Further Studies on the Ciliate Infusoria, Licnophora and Boveria.

By

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(With Plate I—VI.)

Contents.

Introduction.

In an earlier paper [1]), I gave the results of my work on the two new species of Infusoria, Licnophora macfarlandi and Boveria subcylindrica, both found in the respiratory organs of Holothuria californica STIMP., at Pacific Grove, California. Since that time I have had an opportunity to study other species and varieties belonging to these genera, at the Marine Biological Laboratory, Woods Hole, Massachusetts, and at the Naples Zoological Station. The present paper will be devoted: (1) to a comparative study of the species of each genus, and of their relation to other ciliates; (2) to observations on the conjugation, regeneration, and response to electrical stimulus in Licnophora; and (3) to a description of the division of the micronucleus and of the formation of a new peristome in Boveria.

Licnophora.

Historical summary. — As was stated in the historical summary of my former paper ('01), Licnophora was first described, but not named, by CLAUS ('62), as a parasite on Cladonema. The genus Licnophora was created by CLAPARÈDE ('67) for two new species, Licnophora auerbachii COHN and Licnophora cohnii CLAP. GRUBER ('84) gave the name Licnophora asterisci to a form which he found on Asteriscus, and described as very near to Licnophora auerbachii. FABRE-DOMERGUE ('88) found Licnophora on Syllis and Ophiothrix and expressed the opinion that all the species described should be united under the name Licnophora auerbachii; but WALLENGREN ('94), who worked on Licnophora from Doris muricata, the host on which

[1]) Studies on Ciliate Infusoria. Proc. of the Cal. Acad. of Sciences. Ser. III. Zool. III 1901.

it was discovered by AUERBACH, thinks that FABRE had a still different species. Two fresh water forms were described by MASKELL ('86) and GARBINI ('98), bnt these forms evidently do not belong to the genus Licnophora. Licnophora macfarlandi was fonnd in the respiratory organs of Holothuria californica STIMP., by Prof. F. M. Mc FARLAND in 1893. Dnring the summer of 1901, while working at the Marine Biological Laboratory, Woods Hole, I learned from Dr. CONKLIN [1]) that what proved to be another new species of Licnophora occnrred on the eggs of Crepidula plana. This species had also seen by Dr. CALKINS, and is described by him in a recent bnlletin of the U. S. Fish Commission, as a variety of Licnophora macfarlandi ('01[2]).

The species of Licnophora thus far recognized with their hosts are as follows: —

L. auerbachii COHN	on Doris muricata (AUERBACH, COHN, WALLENGREN).
	„ Thysanozoon tubercula (CLAP.).
L. cohnii CLAP.	„ Psyrmobranchus protensns (CLAP.).
L. asteriscns GRUBER	„ Asteriscus (GRUBER).
L. (auerbachii) FABRE	„ Syllis and Ophiothrix (FABRE).
L. macfarlandi STEVENS	„ Holothuria californica (Mc FARLAND, STEVENS).
L. conklini STEVENS	„ Crepidula plana (CONKLIN, CALKINS, STEVENS).

Licnophora macfarlandi. — A brief summary of the description of this species given in my former paper will serve to recall the principal characteristics of the genus together with the specific pecnliarities of the species. This ciliate has an elongated body which is divided into three distinct regions, — the attachment disc, the neck or stalk, and the oral disc (Fig. 1, a, b, c). The attachment disc is nearly circular in ontline, and consists of a shallow circular cup snrrounded by four ciliary membranes (Fig. 1, m^1—m^4), whose cilia, thongh usually united, may be separated at any point without destroying the coördination of their movements. Outside of the membranes is a collar or velum, consisting of a longer dorsal, and a shorter and wider ventral portion (Fig. 1, v). The neck is flattened dorso-ventrally, and has on the ventral side a longitudinal furrow leading to the mouth. On the right-hand side there is a vibrating lateral membrane extending from the point in the border of the attachment disc where the two parts of the velnm meet, to a point

[1]) The name Licnophora conklini has been given to this species.

just within the oral band near its origin over the pharynx (Fig. 1, e). Being very flexible, the neck varies greatly in length and thickness at different times, and in a contracted state shows conspicuous wrinkles and furrows on both dorsal and ventral sides (Fig. 1; and '01, Pl. I, Figs. 2 and 3).

The oral disc is oval in outline, concavo-convex, and has, on the slightly concave ventral surface, a left-turning peristomal circlet, consisting of very long fine cilia disposed in about one hundred and twenty-five short rows at right angles to the direction of the spiral (Figs. 1 and 3). This ciliary band begins at a point near the border between the neck and oral disc on the right-hand side of the animal, extends aronnd the anterior end and left side of the disc, tnrns to the right, and passes with a twist of 180° into the mouth and pharynx, which together form a pear-shaped cavity with a large external opening, variable in form and size (Fig. 1, o). The oral band has a very complicated structure, as is shown by sections and macerations. Each cross row of cilia originates in a basal band which stains like the basal bodies of single cilia (v. LENHOSSÉK '98; PETER '99). The ends of these basal bands are connected by delicate fibres with a long stout fibre which extends from the base of the attachment cup, in which it has root-like branches, to the end of the oral spire in the pharynx (Fig. 3; and '01, Pl. II, Fig. 17). The cilia of each row are usually twisted together when in action so as to resemble membranellae, and are figured as such by WALLENGREN ('94) and CALKINS ('01[2]). When, however, the organism is viewed under a high-power immersion lens, it is evident that the cilia are distinct. The basal structures indicate that we may have here an intermediate stage in the formation of membranellae from distinct cilia, giving such striated structures as form the peristomal circlet of Stentor and of many of the Hypotrichae. It is possible that united cilia may be found in some species of Licnophora, but such is not the case in the forms that I have examined. Another smaller fibre extends from the point in the border of the attachment disc where the lateral vibrating membrane begins, to the oral end of the peristomal band (Fig. 3, f[2]). These fibres, though slightly contractile, seem to function rather as supporting structures than as myonemes.

A single micronucleus occupies a somewhat isolated position at the base of the attachment cup, to the left of the larger neck fibre (Figs. 3 and 16; '01, Fig. 4).

The macronuclear chain consists of from twenty-five to thirty-five segments in adult specimens, and may be continuous or broken

into several sections. The segments are concentrated into one or more spherical masses before division. When writing my previous paper, I thought that the segments were completely separated in the adult; but, after working on other species where connection between the segments is clearly evident, and examining some preparations of Licnophora macfarlandi that had not been carefnlly studied, I have concluded that in this species the yare also connected, either in a single branched chain or in as many groups as there are separate nuclear masses in division.[1]) The connection is, however, very difficult to detect, and, it appears, may be easily broken (Fig. 16; and '01, Fig. 31).

I also thought that, as Wallengren ('94) had stated, the macronuclei unite in pairs before concentration and division; but this I find from study of the other species is not the case: instead, there is a division of the chromatin in each segment before the segments unite (Figs. 6 and 20; and '01, Figs. 15 and 19).

Fission in Licnophora is longitudinal, a new oral spiral being formed on the left side. The division line runs between the old and new peristomes, and through the attachment disc ('01, Pl. III, Figs. 18—28). The new peristome first appears as an oval ciliary field, from which is formed a right-turning spiral that later turns to the left ('01, Figs. 19—27). The micronucleus migrates to the anterior end of the oral disc, takes a position near and usually anterior to the concentrated macronucleus (Fig. 4). There it divides, and the two resulting micronuclei remain near the ends of the elongated macronncleus during its division, then return to their normal position near the base of the attachment cup of each individual. Division of the cell proceeds from the anterior end throngh the oral disc, neck and attachment disc. The macronuclear band or bands, resulting from division, take the adult position in each individual and separate into segments ('01, Figs. 18—31).

A contractile vacuole is not present, and defecation has not been observed.

This ciliate is nsnally attached to its host. The movements of its cilia, ciliary membranes and lateral vibratile membrane are such as to send a food current to the mouth by way of the ventral furrow in the neck, and also from the opposite direction, when the

[1]) In one case eleven such masses were counted, and from two to five are more common than one.

oral cilia are clapped down on the ventral surface, as they are at intervals in feeding.

The animal is usually rotating from left to right, and turning its oral disc this way and that by means of muscular movements of the neck. When distnrbed, it swims rapidly away with the oral disc at right angles to the attachment disc, darting hither and thither; or it whirls round and round with the two discs in nearly the same plane. It also moves short distances on the surface to which it is attached, and occasionally is seen running abont on the host by means of its oral cilia and ciliary membranes with Trichodina-like agility; a method of locomotion recently observed in Stentor by Jennings ('02).

The food of this species is usnally diatoms, bnt may also be desqnamated cells from the epithelial lining of the respiratory orgaus of its host. It has even been known to take in and digest small specimens of Boveria in cases where there was an immense number of infusoria of both kinds in the small terminal branches of the respiratory organs, and "a strnggle for existence" was literally going on.

This species differs from those previously described (1) in being an endoparasite, (2) in having fonr ciliary membranes around the attachment cnp, (3) in having a stout axial fibre connecting the attachment disc, to which it sends branches, with the oral ciliary band with whose rows of cilia its branches are connected, and (4) in having a lateral vibratile membrane. The micronucleus had not been previonsly described in any species of Licnophora.

3. *Licnophora conklini.* — This form, which occurs on Crepidula plana and on its egg-capsules, appeared at first sight to be identical with the Monterey species, bnt more careful observation showed that the vibratile neck membrane, so conspicuous in Licnophora macfarlandi, was not present, thongh the right side of the neck showed something of the same movements, being raised and lowered at intervals to guide the food particles down the neck furrow into the month. This Licnophora is somewhat smaller than the Pacific coast species, and there is a much greater difference in size between young specimens and those in the first stages of division (Figs. 6, 7, 11, 12; and '01 Figs. 28 and 19—21).

The movements of this species, when attached and when swimming, were the same as in Licnophora macfarlandi; but individuals were very often seen running abont over the egg-capsnles, a kind of locomotion rarely observed in the other species. As in the case of

Licnophora macfarlandi, this species differs from that described by Wallingren ('94) in the form of the attachment disc, the number of ciliary membranes, and the structnre of the peristomal spire. Neither of these American forms show the "haftring", membranellae, or "peristomalrinne" of Wallengren's description. If the lateral vibrating membrane *e* is omitted, Fig. 1 is a correct representation of Licnophora conklini.

On fixing specimens of this species and staining with Delafield's haematoxylin, alnm-carmine or picro-carmine, it was fonnd that there is a striking difference between the macronucleus in this case and that of the other forms described. Instead of being broken up into many segments, forming a long chain extending throngh the neck and around the two discs, the macronucleus here is divided into four widely separated parts. A band extends from one half to two thirds of the distance around the attachment disc on the dorsal side; there are two segments posterior to the mouth; and a group of nsually two segments, not widely separated as in the other cases, ies near the anterior extremity of the oral disc on the right side (Fig. 5). The anterior group is occasionally represented by a single segment and sometimes by three; in rare cases all four parts are segmented (Fig. 22). The segments in this species are all connected as shown in Fig. 5, and only one macronnclear mass was observed in division.

The external phenomena of division are essentially the same as in Licnophora macfarlandi. This species increases in size rather more in early stages (Figs. 5—8), and there appears to be less growth and a greater change in form, involving more shifting of the cytoplasm, in later stages (Figs. 8—10). The young Licnophorae are in some cases very short and broad and gradnally lengthen as the macronncleus lengthens and segments (Figs. 11—15), while in other cases lengthening of the body and segmentation of the nucleus occur before the two individnals separate. In this species the two attachment discs always divide before the oral discs.

Either just before or soon after the first cilia of the new peristome appear, there occnrs a pecnliar nuclear change, referred to above and noted in all the forms studied (Figs. 6 and 20). All the chromatin of each segment is separated into two parts, not necessarily eqnal. In the band which partly surrounds the attachment cup, there may be three, fonr or even more such divisions. Between each of the two masses of chromatin thus separated, there is a space where no stainable material is present, and in each mass there is

a nucleolus, while ordinarily only one nucleolus is present in each segment (Fig. 20). The nucleoli are not seen in specimens stained with haematoxylin or alum-carmine, but appear in picro-carmine, osmic acid or potassium bichromate preparations. What the significance of this chromatin separation in the earliest stages of division may be, it is difficult even to guess, for all the segments later unite into a spherical mass which shows no trace of this introductory division (Figs. 7 and 8); nor is there any indication in later stages that individuality of the divided segments may persist during the process of gross division of the concentrated macronucleus, and the individual half segments become the nuclear segments of the two young Licnophorae.

As in Licnophora macfarlandi, the micronucleus migrates to the anterior end of the oral disc, and divides mitotically; the two resulting micronuclei remain near the ends of the elongated macronucleus during its division, and then move posteriorly to their usual position at the base of the attachment cups (Figs. 8—12).

A careful search for Licnophora was made on various mollusks, holothurians and worms at Woods Hole, but these ciliates were found only on Crepidula and its egg-capsules.

4. *Licnophora auerbachii.* — Out of the large number of holothurians, echinoderms, mollusks and worms examined at Naples, Licnophorae were found only on Asterina gibbosa, Ophiothrix fragilis, Thysanozoon tubercula, Capsa fragilis and Tellina exigua. The Licnophorae from all of these hosts resemble closely Licnophora auerbachii as described and figured by WALLENGREN ('94), so far as general form, number of segments in the macronuclear chain, and division phenomena are concerned; they also show many of the distinguishing characteristics of Licnophora macfarlandi and of the Woods Hole species. These characteristics are four ciliary membranes around the attachment cup, a stout fibre connecting the attachment cup with the oral ciliary band, a ventral furrow leading to the month, and the peristomal band composed of short rows of long fine cilia whose basal bands are connected by fibres with the common fibre running through the neck to the attachment cup (Fig. 2). All of these structures I was able to recognize in preparations of Licnophora anerbachii which WALLENGREN kindly sent to me while I was working on Licnophora at Stanford University in 1900. They must, therefore, now be regarded, without much doubt, as generic rather than specific characteristics.

