UNITED STATES DEPARTMENT OF THE INTERIOR, J. A. KRUG, *Secretary*
FISH AND WILDLIFE SERVICE, ALBERT M. DAY, *Director*

STUDIES OF THE PROTOZOAN PARASITES OF FRESH-WATER FISHES

By H. S. DAVIS, Ph. D.

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Studies of the Protozoan Parasites of Fresh-Water Fishes[1]

FOR A NUMBER OF YEARS the author has devoted considerable attention to the protozoan parasites of our fresh-water fishes. During the course of these studies it has become apparent that such parasites are more common than was realized and that they include organisms with a wide diversity of form and habits. In fact, all of the major groups of Protozoa are represented although, exclusive of the Myxosporidia which are typically fish parasites, the most common forms belong to a few families of flagellates and ciliates. As might be expected, the degree of parasitism shown by the various forms differs greatly. Some are evidently more or less accidental associates of the fish which may provide support or protection although some inanimate object would probably serve just as well. None of these forms, however, are considered here. Others have become more or less dependent on the fish, but the degree to which they may injure the host varies widely. Some only use the fish for support and protection, while their food is derived exclusively from other sources. Others, even among the external parasites, obtain their nutriment exclusively from the host. Between these two extremes there are many intermediate forms which derive their nourishment from both the host and external sources.

Several species observed during the course of these studies are not included, as the information available is too incomplete to be of value. The species discussed are grouped by orders and families, but further than that, no systematic arrangement has been attempted.

[1] Approved for publication Aug. 6, 1946. Fishery Bulletin 41.

METHODS

In most cases the organisms were studied in both the living and preserved condition. In studying living forms the external parasites were removed directly to the slide and examined in water. Preserved specimens were studied as whole mounts and in sections. For the former, wet smears were prepared by spreading a little of the material on a coverslip which was immediately exposed to osmic vapor for 15 seconds. Then the coverslip was floated face down on a small amount of fixing fluid in a watch glass for about 30 minutes. The fixing fluid was a modification of Worcester's formol-mercuric-acetic mixture prepared as follows:

Saturated solution mercuric chloride	75 cc.
Formalin (formaldehyde 40 percent)	20 cc.
Glacial acetic acid	5 cc.

This fixing fluid has been used almost exclusively and for general use with protozoans is believed to give as good results as any fluid yet devised. Material for sectioning was also fixed in this fluid.

For staining both whole mounts and sections, chief reliance was placed on Heidenhain's iron hematoxylin and Ehrlich's acid hematoxylin using eosin and Bordeaux red as counter stains. A combination of the iron hematoxylin and Ehrlich's hematoxylin techniques was found to be especially valuable for staining trichodinids. For this purpose Ehrlich's hematoxylin solution was substituted for the 0.5 percent solution used in Heidenhain's method. Otherwise, the procedure was the same. When Bordeaux red is used as a counter stain, this method also brings out cilia and flagella very clearly.

Order PROTOMONADINA Blochmann

Family **Bodonidae** Butschli

Colponema agitans n. sp.

[Plate 1, figs. 1–5; plate 8, figs. 88–89]

In the spring of 1942 fingerling crappies (*Pomoxis annularis* and *P. sparoides*) in several ponds at the Fish and Wildlife Service station, Kearneysville, W. Va., were found to be infested with four species of flagellates, all undescribed. The parasites were confined to the gills and sometimes were present in great numbers, although fish from the same pond differed greatly in this respect. In some instances all four species were present, but more often only two or three species were found on the same host. Fish with a severe infection were noticeably emaciated, and smaller and weaker than those less heavily parasitized.

Colponema agitans was the smallest of the flagellates on the gills of the crappies and it also occurred in the greatest numbers. It has also been found on the gills of the bluegill sunfish (*Lepomis macrochirus*). While ordinarily most numerous on the filaments, in several fish it was fully as abundant on the gill rakers, and sometimes occurred, in groups of several hundred individuals, free in the mucus covering the gills. Superficially, it resembles the common fish parasite, *Costia necatrix* and could easily be mistaken for this organism on cursory examination. With our limited knowledge of the Bodonidae and incomplete descriptions of many species, it is difficult to determine the affinities of this organism, but owing to the presence of a wide ventral groove and the arrangement of the flagella, it is placed in the genus *Colponema*, at least, provisionally.

The body of the free-swimming flagellate is pyriform to oval in shape, although it is very plastic and capable of rapid changes in form. When attached to the gills, it often appears triangular (figs. 88 and 89) with the free end forming the base of the triangle. The anterior end is flattened and rounded with a wide, shallow groove which takes a spiral course to the middle of the body (figs. 1–4) where it may lead into a cytostome, although this could not be determined with certainty. There are two flagella, the anterior being about one-half as long as the posterior flagellum. The latter serves as a hold-fast organ to attach the parasite to the gills. Several small refringent granules can usually be seen in the living organism The body is covered with a thin pellicle and in the living organism is 6–8μ long and 3–4.5μ wide. The posterior flagellum is about 10μ in length.

When fixed and stained with iron hematoxylin, the most conspicuous structure is the deeply stained blepharoplast which is usually in the form of a curved rod (figs. 2–5), but rarely may be oval in shape. Owing to the small size of the organism it was difficult to trace the attachment of the flagella to the blepharoplast, but in several instances they appeared to arise from opposite ends of this structure. The position of the blepharoplast in the body showed considerable variation. Usually it was located near the anterior end (figs. 3 and 5), but sometimes was in the middle of the body. The large vesicular nucleus is located in the posterior half of the body and usually contains a rounded karyosome with a layer of chromatin granules attached to the nuclear membrane. In addition to the nucleus and blepharoplast, there may be one or two relatively large, deeply stained granules in the endoplasm.

When an infected gill is placed on a slide, the parasite immediately attempts to free itself by very active, jerky movements which are in striking contrast with those of the other flagellates present. Once free, it swims about rapidly with quick, darting movements.

Bodomonas concava n. g., n. sp.

[Plate 1, figs. 6–14; plate 8, fig. 87]

This flagellate was found on the gills of the crappies (*Pomoxis annularis* and *P. sparoides*) in company with *Colponema agitans*, but differs from the latter species in several important respects. It also occurred in small numbers on the gills of the bluegill sunfish (*Lepomis macrochirus*). This species differs so greatly from other members of the family that it has been necessary to propose a new genus.

The organism is elongate, tapering to a point at the posterior end, while the anterior end is broad, flattened and slightly rounded. One side of the body, especially at the anterior end, is much thinner than the other, thus forming a broad, shallow groove which fades out posteriorly (figs. 11, 13, and 14). When attached to the gills the broad, free anterior end is frequently curved toward the ventral side so that the animal is roughly spoon-shaped (figs. 7 and 10). A clear area can be distinguished in the middle of the anterior end which is probably the

cytopharynx. Two flagella arise from the anterior end, one, which is about as long as the body, projects anteriorly and is usually motionless but may move slowly about like a whiplash. When motionless the free end of the flagellum is frequently curved like a hook (fig. 12). The other flagellum is considerably longer and extends posteriorly. It serves as a holdfast organ to anchor the parasite firmly to the gill epithelium. This flagellum is invisible when the parasite is attached and consequently the organism appears to be anchored to the gills by the pointed posterior end. There is a small contractile vacuole near the anterior end and scattered through the endoplasm are the usual refringent granules or fat globules.

The nucleus and blepharoplast are invisible in the living animal, but can be distinguished easily after staining. The nucleus is located near the middle of the body and contains coarse granules of chromatin attached to the nuclear membrane. Sometimes a karysome can be distinguished. The blepharoplast forms a circular or oval disk-shaped body anterior to the nucleus. It usually stains deeply and uniformly, but occasionally takes only a grayish stain in iron hematoxylin. As mentioned above, one side of the body is much thicker than the other and the nucleus is always located in the thicker side. The blepharoplast, however, may extend into the thinner expanded anterior end.

The length of the organism, exclusive of the flagella is about 12 μ and the width 4.5–5μ.

Unlike the other flagellates with which it was associated, *B. concava* was most numerous at the ends of the filaments and when the gills are first removed to a slide are usually motionless. Later, while still attached, they begin to free themselves by violent wriggling movements and contortions of the body which may give them a very different appearance. Once free from the epithelium, they progress by rapid undulatory movements of the body, resulting in a slow forward motion in which the flagella appear to have no part. The anterior flagellum waves about aimlessly through the water, while the posterior flagelleum is stretched out straight behind.

Family **Trypanosomatidae** Doflein

Lamellasoma bacillaria n. g., n. sp.

[Plate 2, figs. 15–18; plate 8, figs. 90–96]

This remarkable organism was found in company with the two preceding species of flagellates on the gills of fingerling crappies (*Pomoxis annularis* and *P. sparoides*). It occurred on a large percentage of the fish examined and in some instances was the most abundant parasite present.

This parasite closely resembles the blood trypanosomes in shape. The body is flattened and ribbon-like with a bluntly rounded anterior end. It is wider near the middle of the body and then tapers gradually toward the posterior end from which projects a long flagellum. The flagellum is shaped like a whiplash, thicker at the base and tapering gradually toward the tip. The most striking characteristic of the organism is the presence of several rows of small refringent rods running lengthwise of the body (fig. 15). At the anterior end the rods are arranged at an angle to the long axis of the body. There is also a small contractile vacuole in this region. The extreme anterior end is free of rods, very transparent and usually in active movement, changing shape rapidly. Two individuals were observed without the characteristic rods, but these were the only ones out of hundreds examined. The length of the body was about 15–20μ with a width of 3–4μ. The flagellum is considerably longer than the body, reaching a length of 30μ.

The nucleus, blepharoplast and rods stain deeply with iron hematoxylin while the rest of the organism is colorless or only lightly stained. The remarkable arrangement of the rods then becomes clear. They are closely attached to the body throughout their entire length and usually appear to be at least partially embedded in the ectoplasm. However, owing to the small size of the parasite, this is difficult to determine with certainty. In sectioned material the rod may be wholly or partially dislodged from the body. They are ordinarily present on both sides of the body, although they have a more uniform arrangement on one side where they always occur in parallel rows arranged end to end as shown in the figures. Six to eight rows can usually be distinguished in the middle of the body, although in small individuals the number may be less. On the opposite side the rods not only form a less regular pattern, but are fewer in number and sometimes may be absent altogether. These rods are probably bacteria, but how they become arranged in such a remarkable manner is difficult to understand. Furthermore, they are more refractive than ordinary bacteria. Of course, bacteria are known to occur on the bodies of many protozoans, but such a regular arrangement is very exceptional, to say the least.

The nucleus is located about one-third the distance

from the anterior end. It is rounded or oval with several large masses of chromatin attached to the inner side of the membrane. A rounded karyosome may also be present. Anterior to the nucleus is a somewhat longer oval blepharoplast which stains even more intensely than the nucleus. In the region of the nucleus and blepharoplast the body is thicker than elsewhere, but becomes thinner toward the opposite edge where it is extended into a transparent membrane. This membrane is bordered by the flagellum which arises from the blepharoplast and extends along the membrane the entire length of the body.

When attached to the gills the parasite has a very different appearance than in the free-swimming condition described above. At this time it is saucer-shaped and closely applied to the rounded surface of an epithelial cell (figs. 91–93). When an infested gill is first mounted on a slide, the parasite appears as a granular, crescent-shaped body at the outer end of an epithelial cell. It is motionless at first and consequently may be easily overlooked. Sooner or later, however, it begins to squirm about, rapidly elongates and becomes free-swimming. In the attached stage the nucleus and blepharoeplast are close together at one side which is considerably thicker (fig. 92). The flagellum cannot be distinguished except in some instances near its attachment to the blepharoplast. The bacteria show the same linear pattern as in the expanded form, but the arrangement is less regular. There are always a considerable number of parasites that remain attached to the epithelial cells until they disintegrate which suggests the possibility that the organisms can become free-swimming only at a certain stage in the life cycle, but for the present this must remain pure conjecture. Reproduction has not been observed except possibly in one doubtful case which could be interpreted as fission in the attached stage.

In the free-swimming stage the organism moves slowly about with the flattened surface uppermost. It progresses by a slow undulatory movement somewhat resembling that of a leech, with the rounded anterior end foremost and the long flagellum trailing motionless in the rear.

Order EUGLENOIDINA Blochmann

Family **Euglenidae** Stein

Euglenosoma branchialis n. g., n. sp.

[Plate 2, figs. 19–23; plate 8, figs. 97 and 98]

This is the fourth species of flagellate found on the gills of the crappie (*Pomoxis annularis* and *P. sparoides*). Unfortunately, the parasite was not recognized at the time the fish were collected and, consequently, only preserved material in the form of wet smears and sections has been available for study. The organism was found on several fish, but was not abundant in any case and was greatly outnumbered by the other flagellates present. Later efforts to find this parasite have proved fruitless.

The body is distinctly of the euglenoid type, being long, spindle-shaped and tapering to a sharp point at the posterior end. Although covered with a thin transparent pellicle the body is very plastic and capable of great changes in shape. The anterior end is spatulate and apparently very flexible. The ventral side bears a groove which can be deepened by the sides being raised and folded inwards. This groove leads into the cytostome from which arise two flagella of approximately equal length (fig. 23). Neither flaggelum appears to extend in any particular direction. They are attached to a small, rounded blepharoplast located on the dorsal side of the cytopharynx. Anterior to the blepharoplast is a small, deeply stained granule which is probably the stigma. The ventral groove widens posterior to the cytostome and the entire body is twisted spirally (figs. 19 and 20). Sometimes this flattening and twisting of the body is quite pronounced, in other individuals it may be scarcely noticeable.

The internal structure is frequently difficult to make out, owing to the large amount of deeply-stained material. Except at the extreme anterior end, the endoplasm is strongly vacuolated and coarsely granular. In addition to the nucleus there are one or more rounded bodies composed of granules staining with iron hematoxylin embedded in an unstained matrix (fig. 23). It is possible that these are paramylum bodies. The oval nucleus is located near the middle of the body, and contains a large oval endosome and a layer of chromatin granules on the inner surface of the membrane (fig. 21). Occa-

sionally in wet smears the nucleus fails to stain with iron hematoxylin and has a uniform grayish color (fig. 23). The length of the fully extended organism ranges from 20 to 30μ with a width of 4–5μ.

When attached to the gills the animal has a very different appearance (fig. 22) and in fact, is scarcely recognizable as the same organism. It is contracted into a rounded disk, shaped like a lozenge. In some cases the anterior end can be distinguished at one side and the same internal structures are present, but with a different arrangement. In a few instances the organism was located in a shallow depression, but usually it appeared to lie in contact with the unmodified epithelium possibly held in place by the flagella.

The exact relation of this organism to the host is uncertain. When first noticed, it was thought that its presence was purely accidental, but after further study, it becme evident that it was as truly an inhabitant of the gills as the other flagellates. This is clearly indicated by the fact that it occurred in considerable numbers on one fish, while absent on others collected at the same time. The fact that it assumes a very different form when attached to the gills also lends strong support for the same conclusion. This, of course, does not necessarily mean that it is a true parasite, the evidence for which is inconclusive. There was no evidence of injury to the gills, but this could hardly be expected of an organism present in such small numbers. The same is true to a large extent of the other flagellate present, yet fish with a heavy infection were definitely weakened.

Order HOLOTRICHA Stein

Family **Amphileptidae** Schouteden

Amphileptus voracus n. sp.

[Plate 2, figs. 24–26; plate 8, figs. 99–108]

This interesting ciliate was found on the gills of fishes, from the Mississippi River at Fairport, Iowa, which had been sectioned for studies on Myxosporidia. The fishes infected included the carp sucker (*Carpiodes carpio*), the smallmouth buffalo (*Ictiobus bubalus*), and the channel catfish (*Ictalurus punctatus*) Later, the same organism was found on the gills of the pearl minnow (*Margariscus margarita*) at Kearneysville, W. Va. The ciliate was not common in any instance and the author's observations have been confined almost entirely to sectioned material. Only two or three living specimens have been seen and these died in a short time.

In general, this ciliate resembles a parasite, *Amphileptus branchiarum*, of the gills of tadpoles, which was described by Wenrich [2] (1924), but differs from it in several important respects. It is possible that the differences are sufficient to justify the erection of a new genus, but until our knowledge of these organisms is more extensive, it is believed that nothing would be gained by such action. While the ciliate may swim about freely, it appears that during most of its existence it is attached to the gills of some fish. It is always located in a depression in the gill epithelium which is evidently the result of its own activities. Sometimes the organisms may be almost entirely surrounded by epithelial cells (fig. 99), but frequently the depression is quite shallow (fig. 102). Ordinarily there is no covering over the open side of the concavity, but sometimes this may be closed by a thin membrane which appears to be continuous with the cuticle of the epithelial cells.

MORPHOLOGY

Amphileptus voracus is ovoid—sometimes nearly spherical—in shape and covered with a thin pellicle showing parallel striations formed by alternating ridges and furrows. It has not been possible to follow the exact course of these striations, but apparently they are transverse to the long axis over about two-thirds of the body and then turn abruptly at right angles to their former course. Rows of long delicate cilia arise from the furrows between the concentric ridges and each ridge contains a single row of trichocysts (figs. 106 and 108). There is no evidence of flattening on one side of the body as in other species of *Amphileptus*.

The mouth or cytostome is a long crescentic slit near the anterior end and extends about two-thirds the distance around the body. The posterior lip of the cytostome is formed by a ridge parallel with the other transverse ridges. This lip bears a row of long cilia and there is some evidence

[2] Publications referred to parenthetically by date are listed in Literature Cited.

that in the living animal they may be united to form a membranelle. The rounded anterior end of the body, forward of the mouth, is smooth without either ridges or cilia. In some individuals this region was cone-shaped rather than rounded. Posterior to the mouth the entire body is covered with concentric ridges and ciliated furrows which are progressively less pronounced toward the posterior end.

Long slender trichites extend into the body from the mouth (fig. 24), but no evidence of a cytopharynx could be distinguished. One or more large food vacuoles containing the partially digested remains of ingested protozoans, such as *Trichodina*, were often present. Rounded metaplastic bodies are usually distributed through the endoplasm and are sometimes so abundant as to hide most of the other structures. Two large oval to spherical macronuclei are always present. They are located in the posterior half of the body; are identical in size and appearance; and filled with fine chromatic granules. There is only one micronucleus which lies close to the end of one of the macronuclei (figs. 26 and 101). Frequently no micronucleus can be found and it is possible that in such cases it is so closely attached to the macronucleus as to be indistinguishable. The micronucleus has the usual structure, with a central spherical body or karyosome composed of fine chomatin granules embedded in a homogeneous matrix and surrounded by a clear zone between it and the nuclear membrane. There are a number of small contractile vacuoles near the surface of the body, each with a separate opening to the exterior.

There was great variation in the size of the organism. This was especially noticeable among individuals on the gills of the catfish, some being several times the size of others. Ten individuals in sections ranged from 22 by 25μ to 45 by 45μ, the majority being about 35 by 40μ.

REPRODUCTION

Amphileptus voracus multiplies by binary fission. The first stage in the process appears to be division of the micronucleus which is followed by division of the organism into two daughter cells of equal size. Each contains a macronucleus with a micronucleus at one end. The macronucleus, which is already noticeably elongated, then divides by amitosis (figs. 103 and 104). In this process a lighter zone appears in the middle of the macronucleus and gradually becomes more pronounced as the ends of the nucleus separate. Wenrich found that in *A. branchiarum* the organism is always enclosed in a capsule during fission. It has been impossible to determine if this is the case in *A. voracus*, but in any event it is evident that the daughter cells quickly separate after fission, as it is rare to find two individuals in close contact. No evidence of sexual reproduction was observed.

REMARKS

The relation of *Amphileptus voracus* to the host presents an interesting problem. As shown by Wenrich there is no doubt that *A. branchiarum* is a true parasite since it feeds on the cells of the host. In the present species, however, the situation is very different. While it is possible that the organism may at times feed on epithelial cells, there is no evidence that this is the case. On the contrary it appears that *A. voracus* ordinarily feeds on other protozoans. Whether these are always parasitic it is impossible to say, but there is apparently no reason why they may not prey on nonparasitic forms if they are available. It happens, however, that the gills of all fish harboring *A. voracus* were at the same time infected with one or more species of *Trichodina* or of *Chilodon*. When Trichodina were abundant, *A. voracus* was evidently preying on them to the exclusion of other organisms and a large proportion of those observed contained partially digested remains of trichodinids (fig. 100). Sometimes the diameter of the trichodinid was greater than that of *A. voracus*, indicating that the mouth must be capable of great distension. However, when *Chilodon* was the common parasite, individuals of *A. voracus* present preyed on them with equal unanimity. When this organism is present in any numbers, it must be highly beneficial to the host in freeing it from other protozoans so that the relationship appears to be a true case of commensalism.

Although *A. voracus* must injure the host to some extent in producing the cavity in which it lies, such injuries are slight and not to be compared with the benefits which the fish derives from the association. This cavity is apparently formed by pushing aside the epithelial cells rather than by destroying them. Here *A. voracus* lies in wait for any unwary protozoan that may come its way. How it is able to capture such a large and unwieldy organism as a trichodinid is not apparent. Possibly the prey is immobilized by toxic action of the trichocysts as maintained by some writers. There is also evidence that the posterior lip of the mouth is extensible and may be thrust out to enfold the prey.

Order PERITRICHA Stein

Family **Urceolariidae** Stein

Trichodina sp.

MORPHOLOGY

In addition to descriptions by earlier writers the structure of *T. renicola* has recently been described in some detail by Mueller (1932) and that of *T. spheroidesi* by Padnos and Nigrelli (1942). Nevertheless, in view of the great complexity of the structure of these organisms, which are among the most highly specialized of all parasitic protozoa, it is believed desirable to give a brief description of the anatomy of *Trichodina* in general. The following account is the result of studies on several species parasitic on fish and an attempt has been made to give a composite picture of the structure of trichodinids.

