left, the animals could swim like
normal animals, but in attempting to burrow could succeed in only partially covering their bodies.

By dropping small quantities of ink into the water I found the current through the gill chamber always passing from the posterior to the anterior end as a finely rhythmical flow, nor was I able at any time to discover a change from this habit, though Weymouth and Richardson say that the direction of the current regularly reverses. When at rest the position of the animals in the sand is always with the posterior end downward. Their presence is revealed only by V-shaped ripple marks made by the spreading antennae in the thin film of water that runs down the sand following the dash of each wave. Seldom do the animals betray their presence in any other manner. If a person approaches a bed of sand-crabs the antennae are all withdrawn so that concealment is most complete. Stamping had the effect of producing slight disturbances in the crab beds. These disturbances were probably due to the crawling of the animals deeper into the sand. If one stands for some time in the midst of a bed of sand-crabs, the creatures remain concealed in the sand for an indefinite time. After one withdraws from the vicinity of the bed, apparently the original number reappears. Tossing a spade into a bed of crabs had practically the same effect, excepting that they reappeared sooner than when one stood in their midst.

This concealing action strongly suggested that the animals are frequently the prey of certain beach birds. I suspected the herring gulls, but at no time saw the gulls feeding upon them, nor could I learn from fishermen that such was ever the case. Soon after, however, I noticed flocks of sandpipers and long-billed curlews feeding ravenously upon the crabs and concluded that the concealing reaction is due to the feeding of these birds.

_Emerita_ are usually found in the zone of the beach washed intermittently by the waves. I could see no evidence of their presence in the sand above this zone, although I examined the region carefully. According to Barnhart, they occur in the sand well out beyond the line where the waves strike the beach. Inasmuch as the food of the animals consists of organisms which they entrap with their feathered antennae from the water as it moves to and fro (Weymouth and Richardson), it seems that for this reason they seek those regions where the waves cause a to-and-fro movement of the water.

Here, then, was a complex reaction based upon (1) a to-and-fro movement of the water combined with (2) the burrowing habit. In
Fig. 1. Level plat near the water, showing the migrations of animals towards the water.

Fig. 2. Level plat 200 feet from the ocean, showing the persistent migrations of animals towards the water.

Fig. 3. Same as fig. 2, excepting that the animals used had been gyrated immediately before the experiment.

Fig. 4. Same as fig. 1, excepting that the animals in this experiment had their eyes removed.

Fig. 5. Plat with a 7 per cent slope away from the ocean, showing no special direction tendency.

Fig. 6. Drawing of under-water labyrinth.
an effort to test these reactions further several specimens were deposited on the beach, where the sand was moist, smooth and firm, but sloping toward the sea. Immediately, the creatures all backed down the slope toward the waves, pausing now and then in an effort to burrow. To ascertain whether the direction was determined by the sight of the waves or by the slope, I first made a plat in the sand on the beach sloping decidedly away from the water, and in the center of this placed several individuals. They all moved down the slope away from the water, showing that, in the air at least, they responded decidedly to gravity. I then made in the moist sand, twenty to fifty feet from the water, a smooth, round, firm, level plat about thirty inches in diameter and bounded by a fosse about one inch deep. A carpenter’s level was used in getting the plat level. When Emeritae were placed in the center of this plat, their direction of movement was invariably toward the ocean (fig. 1). I performed this experiment several times, making tracings of the movements of the animals five times, using ten animals each time, always with practically the same result. Even building an embankment of sand about six inches high, entirely obstructing the view of the ocean, created no disturbing effect.

Under these circumstances, a typical reaction consists of a few moments of “death feint” in which the animal lies perfectly still on its back, followed by a quick, righting movement and an attempt to burrow. Not being successful in this, the animal orients itself with its posterior part toward the water and crawls off in a zig-zag route to the fosse on the water side of the plat. Sometimes an individual would never revive from the death feint. It is rather difficult to understand why the physiological make-up of these animals should permit them to prolong a death feint in the hot sun to a stage beyond which there is no recovery, without making efforts to escape into the moist sand. It was noticed that a slight mechanical stimulus, such as being touched with a twig, being rubbed by another sand-crab, or being blown upon, was often sufficient to arouse the animals and to make them right themselves and seek safety.

In order to eliminate any reaction that might obtain from the proximity of the ocean I repeated the experiment on a pile of beach sand that had been hauled and dumped upon an embankment some two hundred feet from the ocean and about thirty-five feet above sea-level, with practically the same result (fig. 2), although in this series of experiments the animals displayed a slight amount of confusion,
as shown by the more circuitous routes from the center to the periphery. In an effort to confuse the animals still further in their sense of direction, I gyrated some individuals more than two hundred revolutions, after which they were quickly transferred to the plat used in figure 2. The animals responded as in previous experiments by moving toward the ocean (fig. 3), although with perhaps less certainty than in the case of the ungyrated specimens.

To ascertain whether or not their direction was determined by sight, I repeated the experiment with specimens from which the eyes had been removed. This time there seemed to be no tendency to move in any one direction (fig. 4). I performed this experiment three times, using a total of forty-five animals. In order to eliminate any shock that might ensue from the removal of the eye-stalks, I kept the blind animals in an aquarium of running sea-water and performed the three experiments at intervals of two and nineteen days respectively, with similar results.

As a consequence of the foregoing experiments and observations it would seem that the eyes of these crabs play an important role in guiding them to their feeding beds, should they by any means be removed therefrom, and in apprising them of the proximity of birds or other enemies.

These animals are positively phototactic to low intensities of light. When kept in the laboratory they congregate in greatest numbers at the side of the aquarium nearest the source of light, unless there be sand in the aquarium.

Inasmuch as the animals were apparently controlled while in the air by two influences, namely a down-hill tendency (geotaxis) and an oceanward tendency, I endeavored to determine what intensity of the former would exactly counteract the latter. In other words, I wished to measure the intensity of the oceanward tropism in terms of the tendency to move downhill, making use of the per cent of slope as a unit of measure. After several trials I discovered that a slope of one and three-fourths inches in twenty-five, or of 7 per cent, most nearly satisfied this equilibrium. In confirmation I tried fifty animals on this slope, with the result that they were fairly evenly distributed in their direction tendencies. Figure 5 represents a typical result of an experiment of this series. Thus it seems that they cannot "believe their own eyes" when confronted by a 7 per cent slope.

It was my desire to make the burrowing instinct of the crabs the basis of a series of labyrinth experiments, to test the habit-forming
capacity after the manner of Yerkes and Huggins, and Spaulding working with crayfish, carcinus and hermit crabs. Accordingly I made a labyrinth consisting of a board twelve inches square with a three-fourths inch guard around the sides. In the middle of one side was an opening through which the sand-erbs might pass into the sea water and sand. The crabs were liberated at the opposite end of the board so that a reasonable amount of activity on their part would cause them to drop through the hole into the water; but the vitality of the animals decreased rapidly, so the experiment in the air was abandoned. Then a series of similar experiments in sea water was attempted, but entirely without positive results. A labyrinth was arranged with an opening through which the crabs could pass into moving water and sand, presumably their optimum habitat. In spite of the fact that the opening was toward the window, the animals showed no capacity to "learn" the way out, but, on the other hand, appeared in successive experiments to lose interest in getting out of the labyrinth, even though a moderate amount of activity would take them out and into the sand and running water. Some of the animals, as soon as liberated, swam directly through the opening; others refused to move for hours and even days. Some died during the experiment, some became indisposed, thus impairing to that extent the value of the experiments. Only eleven of the twenty original animals were used throughout the series.

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It appears that the range of stimuli which Emerita analoga experiences is comparatively narrow. In nature these animals encounter a range of temperature not exceeding 18° F, and the chemical composition and oxygen and carbon dioxide content of the water is presumably rather constant, because the water in the San Diego region comes by an upwelling movement from the bottom of the ocean (McEwen, 1915). Also the physiographic character of the beaches which Emerita inhabits is noticeably uniform. Corresponding to this
narrow range of stimuli and restricted habitat the range of intensity of stimuli to which they respond is comparatively narrow. If the intensity of a certain stimulus transcends the limits of this narrow range no response follows. This seems to explain the frequent indisposition or death of the animals during a series of experiments.

**SUMMARY**

1. *Emerita analoga* lives in the wave-washed parts of the sandy beaches of California.
2. The animals tend (1) to run down slopes, and (2) to go towards the ocean, when within 200 feet at least of it, although their view of the ocean be intercepted.
3. A 7 per cent slope away from the ocean neutralizes their oceanward tendency.
4. Experiments in the air with *Emerita analoga* cannot be continued for many hours with normal responses.
5. *Emerita analoga* is noticeably limited in its habitat, and the range of intensity of stimuli to which it responds is comparatively limited.

**ACKNOWLEDGMENTS**

I am greatly indebted to Mr. Percy S. Barnhart for the use of an unpublished article by him on *analoga*, and for valuable assistance in looking up literature, and also to Dr. W. E. Ritter for many helpful suggestions.

*Transmitted January 12, 1917.*
LITERATURE CITED

Barnhart, Percy S.

Cowles, R. P.

Hay, M. P.

McEwen, Geo. F.

Pearse, A. S.

Smith, S. J.

Spaulding, E. G.

Weymouth, F. W., and Richardson, Charles H., Jr.

Yerkes, R. M.

Yerkes, R. M., and Huggins, G. E.
ASCIDIANS OF THE LITTORAL ZONE OF SOUTHERN CALIFORNIA

BY

WILLIAM E. RITTER AND RUTH A. FORSYTH

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ASCIDIANS OF THE LITTORAL ZONE OF SOUTHERN CALIFORNIA

BY

WILLIAM E. RITTER AND RUTH A. FORSYTH

(Contribution from the Scripps Institution for Biological Research)

The aim of this paper is mainly to contribute to the knowledge of the ascidian fauna of the California coast south of Point Conception. In it are included descriptions of all the new species of which specimens are contained in available collections; also a list, with supplementary notes, of all the species of the region previously described from the same area. This limitation in the scope of the study has led us to exclude from it a considerable number of species which in all probability ought to be included, but are not, because they are known to us only from the localities north of the Point. More exhaustive collecting, particularly on the Santa Barbara Islands and the opposite mainland, will undoubtedly bring to light many species which we now know only from northern localities, where much more collecting has been done than anywhere south of the Point excepting the San Pedro and San Diego regions.

The considerable number of species here recorded as occurring at La Jolla and San Diego only may be taken as indicative of what is to be expected when other portions of the coast have been as well searched for ascidians as this. Nor should it be supposed that even the La Jolla–San Diego region has been exhausted.

The two lists here given of species, with the families to which they belong, include all the ascidians of the southern California littoral zone known to science, and also those not yet known from the littoral, but occurring off shore in depths of water so shallow that they may be expected to be found on shore.
LIST OF SPECIES

Family Molgulidae
1. *Molgula verrucifera*, n. sp.

Family Halocynthiidae

Family Styelidae
3. *Styela montereyensis* (Dall)
4. *Styela gibbsii* (Stimp.)
5. *Styela barnharti*, n. sp.

Family Ascidididae

Family Rhodosomidae

Family Cionidae
8. *Ciona intestinalis* L.

Family Polyzoidae
9. *Metandrocarpa dura* (Ritt.)
11. *Polyzoa translucida*, n. sp.

Family Botryllidae
12. *Botryllus tuberatus*, n. sp.
13. *Botrylloides diegensis*, n. sp.

Family Perophoridae

Family Polycitoridae
15. *Distaplia occidentalis*, n. sp.
17. *Eudistoma diaphanus*, n. sp.

Family Didemnidae
18. *Didemnum cornulentum*, n. sp.
20. *Trididemnum delavallei*, n. sp.
22. *Diplasomoides caulleryi*, n. sp.

Family Synoicidae
23. *Glossophorum planum*, n. sp.
25. *Macrocliniun pellucidum*, n. sp.
26. *Amaroucium californicum*, n. sp.
27. *Amaroucium solidum*, n. sp.
28. *Amaroucium aequali-siphonis*, n. sp.

To be added to these without much doubt, because known from depths of 50 fathoms and less are:

1. *Halocynthia okai* Ritt.
   (Known in depth of 10-80 fath. Ritter, 1907.)
   (Known in 33 fath. Ritter, 1907.)
   (Known in 21 fath. Ritter, 1907.)
   (Known in 29 fath. Ritter, 1913.)
5. *Ascidia (Phallusia) vermiformis* Ritt.
   (Known in 30 fath. Ritter, 1913.)
   (Known in 33 fath. Ritter, 1913.)
   (Known in 33 fath. Ritter, 1913.)

Search for these seven species in the littoral zone will be among
the interesting motives of future ascidian collecting.

**Synoptic Descriptions of Genera**

The following synoptic description and arrangement of the genera,
representatives of which are treated in the paper, has been drawn up
primarily for the use of students, other than specialists on ascidians,
who may want to use the local species in more general zoological or
biological studies. This being the main purpose, questions of the best
system of classification and nomenclature, with which specialists in the
group are much interested at present, are considered no further than
to make sure that all descriptions, definitions, arrangements, and
names have the sanction of at least some of the most experienced
ascidiologists.

**Suborder I. ASCIDIÆ SIMPLICÆ**

Individual animals of considerable size, rarely less than 1 cm. in diameter;
very irregular in form but predominantly massive; sometimes semitransparent,
sometimes leathery in appearance, sometimes coated with sand; sedentary; often
firmly attached to rocks and other objects in adult life; never, as here under-
stood, propagating by budding.

**Genus 1. Molgula (Caesira, some authors)**

*Body* usually unattached because the animal lives on sandy or muddy bottoms,
but sometimes attached to rocks, occasionally pedunculated. Branchial orifice
6-lobed, atrial 4-lobed.

*Outer coat* (test) somewhat cartilaginous, leathery or membranous, fre-
quently covered with sand, which may be attached to hairlike processes or
embedded in the surface layer.

*Branchial tentacles* always compound.

*Branchial sac* with well-developed folds, usually from five to seven on each
side; the branchial slits (stigmata) almost always curved, or even developed
into spirals and arranged in pockets or ampullae in the branchial folds.

*Intestine* always on the left side.

*Sexual organs* usually on both sides but not infrequently on one side only;
when so, almost always on the left. Ovary and testis more or less intimately
associated.

*Excretory organ* on the right side only, with the exception of one genus,
*Rhizomolgula*, in which it is on the left side.
Genus 2. Halocynthia (Pyura, some authors)

Body mostly approaching globular, always firmly attached, sometimes short-pedunculate; surface usually free from foreign substances but often bearing processes of various kinds; both orifices 4-lobed.

Outer coat (test) usually leathery, rather cartilaginous and semitransparent.

Branchial tentacles always compound.

Branchial sac with folds, usually well developed; prevalent number from four to seven, but a few species with a smaller number and a few with as many as fifteen; dorsal lamina always with processes or lauguets.

Intestine on the left side, forming a wide loop.

Sexual organs on both sides.

Genus 3. Styela (Tethyum, some authors)

Body attached, not infrequently pedunculate, sometimes coated with sand; both orifices 4-lobed, often inconspicuously so.

Outer coat leathery, usually thin, surface typically unarmed.

Tentacles both branchial and atrial present, both kinds simple, branchial larger.

Branchial sac with four folds on each side, some of which may be much reduced in size.

Intestine on left side, stomach frequently long with narrow folds in the wall.

Sexual organs on both sides, ovary typically in several sausage-shaped masses with the testes arranged about them in smaller lobes.

Genus 4. Ascidia (Phallusia, some authors)

Body attached, almost always sessile, surface usually smooth and free from foreign substances; branchial orifice 8-lobed, atrial 6-lobed.

Outer coat usually transparent or nearly so, soft or cartilaginous.

Branchial tentacles simple, usually numerous and slender.

Branchial sac never with prominent folds, but often with many small plications; papillae on the inner surface at the intersections of the longitudinal and transverse vessels; dorsal lamina a membrane extending behind the esophageal opening.

Intestine on the left side.

Sexual glands situated within the intestinal loop.

Renal vesicles present, numerous, confined to the wall of the intestine.

Genus 5. Chelyosoma

Body flattened from above, the upper surface covered with tortoise-shell-like plates; both orifices 4-lobed.

Outer coat cartilage-like, translucent, the anterior part differentiated into horny plates.

Branchial sac, general type that of Ascidia and Ciona, but stigmata strongly curved or coiled; dorsal languets as in Ciona.

Intestine located ventrally, sometimes to the right, sometimes to the left; stomach wall, in part, chambered.

Sexual organs forming a network on the intestinal loop.


Body cylindrical, attached; branchial orifice 8-lobed, atrial 6-lobed, lobes not prominent.

Outer coat thin, transparent, soft.

Branchial tentacles simple, slender.
Branchial sac much as in *Ascidia*, but with a series of languets in place of dorsal lamina.

*Intestine* wholly or largely behind the branchial sac.

*Sexual organs* in the intestinal loop.

Suborder II. ASCIDIAE COMPOSITAE

Individuals, called zooids, produced asexually by budding; typically many; small, usually less than 1 cm. long; in most genera connected together by permanent stolons and embedded in a common mass of cellulose mantle or test; but in some genera zooids larger and not fully embedded in the common mass; the whole group of zooids, produced from a single parent, called a colony.

Genus 7. *Metandrocarpa*

*Colony* encrusting on rocks, seaweeds, and other objects; zooids never arranged in systems; cellulose mantle tough; color brick-red.

*Form of zooid* globular, body not divided into sections.