The Naples Licnophorae differ considerably in size on the various hosts, — from 79 μ in length on Thysanozoon to 116 μ on Capsa, the largest specimens, not in division stages, being measured alive when attached to the slide with the two discs in the same plane, as in Fig. 1 and 2. The smallest on Thysanozoon and Asterina were less than half as long as Licnophora macfarlandi, and the largest on Tellina and Capsa slightly smaller than Licnophora conklini. Variations in size may be largely due to differences in habitat, for all of the larger forms are found in protected situations, — in the respiratory organs of Holothuria, or within the shells of Capsa, Tellina and Crepidula, while the smaller forms are on the surface of Asterina, Ophiothrix and Thysanozoon.

The most striking external differences are: (1) the presence or absence of the lateral vibrating membrane characteristic of Licnophora macfarlandi, and (2) the variations in the form of the attachment disc.

As was stated for Licnophora conklini, there is a movement of the thinner right-hand side of the neck which corresponds in some degree to the vibration of the thin extended membrane of the Monterey species.

The attachment disc of the Licnophorae on Asterina and Ophiothrix, when fastened to the slide or cover-glass, always has the form figured by Wallengren ('94) for Licnophora anerbachii (Fig. 2). The diameter is greater in a transverse than in a longitudinal direction, the ratio being 8 : 6½. The disc has an irregular outline on the side toward the mouth, and on the right side there is a definite notch where the two parts of the velum and the right side of the neck meet (Fig. 2, d). This notch is less marked in Licnophora macfarlandi (Fig. 1, d). A few of the specimens on Tellina have a disc of the same form, while others on Tellina and all on Capsa and Thysanozoon have a nearly circular disc. The form and structure of the attachment cup, the ciliary membranes and the velum, as well as the relation of the disc to the two parts of the velum and to the neck at the point *d* are the same in all (Figs. 1, 2, 24—26). In the specimens with a round disc, the neck is somewhat narrower, and usually longer and thinner, while in the broad forms the line of attachment of the neck to the disc is much longer (Figs. 24—26). This difference in form and attachment of the neck may account in part at least for the different appearance of the discs of different specimens when attached to the slide, for the disc always appears circular in preserved specimens not attached to a surface. In Licno-

phora from Thysanozoon the cup is broader in proportion to the diameter of the disc than in any of the others, the ratio being 6 : 8, while in Licnophora macfarlandi it is 6.4 : 11.4, and in Licnophora conklini 5 : 10. This broader cup may be an adaptation to conditions of life on its slimy host.

With the exception of a few specimens from Tellina, which may have been preparing for division, the forms with circular discs all belong to soft-bodied hosts, while those with broader necks and discs are found on the hard spines of Asterina and Ophiothrix. This and the fact that the broader discs differ considerably in form in specimens from the same host, lead me to think that this is not a species characteristic, but an adaptation to the surface to which the organisms attach themselves. It would be an interesting experiment to transfer specimens from Asterina or Ophiotrix to Thysanozoon or to some other soft-bodied host, to see whether adaptive changes in the attachment disc would occur quickly, in which case the variation in form would not be a specific character.

The circular attachment disc, somewhat more slender neck, and shorter oral disc of the Licnophorae ou Thysanozoon tubercula might identify this form with that described by CLAPARÉDE ('67) as Licnophora cohnii. This is probably not the case, however, as he called the form which he found on this species of Thysanozoon at Naples Licnophora auerbachii, and figured Licnophora cohnii with a very long slender pedicle and a circular oral disc.

In the Licnophorae from Ophiothrix, the inner ciliary membrane seems to be permanently united and thickened for use as a grasping organ; and this modification is noticeable, but not to the same extent, in those from Asterina.

In all of the Naples forms the shorter ventral portion of the velum is slightly less transparent and therefore more easily distinguished than in Licnophora macfarlandi. It vibrates much more rapidly than the other part of the velum, or the ciliary membranes, and for that reason is difficult to see, lying as it does between the ciliary membranes and the neck.

The oral disc in the Licnophorae on Tellina and Capsa most closely resembles that of the Monterey species; while in those found on the other hosts the disc as well as the neck is somewhat shorter in proportion to the width (Figs. 1 and 2).

Division stages in the Naples forms do not differ materially from those described for Licnophora macfarlandi and Licnophora conklini. The changes in form and size are more like those observed

in the former. The method of formation of the new peristome is precisely the same, and the division stages of macronucleus and micronucleus are the same as in Licnophora conklini, with the exception that in rare cases the nuclear chain concentrates into two or three masses for division instead of one, indicating breaks in the chain as in Licnophora macfarlandi. Separation of the two attachment discs before that of the oral discs occurs here as in Licnophora conklini.

The one striking difference on which classification may be based is that found in the macronuclear chain. In the Woods Hole species (Fig. 17) we have what appears to be a more primitive form with four distinct and widely separated divisions of the macronucleus distributed to different parts of the body. In the European forms (Fig. 18) the number of distinct segments varies from ten to twenty-five. These segments are, however, separated into four groups corresponding to the four distinct divisions in Licnophora conklini, and the four sections are sometimes seen in resegmentation after division, but more commonly the two middle sections are united as in Fig. 21. Figure 18, a specimen from Asterina, shows a typical number and arrangement of segments, — (*a*) three corresponding to the band in the attachment disc of Licnophora conklini, (*b*) two, and (*c*) two segments corresponding to the right and left middle sections, and (*d*) six to the peristomal section, which is usually divided in Licnophora conklinii. The number of segments in group *a* may vary in specimens from the same and from different hosts, from three to six, that in group *b* from two to three, in group *c* from one to six, and in group *d* from four to eleven. The largest number of segments observed was twenty-three in one large specimen from Tellina, but another equally large one had only eleven, showing that no necessary relation exists between size of the organism and number of segments. In WALLENGREN'S preparations of Licnophora auerbachii from Doris muricata, the variation in number of segments is indicated by the following counts: 13, 16, 17, 20, 21, 22, 25. The variations were from four to seven in the attachment disc and from seven to twelve in the peristomal group. Fourteen and fifteen were the most common numbers in all of the Naples forms. The larger numbers were always seen in large specimens, but such specimens often contained only from eleven to fifteen.

Thus it appears that, if we disregard the differences in size and in form of the attachment disc, all of the Licnophorae studied at Naples may be classed with the one described by WALLENGREN ('94) under one species, Licnophora auerbachii.

Classification. — Though the number of segments in the macronucleus of Licnophora macfarlandi is considerably greater, the same grouping as in the other species can be distinguished, but the groups are not so clearly separated (Fig. 16). The neck is longer in this species than in either of the others, and the middle group of segments is extended toward the attachment disc (Fig. 16, b). The arrangement shown in Fig. 16 is typical, but considerable variation in the number of segments of each group occurs, and the groups are often more closely connected.

The considerably greater size of Licnophora macfarlandi, the larger number of macronuclear segments, and the highly developed vibratile neck membrane lead me to consider it a separate species, until further search can be made on the Pacific Coast, for different species or for the same species on different hosts. A few specimens were found by Dr. HAROLD HEATH at Pacific Grove in 1901 on Cymbulopsis, but they were not observed alive, nor were they compared with the Licnophora from Holothuria. Licnophora may therefore occur on various hosts in Monterey Bay, and if so, it will be interesting to ascertain whether the same or similar variations appear as on the different hosts at Naples.

Until the group is still further studied, I shall refer the forms that I have investigated to the three species, L. conklini, L. auerbachii and L. macfarlandi, and give the following tentative description of genus and species: —

Licnophora. — Length 80—180 μ. Colorless or slightly yellowish. Body flexible and contractile, flattened dorso-ventrally and consisting of three distinct regions, — attachment disc, neck, and oral disc. Attachment disc circular or irregularly oval in outline. Attachment cup nearly hemispherical, and encircled by four concentric ciliary membranes, and by a velum consisting of a longer dorsal and a shorter ventral portion overlapping on the left side and meeting on the right side at a notch in the attachment disc. Neck flattened dorso-ventrally and varying greatly in width and length. A conspicuous furrow on the ventral side extending from the attachment disc to the mouth. Oral disc oval or nearly circular, concavo-convex, with the sinistral peristomal spire on the concave ventral side. Peristomal band composed of short rows of long fine cilia rooted in basal bands connected with a thick, somewhat contractile fibre which extends to the attachment cup, in whose walls its root-like branches ramify. Micronuclens at the base of the attachment cup to the left of the axial fibre. Macronuclear chain consisting

of from four to thirty-five segments, separated into four groups distributed to the attachment disc, neck region and oral disc. Division longitudinal, a new peristome being formed on the left side as a right-turning spiral which later changes to a left-turning spiral. The attachment disc elongates transversely and divides into two equal discs. Conjugation of equal gametes, not permanent. Marine forms. Ectoparasites on various echinoderms, worms, mollusks and medusae; endoparasites in the respiratory organs of Holothuria californica.

L. conklini. — Medium size, 100—135 μ. Attachment disc circular or nearly so. Macronucleus having the four parts usually undivided, except in the peristomal section which, as a rule, consists of two segments. Found on Crepidula plana at Woods Hole, Mass.

L. auerbachii. — Small to medium size, 80—120 μ. Attachment disc either circular or irregularly oval, the ventral side being notched and less curved than the dorsal side. Neck short and broad. Macronuclear chain of from ten to twenty-five segments, separated into four distinct groups containing a variable number of segments; number of segments more commonly fourteen or fifteen. Found on Asterina gibbosa, Ophiothrix fragilis, Thysanozoon tubercula, Tellina exigna, Capsa fragilis and Doris muricata (Wallengren).

L. macfarlandi. — Large, 140—180 μ. Attachment disc circular or nearly so. Macronuclear chain showing the same grouping as in the other species, but divided into more segments, twenty-five to thirty-five. A delicate vibratile membrane on the right side of the neck. Found in the respiratory tree of Holothuria californica in Monterey Bay, California.

Relationship of Licnophora to other Ciliates. — Claparède ('67). who created the genus Licnophora, regarded these ciliates as true Hypotrichae furnished with an attachment disc; and Trichodina as a form derived from temporarily free-swimming Vorticellae. This classification was based on the fact that Licnophora, like the Hypotrichae, has a left-turning peristomal spire, while that of Trichodina and Vorticella is apparently a right spiral.

Bütschli ('89) regards Licnophora as a transitional form between hypotrichous and peritrichous infusoria. He derives the Licnophoridae from the Hypotrichae which they resemble in having an arched dorsal surface, and cilia only on the ventral surface. The peristomal spire is a left-turning one as in the Hypotrichae. The other peritrichous forms with left spirals he derives from the Licnophora-type by loss of the posterior circle of cilia and elevation of the peristome

to a terminal position. The Urceolaria-type, Trichodina-like forms, he regards as formed from the Licnophora-type by extension of the adoral spire around the whole ventral surface dorsal to the attachment disc. The Vorticellidae are then derived from the Trichodina-type by loss of the attaching circle of cilia and extension of the ventral surface giving a conical form, with the adoral spire aronnd the base of the cone. Looked at from the ventral surface the spiral is still a left-turning one, but from the peristome, which according to this theory is morphologically the dorsal side, the spiral turns to the right. The attaching part is drawn out to form a contractile stalk. but the Trichodina-type appears in the free-swimming modification which acquires a ventral circle of cilia. Division in Vorticella is transverse, two new peristomes being formed from the old one by division and partial regeneration; and BÜTSCHLI predicted that division in Licnophora, which had not then been observed, would also be transverse. The discovery that Licnophora divides longitudinally and that the peristome forms as a right spiral which changes to a left spiral, led WALLENGREN ('94) to reject BÜTSCHLI's theory, and express the opinion that Licnophora must be regarded as a highly differentiated form of peritrichous infusoria, and its relations to other Peritrichae remain for the present an open qnestion.

The recent work of WALLENGREN ('01) on Oxytricha indicates that such changes as occur in the formation of the peristome in Licnophora may have a phylogenetic significance. He finds that the reconstructions taking place in division follow the lines of development of the more complex from the simpler Hypotrichae. In the five different species which he studied, the cirrhi were in every case absorbed before division, and six rows of cilia appeared on the ventral surface, as in the more primitive forms; these six rows of cilia were then transformed into the adult cirrhi.

JOHNSON ('93) also calls attention to the probable phylogenetic significance of the change in the new peristomal band in Stentor from a lateral to a terminal position. In the division of the macronucleus of Stentor, JOHNSON observed no structural changes and concluded that, as division may occur during concentration, at the period of greatest condensation or during re-nodulation, the object of concentratiou must be merely to secure a larger number of nodes for the daughter animals. He states in this connection, however, that division at the period of greatest condensation is probably the primitive method, a reminiscence of a time when the nuclens was always spherical.

In Licnophora division and rearrangement of the macronuclear segments in the two new individuals could hardly be effected without condensation of the nnclear chain; but the varying number of segments in the different species from four to thirty-five, the arrangement of the segments in four groups in all the species, together with the method of condensation, division and resegmentation, suggest that the primitive Licnophora-type, or the form from which it was derived, had one spherical macronucleus, and that in the course of the development of the present Licnophora-form, the nncleus first became an elongated band, like that of Trichodina and Urceolaria; the band then segmented into four parts, and further segmentation took place later in varying degrees in the different species. Figures 11—15 may illustrate ontogenetically some of the phylogenetic changes in the Licnophora nuclens, while later changes appear in the adult nnclear conditions of the different species (Figs. 17, 18, 16).

The mnltiple nuclear masses in division, observed in Licnophora macfarlandi and rarely in Licnophora auerbachii, must be attributed to breaks between the segments, due probably to contortions of the organism, and to greater extensibility of the body cytoplasm than of the nnclear membrane. Such breaks would of course be handed down to all descendants of an individual in which they occur, until a new macronncleus is formed after conjugation.