In general the body of a trichodinid is saucer- or bell-shaped, the convex side being ordinarily referred to as anterior and the concave side as posterior. However, these terms when applied to radially symmetrical animals such as the trichodinids are confusing and it is believed more logical to refer to the convex (anterior) surface as adoral, since it contains the opening of the cytopharynx, and to the concave (posterior) surface as aboral. In some species the adoral side may be only slightly elevated while in others it may be dome-shaped or even conical. Moreover, there is considerable variation in this respect among individuals of the same species. The aboral side forms a complicated attachment organ known as the adhesive disk (figs. 109–116). Some writers have used the term "sucking disk," but there is little evidence that the organ is capable of exerting suction and the term adhesive disk is believed to be more in accord with the facts. Encircling the body of the animal just above the adhesive disk is a band of long cilia, forming the ciliary girdle, which is the principal organ of locomotion.

The adhesive disk is the most prominent part of the organism and is composed of a very complicated skeletal structure arranged in the form of three concentric rings. The inner and most conspicuous is the denticulate ring or corona which is made up of a series of horny elements or denticles arranged like beads in a necklace (figs. 53, 54, and 125). Each denticle is shaped like a hollow cone with the smaller end of the cone inserted into the cavity of the adjacent denticle, an arrangement admirably adapted to give both flexibility and strength to the ring as a whole. On the outer side toward the circumference of the disk each denticle bears a flattened, blade-like structure, the hook, which is usually concave on one edge and convex on the other. The concave side is thicker and stains more intensely, while the convex side thins out and is sometimes difficult to distinguish from the surrounding cytoplasm. On the inner side of the denticle and approximately opposite the hook is a short tooth-like process, the ray, which projects toward the center of the disk. There is great variation in the number and shape of the denticles, and for that reason they are of special value in differentiating between the various species. The denticulate ring is located on the surface of the disk and is covered only by a thin, transparent, membrane. Although the rays vary greatly in length in different species, they never extend entirely across the center of the disk which is filled with granular protoplasm.

Overlapping the hooks on the upper or adoral side and extending toward the outer margin of the disk is a circular ribbon-like structure known as the striated band. When viewed from the surface of the disk this band appears as a series of radiating lines extending from the denticulate ring to near the edge of the disk. In sections it is evident that the lines are formed by long, slender rods which lie just above the hooks and extend for about half their length beyond the ends of these structures. The outer end of each rod is attached to the membrane which covers the surface of the adhesive disk. Like the hooks the number of rods varies, but is constant for each species. At the outer edge of the striated band is a distinct line which separates it from the third and outer ring, the "saum" of German writers which forms the border of the adhesive disk. This border is composed of a thin flexible membrane which is joined to the striated band by an articulation upon which it moves freely. The border membrane is finely striated, but the striations are not continuous with those of the striated band, the number usually being somewhat greater (fig. 109). Just above and anterior to the border membrane is a row of fine cilia that are united for the greater part of their length to form a membranelle. This ring of cilia is difficult to make out and has been overlooked by most investigators. According to Padnos and Nigrelli (1942) it occurs in *T. spheroidesi* and is one of the characters they use to distinguish this species from other marine tri-

chodinids. The writer has found this ring of cilia in all species of *Trichodina* described in this paper and believes that it is of universal occurrence. It is, however, more easily distinguished in some species than in others and sometimes can be made out only in cross sections.

A marginal fold of granular cytoplasm known as the velum (figs. 36–42) forms the outer edge or rim of the body proper. The shape and thickness of the velum varies greatly, even in the individuals of the same species, and sometimes it appears to be practically nonexistent (fig. 50)[3]. At the base of the velum there may be several folds or wrinkles between it and the central, more elevated portion of the adoral surface (figs. 36–38 and 46–48). The presence of these wrinkles depends largely on the condition of the adhesive disk. When it is curved downwards, they tend to disappear. Underneath the velum is a shallow groove to which is attached a band of long cilia, forming the so-called ciliary girdle. The structure of the ciliary girdle is difficult to make out, but as described by Wallengren (1897) and Fulton (1923), consists of a series of membranelles, each composed of several cilia which arise from a row of basal granules and unite to form a thin plate. Each membranelle is attached obliquely to the floor of the groove like the teeth on a worm-gear wheel. The membranelles show a tendency to fray out at the ends into their component cilia. The parasite moves about over the body and gills of the host by means of the ciliary girdle which also serves to propel it through the water. When swimming freely, the concave adhesive disk is always in advance in utter disregard of the principles of streamlining.

In some species (*T. fultoni*, *T. discoidea*, *T. platyformis*, *T. vellata*, and *T. tumefaciens*) there is a row of shorter cilia just above the membranelles, which appears to have escaped the attention of previous observers. These cilia, which are very fine and delicate, curve upward past the velum and show a rapid, vibratory movement in striking contrast with the wave-like movement of the membranelles. They are a short distance apart and can best be seen in the living animal when viewed from the adoral surface. It seems probable that these cilia, which will be called marginal cilia, are homologous with the cirri which are characteristic of the genus *Cyclochaeta*.

In *T. myakkae* and *T. symmetrica* it is evident that for each rod in the striated band, there is a corresponding membranelle in the ciliary girdle. While a similar arrangement cannot be demonstrated for other species, there is no reason to believe that this is not the case, but that owing to their larger size, the correspondence in numbers is not so evident. In all species, however, each rod of the striated band is connected with the ciliary girdle by a strand of more deeply staining material (figs. 46–48) and it is assumed that in this way the rods are connected with the corresponding membranelles. Mueller (1938) noted this connection between the rods and ciliary girdle in *Vauchomia* (*Trichodina*) *renicola* and *V. nephritica*. He believed, however, that such a connection was lacking in other species and used this as one of the distinguishing characteristics of the genus *Vauchomia*. On the contrary, the writer has been able to find such a connection in all species of *Trichodina* studied, but it is more easily distinguished in some species than in others. Mueller believed that these structures are myonemes which appears to be a logical assumption. The adhesive disk is very flexible and readily conforms to the contours of the surface to which the organism happens to be attached so that the need of contractile elements is obvious.

The mouth is located at one side of the body just above the velum and opens into the gullet or cytopharynx which extends for some distance into the body (figs. 47 and 52). The cytopharynx bears two parallel rows of long hair-like cilia which follow a spiral course and are continued through the mouth into a groove which also takes a spiral course clockwise around the adoral surface. The character of this spiral band known as the adoral spiral varies greatly in different species. In some, as in *T. myakkae* and *T. symmetrica* it may extend only a little more than half the distance around the adoral surface, while in other species it may make a complete circuit or even more. In either case the adoral spiral contains two rows of cilia which are usually long, but in some cases (*T. fultoni*) may be quite short. Except in the cytopharynx and in the immediate vicinity of the mouth, the cilia of the adoral spiral are usually motionless and instead of being erect are frequently folded over the adoral surface.

Near the center of the body is a large contractile vacuole with a wall composed of modified endoplasm and a duct leading to the exterior. The contractile vacuole is ordinarily described as opening into the cytopharynx, but this is certainly not the case in some species of *Trichodina*. In *T. fultoni*, for example, where on account of its size the duct from the contractile vacuole can be traced easily (figs. 118

[3] It provides a convenient line of demarkation between the adoral and aboral surfaces.

and 127), there is no question that it follows a separate course from the cytopharynx to the surface and opens independently a short distance to the left of the mouth where viewed from the oral surface. Extrusion of liquid from the contractile vacuole through a separate opening has also been observed in the living animal. A similar conditon can be easily demonstrated in *T. platyformis* and *T. tumefaciens* (fig. 147). It is possible that the species differ in this respect, but the writer has been unable to find a clear case of a connection with the cytopharynx.

The most conspicuous object in the endoplasm is the large, horseshoe-shaped macronucleus which is so located that the cytopharynx and the duct leading from the contractile vacuole lie between the open ends of the horseshoe. The macronucleus is filled with finely granular chromatic material with occasional rounded bodies of denser material. The micronucleus is small, rounded, or more commonly ovoid and sometimes greatly elongated. It is located near the open ends of the macronucleus and frequently lies in a shallow depression on the outside of one arm (fig. 57). The micronucleus, as in other ciliates, contains a deeply staining central body separated from the delicate membrane by a clear area. The remainder of the endoplasm is filled with food vacuoles in different stages of digestion, granular material, and fat globules.

REPRODUCTION

Binary Fission

As in other ciliates, the principal method of multiplication in *Trichodina* is by binary fission. As might be expected in an animal of such complexity, the process is by no means a simple one. Nevertheless, it takes place while the organism is moving about as usual and there is no evidence of an encysted or resting stage. Fission has been described in detail by several writers the most recent accounts being by Diller (1928) and by Padnos and Nigrelli (1942). The writer's observations are in essential agreement with those of these investigators.

One of the first indications of fission is a change in the macronucleus, which loses its horseshoe shape and becomes shorter and thicker. At the same time, the velum becomes thickened and the margin somewhat irregular or even crenated in some species. This is accompanied by the appearance of slight indentations in the velum and adoral side which mark the beginning of the constriction that will eventually divide the organism into two equal parts. While these changes are taking place, breaks appear in the striated band immediately beneath the indentations in the velum (figs. 170 and 176), which eventually extend to the denticulate ring. As the macronucleus becomes shortened, the micronucleus moves to one side of the body and divides by mitosis, the plane of division coinciding with that which divides the ciliate into two equal parts (fig. 177). Division of the macronucleus which lags somewhat behind that of the micronucleus is accomplished by amitosis, the nucleus becoming dumb-bell shaped and gradually separating into two equal parts in the usual way.

The two daughter individuals resulting from fission (figs. 171 and 172) are identical with the parent except that they are only one-half the size and contain only one-half the number of skeletal parts. Padnos and Nigrelli found that in *T. spheroidesi* the adoral and aboral bands of cilia were retained, but that the cytopharynx disappeared during fission. The contractile vacuole apparently divided with the macronucleus. This appeared to be the sequence of events in the species observed by the writer.

The skeleton is restored to normal after fission by a complicated process which is initiated when the ciliate first starts to divide. At the time the macronucleus contracts in preparation for division, a series of thin, overlapping plates appear on the lower (aboral) side of the striated band just outside the hooks of the denticulate ring (figs. 170 and 176). After fission is completed, these plates rapidly enlarge and each developes into a denticle similar to those in the original denticulate ring the hooks being formed first and later the rays (figs. 173–176 and 179–181). At the same time the old denticulate ring is gradually absorbed and eventually disappears entirely. Since the number of plates which develop into denticles in each daughter ciliate is twice that of the number of denticles derived from the parent, the original number is thus restored. Furthermore, since the striated band is also divided in half during fission, it is obvious that the number of rods in the band is reduced accordingly. The original number, however, is restored, not by the formation of a new striated band, but by the formation of new rods between those of the original band. In this process a faint line appears between each two striations (figs. 110, 113, and 114) which gradually increases in density until it is indistinguishable from the older striations on either side. Since the new rods develop gradually, the appearance of the new striations provides a convenient means of determining the relative age of trichodinids.

Conjugation

Sexual reproduction by conjugation has been observed by several investigators, but only recently has the process been described in any detail. Contrary to general belief, conjugation is by no means rare and the writer has observed it in 8 of the 11 species considered in this paper. It is probable that failure to find conjugation in the other 3 species was due to the fact that only a limited amount of material was available for study.

A detailed account of conjugation in *Trichodina spheroidesi*, a parasite of marine fishes, is given by Padnos and Nigrelli (1942) although they failed to observe some stages of the process. According to these writers, conjugation in *T. spheroidesi* is anisogamous and the aboral surface of the microconjugant is fitted over the adoral surface of the macroconjugant. The micronucleus of each then begins to swell and eventually divides mitotically. At the same time the macronucleus shows signs of vacuolization and breaks down into large, coarse fragments. These continue to break up into smaller and smaller parts until minute spherical bodies with deeply staining granules are formed. Padnos and Nigrelli were unable to follow changes in the micronucleus immediately preceding the metaphase, the latter, however, was well defined. During the final fragmentation of the macronucleus, two micronuclear divisions take place in each conjugant. Following division of the micronuclei, the protoplasm of the two conjugants becomes continuous and the contents of the smaller individual passes into the larger. The investigators assume that the gametic nuclei then unite to form the synkaryon and the remaining nuclei are resorbed. The ensuing processes are confined to the single large exconjugant.

The exconjugant now contains only one functional nucleus, the synkaryon, and remains of the broken down macronucleus and degenerating micronuclei. Padnos and Nigrelli assume that the zygotic nucleus divides three times, resulting in the formation of eight micronuclei. Seven of these become macronuclear anlagen and one the functional micronucleus. The latter divides in the usual way and in the division of the cell the macronuclear anlagen are distributed between the daughter cells. The most frequent distribution is three and four, but sometimes may be two and five or even one and six. In any case, cell division continues until each of the daughter individuals contains one macronuclear anlage and a micronucleus. The macronucleus then increases in size and assumes the characteristic horseshoe shape.

Reorganization of the denticulate ring occurs in the macroconjugant shortly after fusion of the protoplasmic contents of the two conjugants. A new ring is formed outside the old in the same manner as in fission, but in this case the number of denticles in the new ring is the same as in the old.

While, as previously mentioned, the writer has observed conjugation in eight species, considerable numbers of conjugants have been available for study only in *T. symmetrica*, *T. californica*, and *T. discoidea*. However, it is evident that the process is essentially the same in all species studied. The greatest handicap to a study of the details of conjugation is the virtual impossibility of following the micronuclei throughout the process. During mitosis the micronuclei can be recognized without great difficulty, but at other times they are usually indistinguishable from the rounded remnants of the macronuclei which are scattered throughout the endoplasm. Consequently, there are many breaks in the sequence of events which can only be bridged by assumptions.

In general, conjugation in the species studied is similar to the process in *T. spheroidesi*, as described by Padnos and Nigrelli, but there are some important differences. These investigators emphasize the fact that in *T. spheroidesi* the conjugants are always unequal in size and their account of the entire process is based on this conception. On the other hand, Diller (1928) found that in the few conjugating pairs seen by him, the two conjugants were of the same size. The writer has found both conditions, sometimes even in the same species. In *T. discoidea* and *T. symmetrica* (figs. 182 and 184) the upper conjugant is usually smaller than the lower, but the difference in size is not great and occasionally the two conjugants may be practically equal in size. In *T. californica*, however, the conjugants are normally of the same size and no distinction can be drawn between macro- and micro-conjugants. It seems probable that where the conjugants differ in size, this difference is due to their relative age. This can best be seen in *T. discoidea*, since in this species the striations in the striated band stain more deeply than usual. In all conjugants of this species observed it was found that the smaller conjugant was younger than the larger conjugant (figs. 187 and 188). On the other hand in *T. californica* where the two conjugants are of the same size, the condition of the striated band in each conjugant indicated that they were approximately the same age.

As found by Padnos and Nigrelli, the conjugants in all species unite before there is any noticeable

change in the structure of either conjugant (figs. 182–184) and protoplasmic continuity soon becomes established between the two. The micronuclei begin to swell and soon leave their position near one arm of the macronucleus, moving to the center of the body just underneath the adoral surface where they divide mitotically. During the prophase the greatly enlarged micronuclei are characterized by a central rounded mass of chromatin surrounded by a large, clear area. Later the mitotic figure is formed within this space in the usual manner. The most striking aspect of mitosis is the extraordinary length of the spindle during late anaphase and telophase when it may become considerably longer than the diameter of the body. A similar condition has been described in other ciliates.

During division of the micronucleus, the macronucleus undergoes extensive changes. It becomes greatly elongated and attenuated with the formation of slender arm-like processes (figs. 186 and 189). Later the entire nucleus breaks up into small, rounded structures, each resembling a small nucleus (fig. 192). These may be uniformly granular or they may contain a rounded body in the center.

It has been impossible to follow the divisions of the micronucleus in the two conjugants, but there is no reason to doubt that Padnos and Nigrelli are correct in assuming that there are two divisions, one following immediately after the other. It is worth mentioning, however, that the nuclei in each conjugant are always in practically the same stage.

Fusion of a micronucleus from each conjugant to form the synkaryon is evidently followed by three divisions of the latter in quick succession, resulting in eight nuclei, seven of which form the macronuclear anlagen, while the other forms the functional micronucleus. At first it is impossible to distinguish these nuclei from the fragments of the original macronucleus since they are practically identical in appearance (fig. 192). At a little later stage, however, the macronuclear anlagen can be easily recognized by their larger size and finely granular contents (fig. 193). While these changes are taking place in the nuclei a new denticulate ring is formed to replace the old (figs. 190 and 191), but in this case the new ring has the same number of denticles. Following the development of the macronuclear anlagen and new denticulate ring, the exconjugant divides in the usual way except that only the micronucleus divides, while part of the macronuclear anlagen pass into each daughter cell along with fragments of the macronucleus. Figure 196 shows an individual with two macronuclear anlagen and figure 197 a later stage with one which is already developing into the macronucleus.

The writer has been unable to find any evidence that following division of the micronuclei in each conjugant the two fuse as described by Padnos and Nigrelli. On the contrary it is believed that a mutual exchange of micronuclei takes place between the two conjugants, although it is realized that the evidence is by no means conclusive. If this is the case, conjugation in *Trichodina* more nearly resembles that of *Paramecium* than of *Vorticella*, as held by Padnos and Nigrelli. In addition to the fact that no evidence has been found of the absorption of one conjugant by the other, strong support for the view that union of the conjugants is temporary has been found in *T. discoidea*. Two pairs in a late stage of conjugation were observed in which both conjugants showed a ring of overlapping plates in the striated band, which is the first indication of the formation of a new denticulate ring. Moreover, several juvenile exconjugants were found in which a new ring was being formed with the same number of denticles as the old. These individuals also contained macronuclear anlagen and rounded fragments of the macronucleus (figs. 194 and 195) as found in the larger exconjugants.

Diller (1928) has described in detail nuclear changes in a species of *Trichodina* from tadpoles which he believed to be endomixis. Padnos and Nigrelli point out that the endomictic stages described by Diller conform closely to the postconjugative stages in *T. spheroidesi* and conclude that he has misinterpreted postconjugation for endomixis. With this conclusion, the writer is in complete agreement. Stages similar to those described by Diller have been observed in all species which contained conjugating forms and are undoubtedly normal postconjugative stages. Diller states that there is little danger of confusing conjugation with endomixis in the *Trichodina* from tadpoles, since the macronuclear fragments instead of being spherical as in endomixis are rectangular, linear, or spindle-shaped. As a matter of fact, in all species observed by the writer the macronuclear fragments were elongated in early stages of conjugation, but later became rounded and similar to those described by Diller as characteristic of endomixis. In a later paper Diller (1936) admits the possibility that "hemixis and exconjugant stages" were lumped together in his account of endomixis in *Trichodina*.

Effects of Trichodinids on the Host

The trichodinids are among the most common protozoan parasites of fish and have been the cause of serious mortalities among trout and pond fishes at hatcheries. However, since they are frequently accompanied by other protozoan parasites such as *Chilodon*, *Costia*, and *Ichthyophthirius*, it is sometimes impossible to determine the relative importance of the trichodinids as pathogenic agents. Trichodinids may occur on the body, fins, and gills, although several species are confined to the latter. Two species have been described by Mueller (1932, 1938) from the urinary tract of fishes, but they should be relegated to his genus *Vauchomia* and are not considered here. Trichodinids can be seen moving about over the integument and gills by means of the membranelles in the ciliary girdle and when dislodged are propelled through the water by the same organelles. Individuals of some species, such as *T. bursiformis* and *T. bulbosa*, probably may remain anchored in one spot for some time.

Trichodiniasis, the disease caused by these parasites, is characterized by white irregular blotches on the head and dorsal surface of the body, especially near the base of the dorsal fin. The fins may also become badly frayed in heavily infected fish. This is accompanied by sluggishness and a partial or complete loss of appetite. When an infected fish is viewed in a bright light at the proper angle, a white translucent covering or film can be seen extending over the body which in places reaches a considerable thickness, forming the white blotches previously mentioned. This film is composed of epithelial cells in various stages of disintegration. The scales may also become loosened and in extreme cases the skin may show a reddish tinge due to congestion of the blood vessels. A similar hyperplasia of the epidermis may be caused by infection with other parasites and is evidently the result of a general irritation and not a specific reaction to this particular parasite.

It is interesting to find that the hyperplasia of the epithelium, which is basically a protective reaction on the part of the host, actually benefits the trichodinid parasites since they feed on the epithelial cells thus formed. In some species the epithelial cells appear to form the principal if not the only food of the parasites. Many instances of the ingestion of epithelial cells by *T. fultoni* (fig. 120) have been noticed and epithelial cells in various stages of digestion have been observed in the food vacuoles of several species. A similar hyperplasia, though not as pronounced, may occur on the gills and no doubt explains an interesting observation by Mueller (1937) on infected bass in the Myakka River near Sarasota, Florida. At the end of the dry season, the river consisted of a series of separate pools in which the fish were concentrated in large numbers. In such a pool a number of largemouth bass were taken with gills heavily infected with *Trichodina*. These fish appeared on the verge of suffocation and were unable to swim, so that they could be readily caught in the hand. Other bass in the same pool were active and healthy and when taken with a net, showed no *Trichodina*.

Padnos and Nigrelli (1928) noted the presence of red blood cells in food vacuoles of *T. spheroidesi* and concluded that these parasites were capable of considerable tissue destruction. They also found that in exceptionally heavy infestations, the gill epithelium was completely destroyed, leaving large denuded areas among the filaments. Such a condition resulted in the death of the host. According to Guberlet (1926) tench (*Tinca tinca* Linné) in aquaria which were heavily infected with *Cyclochaeta domergui* (*C. guberleti*, McLennan) showed in addition to the white film on the body hemorrhagic areas in the skin, and in many cases the skin sloughed off in large patches.