*Branchial sac* without folds but with internal longitudinal vessels; branchial and atrial tentacles present, all simple.

*Intestine* short, situated on the left-ventral side of the branchial sac; stomach with a series of folds and a large caecum.

*Sexual glands* two series of masses called polycarps, one on each side of the endostyle, each polycarp with its own short duct opening into the perbranchial chamber; the anterior polycarps of each series female, the posterior male.

*Budding* thoracic.

Genus 8. *Polyzoa*

Like *Metandrocarpa* except:

*Colony* with zooids united only by strands; no common investing cellulose mass; colorless, transparent.

*Sexual glands* all hermaphroditic polycarps.

Genus 9. *Botryllus*

*Colony* thin and encrusting; zooids wholly embedded in common cellulose mass, arranged in regular or somewhat elongated "systems" around a common atrial opening; cellulose mass containing many vessels terminating in ampullae; color various, often very conspicuous.

*Form of zooids* generally elliptical, body not divided into sections.

*Branchial sac* without folds but with a few internal longitudinal vessels.

*Intestine* on left side at posterior end; stomach with folds and a caecum.

*Sexual glands* a single pair of hermaphroditic masses, one on each side of a branchial sac.

*Budding* thoracic.

Genus 10. *Botrylloides*

Like *Botryllus* except:

*Colony* with zooids arranged in long-elliptical or tortuous systems.

Genus 11. *Perophora*

*Colony* composed of zooids connected by stolons, partly or wholly embedded in cellulose mass; zooids never in systems; transparent to yellowish white.

*Form of zooids* spheroid, body not divided into sections; branchial orifice 6-lobed, atrial 5-lobed.

*Branchial sac* without folds, with internal longitudinal vessels or papillae; a series of languets along mid-dorsal line.
Intestine on left side; stomach wall not folded, intestine divided into several distinct sections.

Sexual organs on left side in the intestinal loop, ovary a single mass, testes in numerous lobes.

Budding stolonic.

Genus 12. Distaplia (Holozoa, some authors)

Colony usually consisting of short club-shaped or capitate masses closely united at base; zooids typically in systems with distinct common atrial orifces; cellulose mass soft, usually highly colored.

Form of zooids: body elongated, separated into thorax containing branchial sac, and abdomen containing intestine and sexual organs; branchial orifice 6-lobed, atrial with a long dorsal tongue, or languet.

Branchial sac with neither folds nor internal vessels, four series of stigmata.

Intestine a simple, elongated loop; stomach ovate, wall not folded but with a network of fine ridges on inner surface.

Sexual organs: gonads situated on the right-posterior part of the intestinal loop, ovary very simple, ripe ova large; testis composed of a few masses; larvae develop in an incubating pouch.

Budding stolonic.

Genus 13. Eudistoma

Colony massive and variously lobed; dull white or dark from covering of sand; zooids not usually grouped in systems.

Form of zooids: body elongated, divided into thorax and abdomen; a long ectodermal process usually given off from posterior end of abdomen; orifces each 6-lobed.

Branchial sac simple with few (three to five) series of stigmata.

Intestine a long, nearly straight, simple loop; stomach smooth-walled, frequently far back in the abdomen.

Sexual organs: gonads alongside the intestinal loop, far back; ovary simple; testis of numerous lobes; no incubating pouch.

Genus 14. Didemnum

Colony always closely encrusting, usually very thin, rarely somewhat massive; cellulose material containing stellate calcareous spicules; zooids not in systems: color white or variously tinted.

Form of zooid: divided into thorax and abdomen; branchial orifice 6-lobed, atrial orifice plane, situated on dorsal side of thorax.

Branchial sac with four rows of stigmata.

Intestine a simple loop, its posterior part usually containing the stomach; stomach smooth-walled.

Sexual organs situated on the left-posterior side of the intestinal loop; ovary very simple, ripe ova very large; vas deferens wound in a close spiral around the testis.

Genus 15. Trididemnum

Scarcely differing from Didemnum except in the number of stigmatic series, these being here three; colony perhaps typically somewhat more fleshy.

Genus 16. Diplosoma

Colony mostly thin and encrusting, soft and lax because of numerous great spaces in the transparent cellulose mass; systems where present very irregular.

Form of zooids: divided into thorax and abdomen; not sharply separated from each other; branchial orifice 6-lobed, atrial a large, simple opening.

Branchial sac with four rows of stigmata.
Genus Kittei-Forsyth: Ascidians of Southern California

Intestine a simple loop; stomach smooth-walled.

Sexual organs: gonads on right-posterior side of intestinal loop; ovary simple, eggs very large, few in number; testis not many-lobed, vas deferens not coiled.

Budding intestinal.

Genus 17. Diplosomoides

Similar to Diplosoma but calcareous spicules in the cellulose mass; a languet over the atrial orifice.

Genus 18. Glossophorum

Colony massive, sub globular or lobed, sometimes pedunculate; zooids mostly in distinct systems.

Form of zooid much elongated; body divided into three sections: thorax, abdomen, and postabdomen; branchial orifice 6-lobed, atrial with a languet.

Branchial sac well-developed, as many as twenty rows of stigmata; papillae on the inner surface of the branchial membrane along the interserial vessels.

Intestine a close, twisted loop situated close behind the thorax; stomach smooth-walled.

Gonads, ovary and testis commingled, making a rather compact mass, situated in the stalked postabdomen.

Budding stolonic.

Genus 19. Macroclinum

Colony massive or divided into club-shaped pieces; sometimes coated with sand.

Form of zooid elongated, divided into thorax, abdomen, and postabdomen, the last not constricted from the abdomen and variable in size and make-up; branchial orifice 6-lobed, atrial with a well-developed languet.

Branchial sac: many rows of stigmata, twenty in some species.

Intestine: loop not twisted, stomach wall not folded but inner surface uneven in some species.

Gonads close behind the intestinal loop; ovary simple, surrounded by the numerous masses of the testis.

Budding stolonic.

Genus 20. Amarouclum

Colony variable, thin and encrusting, massive or divided into pedunculated sections.

Form of zooid elongated, divided into thorax, abdomen, and postabdomen, the latter often long and cylindrical; branchial orifice 6-lobed, atrial with a languet.

Branchial sac: rows of stigmata, mostly less than twenty.

Intestine: loop simple, stomach wall folded.

Gonads in the postabdomen, usually somewhat removed from the intestinal loop; ovary simple, testis many-lobed, scattered along the postabdomen.

Genus 21. Euherdmama

Colony consisting of large, elongated zooids, wholly separated from one another except for the basal stolonic attachments, making the condition commonly known as social; color opaque white, or transparent.

Form of zooids; length as great as 3 cm.; divided into thorax, abdomen, and postabdomen, the last very short; both orifices 6-lobed.

Branchial sac with twelve rows of stigmata.

Intestine a very long, simple loop, the esophagus making one of its limbs; stomach with folded wall situated near the loop.

Sexual organs: gonads in the short postabdomen; ovary simple; testis in many separate masses; embryos developed in the long, straight oviduct.

Budding not known, probably stolonic.
DESCRIPTION OF SPECIES

Note.—The formula for the number and arrangement of vessels on the inner surface of the branchial membrane of simple ascidians, adopted by Ritter in his later papers on ascidian taxonomy, is used in the descriptions. Thus:

\[
\begin{align*}
\text{e'd's} &\quad \{ \begin{array}{ll}
1-7 & 2-6, \text{ etc.} \quad \text{R.} \\
2-8 & 1-7-2, \text{ etc.} \quad \text{L.}
\end{array} \end{align*}
\]

means that counting from the endostyle (e’d’s) the vessels of the right side (R) number 7–6, etc., on the folds and 1–2–60, etc., between the folds. The other row of numbers is self-explanatory.

Molgula verrucifera, n. sp.

Pl. 38, fig. 5; pl. 40, figs. 15–20

Superficial characteristics.—Regular in outline except for adhering foreign bodies, nearly spherical but usually somewhat depressed and covered with heavy coating of sand. Siphons not far apart and rather conspicuous though entirely covered with sand (pl. 38, fig. 5). Size: 8 by 6 by 5 mm.; 8 by 7 by 6 mm.; 8 by 7 by 5 mm.; 8 by 6.5 by 6.5 mm.; 8 by 6 by 6 mm.; 8 by 6.5 by 9 mm.; 10 by 8 by 7.5 mm. Test thin but firm, sandy covering adhering to its numerous fine processes. Mantle thin; the longitudinal muscle bands radiate from the siphons and spread out on the sides of the body so as to be separated by considerable spaces, extending to about the middle of each side. Finer circular muscle bands are confined to the siphonal regions. Musculature of the two siphons about the same in strength.

Branchial system.—When removed from the test, the two siphons are of about equal length although atrial is usually more slender. Branchial orifice 6-lobed with tentacle-like processes inserted around its edge. Of these processes six long ones are arranged symmetrically and alternating with them are two smaller processes (pl. 40, fig. 18). Atrial orifice 4-lobed, also with tentacle-like processes inserted on its edge (pl. 40, fig. 17). Branchial tentacles 16–20, usually of two sizes alternating with each other and closely crowded around branchial orifice. Each tentacle bipinnate, the branches ending bluntly and often swollen at tips (pl. 40, fig. 19). Hypophysis opening an elongated slit, the ends of which may curve one way or the other to give rise to the variations which appear in different individuals, situated on right side of anterior end of the elongated ganglion (pl. 40, fig. 20). Branchial sac with seven well-developed folds on each side (b.f., pl. 40, fig. 15), those next to the dorsal lamina very short. Formula of internal longitudinal vessels:

\[
\begin{align*}
\text{e'd's} &\quad \{ \begin{array}{ll}
1-0-6-0-6-0-6-0 & 3-0-0-3-0-0-0-3-0-0 \quad \text{R.} \\
3-0-5-0-6-0-6-0 & 5-0-3-0-0 \quad \text{L}
\end{array} \end{align*}
\]

The vessels are confined to surface and upper halves of the curved folds, usually the same number on each side, but those on convex sides always stronger. Five primary transverse vessels occur, intercepting two infundibula on each fold. Secondary transverse vessels present.
one between each of the two infundibula thus intercepted. Finally tertiary vessels are often found on the faces of the infundibula, where they divide the stigmata of the faces and separate the two short spirals which, in such cases, often occur at the spaces of the infundibula. Usually the stigmata at the apex of an infundibulum form a single short spiral (pl. 40, fig. 16). At the posterior end of the sac, the stigmata become irregular, assuming s-shaped and spiral forms. Dorsal lamina a plain membrane with thickened edge. Endostyle (end., pl. 40, fig. 15) long and slender.

Digestive system.—Situated on left side of the body, forming a long closed loop which in turn forms a regular curve on posterior dorsal portion of that side (pl. 40, fig. 15). Esophagus (e), not quite as long as stomach, emerges from dorsal posterior portion of branchial sac. Stomach (s.) thin-walled, smooth, over twice as long as wide, and of not much greater diameter than intestine. On inner surface of esophagus and stomach, and extending beyond them is a voluminous rosette-shaped reddish-brown liver (l., pl. 40, fig. 15). This made up of two distinct portions, each consisting of numerous radiating elongated caeca. Anus plain-edged, situated in peribranchial cavity near the emergence of esophagus and near atrial siphon. Kidney bean-shaped, and located on right side and ventral portion of the body just posterior to ovary, to which it is almost equal in size.

Reproductive system.—Consists of two hermaphroditic gonads, one on each side of the body. Ovaries large, irregularly oval, somewhat flattened bodies, that on left side situated just anterior to intestinal loop (pl. 40, fig. 15). Short oviduct arises from anterior edge of ovary and opens into peribranchial cavity where the larvae develop. Specimens taken in July and in October had numerous tadpoles. Testicular lobes (t., pl. 40, fig. 15) comparatively few, situated on posterior border of ovary.

Habitat and distribution.—On the exposed surfaces of rocks usually, but sometimes on the under surfaces, in the littoral zone, La Jolla, California. M. verrucifera undoubtedly occurs at other places than La Jolla but has not yet been collected elsewhere.

This species belongs to the small group of molgulids in which both branchial and atrial orifices are armed with well-developed processes. On this basis it would fall into the genus Ctenicella as defined by Lacaze-Duthiers. We agree, however, with those recent asciidiologists who consider the group recognized by this author as too small and heterogeneous to be profitably accepted as a genus. Nor does our species come near to accommodating itself to Ctenicella as redefined by Hartmeyer. There is no course open to us, therefore, but to place it in the genus Molgula, in which group it occupies a fairly distinct place by virtue of the possession of the siphonal processes.

The specific name has reference to the wart-like appearance of the siphons even in preserved specimens.
Halocynthia johnsoni Ritt.


A striking thing about this species is its great abundance in San Diego Bay, and the large size reached there by the individuals, as compared with what one finds on the open shores. Its favorite habitat appears to be the piles of wharfs where, at times, it makes almost a solid coating. Although it must be counted as a native of the whole littoral zone, we have found only occasional small specimens at outside points.

Mention may be made of the fact that before San Pedro Bay was completely dredged to make it a harbor, *H. johnsoni* occurred there in the shoaler waters in enormous numbers on the bottom, associated with several species of lamellibranch mollusks.

**Styela montereyensis** (Dall)

Pl. 38, fig. 1; pl. 41, fig. 28-34

*Cynthia(?) montereyensis*, Dall, 1871, p. 157.
*Caviolinopsis rubra*, Fewkes, 1889.
*Botenia(?) rubra*, Herdman, 1891, p. 599.
*Styela montereyensis*, Banerof, 1899, pp. 73 and 92.
*Styela (sens. restr.) montereyensis*, Huntsman, 1911, p. 131.

Although *Styela montereyensis* was, so far as we know, the first ascidian ever described from the California coast, and though it has been studied more, probably, than any other, because of the meagerness of the original description we describe it as fully as though it were a new species.

*Superficial characteristics* (pl. 38, fig. 1).—Long, club-shaped, pedunculated; prevailing color dark red. Peduncle at least as long as body, often twice as long. Test firm, thick, opaque, with about twelve corrugations. Transverse wrinkles often occur, particularly at anterior end and along peduncle. Both orifices 4-lobed; siphons always distinct, the branchial being directed ventrally with a pronounced uniform curve, the atrial directed anteriorly. Body merges gradually into peduncle. Mantle fairly muscular but semitransparent, containing two layers of muscle, an inner one of longitudinal bands overlaid by a more delicate layer of transverse fibers crossing it at right angles; both layers become feeble posteriorly and wholly disappear in the peduncle.

Table 1 gives measurements for *Styela montereyensis* from different localities.
TABLE 1

Styela montereyensis

<table>
<thead>
<tr>
<th>Locality</th>
<th>Length of body</th>
<th>Length of peduncle</th>
<th>Longitudinal vessels of sac</th>
<th>Number of branchial tentacles</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Diego Bay</td>
<td>10 cm.</td>
<td>19 cm.</td>
<td>end 7-14-10-16-13-16-9-22-7 R.</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-14-15-16-12-16-11-22-7 L.</td>
<td></td>
</tr>
<tr>
<td>Pacific Grove</td>
<td>8 cm.</td>
<td>8 cm.</td>
<td>end 6-13-9-11-7-14-8-17-6 R.</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6-12-9-11-8-15-9-13-6 L.</td>
<td></td>
</tr>
<tr>
<td>Trinidad</td>
<td>3.5 cm.</td>
<td>8 cm.</td>
<td>end 6-9-8-4-8-4-14-6 R.</td>
<td>80</td>
</tr>
<tr>
<td>Northern Mendocino</td>
<td>3.5 cm.</td>
<td>5 cm.</td>
<td>end 3-6-4-8-4-7-5-10-3 R.</td>
<td>60</td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Mendocino</td>
<td>3 cm.</td>
<td>3.5 cm.</td>
<td>end 2-4-4-5-5-4-4-10-3 R.</td>
<td>55</td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patrick's Point</td>
<td>2.5 cm.</td>
<td>8 cm.</td>
<td>end 3-7-5-8-5-8-5-12-5 R.</td>
<td>65</td>
</tr>
<tr>
<td>Coronado</td>
<td>2.5 cm.</td>
<td>2.5 cm.</td>
<td>end 3-4-5-6-3-4-3-8-2 L.</td>
<td>65</td>
</tr>
<tr>
<td>Half Moon Bay</td>
<td>1.8 cm.</td>
<td>2 cm.</td>
<td>end 4-4-5-3-4-3-6-2 R.</td>
<td>50</td>
</tr>
<tr>
<td>Point Conception</td>
<td>1.6 cm.</td>
<td>1.8 cm.</td>
<td>end 2-7-3-10-3-7-3-12-3 R.</td>
<td>56</td>
</tr>
<tr>
<td>Pebble Beach</td>
<td>1 cm.</td>
<td>1 cm.</td>
<td>end 2-5-3-6-3-5-2-8-3 R.</td>
<td>40</td>
</tr>
</tbody>
</table>

Branchial system.—Branchial tentacles numerous, long, slender, inflated, varying in size and number, maximum being about 130 (pl. 41, fig. 30). Atrial tentacles numerous, filiform, scattered over inner surface of a velum near base of atrial siphon; outer surface of velum applied to wall of atrial siphon (pl. 41, fig. 32). Dorsal tubercle prominent, hypophysis mouth of horseshoe type, varying greatly in different individuals. Branchial sac with four folds on each side, those next the dorsal lamina having the greatest number of longitudinal vessels. Longitudinal vessels on folds and in spaces vary greatly in number with size of individual. Transverse vessels cross longitudinal vessels and produce the meshes which are about square. Number of stigmata in a mesh from three to ten. Dorsal lamina a plain membrane. Endostyle tortuous at anterior end. Spinules occur on inner surface of siphons, their free margins rounded and their surfaces longitudinally striated (pl. 41, figs. 33 and 34). Each spinule a single cell with a single nucleus situated somewhat nearer the base than the apex of the cell. The longer, more or less pointed striated part of the cell is a thin, indurated layer making something like a shield on the cell’s back. The existence, in an animal as high in the animal kingdom as the ascidians, of structures which consist of a single cell and present differences in different species, is a fact deserving special mention. Huntsman (1911) was the first to describe the spinules in detail, and we are glad to be able to confirm his observation that the structures are regularly different, at least as between S. montereyensis and S. yaculatensis.
Digestive system (pl. 41, fig. 29).—On left side of animal. Stomach, in lower left half of body near ventral side-wall, possessing about thirty close, regular, ridges or folds. Esophagus somewhat less than half as long as stomach and joins latter at its posterior end. Long axis of stomach parallel with that of body. Intestine, immediately upon emerging from the pylorus, bends posteriorly and lies along upper half of stomach; it makes U-shaped bend anteriorly to form the rectal arm, which is about twice as long as the descending portion; anus with from six to sixteen blunt lobes. Inside the intestine, running its entire length, is a broad, fleshy fold that rolls up to form a tube. At the pylorus this expands into a bulb cleft on its surface. Running the entire length of the intestine is a clear, thin strip of wall about opposite the fold. In the stomach near the dorsal side is a foldless strip, the width of several folds. A larger and broader fold than the others borders this plain surface on one side.