If the changes in the new peristome have such phylogenetic significance as Wallengren suggests for Oxytricha and Johnson for Stentor, then the three snccessive stages, — (1) an oval field covered with short cilia of equal length, (2) development of a right-turning spiral by growth and definite arrangement of the outer cilia of the field, while the cilia in the center of the field degenerate ('01, Figs. 11, 16, 19, 20), and (3) change of the right spiral to a left spiral, — wonld indicate that the present type of Licnophora has been derived from a holotrichous form covered with short cilia of equal length, that the first differentiation of cilia in the oral region took the form of a right spiral, and that the change from a right to a left spiral was probably coincident with a gradual change in the form of the organism, which is repeated in the development of each new individual, but is somewhat obscured by the retention of the old left-turning spiral in the parent organism.

As to the phylogenetic development of the attachment apparatus, nothing is indicated in the phenomena of fission, since the disc, membranes, velum and cup are equally divided between the two

individuals; and no regeneration occurs when the attachment disc is removed.

Conjugation in Licnophora auerbachii. — During four summers, three at Pacific Grove and one at Woods Hole, I had watched in vain for conjugation in Licnophora macfarlandi and Licnophora conklini. While examining Licnophora auerbachii on Thysanozoon tuberculа at Naples, I discovered one pair in conjugation, but was unable to fix and stain them satisfactorily on account of the slime in which they were embedded. The following day I found a second pair in material taken from Asterina gibbosa, and the next day several pairs were obtained from the same host, an individual that had been in the laboratory six weeks, and on which the infusoria were very abundant. A few days later two pairs were found on Ophiothrix fragilis. There seemed to be no difference in the mode of union of the Licnophorae on the different hosts, and those from Asterina were much more convenient to work with, as the conjugating pairs very quickly attached themselves to the slide and remained in one place until they separated. The slides could therefore be placed in a moist chamber, the pairs observed at intervals, and fixed as desired. In all cases, however, the union was effected before the material was removed from the host, so that it was impossible to tell from the living material what stages one might have, or how long the period of union lasts. One pair which was observed at intervals for seventeen hours, was just separating at the end of that time, but how long they had been united before this period of seventeen hours was of course not known.

At different times during the winter more conjugates were found, in each case on only one Asterina out of several kept in the same aquarium and examined every two or three days. In every case the host was one that had been kept in the laboratory for from four to six weeks, supplied with fresh sea water every day, but with no food.

Methods. — It was found by experiment that the conjugating pairs, after they had attached themselves to the slide, could be fixed so that they would remain fastened to the glass during the processes of hardening, staining, dehydrating, clearing, and mounting in balsam. The method used was to drain off most of the water and pour on a mixture of absolute alcohol and 5% glacial acetic acid, or BOVERI's picro-acetic. Any fixing fluid containing osmic acid caused the Licnophorae to loosen their hold on the glass, and corrosive-acetic (3%), though at first apparently successful, resulted in loss

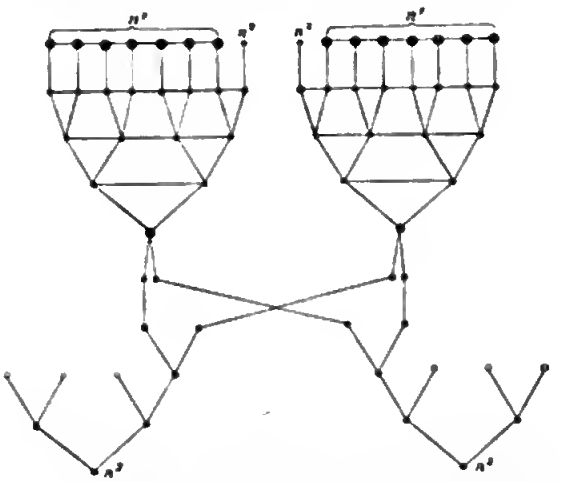
of the specimens in the alcohols. MAUPAS's glycerine methods were not successful with this form. The micronucleus of Licnophora is difficult to stain well with anything except iron-haematoxylin, but that can be used with satisfactory results only for sections, and here it was necessary to work with whole material. Several stains were tried but nothing else gave better results than staining about twelve hours with alum-carmine and decoloring under the microscope with acid alcohol. The specimens were then dehydrated, cleared with xylol or clove oil and mounted in balsam.

Stages of conjugation. — On account of the limited number of conjugates found, and the difficulty in distinguishing the faintly staining micronucleus among the many deeply staining macronuclear segments, not all of the stages were obtained, but enough, I think, to show what the formula of micronuclear changes must be for this form.

Figure 30 is a free-hand sketch of the first pair of conjugates seen in material from Thysanozoon. Here the attachment discs are toward the observer, while in all other cases they were attached to the slide with the dorsal surface toward the observer (Figs. 31—39). The gametes are equal in size and are united by the central portion of their ventral surfaces within the peristomal band. The field of union does not include the mouth, but I have never observed any feeding during the period of conjugation. The adoral cilia are usually curved over ventrally so that their tips meet or interlace, as shown in Figs. 31—36, and there is very little ciliary motion after the conjugates are attached to the slide, unless they are disturbed. The oral disc, instead of being flattened dorso-ventrally and somewhat concave on the ventral side, is contracted into a nearly spherical form (Fig. 30). The macronuclear segments lose their regular arrangement, and are variously disposed in the dorsal region, leaving the now convex ventral peristomal field free for the evolutions of the micronucleus.

Figure 31 shows a very early stage where the micronuclei have moved but little from the usual position near the attachment cup. In Fig. 32 we have a slightly later stage with the micronuclei in the ventral region, and in Fig. 33 is shown the only case of micronuclear division observed. The spindles are similar to those seen in fission of Licnophora macfarlandi (Fig. 4). Figure 34 shows the beginning of the second micronuclear division. Figure 35 is a stage in which the third division has occurred and the pronuclei are seen in the ventral region, while the three other micronuclei in each

gamete are among the macronuclear segments in the dorsal region. The macronuclear material is more granular in this stage than in the preceding stages. Figure 36 shows the only specimen seen in which preparation for exchange of micronuclear elements was evident, but it proved to be impossible to demonstrate the micronuclei. Figure 37 shows a pair on the point of separating, and Fig. 38 an exconjugate just after separation. In this stage the micronucleus is always in the position indicated, and is always very large. During the separation of the gametes the cilia are again in rapid motion. Figure 39 shows the first division of the conjugation micronucleus, and Figs. 40—43 later stages. Nothing was found between the stage shown in Fig. 39 and that in Fig. 40, where three divisions of the conjugation nucleus have occurred, the eight resulting nuclei remaining connected. At one end of the chain is the micronucleus, of about the usual size; the remaining seven segments are much larger, but have not yet reached the normal size. The old macronuclear segments are only faintly visible. Figure 41 is a similar stage where a few of the old segments are still distinguishable. In these stages the micronucleus is not always found near the attachment end. Figures 42 and 43 show the macronuclear segments in division, and in Fig. 43 connection between the micronucleus and the macronuclear chain has disappeared.



Textfigur A. Diagram showing the evolutions of the micronucleus of Licnophora during conjugation. n^1 = macronuclear chain, n^2 = micronucleus.

The micronuclear phenomena described may be represented as above, the formula being similar to that for other ciliates, but differing from all other cases recorded in that seven of the nuclear

elements resulting from the three divisions of the conjngation nncleus remain connected as the macronuclear chain of the exconjugate.

Physiological considerations. — The conjngation of Licnophora was stndied mainly from a morphological point of view. A few physiological points were noted incidentally, bnt material was lacking for an extended series of experiments, even if that were possible with marine forms. Althongh, as was stated above, conjngating gametes were found only on animals that had been for some time in the aquaria, the infusoria did not appear to be in a starving or otherwise unhealthy condition. There was no more variation in size than usual, and food masses were present in early stages of conjugation. A few cases of slightly abnormal division were observed: either cell-division was delayed, or it occurred before division of the macronncleus was complete. Figure 19 shows a case of conjugation in which one of the gametes was one of a pair undergoing ordinary division or fission, but they had not separated at so early a stage as nsual, as appears from the condition of the macronnclens in the two danghter individnals. Conjngation nsually occurred between individnals of approximately the same size and nnclear conditions, — the ordinary adult size before any signs of approaching division appear. Occasionally one or both gametes was larger and showed in the macronnclear segments the separation of the chromatin which is characteristic of early stages of division. Conjugation, therefore, appears to be possible at any time in the cycle between one division and the next.

After the first pair was discovered, many preparations were made by mixing material from different hosts, but no cases of conjngation resnlted. All nnions were effected before the specimens were transferred from the host to the slide, and may, therefore, have been exogamous or endogamons.

One point of interest is the fact that all of the exconjugates observed were small, emaciated, and free from food masses. This snggested that, at least in Licnophora, conjugation may be an exhausting process, leaving the exconjugates in a weak condition which they may or may not survive, according as circumstances are favorable or unfavorable. If this on further observation should prove to be true, it may acconnt for the fact that Calkins ('02) found that only about 6 % of the exconjngates in his hay cnltures survived, while 83 % of the wild exconjugates lived and multiplied, and one endogamous exconjngate, isolated and treated with beef extract, went on dividing up to the three hundred and fiftieth generation.

The real significance of conjugation in Protozoa and its relation, if any, to fertilization in Metazoa are questions of vital interest and much discussed at present.

BÜTSCHLI, ENGELMANN, GRUBER, MAUPAS, HERTWIG, BOVERI and others agree that nnion of the micronuclei of the two gametes is the essential thing in the process of conjugation, bnt very different opinions are held as to why conjngation occurs.

MAUPAS ('89) considers that conjngation is a rejuvenating process necessary to prevent senile degeneration and death. BÜTSCHLI ('76) and ENGELMANN (76) have described the object and the result of conjngation as "Verjüngung" and "Reorganization" respectively.

R. HERTWIG ('99), finding that when either in overfed or in starving Actinosphaerium, the nnclens becomes disproportionately large, a redncton in size is accomplished by absorption of a part of the nuclear substance by the cytoplasm, suggests that degeneration of the macronucleus in ciliates, and reduction of the micronucleus in ciliates and of the nucleus in other Protozoa, may have a similar explanation; i. e., reconstrnction of the nuclens is rendered necessary by a disproportion between nncleus and cytoplasm. With this interpretation reduction has a physiological rather than a phylogenetic significance.

GRUBER ('86) agrees with WEISMANN that the object of fertilization and of conjugation is to introduce variability of individuals and to build np new species. According to this theory, the micronncleus contains only "Keimplasma", while the macronucleus consists of a very small amount of "Keimplasma" with a large amount of "Histogenicplasma" taken up from the cytoplasm after conjugation and subseqnent division of the conjugation nucleus. The macronncleus, in their opinion, controls all the functions of the cell, the micronuclens being active only in conjugation.

CALKINS ('02), as a result of his recent experiments with Paramoecium, maintains that conjugation is not necessary to prevent senile degeneration, since the so-called rejuvenescence may be accomplished by other means. Degeneration and death in his cultures appeared to result from weakeued powers of digestion and assimilation; the infusoria were restored to health and reprodnctive activity by the use of various stimulating agents, notably beef-extract and extract of sheep's brain. On one occasion the infusoria recovered from a period of depression apparently as a result of slight mechanical stimulation. It is to be remembered, however, that the conditions in artificial cultures are quite different from those in nature; and

these experiments afford no convincing proof that what CALKINS designates as "parthenogenetic" recovery from a period of depression, is in any way comparable to the results of conjugation.

CALKINS also finds that no one of MAUPAS' three conditions, — hunger, diverse ancestry and sexual maturety, are necessary for "fertile" conjugation. Another interesting observation which he made was that in nearly every case one of a pair of exconjugates was more fertile than the other, indicating the possibility of "incipient fertilization". WATASÉ's suggestion that difference in sex is a temporary differentiation of protoplasm in one of two different directions, is significant in this connection.

It is very evident that much more extended observations of conjugation in many different species of Protozoa under as nearly normal conditions as possible, are necessary before any definite conclusions can be reached as to the whole significance of conjugation in the life history of the Protozoön, and as to the relation between conjugation in Protozoa and the processes of maturation and fertilization in Metazoa.

Regeneration in Licnophora. — While working with Licnophora anerbachii at Naples, it occurred to me that it would be interesting to ascertain whether so highly organized a ciliate as Licnophora would regenerate to the same extent as Stentor. Like Stentor, Licnophora has a segmented macronuclens, making it possible to cut the animal into several pieces each of which contains one or more nuclear segments; but unlike Stentor, this ciliate has but one micronuclens and that in a definite and somewhat isolated position close to the wall of the attachment cup, as shown in Fig. 44. Licnophora also differs from Stentor in having a highly complicated suction apparatus, or attachment disc at the posterior end, and a large ventral peristome, leaving only a small portion of comparatively undifferentiated cytoplasm between the two discs.

Methods. — The Licnophorae were taken from Asterina with a pipette, placed on a glass slide and allowed to attach themselves to the glass. The cutting was done with a sharp lancet needle under a BAUSCH and LOMB 1 inch objective and ocular Cap. C. The slides were then placed in a moist chamber similar to that described by MAUPAS ('89) for his experiments with Protozoa; or in some cases the fragments were removed to watch glasses so that the water could be changed frequently, and these were kept in the moist chamber. A few were cut on the paraffined slides used in my experiments on Echinus eggs ('02), but this method did not prove to be as advantageous

as cutting on the glass and leaving the animals attached to the slide when possible. In some of the experiments the specimen after the operation was kept under constant observation for several hours, more water being added at intervals to connteract as far as possible the plasmolyzing effect of the evaporating sea water, which makes it more difficult to experiment successfully with marine than with fresh water organisms.