Fish are infected by direct transmission and there is no evidence that the parasites can spread by any other means. Richardson (1937) found that 12 hours after the introduction of an infected trout into a container with 2 clean fish, the latter were as heavily infected as the former. He also found that the length of time the parasite can live off the fish depends upon the temperature. When a freshly dead or dying trout is placed in a container, the parasites will escape slowly from the host and settle on the bottom of the dish. This process continues for several hours and the parasites may live for 2–3 hours after leaving the host. At room temperature (22°–25° C.) live *Trichodina* escaped from the dead host during a period of 8 to 10 hours and were still capable of infecting other trout at the end of this time. At lower temperatures the process was greatly prolonged. At 11° C. live trichodinids were present on the dead host up to 72 hours and at 4.5° C. they survived for 140 hours after the death of the host. All parasites died quickly on drying and experiments to produce a resistant form were unsuccessful.

The descriptions that follow include all species of *Trichodina* known to occur on freshwater fishes in this country. It is believed, however, that these are only a small fraction of the total number.

Trichodina discoidea, n. sp.

[Plate 3, figs. 27–30; plate 9, figs. 109–116]

This trichodinid apparently occurs on a considerable variety of hosts. It has been found on the gills of the bluegill sunfish (*Lepomis macrochirus*), the black crappie (*Pomoxis sparoides*) and the rock bass (*Ambloplites rupestris*) at Kearneysville, W. Va. It was also found on the gills of the channel catfish (*Ictalurus punctatus*) from the Mississippi River at Fairport, Iowa. In every instance it was associated with other species of *Trichodina* including *T. myakkae*, *T. symmetrica*, *T. fultoni*, and *T. bursiformis*. In most cases it was present in only relatively small numbers, but it was abundant on the gills of three bluegill sunfish from a hatchery pond at Kearneysville, W. Va. in April 1944.

Trichodina discoidea well exemplifies the characteristic features of the genus. The adoral surface is usually only slightly elevated and may be perfectly flat. Occasionally, however, the central part may form a rounded dome in striking contrast with the flattened margin of the body (fig. 115). The adoral spiral makes slightly more than one complete turn around the body and bears throughout two rows of long cilia. The margin of the body supporting the velum varies greatly, but is usually thicker than usual with a well-defined shallow groove which bears the membranelles of the ciliary girdle. A row of marginal cilia is present just above the membranelles. The adhesive disk is large and flattened with a diameter greater than that of the rest of the body. The denticulate ring may contain from 18 to 30 denticles, the usual number being 20 to 25. The hooks have the usual broad blade-shape, but this can be made out only in the adults (fig. 109). In juveniles the hooks appear much narrower (figs. 113 and 114). At all ages the outer edge of the hooks is less rounded than usual so that they appear to be truncated, a characteristic of this species. The rays are long, slender and sharply pointed. In the striated band there are 6 to 8 rods to each denticle. The border membrane (fig. 109) is wider and more distinct than in most species. The row of cilia above the membrane is well developed and easily distinguishable in sections. The diameter of the denticulate ring in adults varies from 19 to 29 μ and that of the striated band from 35 to 50 μ.

The macronucleus has the usual structure with the small, oval micronucleus located in a slight depression in the outer side of one arm. The contractile vacuole is well-defined, and can be seen to open to the exterior at the left of the mouth.

Trichodina discoidea is more variable than most species with respect to size and number of denticles. In fact, it was thought at first that two distinct species were present, but further studies showed that in other respects there was no appreciable difference between the large and small forms and that all intermediate stages could be found. As might be expected, the larger individuals usually had more denticles than the smaller. There is evidence of the presence of different strains or races in which the number of denticles is relatively constant. For example, denticle counts in 10 trichodinids selected at random from the gills of 3 sunfish from the same pond were as follows: fish A, 21, 22, 20, 20, 20, 22, 21, 22, 20, 20; fish B, 21, 22, 21, 21, 19, 21, 19, 21, 22, 22; fish C, 26, 23, 22, 22, 23, 23, 23, 26, 23, 24.

Conjugating pairs and exconjugants were common on the gills of bluegill sunfish examined in April 1944. Individuals in various stages of fission were also numerous. It is an interesting fact that in all species when conjugating forms are present, vegetative forms undergoing binary fission are also common. No conjugating or dividing stages were found on the rock bass and crappie. Only small numbers of this species were present and these averaged larger than those on the bluegills. Apparently trichodinids continue to grow after they mature and if for any reason fission is delayed, may become larger than usual. This would explain the presence of exceptionally large individuals which have been observed in several species.

Trichodina platyformis, n. sp.

[Plate 3, fig. 34; plate 9, figs. 117, 119, and 121]

Trichodina platyformis occurred on the gills of the pearl minnow (*Margariscus margarita*) and the dace (*Rhinichthys atronasus*) at Kearneysville, W. Va., during the spring of 1944. While a few specimens were found on most of the minnows examined, it was never abundant, and occurred even less frequently on the dace. Probably on account of its large size, it was more common on the gill arches than on the filaments and only very rarely was found between the lamellae.

This species is larger than most with a saucer-shaped body which is elevated into a low, rounded dome on the adoral side and shows little variation in form. The adoral spiral extends about one and one-fourth times around the body with two rows of

long cilia which, as usual, are motionless except near the mouth. Sometimes these cilia were folded back against the surface of the body and in that position were difficult to distinguish. The wrinkles around the margin of the adoral surface are usually prominent and 3 to 4 in number.

A row of marginal cilia is present. The adhesive disk is flat with the border membrane extending beyond the velum. The denticulate ring is well-developed and contains from 26 to 35 denticles, the usual number being 28 to 31. One exceptional individual was observed with only 20 denticles which were the usual shape and size. The broad, flat hook is so wide that the convex edge is nearly in contact with the concave side of the adjoining hook. The rays are long (10μ) and slender, sharply pointed at the end. The striated band has 10 rods to each denticle. The diameter of the denticulate ring is from 31 to 50μ and that of the striated band from 56 to 70 μ.

The macronucleus has the usual form with a comparatively large micronucleus near one arm. The latter contains an oval endosome with broadly rounded ends. The contractile vacuole is easily seen in the living organism and opens to the left of the mouth by a separate duct.

This species resembles *T. truttae* in many respects, but can be easily distinguished by its much smaller size. There are also fewer bars in the striated band.

Trichodina vallata, n. sp.

[Plate 3, fig. 32; plate 9, figs. 123 and 124; plate 10, fig. 126]

Some years ago fingerling channel catfish (*Ictalurus punctatus*) in a rearing pond at the Fishery Biological Station, Fairport, Iowa, became severely infected with *Costia necatrix*. An examination of these fish disclosed that, in addition to the flagellate, numerous specimens of an undescribed trichodinid was present on the body. That is the only time this species has been seen.

The adoral surface is moderately convex and presents no distinctive features with the exception of the adoral spiral which forms a circular ridge around the body (fig. 32). This ridge bears a narrow groove from which arise two rows of long cilia. Since the adoral spiral is so different from that of any other trichodinid, it provides a convenient means of distinguishing the species. The extent to which the adoral spiral is elevated varies, the two extremes being represented in figures 123 and 124. It makes about one and one-fourth turns around the body. The marginal cilia are well-defined.

The adhesive disk is very similar to that of *T. discoidea*. The average number of denticles, however, is less and the number is remarkably constant. A count of 25 specimens taken at random showed a range of 18 to 21 with the great majority having 19 or 20 denticles. There are 10 rods to each denticle in the striated band as compared with 6 to 8 in *T. discoidea*. The diameter of the denticulate ring ranges from 25 to 30μ, and that of the striated band from 38 to 48μ.

Trichodina fultoni, n. sp.

[Plate 5, figs. 46–48 and 54; plate 9, figs. 118, 120, 122, and 125; plate 10, fig. 127; plate 12, figs. 170–175]

This large species of *Trichodina* is common at the Fish and Wildlife Service hatchery, Kearneysville, W. Va. It has been found on several species of fish including the largemouth black bass (*Huro salmoides*), the smallmouth bass (*Micropterus dolomieu*) the bluegill sunfish (*Lepomis macrochirus*), the rock bass (*Ambloplitis rupestris*), the rainbow trout (*Salmo irideus*) and several species of minnows. It is possible that the occurrence of this trichodinid on such a variety of hosts was due to the crowded conditions characteristic of all hatcheries and that in nature its range is more restricted. It is ordinarily found on the body and fins, but may occur on the gills, especially in large fish. It is the most common species at the Kearneysville hatchery and has at times caused considerable mortality among pond fishes.

Trichodina fultoni is one of the largest of the trichodinids, being exceeded in this respect only by *T. truttae*. The adoral surface of the body may be flat or slightly convex. The adoral spiral makes an almost complete circuit of the body, but is not present in the space between the arms of the macronucleus. Along most of its length the cilia are very short and difficult to distinguish. Between the adoral spiral and the velum there are several concentric folds which are better developed than usual. The marginal cilia are also more distinct than in most species and in the living animal can be made out without difficulty when it is viewed from the adoral side. The number of denticles ranges from 25 to 30, the usual number being 27 to 29. The hooks are broad and well defined. The rays are short and curved in the same direction as the hooks (figs. 54 and 125). There are 12 to 14 bars in the

striated band to each denticle. In the adults the diameter of the denticulate ring ranges from 50 to 58μ, and that of the striated band from 75 to 90μ. The over-all diameter of the body in the living organisms is about 100μ.

The large horseshoe shaped macronucleus has the usual structure, but the micronucleus is very difficult to locate. In all other trichodinids studied the micronucleus could be identified without difficulty, but in the great majority of the specimens of *T. fultoni* it has been impossible to distinguish this structure with certainty. It is of the usual vesicular type with a large endosome and may lie underneath the macronucleus where it is not readily seen. To add to the difficulty, the endoplasm contains numerous food vacuoles filled with fragments of tissue cells which stain deeply. The large contractile vacuole is located in the center of the body just above the adhesive disk and is provided with a small duct which opens to the exterior through a narrow slit slightly above and to the left of the mouth (fig. 118).

Trichodina fultoni is believed to be identical with the form described by Fulton (1923) from the gills of *Necturus*. Fulton's description of the parasite is incomplete and his only figures are photomicrographs which fail to bring out some of the essential details, but in size and general appearance there is a close resemblance between the two organisms. Fulton transferred specimens to the body of Hydra and "found that they crawled on the surface of the polyp quite as in the forms normally there." On this entirely inconclusive evidence he identified his form as *T. pediculus*. Furthermore, it is evident that several species have been included in *T. pediculus* by earlier writers, none of which appear to be identical with the present form. Since Mueller (1937) has discussed in some detail the questionable basis for Fulton's identification it is unnecessary to go into the matter further. Nevertheless, Mueller followed Fulton, though with some misgivings, and identified a trichodinid which he found on the gills of the largemouth black bass from the Myakka River near Sarasota, Florida, as *T. pediculus*. Mueller's description of the parasite is more complete and while his form differs in some details from that found at Kearneysville, it is not believed that the differences are sufficient to justify considering them to be specifically distinct. The size is approximately the same, but the number of denticles averaged slightly less (23 to 26), although the range overlaps. Also in Mueller's figures the rays are somewhat longer and less curved than in the writer's material. Finally, Mueller found about 9 bars in the striated band to each denticle while the Kearneysville form has 12 to 14 bars. In other respects the two forms appear to agree.

Trichodina truttae Mueller

[Plate 5, fig. 53; plate 10, figs. 129 and 130]

Specimens of a trichodinid preserved in formalin were received from G. C. Webb, Philomath, Oregon. These parasites occurred on the body of fingerling cutthroat trout (*Salmo clarkii*) at the Alsea hatchery of the Oregon State Game Commission. They agree very closely with a trichodinid described by Mueller (1937) from the gills of cutthroat from Oregon.

The body of the parasite is flattened and saucer-shaped. The denticulate ring contains from 28 to 31 denticles while Mueller found from 28 to 30 in his material. The striated band has 20 rods to each denticle. The denticles bear a flattened blade-like hook which is even broader than usual. Mueller, on the contrary, describes the hook as much narrower, but it is believed the discrepancy can be explained by the fact that he had only stained whole mounts for study. The blade part of the hook is thinner than usual and even in formalin material is so transparent that it is easily overlooked. The rays are long and slender as described by Mueller. He emphasizes the fact that the hook and ray of each denticle are not opposite each other as in other species, but that the hook is ahead of the ray. While this is frequently the case, there is considerable variation in this respect and both conditions may occur in the same individual. The adoral surface is only slightly elevated and the adoral spiral makes slightly more than one complete circuit of the body. The cilia are short throughout, except in the immediate vicinity of the mouth.

This species is notable for its size. In full-grown individuals the denticulate ring has a diameter of 75 to 85 μ, and the striated band a diameter of 110 to 125 μ. The overall diameter of the body, exclusive of the ciliary girdle is about 130 to 140 μ in preserved specimens.

Trichodina symmetrica, n. sp.

[Plate 5, figs. 49–52; plate 10, figs. 134–136, 138, and 139]

Trichodina symmetrica was first found on the gills of the channel catfish (*Ictalurus punctatus*) from the

Mississippi River at Fairport, Iowa. Several fish taken during July and August were infected. Later it was found on the gills of the pearl minnow (*Margariscus margarita*) and the dace (*Rhinichthys atronasus*) collected during the spring and early summer at Kearneysville, W. Va. The parasite was not abundant but a large percentage of the fish examined were infected.

This species shows less variation in shape than most trichodinids. The adoral surface forms a low, rounded dome with the mouth located somewhat higher on the surface than usual. The adoral spiral extends about two-thirds of the distance around the body. The cilia are relatively long and vary but little in length throughout the course of the spiral which ends abruptly instead of fading out gradually. The velum is thick, frequently poorly defined, and without wrinkles on the adoral surface. There are from 21 to 28 denticles in the denticulate ring, the usual number being from 24 to 26. The hooks are spatulate and rounded at the end, the rays are slender and frequently difficult to distinguish. The striated band contains about 5 rods to each denticle and in suitably stained specimens it is evident that there is a striation or bar for each membranelle in the ciliary girdle. The row of cilia on the adoral side of the border membrane is better developed than usual and extends a short distance beyond the edge of the membrane. The diameter of the denticulate ring ranges from 13μ to 22μ and that of the striated band from 24μ to 35μ.

The macronucleus has the usual horseshoe shape, but shows a tendency to assume unusual forms, such as an outgrowth of one arm which, in one instance, was connected with the opposite arm above the mouth. The micronucleus is spindle-shaped, even in the resting condition, and frequently lies in a depression in one arm of the macronucleus.

During binary fission the velum disappears and the margin of the body becomes noticeably thicker, so much so in fact that sometimes the body has the same thickness throughout. In such individuals the adoral spiral forms a border for the flattened adoral surface. Conjugating individuals were common both at Fairport and at Kearneysville.

Trichodina californica, n. sp.

[Plate 4, figs. 39, 41, 43, and 45; plate 10, figs. 131–133, 137, and 140]

During the fall of 1943, adult chinook salmon (*Oncorhynchus tschawytscha*) from the Sacramento River, Calif., showed a heavy infection of the gills with this trichodinid. The fish had been held for several weeks in ponds at the Coleman hatchery on Battle Creek. Only preserved material has been available for study.

Trichodina californica is characterized by an unusual plasticity resulting in marked changes in form so that the general appearance of the organism may vary greatly. The adoral surface of the body is raised into a rounded dome which is usually unsymmetrical, the greatest bulk being at one side. Occasionally the adoral surface is flat instead of convex and all intermediate stages can be found. In such individuals the body may project some distance beyond the adhesive disk on all sides, as in figure 41. More often, however, the bulk of the body is on one side (fig. 43), so that in sections it appears very unsymmetrical. The adoral spiral makes a complete circuit of the body and may, as in other species, be located entirely on the adoral surface, but usually during part of its course it occupies a groove in the margin (fig. 41). The cilia are relatively short and inconspicuous. Concentric wrinkles may be present on both sides of the adoral spiral, but there is great variation in their number and shape. The denticulate ring contains from 25 to 32 denticles, the usual number being 26 to 28. The hooks are wide and blade-shaped, the rays straight and pointed at the end. The striated band contains 8 to 10 bars to each denticle. The denticulate ring has a diameter of 25 to 33μ, and the striated band a diameter of 38 to 50μ.

The macronucleus has the usual horseshoe shape and is filled with fine chromatic granules among which are numerous rounded nucleoli. The ovoid, vesicular micronucleus is located at the side or underneath one arm of the macronucleus. Food vacuoles are numerous in the endoplasm.

Conjugating forms and individuals in various stages of binary fission were common, but owing to poor fixation were not as valuable for study as similar stages in other species.

A remarkable condition, which has not been found in other species, was observed in a small percentage of the parasites. These individuals contained a deposit of amorphous material in the endoplasm which was usually first noticeable near the adoral surface. Eventually, the entire organism became filled with this material (figs. 133 and 137) and then appeared to be enclosed in an opaque covering which was a glistening white by reflected light. The composition of this material is not known, but it is ap-

parently an organic substance since it is not affected by hydrochloric acid. It was most noticeable in specimens preserved in formalin, but was not affected when they were embedded and sectioned. In sections of parasites fixed in formol-mercuric-acetic fluid the deposit had largely disappeared. No explanation could be found for this condition.

Trichodina californica can be easily recognized by the small size of the adhesive disk as compared with the rest of the body. In this respect it most closely resembles *T. tumefaciens*, but is considerably larger and the denticulate ring is quite different in the two species.

Trichodina tumefaciens, n. sp.

[Plate 4, figs. 36 and 37; plate 10, figs. 141 and 142; plate 11, figs. 143–148]

The gills of sculpins (*Cottus bairdii*) taken from a small brook near Kearneysville, W. Va., were infected with a trichodinid which may be restricted to this host, since it was not found on other fishes collected at the same time. It was present on practically every sculpin examined and in many instances was abundant.

Trichodina tumefaciens, like *T. californica*, is characterized by the relatively large size of the adoral part of the body, which is usually strongly convex, but has an unusual capacity to change its shape. It shows the same tendency as *T. californica* for the bulk of the adoral dome to be located at one side instead of over the center of the adhesive disk. The cilia of the adoral spiral, which extends about one and one-fourth times around the body, are exceptionally long. Outside the adoral spiral are several concentric wrinkles which are more prominent than usual. When the adhesive disk is curved downwards so as to form a cup-shaped structure these wrinkles are smoothed out. There is a well-defined row of marginal cilia. The denticulate ring contains from 19 to 26 denticles, the usual number being 21 to 25. The striated band has about 7 bars to each denticle.

This species is notable for the marked change that occurs in the denticulate ring during the life of the individual. No doubt similar changes occur in all trichodinids, but in *T. tumefaciens* they are more noticeable than in most species. In fact, the difference between juveniles and adults is so great that they were at first thought to be different species. In the juveniles the hooks are narrow, and the rays slender and pointed (figs. 143 and 144). Figure 145 represents an intermediate stage in which both hooks and rays are larger and wider. Finally, in the adults (fig. 146) the hooks have the usual blade-like shape while the rays are broad and rounded at the end.

The diameter of the denticulate ring varies from 18 to 23μ and that of the striated band from 29 to 38μ.

The macronucleus and micronucleus have the usual structure and there is nothing distinctive about either. The cytopharynx forms a relatively large tortuous tube nearly as long as the diameter of the adoral dome. The contractile vacuole is more conspicuous than in most species with a separate duct to the exterior which is easily seen. Various stages in conjugation were observed but they were not common.

Trichodina bulbosa, n. sp.

[Plate 3, figs. 31, 33, and 35; plate 11, figs. 149–157]

This interesting trichodinid was found on the gills of the pearl minnow (*Margariscus margarita*) at Kearneysville, W. Va., during the spring and early summer of 1944. These fish were collected from the same brook as the sculpins infected with *T. tumefaciens*, but only the pearl minnows carried the present species which was found on nearly every fish examined.

Trichodina bulbosa is quite different from most species of *Trichodina*. It is shaped like a turban rather than the usual saucer shape. The adoral surface may be rounded or cone-shaped and the adhesive disk is strongly curved to form a cup-like structure. The adoral spiral extends about three-fourths of the distance around the body, but follows an unusual course. From the mouth it extends upwards along one side of the body until it reaches the crest of the dome and then drops down and terminates just above the denticulate ring. Throughout its course the cilia are exceptionally long and usually erect, but may be bent toward the center so as to lie in contact with the adoral surface. Sometimes there may be one or more wrinkles near the base of the velum, but they are never a prominent feature. There are 19 to 24 denticles in the denticulate ring, the usual number being 21 or 22. The hooks are exceptionally long and paddle-shaped with their greatest width near the outer end. The rays are slender and pointed. The striated band is wider than usual with 5 to 6 bars to each denticle. The diameter of the denticulate ring when flattened is 10μ to 12μ, and that of the striated band 22μ to 26μ.

The macronucleus has the usual horseshoe shape

and lies just above the denticulate ring with the vesicular micronucleus underneath one arm (fig. 31).

The parasites cling to the gills in a very characteristic manner by means of the adhesive disk which encloses the edge of a lamella. Considerable pressure is evidently exerted by the edge of the disk, since the epithelial cells may be forced to one side as in figure 154 so that in sections the margin of the lamella appears to be separated from the main part by a narrow neck. This, however, is not so marked as with *T. bursiformis*.

Conjugating individuals were observed in several instances (fig. 185), the process being essentially the same as in other species. Both conjugants were of approximately the same size, and the upper clung so closely to the lower as to narrowly constrict the body of the latter.