Reproductive system (pl. 41, figs. 28, 29, and 31).—Ovaries much elongated, cylindrical masses, two on each side of the body; those on the right longer, extending nearly entire length of the body; those on the left considerably shorter. Of these latter the larger lies in the loop of the intestine and follows the rectum. The smallest ovary extends diagonally from the pyloric end of the stomach to end near its partner. Ovarian cylinders narrow down to short necks or oviducts near base of atrial siphon. Arranged along both sides of the elongated ovaries are series of testicular lobes, whose ducts unite on the mid-line of the inner surface of the ovary, and the common vas deferens thus formed ends as a papilla, a little short of the termination of the oviduct. The shape of the testicular lobes varies considerably, being simple and club-shaped in the younger individuals, but becoming bifurcate and irregularly branched in the larger ones.

Breeding time.—The summer months at least, in Monterey Bay. Observations on the point have not been made at other times and in other localities.

Habitat and distribution.—The littoral zone from British Columbia at the north to the southern limit of the United States at the south, according to present knowledge.

That this, one of the earliest known and most familiar ascidian species of Pacific North America, should have remained to this time without a detailed description is one of the vicissitudes in the progress of knowledge of our local marine fauna.

Although the specifications as to color in the diagnosis is the simplest statement that can be made, it would have to be much modified to make it apply to all individuals. Rarely if ever does it happen that a grown specimen is uniformly colored. The anterior part and one whole side of the animal are frequently more highly colored. Occasional specimens are almost devoid of the red color.

The flutings of the test are real structural differentiations and not mere folds, the test being much thicker in the ridges than in the
valleys. A cross-section of the ridges reveals, even to cursory inspection, the fact that the outer half, approximately, of the test-substance of the ridge is denser and more opaque than the inner part. This ease of sharp differentiation within the mass of a structure produced mainly by secretion should repay investigation.

Particular attention may be called to the fact so clearly brought out in the table, that the number of inner longitudinal vessels of the branchial sac, both on and between the folds, increases regularly and continuously with the increase in size, and hence presumably with the age of the animals.

Equally clear is it that the branchial tentacles also increase in number. This result corresponds with what was found by Ritter (1913) in several other species, but it is interesting to notice that certain differences in the mode of increase of parts in different species is indicated. For example, while the addition of new branchial tentacles in *S. montereyensis* is obvious for a large portion, at least, of the individual's life, this seems to be rather exceptional; for little or no increase in number occurs in *Halocynthia aurantium*, *Boltenia orifera*, and *Styela macronteron*, species previously studied with reference to the same point.

Although the three stalked species of *Styela* occurring on the Pacific Coast of North America are well known to the senior author of this paper, two of them, *S. greeleyi* and *S. yacatatensis* having been described by him, until recently there has been some doubt in his mind about the specific distinctness of the three. But the studies of Huntsman and our own have removed the doubt.

It is noteworthy that this species, like several other ascidians, seems to flourish much better on the piles and other similar objects introduced into the water than on the natural shore rocks. The senior author has collected *S. montereyensis* from many points on the coast from San Diego to Mendocino, but has never seen a specimen of anything like maximum size growing on native rocks. The largest individuals seen were on the piles of the wharf at Santa Barbara; and at no other point has it been found in such abundance as there. However, it occurs in abundance and large size on the wharfs and breakwaters in the vicinity of Los Angeles.
Styela gibbsii (Stimp.)

Cynthia gibbsii, Stimpson, 1864, p. 159.
Styela gibbsii, Herdman, 1898, p. 261.
Styela gibbsii, Ritter, 1907, p. 23.
Tethyum gibbsii, Hartmeyer, 1909, p. 1359.
Styela (sens. restr.) gibbsii, Huntsman, 1911, p. 131.

This is one of the most widely distributed ascidians of the west coast of North America, it being now recorded from British Columbia to San Diego, and from the littoral zone to a depth of forty fathoms. On the coast of southern California it appears to be rare along shore, but fairly common down to a depth of forty to fifty fathoms.

Styela barnharti, n. sp.

Pl. 38, fig. 2; pl. 42, figs. 39 and 40

Superficial characteristics.—Roughly elliptical in outline, almost twice as long as broad, with branchial siphon sessile and directed anteriorly; atrial orifice a short distance below it on dorsal side. Each orifice 4-lobed and surrounded by four flattened, smooth mammillae corresponding to the lobes. Entire surface of the body mammillated with large rounded protuberances, except for the flattened disc of attachment (pl. 38, fig. 2). Test thick, firm, tough and semitransparent. Color reddish yellow, the red being most intense on the anterior end. Mantle strongly muscular, musculature consisting of longitudinal overlaid by weaker circular bands. Circular muscles most strongly developed in siphonal regions. Size of largest animal investigated: length 4 cm., diameter 2.25 cm.

Branchial system.—Branchial tentacles of several sizes, about forty. Atrial tentacles numerous, very small, slender, and tapering; in a single circle on inner surface of a narrow velum which is folded up against the wall of atrial siphon but not smoothly, the result being that the atrial tentacles are inserted in the bottom of a groove formed by a fold in the velum. The free edge of the velum and the rounded edge of the fold, between and beyond which the tentacles extend, are on about the same level. Spinules, each consisting of a single cell, occur on inner surfaces of siphons; rounded and toothed at their anterior ends with striations corresponding to the teeth on the dorsal surface; nucleus of the cell situated toward the posterior end. Spinules very similar to those of S. montreyensis. Dorsal tubercle horseshoe-shaped with ends curled inward. Branchial sac with four folds on each side. The distribution of the longitudinal vessels on the two sides for the large individual was:

\[
\begin{array}{l}
\text{R.} \\
\{ 6-16-9-14-6-14-8-15-6 \} \\
\{ 6-16-10-16-6-19-7-16-6 \}
\end{array}
\]

Transverse vessels of four orders and regularly arranged. Order of occurrence: 1-4-3-4-2-4-3-4-1; those of fourth order crossing the stigmata. Dorsal lamina a plain broad membrane.
Digestive system.—On left side of body. Esophagus (e., pl. 42, fig. 40) emerges from dorsal posterior portion of branchial sac and curves into stomach, which is over twice as long as wide, and has about thirty-five longitudinal folds. Stomach lies along ventral and posterior portion of the animal; from its pyloric end the intestine runs anteriorly considerably past the middle of the body, then forms a loop and runs posteriorly parallel to itself and the stomach and to the left of the cardiac portion of the stomach, where it again forms a wide loop and runs anteriorly along dorsal portion of the animal, becoming somewhat convoluted just before reaching atrial orifice, where it ends in an anus bordering by many rounded lobes (pl. 42, fig. 40). Endocarps very numerous on intestine as well as on mantle.

Reproductive system.—Gonads probably nine in number on the right side and three on left side; those on the right in two groups. On the right side toward the dorsal surface are six parallel ovarian cylinders (e., pl. 42, fig. 39). Of these the two middle ones are largest and seem to be continuous with each other at their posterior ends, although this may be due to crowding, as the glands were distended with ripe eggs. The two pairs of outside ovarian cylinders are less than half the diameter of the middle ones, all ending in tubular oviducts at their anterior ends somewhat below the atrial orifice. Just posterior to and partly concealed by the most ventral of these ovaries is a very small cylinder making the seventh of the group. Testicular lobes are thickly attached to the inner surfaces of the cylinders and their vasa efferentia join the vas deferens running along the center of each cylinder to end in a free tubular portion similar to and just back of the oviduct. The second group of two gonads of the right side is anterior to the group just described and transverse in position. The testicular lobes in this group extend considerably beyond the ovaries (pl. 42, fig. 39). On the left side the longest ovarian cylinder lies in the last loop in the intestine. The second longest one lies diagonally from the top of the first intestinal loop toward the atrial orifice. Between these two is a much shorter cylinder, ending in an oviduct located considerably farther back than the other oviducts (pl. 42, fig. 40).

One large specimen of this species was taken in July, 1915, from piles in San Diego Bay. Two small specimens were found in February on the carapace of a crab, *Rhodéa parvafrons*, taken at the end of the wharf of the Scripps Institution.

*Styela barnharti* belongs to the comparatively small section of the genus which have more than five gonads on a side. Its nearest of kin seems to be *S. elsia* Hartmeyer of the Japanese waters. So far as the gonads of the right side are concerned, *barnharti* and *elsia* appear to be considerably alike, the resemblance pertaining not only to the number but also to the disposition; for, according to Hartmeyer, those of this side form two groups. However, the anterior group in *elsia* contains three instead of two as in *barnharti*. But the similarity between the two species beyond this point is not close. In *elsia* the
gonads of the left side are as numerous as those of the right, though
in the form of the digestive tract and the number of the internal
longitudinal vessels the two are not widely separated. But in body
form, character of the external surface, and number of branchial ten-
cacles, as well as in the gonads of the left side, the two species are
sharply separated.

Worthy of note is the fact that a majority of the Styelas having
a high number of gonads apparently belong to the Pacific Ocean.
But should more extensive study prove this to be actually so, the fact
could hardly be considered as anything more than a coincidence. So
far as we have been able to ascertain from the literature, the differ-
ence in thickness of the ovaries of the right side of S. barnharti, as
shown in figure 39 of plate 42, is unique. It should be remarked that
it is not due to difference in the stage of growth in the ova, these not
being of recognizably different size in the larger ovaries. The mean-
ing of this difference is not clear, but the fact that on the right side,
where the number of ovaries is greatest, there are three distinct sizes,
the smallest being relatively quite small, suggests that these smaller
ovaries are in process of becoming rudimentary.

We take pleasure in naming this interesting Stylca for Mr. P. H.
Barnhart, curator of the Scripps Institution, to whom we are indebted
for all the specimens so far seen.

Ascidia californica, n. sp.
Pl. 38, fig. 6; pl. 41, figs. 24 to 27

Superficial characteristics (pl. 38, fig. 6).—Elliptical in outline
but somewhat narrower anteriorly and quite depressed. Attached
by the entire left side. Test thick, gelatinous, containing many
anastomosing vessels, translucent but not sufficiently transparent to
permit much of the internal organs to be seen; surface generally
smooth and even. Siphons usually not prominent; the branchial
directed forward and frequently somewhat to the right; the atrial
located at half or a little more than half the animal's length toward
posterior end on the upper surface but toward the left edge. The
eight lobes of the branchial orifice are regular and somewhat long and
pointed, with a brick-red pigment spot between the lobes. Lobes of
the atrial orifice, almost invariably six in number, are bordered by a
series of minute teeth; shorter and more rounded than the branchial
lobes and similarly possessing pigment spots (p.s., pl. 41, fig. 27).
Largest specimen about 3.5 cm. long and twice as long as wide; usually
smaller. Mantle thin and transparent on left or under side, with no
muscle bands except in siphons and anterior part of branchial sac;
on right or upper side muscle bands, running in all directions, form a
thick pad.
Branchial system.—Siphons described above. Branchial tentacles very long and slender and of about the same size, varying in number, the larger individuals having as many as 150. Hypophysis (hy., pl. 41, fig. 25), small and either oval or horseshoe-shaped with opening directed forward. Ganglion mass (gl.) separated by several times the length of hypophysis-mouth from the hypophysis (pl. 41, fig. 25). Dorsal lamina (d.l.) a broad membrane, broader posteriorly than anteriorly, provided with transverse ribs which project slightly past the edge; a few minute teeth between these projections. The lamina extends beyond the opening of the esophagus to the end of the sac. Branchial sac extends the entire length of the animal. Internal longitudinal vessels bear papillae at their intersections with the transverse vessels. In a large individual sixty internal longitudinal vessels and seventy transverse vessels were counted. No intermediate papillae present; ends of papillae curve toward the dorsal lamina and have a bulge on the concave surface. Plications in branchial membrane few than the longitudinal vessels. Meshes rectangular, a little longer than wide, each containing about three stigmata.

Digestive system (pl. 41, fig. 24).—On left side of branchial sac. About one-sixth of the branchial sac extends behind the digestive apparatus and about one-third in front of it. Mouth of esophagus about one-fourth the length of branchial sac from its posterior end. Esophagus sharply curved to enter the stomach. Stomach (s., pl. 41, fig. 24) about twice diameter of intestine at its esophageal end, but gradually tapering to intestine at the other end; long axis at right angles to that of sac; wall with about twelve, wide, orange-colored, longitudinal folds. From the stomach the intestine runs anteriorly and then curves in such a fashion as to form the letter S reversed. Smooth-edged anus close to base of atrial siphon. A renal gland ramifies over the rectal limb of the intestine.

Reproductive system (pl. 41, figs. 24 and 26).—Peculiar widely branched ovary ramifies over whole inner surface of that part of intestinal loop which lies anterior to stomach. Testis lobes, much smaller and more finely branched than those of ovary, spread especially on inner surface of stomach and to some extent on both surfaces of intestinal loop. White vas deferens and oviduct run side by side along posterior side of rectum, the vas deferens lying between oviduct and rectum, both ending near the anus.

Specimens with sperm ducts enlarged and with eggs in their oviducts were taken in February at San Pedro, Santa Cruz, and La Jolla; in May at Half Moon Bay; in June at San Clemente, and in July at San Pedro.

A. californica belongs to the Mentula section of the genus Ascidia, this section being considered as characterized primarily by the extension of the branchial sac and dorsal lamina behind the esophageal mouth. But within this section it seems to be sharply set off from any species hitherto described. So far as we are able to ascertain, the fine pectination of the lobes of the atrial orifice (pl. 41, fig. 27) is entirely unique. The form and distribution of the ovary also con-
stitute good diagnostic features. In form, the ovary seems to resemble that of *A. aperita* Sluiter more than that of any other species; but the distribution of the lobes in the two is quite different, the broad, simple loop of the intestine of *aperita* making it impossible for the ramifications to implicate so large a portion of the loop as it does in *californica*. But in most respects *aperita* and *californica* are very distinct.

*A. californica* probably resembles *Ascidia (Phallusia) ceratodes* Huntsman from the coast of British Columbia more closely than any other species, but from this it is distinguished by its smaller size, larger number of internal vessels, both longitudinal and transverse, smaller number of folds in the stomach, and most positive of all, more diffuse character of the ovarian lobes.

In view of the fact that the species is fairly common in the whole California littoral and seems not to occur much beyond this region, to the north, at least, we have felt that it is sufficiently typical of the region to merit the specific name *californica*.

*Habitat and range.*—On under sides of rocks at extreme low tide, the California coast from San Diego to Half Moon Bay (Mendocino County); also on kelp holdfasts (La Jolla) and "eel-grass" (Tomales Bay). Also to depth of thirty meters off San Diego.

From the material and data at our disposal the species seems to reach its best development at Santa Cruz.

*List of localities.*—San Diego region, almost everywhere, including San Diego and False Bays as well as points on the open coast, especially at La Jolla; Laguna Beach; San Pedro; San Clemente Island; Monterey Bay; mouth of San Francisco Bay; Tomales Bay. But at none of the stations except in Monterey Bay, at Santa Cruz, has it been taken in large numbers massed together in the fashion characteristic of many ascidian species.

*Type locality.*—La Jolla.

**Cheylosoma productum** (Stimp.)

*Cheylosoma productum*, von Drasche, 1884, p. 281.
*Cheylosoma productum*, Bancroft, 1898, p. 309.
*Cheylosoma productum*, Huntsman, 1911, p. 124.

The prevailing size and elevation of specimens of this species, occurring on the California coast, are so much less than similar dimensions of animals from Puget Sound, the type locality, as to seem almost to justify the recognition of a subspecies for the southern forms. However, neither the detailed anatomical studies by Bancroft (1898)
nor the examinations made later by several observers have succeeded in finding any constant structural characteristics on which to base such a group; and as both size and height are subject to much variation it appears best, on the whole, not to give our forms a separate name. Specimens are not infrequently taken in the littoral zone, but are more common in depths of a few fathoms.