Experiments. — The first set of experiments had for its object to determine whether the oral disc, if removed from the attachment disc, would regenerate a new attachment disc. The animals were cut through the neck region as in Fig. 44, $x \ldots x$, leaving the smaller posterior piece, containing the micronuclens and three or four segments of the macronuclens, attached to the glass, and the larger anterior portion, containing from eight to ten macronnclear segments free in the water. The two separated discs behaved quite differently. The attachment disc continued to rotate as usual, but more rapidly for a time. The oral disc contracted and ceased to move for a short time as through paralyzed, then expanded and began to whirl about by means of the usual movements of the peristomal cilia. The attachment disc often lived for several days, but showed no sign of regeneration; that was hardly to be expected, however, on account of the complicated suction apparatus and the small amount of undifferentiated cytoplasm. There appeared to be no reason why the oral disc should not develop a new attachment disc; but, though these pieces lived for from six to nine days, they never showed any indication of regeneration. The cut surface closed quickly, and the pieces lay on the surface of the glass, usually ventral side up, sometimes quiet, at other times whirling about or even tnrning over repeatedly. There was no evidence that they took any food. After several days they became very transparent and seemed to be in a starved condition though there was plenty of food material at hand; they moved less frequently and soon died. Three possible interpretations of the failure of these pieces to regenerate an attachment disc presented themselves. (1) These ciliates might not be capable of regeneration. (2) Failure to regenerate an attachment disc might be due to the fact that there was no micronuclens present in the oral disc. (3) Licnophora may not be able to form a new attachment disc in any other way than by division of the old disc as in the process of fission.

The next thing to be done was to find out whether regeneration would take place if the animals were cut in some other region.

Several specimens were cut as in Fig. 45, *a*....*a*, *b*....*b*, and left in the moist chamber over night. The small pieces rounded up in various forms (Fig. 46, *a* and *b*), and moved about over the surface of the glass, but no regeneration occurred. The next morning after the operation, the larger part of the animal had closed in at the anterior cut-end, so that the two ends of the oral band were united, and the cytoplasm had shifted so as to restore the usual form of the oral disc. Whether the oral disc and the peristome would later grow to the normal size I was unable to determine. The changes that did occur could hardly be called regeneration, but rather repair and "regulation". Animals cut in this way were watched under the microscope, and seen to close in as in Fig. 47, with the ends of the ciliary band united, but with a notch at the point of union. This notch disappeared later (Fig. 48). One such specimen, cut as in Fig. 45, *c*...*c*, had closed in completely and was feeding after fifteen minutes, making perfectly normal, coördinated movements of its shortened band of cilia, the oral spire being approximately one half of its usual length, and the disc two thirds of its former size. The smaller piece in this case, at first hung by a thread of protoplasm, but was detached after five minutes. The animal twice loosened itself from the slide and finally shook off the piece, as it does any foreign object which may have become entangled in its cilia.

When the line of separation of the two parts passes through the mouth region (Fig. 45, *d*....*d*, *e*....*e*), the two cut ends of the ciliary band do not come together, but new cilia form between the ends (Fig. 49), and later the disc assumes approximately its normal form, but both mouth and peristomal spire are, of course, smaller than usual. Here we have regeneration in the production of new cilia to complete the peristomal ciliary band, and reorganization or shifting of the cytoplasm to bring the mouth and spire into normal relations.

If the cut is made at *f*...*f*, removing the whole, or all but a few rows of the peristomal band, a movement of the cytoplasm from the attachment disc into the neck and the small part of the oral disc remaining, occurs, and a small new oral spire is formed (Fig. 50). This result was also obtained in one case where a small part of the pharynx remained. The new peristome being formed on the ventral side next to the glass, I could not ascertain whether the method of development was the same as in fission or not. When first seen these new peristomes appeared to be complete circles of short cilia, and the intermediate stages between this and the left-turning spiral

were not observed. At first these specimens were very short and broad (Fig. 50), but in the course of twenty-four hours the attachment disc grew smaller and the oral disc longer and narrower, giving nearer normal proportions (Figs. 51 and 52). Figure 52 shows the smallest new peristome observed.

When pieces were cut off diagonally (Fig. 45, *g* .... *g*, *h* .... *h*, *i* .... *i*), new cilia were formed to complete the spire (Figs. 54 and 55).

From these experiments it appears that repair, reorganization and regeneration, so as to produce a complete and fairly normal organism, are possible in pieces of Licnophora consisting of the attachment disc, neck, and one fourth or more of the oral disc. The smallest pieces that produced a new peristome were much smaller than the whole oral discs which did not regenerate an attachment disc.

None of the pieces removed from the oral disc showed any sign of regeneration, though they lived and moved about for several days.

Various experiments were made to see whether a cut extending some distance into the oral disc would close. In nearly every case, even when some cytoplasm escaped, the parts came together almost instantly, and in a few minutes no trace of the injury was visible. In some cases the ends of the ciliary band did not meet exactly, but this did not prevent perfectly coördinated movements of the cilia.

The attachment disc was also cut in various ways. Cuts extending from two thirds to three fourths of the distance across the disc usually closed very quickly, and after fifteen to thirty minutes no trace of the cut remained. The attachment disc of such a specimen is shown in Fig. 56. The edges of the cut separated very widely at first (*b*), but came together quickly, and at the end of a minute just a trace of the cut was visible (*c*). In the case shown in Fig. 57, the cut closed quickly, but a notch and traces of the injury remained after twenty minutes; these completely disappeared in the course of four hours. Figure 58 was drawn from a specimen in which the cut closed more slowly from the inner end outward. At the end of five minutes the cut was still open, as in *b*; after thirty minutes it had closed, but imperfectly, leaving the edge of the disc jagged, and the velum not united. Three hours later the irregularity in the outline of the disc was still noticeable.

Cutting off a small portion of the disc gave such results as appear in Figs. 59 and 60. The cytoplasm rounded out at the cut edge, and the ends of the velum came gradually nearer together,

giving such appearances as are shown in Figs. 59, *c* and 60, *c*, at the end of fifteen and twenty-five minutes respectively. Perfectly normal discs were observed the next morning in several such cases, indicating slight regeneration in the velum and ciliary membranes. If half or more of the disc was removed, the animal loosened itself from the glass, the mutilated disc was more or less absorbed, and no regeneration occurred.

In one case the pellicula and most of the cytoplasm from the dorsal side of the disc was accidentally removed, leaving the cnp, velum and membranes as seen in Fig. 61. The cytoplasm moved forward from the neck region over the exposed cnp until at the end of thirty minutes it was completely covered (Figs. 62 and 63) with a thin layer of cytoplasm. Four hours later the outline of the disc remained somewhat irregular, but the animal had resumed its usual rate of rotation which during the first half hour after the injury had been very slow.

When the animals were cut lengthwise in halves, the pieces rounded up and lived for some time, but did not regenerate, though each half must have contained several nuclear segments.

The whole series of experiments shows that regeneration in Licnophora is very limited, being confined to the production of a few new oral cilia, a new peristome and possibly a very small portion of the attachment disc. The organism, however, possesses marked powers of repair and "regulation" in the sense used by Driesch. Further experimentation, together with histological study of the regenerating pieces, is necessary in order to determine whether regeneration in this form is in any way dependent on the presence of the micronucleus, as the failure of the isolated oral disc to regenerate a new attachment disc suggested. Since the formation of new parts in Protozoa usually follows the same method as the development of those parts in the process of fission, and the new attachment discs are formed by elongation and division of the old disc, and not independently as is the new peristome, it seems probable that Licnophora is incapable of regenerating an attachment disc; but further study of this and related forms is necessary to prove this point.

In connection with these experiments a curious case was noticed where a specimen, otherwise apparently normal, had a second oral disc, somewhat smaller and attached to the left dorsal side. This second disc sometimes lay with its ventral side against the dorsal surface of the larger disc as in Fig. 64; but, when feeding, the disc

was thrown out exposing the peristome as in Fig. 65. It was first seen on Feb. 28th; on March 1st both discs were feeding normally; but on the following morning the abnormal disc appeared to be growing smaller, the cilia disappeared, and the disc seemed to be sinking into the normal disc. At noon it was redneed to less than half its former size. On killing the specimen with aceto-carmine, it was fonnd that the macronuclear segments of the attachment disc were in normal positiou, but there were only three segments in the peristomal group, and two of those were in the abnormal part (Fig. 66). The position of this secondary disc did not indicate an abnormal form of fission, nor was there any possibility of its being a case of conjugation of uneqnal gametes. The most probable explanation seemed to be that it was an abnormal growth dne to injury.

The literature on regeneration in Protozoa deals mainly with the bearing of the experiments on the functions of the nucleus. (EICHHORN 1783; HAECKEL '68; GREEF '67; BRANDT '77; NUSSBAUM '84; GRUBER '85, '86 and '87; VERWORN '88 and '91; BALBIANI '88 and '92—93; HOFER '89; LILLIE '96; MORGAN '01.) No experiments on the Peritrichae are recorded. GRUBER alone mentions the micronncleus in connection with regeneration, expressing the opinion that it plays no active part in the process. He cites as evidence that it is the macronucleus and not the micronncleus that is essential in regeneration, the fact that pieces of conjngating Stentors do not regenerate nntil a stage is reached in which one of the micronnclei has assumed the form and functions of a macronucleus. These experiments are not convincing, however, since the micronucleus, in the stages of conjugation in which regeneration does not occur, is undergoing such a series of changes connected with the phenomena of conjugation that it could hardly be expected to take part at the same time in another process.

Reaction of Licnophora to electrical stimulus. — Three years ago at Pacific Grove, I tried the effect of the constant cnrrent on Licnophora, and the few observations made then promised interesting results; but tlie apparatns at hand was not suited to the work. At Naples it was possible to continue the experiments and obtain definite results.

Methods. — The apparatus used was a battery for physicians, combining in one instrument a battery of thirty elements, induction apparatus, rheostat, indicator, and keys for shifting the current and for turning it on or off, as desired. This instrument, manufactured by REINIGER, GIBBERT and SCHALL, of Erlangen, is the most con-

venient piece of apparatus for such experiments that I have seen, provided that a strength of not more than 30 M. A. is required.

Pieces of filter paper abont 1,5 cm sqnare were placed on a wide glass slide, leaving a space of from 0,5 cm to 0,7 cm between them, and on these rested non-polarizable clay electrodes. The water containing the Licnophorae was placed on the slide with a dropper, the animals allowed to attach themselves, and a favorable individual brought to the center of the field of the microscope before the electrodes were adjusted and the current turned on. Observations were made with a Zeiss A objective and ocular 4; no definite resnlts could be obtained with a cover-glass and a high power.

Normal movements. — In connection with the following experiments, it was found necessary to make a careful analysis of the normal movements of Licnophora for comparison with the movements noted when the organism was subjected to the action of the current.

A. Movements when the animal is attached: —

1. Slow rotation on the longitudinal axis, effected by the ciliary membranes beating against the slide or any other surface.
   (a) Oral cilia moving with the effective stroke toward the mouth.
   (b) Oral cilia suddenly clapped down on the ventral surface sending a current toward the mouth.
   (c) Oral cilia at rest except in the mouth and pharynx.
   (d) Oral cilia beating either ventrally or dorsally.
   (e) Oral disc bending dorso-ventrally or ventro-dorsally through an arc of 120° or more, with the oral cilia at rest, the bend being made at the neck.
2. No rotation, ciliary membranes and velum not vibrating.
   (a) Oral cilia at rest except in the mouth and pharynx.
   (b) Oral cilia sending food currents to the mouth as in *a* and *b* above.
   (c) Oral disc turning now this way now, that by bending or twisting the neck.

B. Swimming movements: —

1. Forward movement for a short distance, probably effected by the ciliary membranes beating against the water in the same manner as against a surface; rotation from left to right as when attached.

2. Whirling round and round, the two discs in the same plane, dne to the action of the oral cilia turning the animal toward the aboral side.
3. Turning in various directions according to the comparative violence of the vibration of the cilia of the two discs, and their relative position.

Experiments and observations. — In general the orientation of Licnophora by the constant current was as follows: —

A. Attached to the slide.

1. Aboral side toward the anode and oral disc at right angles to the slide (Fig. 27).
2. Violent vibration of the oral cilia on the anode side and a turn of 180° at each change of the current (Fig. 28).

B. Swimming: —

1. Movement toward the kathode, oral disc forward, often with many turns, but sometimes straight across the field, whirling on the longitndinal axis, as when attached or when swimming without the influence of the electric current.
2. Stimulation of the oral cilia on the anode side, longitudinal turn through 180°, and swimming toward the kathode, at each change of the current.

Effective current. — For most individnals a current of abont 20 M. A. was necessary to orient them when they were fresh from the host and very active; for a few 25 M. A. was required, and after an individual had been experimented with for an honr or more, 10 M. A. was sufficient, and in one case 9 M. A. A few examples are given below.

I.

Two fresh specimens.

20 M. A. effective for one.
25 M. A. effective for the other.

II. Fresh specimen.		III. Fresh specimen.	
9 M. A.	0[1]	20 M. A.	0
10 „ „	0	25 „ „	+
15 „ „	0	15 „ „	+ (after 15′)

[1]) 0 = not effective. + = effective.

20 M. A.	+	10 M. A.	0
17 „ „	+	12 „ „	+ (after 30')
16 „ „	+	11 „ „	+ („ 40')
15 „ „ .. .	0	10 „ „	+ („ 60').

Individual variations. — Some specimens turn through 180° at each change of the current and remain at rest in the position shown in Figs. 27 and 28, with the oral disc at right angles to the slide and to the direction of the current, aboral side toward the anode, as thongh held there by the force of the current, the reaction being the same for any effective strength of current. Some, after turning to the position shown in the figures, turn back 40°—50°, then forward again and repeat these movements until the current is changed, when they immediately tnrn 180° and go through the same backward and forward movements.