Trichodina bursiformis, n. sp.

[Plate 4, figs. 38, 40, 42, and 44; plate 11, figs. 158–165]

This interesting species has been found only on the gills of the rock bass (*Ambloplites rupestris*) from Opequon Creek near Kearneysville, W. Va. Two fish with a severe infection with this trichodinid were taken in March 1943. In both fish a much smaller number of *Trichodina discoidea* were also present on the gills.

Trichodina bursiformis is very different from other known trichodinids and most nearly resembles *T. bulbosa*. In fact, this species shows an extreme development of the features which differentiate the latter from other forms. The structure of this bizarre organism is difficult to describe, especially since the body is very plastic and the shape very variable. When free-swimming, the animal resembles an old-fashioned sunbonnet with the elevated adoral surface forming the crown. The adhesive disk, instead of being expanded in the usual manner, is compressed, bringing the border of the opposite sides close together with a long, narrow opening between (figs. 42 and 162). This apparently always takes place in the plane which passes through the mouth. The adoral surface is also compressed in the same plane so that instead of the usual dome it forms a ridge extending from the mouth to the opposite side of the disk. This ridge may vary greatly in shape; sometimes it is low and comparatively broad, sometimes high and narrow. A band of long cilia, the adoral spiral, extends along one side of the ridge and around each end. At one end it enters the mouth, at the other end it terminates in a tuft of longer cilia which is very conspicuous. As usual, the cilia in the adoral spiral show no movement, except near the mouth, and are frequently folded inwards over the adoral surface instead of standing erect. As in *T. bulbosa*, the adoral spiral extends upward from the mouth along the ridge and then drops down to the level of the denticulate ring.

In cross sections of the organism (figs. 40, 160, 161, and 164) the adhesive disk appears as long arm-like structures projecting from each side of the body. As shown in the figures, one arm is usually somewhat shorter than the other. The velum is well-defined and in sections appears as a thick lobe projecting over the base of the ciliary girdle. At the base of the velum there are several narrow, concentric wrinkles. The denticulate ring is well-developed, but evidently has even greater flexibility than usual. The hooks are spatulate, very similar to those of *T. bulbosa*. The rays are long and slender, but somewhat enlarged at the base. The ring is composed of 24 to 27 denticles with a diameter of 14 to 18μ. The striated band is about 25 to 35μ in diameter with 5 bars to each denticle. The macronucleus has the usual horseshoe shape, but one arm is frequently longer than the other. The vesicular micronucleus is ovoid and may lie between the arms of the macronucleus or at one side.

When attached to the gills the adhesive disk serves as a clasping organ which grasps the edge of the lamella with such force as to squeeze the epithelial cells out of position (figs. 160, 161, and 164). This method of attachment is more highly developed than in *T. bulbosa* and apparently the parasites may remain in one position for some time. However, when the gills are removed from the fish, they quickly become detached and swim about rapidly with the narrow opening of the adhesive disk foremost.

As already pointed out, *T. bursiformis* resembles *T. bulbosa* in many respects, but can easily be distinguished by its larger size and compressed body. The adoral spiral is also more prominent with longer cilia.

Trichodina myakkae Mueller

[Plate 5, figs. 55–58; plate 11, figs. 166–169]

This trichodinid was found by Mueller (1937) on the gills of the largemouth black bass from the Myakka River near Sarasota, Fla. Later (Mueller 1938), he reported its occurrence on carp and suckers from Chautauqua Lake, N. Y. The writer first

found *T. myakkae* on the gills of the smallmouth buffalo (*Ictiobus bubalus*) and the carp sucker (*Carpiodes carpio*) from the Mississippi River at Fairport, Iowa. During November and December 1944 it was present in considerable numbers on the gills of brook trout (*Salvelinus fontinalis*) in a hatchery pond at Kearneysville, W. Va. Evidently this species can live on a wide variety of hosts.

The organisms observed by the author agree in all essential respects with Mueller's description. The adoral surface is usually strongly convex, but may be much lower in some individuals than in others. The adoral spiral extends from one-half to two-thirds the distance around the body. The cilia are exceptionally long and near the mouth may equal the membranelles of the ciliary girdle in length. The groove bearing the ciliary girdle is shallow and poorly developed. The denticulate ring has a very characteristic structure which serves to distinguish this species from other known forms at a glance. The hooks are straight and narrow and the rays are entirely lacking. The striated band has 4 to 5 bars to each denticle and when properly stained can be plainly seen to have a bar for each membranelle (fig. 58). The number of denticles may vary from 17 to 24. In specimens from Fairport the number was somewhat less than in those from Kearneysville. In the former the usual number was 19 to 20, while in the latter it was 20 to 23. Mueller found 18 to 22 denticles in his specimens. The diameter of the denticulate ring in adults was remarkably constant, ranging from 11 to 12μ, while the diameter of the striated band varied from 21 to 25μ.

The macronucleus has the usual granular structure and horseshoe shape. The micronucleus is greatly elongated (fig. 57), much more so than in other species. It is usually located in a depression in an arm of the macronucleus and may be so closely attached as to be difficult to distinguish. Food vacuoles containing bacteria are usually present and it is probable that they form the principal food of this species. This is to be expected, since *T. myakkae* is too small to ingest epithelial cells.

Family **Scyphidiidae** Kahl

Scyphidia macropodia, n. sp.

(Plate 6, figs. 67–72; plate 13, figs. 198–208; plate 14, figs. 209, 217, and 218)

A number of fingerling bullheads (*Ameiurus nebulosus*) which were confined in a concrete tank at the Fish and Wildlife Service Station, Kearneysville, W. Va., during August 1943, became heavily infected with an undescribed scyphidian for which the name *Scyphidia macropodia* is proposed. The parasite was abundant on the gills and body of the fish and caused considerable mortality. A few scyphidians were found on the gills of fingerling bluegills (*Lepomis macrochirus*) in an adjoining tank, but it seems probable that the infection in this case was accidental. Several specimens of the same parasite have been found on the gills of the channel catfish (*Ictalurus punctatus*) from the Mississippi River at Fairport, Iowa.

Morphology

The body of *Scyphidia macropodia* is cylindrical with approximately the same diameter throughout, but occasionally may be somewhat constricted near the base. It is attached to the epithelium by means of a holdfast organ, the scopula, which in this species is exceptionally large. The scopula is a very flexible expansion of the aboral or posterior end of the animal and conforms to the surface contours of the epithelium. The peristome at the adoral or anterior end has a slightly greater diameter than the body proper. About one-third of the distance between the scopula and the peristome there is a membranelle which extends around the body like a collar. In the living organism it is difficult to distinguish the cilia in the membranelle, but sometimes they are separated along the outer margin. When viewed from the adoral surface the membranelle shows a slow, undulatory movement. On both sides of the central membranelle the pellicle is marked by parallel transverse striations extending around the body. There are about 16 of these striations between the peristome and the membranelle.

The border of the peristome consists of a thick, lip-like structure which by constriction closes over the disk when the animal retracts (figs. 70 and 199). Just within the border is the adoral groove bearing two parallel rows of cilia. As found by Noland and Finley (1931) in Vorticella, the cilia are united at the proximal end to form a "semi-membranelle," but are usually separated at the tips so that the membranelle has a ragged edge. The cilia in the outer row, however, sometimes appear to be united throughout their length. The cilia in the inner row are much longer and thicker than those in the outer. The two rows of cilia form the adoral spiral which, when viewed from above (fig. 67), can be seen to follow a spiral course anticlockwise around the peristomial

disk. The course of the adoral spiral is the same as in *Trichodina* and makes about one and one-fourth turns around the disk. At one side the adoral spiral leads into the vestibule, and as they enter the membranelles diverge from each other for a short distance, but soon come together again to follow a spiral course around the vestibule. Since the adoral spiral makes more than a complete circuit of the peristomal disk, four rows of cilia are present for a short distance near the opening into the vestibule (fig. 71). Within the adoral spiral the surface of the peristome is smooth and convex.

The contractile vacuole is located just beneath the central convex surface of the peristome and opens at one side into the vestibule. The most conspicuous structure within the body is the large, deeply stained macronucleus which is very similar to that of *S. tholiformis* (Surber 1943). The macronucleus is a long, sausage-shaped structure with two arms extending the length of the body and connected by an open loop around the vestibule. The ends of the nucleus lie just above the scopula in a tangled mass, the exact arrangement depending on the amount of retraction. There is, however, no branch or "prong" from an arm of the nucleus as in *S. tholiformis*. The macronucleus is uniformly and finely granular with practically the same structure throughout. The micronucleus is located among the tangled ends of the macronucleus and is composed of a rounded mass of deeply staining granules embedded in a homogeneous matrix which is separated from the nuclear membrane by a clear halo. Numerous food vacuoles are usually present in the finely granular endoplasm.

The living organism when fully extended is about 35 to 45μ in length with a width of 20 to 25μ.

In addition to the attached stage described above, the organism may change into a free-swimming or telotroch stage (figs. 201 and 202) as described by Surber (1943) for *S. tholiformis*. The transformation takes place very rapidly when the parasites are removed to a slide, but occurs in only a small percentage of the organisms. The change is accomplished by an extreme shortening of the body so that it becomes disk-shaped and has a striking superficial resemblance to a trichodinid. The scopula is retracted to form the lower surface of the telotroch, while the convex upper surface is formed by the constricted border of the peristome which covers the peristomal disk, except for a small opening in the center. At the same time the cilia in the central membranelle become free, increase in length, and form the locomotive organ in the same manner as the ciliary girdle of trichodinids.

Reproduction

Like other scyphidians, *S. macropodia* reproduces asexually by binary fission. In this process (fig. 205) the organism divides lengthwise, the micronucleus dividing mitotically, followed by amitotic division of the macronucleus, which is first greatly shortened and then divides into two equal parts.

Although individuals in various stages of conjugation were not uncommon, it has been impossible to follow the entire process in detail. However, it appears to agree in most respects with conjugation in *Vorticella microstoma* as described by Finley (1943). The small free-swimming microconjugants are apparently formed by budding (fig. 218) from an ordinary individual.[4] The newly formed microconjugant is surrounded by a band of cilia and contains a U-shaped macronucleus and a single micronucleus. The microconjugant becomes attached to the macroconjugant near the peristome (figs. 70 and 217), but does not unite with it immediately. The micronucleus then forms eight nuclei of equal size by three rapidly recurring divisions. At the same time, the micronucleus in the macroconjugant divides twice to form four nuclei of equal size. During these divisions of the micronuclei the macronuclei of both conjugants remain unchanged.

The next stage in the process has not been followed but from analogy with other vorticellids we may assume that the eight micronuclei pass from the microconjugant into the macroconjugant. One of these becomes a pronucleus and unites with the pronucleus of the macrogamete to form the synkaryon. The remaining seven micronuclei of the microconjugant and three micronuclei of the macroconjugant then degenerate and take no further part in the process. During formation of the synkaryon the macronuclei of both conjugants break down into rounded structures similar to those formed in *Trichodina*. The synkaryon then divides twice to form four nuclei, three of which increase in size to form macronuclear anlagen while the fourth becomes the functional micronucleus (figs. 203, 204, 207, and 208). This does not agree with the findings of Finley and other investigators of conjugation in the peritrichous

[4] Penard (1922) found that the microconjugants in *Glossatella tintinnabulum* are formed by two rapidly recurring divisions resulting in the formation of four small individuals. A similar process has been observed in *S. macropodia*, but, as will be explained later, is believed to follow conjugation rather than precede it.

ciliates who have found that the postconjugants contain eight nuclei, seven of which develop into macronuclear anlagen as in *Trichodina*. The only exception is Enriques (1907) who found that in *Opercularia* only four nuclei were present in the post conjugant, three of which became macronuclear anlagen. Enriques gave as an illustration one with seven macronuclear anlagen and one micronucleus, which he believed to be exceptional, but Finley (1943) argues with some justification that this was probably a normal stage rather than an exception.

Postconjugants in *S. macropodia*, containing three macronuclear anlagen and one micronucleus were quite common, but although a special search was made, no individuals containing more than four nuclei could be found. Although the Feulgen stain was not used there was no difficulty in distinguishing the nuclei from the fragments of the degenerating macronuclei which were the only other structures taking the nuclear stain. Various stages in the development of the macronuclear anlagen were observed, but there were never more than three in any individual. They are spherical at first, finely granular and stain less deeply than the rounded remnants of the macronuclei. As the macronuclear anlagen increase in size, they become elongate and eventually U-shaped.

The postconjugant then divides by two rapidly recurring divisions into four individuals which are much smaller than usual and have a different appearance (figs. 68 and 206), due to the peristomal disk being entirely enclosed by the border. Figure 68 is a section of one of the daughter cells formed by the first division of the postconjugant. This individual contained a U-shaped macronucleus and a micronucleus in the anaphase preparatory to the second division. In the next section there was a similar individual with a dividing micronucleus, but with two U-shaped macronuclei. When these two individuals divide it is believed that in the one figured both the micro- and macronuclei divide, while in the other only the micronucleus would divide, one of the two macronuclei passing into each daughter cell. This would result in the formation of four individuals in a group as in figure 209. In support of this explanation of the formation of the group is the obvious fact that in the two individuals above and at the left the macronuclei are longer than in the other two. The former are believed to be the daughter cells of an individual with two macronuclei, while the latter were formed from an individual with one macronucleus which divided along with the cell. Although, as previously pointed out, Penard (1922) thought these small individuals became microconjugants, it is believed that the explanation given above is more in accord with the evidence.

Some attention was given to conjugation in *S. tholiformis* which appears to be essentially the same as in *S. macropodia*. The macronuclei, however, break down before the conjugants unite and consequently it is very difficult to follow the divisions of the micronuclei. Only a few postconjugants were found, but no more than three macronuclear anlagen could be distinguished in any individual.

Class SUCTORIA Clarapede and Lachmann

Trichophyra ictaluri, n. sp.

[Plate 6, fig. 73; plate 14, figs. 214, 222, and 223]

This suctorian was found on the gills of channel catfish from the Mississippi River at Fairport, Iowa. It was present on several fish out of a small number examined and although not abundant in any instance, is evidently not uncommon. This species was figured and described very briefly in a previous publication (Davis 1942). Since that time additional material has become available which makes a more complete description of the parasite possible. This description, however, is based entirely on preserved material.

In sections the parasites appear somewhat elongated with one side in close contact with the surface of the gill. The tentacles are arranged in bundles or fascicles and there is usually a fascicle at each end of the body. It was thought at first that two fascicles of tentacles was characteristic of this species, but an examination of unsectioned gills preserved in formalin disclosed that there may be from one to four fascicles of tentacles (fig. 214). The number appears to depend on the age of the parasite, small—and presumably young—individuals having only one bundle of tentacles. Individuals intermediate in size may have two fascicles, while full-grown suctorians may have four distinct fascicles of tentacles

evenly spaced around the body. No more than four have been observed in any individual. The tentacles are usually borne on smooth, rounded elevations, but sometimes the surface to which they are attached is quite irregular. The tentacles are somewhat enlarged at the free end and at the proximal end can be traced for some distance into the body where those of each fascicle converge toward a common point. It was impossible to determine their number, but there may be at least 10 to 12 tentacles in each fascicle. The body is covered by a thin, dense layer of ectoplasm, but there is apparently no distinct pellicle. The side of the body in contact with the epithelium may be convex or flattened and is closely attached to the lamella. Frequently the epithelial cells have been destroyed or pushed aside so that the parasite may be in direct contact with the capillary network (figs. 73 and 223).

The endoplasm is coarsely granular and ordinarily contains a number of rounded metaplastic bodies. In some instances these bodies were so abundant as to obscure all other structures. The macronucleus is large and may be rounded or sausage-shaped. It is filled with spherules of equal size which stain intensely with chromatic stains. In addition, there are usually one or more rounded bodies several times the size of the spherules which also stain deeply. No membrane could be distinguished around the macronucleus. The micronucleus is vesicular with a rounded endosome composed of chromatin granules in a nonstaining matrix which is separated by a clear area from the nuclear membrane.

The organisms vary greatly in size. The largest individual observed in formalin material with 4 fascicles of tentacles was 50 by 65μ. In sections the longest diameter was usually about 35–40μ and the thickness 15–20μ.

The parasite multiplies by endogenous budding. Several individuals were observed with buds nearly ready to emerge.

The only suctorian previously described from fish is *Trichphrya micropteri* (Davis 1942) from the gills of the smallmouth black bass. This species has only one bundle of tentacles so there is no possibility of confusing the two forms. There is no doubt that many other suctorians occur on the gills of fishes. The writer has specimens of several species, but the material is too fragmentary to permit a detailed description.

Order MYXOSPORIDIA Butschli

The Myxosporidia form a large and well-known group of Protozoa which are typically fish parasites, although a few species occur in amphibians and reptiles. It is, therefore, to be expected that any extensive study of protozoan parasites would disclose many members of this group. They are especially common on the gills of freshwater fishes and although many species have been observed, only two which are of more than ordinary interest, are considered here.

Family **Chloromyxidae** Thelohan

Chloromyxum externum, n. sp.

[Plate 7, Figs. 74–80, 82, and 83; Plate 14, Figs. 215, 216, 219–221, and 224]

Myxosporidians are typical endoparasites, being found in the tissues and cavities of the body, and their structure and life cycle are especially suited to this mode of life. It is, therefore, of considerable interest to find a myxosporidian which, while exhibiting the typical structure of the group, is a true ectoparasite. This species, for which the name *Chloromyxum externum* is proposed, lives on the surface of the gills and so far has been found only on the pearl minnow (*Margariscus margarita*) and the black-nosed dace (*Rhinichthys atronasus*). Infected fish of both species were collected from the same brook near Kearneysville, W. Va., during March, April, and May, 1944. The parasites are small and easily overlooked unless present in considerable numbers.

The trophozoites are very similar to those of many other species of *Chloromyxum*. They are colorless with a clear, hyaline layer of ectoplasm surrounding a finely granular endoplasm. The latter is faintly vacuolated and contains numerous small, refringent, fat globules (fig. 74). The trophozoites move slowly by means of a short conical pseudopodium formed of ectoplasm. In fixed material the ectoplasm is more distinctly reticulated than the endoplasm.

The nuclei cannot be seen in the living trophozoite, but after fixation and staining, two types of nuclei

can be distinguished without difficulty. The more numerous generative nuclei are surrounded by a dense layer of cytoplasm to form the generative cells (fig. 79). These cells are clearly set off from the undifferentiated endoplasm by the coarsely granular cytoplasm which takes the hematoxylin stain. Each nucleus contains a large, centrally located nucleolus or plasmosome which is surrounded by a clear area. There is no chromatic network, the chromatin, as is frequently the case in the Myxosporidia, being limited to a layer of fine granules attached to the nuclear membrane. The generative cells vary in size and are rounded to irregular in shape. Occasionally a generative cell may contain two nuclei, but usually each cell is distinct with only one nucleus until the spores start to develop. The vegetative nuclei can be easily recognized by the fact that they are not surrounded by a differentiated layer of cytoplasm. Otherwise, their structure is essentially the same as that of the generative nuclei except that they may be slightly smaller.

Naturally, the trophozoites show considerable variation in size. The dimensions of several large trophozoites selected at random in sections and smears were as follows: 14 by 32μ; 15 by 28μ; 16 by 19μ; 14 by 24μ; 15 by 20μ; 17 by 19μ; 16 by 27μ. Rounded trophozoites were 15 to 17μ in diameter.

The spore (fig. 80) is approximately spherical to ovoid with four polar capsules of approximately the same size in which the coiled filaments can be seen indistinctly. Each valve bears about six concentric ridges which are parallel with the sutural ridge. The fresh spore is about 8μ in diameter, but in sections they show considerable shrinkage, the diameter being only about 6μ.

REPRODUCTION

Sporulation is essentially the same as in other Myxosporidia. The generative cells become aggregated in groups of eight cells to form a sporoblast which develops into the mature spore in the usual manner. No pansporoblasts are formed and each sporoblast becomes a spore independent of other sporoblasts. Furthermore, it appears that, in most cases at least, only one spore matures at a time, although two or three spores in different stages of development may be present in the same trophozoite (fig. 77). How many spores may be produced by a trophozoite is not known, but it is evident that the species must be considered polysporous, even though no trophozoite has been found with more than two well-developed spores.

Although numbers of infected fish were collected during March, April, and May, sporulating trophozoites were found in only one instance. A black-nosed dace, collected on April 10, was heavily infected with vegetative and sporulating trophozoites. No evidence of sporulation could be found among the parasites of several pearl minnows collected at the same time.

It seems probable that, in addition to the production of spores, the trophozoites multiply by plasmotomy. This is a common method of reproduction among the Myxosporidia and while it has not been observed in *C. externum*, there is indirect evidence that it does occur. Naturally, plasmotomy is difficult to demonstrate under the best of circumstances, but with trophozoites living on the surface of the gills, the possibility of observing it is slight indeed. The best evidence that such a process must occur is furnished by the distribution of the trophozoites on the gills. Instead of being evenly distributed, they are always much more abundant on some gills than on others in the same fish and they are also more numerous on certain parts of the same gill. A few adjoining filaments, for instance, may have large numbers of trophozoites, while the rest of the gill is almost free from them. This cannot be due to differences in the structure or location of the filaments, since the heaviest infection may be found on almost any part of the gill.