*Ciona intestinalis* (L.)

*Ascidia intestinalis*, Linn., 1767, p. 1087, no. 3.
*Ciona intestinalis*, Flemming, 1828, p. 468.
*Ciona intestinalis*, Roule, 1884, p. 13.
*Ciona intestinalis*, Hartmeyer, 1903, p. 297.

(For an exhaustive bibliography, see Hartmeyer, 1903.)

We have subjected specimens from San Diego Bay to a thorough-going comparison with the descriptions and figures of Mediterranean specimens given by M. Roule in his well-known monograph of 1884, and find nothing to suggest even a race distinction for the west American animals. Indeed, the perfect identity and the fact that the species appears to be distributionally restricted in this region almost entirely to localities frequented by ships, leads one to wonder if it is not an immigrant to these parts. This surmise is the more warranted by the habits of the animal, it being especially given to clinging to the under sides of floats, buoys, and the like. In these situations it flourishes most luxuriantly and occurs in enormous numbers. We would not however, make too much of this suggestion as to distribution, since the species has been reported (Huntsman, 1911, and Ritter, 1913) from a rather wider range in Pacific waters than is altogether consistent with this theory. *Ciona* is sexually ripe in San Diego Bay through the later summer, the entire autumn, and to mid-winter at least; probably ripe animals may be found throughout the year.

*Metandrocarpa dura* (Ritter)

*Metandrocarpa dura*, Michaelson, 1904, p. 70.

Although this species is not very often taken in its original habitat, it is still one of the most conspicuous of our ascidians—this from its favorite habit of forming incrusting masses on various of the larger seaweeds, which, though usually growing below tide, are often washed up decorated with the bright red colonies.
Metandrocarpa michaelseni, n. sp.

Pl. 38, fig. 8; pl. 39, fig. 14; pl. 42, figs. 41-45

Superficial characteristics (pl. 38, fig. 8).—Zooids appearing as rounded mounds, the larger ones averaging about 4 mm. in diameter through base and from 2 to 3 mm. high; never embedded in a common test but probably always a film of test passing between them; adherence to substratum, usually the under side of rocks, very close. Color bright, cherry-red to hardly more than a tinge of that color; cherry far more common. Siphons short and always deeper red than body. In large colonies, which may be half a square foot in extent, zooids come to be close together, almost covering the substratum, but these irregularly distributed with no intervention of common test. Blastozoids seem always to move away some distance, three, four, five or more millimeters and a delicate trail of test with a strand can be made out connecting bud and parent (t.t., pl. 39, fig. 14). Large, numerous ectodermal vessels in test film around blastozooid (c.p., pl. 39, fig. 14).

Zooids.—Test thick, tough, and not transparent. Mantle with many circular and longitudinal muscle fibers. Body rests on its left and ventral side with its anterior or disc shifted toward right and dorsal so as to bring branchial orifice to top of mound (pl. 42, fig. 42); length about 3 mm.; consisting of large branchial sac with digestive and reproductive organs on the sides.

Branchial system.—Siphons fairly close together in central portion of the upper surface of mound and very little elevated above general surface; orifices not bordered by definite lobes. Branchial tentacles from thirty to forty, alternating long and short (pl. 42, fig. 43). About twenty very small atrial tentacles (pl. 42, fig. 44). Branchial sac at most with nine rows of stigmata; four or five internal longitudinal vessels on each side; largest individuals always with five on each side; very fine transverse vessels cross most of stigmatic series midway between the primary vessels. Much pigment in blood cells throughout branchial sac as in other tissues of animal. Number of stigmata about thirty in each half series. Table 2 shows the distribution of the stigmata in five individuals.

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Dorsal lamina, a plain fold growing wider and thicker posteriorly. Hypophysis a single elliptical opening just anterior to the elongated ganglion (hy. and g.g., pl. 42, fig. 43).
Digestive system (pl. 42, figs. 41 and 42).—Situated on left posterior half of branchial sac. Esophagus (c., pl. 42, fig. 41) emerges from dorsal posterior part of branchial sac. Stomach wider than long, its wall thrown into twelve to seventeen looped folds of varying lengths depending on their position; sight of these folds heading around the esophagus, the others, shorter, on opposite sides of organ, point toward a seam constituting a part of the cardiac end of stomach. A sac-like caecum (c., pl. 42, fig. 41) on stomach near end of seam, joined by a fine duct to pyloric gland which ramifies over intestine (p. gl., pl. 42, fig. 41). Intestine emerging funnel-wise from stomach, regularly S-shaped, the dorsal half of the S twisted nearly to a right angle with ventral half (int., pl. 42, figs. 41 and 42); a narrow, thin strip of epithelium running along entire convex side of intestine; anus with a thickened bilobed margin, situated in atrial chamber near atrial orifice.

Reproductive system (c., t., pl. 42, fig. 41).—Gonads in form of "polyearps" attached to mantle on both sides of body. About three ovaries anteriorly situated on each side of endostyle (c., pl. 42, fig. 41). About five testis masses (t., pl. 42, fig. 41) on right side in a row along the endostyle; on the left side usually four or five masses arranged around end of intestinal loop. From the summits of male gonads extend short vasa deferentia into the peribranchial cavity (v. d., pl. 42, fig. 45).

Breeding habits.—Embryos and advanced tadpoles retained in peribranchial chamber; breeding during midwinter months.

This species, the name of which we are glad to make stand as a testimonial to Dr. Michaelsen's good work on this group of ascidians, is rather sharply distinct from M. dura Ritter, its neighbor in habitat, as well as in a number of structural features. While dura never, so far as our observations go, departs much from the completely aggregated and fused type of colony (Ritter, 1896, especially figures 1 and 2, pl. 12), the colony of michaelseni seems never to assume this form. Not infrequently the zooids of the middle portion of the colony of michaelseni are so close together as to be nearly or quite in contact with one another. This seems to arise entirely from the intercalation through budding of new zooids and never from the approximation of the buds in the new parts of the colony. The trailing off of zooids in these parts of the colony as shown in figure 14 of plate 39, is entirely characteristic of this species. This difference may depend upon the difference in habit of the two species, dura growing typically on seaweeds where, as a consequence, the room for expansion of a given colony is limited, while michaelseni is almost if not quite restricted to the surfaces of rocks and molluscan shells. As to the zooids themselves, the most positive difference is in the number of series of stigmata, dura having twelve and michaelseni nine.
Habitat and distribution.—Typically the under sides of rocks in littoral zone on rocky shores everywhere on coast of southern California and probably, though not certainly, as far north as San Francisco, at least.

Type locality.—La Jolla.

Polyzoa translucida, n. sp.

Pl. 38, fig. 7; pl. 42, figs. 36 to 38

Superficial characteristics of the colony.—Composed of zooids joined by short strands to a basal network and, in older colonies, with individuals so close together that considerable portions of the tests of neighboring zooids adhere to each other. Zooids generally roughly egg-shaped although in the largest individuals the two siphons on anterior end may be quite prominent. Zooids colorless and semi-transparent (pl. 38, fig. 7).

Zooids.—Largest 5 to 6 mm. long and 3 to 4 mm. thick. Tests tough, but thin and transparent. Mantle with numerous fine longitudinal and transverse muscle fibers. Body consisting of large branchial sac with digestive and reproductive organs located at side; on each side about opposite the third stigmatic series, an elliptical sac-like body attached to mantle, probably corresponding to the endocarp of styelids (cn., pl. 42, figs. 36 and 37).

Branchial system.—Branchial siphon 4-lobed, located in middle of anterior end. Atrial siphon also 4-lobed, near branchial siphon on dorsal side of anterior end. Branchial tentacles about thirty, alternating long and short. Atrial tentacles about twenty, very small. Branchial sac without folds, with twelve rows of stigmata, each having from thirty to forty stigmata in a half-series; three longitudinal vessels on each side; greatest number, about fourteen, of stigmata between endostyle and first longitudinal vessel; about eight stigmata in each of remaining intervals. In largest individual fine longitudinal vessels cross the stigmatic series midway between the primary vessels (pl. 42, figs. 36 and 37). Endostyle narrow. Dorsal lamina a plain membrane rolled into a tube. Hypophysis a slit-like opening just in front of ganglion.

Digestive system.—Situated on left side and posterior half of branchial sac. Esophagus (e. pl. 42, fig. 36) about equal in length to stomach; emerging from branchial sac near its dorsal edge, removed about two stigmatic series from its posterior end. Stomach (s., pl. 42, fig 36) considerably broader than long, made up of eleven or twelve prominent folds shorter on side turned away from sac. A section of intestinal tract consisting of the esophagus, stomach, and a portion of the intestine about as long as the esophagus, lies in a horizontal position; organ then bends and runs parallel to its previous course nearly to the dorsum of animal, where it turns anteriorly a short distance and ends in the bilobed anus. In wall of intestine is a narrow strip running its length, thinner than the rest of the wall and free from pigment, which is seen to be a groove when viewed from within the cavity of the intestine. A cæcum from near pyloric end of stomach is joined by a fine duct to a pyloric gland which ramifies over intestine (pl. 42, fig. 36).
Reproductive system.—Gonads hermaphroditic "polyoarps," those on right disposed in row along the endostyle, as many as twelve in large individuals; on left about five, situated near endostyle in anterior half of sac (o. and t., pl. 42, figs. 36 and 37). In large individuals peribranchial cavities contain numerous eggs and larvae. In such cases the large oviduct forms a conspicuous portion of hermaphroditic gonad (ov. d., pl. 42, fig. 38). A small tentacle-like sperm duct terminates the male gonad near oviduct (v. d., pl. 42, fig. 38).

Habitat and distribution.—So far as known, only from wharf piles in San Diego Bay where it occurs interwoven with hydroids and other animals which inhabit the piles. The only specimens were taken in June.

Although the presence of eight internal longitudinal vessels on the branchial sac is held to be a generic character in Polyzou, we cannot believe that the much smaller number in translucida ought to bar it from the genus, so well does it agree with the other more important generic characters.

Botryllus tuberatus, n. sp.

Pl. 39, figs. 10 and 12; pl. 40, fig. 22

Superficial characteristics of the colony.—Thin, encrusting, usually not more than 3 or 4 cm. in expanse and 1 to 2 mm. in thickness. Number of zooids in circular systems varies between three and ten; systems close together. Zooids usually black from pigment although variations occur, and colonies with comparatively little pigment are found. Zooids of a system communicate with common atrial orifice by long, spout-like siphons (a.s., pl. 40, fig. 22); openings of siphons varying from small and oval in younger zooids to large and gaping in older ones; edges of upper portions of openings unite to form the common cloacal orifice, lower lips often extending a little beyond the cloacal orifice, so as to be visible through it from above (pl. 39, fig. 12). At intervals along margin of colony occur exceedingly dense, pedunculated bunches of ectodermal ampullae, each ampulla having its own long ectodermal vessel running into body of colony; young zooids often occurring among the ectodermal ampullae (c.a. and y. z. pl. 39, fig. 10); the ectodermal vessels generally branch and send out smaller, less darkly pigmented ampullae. Test gelatinous and transparent.

Zooids.—Length about .8 mm. General shape cylindrical but curved, conave side being dorsal; length a little greater than width. Mantle strongly pigmented, and containing a few widely separated extremely delicate longitudinal muscle fibers (pl. 40, fig. 22).

Branchial system.—Sacc cylindrical, a little longer than broad; four series of stigmata; three longitudinal vessels on each side; about fourteen stigmata in each half-series; usually three stigmata in spaces between longitudinal vessels and four between endostyle and its adjacent vessels, and four between dorsal lamina and its adjacent vessels; stigmata five or six times as long as broad. Endostyle wide and straight. Dorsal lamina a plain membrane rolled into a tube. Sixteen small branchial tentacles of which the eight larger ones alternate with eight very small ones.
Digestive system.—Situated on left side and posterior end of branchial sac. Stomach longer than broad, and tapering toward esophageal end, its long axis being horizontal and its lower edge about even with lower edge of sac; its wall thrown into seven distinct longitudinal folds; a tubular caecum attached to stomach near its posterior end and upper side (c., pl. 40, fig. 22). Intestine tapers from stomach and almost immediately makes a sharp bend and runs dorsally parallel to and above stomach; upon reaching dorsal edge of body, it turns anteriorly for a short distance; anus situated in lower part of atrial chamber.

Reproductive system.—No reproductive organs were seen in the colonies investigated. The fact that the specimens were collected in January and December may account for this. Very young zooids had two great protruding buds, one on each side. In young zooids the atrial orifice is similar to the branchial but takes on the spout-like character as the animal matures.

Habitat and distribution.—So far as known, B. tuberatus is confined to the coast of southern California. No Botryllid has yet been seen north of Point Conception. The type locality of this species is Santa Barbara, and the specimens taken there were on the leaves of kelp, Macrocystis pyrifera. Despite much search in the kelp beds off San Diego, the animal has not been found there. At La Jolla the species occurs in considerable abundance at times on rocks at extreme low tide. This difference in habitat and the fact that the San Diego specimens are, at least in some cases, much more deeply pigmented than those from Santa Barbara and usually devoid of the peculiar tube-like masses of ectodermal ampullae, have caused us to hesitate much as to the propriety of regarding them as belonging to the same species. However, the absence of differences in the zooids of the colonies from the two localities, the well-known color variation in the genus, and the presence of small and somewhat protruding clusters of ampullae on a few specimens from points midway between Santa Barbara and San Diego have led us to follow the more conservative course and treat all the specimens as of the same species. Should future study prove this to be unjustifiable, the San Diego form should be the basis for another species.

The great pedunculated masses of ampullae are sufficient in themselves to set tuberatus off sharply from the other species of the genus.

Botrylloides diegensis, n. sp.

Pl. 43, figs. 46–49

Superficial characteristics of the colony.—Flat, inerustling, irregular in outline; may be several centimeters in expanse with a thickness of 5 mm. Color in life varying from pinkish yellow to purple. Systems elongated; number of zooids in a system variable, ten to fifteen occurring in the older systems; islands of test (i. e., pl. 43, fig. 49) in the colony surrounded by zooids give impression of systems; atrial orifices not found. Ectodermal vessels and ampullae numerous near margins of colony and in island of test; ampullae oval, and black from accumulation of pigmented cells.
Zooids.—Cylindrical, standing vertically in test; length 2.5 mm., width about 1 mm.; length of languet and size of atrial orifice varies depending upon position of zooid in system; atrial orifice often wide and gaping. Mantle thin, with feeble musculature, only a few circular and longitudinal bands occurring near branchial and atrial orifices, but pigment very abundant. Alimentary organs and stigmata easily seen through mantle if some of pigment is removed with strong alcohol (pl. 43, figs. 46 and 47).

Branchial system.—Branchial orifices large and circular, projecting slightly above surface of colony. Branchial tentacles sixteen, symmetrically arranged. Tentacles next to endostyle and lateral ones at a quadrant's distance from this longest, with enlarged pigmented bases, insertion deeper in the siphon than the others. Dorsal tentacle, inserted just above the hypophysis, long but its pigmented base not as large as in the others; alternating with the major tentacles, four tentacles about half as long; and finally alternating with the eight large and medium-sized tentacles, eight very short ones (pl. 43, fig. 48). Twelve series of stigmata in the branchial sac with about fourteen stigmata in a half-series; three internal longitudinal vessels on each side; two or three stigmata in a mesh except next to the dorsal lamina where there are from four to six. Dorsal lamina a plain straight membrane, wider toward the posterior end of the body. Endostyle large, conspicuous, straight. Neural gland situated over anterior end of dorsal lamina just outside peripharyngeal band; duct of gland opening conspicuously on surface of the oval dorsal tubercle (fig. pl. 43, fig. 48).

Digestive system.—Stomach (s., pl. 43, fig. 46) seeming to consist of nine or ten large drawn-out loops, these being clustered at esophageal end and from thence tapering back to pyloric end; loops concentrated on right side, only three reaching around on left side so that the latter presents a plain space from which a sac-like caecum (c., pl. 43, fig. 46) is given off toward the intestine where it connects with a pyloric gland located on that organ; posterior to branchial sac and toward right side of body. Esophagus emerging from branchial sac at its posterior end near dorsal lamina; about as long as stomach, inserted into a depression surrounded by rounded ends of gastric loops. Intestine on left side and posterior end of branchial sac, bending abruptly forward and dorsalward a short distance behind the stomach; anus on dorsal side of branchial sac about midway the length of the zooids; two narrow, thin, non-pigmented strips on opposite sides of intestine extend whole length of rectal portion. Rectum somewhat compressed, strips placed at edges of compression; strip along outer convex side of intestine broader (pl. 43, fig. 46).

Reproductive system.—One ovary and one spermary on each side of posterior half of body; these appearing as conspicuous protuberances, the spherical ovary especially prominent when containing a nearly mature ovum (o., pl. 43, fig. 47); lobulated spermary anterior to ovary of same side; ovary and spermary of left side farther forward than those of right.

Habitat and distribution.—The species occurs in great abundance on piles, floats, etc., in San Diego Bay, but so far has not been taken elsewhere, though it will undoubtedly be found at other points on the coast of southern California at least.
B. diegensis appears to be more like Botrylloides purpureum, Herd. (Herdman, 1886) from near the Philippine Islands than any other species. The conspicuous gastric loops are, however, quite distinctive of diegensis. Furthermore, purpureum is described as presenting a lobing of the mantle at the branchial aperture which does not occur in diegensis. Again, the transverse musculature of the mantle of purpureum is considerably stronger than that of diegensis.