One case was observed where the oral disc was held nearly parallel to the slide as in Fig. 29. A current of 20 M. A. caused the animal to come to rest with the anterior end toward the kathode. With a cnrrent of 25 M. A. it swung around 15°—20° to the position $b^1 \ldots b^2$, but always came back to $a^1 \ldots . a^2$. After an hour, 10 M. A. was sufficient to prevent it from turning beyond a^2; it swung back and forward between a^1 and a^2, remaining most of the time in the quadrant $a^1 \ldots . a^1$. With 8 M. A. it succeeded in turning completely aronnd after several trials, bnt was very slow in responding to a change in the current when it was at rest.

In every case the oral cilia on the side toward the new anode beat violently the instant the current was changed, but in some cases, after tnrning 45°—50°, these cilia all came to rest, and still the animal turned through the remainder of the 180° by the action of its ciliary membranes against the glass, and freqnently rotated backward and forward through a qnadrant or more. A dorso-ventral bending of the neck carrying the oral disc through an arc of 45° —50° was also observed while the oral cilia were at rest and the animal was swinging through a qnadrant, but not beyond the position shown in Figs. 27 and 28.

In some cases there seemed to be a violent muscular effort, when the ciliary membranes were at rest, to turn the oral disc beyond the critical point, but without effect. This movement is identical with normal movements of the oral disc in feeding when the attach-

ment disc is not rotating, but is held fast to the host by the attachment apparatus (A. 2, *b* [1]).

The rotation on the longitudinal axis of the animal is an almost constant normal movement, and is easily brought about, when not occurring, by such a slight mechanical stimulus as jarring of the slide or by a current of water. The special action of the electric current seems to be confined to orientation in a certain direction, — the longer axis in line with the current, the aboral side toward the anode and the mouth opening toward the kathode. The ciliary membranes seem to be powerless to turn the oral disc beyond a certain point. After reaching this point, their action may be reversed so as to turn the disc backward, a movement which I have not been able to detect under ordinary conditions, but noticed once in a case where an individual entangled in some debris on the slide was trying to free itself.

The response to the current, both when attached and when swimming, is complicated by the ordinary feeding movements of the oral cilia, and by the various turns and twists of the neck; but it seems to me that all of these movements may be disregarded and the effect of the current, when the oral cilia are at rest and the position of the oral disc relative to the attachment disc is constant, may be regarded as the true response. The vibration of the oral cilia on the anode side, too, seems to have no necessary relation to the change in the position of the animal occasioned by a change of the current; it is probably an expression of the first effect of the changed current on the anode side of the organism. The fact that the ciliary membranes beat in a manner to produce the usual rotation, and when that proves ineffective, beat so as to rotate the body backward and then forward again, shows that the membranes are not held to any forced position by the current, but that, in the last analysis the effect of the current is to hold the body in a certain position relative to the current. This position is apparently determined by the form and structure of the body, since the long axis of a horizontal section is always in line with the current, and the mouth opening toward the kathode.

When the animal is swimming, it is difficult to tell whether the sudden longitudinal turning through 180° at each change of the current is due wholly to the action of the stimulated cilia on the anode side or in part to muscular action. Orientation is always as

[1]) page 27.

in Fig. 29, and the action of the oral cilia and ciliary membranes is the same as in ordinary swimming except that movement in a definite direction is long continued, while ordinarily the animal darts hither and thither, whirling and turning in every direction. Here, too, the effect of the current seems to be limited to orientation of the organism with the long axis in line with the current, and the anterior end and mouth opening toward the kathode, so that the animal continues to swim in one direction by means of the ordinary action of its ciliary membranes.

As in the case of Amoeba, Actinosphaerium, Paramoecinm and other ciliates, and of flagellates [recently shown by PEARL ('00)], Licnophora nnder the action of the constant current swims toward the kathode. Comparison of the behavior of Licnophora under electrical stimulation when attached and when swimming, with its movements when not subjected to the action of the electrical current, leads me to think that orientation in both cases is connected rather with bodily form and structure than with any special effect on the cilia, cansing them to take "forced positions" as described by PEARL ('00) for Paramoecinm and other ciliates. The responses of Licnophora are especially interesting on acconnt of its structural peculiarities, localized ciliary apparatus, and the fact that withont change in structure it may be either attached or free-swimming.

Few if any experiments of this kind upon peritrichous Infusoria have been described. The simpler forms of Protozoa have been used for experiments in galvanotaxis, and the attention of investigators has been directed mainly to orientation, to changes in the form of the body, and to the action of the cilia. (KÜHNE '64; VERWORN '97; LUDLOFF '95; JENNINGS '97; LOEB and BUDGETT '97; BIRNKOFF '99; CARLGREN '00; PEARL '00.) For comparison with the results obtained with Licnophora, experiments should be made with other highly differentiated ciliates, with permanently fixed forms, and with other forms which may be either fixed or free-swimming, as Trichodina, Stentor and Boveria.

Boveria.

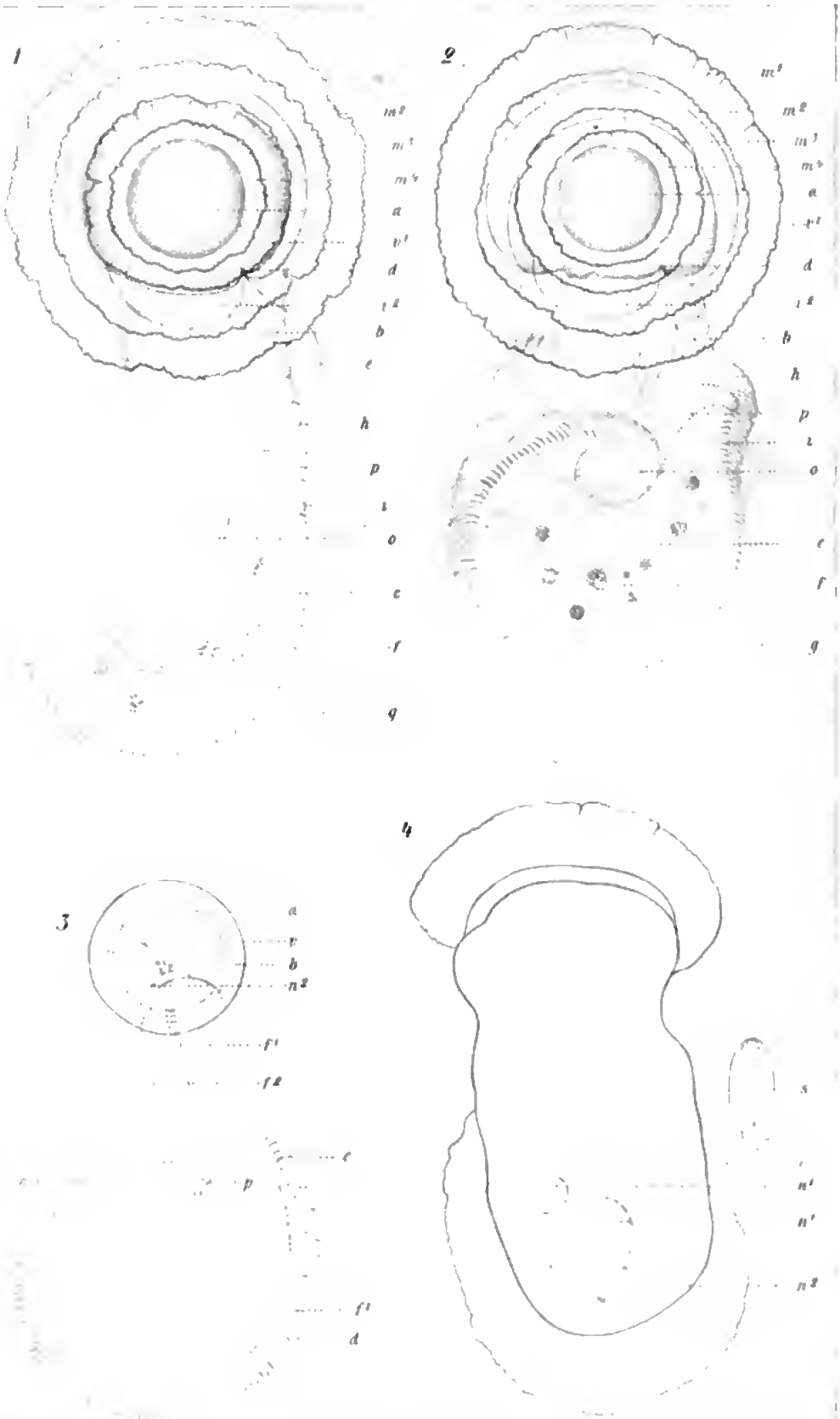
A short time before I left Naples, I learned that a species of Boveria, apparently identical with Boveria subcylindrica (STEVENS '01), occurs in abnndance on the bivalves, Tellina exigua and Capsa fragilis. I obtained the material and was able to clear up several donbtful points in my previous work, notably the formation of the oral spire in fission, and the number of chromosomes that appear

in divison of the micronucleus. Doubtless Boveria occurs on other hosts in the Bay of Naples, but I did not have time to investigate further. No cases of conjugation were observed, but it may be possible to obtain them by keeping the hosts in the laboratory several weeks as was done with Licnophora.

Comparison of the Monterey and Naples forms. — To summarize briefly the description of Boveria subcylindrica given in full in my "Stndies on Ciliate Infusoria" ('01), this species is characterized by an elongated body varying from a cylindrical form to that of a truncated cone with ronnded ends. The length is from 54 μ to 81 μ, the width at the oral end 18 μ to 21 μ, and at the aboral end 9 μ to 18 μ. The terminal oral spire consists of a double row of long rather coarse cilia, making one complete turn and abont 290° of a second turn. The mouth is within the loop formed by the union of the two rows of oral cilia at the inner end of the spiral (Figs. 67—69, and '01, Pl. IV and Pl. V). The whole surface of the body, with the exception of the peristome and a small circle at the aboral end is covered with longitudinal rows of fine cilia, much shorter than the oral cilia. Just posterior to the peristome and about 100° from the onter end of the ciliary spiral is a large contractile vacuole with a variable period averaging from two to three minntes (Figs. 67 and 69, *v*). The nucleus, which is faintly visible in the living animal, is a large oval strncture, centrally placed, and showing in sections a linin network, coarse chromatin granules, and often from two to four large nncleoli ('01, Pl. V, Figs. 48, 53, 54). Near the aboral end is a large micronucleus which stains deeply with iron-haematoxylin, safranine, carmine, methyl green, and other nnclear stains. Between the micronucleus and the aboral end is a lenticular disc of very dense cytoplasm, observable in the living animal and in all preparations (Fig. 69).

Boveria is, when undisturbed, essentially an attached form. In the respiratory organs of Holothuria, it appears to hold itself against its host by the constant motion of the body cilia with the effective stroke toward the peristome, but in live cultures it is frequently seen attached by the tips of its aboral cilia to the glass or to other objects on the slide (Figs. 67 and 68). When disturbed, Boveria swims very rapidly in a slightly serpentine course, aboral end forward, with a slow rotary motion and slight flexions of the oral end of the body.

Division in Boveria is of the variety known as oblique fission ('01, Pl. IV, Figs. 38—45). The peristome and contractile vacuole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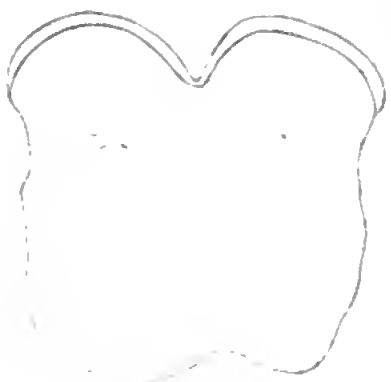

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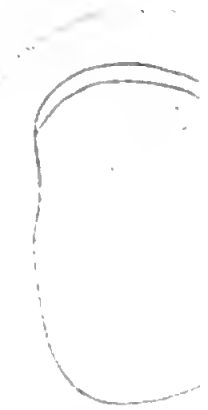