Nothing is known regarding the means by which the parasites are transmitted from one fish to another. From analogy with other species of Myxosporidia, it must be assumed that the spores after dropping from the gills are ingested accidentally by other minnows and germinate in the stomach or intestine. The ameboid sporoplasm then enters a blood vessel and is carried to the gills. In fresh-water fishes the gills are among the organs most commonly infected by Myxosporidia. Most of the gill parasites are so-called histozoic forms which form cysts on the gill tissues, but, nevertheless, reach the gills by means of the circulatory system (Davis 1923, p. 431). Having reached the gills, it is assumed that the young trophozoites of *C. externum* leave the capilliaries and make their way through the epithelium to the surface. Although sections of infected gills have been searched carefully, trophozoites which were entirely surrounded by epithelial cells have been found in only one instance. In this case a group of several small trophozoites were located in the stratified epithelium lying between the lamellae. The appearance of the trophozoites in close contact

with each other suggests that they may have been formed by division of a single individual. Of course, the possibility is not ruled out that the spores may germinate on the gills after having become entangled in the mucus, but there is certainly no evidence that this ever occurs.

Relation of Parasite to the Host

As already pointed out, this species is of exceptional interest since it is the only known ectoparasite in the entire group of the Myxosporidia. The trophozoites are found attached to the surface of the epithelium or in the mucous coating surrounding the gills. In some instances they may be attached to the epithelium simply by a pseudopodium (fig. 78), but usually the entire organism is in close contact with the epithelial cells. Frequently the trophozoite lies in a saucer-shaped depression in the epithelium which appears to have been formed by lysis of the epithelial cells (figs. 83 and 215). Sometimes the cells have been entirely destroyed so that the trophozoite is in direct contact with the capillary network of the lamella (figs. 82 and 220). The trophozoites may fit so closely into the space formerly occupied by tissue cells as to appear at first glance to be an integral part of the epithelium.

In severe infections large numbers of trophozoites are found in the mucus which forms a protective coating over the gills. In such cases the secretion of mucus is noticeably increased and it is filled with tissue cells in various stages of disintegration. Frequently, as shown in figure 76, such cells may be partially surrounded by closely attached trophozoites, but whether this occurred before or after the cell became detached from the epithelium, it is impossible to say. Many trophozoites, however, lie free in the mucus, probably moving slowly about by means of pseudopodia. It is evident that when the parasites are abundant the gills may be seriously injured and in one extreme case the ends of several adjoining filaments were entirely destroyed, the space being occupied by mucus filled with trophozoites and disintegrating tissue cells.

The food and nutrition of the parasite present an interesting problem. Other species of Myxosporidia live in the tissues or in cavities of the host, such as the gall and urinary bladders, and consequently, are surrounded by fluids containing nutritive substances, which they presumably absorb. There are, however, several instances (Davis 1916, p. 336) where myxosporidian trophozoites have been observed to ingest solid food particles so that this method of feeding is not impossible. Nevertheless, no evidence has been found that this occurs in *C. externum*, although special attention has been paid to this possibility. We must assume, therefore, that the only nutrient the trophozoites obtain is absorbed in liquid form from the surrounding medium. The surface of the gills, which are continually bathed by flowing water, would seem to afford little opportunity for the accumulation of nutritive fluids. However, it is possible that the mucus may have a tendency to retain such substances. Furthermore, it seems probable that trophozoites in close contact with the epithelium may absorb directly, products produced by lysing the cells and that the condition shown in figure 76 is not simply for support. On the contrary, the primary purpose may be to digest the cell to which the trophozite is attached. It is common to find trophozoites clinging to epithelial cells in various stages of disintegration although most of them do not enclose the cells as closely as shown in the figure.

General Discussion

One of the most interesting problems in connection with *C. externum* is the obvious fact that here we have a reversal of the usual course of evolution, although there is evidence that it may have occurred in other groups such as the nematodes. So far as the writer is aware, however, no other case of a similar change in its mode of life is known among the Protozoa.

It is interesting to find that within the Myxosporidia there are species which indicate how this transformation from an internal to an external parasite may have been brought about. Although most myxosporidians found on the gills are cyst-forming, this is not true of all species. *Myxosoma endovosa* occurs only in the form of ameboid trophozoites similar to those of *C. externum*. In this species, however, the trophozoites are found in the capillaries of the lamella in which they sporulate. The trophozoites of a second species *Myxobilatus* (Henneguya) *plasmodia* (Davis 1922) leave the capillaries and move about among the cells of the respiratory epithelium. However, as shown in figure 85, this species does not normally come into contact with the water which bathes the gills, although the epithelial cells covering the trophozoites may be stretched to a very thin envelope. In exceptional cases the enclosing membrane may be stretched to the breaking point, but this condition cannot be considered normal. Finally, the next step is illustrated by

C. externum, which has left the respiratory epithelium and the protection it affords in favor of an existence on the surface of the gill.

Family **Myxosmatidae** Poche

Myxosoma endovasa, n. sp.

[Plate 7, figs. 81, 84, and 86]

While examining sections of the gills of a smallmouth buffalo (*Ictiobus bubalus*) from the Mississippi River at Fairport, Iowa, small trophozoites of a myxosporidian were found in the capillaries of the lamellae. Both vegetative and sporulating trophozoites were abundant and it was evident that this species, unlike other myxosporidians, spends most of its life in the blood vessels.

The trophozoites usually occurred in the large capillary extending around the edge of the lamella. Their relation to the capillary could best be seen in cross sections of the lamella (fig. 84). In such sections the trophozoites were roughly crescent shaped with the convex side of the organism toward the edge of the lamella and in close contact with the capillary wall. On the concave side of the trophozoite blood corpuscles could frequently be seen and it was evident that at first the capillary was not completely blocked by the trophozoite and that the blood continued to circulate past the concave side. This was made possible by distension of the capillary and consequent thinning of the wall so that it was very difficult to distinguish. It is probable that the larger sporulating trophozoites (fig. 86) may eventually stop the movement of blood in the capillary.

The trophozoite is composed of a thin, dense layer of ectoplasm surrounding the finely granular endoplasm which contains a larger number of nuclei. The vegetative nuclei are larger with a nucleolus, and with the chromatin granules more distinct than in the smaller generative nuclei. No specially differentiated layer of cytoplasm can be distinguished around the latter until they become segregated to form the sporoblasts. The trophozoites are polysporous.

When viewed from above, the spore is nearly spherical and contains two relatively large pyriform polar capsules. The shell valves are thin and without markings. Spores preserved in formalin were about 9μ long with a width of 8μ. The polar capsules were 3.5 by 5μ.

Order HAPLOSPORIDIA Caullery and Mesnil

Dermocystidium salmonis, n. sp.

[Plate 6, figs. 59–66; plate 14, figs. 210–213]

The gills of an adult chinook salmon (*Oncorhynchus tschawytscha*) from the Sacramento River at Balls Ferry, Calif., and confined for several weeks in a holding pond at the Coleman hatchery, showed a number of small, white cysts on the filaments. As they closely resembled myxosporidian cysts, it was assumed at first that they were formed by a member of this group. This was soon disproved by an examination of the contents. Although the cysts were filled with spherical bodies about the size of myxosporidian spores, the resemblance was only superficial, the internal structure being very different. Instead of the conspicuous polar capsules the greater part of each spore was occupied by a large, rounded refringent body which is the distinguishing character of the genus *Dermocystidium*.

Several species of this genus have been described from fish and amphibians in Europe, but none have been reported previously from this country. Two of the four European species occur in the skin of frogs and salamanders while the other two species are fish parasites. Dunkerly (1914) found the cysts of a *Dermocystidium* on the gills of the brown trout (*Trutta fario*) in Ireland which he believed was identical with a species previously described by Perez from salamanders.

A little later, Leger (1914) found the same parasite on gills of trout in the Alps. He showed that the species is distinct from that on salamanders and called it *Dermocystidium branchialis*. Recently Jirovec (1940) has described another species *D. vejdovskyi* which occurs on the gills of *Esox lucius*. This species differs in several respects from that found on trout, especially in the structure of the cysts. Jirovec believes that a parasite of *Daphnia magna* described by Ruhberg (1933) should be included in the genus.

There is no doubt that the species found on salmon is distinct from any of the European forms and con-

sequently the name *Dermocystidium salmonis* is proposed for it. Unfortunately, only preserved material was available for study.

The cysts (fig. 210) are located on the sides of the gill filaments where they occupy a cup-shaped cavity between the lamellae. They are rounded, glistening white and may reach a diameter of one millimeter. The mature cysts are easily dislodged from the gills and then can be seen to be composed of a thin transparent membrane surrounding a mass of spores. The loose connection of the cysts with the gill tissues sharply differentiates them from myxosporidian cysts which are attached more firmly. In section (fig. 212) the cyst wall can be seen in close contact with epithelial cells which surround it on all sides. From a study of young cysts (fig. 213) it appears that they develop in the thick epithelial layer between the bases of the lamellae. As the cyst grows the epithelial cells increase and there is also some hyperplasia in the epithelium of adjoining lamellae. This results in fusion of the lamellae to form the characteristic cup surrounding the cyst (fig. 211). Sometimes not more than three or four lamellae on each side are involved, in other cases as many as ten or twelve lamellae may be affected. Even the fully matured cyst is entirely surrounded by epithelial cells, although on the outer side they may be stretched to a single layer of thin, flattened cells. In sections, the cyst wall is more or less wrinkled and distorted, evidently due to shrinkage in the course of preparation.

In fully matured cysts the contents are composed of spherical spores, each containing a large, rounded body which in formalin material is homogeneous and highly refringent. The spores (fig. 66) vary considerably in size, the majority being about 8 to 10μ in diameter, but a few may reach a diameter of 12μ. The rounded body within the spore is about 6 to 7μ in diameter. Each spore is surrounded by a thin, transparent, structureless membrane without markings of any kind. The rounded body is always located excentrically, the wider space between the body and the spore wall being occupied by the nucleus, surrounded by cytoplasm which in this region is denser than in other parts of the spore. The nucleus is of the vesicular type with a large deeply stained karyosome surrounded by a clear halo. The rounded body stains deeply with plasma stains such as eosin and Bordeaux red. It does not stain with Sudan III and stains a light pink with scarlet R. It fails to give the reaction for glycogen in Lugol's solution.

In two instances young cysts were found in which spores had not yet developed. One was only 60μ in diameter (fig. 213) with a comparatively small number of cells; the other was somewhat larger. Both cysts contained only loose, rounded cells. Each cell contained one or two nuclei (figs. 59–64) and was completely separated from its neighbors. There was nothing resembling a multinucleate plasmodium such as was found in *D. vejdovskyi* by Jirovec. The cells appeared to be multiplying rapidly, the nucleus first dividing to form a binucleate cell (figs. 62 and 63) which later divided into two equal parts (fig. 65). Several dividing binucleate cells were observed, but no case of nuclear division could be found. With the exception of the number of nuclei, the cells all showed the same structure. The cytoplasm was vacuolated with a central nucleus containing a large rounded karyosome. The karyosome appeared to be surrounded by achromatic material which was more abundant on one side and extended in strands to the nuclear wall.

All other cysts were much larger and the bulk of the contents was composed of fully developed spores. In several cysts there were certain areas containing cells which were evidently developing into spores. Various stages in the transformation of sporoblasts into spores (fig. 212) could be found without difficulty. The smallest sporoblast contained a central nucleus surrounded by granular vacuolated cytoplasm and were similar to the cells in young cysts, but smaller. Somewhat larger cells contained one or two—sometimes more—small, rounded, refringent bodies which are the forerunners of the large spheroidal bodies in the mature spore.

The history of the spores after leaving the cyst is unknown. A number of free spores were noticed on the gills which had evidently become entangled in the mucus, but they were in the same condition as when they left the cyst.

Since only the encysted stage of the various species of *Dermocystidium* is known and the structure of the spore is essentially the same in all, it is evident that characters for distinguishing the different species are very limited. Nevertheless, there can be little doubt that *D. salmonis* is specifically distinct from the European species. In *D. branchialis* the spore is smaller, with a diameter of 7μ to 8μ. The most marked difference, however, is in the character of the cyst wall, which is much thicker than in *D. salmonis*. Furthermore, there is no such regular arrangement of the lamellae surrounding the cyst. Also, according to Dunkerly,

the protoplasm in the cyst is only indistinctly divided into cells, which later become the sporoblasts. In *D. vejdovskyi* there is an even greater difference in the structure of the cyst, which is divided by a thin structureless membrane into numerous compartments, each containing one or more multinucleate plasmodia. The latter divide into uninucleate sporoblasts which develop into spores much as in *D. salmonis*.

There is much difference of opinion regarding the affinities of the genus. Alexeieff and Grassi have suggested a relationship to *Blastocystis*, but this appears to be based on a superficial resemblance rather than the possession of any fundamental characters in common. Leger, on the other hand, links *Dermocystidium* with the Haplosporidia which is such a heterogeneous group with indefinite limitations that it can easily be stretched to accommodate almost any taxonomic orphan. To the author it appears fruitless to speculate about the affinities of the genus until something is known regarding other stages in the life cycle.

CONTROL MEASURES

The control of ectoparasitic Protozoa presents no serious problem when fish are held in tanks or small pools, but in large ponds or under natural conditions little can be done to reduce their numbers. The most practicable method of control is to place the fish in a solution which will kill the parasites without material injury to the host. A number of chemicals have been used for this purpose, the most common being salt and acetic acid. Immersion of the fish in a 3 percent solution of salt (sodium chloride) for 3 to 10 minutes will kill or so weaken most protozoans that they will leave the host. A more effective treatment, however, is to dip the fish in a 1 to 500 solution of glacial acetic acid. Both of these methods necessitate considerable handling of the fish which, especially when several treatments are required, may be quite injurious. Furthermore, when large numbers of fish are to be treated the dipping method requires much time and labor.

Recently Fish (1940, 1940a) has developed a formalin treatment, especially designed for use in raceways and small pools, which is the most effective means yet devised for ridding fish of ectoparasitic Protozoa. In this treatment the fish are held for 1 hour in a solution composed of 1 part formalin (40 percent formaldehyde) to 4,000 parts water. The formalin is added directly to the tank or pool containing the fish. Since it diffuses very slowly through the water, special precautions must be taken to obtain a uniform concentration. An effective method is to dilute the formalin 1 to 100 and then spread the solution as uniformly as possible over the surface of the water. If the fish are crowded it will be necessary to aerate the water during treatment. This treatment will kill all external protozoans and will not injure the fish appreciably unless they have already been greatly weakened by the parasites.

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EXPLANATION OF PLATES

Plates 1 to 7 are from figures by the author, drawn to scale with the aid of the camera lucida. Plates 7 to 14 are from photomicrographs by the author. The approximate magnification is given for each figure.

ABBREVIATIONS

ad. s., adoral spiral

ba., bacteria

bl., blepharoplast

b. m., border membrane

c. g., ciliary girdle

cil., cilia

cp., cytopharynx

c. v., contractile vacuole

c. v. d., duct from contractile vacuole

dt., denticle

fl., flagellum

la., gill lamella

m., mouth

ma., macronucleus

mb., membranelle

m. c., marginal cilia

mi., micronucleus

mng., macronuclear anlagen

my., myoneme

n., nucleus

p. b., peristome border

scp., scopula

st., stigma

st. b., striated band

tr., trichites

ve., velum

vt., vestibule

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ILLUSTRATIONS

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PLATE 1

FIGURES 1–4.—Free-swimming individuals of *Colponema agitans*. The nucleus and elongated blepharoplast are deeply stained. × 2,500.

FIGURE 5.—Three individuals of *C. agitans* attached to gill epithelium. Drawn from section. × 2,500.

FIGURE 6.—*Bodomonas concava* attached to gill epithelium by posterior flagellum. × 2,500.

FIGURE 7.—*B. concava* attached to epithelial cell in position usually found when first placed on slide. Drawn from whole mount. × 2,500.

FIGURE 8.—Side view of *C. agitans* attached to epithelium. Drawn from section. × 2,500.

FIGURE 9.—Attached form of *B. concava* viewed from flattened ventral side. The blepharoplast is in thinner part of body. × 2,500.

FIGURE 10.—Group of *B. concava* attached to epithelium in usual position. Drawn from section. × 2,000.

FIGURES 11–13.—Free-swimming specimens of *B. concava*. Drawn from whole mount. × 2,500.

FIGURE 14.—Living *B. concava* attached to gill. × 2,500.

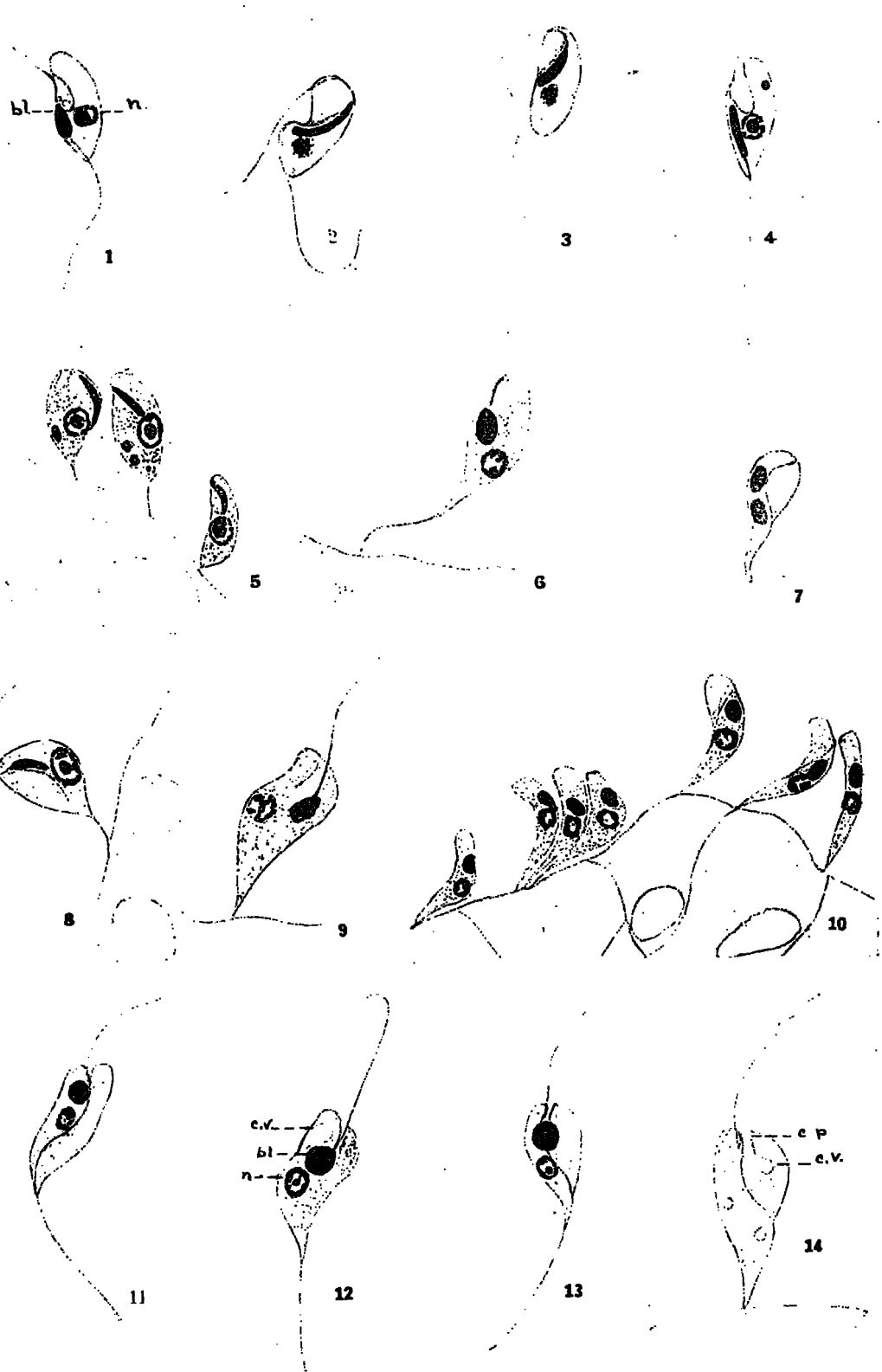
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PLATE 2

FIGURE 15.—Free-swimming form of *Lamellasoma bacillaria*. × 2,500.

FIGURE 16.—Attached form of *L. bacillaria* viewed from the side. Drawn from unstained specimen killed in osmic vapor. × 1,340.

FIGURE 17.—Attached form of *L. bacillaria* viewed from above. × 2,500.

FIGURE 18.—Free-swimming form of *L. bacillaria* drawn from unstained specimen killed in osmic vapor. × 1,340.

FIGURES 19 and 20.—*Euglenosoma branchialis* to show spiral twist to body and ventral groove. Internal structure is not shown. × 2,000.

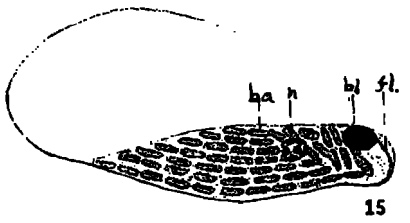
FIGURES 21 and 23.—Free-swimming form of *E. branchialis*. × 2,500.

FIGURE 22.—*E. branchialis* attached to gill epithelium. × 2,500.

FIGURE 24. Section of *Amphileptus voracus* through mouth. Only one macronucleus in section.

FIGURE 25. *A. voracus*, drawn from specimen preserved in formalin. × 1,000.

FIGURE 26. Section of *A. voracus* showing both macro- and micronuclei. × 1,000.