**Perophora annectens** Ritt.

*Perophora annectens*, Ritter, 1893.
*Perophora annectens*, Huntsman, 1911, p. 118.

A careful study of the asexual reproduction and relation of the zooids in the colony in *P. annectens* relative to its geographic distribution and general habits of life ought to be made; for nowhere in its range, as it now seems, excepting in central California, do the colonies reach the complete "compound ascidian" condition described for some specimens by Ritter, 1893. Although the species is by no means rare on the southern coast, we have never found it in any such abundance as that in which it occurs at Pacific Grove, and the zooids are always, so far as our observations have gone, quite distinct from one another. Huntsman (1911) reports the same to be true of specimens from the coast of British Columbia.

**Distaplia occidentalis**, n. sp.

Although *D. occidentalis* has already figured to a considerable extent in writings on ascidians, notably in the paper by Bancroft (1899), it has always appeared, so far as taxonomy is concerned, as a manuscript species by Ritter. But since neither diagnosis nor species-figure have been published hitherto, its career as a known species ought to date from the publication of this paper, and Ritter and Forsyth recognized as responsible for it (pl. 45, figs. 64 and 65).

*Superficial characteristics of the colony.*—Either flat and encrusting, or pedunculated and mushroom-shaped with all gradations between; flat, from 3 or 4 mm. thick and several centimeters in expanse; pedunculated forms varying from 2 mm. to 1 cm. or more across flattened heads, peduncles being of same length or longer; flat form often pedunculated at margins of colonies. Systems plain, several in a head; zooids closely arranged around a large, cylindrical, lobed, common atrial orifice, which extends considerably above the general
surface as a delicate-walled short pipe. Color light green, variable, dark brown, cadmium-yellow, brick-red, dirty white. Test consisting of a thin, tough outer layer covering the less resistant inner portion, both having many bladder cells; ectodermal vessels running parallel with long axis of peduncle and not branching or anastomosing.

Zooids (pl. 45, fig. 64).—Small, from 2 to 3 mm., average of twelve individuals 2.5 mm. Mantle delicate, containing many diagonally running muscle fibers; two strong dorsal longitudinal muscle bands extending from the vicinity of branchial siphon to the esophagus, causing branchial sac to shrink along dorsal sides in preserved specimens. The atrial languet long, but varying in shape and size depending on position of zoonid in colony; one or three-lobed.

Branchial system.—Branchial siphon large with six broad blunt lobes. At base of siphon sixteen tentacles, four placed symmetrically at the quadrants of circle, much larger; alternating with these, four about half as long; finally alternating with the eight, eight very short ones (pl. 45, fig. 65). Branchial sac with four series of stigmata; the most posterior series having the longest stigmata, those of the other series gradually shorter in order; the stigmata long and slender, about twelve in a half-series; each series crossed by a delicate intermediate transverse vessel which does not interrupt stigmata; in young individuals stigmata become gradually smaller toward endostyle, leaving triangular spaces between series and main transverse vessels. Dorsal languets three, about half the length of the stigmata, located a little to left of mid-dorsal line.

Digestive system.—Esophagus about equal in length to stomach and very slightly twisted. Stomach inflated in appearance, egg-shaped, long in proportion to its breadth; smooth on outer surface, slight, discontinuous, branching ridges generally longitudinal on inner surface, giving it a reticulated appearance; long axis of stomach forming a slight angle with long axis of zoonid. On emerging from stomach, intestine runs ventrally for a distance equal to width of stomach, then bends anteriorly and maintains a straight course until it ends as a lobed anus near the middle of branchial sac. Ramifying over the intestine from the pylorus halfway up to anus is the pyloric gland (p. gl., pl. 45, fig. 64). Within intestinal loop is a clear bulb which is part of this system; this connected with stomach by a fine duct (pl. 45, fig. 64).

Reproductive system.—Testicular lobes and ovary on right side of intestinal loop in young individuals, but in adults ovary always posterior to loop and testis largely so, though owing to its great size it may extend beyond the loop in both directions; testicular lobes all communicating with the vas deferens at one point by delicate vasa efferentia; the vas deferens running along intestine nearly to the anus. Oviduct a thin-walled tube lying immediately over the vas deferens, bending to enter incubatory pouch at right dorsal posterior corner of branchial sac. Incubatory pouch (i. p., pl. 45, fig. 64) containing two or three eggs, very rarely four; larvae present in colonies taken in June and July; absent in colonies taken in January.

Habitat and distribution.—On rocks in littoral zone from San Diego to Puget Sound, common in many places.

Type locality.—San Diego, California.
As pointed out by Bancroft (1899), *D. occidentalis* is easily distinguishable from all the other species of the genus, with the possible exception of *D. rosea*. There seems, however, no doubt about the distinctness of these two. Although the color of *occidentalis* is so varied that not much reliance can be placed upon it for taxonomic purposes, yet since rosaceous is one shade that seems not to occur in *occidentalis*, the color distinction is worth something. The most positive differences between them so far as recognized are the absence in *rosea* of internal vessels crossing the stigmata; the smaller number, usually twelve, of stigmata in a half-series in *occidentalis*; *rosea* having, according to Herdman, from twenty to thirty; and the presence on the inner surface of the stomach wall of *occidentalis* of the network of low but distinct ridges.

*Color.*—Examination of an abundance of colonies by the senior author at Point Conception in January, 1908, some of which were nearly white while others were brick-red, led to the supposition that the red color was an old-age mark. There was ample evidence of degeneration in the red colonies, but not in the light ones. But besides the degenerated zooids in the red colonies, there were also many partly grown ones, indicating that the colonies were undergoing degeneration and regeneration simultaneously. On the whole the zooids of the light-colored colonies were considerably larger than those of the red ones, though partly grown zooids were also present in the light colonies. The pigment of the red colonies was confined to the test. No larvae were found in any of the animals at this time.

*Embryogenesis.*—The escape of the larvae from the parents and their behavior during their brief existence before becoming attached were observed by the senior author on July 24, 1896. The young are retained in the parent until the tadpoles are fully formed. They emerge from the common cloacal orifice posterior end foremost. Their exit is entirely passive, the egg membrane being still intact and the larva’s tail closely folded around the body. Frequently the individuals are shot out with considerable force. So far as could be ascertained by watching the escaping larvae, they are forced into the common atrial chamber by muscular contraction of the individual parent zooids, and are then expelled to the outside by a contraction of the common chamber. The exact nature of this second phase of the expulsion is not clear and merits study, particularly as to the mechanism by which it is accomplished.

Immediately upon reaching the outside world most of the larvae
fall inertly to the bottom, though an occasional one escapes from the egg membrane and swims with a wriggling motion immediately on emerging from the parent. Although the larvae have a free swimming period, this is very short. It lasts but a few hours at the most, nor is the swimming executed during the period sufficiently vigorous and determinative to make it count for much if anything in the distribution of the species. In view of the very brief swimming period in the larval life of this and of several other species of Ascidians, the general question of the significance of larvae in the group becomes of interest and suggests a systematic study of the point.

Bancroft (1899) describes some interesting features of the reproductive system of Distaplia occidentalis, from which we quote:

The most striking peculiarity of the oviduct is that its diameter, even when distended by the passage of the ovum, is very much less than the normal diameter of the ripe egg. Accordingly, when the egg is passing through the duct, it is greatly distorted, assuming the shape of a sausage.

With regard to the incubatory pouch he says:

A careful examination of the structure of the pouch shows that it is not merely a diverticulum from the peribranchial sac, but consists of two parts which, for descriptive purposes, may be called the oviduct and the peribranchial portions, though I do not know that they have been developed from the oviduct and peribranchial sac respectively. The oviducal part is a narrow tube, the anterior end of which connects with the oviduct, and the posterior end with the bottom of the pouch. Anteriorly the peribranchial portion is a narrow tube opening into the posterior dorsal corner of the right peribranchial sac. Posteriorly, it is enlarged to form the pouch proper, in which the developing embryos are lodged. The oviducal portion of the pouch is a continuation of the oviduct into the pouch, and the egg never reaches the peribranchial sac at all, but is conveyed directly to the bottom of the pouch. . . . As the pouch is completely separated from its zoonid long before the larvae are mature, the only function of this peribranchial orifice is to serve as a passage of the spermatozoa.

Our observations, as reference to plate 45, figure 64 will show, confirm Bancroft’s account of the incubatory pouch, but leave the question as to the exact method by which the eggs get into it unanswered.

The two species of the old genus Distoma treated in this paper belong to Caullery’s subgenus Eudistoma.

Eudistoma psammion, n. sp.

Pl. 44, figs. 52 and 53

Superficial characteristics of the colony.—Massive, though encrusting; hard, largely because of much sand in deeper layers of test; thickness in thickest part about 2 cm.; not thin in any part; expanse
several centimeters but extremely variable. Considerable sand adhering to tougher surface layer but a stratum of test just beneath freer from sand than other parts. Color varying from brown to brown with a distinct tinge of claret. Zooids in systems with about eight animals in a system; common cloacal orifices on small rounded elevations regularly distributed over surface.

Zooids.—Not thickly crowded and apparently at different levels in the test, perhaps due to shrinkage; consisting of thorax and abdomen, a slight constriction at base of branchial sac marking separation. Abdomen containing intestinal loop, reproductive organs, and heart; from two to four times as long as thorax (pl. 44, fig. 52). Length of zooid from 4 to 5 cm. in shrunken preserved condition. Muscle bands of mantle in two strong sheets (m.b. and m.b.' , pl. 44, fig. 52) on right and left sides of body and extending its entire length; on both sides the longitudinal muscles stronger than the transverse; the portion of mantle free from muscle fibers often puckered between muscle sheets. The visceral organs protrude, hernia fashion, through this less resistant portion of mantle, especially when reproductive organs are at their fullest development. Ectodermal processes (e.p., pl. 44, fig. 52) borne at posterior ends of zooids, two or three times length of body.

Branchial system.—Siphons long and tubular, each 6-lobed; the atrial usually longer than the branchial, situated on dorsal side of branchial sac, often some distance from its anterior end; remoteness of the zooids from common cloacal orifice in the systems accounts for long “goose-neck” character of the atrial siphon (pl. 44, fig. 53). Branchial tentacles of different sizes, about thirty in number, scattered over the surface of siphon instead of being in a circle. Branchial sac with three series of stigmata and ten stigmata in a half-series. Endostyle usually very convoluted, doubtless due to shrinkage. Dorsal languets two, short and blunt.

Digestive system.—Consisting of long loop occupying entire length of abdomen; plane of loop extending from right to left of zooid. Emerging from greater part of posterior end of branchial sac is the large, long esophagus which enters stomach (s., pl. 44, fig. 52) located past the middle of abdomen. Stomach somewhat flattened from right to left and straight to outline across posterior end; remaining outline a regular curve, except for a depression located anteriorly into which the esophagus enters. Stomach wall smooth on outer surface, fairly thick; granular on inner surface. Intestine proper differentiated into three distinct parts: the first a little longer than the stomach, enters an enlarged second portion of about the same length, having firm thick walls, and entering the rectal limb of the loop by a narrow tubular constriction; third is the rectal limb, the first portion of which is enlarged and firm-walled for a distance equal to all the preceding intestine; anus at about level of posterior one-third of atrial chamber. The two arms of the intestinal loop almost parallel throughout their course (pl. 44, fig. 52).

Reproductive system.—Ovary and testicular lobes on dorsal side of intestinal loop and when fully developed, so voluminous as to make zooid flask-shaped (t., pl. 44, fig. 52). Eggs develop in atrial cavity and become almost as large as branchial sac and produce a great protuberance.
Breeding season.—June and July. Colonies collected in these months had large eggs in the atrium while in colonies taken in January the ovarian eggs were still small.

Habitat and distribution.—In the littoral zone, usually on the underside of rocks. According to our present information extending from San Diego to Dillon’s Beach near the mouth of Tomales Bay. The species has been taken on the rocks north of the Scripps Institution, La Jolla, though not in abundance.

Type locality.—La Jolla, California.

Eudistoma diaphanes, n. sp.

Superficial characteristics of the colony.—Flat and encrusting, soft, usually even surfaced and regular in outline. Rarely exceeding 10 cm. in greatest expanse, usually much smaller; seldom if ever more than 1 cm., frequently only a few millimeters thick. Color varying from white to pale vermillion; test transparent, containing a great quantity of cellular material but no bladder cells or spicules; almost entirely free from sand.

Zooids.—Uniformly distributed; not disposed in systems; inconspicuous by reason of small size and meagerness of pigment; placed at various angles to surface of colony. Length about 3 mm.; less in preserved condition. A distinct capsule of test enveloping each zooid. Mantle musculature similar to that of Eudistoma psammion. Ectodermal processes present but not as long and numerous as in E. psammion.

Branchial system.—Siphons relatively shorter than those of E. psammion, the two of about equal length; both opening on surface. As far as could be made out, branchial sac very similar to that of E. psammion. Branchial tentacles about twenty in number, of two sizes, not all in same circle but scattered somewhat over surface of siphon.

Digestive system.—Similar to that of psammion but violent contraction often obscures similarity.

Reproductive system.—Similar to that of psammion. Specimens from La Jolla collected in July had large eggs in atrial cavity.

Habitat and distribution.—On under side of rocks in littoral zone from San Diego to San Francisco, according to our present knowledge.

Type locality.—La Jolla, California.

These two species of Eudistoma are quite similar as far as the zooids are concerned; but the striking differences in the colonies, and the fact that E. diaphanes does not have systems, and hence its two siphons are of about equal length is our basis for recognizing two species.

The senior author has more or less carefully examined specimens presumably of Eudistoma diaphanes from many points on the California coast between Cape Mendocino and San Diego. Unquestionably much variation occurs within the range, particularly in the size and color of the colonies, and it is by no means impossible that more
exhaustive collecting and more extensive comparative studies will find that the animals living farther to the north are specifically distinct from those of the south on which we are chiefly relying for the establishment of the present species. In connection with the description of Distoma lobata from Puget Sound, Ritter (1900) remarks upon the similarity of lobata to a Distoma "widely distributed on the California coast." The species occupying us is one of the group of Californians to which reference was made in this quotation.

These two Eudistomas furnish a good case of coincident distribution of two closely related species. That they are very much alike as far as the zooids are concerned is obvious; and so far as we know there is nothing at all differential in their habitats. However, we would not be too positive on this latter point. Wider and more detailed knowledge of the range of both may bring to light habit differences which we do not now recognize.

If Ritter (1900) is right in supposing the zooids of D. lobata to possess five instead of three series of stigmata, the two closely allied California species now before us seem to be considerably less similar to the Puget Sound species than to E. plumbium Della Valle, a Mediterranean species. E. diaphanes in particular has much in common with the European species; but diaphanes differs from plumbium according to our present knowledge, in the larger number of branchial tentacles, in the relatively longer esophagus, and probably in the sharp division of the post-gastric intestine into sections.

Didemnum carnulentum, n. sp.

Pl. 39, fig. 11; pl. 44, figs. 57 to 59

Superficial characteristics of the colony.—Thin and encrusting and of considerable expanse, often half a foot or more; thickness 4 mm. or less. Color typically the pink of the human skin but varying to opaque white. Position of the zooids indicated by small spots caused by accumulation of spicules; these spots often appearing in double rows which surround islands of gelatinous-appearing semitransparent test and giving surface a reticulated appearance (pl. 39, fig. 11). Spicules varying in diameter from .19 to .075 mm., their blunt rays springing from a spherical nucleus (pl. 44, fig. 59); confined mainly to uppermost stratum of colony. Bladder cells, usually polygonal from mutual pressure but free from spicules, make up lower layer of test. Branchial sacs of zooids embedded in upper spicule-bearing stratum, their abdomens extending about halfway down into bladder-cell stratum. Upper stratum of test having spaces in it which seem to serve for communication with the common cloacal orifices, zooids not being arranged with any reference to these openings. Cloacal
orifices comparatively few in number and large; branchial orifices small openings in center of spots caused by closely set spicules.

Zooids (pl. 44, fig. 57).—From 1 to 2 mm. long, depending upon thickness of colony; made up of thorax and abdomen. Mantle with no muscle fibers visible. Color orange.

Branchial system.—Branchial siphon with six distinct, usually pointed lobes, located in middle and anterior part of branchial sac. Atrial siphon a plain round opening on dorsal side opposite middle of sac. Branchial sac with four series of stigmata, each having about six in a half-series; stigmata elliptical. Endostyle proportionately very broad. Dorsal languets three. Branchial tentacles eight, very small. Two strong muscle bands from dorsal part of branchial sac continue to near the recto-esophageal collar where they terminate in a free process (m.b., pl. 44, fig. 57).

Digestive system.—An elongated loop with arms closely applied but open at bend. Esophagus (e.. pl. 44, fig. 57) proportionately large, emerging from middle posterior part of branchial sac; often as much as three times the length of sac. About two-thirds of its length from sac, esophagus bound to rectum by a band (c.c., pl. 44, fig. 57). Stomach (s., pl. 44, fig. 57) almost globular with smooth surface; its long axis slightly inclined to that of zooid. First part of intestine, about equal in length to stomach, connected by a portion equally long but of smaller diameter with rectal arm of loop; anus very near atrial opening.

Reproductive system.—Large undivided testis situated on left side of intestinal loop; on its rounded mound-like surface is the coiled vas deferens (v.d., pl. 44, fig. 57), coil having six turns. Ovary, usually containing one large egg and several very small ones, located between stomach and testis (pl. 44, fig. 57); ripe eggs almost as large as branchial sac. No oviduct appears to be present and how eggs are discharged is not definitely known. Large tadpoles found in the test of some colonies. Budding the same type as that which will be described below for Trididemnum della vallae.