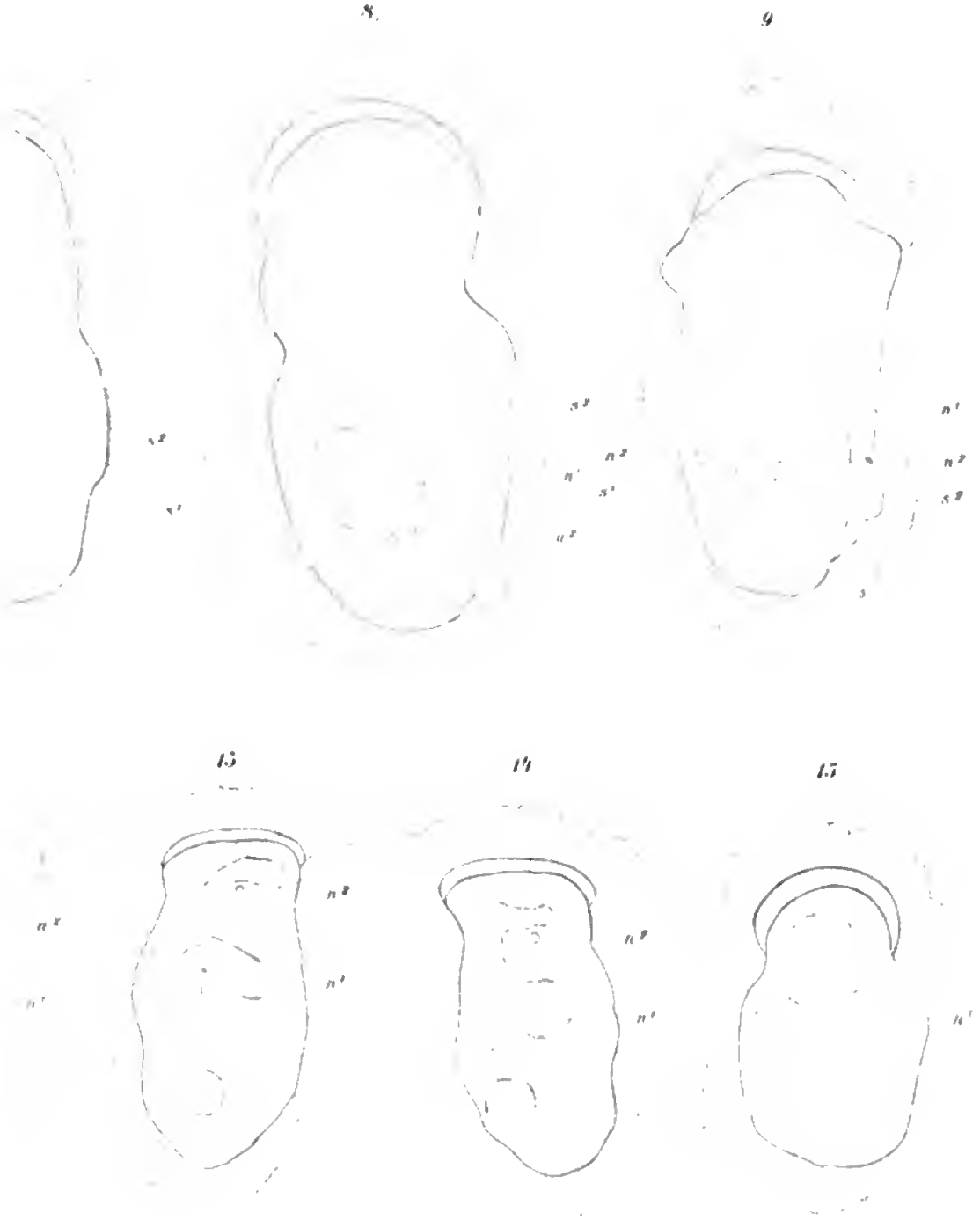
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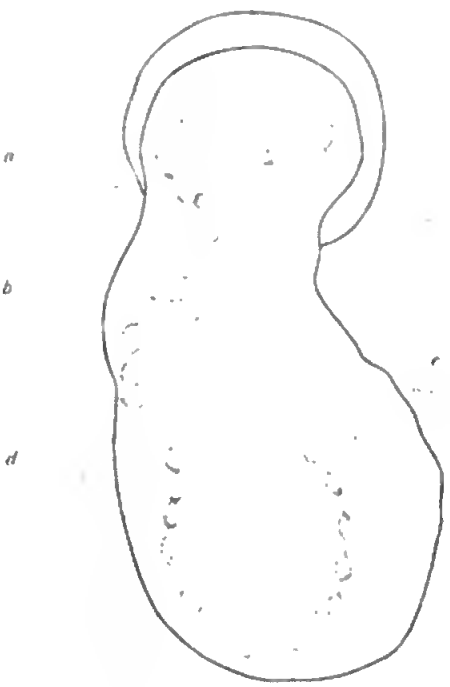

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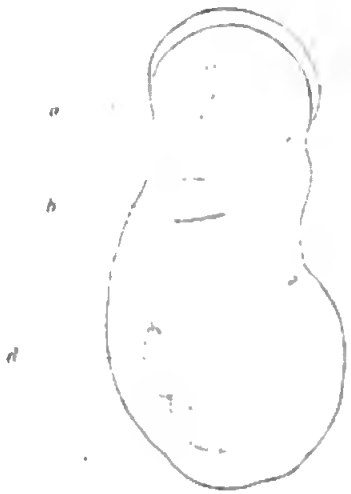

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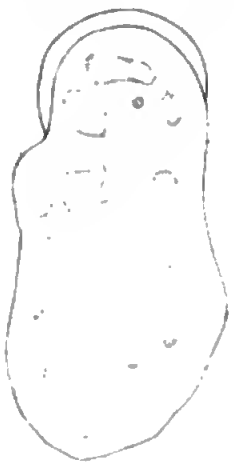

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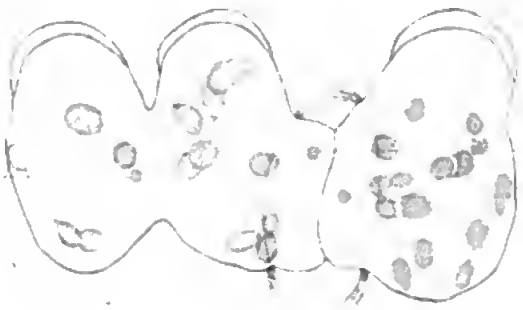

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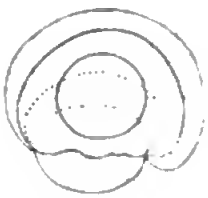

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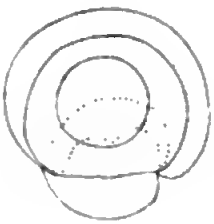

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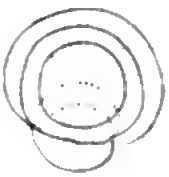

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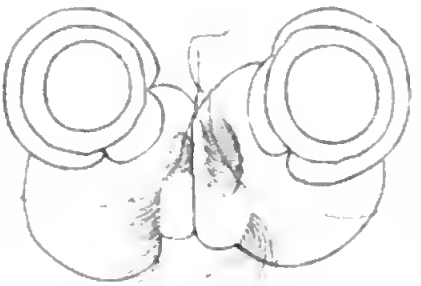

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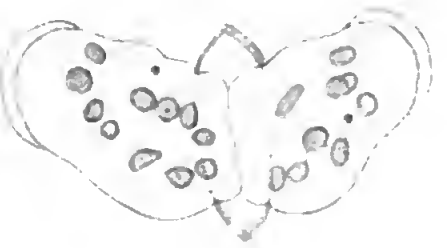

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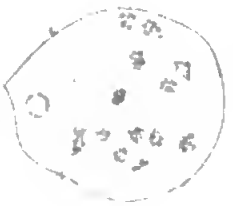

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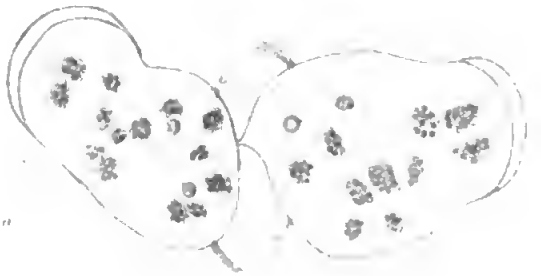

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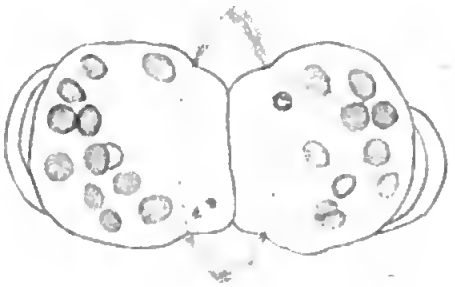

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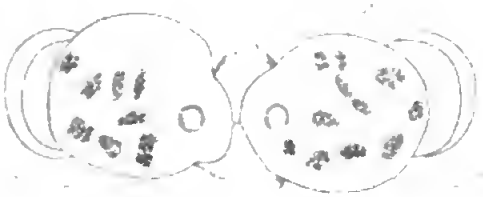

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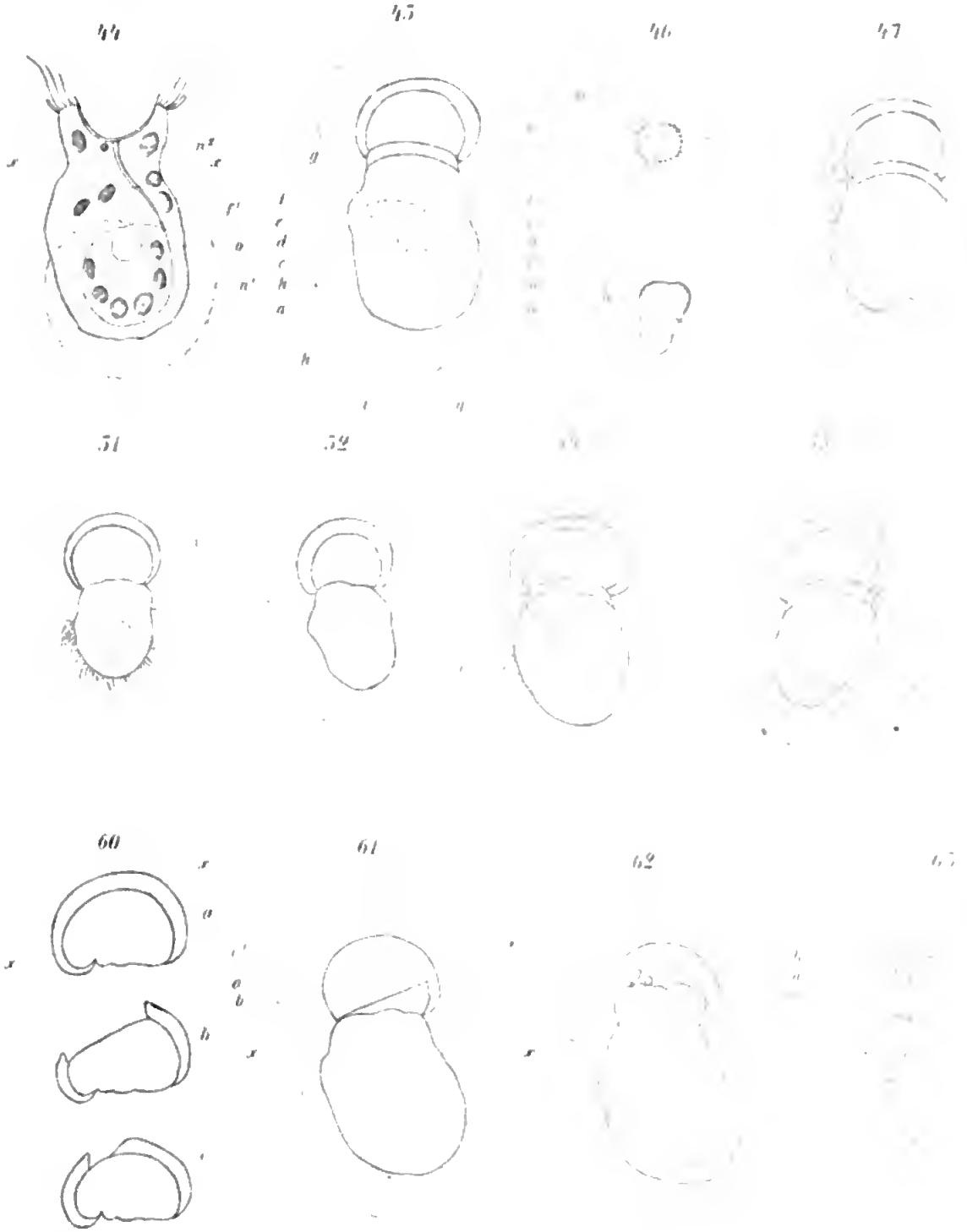
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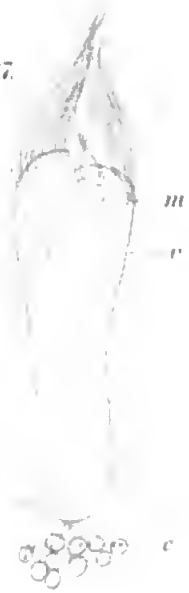

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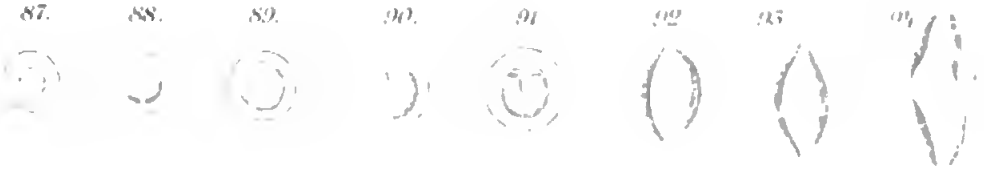

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disappear and new ones are formed in the two daughter animals. The micronucleus divides first, and the two resulting micronuclei take positions at the two ends of the elongating macronucleus, which then divides without the formation of a distinct spindle or chromosomes. In the division of the micronucleus, a peculiar series of stages was observed in sections, and two cases are figured where distinct chromosomes were seen at each pole of the micronuclear spindle ('01, Pl. V, Figs. 57, 58, 67); but the number of cases of division where the chromosomes were clearly seen was too small for any definite conclusions.

The Neapolitan Boveria differs but little from the above description, in structure, form or movements. The specimens found on Tellina varied in length from 37 μ to 102 μ, and those on Capsa from 65 μ to 121 μ, some of the latter being longer than the Licnophorae on the same host. The longest individuals are considerably longer and more slender than those whose nuclei indicate approaching division, and the difference in length between the youngest individuals and the longest is much greater than in the Monterey form. Evidently there must be a change in proportion just before division.

Figure 73 shows one of the long slender individuals drawn to scale, 116 μ long. 20.9 μ wide at the oral end and 13.95 μ wide midway between the two ends. In Fig. 72 a specimen whose micronucleus shows the first indications of division, is represented, drawn to the same scale. Figures 70, *a* and *b* show young Boveriae just after division. Comparing these figures with Figs. 67—69, it will be seen that the aboral end of the Monterey form is rounded, while that of the Naples form is pointed. The peristome of the two forms differs only in the length of the spire, that of the Naples variety being from 80° to 90° shorter (Fig. 74, *a* and *b*). The contractile vacuole, however, is in about the same position relative to the mouth, and therefore nearer to the outer end of the spire in the Naples form. The arrangement of the cilia, and the structure of cytoplasm, nucleus, and micronucleus seem to be identical in the two forms; but the denser lenticular disc of cytoplasm near the aboral end is not found in the Naples variety (Figs. 69 and 73). Division phenomena in the two forms are the same. Conjugation has not been observed.