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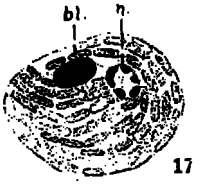

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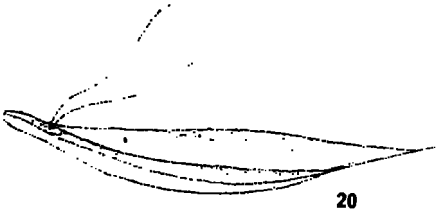

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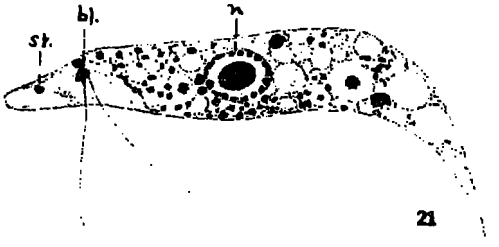

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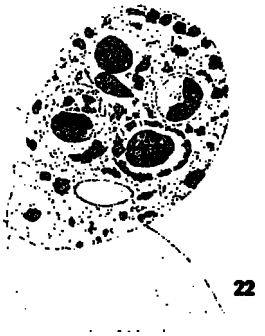

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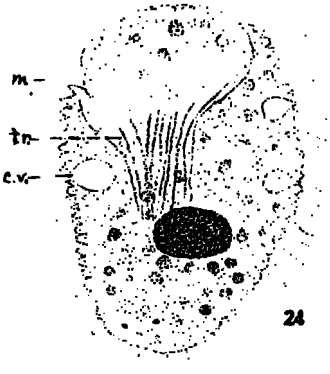

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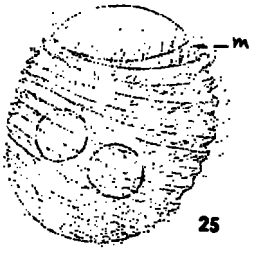

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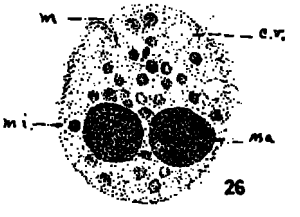

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PLATE 3

FIGURE 27.—Section of *Trichodina discoidea* through mouth. × 1,340.

FIGURE 28.—Part of denticulate ring, *T. discoidea*. × 2,000.

FIGURES 29 and 30.—Sections of *T. discoidea*. × 1,340.

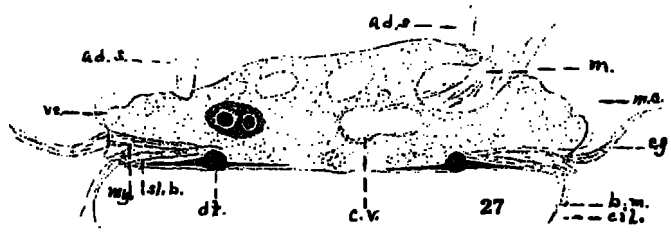
FIGURE 31.—Section of *T. bulbosa*. × 1,340.

FIGURE 32.—Section of *T. vallata*. × 1,340.

FIGURE 33.—*T. bulbosa* viewed from side with adoral spiral. Drawn from whole mount. × 670.

FIGURE 34.—Section *T. platyformis*. × 1,000.

FIGURE 35.—*T. bulbosa*, seen from opposite side to that shown in figure 33. × 670.



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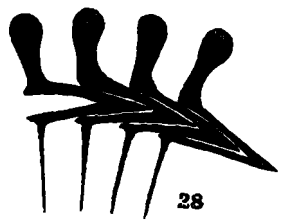
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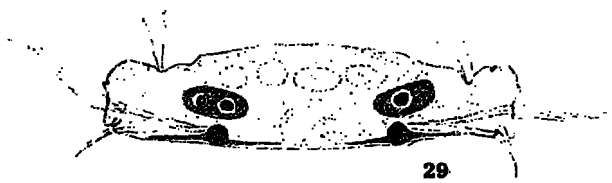
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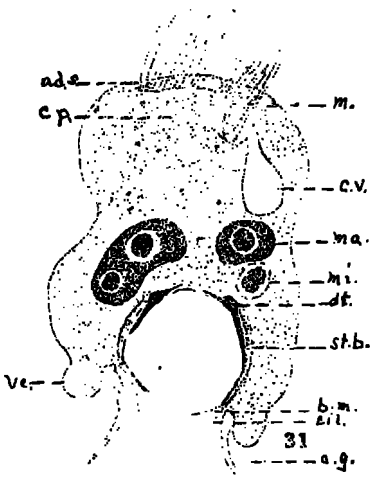

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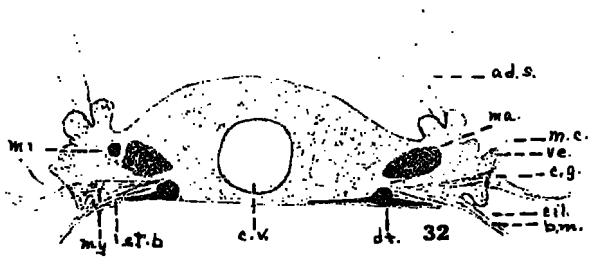

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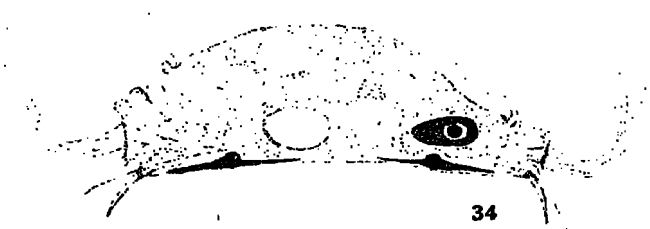
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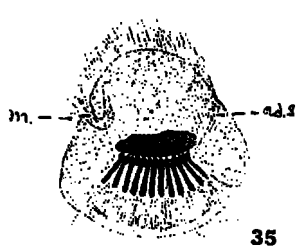

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PLATE 4

FIGURE 36.—Section of *Trichodina tumefaciens* through mouth. × 1,340.

FIGURE 37.—Section of *T. tumefaciens* showing usual shape of body. × 1,340.

FIGURE 38.—Side view of *T. bursiformis* drawn from whole mount. × 1,340.

FIGURE 39.—Section of *T. californica*. An epithelial cell is being taken into the mouth. × 1,000.

FIGURE 40.—Section of *T. bursiformis*. × 1,340.

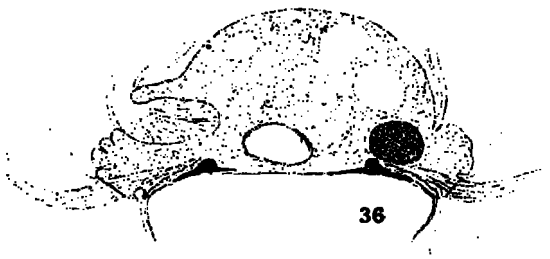
FIGURE 41.—Section of *T. californica* with flattened adoral surface. × 1,000.

FIGURE 42.—Side view of *T. bursiformis*. Drawn from unstained specimen killed in osmic vapor. × 670.

FIGURE 43.—Section of *T. californica* with bulk of body on one side of adhesive disk. × 1,000.

FIGURE 44.—Part of denticulate ring of *T. bursiformis*. × 2,000.

FIGURE 45.—Part of denticulate ring of *T. californica*. × 2,000.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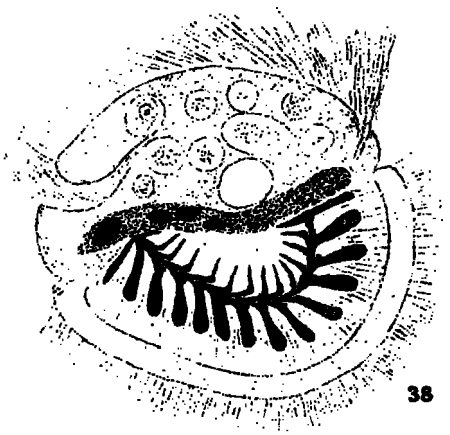
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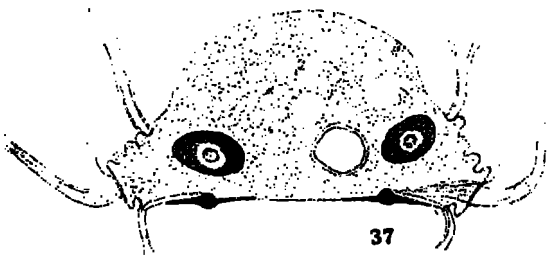
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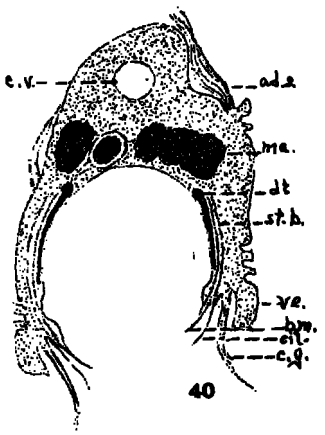

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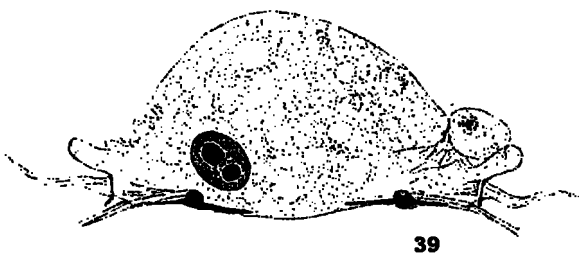
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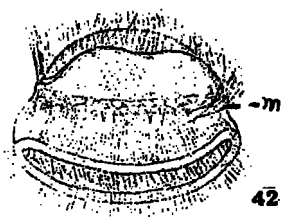

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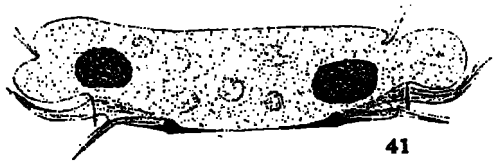
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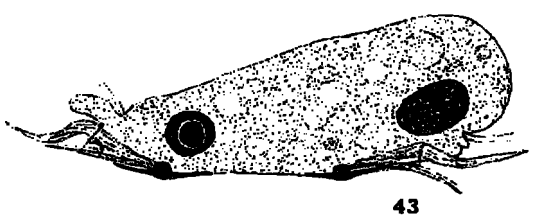
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PLATE 5

FIGURES 46 and 48.—Sections through part of body of *Trichodina fultoni*. × 1,340.

FIGURE 47.—Section through body of *T. fultoni*. Note food vacuole containing epithelial cells. × 670.

FIGURES 49, 50, and 51.—Sections of *T. symmetrica*. In fig. 50 the micronucleus can be seen by the side of the macronucleus with the contractile vacuole on the other side. × 1,340.

FIGURE 52.—Section of *T. symmetrica* through mouth. Such extreme distension of the cytopharynx is very unusual. × 1,340.

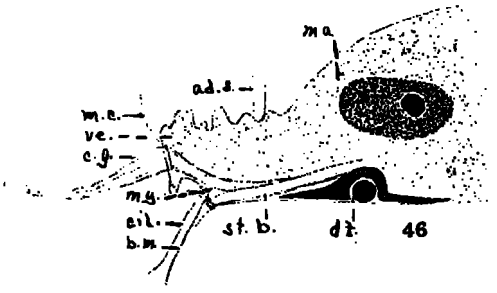
FIGURE 53.—Part of denticulate ring, *T. truttae*. × 1,000.

FIGURE 54.—Part of denticulate ring *T. fultoni*. × 1,000.

FIGURES 55 and 56.—Sections of *T. myakkae*. × 1,340.

FIGURE 57.—Adoral view of *T. myakkae*. × 1,340.

FIGURE 58.—Part of adhesive disk of *T. myakkae*, Semi-diagrammatic. × 2,000.



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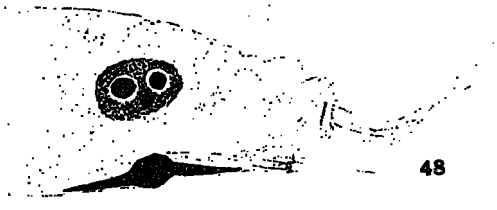
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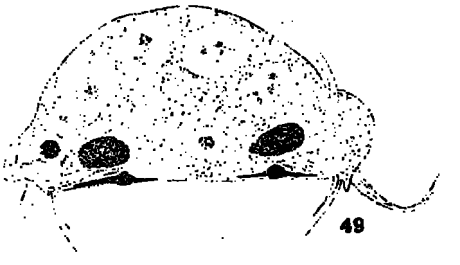
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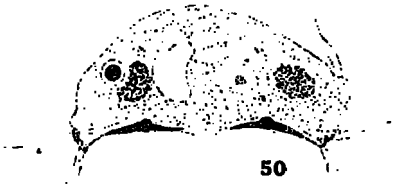
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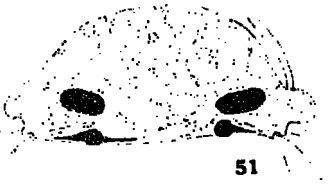
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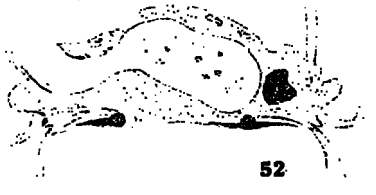
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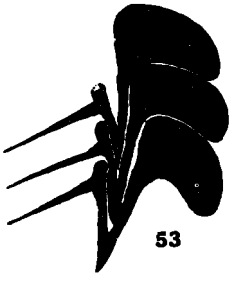
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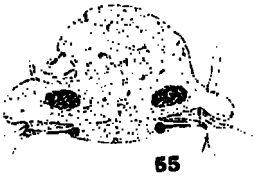
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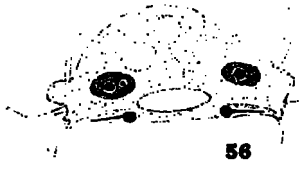
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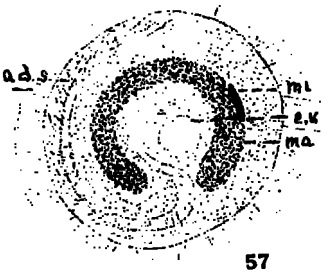

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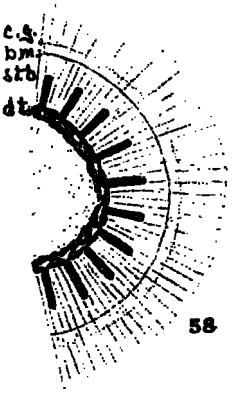

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PLATE 6

FIGURES 59–63.—Cells from young cyst of *Dermocystidium salmonis*. × 2,500.

FIGURE 64.—Developing spore of *D. salmonis*. Drawn from formalin material. × 1,340.

FIGURE 65.—Dividing binucleate cell of *D. salmonis*. × 2,500.

FIGURE 66.—Mature spore *D. salmonis*. × 2,500.

FIGURE 67.—Adoral view of peristome of *Scyphidia macropodia*. Semidiagrammatic. × 1,340.

FIGURE 68.—Small form of *S. macropodia* with micronucleus in anaphase. × 1,000.

FIGURE 69.—*S. macropodia*. Drawn from whole mount. × 1,000.

FIGURE 70.—Section of *S. macropodia* during conjugation. The microconjugant has 8 micronuclei, the macroconjugant, 4 micronuclei. × 1,000.

FIGURE 71.—Section of expanded *S. macropodia*. Part of the macronucleus was in the next section. × 1,000.

FIGURE 72.—Section of *S. macropodia* through vestibule. Only part of macronucleus is shown. × 1,000.

FIGURE 73.—Section of *Trichophrya ictaluri* attached to lamella. The parasite is in contact with the capillary network which forms the middle layer of the lamella. The epithelium on the opposite side of the lamella is not shown. × 1,340.

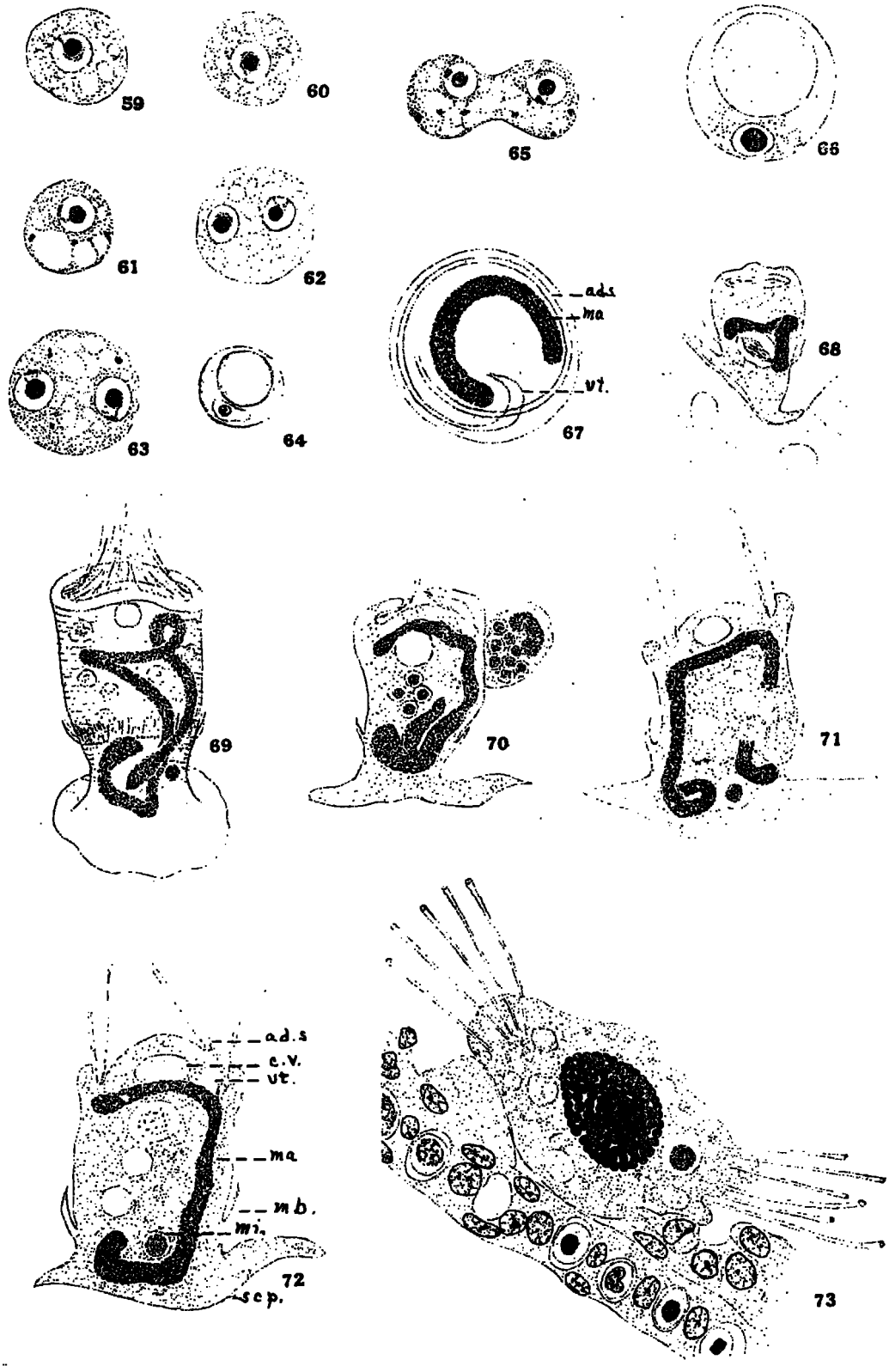
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PLATE 7

FIGURE 74.—Trophozoite of *Chloromyxum externum*. The granular endoplasm is surrounded by the hyaline ectoplasm. Drawn from living organism. × 1,340

FIGURE 75.—Similar to figure 74, but drawn from a stained specimen. Each generative nucleus is surrounded by a dense layer of cytoplasm. × 1,340.

FIGURE 76.—Trophozoite of *C. externum* closely attached to epithelial cell. × 1,340.

FIGURE 77.—Sporulating trophozoite of *C. externum* with two spores, one not yet fully developed. × 1,340.

FIGURE 78.—Trophozoite of *C. externum* attached to epithelium of lamella. The epithelial cells are disintegrating. × 1,340.

FIGURE 79.—Trophozoite of *C. externum* more highly magnified. × 2,500.

FIGURE 80.—Spore of *C. externum* viewed at right angles to sutural plane. × 1,340.

FIGURE 81.—Spore of *Myxosoma endovasa*. × 1,340.

FIGURE 82.—Trophozoite of *C. externum* in epithelium at edge of lamella. × 1,340.

FIGURE 83.—Trophozoite of *C. externum* attached to epithelium of gill filament × 1,340.

FIGURE 84.—Cross section of edge of gill lamella showing trophozoite of *Myxosoma endovasa* in capillary. × 1,340.

FIGURE 85.—Section of lamella with trophozoite of *Myxobilatus plasmodia* in epithelium. × 1,340.

FIGURE 86.—Section of edge of lamella showing capillary with vegetative and sporulating trophozoites of *M. endovasa*. × 1,340.