Breeding season.—Colonies containing many tadpoles in June, but few or none in January and February.

Habitat and distribution.—In littoral zone but never at limit of high tide; usually on under sides of rocks. Abundant at La Jolla and on all rocky beaches of the San Diego region. Although this species has not been found at any other point, in all probability this is due to insufficient collecting.

Type locality.—La Jolla, California.

The specific name refers to the prevailing flesh color of the living colonies.

**Didemnum carunculatum var. lacteolum**

Pl. 40, fig. 23; pl. 44, fig. 60

*Superficial characteristics of the colony.—Encrusting and very thin, never of the great extent of Didemnum carunculatum; 1 mm. and less in thickness. Color pure white, due to numerous calcareous spicules varying in size from .15 to .065 mm. (pl. 44, fig. 60); spicules
very numerous in lowermost stratum of colony, zooids extending into both upper and lower limy strata. Bladder cells not conspicuous but present around closely packed zooids. Zooids very similar to those of Didemnum carnulatum but smaller. The atrial orifice proportionately much larger, often over half the length of branchial sac. Testis almost spherical when fully ripe and half as large as sac. Coil of vas deferens contains seven turns. Ripe ova gigantic, even larger proportionately than in carnulementum. Budding similar to that of Tridemnum della vallei. Breeding season in June.

Habitat and distribution.—On under sides of rocks in the littoral zone and in the holdfasts of kelp, hence from depths of a few fathoms. Common at La Jolla and in the San Diego region.

The variety or sub-species here recognized differs from the typical species in color chiefly, it being opaque white. We do not, however, depend on this difference exclusively for recognizing a group that deserves a separate name. The other differences that seem significant are in the character of the atrial orifice, this being considerably larger in lactocolum; and in the spicules, these being blunter in lactocolum. Possibly significant, too, is the fact that what we are regarding as a variety lives typically in the great masses of kelp holdfasts, at a depth, consequently, of several fathoms.

The species and variety of Didemnum here described have much in common with several previously described species; but of their distinctness from all those that have been treated with sufficient detail to enable us to judge, there seems little doubt.

It appears almost incredible that the one hundred species, more or less, of Didemnum now recognized, can all be really distinct. Yet we cannot assume that specimens from a geographical region as remote as ours from other regions which have furnished the known species, are the same as any that have been named but not described with sufficient definiteness to enable us to make sure of the identity of ours with these. There seems no course open, therefore, but to add still another to the list of supposed species.

A thorough revision of this group of ascidians based on ample material of all recognized species is much to be desired.

The milk-whiteness of the colonies suggested the name..

Tridemnum della vallei, n. sp.

Pl. 44, figs. 54 to 56

Superficial characteristics of the colony.—Encrusting on the under surfaces of rocks, colonies being half a foot or more in expanse in some cases; thickness from 1 to 2 mm. Color gray with tinge of yellow.
Common cloacal orifices large and comparatively few, scattered among the numerous zooids. Test immediately around cloacal openings free from spicules. Spicules of usual *Didemnum* type but differing from those of *D. carunculatum* in that the rays are distinctly longer and relatively slenderer (pl. 44, fig. 56); size from .2 mm. to .08 mm., confined mainly to surface layer of test, but sparingly present throughout. Usually in preserved specimens branchial orifices marked by white spots due to accumulation of spicules; these spots uniformly and thickly scattered over surface of colony without definite arrangement with reference to the cloacae. In expanded condition six branchial lobes showing as clear oval spots in middle of each lobe near its base. No bladder cells observed in test.

**Zooids** (pl. 44, fig. 54).—Length 1 mm. or less; divided into thorax and abdomen, the two being equal in size; esophagus and rectum girdled by a constricting band about midway the length of zooid (pl. 44, fig. 54); placed at all angles to surface of colony. Ectodermal processes, (e.p. pl. 44, fig. 54) given off from mantle near stomach, extending for varying distances into test, ending in pigmented swellings. Mantle very thin and delicate, a few weak, longitudinal and horizontal fibers being visible over branchial sac.

**Branchial system.**—Branchial siphon with six distinct lobes; atrial orifice plain, at the end of a tubular siphon which protrudes from middle of dorsal side; its sphincter muscle strong and distinct. Three series of stigmata, about ten orifices in first series, eight in second, and seven in third; considerable area of unperforated branchial membrane at both ends of sac. Endostyle straight and proportionately very broad. Two dorsal languets placed at intervals between stigmatic series. Two muscle bands in branchial sac, one on each side of mid-dorsal line, course ventralward toward posterior end of sac and, meeting each other behind sac between esophagus and endostyle, are carried out into a blunt process with an epithelial covering, this process penetrating into the test (m.b., pl. 44, fig. 54). Endostyle often arched at both ends in preserved specimens, due to contraction of these muscles. Branchial tentacles equal in length, variable in number, twenty being found in one zooid; about sixteen the usual number (pl. 44, fig. 55).

**Digestive system.**—A loop in which the esophageal and rectal limbs are closely applied, loop being wide and nearly circular. Recto-esophageal collar almost midway the length of the esophagus. Stomach almost globular, smooth-walled. First part of intestine, a little shorter than stomach, tapering from large pyloric opening; connected by a piece of about equal length but of much smaller diameter to rectal arm of loop; this latter of greater diameter than middle piece; anus opposite atrial orifice (pl. 44, fig. 54).

**Reproductive system.**—Single conical mound-shaped testis situated on bend of intestinal loop on left side; coil of vas deferens containing six turns. The ovary, apparently consisting of single egg when ripe as large as branchial sac; but in reality a small number of minute ova always present. No oviduct found and the mode of escape of eggs not known; the relatively enormous eggs (fully half the size of full-grown zooids) after escape from ovary, scattered through test at rather regular intervals in layer immediately in contact with the sub-
stratum. Budding abundant in some colonies. (See section on this subject at end of description.)

Period of egg-production.—January.
Period of budding.—July.

Habitat and distribution.—Under sides of rocks in the littoral zone at La Jolla and in the San Diego region; Dredging Station II off San Pedro, depth six meters.
Type locality.—La Jolla, California.

This species seems to resemble Trididemnum (Didemnum) strangulatum Ritt, as closely as any other but is well separated from it by the shorter stigmata, unlobed atrial orifice, and presence of the muscular process at the posterior end of the branchial sac.

We are glad to name this species, a study of the budding of which has received considerable of our attention, after Professor Della Valle, whose work on budding in this group is distinguished.

The budding is very similar in this species to that described later on in detail for Diplosoma pizoni. As seen in plate 44, figure 54, the budded intestinal loop grows out just beneath the recto-esophageal collar from two sources, the mother esophagus and the mother rectum. Likewise the budded thorax is connected to the same two sources of the mother zooid posterior to the budded intestinal loop. The supposition that a division takes place so as to give the mother thorax the newly budded intestinal loop and the budded thorax the old intestinal loop is supported by the fact that many zooids were seen with large thoraces and small intestinal loops and vice versa.

Diplosoma pizoni, n. sp.
Pl. 43, figs. 50 and 51; pl. 45, figs. 66 to 68

Superficial characteristics of the colony.—Exceptionally soft, encrusting, several centimeters in expanse; thickness about 3 mm. Color of preserved specimen mottled light and dark gray; closely set zooids easily seen through transparent test. Common cloacal orifices few in number, large and chimney-shaped. Each zooid surrounded by thin layer of test and joined to lower surface of colony by a strand of about the same length as zooid. The test consisting of upper and lower thin layers, and strands surrounding zooids joining these layers, containing great spaces between and among zooids; many small colorless cells and fewer but much larger round pigmented cells; but no bladder cells.

Zooids.—About 1.5 mm. long, consisting of thorax and abdomen. Mantle containing much dark pigment especially over stomach and intestine, the epithelial layer here consisting of large, flat, polygonal cells with small, round, clear nuclei usually near one end of darkly pigmented cell body (pl. 43, figs. 50, 51); adheres closely to surround-
ing test. Muscle fibers in transverse vessels and two strong bands on each side of dorsal lamina coalescing at posterior end of branchial sac into a band that continues posteriorly through test strand nearly to lower surface of colony. A few ectodermal vessels with enlarged extremities present on ventral side of zooid in angle between sac and intestine (e.g., pl. 43, fig. 50).

**Branchial system.**—Branchial siphon plainly 6-lobed; atrial orifice a great opening usually longer than half the branchial sac and extending at least halfway across sides of sac. Branchial sac with four rows of stigmata, each containing seven or eight large stigmata in a half-series. Three tentacle-like dorsal lamngets. Branchial tentacles sixteen, of three sizes, and symmetrically arranged so that the four largest alternate with the four or second size and finally the eight smallest alternate with the eight larger ones.

**Digestive system.**—Intestinal loop twisted and turned up at a right angle to long axis of zooid so that reproductive organs, belonging typically to right side, became located at posterior end of zooid. Short esophagus given off from posterior dorsal part of branchial sac; stomach globular, smooth-walled; portion of intestine immediately following stomach and about equaling it in length connected by a piece of less diameter to a rigid bulbous portion at beginning of rectal arm; rectum oblique; anus situated less than half the distance up branchial sac. Pyloric gland (p.g.l., pl. 45, fig. 68) and its bulbiferous duct well-developed.

**Reproductive system.**—Situated on right side of intestinal loop, but bend of intestine brings gonad to posterior of zooid. Testis conical, two-lobed, the vas deferens emerging from between the lobes. Ovary usually containing one large egg and a few small ones (o., pl. 43, fig. 50). Ripe discharged eggs and embryos scattered thickly on lower layer of test, the eggs apparently making their way down through the strand of test to reach this position; eggs observed at different levels in these strands seeming to support this theory (o.v., pl. 44, fig. 50). Tadpoles probably break through test and escape into common atrial cavity and pass out through common atrial openings.

**Habitat and distribution.**—Known only from San Diego Bay where but a single colony has yet been taken, this having been found on piles.

*D. pizoni* is undoubtedly very close of kin to *D. listeri*, but there is hardly a doubt about their specific distinctness. *Listeri* has bladder cells in the tests; *pizoni* has not. *Listeri* has ten stigmata in a half-series, while *pizoni* usually has seven and seems never to have more than eight. The stomach of *pizoni* is more spherical than that of *listeri*, it being figured as heart-shaped in the European species.

**Assexual reproduction in *D. pizoni*.**—The larva of this species presents the same aspects as those pictured and described by Salensky and Caullery for *Diplosoma listeri*. In the diplosomic larva the zooid and blastozoid are distinguishable here as there, the former being characterized by its vascual appendages and pigmented larval sense...
organ. The opinion of Caullery (1895) and Pizon (1905) is that the double larva is due to precocious budding; and this view seems unescapable from our observations. Pizon (1905) has so fully described all stages in the budding of Diplosoma listeri, from which the course of things in our species varies but little, that the barest outline is all we have thought necessary to give.

Various stages of bud development in the colony were observed in this species. Examining plate 45, figure 66, one sees that the new intestine (b.i.) arises from two sources. The new esophagus comes from the mother esophagus and the new rectum is joined to the mother rectum. According to Pizon, who watched growing colonies in all stages of development, a bud from the mother esophagus produces a new esophagus, stomach, and intestine, and these curl around to meet and join a little rectal bud from the mother rectum. The budded branchial sac and rectum (b.b.) likewise arise from two sources. The budded esophagus is joined to the mother esophagus and the new rectum is joined to the mother rectum. According to Pizon, the branchial sac is formed from the epicardium and its accompanying rectum grows from a bud on the mother rectum up into place alongside the new sac. Plate 45, figure 67 shows at d, i, the degenerating piece of intestine the disappearance of which severs the intestine of the budded zooid (b.b.) from the intestine of the mother zooid (m.s.). After the break there are two zooids in one of which the original mother branchial sac (m.s.) has the newly budded intestinal loop and the newly budded branchial sac (b.b.) has the old original intestinal loop (m.i.).

Diplosomoides caulleryi, n. sp.

Pl. 40, fig. 21

Superficial characteristics of the colony,—Thin, encrusting, and comparatively firm; thickness about 2 mm.; expanse several centimeters. Color of preserved specimens dark gray sprinkled with white calcareous spicules. Zooids numerous, each branchial orifice 6-lobed, light colored, circular. Common atrial orifices few, large, rounded or elongated, flush with surface; test immediately around them free from spicules. Spicules fairly numerous and evenly scattered in surface of test. Little test immediately around branchial sacs, this stratum of colony being cavernous; lower stratum, containing abdomens of zooids and large eggs, more continuous and solid. Spicules sparingly scattered throughout test between bounding layers; somewhat resembling the large round test cells to which they are about equal in size.
Zooids.—Length about 1.5 mm., consisting of thorax and abdomen (pl. 40, fig. 21); long axis of abdominal loop usually continuous with that of thorax, though often flexed laterally; mantle adhering very closely to test. A few ectodermal vessels (e.g., pl. 40, fig. 21) with club-shaped extremities present just anterior to stomach and toward ventral side of zooid, always in close proximity and just ventral to budded branchial sac.

Branchial system.—Branchial orifice with six small pointed lobes. Atrial orifice a great opening extending almost the length of sac and reaching halfway across sides; atrial languet present, large and scoop-shaped. Branchial sac with four series of stigmata each containing eight stigmata in a half-series; dorsal languets three, very slender. Branchial tentacles very slender, about sixteen, of different sizes, not arranged in any regular order.

Digestive system.—Esophagus emerging from central portion of posterior end of branchial sac, about one and one half times as long as stomach, curved. Stomach smooth-walled, globular-elliptical. Intestine consisting of a piece immediately following stomach, large in diameter and about equalling stomach in length; then a shorter connecting piece of smaller diameter, situated at lowest part of loop; next a large, smooth-walled, bulbous portion; finally the rectal arm of about same diameter as esophagus, lying about parallel with stomach and esophagus. The smooth-edged anus situated just opposite middle of posterior series of stigmata.

Reproductive system.—Testis (t., fig. 21) double, the two parts forming rounded protuberances on right side of intestinal loop; vas deferens arising between lobes and running along side intestine, vas deferens arising between lobes and running alongside intestine. Ovary an elongated sac between stomach and rectum; the small ovarian eggs arranged in a row. It would seem that the ovarian sac allows eggs to escape by rupture since in some colonies large eggs studded the lowermost layer and apparently had no connection with zooids.

Budding.—Intestinal and similar to that described for Diplosoma pizoni. In this species esophagus of budded sac joins mother zooid very close to junction of mother esophagus and stomach.

Habitat and distribution.—Known only from upper part of San Diego Bay, near National City.

The combination of characters in this species makes difficult its inclusion in any of the numerous genera that have been created for ascidians of the Didemnum type. In fact, as the family Didemnidae has been defined by some authors, e.g., von Drasche (1883) and Van Name (1910), it would be excluded from the family by its possession of a straight vas deferens according to both these authors, and by its atrial languet according to Van Name. We, however, agree with Hartmeyer (1909) that the definition of the family ought to be sufficiently elastic to admit this species. It seems to us that in the general character of the colony, especially in its being hardened by the possession of calcareous spicules and still more in the type of budding.
its closer affinity to the Didemnidae through Trididemnum than to the Polycitoridae through Distaplia is undoubted. Having decided that it belongs to the Didemnidae, the question as to what genus of that family it should be assigned remains to be decided. Accepting the genera of the family recognized by Hartmeyer (1909) only two of the nine of these, Didemnum and Polysyncraton, are regularly characterized by the possession of an atrial languet, and in both of these the testis is single-lobed and the vas deferens is coiled; so it does not seem possible to regard our species as belonging to either of these genera. All things considered, we conclude that the species can be placed in Diplosomoïdes Herdman with less violence than in any other recognized generic group. In all respects other than that of its atrial languet, caulleryi conforms very well to the characterization of this group; and we think Hartmeyer (1903) justified in redefining Diplosomoïdes as as to include species which, like his D. dubium, possess an atrial languet. The languet of dubium is much shorter than that of D. caulleryi, and the difference between the two may be taken, as we have contended in discussing the structure in Amaroucium, as indicating its modernness and, perhaps for this reason, undependableness for the characterization of genera.

We take pleasure in naming this especially interesting species after Professor Caullery, whose observations on the reproduction of various species of the Didemnidae has contributed so much to our knowledge of the remarkable phenomena here presented.

Glossophorum planum, n. sp.

Pl. 59, fig. 13; pl. 46, fig. 71

Superficial characteristics of the colony.—Larger colonies pumpkin-shaped, smaller ones spherical, all having short, thick, cylindrical peduncles; outline regular, surface smooth, free from sand or other foreign substances; systems distinct and regular, zooids plainly seen through test; common cloacal orifices open and very distinct even in preserved specimens (pl. 46, fig. 71). Color grayish brown, much the same in living and preserved specimens. Length of about maximum-sized colony 10 cm., width 5 cm., thickness 1.5 cm. The test consisting chiefly of a well-defined central core into which zooids do not extend; small in quantity among zooids; matrix semicartilaginous and transparent, but containing a great number of very small pigmented cells which impart to it a somewhat dirty tinge; portions among zooids containing scattered, rather small bladder cells; central core thickly penetrated by thin-walled transparent stolons to which zooids are always attached.
Zooids.—Numerous, distinctly seen through test, arranged in very regular systems, on an average about a dozen individuals in a system, occasionally as many as twenty, in a system. The three regions of body, thorax, abdomen, and postabdomen, distinctly set off from one another, making it difficult to extract zooids entire; never much contracted; little pigment matter in tissue; musculature very feeble; mantle thin and transparent; entire structure easily made out by examining animals in situ in slices of colony. Length of zooids about 4.5 mm.; length of branchial sac about 3 mm.; postabdomen slightly longer than abdomen; musculature very weak. About sixteen delicate longitudinal muscle bundles extending from branchial siphon backward to terminate about midway the length of branchial sac; a few delicate encircling fibers in branchial siphon; stronger fibers constituting a sphincter muscle around atrial orifice.