Formation of the oral spire. — In the Monterey form of Boveria, it was noted that the peristomal cilia disappear in the early stages of division, and that a new ciliary spire is formed for each individual in the later stages before they separate. The manner in which the

new peristomal band develops was not observed, but can now be described for the Naples variety.

The peristomal cilia first appear on the side as a straight band (Fig. 75), which gradually assumes a terminal position, beginning to coil at the distal end (Figs. 76—78). The earliest stage in which I have been able to detect the lateral ciliary band is shown in Fig. 75, where the cilia are hardly longer than the ordinary body cilia, which are omitted in the figure for the sake of clearness. A slightly later stage is shown in Fig. 76, where the distal end of the band has begun to coil, but the cilia are still short, and the two rows are not distinct. Later stages are shown in Figs. 77 and 78. When the two individuals separate, the peristome is usually still somewhat oblique; the outer end of the spire still having a lateral position (Fig. 70, *a*). In the largest adult specimens, an obliquity in exactly the opposite direction is very often noticeable, the outer end of the spire being considerably elevated above the mouth region (Figs. 68 and 73). The method of development of the peristome as a lateral band may ultimately have some bearing on the classification of Boveria, which is at present not settled.

Division of the nuclei in the Naples variety. — Division of the micronucleus is a point of special interest, as Boveria is the only ciliate yet studied which has been found to have a very small number of clearly defined chromosomes in the micronuclear spindle. Figures 79—86 show in outline the principal stages of micronuclear and macronuclear division, and Figs. 87—96 micronuclear division in greater detail. The position of the micronucleus when it first shows signs of division varies from the usual position near the aboral end to a position in contact with the macronuclear membrane (Fig. 79). The spindle usually appears at one side, but near the posterior end of the macronucleus (Fig. 80); in later stages it stretches along the nuclear membrane with its poles approaching the ends of the macronucleus; and the two micronuclei when separated are located at or very near the poles of the dividing macronucleus (Figs. 81—86).

The micronucleus before dividing increases in size to about three or four times its original volume (Figs. 87—89), a notch appears in one side (Figs. 72 and 90), and later the partly separated halves are divided by clefts at right angles to the first division (Fig. 91). The four quarters of the micronucleus lengthen greatly (Figs. 92 and 93), and divide transversely (Fig. 94). The two groups of four chromosomes each then separate, each pair of chromosomes remaining

connected by a single fibre (Fig. 96). In some cases the whole spindle must move forward toward the oral end of the animal, and in others the anterior end of the spindle must move further than the posterior end, for the two micronuclei eventually in all cases reach positions near the poles of the macronucleus. The latest stage observed in which the separate chromosomes were evident is shown in Fig. 95. The indications are that the four chromosomes unite as quarters of a sphere, and possibly we have here a demonstration of individuality of chromosomes, as well as a case of a central spindle formed from material derived from the dividing chromosomes.

This micronucleus stains very clearly in all stages with SCHNEIDER's aceto-carmine, and if the infusorian can be found in conjugation in sufficient numbers, it ought to throw some light on the question of maturation, or reduction of chromatin in Protozoa. Unfortunately, I have as yet never seen the conjugation of this form, and did not have time to experiment with it before leaving Naples. I hope, however, to have an opportunity soon to study the phenomena of conjugation in Boveria either at Pacific Grove or at Naples.

The method of division of the macronucleus is shown a little more clearly by the aceto-carmine preparations (Figs. 79—86) than by my earlier figures. At first the macronucleus becomes much longer and the chromatin appears to be considerably increased in amount (Figs. 79—80). Soon a separation of the granules into a central sphere and two elongated polar masses occurs (Figs. 81—82). As the nucleus lengthens, fibres appear between the central and the polar groups of granules (Fig. 83); the central group lengthens and divides into two (Fig. 84). Figure 85 is a later stage where the two nuclei are still connected by the nuclear membrane: the two groups of granules in each nucleus are still distinct; but in a slightly later stage (Fig. 86), no separation of the granules can be detected. In all stages the granules of the polar and central groups appeared to be of the same form and staining quality, the only difference being that in the central group they were more densely packed together. It is possible that some such division center exists here as that figured by SCHAUDINN ('94) for Amoeba crystalligera, GRUBER, and that it owes its origin to the large nucleoli sometimes seen in the resting nucleus ('01, Figs. 54—55); but, if this is so, it is obscured by a covering of chromatin granules. No division center was discovered in the micronucleus.

The apparent interrelation between the micronucleus and the

macronuclens of Boveria in division stages recalls again the question of homology between the macronucleus and micronucleus of ciliates, and the nucleus and division center of the Metazoa.

In the discussion of the centrosome question participated in by BÜTSCHLI ('92), HEIDENHAIN ('94), R. HERTWIG ('95, '98, '99), LAUTERBORN ('95, '96), BOVERI ('95, '00), and ISHIKAWA ('00), BOVERI has shown conclusively that the micronucleus of ciliates cannot be homologized with the centrosome of Metazoa; but the behavior of the micronucleus of Boveria still indicates that it must have some influence over the macronucleus during division. There seems to be no other explanation for the constant position of the two micronuclei at the poles of the dividing macronucleus, when they might reach the usual position in the aboral end of each individual by a much shorter path. In Licnophora also, the micronucleus comes into close relations with the macronucleus during division, and the conditions in Kentrochona (ROMPEL [1]) '94) very closely resemble those in Boveria.

Classification. — In my earlier paper on Boveria, I placed it in the order Heterotricha, but was unable to determine the family. If it is to remain there, the limits of the order must be extended to include a form which has long oral cilia instead of membranellae. (BÜTSCHLI '89; LANG '01; CALKINS '01.) The method of formation of the new peristome as a lateral band might indicate relationship to the Stentors; but the oral spire in Boveria is a right-turning one, the nuclei are very different from those of Stentor, no myonemes are present, nor are there body cilia within the peristomal field. The very pronounced band of oral cilia, and the absence of trichocysts prevents this form from being placed under the Holotrichae. and the presence of body cilia separates it from the Peritrichae. which it resembles more closely in the structnre of its peristome.

Leaving the question of order and family open for the present, I add the following genus and species descriptions. making the form found at Naples a variety of Boveria subcylindrica: —

Boveria (n. gen. STEVENS '01). — Size variable, 80 μ—120 μ for adults. Form cylindrical or tapering. several times longer than broad. Cilia of two kinds: (1) a general body system of fine cilia arranged in slightly curved longitudinal rows; (2) a terminal peristomal spiral of long coarse cilia in a double row, closed at both

[1]) ROMPEL describes the two bodies at the poles of the macronuclear spindle as centrosomes; but both BALBIANI ('95) and HERTWIG ('95) regard them as micronuclei.

ends, and opening out at the inner end to enclose the mouth. Macronucleus oval, central; micronucleus nearer the aboral end. Contractile vacuole near the peristome. Reproduction by oblique fission. Marine forms, parasitic or commensal, usually attached by the cilia of the aboral end.

B. subcylindrica. — Length of individuals varying from 54 μ just after a division to 81 μ just before division. Oral cilia about one half the length of the body; body cilia one third or one fourth as long, somewhat shorter on the aboral end than on the sides. Aboral end rounded. A disc of denser cytoplasm between the micronucleus and the aboral end. Oral spire consisting of one turn and 290° of a second turn. Found in te respiratory tree of Holothuria californica in Monterey Bay, California.

B. subcylindrica, var. neapolitana. — Length from 37 μ to 116 μ. Aboral end pointed. No disc of denser cytoplasm near the aboral end. Oral spire consisting of one turn and 210° of a second turn. Found on Tellina exigua and Capsa fragilis in the Bay of Naples.

Summary.

1. The European and American forms of Licnophora closely resemble one another in nearly all structural characters.

2. These forms are to be classified, mainly on the basis of macronuclear differences, under three species, — L. conklini, L. auerbachii Cohn, and L. macfarlandi Stevens.

3. Conjugation of equal gametes occurs, resulting in one micronucleus and a macronuclear chain of seven segments in Licnophora auerbachii.

4. The exconjugates are small and emaciated, indicating that conjugation is an exhausting process.

5. Regeneration in Licnophora is limited to a small part of the attachment disc, a part of the oral ciliary band, or a new peristome.

6. All of the pieces that regenerated contained both macronuclear segments and the micronucleus, while the whole oral disc or parts of it, containing several macronuclear segments but not the micronucleus, did not regenerate.

7. The failure of Licnophora to regenerate an attachment disc is probably due to inability to form such a disc in any other way than by equal division of the old disc.

8. Orientation of Licnophora by the constant current appears to be more closely connected with bodily form and structure than

with any special effect on the cilia causing them to take "forced positions".

9. The Naples form of Boveria appears to be only a variety of the species described as B. subcylindrica.

10. The new peristome in Boveria is first a lateral band, which gradually assumes a spiral form and a terminal position.

11. The micronucleus of Boveria has four distinct chromosomes in division stages.

12. The position of the micronuclei indicates some influence over macronuclear division.

In conclusion I desire to express my gratitude to the "Association for maintaining the American WOMAN'S Table at the Zoological Station at Naples and for promoting Scientific Research among Women" for the use of tables both at the Marine Biological Laboratory, Woods Hole, and at the Zoological Station, Naples; also to thank the staff of both laboratories for many courtesies and for untiring effort to secure for me the desired material. I am also much indebted to Prof. MORGAN and Prof. WARREN for valuable criticism.

Literature.

'88. BALBIANI, E. G.: Recherches expérimentales sur la mérotomie des infusoires ciliés. Recueil zool. de la Suisse. V. p. 1. (1888.)

'92—93. —: Nouvelles recherches expérimentales sur la mérotomie des infusoires ciliés. Ann. d. Microgr. IV. p. 79. (1892.) — 2. Partie. V. p. 85. (1893.)

'95. —: Sur la structure et la division du noyau chez le Spirochona gemmipara. Ann. d. Microgr. VII. p. 289. (1895.)

'99. BIRNKOPF, B.: Untersuchungen über Galvanotaxis. Arch. f. d. ges. Physiol. LXXVII. p. 555. (1899.)

'95. BOVERI, TH.: Über das Verhalten der Centrosomen bei der Befruchtung der Seeigeleier nebst allgemeinen Bemerkungen über Centrosomen und Verwandtes. Verh. d. Phys.-Med. Ges. zu Würzburg. XXIX. p. 23. (1895.)

'00. —: Zellenstudien. IV. Über die Natur der Centrosomen. p. 176. Jena. (1900.)

'77. BRANDT, K.: Über Actinosphaerium eichhornii. Diss. Halle. (1877.)

'76. BÜTSCHLI, O.: Studien über die ersten Entwicklungsvorgänge der Eizelle, der Zellteilung und die Konjugation. Abh. d. Senckenb. naturf. Gesellsch. Frankfurt a. Main. X. p. 213. (1876.)

'89. —: BRONN's Klassen und Ordnungen des Tierreichs. I. p. 1757. (1889.)

'92. —: Über die sogenannten Centralkörper der Zelle und ihre Bedeutung. Verh. d. Nat.-Med. Verh. zu Heidelberg. (N. F.) IV. p. 535. (1892.)

'01[1]. Calkins, G. N.: The Protozoa. Columbia Univ. Biol. Series. (1901.)
'01[2]. —: Marine Protozoa from Woods Hole. U. S. Fish Com. Bulletin. p. 459. (1901.)
'02. —: Studies on the Life-History of Protozoa. I. The Life-Cycle of Paramoecium caudatum. Arch. f. Entwicklungsmech. XV. p. 139. (1902.)
'02. —: Studies on the Life-History of Protozoa. III. The six hundred and twentieth generation of Paramoecium caudatum. Biol. Bulletin. III. p. 192. (1902.)
'00. Carlgren, O.: Über die Einwirkung des konstanten galvanischen Stromes auf niedere Organismen. Arch. f. Physiol. p. 49. (1900.)
'67. Claparède, E. K.: Sur les Licnophora, nouveau genre de la famille Urceolarina. Ann. des Sci. Nat. (Zool.) Tome VIII. Ser. 5. p. 30. (1867.)
'62. Claus, C.: Ein neues auf Cladonema parasitisch lebendes Infusorium. Würzburger Natur. Zeitschr. III. p. 252. (1862.)
'66. Cohn, J.: Neue Infusorien im Seeaquarium. Zeit. f. wiss. Zool. XVI. p. 253. (1866.)
'91. Cuénot, L.: Protozoaires commensaux et parasites des Echinoderms. Note prelim. Rev. Biol. du Nord de la France. III. p. 285. (1891.)
1783. Eichhorn, J. C.: Zugabe zu meinen Beiträgen zur Naturgeschichte der kleinsten Wassertiere. Danzig. (1783.)
'76. Engelmann, Th. W.: Über Entwicklung und Fortpflanzung von Infusorien. Morph. Jahrb. p. 573. (1876.)
'84. Entz, G.: Über die Infusorien des Golfes von Neapel. Mitt. d. zool. Stat. zu Neapel. V. p. 289. (1884.)
'88. Fabre-Domergue, P.: Études sur l'organisation des Urcéolaires et sur quelques genres d'Infusoires voisins de cette famille. Journ. de l'Anat. et de la Physiol. XXIII. p. 243. (1888.)
'98. Garbini, A.: Di una seconda Licnophora di acqua dolce (L. europaea, n. sp.). Zool. Anz. XXI. p. 513. (1898.)
'67. Greef, R.: Über Actinophrys eichhornii und einen neuen Süßwasserrhizopoden, besonders in Rücksicht auf Teilbarkeit derselben resp. Vermehrung durch künstliche Teilung. Arch. f. mikr. Anat. III. p. 396. (1867.)
'84[1]. Gruber, A.: Über Kern und Kernteilung bei den Protozoen. Zeit. f. wiss. Zool. XL. p. 121. (1884.)
'84[2]. —: Die Protozoen des Hafens von Genua. Nova Acta Leop.-Carol. XLVI. p. 519. (1884.)
'85. —: Über künstliche Teilung bei Infusorien. I. Biol. Centralbl. IV. p. 717. (1885.) II. Ibid. V. p. 137. (1885.)
'86. —: Beiträge zur Kenntnis der Physiologie und Biologie der Protozoen. Ber. d. Naturf. Ges. Freiburg. I. p. 33. (1886).
'87. —: Mikroskopische Vivisektion. Ber. d. Naturf. Ges. Freiburg. II. p. 47. (1887.)
'68. Haeckel, E.: Monographie der Moneren. Jen. Zeit. IV. (1868.)
'94. Haidenhain, M.: Neue Untersuchungen über die Centralkörper und ihre Beziehungen zu Kern- und Zellenprotoplasma. Arch. f. mikr. Anat. XLIII. p. 423. (1894.)
'89. Hertwig, R.: Über die Konjugation der Infusorien. Abh. d. k. bayr. Akad. d. Wiss. München. Cl. II. XVII. p. 151. (1889.)
'92. —: Über Befruchtung und Konjugation. Verh. d. deutsch. zool. Ges. Berlin. p. 95. (1892.)