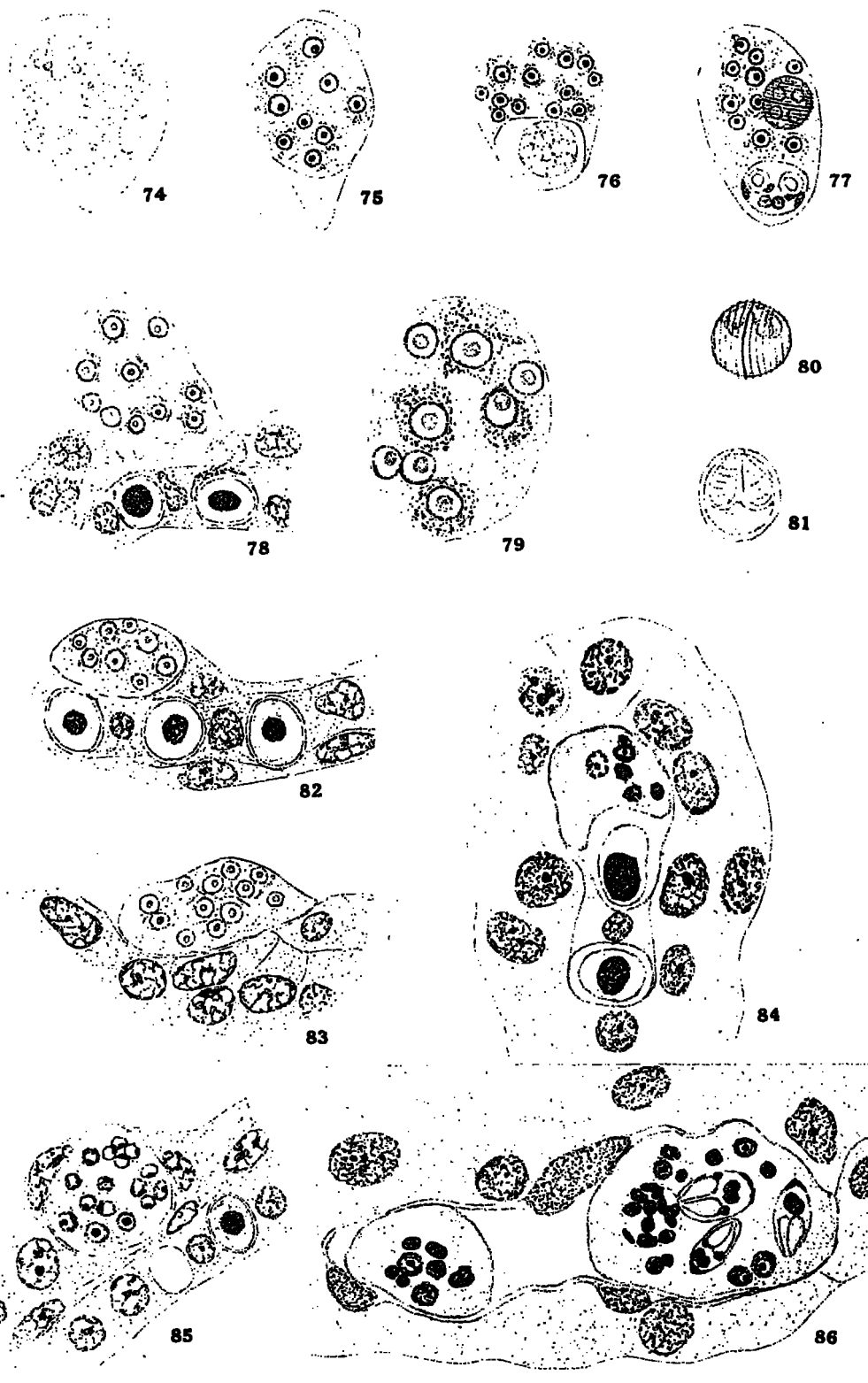
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PLATE 8

FIGURE 87.—Section of gill epithelium infected with *Bodomonas concava*. × 1,270.

FIGURES 88 and 89.—Sections of gill epithelium infected with *Colponema agitans*. × 1,270.

FIGURES 90, 94, and 95.—Free-swimming form of *Lamellasoma bacillaria*. From whole mounts. × 1,270.

FIGURE 91. *L. bacillaria* attached to epithelial cell. From whole mount. × 1,270.

FIGURES 92 and 93. Attached form of *L. bacillaria* viewed from side. From whole mounts. × 1,270.

FIGURE 96. Attached form of *L. bacillaria* viewed from above. From whole mounts. × 1,270.

FIGURES 97 and 98. *Euglenosoma branchialis*. From whole mounts. × 1,270.

FIGURE 99.—Section of *Amphileptus voracus* nearly surrounded by epithelial cells. Only one macronucleus is shown. × 680.

FIGURE 100. Section of *A. voracus* which has just ingested a trichodinid. The endoplasm is filled with metaplastic bodies. × 680.

FIGURE 101.—Section of *A. voracus* showing small micronucleus at end of macronucleus. × 680.

FIGURE 102. Section of *A. voracus* showing concentric ridges on upper side. × 680.

FIGURE 103. Section of *A. voracus* showing macronuclei just beginning to divide. The organism has already divided into two daughter cells, but this is not visible in photograph. × 680.

FIGURE 104. Later stage in division of macronucleus. × 680.

FIGURE 105. Section of *A. voracus* attached to lamella showing both macronuclei in resting stage. × 680.

FIGURE 106.—Section of one side of body of *A. voracus* showing ridges running in two directions. × 680.

FIGURE 107.—Whole mount of *A. voracus*. × 680.

FIGURE 108.—Section of *A. voracus* showing one end of organism with mouth opening and transverse ridges containing trichocysts. × 680.

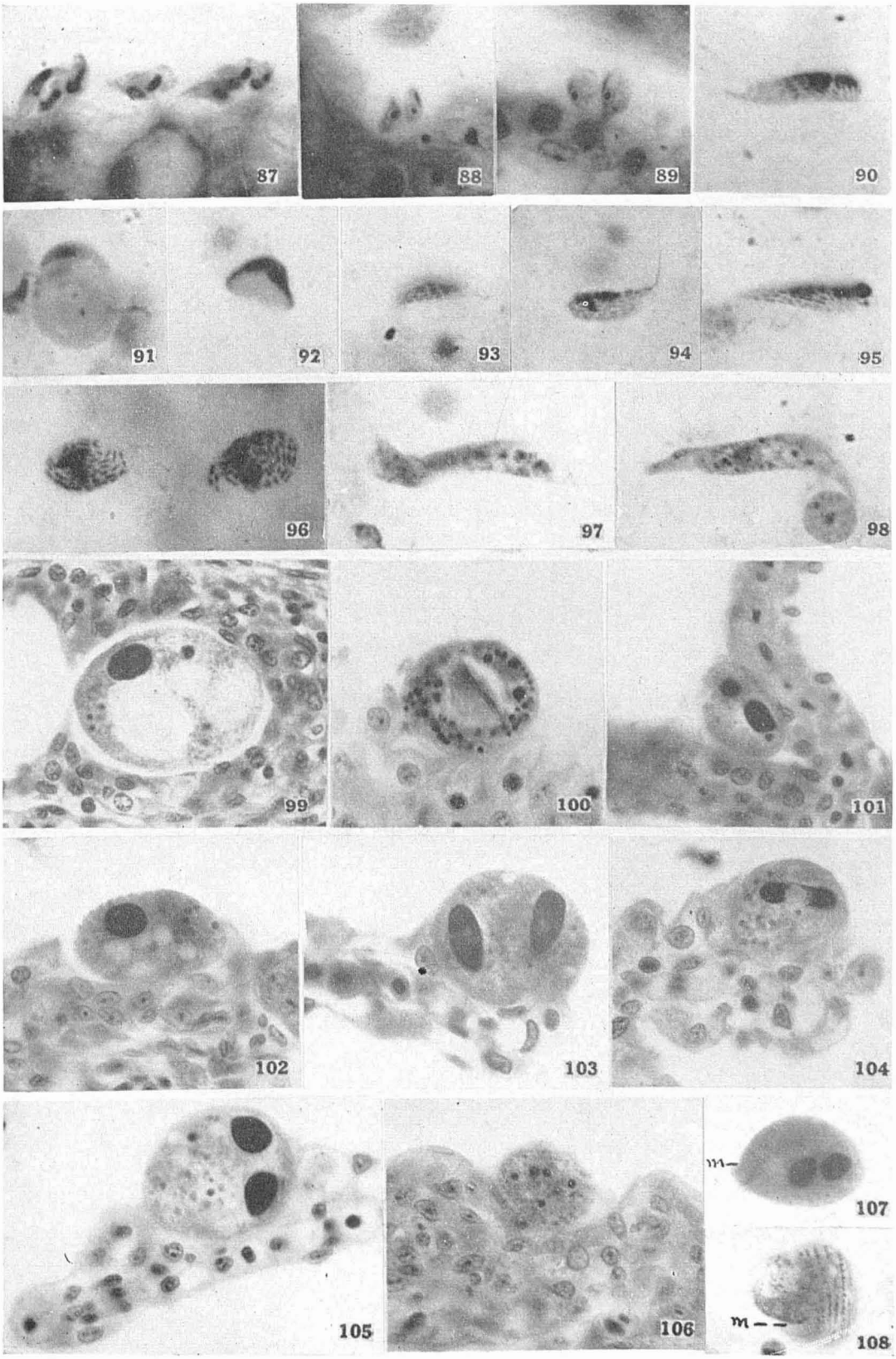
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PLATE 9

FIGURE 109.—Adhesive disk of adult *Trichodina discoidea* showing denticulate ring encircled by striated band. Outside the striated band is the faintly striated border membrane. × 680.

FIGURE 110.—Adhesive disk of a somewhat younger stage of *T. discoidea*. Alternate bars in the striated band are less distinct and the hooks and rays of the denticles are more slender than in figure 109. × 680.

FIGURES 111 and 112.—Section of *T. discoidea* attached to gill epithelium. × 680.

FIGURES 113 and 114.—Adhesive disks of juvenile *T. discoidea*. The new bars in the striated band are just beginning to appear. × 680.

FIGURES 115 and 116.—Sections of *T. discoidea* on gills. × 680.

FIGURES 117 and 119.—Sections of *T. platyformis*. × 680.

FIGURE 118.—Section of *T. fultoni* showing duct from contractile vacuole to exterior. × 680.

FIGURE 120.—Section of *T. fultoni* ingesting an epithelial cell. × 680.

FIGURE 121.—Adhesive disk of adult *T. platyformis*. × 680.

FIGURE 122.—Adhesive disk of *T. fultoni*. × 510.

FIGURE 123.—Section of *T. vallata* showing extreme development of ridge bearing adoral spiral. × 680.

FIGURE 124.—Section of *T. vallata* with less prominent adoral ridge. × 680.

FIGURE 125.—Part of adhesive disk of *T. fultoni* to show structure of denticulate ring and striated band. × 680.

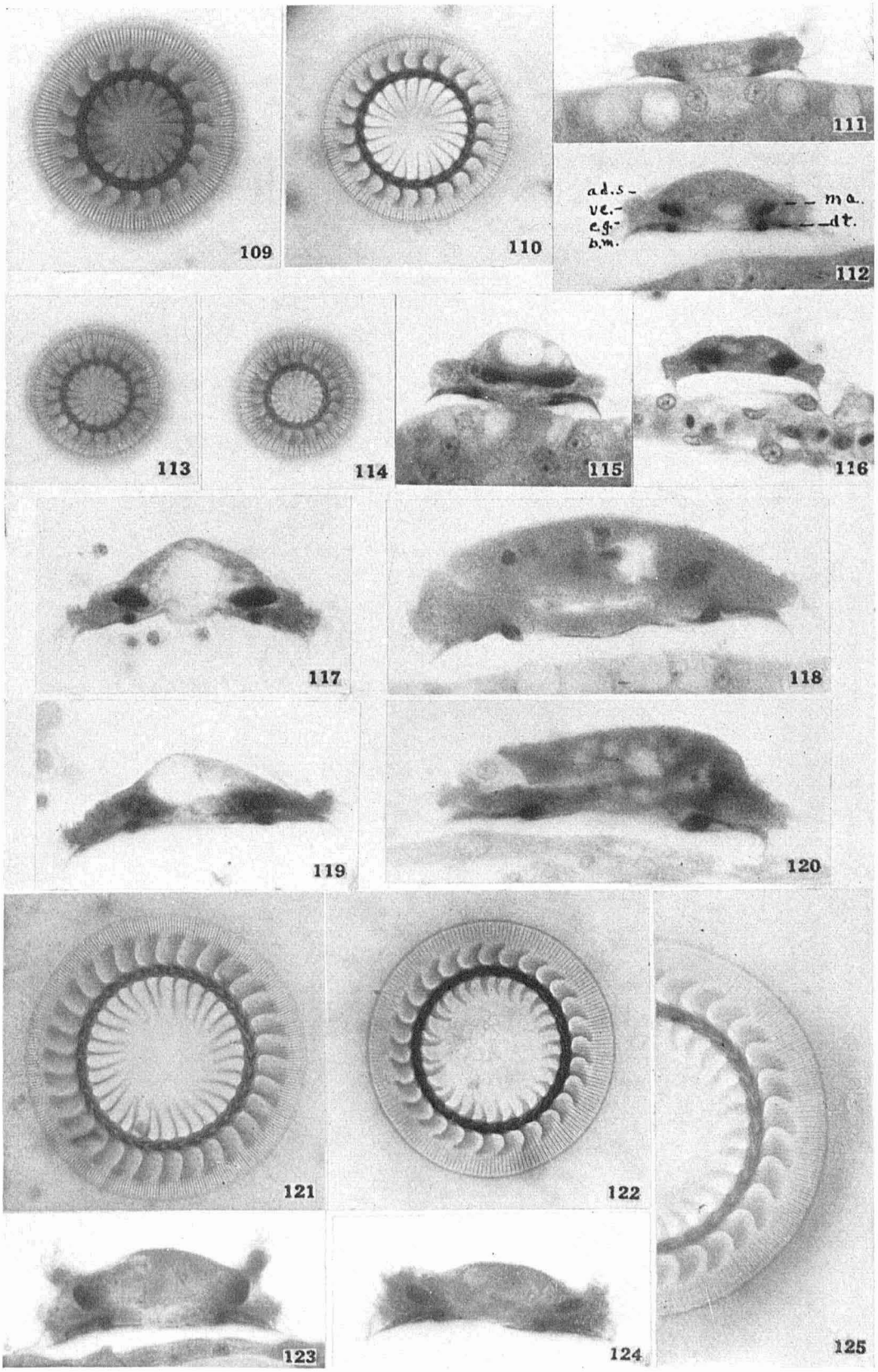
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PLATE 10

FIGURE 126.—Adhesive disk of *Trichodina vallata*. × 680.

FIGURE 127.—*T. fultoni* from adoral side to show separate openings of cytopharynx and duct from contractile vacuole. × 340.

FIGURE 128.—Part of adhesive disk of *T. platyformis* with lens focused just below striated band to show myonemes extending to ciliary girdle. × 680.

FIGURES 129 and 130.—Adhesive disks of *T. truttae*. × 340.

FIGURES 131 and 132.—Adhesive disks of *T. californica*. × 680.

FIGURE 133.—Surface view of *T. californica* covered by deposit of amorphous material. × 680.

FIGURE 134.—Adoral view of *T. symmetrica*. The small micronucleus can be seen near the lower arm of the macronucleus. × 680.

FIGURES 135 and 136.—Sections of *T. symmetrica*. × 680.

FIGURE 137.—Section of *T. californica* filled with amorphous material. The macronucleus has been broken across one arm. × 680.

FIGURES 138 and 139.—Adhesive disks of *T. symmetrica*. × 680.

FIGURE 140.—Section of normal *T. californica* through mouth opening. × 680.

FIGURES 141 and 142.—Sections of *T. tumefaciens*.

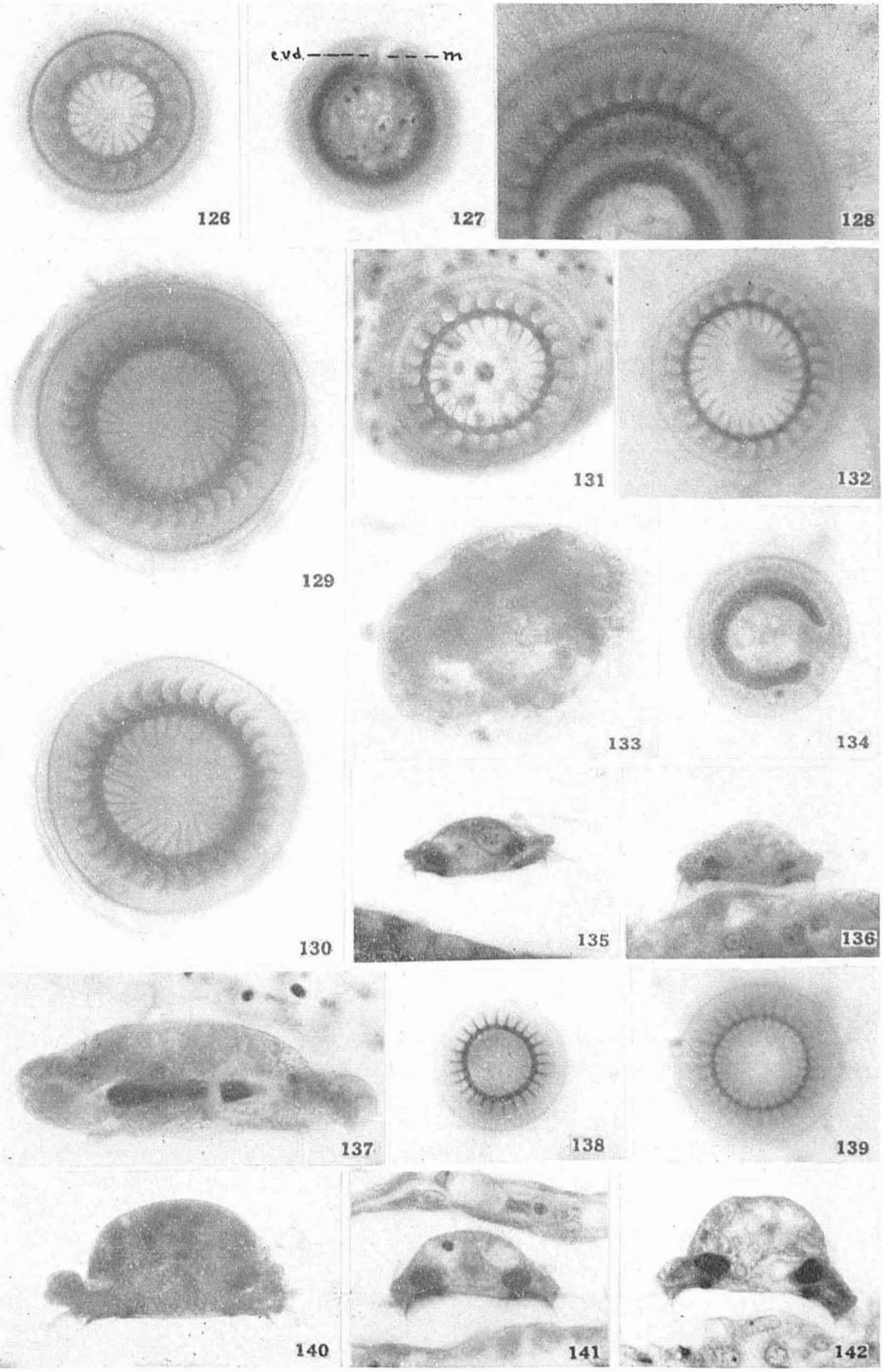
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PLATE 11

FIGURES 143 and 144.—Adhesive disks of juvenile *Trichodina tumefaciens*. × 680.

FIGURE 145.—Adhesive disk of intermediate stage, *T. tumefaciens*. × 680.

FIGURE 146.—Adhesive disk of adult *T. tumefaciens*. Hooks and rays of denticles are much broader than in earlier stages. × 680.

FIGURE 147.—Section of *T. tumefaciens* showing opening of contractile vacuole to exterior. The mouth is at a lower focus. × 680.

FIGURE 148.—Section of *T. tumefaciens* with most of body at one side of adhesive disk. × 680.

FIGURE 149.—Side view of *T. bulbosa* with adoral dome more elongate than usual. × 680.

FIGURES 150 and 151.—Adhesive disks of *T. bulbosa*. × 680.

FIGURE 152.—View of *T. bulbosa* from side showing adoral spiral. × 680.

FIGURE 153.—*T. bulbosa* from side opposite to that shown in figure 152. × 680.

FIGURES 154–157.—Sections of *T. bulbosa*. × 680.

FIGURE 158.—Adhesive disk of *T. bursiformis*. × 680.

FIGURE 159.—Side view of *T. bursiformis*. × 680.

FIGURES 160 and 161.—Sections of *T. bursiformis* attached to lamellae. × 680.

FIGURE 162.—*T. bursiformis* seen from side opposite to that shown in figure 159. × 680.

FIGURE 163.—Side view of *T. bursiformis* photographed at higher focus to show opening formed by edges of adhesive disk. × 680.

FIGURE 164.—Section of *T. bursiformis* attached to edge of lamella. × 680.

FIGURE 165.—*T. bursiformis* viewed from adoral side. The dark circle at a lower focus is the outline of the adhesive disk. × 680.

FIGURE 166.—Adhesive disk of *T. myakkae*. × 680.

FIGURES 167–169.—Sections of *T. myakkae*. × 680.