Branchial system.—Branchial orifices easily seen on surface of colony; each with six broad scallops when fully expanded, but becoming pointed lobes upon contraction. Atrial orifice overarched by a long broad languet often truncated but sometimes with three delicate terminal lobes; size and shape of languet depending upon age of zooid and its position in system. Tentacles eighteen to twenty-six, of three lengths, the six longest nearly as long as the half-diameter of circle in which they are situated. Number of series of stigmata from thirteen to seventeen; about thirteen or fourteen very regular stigmata in each half-series; number of series as well as number of stigmata increasing with age of zooid; a small muscle band in each interstigmatic vessel; papillae on interstigmatic vessels regular in size and arrangement, there being one for the interval between every two stigmata. Dorsal languets very regular, one for each interstigmatic vessel. Endostyle narrow and straight. Atrium exceptionally large and well-defined.

Digestive system (pl. 46, fig. 71).—Small in proportion to size of branchial sac; lateral flexure of intestinal loop pronounced, the antero-posterior axis of stomach being brought nearly to a right angle with the long axis of branchial sac. Esophagus emerges from dorsal posterior angle of branchial sac and bends abruptly ventrally to a right angle to enter the smooth-walled stomach which is slightly asymmetrical and a little longer than broad. Intestine usually divided into three portions; first, the part immediately behind stomach which is a little longer than stomach and has a bulge about midway of its length; next, a connecting piece that lies on left side and enters enlarged rectal limb by a very small, short, cylindrical tubule; and third, the long rectal piece having two blunt ceca on each side of proximal end. Rectum runs first parallel with stomach, then passes to left of esophagus and ends in a constricted anus a little less than half way up branchial sac.

Postabdomen elongated, pear-shaped, connected with abdomen by a narrow peduncle near intestinal loop; containing but little mesenchymatous tissue, and a large and distinct heart (h., pl. 46, fig. 71) situated in its posterior end.

Reproductive system (a. and t., fig. 71).—Not voluminous, situated in postabdomen about midway of its length, compact, the testicular lobes (t.) and ovary (a.) closely intermixed; vas deferens (v.d.) con-
spicuous, passing around left side of intestinal loop to right side and following rectum to end near the anus; its last portion swollen when filled with sperm. Tadpoles develop in atrium.

_Habitat and distribution._—Species widely distributed on California coast, it having been found at almost every point where ascidian collecting has been done, from Mendocino to San Diego. It is confined to rocky localities, but not to the littoral zone, judging from the frequency with which it is washed ashore. To what depths it extends is not known as it has never been taken by dredge or trawl.

At San Diego it occurs on the United States Government breakwater at the entrance of the bay; also on the breakwater at Coronado. In these localities it is not confined to the under surfaces of rocks, but grows on their tops and sides.

_Type locality._—San Diego, California.

This species is undoubtedly closely related to _Glossophorum humile_ Lahille; but the following differences seem fully to establish the specific independence of the two; _G. humile_, according to Lahille, is entirely covered with fine sand, while our species is peculiarly free from sand. The colony of _G. humile_ is relatively thinner than that of _G. planum_ and no mention is made by Lahille of a peduncle of _G. humile_. The atrial languet is pointed in _G. humile_ while it is usually broadly truncate in _G. planum_. There are about twenty stigmata in a half-series in _G. humile_ while fourteen is the highest number found in _G. planum_. According to Lahille and Herdman, _G. humile_ possesses sixteen tentacles; never less than eighteen have been found for _G. planum_.

Perhaps no species of compound ascidian in our fauna is so well adapted for laboratory demonstration as is this, and since it is fairly abundant and obtainable with little difficulty, it should be useful in this way.

_Macroclinum par-fustis_, n. sp.

_Pl. 38, fig. 3; pl. 45, fig. 63_

_Superficial characteristics of the colony._—Sand-encrusted, consisting of a few or numerous club-shaped masses each having a peduncle usually about twice as long as the more or less rounded head; masses connected together in colony by a stolonic basal network (pl. 38, fig. 3). Length various, the largest colony investigated 6 cm. long, with head 2 cm. long and 1.5 cm. thick. On smaller heads one common cloacal orifice present in center of anterior portion; on larger heads several such orifices occur, each surrounded by about twelve zooids. Branchial orifices indicated by slight, sand-covered elevations in preserved specimens. Test gelatinous and transparent, containing many small test cells scattered throughout its substance. Surface layer tougher and usually thickly embedded with sand grains.
Zooids.—Consisting of thorax, abdomen, and postabdomen; thorax a little longer than abdomen; total length about 1 cm.; long postabdomen terminating in an elongated granular mass, probably stored-up nutrient material. Mantle very thin with numerous delicate longitudinal muscle bands extending entire length of animal; circular fibers in branchial siphon and a few irregular ones in mantle over upper part of branchial sac.

Branchial system.—Branchial orifice with six blunt lobes; atrial with flat truncated languet ending in three lobes (pl. 45, fig. 63). Branchial tentacles about thirty, of three sizes. Branchial sac with sixteen series of stigmata, from twenty-five to thirty stigmata in a half-series; a small space near the endostyle free from stigmata; stigmata next to endostyle smaller than others of a series. Endostyle straight and narrow. Dorsal languets with flattened bases which run into transverse vessels upon which they are situated, about as long as stigmata in anterior part of the sac, becoming a little longer and heavier toward posterior end of series.

Digestive apparatus.—Esophagus emerging from middle of posterior end of branchial sac, about equal to stomach in length. Stomach roughly cylindrical, a little longer than wide, with a seam on left surface; wall granular on its inner surface, but not folded. Intestine extends posteriorly from stomach for a distance about equal to length of stomach, then makes a loop and after running parallel to stomach crosses esophagus on left side and ends in a bilobed anus less than halfway up atrial chamber. In base of loop intestine narrows abruptly to enter rectal arm between two blunt caeca (c., pl. 45, fig. 63).

Reproductive system.—Gonad a pyriform elongated mass just behind intestinal loop, the numerous testicular lobes composing the great bulk of it; usually one well-developed egg and several much smaller ones, yellow in preserved specimens, situated in anterior of gonad, surrounded by testis lobes.

Habitat and distribution.—M. par-fustis is known from rocky shores at La Jolla, Santa Monica, and Pacific Grove; and from Dredging Station LIX, off San Pedro, in twenty-eight meters water.

Type locality.—La Jolla.

The name is derived from Latin fustis, a cudgel. The systematic position of this and the next species, M. pellicidum, is by no means clear. M. par-fustis might be placed in the genus Pasammaplidium, its resemblance to P. rectiforme Herdm., one of the species of this genus with a smooth-walled stomach, being close. However, on the basis of the single character, the sandiness of the test distinctive of Pasammaplidium, it would be necessary to assign par-fustis and pellicidum to different genera, a course which in our judgment would be unjustifiable in view of the very close resemblance of the two so far as the zooids are concerned.

All things considered, we conclude that MacrocUnum Verr. as redefined by Hartmeyer (1903) is the generic group with which our
species is most akin. According to this revised definition, the only difficulty in the way of this disposition of the species is the breaking-up of the colony of both par-fustis and pellucidum into club-shaped masses. In view, however, of what occurs in the various other allied genera, this difficulty can hardly be regarded as serious. What may be of more importance as touching the integrity of Macroclinum is the discovery by Van Name (1910) that the inner surface of the stomach of M. ponum (SAR), which should be the type species of the genus, is areolated. This fact raises the question of whether further study may not make it advisable to retain the genus Aplidiopsis Lah., which Hartmeyer has assumed to be synonymous with Macroclinum, for the smooth-stomached species. Should this turn out to be so, par-fustis and pellucidum would be transferred to Aplidiopsis, for there is no trace of areolation in the stomachs here.

Macroclinum pellucidum, n. sp.
Pl. 39, fig. 9; pl. 45, fig. 62

Superficial characteristics of the colony.—Pyriform with peduncle as long as and often longer than globular portion; entire length about 1.5 cm. Anterior surface somewhat flattened with one large, centrally located, common atrial orifice; around this usually three to six branchial orifices; branchial orifices 6-lobed; common atrial orifice indistinctly 12-lobed. Colony colorless and transparent, making zooids distinctly visible through test; test tough on outer surface and only thin partitions between zooids within (pl. 39, fig. 9).

Zooids.—Occupying greater part of globular portion of colony, though unusually contracted in preserved specimens. Branchial sacs often broken loose at orifice and forced out through opening by shrinkage of colony. Thorax, abdomen, and postabdomen each about 3 mm. long. Mantle with about twelve wide, longitudinal muscle bands on a side, these separated by spaces several times wider than bands; bands running together just below branchial sac; lodged in a depression on ventral side of zooid between arms of intestinal loop, and continuing posteriorly into peduncle, where they disappear in a large lobe of granular material, probably accumulated food (m.b., f.m., pl. 45, fig. 62).

Branchial system.—Branchial siphon indistinctly 6-lobed; atrial with flat, truncated languet having usually three or four lobes. Branchial sac with eleven series of long, narrow stigmata, about twenty in half-series; most posterior series having longer stigmata than others; stigmata of last series gradually shorter toward endostyle; in younger zooids number of series less than eleven, stigmata at the ends of a half-series small, oval, gradually becoming longer toward middle of series. Dorsal languets long and flat, with broad flat bases which merge into membrane of transverse vessels upon which
they are situated; as long as the stigmata in anterior part of sac, and increasing in length toward posterior end. Endostyle much convoluted, doubtless due to shrinkage. About twenty closely set, branchial tentacles of three sizes.

Digestive system.—Esophagus emerges from dorsal posterior part of branchial sac and makes a short curve to enter large, globular, smooth-walled stomach. Stomach with long axis almost horizontal, asymmetrical by entrance of esophagus on median line of under side. Plane of intestinal loop placed nearly at a right angle to sagittal plane of zooid; anus about halfway up branchial sac (pl. 45, fig. 62).

Reproductive system.—Ovary and testis closely associated; mostly on dorsal side of intestinal loop when immature, but extending behind it in maturity. Testicular lobes numerous and surrounding the comparatively few eggs located in anterior part of gonad. Tadpoles found in atrial cavity in July.

Habitat and distribution.—Known only from La Jolla, where it occurs on rocky shores between tides. Only one lot of specimens has so far been found. The almost glass-like transparency of test of animal in life has suggested the specific name. For remarks on the systematic position of this and the preceding species, see under *M. par-fustis.*

Amaroucium californicum, n. sp.

Pl. 46, fig. 72

This species has been referred to several times in papers by the senior author as a MS. species by Ritter; but in the absence of a diagnosis or a figure hitherto published, from now on it should be assigned to Ritter and Forsyth.

Superficial characteristics of the colony.—Exceedingly irregular, usually encrusting, variable in the thickness even in same colony; frequently lobulated, in some cases pedunculated. Very variable in size, often reaching an expanse of from 10 to 20 cm.; thickness varying from .5 to 2 or 3 cm., even in same colony. Never encrusted with sand, but sand sometimes scattered through deeper portions. Color various, from opalescent white to reddish brown.

Zooids.—Usually very numerous; frequently, though by no means invariably, distinctly visible through test, both in living and preserved colonies. Systems sometimes present, distinct; sometimes absent, the same colony at times showing both conditions. In an encrusting colony from San Diego jetty, the systems very plainly oval when zooids were few in number but became elongated as number of zooids increased. These zooids were orange-colored especially anteriorly; buried in the clear, colorless, transparent test, systems separated on the surface by whitish, almost opaque, elevated ridges. In another colony devoid of systems, the individuals were light brown, set in the still lighter, opalescent, gelatinous test. Still another colony was opalescent white, individual zooids opaque white. Rarely large, not often exceeding 6 mm. in length, usually shorter, only a few zooids observed whose long, slender postabdomens brought them near to 1 cm.
in length; consisting of thorax, abdomen, and postabdomen, marked off from each other by slight constrictions; thorax and abdomen usually about same length. Musculature consisting of about fifteen longitudinal muscle bands on a side, extending into postabdomen where they are closer together and stronger; usually considerable pigment in mantle.

**Branchial system.**—Branchial orifice with six well-developed lobes; atrial without lobes, but having a long, tapering, pointed langet. Branchial sacs with eight to fourteen series of stigmata, ten seemingly most common; about twelve stigmata in a half-series. Endostyle narrow and straight. Dorsal languets, one to each transverse vessel, placed to left of a clear strip of sac free from stigmata; about as long as stigmata and gradually increasing in length toward posterior end of series. Branchial tentacles short, blunt, about ten, somewhat irregularly scattered.

**Digestive system.**—Plane of intestinal loop transverse to median plane of zooid; esophagus large and almost twice the length of stomach; stomach cylindrical, about as wide as long, with longitudinal folds each presenting a loop turned toward anterior end of stomach; folds sometimes branching or discontinuous, making the number variable for different colonies, twenty-two and fourteen being the average for a large number of zooids. Intestine divided into three divisions; anus not quite halfway up branchial sac.

**Postabdomen and reproductive system.**—Varying greatly in length, short in young zooids. Testicular lobes (t., pl. 46, fig. 72) in two rows, right and left. Ovary situated a short distance behind intestinal loop just anterior to testicular lobes, much less voluminous than the latter. Larvae retained in atrial chamber till a late stage of development.

**Breeding season.**—June and July, at least.

**Habitat and distribution.**—A. *californicum* is probably the most common ascidian on the California coast. It occurs on rocks, usually on the under sides, at all points where collecting has been done, and is found everywhere on piles, floats, breakwaters, etc., except in the upper portions of bays where the density and temperature of the water are distinctly different from normal sea water. Dredgings do not indicate that it flourishes in depths beyond a very few fathoms. It also occurs at Puget Sound (Ritter, 1900), and, with some doubt, on the Alaskan coast as far to the north and west as the Shumagin Islands (Ritter, 1901).

**Type locality.**—San Diego.

Although *A. californicum* possesses no strikingly distinctive characteristics, is very variable and has a wide distribution, it cannot be identified with any hitherto described species. Its closest relative appears to be *A. glabrum* Verrill of the north Atlantic coast of North America. The detailed examination of *A. glabrum* and also of the allied species *A. pellucidum* by Van Name (1910) enables us to make a pretty satisfactory comparison of *californicum* with these species.

To the general form of the colony of *californicum*, the reverse of
the statement made by Van Name for glabrum may be applied. Concerning the Atlantic species he writes: "The tendency to irregular and individual variation in the shape of the colony is considerable, but nearly all the shapes are readily recognized as modifications of the capitate form above described." For californicum we might transcribe this sentence, but with the word "capitate" changed to "encrusting." Undoubtedly the typical colony of californicum is flat and cake-like, but deviations from this in various directions are numerous.

As to the zooids, the following differences seem to be differential: the average number of stigmata, twelve, in a half-series in californicum is so much fewer than the number, eighteen, given for glabrum, as to leave no doubt about the distinctness in this point. The number of folds in the stomach wall is also undoubtedly sharply different in the two species. As our table 3 shows, the folds of the californicum stomach vary widely in number, the lower limit being fourteen and the upper limit twenty-two; and a cursory inspection of the table reveals that the prevalent number is much nearer the upper than the lower limit. Concerning the folds of glabrum, Van Name says: "The stomach has as a rule a rather small number, 12 to 15, of deep longitudinal folds; but in some colonies the number averages higher, about 18 or 20 or even more." An exhaustive statistical comparison of the two species would be interesting, but there is little question as to what, in general, the result would be. Further, the sharp division of the loop of the intestine into a portion of large and smaller diameter found in californicum must be wanting or nearly so in glabrum, judging from Van Name's figure. There are probably minor differences in the lobings of the branchial and atrial orifices, but this point requires further examination.

**TABLE III**

**Folds in the Stomach Wall of Different Zooids of the Same Colony of Amaronium californicum**

<table>
<thead>
<tr>
<th>Colony</th>
<th>Folds</th>
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<tr>
<td></td>
<td>16-21-22-21-23-22-22-21-16-20-16-19</td>
</tr>
<tr>
<td>4. Santa Monica, 2d colony.</td>
<td>14-12-12-12-12-14-12-12-14-13-14-12</td>
</tr>
</tbody>
</table>
The data contained in the above table are presented as a little study on the problem of variation of organisms produced monogenetically. The two facts revealed by them are sufficiently significant to warrant their publication, indicating as they do the desirability of subjecting the point to a special investigation. First there is a considerable range of variation among the zooids in a colony, the extremes being 16 and 23 in colony number 2. This result is not in harmony with the widely held notion that variation does not take place to any extent among individuals produced asexually from the same parent. The second notable fact is that some of the colonies, especially number 4, have a very distinct mode of their own. The question as to whether we have here a case of what may be called somatic heredity obtrudes itself and invites experimental study.