'95. —: Über Centrosoma und Centralspindel. Sitz.-Ber. d. Ges. f. Morph. u. Phys. München. I. p. 41. (1895.)

'98. —: Über Kernteilung, Richtungskörperbildung und Befruchtung von Actinosphaerium eich. Abh. d. k. bayr. Akad. d. Wiss. München. Cl. II. XIX. p. 1. (1898.)

'99. —: Was veranlaßt die Befruchtung der Protozoen? Sitz.-Ber. d. Ges. f. Morph. u. Phys. München. I. p. 1. (1899.)

'89. HOFER, B.: Experimentelle Untersuchungen über den Einfluß des Kerns auf das Protoplasma. Habilitationsschrift. Jena. (1889.)

'00. ISHIKAWA, C.: Further Observations on the Nuclear Division of Noctiluca. Journ. Col. Sci. Imp. Univ. Tokyo. XII. p. 243. (1900.)

'97. JENNINGS, H. S.: Studies on Reactions to Stimuli in Unicellular Organisms. I. Reactions to Chemical, Osmotic and Mechanical Stimuli in Ciliate Infusoria. Journ. of Physiol. XXI. p. 305. (1897.)

'02. —: Ibid. IX. On the Behavior of Fixed Infusoria (Stentor and Vorticella), with Special Reference to the Modifiability of Protozoan Reactions. Am. Journ. of Physiol. VIII. p. 23. (1902.)

'93. JOHNSON, H. P.: A Contribution to the Morphology and Biology of the Stentors. Journ. of Morph. VIII. p. 467. (1893.)

'82. KENT, W. S.: A Manual of Infusoria. London. II. p. 651. (1882.)

'64. KÜHNE, W.: Untersuchungen über das Protoplasma und die Kontraktilität. Leipzig. (1864.)

'01. LANG, A.: Lehrbuch der vergleichenden Anatomie der wirbellosen Tiere. II. Protozoa. Jena. (1901.)

'95. LAUTERBORN, R.: Protozoenstudien. I. Kern- und Zellteilung von Ceratium hirundinella. O. F. M. Zeit. f. wiss. Zool. LIX. p. 167. (1895.)

'96. —: Untersuchungen über Bau, Kernteilung und Bewegung der Diatomeen. Leipzig. (1896.)

'98. v. LENHOSSÉK, M.: Über Flimmerzellen. Verh. d. Anat. Ges. XII. p. 106. (1898.)

'96. LILLIE, F.: On the smallest parts of Stentor capable of regeneration. Journ. of Morph. XII. p. 239. (1896.)

'97. LOEB and BUDGETT: Über die Ausscheidung electropositiver Ionen an der äußeren Anodenfläche protoplasmatischer Gebilde als Ursache der Abweichungen vom PFLÜGER'schen Erregungsgesetz. Arch. f. d. ges. Physiol. LXV. p. 518. (1897.)

'95. LUDLOFF, K.: Untersuchungen über den Galvanotropismus. Arch. f. d. ges. Physiol. LIX. p. 525. (1895.)

'86. MASKELL, W. M.: On the Fresh-water Infusoria of the Wellington District. Trans. New Zealand Inst. XIX. p. 57. (1886.)

'89. MAUPAS, M.: Le rajeunissement karyogamique chez les Ciliés. Arch. Zool. exp. VII. Ser. 2. p. 149. (1889.)

'65—72. MOEBIUS and MEYER: Fauna der Kieler Bucht. I. p. 22. (1865—72.)

'01. MORGAN, T. H.: Regeneration of Proportional Structures in Stentor. Biol. Bulletin. II. p. 311. (1901.)

'84. NUSSBAUM, M: Über spontane und künstliche Zellteilung. Sitz. d. niederrh. Ges. p. 259. (1884.)

'00. PEARL, R.: Studies on Electrotaxis. I. On the Reactions of certain Infusoria to the Electric Current. Ann. Journ. of Physiol. IV. p. 96. (1900.)

'99. Peter, K.: Das Centrum für die Flimmer- und Geißelbewegung. Anat. Anz. XV. p. 271. (1899.)
'94. Rompel, J.: Kentrochona nebaliae n. g. u. sp., ein neuer Infusor aus der Familie der Spirochonien, zugleich ein Beitrag zur Lehre von der Kernteilung und Centrosoma. Zeit. f. wiss. Zool. LVIII. p. 618. (1894.)
'94. Schaudinn, F.: Über Kernteilung und nachfolgender Körperteilung bei Amoeba crystalligera Gruber. Sitz.-Ber. d. Akad. d. Wiss. zu Berlin. XXXVIII. p. 1036. (1894.)
'01. Stevens, N. M.: Studies on Ciliate Infusoria. Proc. of the Cal. Acad. of Sci. Ser. III. Zool. III. p. 1. (1901.)
'02. —: Experimental Studies on Eggs of Echinus microtuberculatus. Arch. f. Entwicklungsmech. XV. p. 1. (1902.)
'88. Verworn, M.: Biologische Protistenstudien. I. Zeit. f. wiss. Zool. XLVI. p. 455. (1888.)
'91. —: Die physiologische Bedeutung des Zellkerns. Arch. f. d. ges. Physiol. LI. p. 1. (1891.)
'97. —: Die polare Erregung der Protisten durch den galvanischen Strom. Arch. f. d. ges. Physiol. LXV. p. 47. (1897.)
'94. Wallengren, H.: Studier öfver Ciliata Infusorier. I. Slägtet Licnophora Claparède. Lund. p. 1. (1894.)
'01. —: Zur Kenntnis der Neubildungs- und Resorptionsvorgänge bei der Teilung der hypotrichen Infusorien. Zool. Jahrb. XV. p. 1. (1901.)

Explanation of Figures.

Plate I.

Fig. 1. Freehand sketch of living Licnophora macfarlandi, ventral view, showing attachment disc and cup (*a*), neck (*b*), oral disc (c), point of meeting of velum and vibratile membrane (*d*), vibratile membrane (*e*), oral cilia (*g*), ventral furrow (*h*), basal bands of the oral cilia (*i*), food masses (*f*), ciliary membranes (m^1, m^2, m^3, m^4), mouth (o), pharynx (p), and velum (v^1, v^2).

Fig. 2. Similar sketch of Licnophora auerbachii from Asterina, showing the different form of the attachment disc, shorter neck, and absence of the vibratile membrane (*e*).

Fig. 3. Reconstruction from macerations of Licnophora auerbachii, showing the relation of the fibres (f^1, f^2) to the attachment cup and oral spire. n^2 = micronucleus. *b* = basal bodies of the ciliary membranes. c = basal bands of the peristomal cilia. *d* = connecting fibres between the fibre (f^1) and the ends of the basal bands (*c*).

Fig. 4. Outline drawing from a whole mount of Licnophora macfarlandi to show the micronuclear spindle (n^2). n^1 = macronucleus. *c* = the new peristome. *s* = micronuclear spindle more highly magnified. Bausch and Lomb objective $^1/_5$, oc. C, camera. *s* was drawn with oil immersion $^1/_{12}$, oc. C.

Plate II (Division).

Fig. 5. Outline drawing of Licnophora conklini, adult form, showing macronuclear segments (n^1), micronucleus (n^2), outer ciliary membrane (m^1), and oral cilia (s^1). B. and L. obj. $^1/_{12}$, oc. A, camera.

Fig. 6. An early division stage of the same species, showing enlarged micronucleus (n^2), separation of chromatin in the macronuclear segments (n^1), and the beginning of a new peristome. Same magnification.

Figs. 7—10. Later stages showing division of the nuclei (n^1, n^2), change in form, and development of a new peristome. Same magnification.

Figs. 11—15. Young Licnophora conklini, showing resegmentation of the macronucleus. Same magnification.

Fixation with alsolute acetic (5%).
Staining with alum carmine.

Plate III.

Figs. 16—18. Camera drawings of Licnophora macfarlandi (Fig. 16), L. conklini (Fig. 17), and L. auerbachii Fig. 18), to show the segmentation of the macronucleus and the grouping of the segments. B. and L. obj. 1/3, oc. C.

Fig. 19. Conjugation between one gamete whose nuclear segments show approaching division, and another gamete not yet separated from its sister Licnophora. ZEISS obj. D, oc. 6.

Fig. 20. Macronuclear segment, showing separation of chromatin and two nucleoli. B. and L. obj. 1/12, oc. C.

Fig. 21. Licnophora auerbachii, showing separation of the macronucleus into three parts in the early stages of segmentation. B. and L. obj. 1/3, oc. C.

Fig. 22. Licnophora conklini, showing the macronucleus segmented irregularly and to an unusual extent. B. and L. obj. 1/12, oc. A.

Fig. 23. Young L. conklini, next stage after Fig. 15, Pl. II. Same magnification.

Figs. 24—26. Attachment discs of L. auerbachii from Asterina (Fig. 24), Capsa (Fig. 25), and Thysanozoon (Fig. 26), showing difference in form of the disc (t), velum (v^1, v^2), attachment cup (a), attachment of the neck to the disc (b), and notch in the disc (d).

Figs. 27—29. Sketches of L. auerbachii under the influence of the constant electric current. + = anode. − = kathode.

Plate IV (Conjugation).

Fig. 30. Sketch of a living pair of conjugates, L. auerbachii from Thysanozoon.

Figs. 31—39. Various stages in the conjugation of L. auerbachii from Asterina, showing macronuclear and micronuclear changes. n^1 = macronucleus. n^2 = micronucleus. a in Fig. 35 = degenerating micronuclei. x in Fig. 36 = region of micronuclear exchange, pronuclei not visible. a in Fig. 37 = conjugation nucleus. ZEISS obj. D, oc. 6.

Figs. 40—43. Exconjugates, showing degeneration of the old macronucleus, and development of new macronuclear segments. Same magnification.

Plate V (Regeneration).

Fig. 44. Diagrammatic drawing of L. auerbachii to show the relative position of the macronuclear segments (n^1), the micronucleus (n^2), the mouth (o) and the neck fibre (f^1). $x \ldots . x$ = the line of cutting in the first experiments.

Fig. 45. Dorsal view of L. auerbachii showing how the specimens were cut in the various experiments.

Fig. 46. Small pieces from the anterior end of the oral disc.

Figs. 47—48. Results from cutting at *b* . . . *b*.

Fig. 49. Result from cutting at *d* . . . *d*; new cilia at *c*.

Figs. 50—53. Individuals with new peristomes, resulting from cutting at *f* . . . . *f*.

Fig. 54. Result of cutting at *g* . . . . *g*; new cilia at *c*.

Fig. 55. Result from cutting at *i* . . . . *i*; new cilia at *c*.

Figs. 56—60. Results from cutting the attachment disc. Neck and oral disc not shown in the sketches.

Figs. 61—63. A case where the pellicula and cytoplasm were removed from the dorsal side of the attachment disc posterior to *x* . . . . *x*. Injury repaired by shifting of cytoplasm from the neck region.

Figs. 64—66. Abnormal specimen with a second oral disc (c^2). Fig. 64 with ventral surface of the second disc (c^2) against the dorsal surface of the normal disc (c^1); Fig. 65, ventral surface exposed; Fig. 66 specimen killed and stained with aceto-carmine after partial absorption of the abnormal disc, to show the nuclear conditions.

Plate VI (Boveria).

Figs. 67—69. Sketches of Boveria subcylindrica from Holothuria, showing mouth (*m*), vacuole (*v*), attachment of aboral cilia (*c*), food mass (*f*), macronucleus (n^1), micronucleus (n^2), and denser stratum of cytoplasm near the aboral end (*a*). Figs. 67 and 68 were from living specimens, and Fig. 69 from a section. Hermann fixation, iron-haematoxylin staining, B. and L. obj. $^1/_5$, oc. C.

Fig. 70. Young Boveriae, Naples variety.

Figs. 71—73. Adult Boveriae, Naples variety.

Fig. 74. Diagrams of the peristomal spirals of the Monterey variety (*a*), and of the Naples variety (*b*).

Figs. 75—78. Sketches from living specimens, showing formation of new peristomes. Body cilia omitted for the sake of clearness.

Figs. 79—86. Division stages from the Naples variety. Fixation and staining with Schneider's aceto-carmine. B. and L. obj. $^1/_5$, oc. C.

Figs. 87—96. Division stages of the micronucleus from aceto-carmine preparations. B. and L. obj. $^1/_{12}$, oc. C.

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