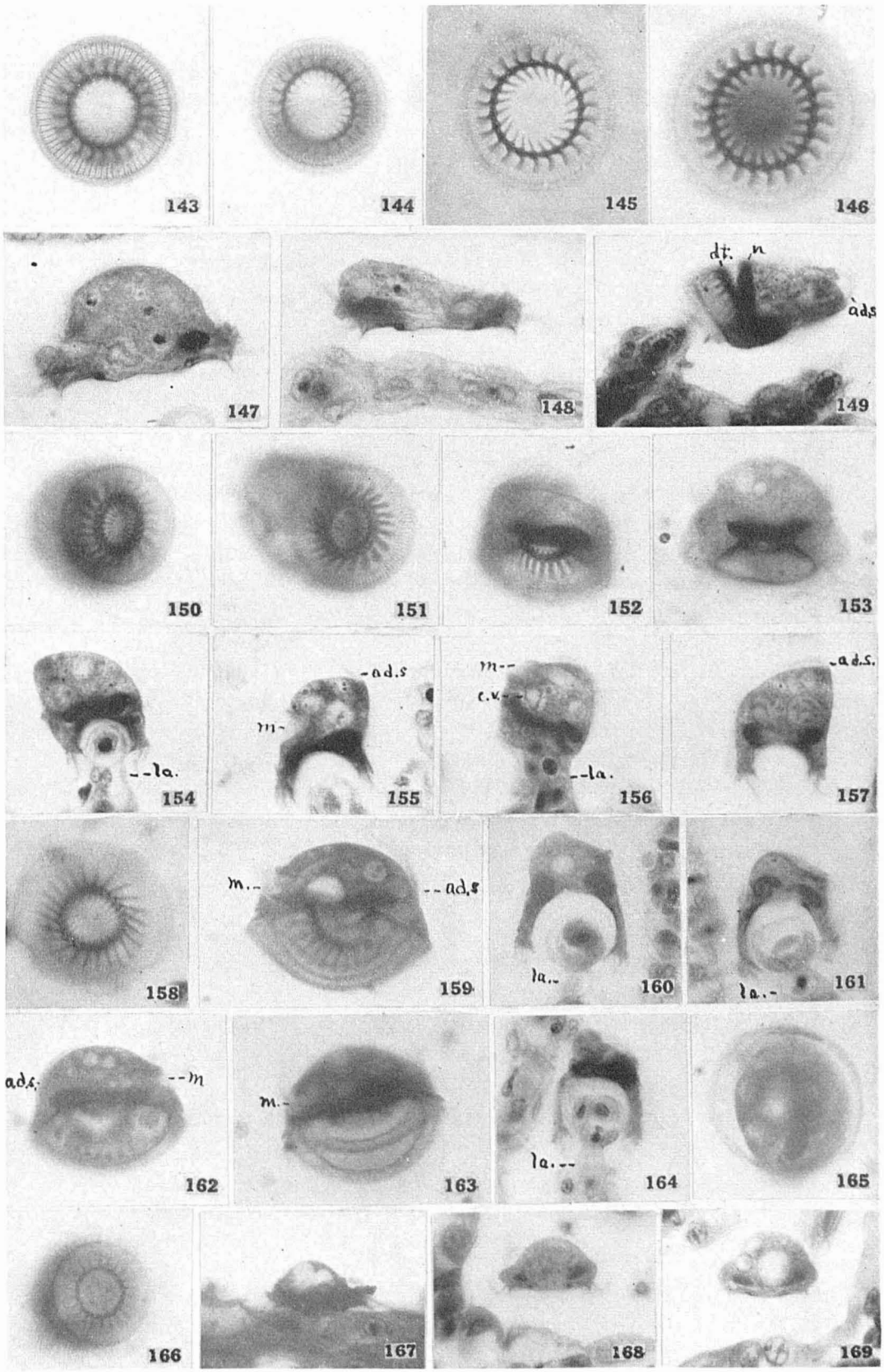
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PLATE 12

FIGURES 170–175.—Successive stages in division of the adhesive disk of *Trichodina fultoni*.

FIGURE 170.—Early stage, showing split in striated band, but denticulate ring not yet affected. The dark line in the striated band is the new denticulate ring formed of overlapping plates. × 510.

FIGURE 171.—Later stage showing division of denticulate ring. The dividing macronucleus can be seen indistinctly at a lower focus. × 510.

FIGURE 172.—Daughter individual formed by fission in which denticulate ring is just closing. × 510.

FIGURE 173.—Slightly later stage than figure 172. New denticulate ring of overlapping plates can now be seen clearly. The old ring is being resorbed. × 510.

FIGURE 174.—Later stage with old denticulate ring nearly resorbed. Hooks are forming on the new ring, but the rays have not yet appeared. × 510.

FIGURE 175.—Still later stage in which development of new denticulate ring is nearly completed. Remains of the old ring are still visible. The first indication of new bars can be seen in the striated band. × 510.

FIGURE 176.—Adhesive disk of *T. discoidea* in early stage of fission. × 680.

FIGURE 177.—Binary fission in *T. symmetrica* showing dividing macronucleus. Just below is the micronucleus in later stage of division. Photographed from section. × 680.

FIGURE 178.—Dividing micronucleus of *T. symmetrica*. The macronucleus in adjoining section formed a rounded mass, but had not yet started to divide. × 680.

FIGURES 179–181.—Formation of new denticulate ring in *T. discoidea* shortly after fission. × 680. Compare with figures 172 to 175.

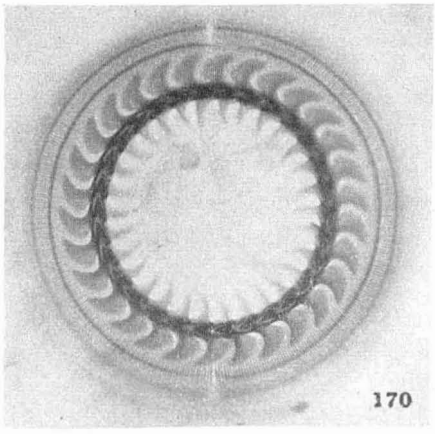
FIGURE 182.—Section of conjugating *T. discoidea* in early stage. The macronuclei have not yet begun to break down. × 680.

FIGURE 183.—Section of conjugating *T. tumefasciens* in same stage as figure 182. × 680.

FIGURE 184.—Section of conjugating *T. symmetrica* in same stage as above. × 680.

FIGURE 185.—Section of conjugating *T. bulbosa* at a somewhat later stage than in preceding figures. The macronuclei have now broken down into rounded bodies. × 680.

FIGURE 186.—Section of conjugating *T. discoidea* in late stage. A dividing micronucleus in late anaphase can be seen in lower conjugant. × 680.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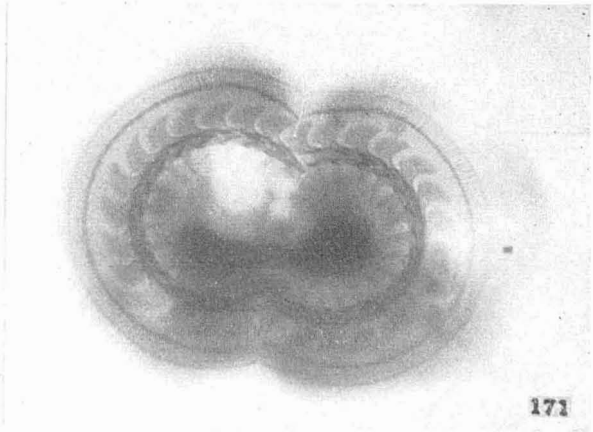
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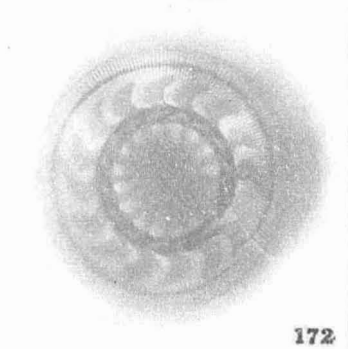
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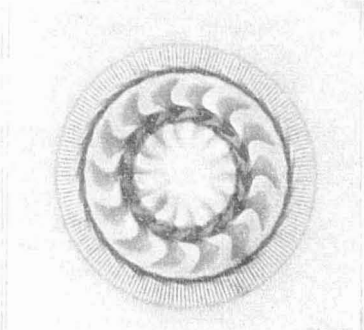
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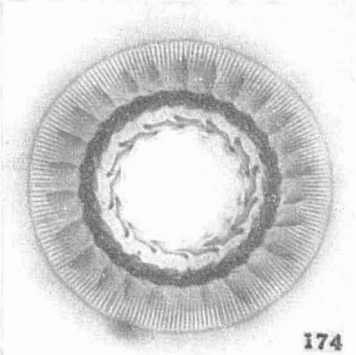
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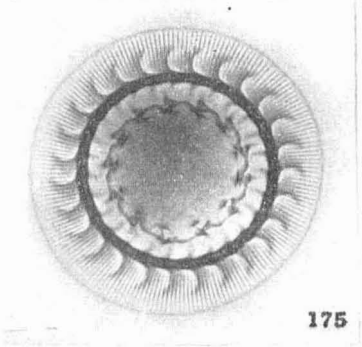
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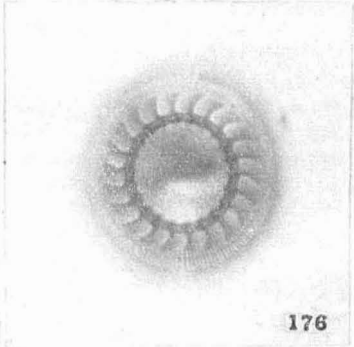
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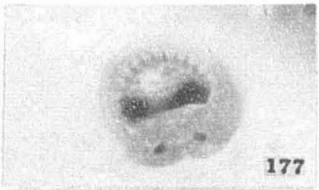
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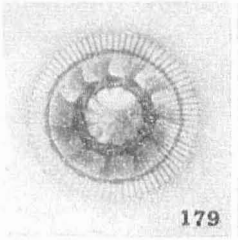
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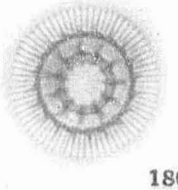
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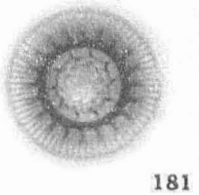
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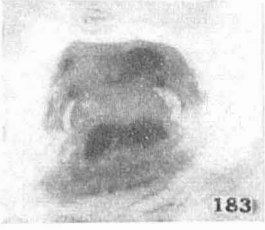
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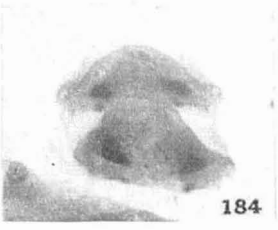
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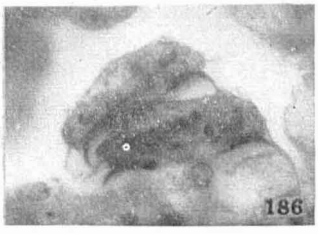
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PLATE 13

FIGURE 187.—Conjugating pair of *Trichodina discoidea* viewed from lower side. The smaller conjugant is plainly a juvenile. The dark line in the adhesive disk separates the striated brand from the border membrane, which is more distinct than usual. × 680.

FIGURE 188.—Conjugating pair of *T. discoidea* viewed from lower side. The lower conjugant (uppermost in photograph) is an adult, while the other is evidently a juvenile. The rudiments of a new denticulate ring can be seen in the striated band of each conjugant. × 680.

FIGURE 189.—One of a conjugating pair in *T. symmetrica* viewed from the adoral side. The macronucleus is becoming elongated with the formation of branches and will soon break up into rounded fragments. × 680.

FIGURES 190 and 191.—Exconjugants of *T. discoidea* in which a new denticulate ring is developing with the same number of denticles as the old. × 680. Compare with figures 172 to 181.

FIGURE 192.—Exconjugant of *T. tumefaciens* in which the macronuclear anlagen are just beginning to enlarge and become distinguishable among rounded fragments of the macronucleus. × 680.

FIGURE 193.—Exconjugant *T. symmetrica* with macronuclear anlagen clearly distinguishable. × 680.

FIGURES 194 and 195.—Photographs of exconjugant of *T. discoidea* taken at different levels. The adhesive disk in fig. 194 is evidently that of a juvenile. A new denticulate ring is forming with the same number of denticles as the old. At a lower focus macronuclear anlagen can be seen among the rounded fragments of the macronucleus. × 680.

FIGURE 196.—*T. discoidea* with two large macronuclear anlagen, the functional micronucleus, and rounded fragments of the original macronucleus. ×680.

FIGURE 197.—A later stage than figure 196 showing U-shaped macronucleus, micronucleus, and fragments of original macronucleus. × 680.

FIGURE 198.—Living *Scyphidia macropodia* attached to gill epithelium. × 340.

FIGURE 199.—Section of *S. macropodia* with retracted peristome. × 680.

FIGURE 200.—Section of *S. macropodia* with peristome expanded. × 680.

FIGURE 201.—Side view of living free-swimming telotroch stage of *S. macropodia*. × 340.

FIGURE 202.—Whole mount of telotroch stage viewed from above to show condition of macronucleus. × 680.

FIGURES 203 and 204.—Postconjugant of *S. macropodia* photographed at two levels to show macronuclear anlagen. × 680.

FIGURE 205.—*S. macropodia* dividing by binary fission. × 680.

FIGURE 206.—Living *S. macropodia* attached to gill filament. Two individuals of small form at extreme left. × 340.

FIGURES 207 and 208.—Postconjugants of *S. macropodia* with three macronuclear anlagen and functional micronucleus. × 680.

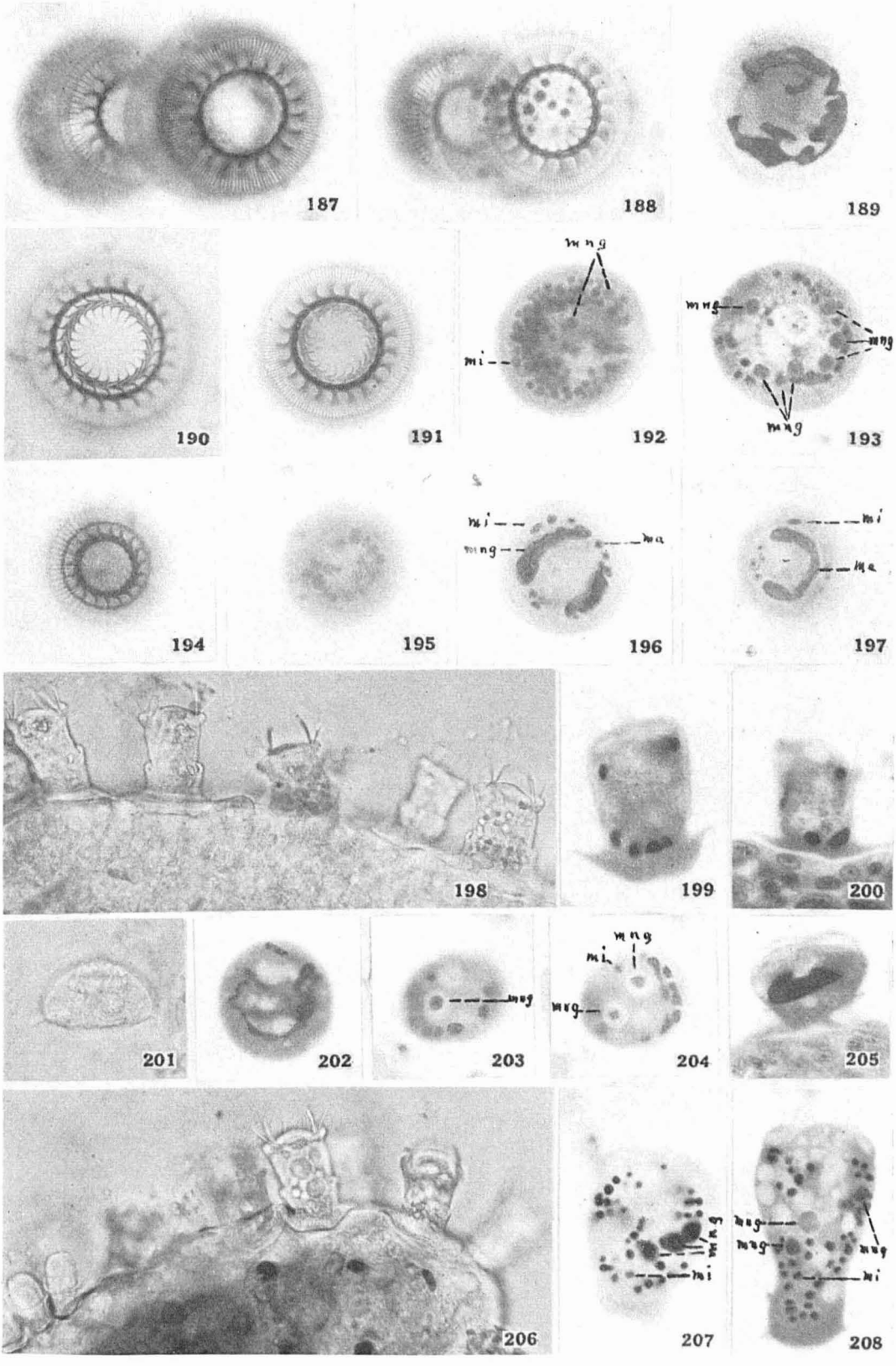
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PLATE 14

FIGURE 209.—Four small individuals of *Scyphidia macropodia* which are believed to have been formed by two successive divisions of postconjugant. The micronuclei are not shown. × 680.

FIGURE 210.—Cysts of *Dermocystidium salmonis* on gill filaments. ×7.

FIGURE 211.—Section of cyst of *D. salmonis* showing fusion of lamellae surrounding cyst. The space between the spores and the cyst wall is due to shrinkage. × 85.

FIGURE 212.—Section of part of cyst of *D. salmonis* showing sporoblasts and mature spores. The thin cyst wall is in close contact with epithelial cells. × 680.

FIGURE 213.—Section of young cyst of *D. salmonis*. The irregularities in the cyst wall are probably due to shrinkage. × 680.

FIGURE 214.—Adult *Trichophrya ictaluri* with four fascicles of tentacles. Photographed from unstained specimen preserved in formalin. × 510.

FIGURES 215 and 216.—Trophozoite of *Chloromyxum externum* attached to gills. × 680.

FIGURE 217.—Conjugation in *Scyphidia macropodia*. The micronuclei are dividing in both conjugants. × 680.

FIGURE 218.—Formation of microconjugant in *S. macropodia*. × 680.

FIGURE 219.—Exceptionally large trophozoite of *C. externum*. × 680.

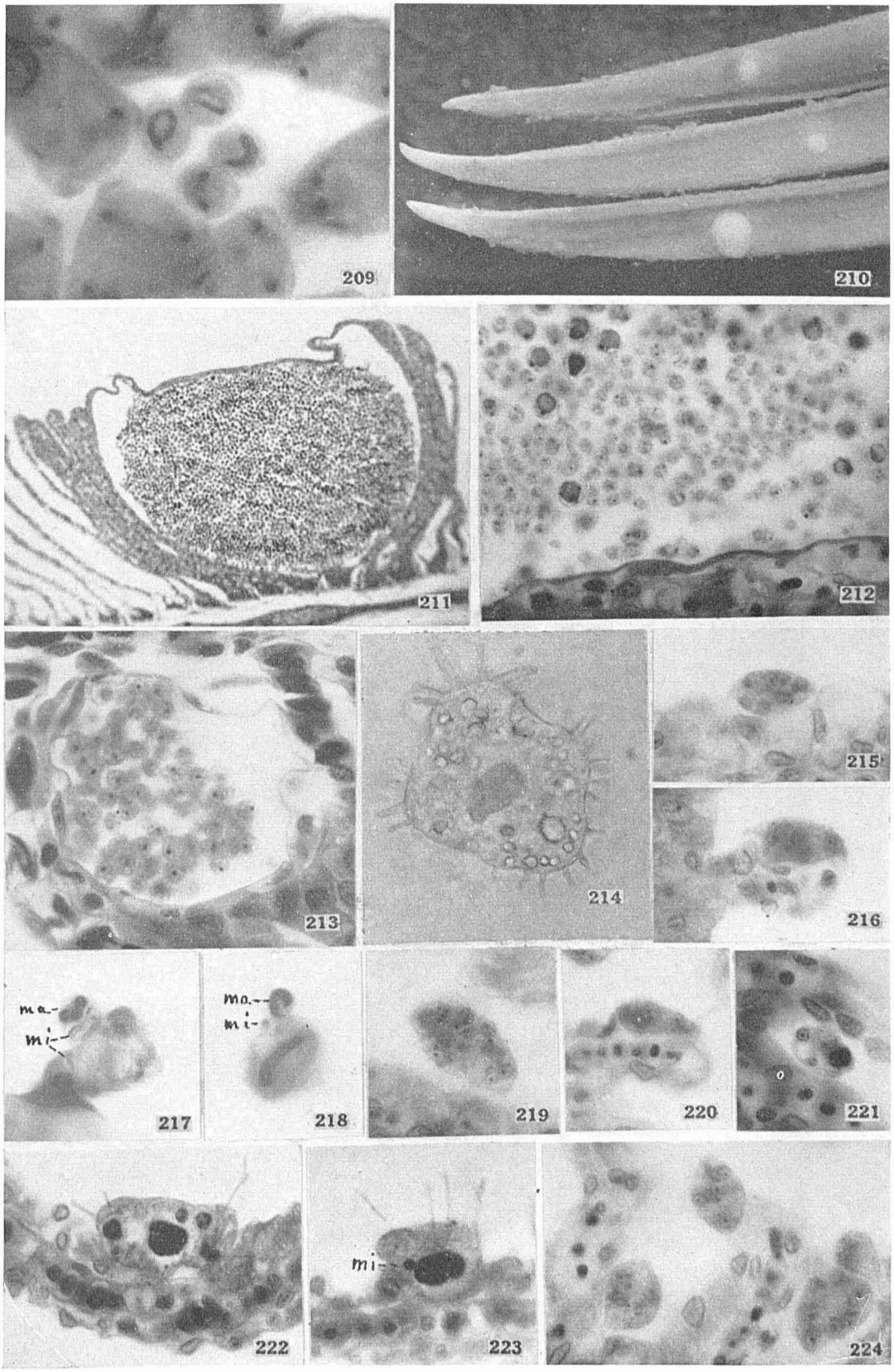
FIGURE 220.—Trophozoite of *C. externum* attached to lamella. Note that it appears to be continuous with the epithelium. × 680.

FIGURE 221.—Sporulating trophozoite of *C. externum*, containing a mature spore (deeply stained) and developing spore just above. × 680.

FIGURE 222.—Section of *Trichophrya ictaluri* attached to lamella. The endoplasm contains rounded metaplastic bodies in addition to the macronucleus. × 680

FIGURE 223.—Section of *T. ictaluri*. The epithelial cells have been destroyed or forced to one side, so that the parasite is in direct contact with the capillary network of the lamella. × 680.

FIGURE 224.—Section of gill of minnow showing several trophozoites of *C. externum*. × 680.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