**Amaroucium solidum, n. sp.**

Pl. 46, figs. 69 and 70

*Superficial characteristics of the colony.*—Large, fleshy, potato-shaped lobes; young colonies more globular; largest colony studied, length 16 cm., width 7 cm., thickness 3.5 cm. Some living colonies opalescent white, the zooids showing as opaque white objects; others opalescent with a yellowish blue tinge, the zooids distinctly yellow. Zooids very numerous, standing at various angles in colony. Systems seemingly never present. Test gelatinous with many variously shaped pigment grains; surface layer tougher than underlying portion.

*Zooids.*—A little pressure on a living colony forces zooids out upon surface; easily removed from test in preserved specimens; varying greatly in length, some very long, extending well back into central gelatinous core; average length in large colony, thorax 3 mm., abdomen 2 mm., postabdomen 8 mm. Mantle delicate and transparent, containing about twenty-four longitudinal muscle bands on a side, these separated by considerable spaces in the thorax but closer together in the abdomen and postabdomen; a few transverse fibers in the siphonal region.

*Branchial system.*—Branchial siphon with six blunt lobes grooved so as to appear twelve when viewed from above; atrial siphon with short, overhanging, triangular langet and five small, pointed lobes. Branchial sac with from thirteen to fifteen series of stigmata, each series having about fifteen stigmata on a side in middle of sac (pl. 46, fig. 69). Endostyle wide and straight, with a narrow space on each side free from stigmata. Branchial tentacles about twenty, of varying sizes.

*Digestive system.*—Plane of intestinal loop, transverse; esophagus almost as long as stomach; stomach cylindrical, one and one-half times longer than wide, having about eight folds, these sometimes discontinuous (s., fig. 69); intestine divided into several parts (pl. 46, fig. 70); first, a piece a little shorter than stomach with a bulge about mid-
way of its length; second, a larger section which makes the loop; third, a short, very narrow isthmus connecting the large piece just described with rectal limb, the beginning of which is provided with prominent caeca; and finally, the rectal piece running up left side of body to end as a constricted anus about one-third the distance up branchial sac.

Postabdomen and reproductive system.—The postabdomen variable in length, often three or four times as long as thorax; about half the diameter of thorax, tapering toward posterior end. The conspicuous cloison, or partition, halves the cavity of postabdomen from right to left; many round pigment granules occurring in walls of this partition. Numerous regular testis lobes occupy posterior half of postabdomen. The ovary (a., pl. 46, fig. 69) just anterior to the latter, quite remote from intestinal loop.

Breeding season.—Summer months at least, the atrial chambers of zooids examined at this time being filled with tadpoles.

Habitat and distribution.—The only localities from which specimens have been certainly identified as belonging to A. solidum are San Diego Bay and Santa Monica, where they were taken from piles in both localities. Almost certainly, however, the species occurs in Monterey Bay and other northerly points.

Type locality.—San Diego Bay.

The great variability in the colonies of A. californicum makes the species seem to include solidum so far as external appearance is concerned, and since the two overlap a good deal in distribution, if indeed they are not coincident, the collector is likely to confuse them at times. In general, however, the much greater massiveness of solidum will distinguish them even to cursory observation. But examination of the zooids leaves no question about the specific distinctness of the two. Perhaps the most accessible point of distinction between the zooids of the two is in the secondary lobing of the lobes of the branchial siphon of A. solidum and the teeth around the atrial orifice in the same species.

A. solidum has much in common with A. obesum Sluit. (Sluiter, 1900) from the Chatham Islands. But the two are well distinguished, seemingly, by a number of characteristics, notably the undivided branchial lobes and the absence of denticles around the atrial orifice in obesum. In fact, the two positive characteristics in solidum are very exceptional in the genus Amaroucium.

Amaroucium aequali-siphonis, n. sp.

Pl. 38, fig. 4; pl. 45, fig. 61

Superficial characteristics of the colony.—Consisting of long, pedunculated, club-shaped lobes, completely encrusted with sand, each growing from a basal network. Both head and peduncle of each lobe
usually flattened throughout its entire length; heads not sharply set off, but tapering gradually into peduncle which becomes gradually smaller until attached end is reached. New lobes spring at times from substratum and result in a dense growth of more or less parallel, slender lobes (pl. 38, fig. 4). Sometimes many lobes arise from a common center, resulting in a more or less spherical body whose surface is made by the heads of the lobes. In this form of growth the heads of the lobes have a broader anterior expanse than in the form first described; the lobes themselves sometimes branched. Test gelatinous and transparent, but having a dirty tinge due to innumerable small test cells. No common cloacal orifices present. Length of longest lobes about 2 cm., width of heads about 7 mm., thickness 3 mm.

Zooids (pl. 45, fig. 61).—Not arranged in systems; both siphons opening on surface of distal ends of lobes of colony; long and slender, the postabdomen extending through almost entire length of peduncle to end in a slight swelling which contains heart; abdomen somewhat longer than thorax. Mantle contains longitudinal muscle bands which run length of zooid and are separated by considerable spaces over thorax and abdomen, but are closer together in postabdomen.

Branchial system.—Both orifices distinctly 6-lobed. Branchial tentacles about twenty, alternating long and short. Branchial sac with eight series of stigmata, about fifteen stigmata in a half-series. Dorsal languets about as long as stigmata (pl. 45, fig. 61).

Digestive system.—Intestinal loop somewhat longer than branchial sac, its plane oblique to sagittal plane of zooid; esophagus about equal in length to stomach, tapering to a small diameter at its entrance into stomach; stomach somewhat longer than broad, wall longitudinally folded, the folds, never more than six or seven, sometimes broken, often one whole side practically foldless; portion of intestine between stomach and loop about twice as long as stomach, constricted about midway in its course; a small, short tube intercalated into intestine at beginning of rectal limb; lobed anus located a little anterior to middle of branchial sac (a., pl. 45, fig. 61).

Reproductive system.—Ovary posterior to intestinal loop, not in contact with it; testis lobes numerous, beginning just posterior to ovary and extending through entire postabdomen (a., t., pl. 45, fig. 61).

Habitat and distribution.—So far the species has been taken only at Rincon Point, Santa Barbara, California. It belongs to the littoral zone alone, so far as we know.

In spite of the violence done to the genus Amaroueium by forcing this species into it, after much deliberation we have decided that for the present, at least, to do so is justifiable. As will be seen from the description and figures, the animal is a perfectly typical member of the genus in every respect except possibly the character of the colony and certainly the form of the atrial orifices of the zooids. The pedunculation and lobulation, and the sand-encrustation of the colony, though not altogether typical are by no means unique, the type species
proliferum M. Edw. of Amaranicum being, as is well known, pedunculated. And numerous species are more or less sand covered.

The serious difficulty is in the entire absence of an atrial languet and the regular 6-lobing of the orifice. But although the possession of a languet is rightly regarded by most authors as one of the best generic characters of Amaranicum, a comprehensive review of the genus reveals the fact that while in the great majority of the species the languet is distinct and large, a fairly complete series from the langueted orifice to the regularly lobed type like that in our animal, exists.

A. complanatum, Herdm., and A. pallidum, Herdm. are described by their author (Herdman, 1891) as having the atrial languet short and inconspicuous. In A. pribilovense Ritt. (Ritter, 1899), the languet is "very variable in length, in some zooids the siphon departing but slightly from the normal 6-lobed condition." And finally in A. anomalum Herdm., the atrial siphon is wholly absent, judging from the author's figure; the point is not mentioned in the text (Herdman, 1899, pl. Pel III, fig. 14).

Nor could one expect otherwise than that a range of variety like this would occur in a genus of many species when he considers the abundant evidence that the atrial languet is, phylogenetically speaking, a late differentiation from the normal, evenly bordered siphon. On the whole, therefore, we have thought it more warrantable to place the species in this genus than to adopt the next best alternative—that of establishing a new genus—a course which may be necessary at some future time when a study shall have been made of the kindred animals from the whole California coast. We have observed several other closely similar ascidians from points north of Point Conception, and an exhaustive study of all these may necessitate a change in the present disposition of the species now under consideration.

In concluding, for the present, these remarks on the taxonomy of this species, we would point out that were the genus Sigillina to stand substantially as described by Savigny, these California species would probably accommodate themselves more easily to it than to any other of the numerous small off-side genera of the family Synoicidae (Polyclinidae). If, however, Sigillina really belongs to the Polyclinidae (Distomidae) as is now held to be the case, there would be no possibility of placing our species in this genus, for its polyclinid characteristics are unequivocal.
Euherdmania claviformis (Ritt.)

Herdmania claviformis, Ritter, 1903, p. 237.
Euherdmania claviformis, Ritter, 1904, p. 650.
Euherdmania claviformis, Hartmeyer, 1909, p. 1470.

This is one of the abundant ascidians on the southern as well as on the central and northern California coast. In the vicinity of La Jolla, for example, it is found in great abundance on the under side of rocks at low tide. Apparently it is confined to the littoral zone.

On the whole the zooids are somewhat smaller in southern than in northern colonies.
BIBLIOGRAPHY

BANCROFT, F. W.

CAULLERY, MAURICE.

DALL, W. H.
1871. Description of sixty new forms of mollusks from the west coast of North America and the north Pacific Ocean, with notes on others already described. Amer. Jour. ConchoL, 7, 93.

DAUMÉZON, GEORGES.

VON DRASCHE, R.

FEWKES, J. W.

HARTMEYER, R.

HERDMAN, W. A.

HUNTSMAN, A. G.

MICHAELSEN, W.

PIZON, A.
RITTER, Wm. E.
1903. The structure and affinities of Herdmania claviformis, the type of a new genus and family of ascidians from the coast of California. Mark Memorial Volume, 12, 237-261.

ROULE, Louis.

SALENSKY, W.

SAVIGNY, J. S.
1816. Mémoire sur les animaux sans vertèbres, 2me. partie.

Sluiter, C. P.

STIMPSON, WM.

VAN NAME, W. G.

VERBIL, A. E.
EXPLANATION OF PLATES

PLATE 38

Fig. 1. *Styela montereyensis*. Whole animal. Natural size.

Fig. 2. *Styela barnharti*. Whole animal, a little larger than natural size.

Fig. 3. Colonies of *Macroclinum par-fustis*, joined by basal stolons of test. Natural size.

Fig. 4. Colonies of *Amarouciun aequali-siphonis* joined by basal stolons of test. About twice natural size.

Fig. 5. *Molgula verrucifera*. Three animals. × 2.

Fig. 6. *Ascidia californica*. Whole animal. Natural size.

Fig. 7. *Polyzoa translucida*. Zooids joined by basal stolonic network and growing among the branches of a young hydroid. Natural size.

Fig. 8. *Metandrocarpa michaelseni*. Zooids connected by trails of test. Natural size.

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Fig. 9. Colony of *Macroclinum pellucidum* with zooids seen through transparent test. × 6.

Fig. 10. Small portion of a colony of *Botryllus tuberatus* showing a single system of zooids, two tuberlike masses of ectodermal ampullae, c.a., and ectodermal vessels. × 8.

Fig. 11. Colony of *Didemnum carnulentum*. × 2.

Fig. 12. A single system of *Botryllus tuberatus*, showing character of the common cloacal orifice. The lower lip of atrial siphon extends beyond edge of common orifice. × 8.

Fig. 13. Colony of *Glossophorum planum*. Natural size.

Fig. 14. Three zooids of *Metandrocarpa michaelseni* showing trails of test and ectodermal processes.
Fig. 15-20, *Molgula verrucifera.*

Fig. 15. Animal viewed from left side, test removed. $\times 4\frac{1}{2}$.

Fig. 16. Portion of branchial sae showing three folds.

Fig. 17. Tip of atrial siphon showing processes.

Fig. 18. Tip of branchial siphon showing processes.

Fig. 19. Branchial tentacles.

Fig. 20. Hypophysis and ganglion.

Fig. 21 *Diplosoma caulleryi.* Zooid viewed from right side.

Fig. 22 *Botryllus tuberatus.* Zooid enlarged, view from left side especially to show the snout-like atrial siphon, a.s.

Fig. 23 *Didemnum carneulentum, var. lacteolum.* Zooid much enlarged, viewed from left side.

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Fig. 24. Animal viewed from left side, test removed. Natural size.

Fig. 25. A portion of tentacular cirelet and hypophyseal region seen from within.

Fig. 26. Stomach and intestine viewed from right side to show ramifications of ovary and testis.

Fig. 27. Two lobes of atrial siphon showing the bordering teeth and pigment spots.

Figs. 28-34, *Styela montereyensis*.

Fig. 28. Animal, with test removed, viewed from the right side, showing position of the reproductive organs. Natural size.

Fig. 29. Same viewed from left side.

Fig. 30. A portion of the tentacular cirelet and hypophyseal region seen from within.

Fig. 31. Anterior portion of ovary, viewed from the inner surface.

Fig. 32. Atrial siphon cut so as to expose part of velum with atrial branches.

Fig. 33. Spinule

Fig. 34. Spinule.

Fig. 35. *Styela yakutatensis*. Spinule.
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Figs. 36-38, Polyzoa transiucida. Animal much enlarged

Fig. 36. Viewed from left side with test removed.
Fig. 37. Same viewed from right side.
Fig. 38. Hermaphroditic gonads.

Figs. 39 and 40, Styela barnharti.

Fig. 39. Animal with test removed, viewed from the right side and with muscle bands of mantle indicated only in siphonal regions. Natural size.
Fig. 40. Same viewed from left side, with muscle bands of mantle indicated only in siphonal regions. Natural size.

Figs. 41-45, Metandrocarpa michaelsemi.

Fig. 41. Animal much enlarged, viewed from left (lower) side, test removed.
Fig. 42. Same viewed from right (upper) side.
Fig. 43. Branchial tentacles, hypophysis, ganglion and gland viewed from inside.
Fig. 44. Atrial tentacles.
Fig. 45. Testis masses attached to mantle.
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Figs. 46-49, *Botrylloides diegensis*.

Fig. 46. Zooid much enlarged, viewed from left side.

Fig. 47. Same viewed from dorsal side.

Fig. 48. Branchial tentacles, hypophysis, ganglion and gland.

Fig. 49. Colony of *Botrylloides diegensis*. Natural size.

Figs. 50 and 51, *Diplosoma pizoni*.

Fig. 50. Zooid much enlarged, viewed from right side.

Fig. 51. Portion of pigmented epithelium of the mantle which covers the stomach.
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Figs. 52 and 53, Eudistoma psammion.

Fig. 52. Zooid much enlarged, viewed from dorsal side.
Fig. 53. Branchial sac showing elongated atrial siphon.

Figs. 54–56, Trididemnum della vallei.

Fig. 54. Zooid much enlarged, viewed from right side.
Fig. 55. Branchial tentacles.
Fig. 56. Spicule.

Figs. 57–59, Didemnum carnulentum.

Fig. 57. Zooid much enlarged, viewed from left side.
Fig. 58. Branchial tentacles.
Fig. 59. Spicule.
Fig. 60. Didemnum carnulentum, var. lacteolum. Spicule.
Fig. 61. *Amaroucium aequale-siphonis*. Zooid much enlarged, viewed from right side.

Fig. 62. *Macroclinum pellucidum*. Zooid much enlarged, viewed from left side.

Fig. 63. *Macroclinum par-fustis*. Zooid much enlarged, viewed from left side.

Fig. 64 and 65, *Distaplia occidentalis*

Fig. 64. Zooid, much enlarged, right side.

Fig. 65. Anterior end of zooid, seen from within.

Figs. 66-68, *Diplosoma pizoni*.

Figs. 66-67. Zooid, showing stage in budding.

Fig. 68. Digestive system with pigmented epithelium removed and spread out to show parts.
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Figs. 69 and 70, *Amaroucium solidum*.

Fig. 69. Zooid much enlarged, viewed from right side.

Fig. 70. Stomach and portion of intestine.

Fig. 71. *Glossophorum planum*. Zooid much enlarged, viewed from right side.

Fig. 72. *Amaroucium californicum*. Zooid much enlarged, viewed from right side.
ABBREVIATIONS

a.—anus.
a. l.—atrial languet.
a. o.—atrial orifice.
a. s.—atrial siphon.
a. t.—atrial tentacles.
b. h.—budded branchial sac.
b. f.—branchial folds.
b. o.—budded intestine.
br. a.—branchial orifice.
br. s.—branchial sac.
b. s.—branchial siphon.
c.—caecum.
d. i.—Degenerating piece of intestine.
d. l.—dorsal lamina.
d. la.—dorsal languet.
e.—esophagus.
e. c.—esophageal collar.
e. a.—ectodermal ampullae.
e. n.—endocarp.
e. n.—endostyle.
e. p.—ectodermal process.
f. m.—food mass.
f. p.—fecal pellets.
g.—gland.
g. g.—ganglion gland.
gl.—ganglion.
h.—heart.
hv.—hypophysis.
i. l. v.—internal longitudinal vessel.
inf.—infundibulum of branchial sac.
int.—intestine.
i. p.—incubatory pouch.
i. t.—island of test.
l.—liver.
m.—mantle.
m. b.—muscle band.
m. i.—mother intestine.
m. s.—mother branchial sac.
v.—ovary.
v'.—rudimentary ovary.
ov.—ovum.
ov. d.—oviduct.
p. g.—peripharyngeal groove.
p. gl.—pyloric gland.
p. s.—pigment spot.
p. t. v.—primary transverse vessel.
r.—renal organ.
s.—stomach.
sp.—spinule.
s. t. v.—secondary transverse vessel.
t.—testis.
t. t.—trail of test.
tst.—test.
v.—velum.
v. d.—vas deferens.
y. z.—young zooid.
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