From the Biological Laboratories of Bard College and the College of the City of New York.

Taxonomic and cytological studies on the ciliates associated with the amphipod family Orchestiidae from the woods hole district.

II. The Coelozoic Astomatous Parasites.

By

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(With plates 9—10.)

While examining marine amphipods of the genus *Orchestia* for ectocommensal ciliates (KIDDER and SUMMERS, 1935) our attention was drawn to the astomatous ciliates of one particular amphipod, *O. agilis* SMITH.

In this arthropod host we have found a single but highly variable ciliate species which we will name *Anoplophrya orchestii* sp. nov. At the outset of this study we were impressed by the preponderance of individuals of several different classes from a given host. We were inclined to regard each class as a distinct species but we are now convinced, however, that there is but one species, which is highly polymorphic for a ciliate type. In order to validate our observations on the life history of *A. orchestii* it will be necessary to devote some time to a discussion of the facts pertaining to this question.

Part A of this paper deals with the systematic position, morphology, and division of the new species while part B concerns conjugation and reorganization.

The study was begun at the Marine Biological Laboratory, Woods Hole, Massachussets and continued in the zoological laboratories of Bard College and Columbia University.

Materials and Methods.

The sandfleas, *Orchestia agilis*, were found in extreme numbers under moist seaweed on the beaches near Woods Hole, Mass. They were taken from two different localities: Nobska Beach on Vineyard Sound and from Sippewissett Beach on BUZZARD'S Bay. Although the environmental conditions differ somewhat between these two places we have been unable to make any distinction between the amphipods from the two localities as regards either the hosts themselves or their parasites.

In heavily infected hosts the parasites occur in countless numbers, sometimes literally packing the lacunae or blood-vascular spaces which represent the body cavity. In macerated preparations they may be seen moving about in the transparent distal portions of the appendages, in the epipodial (branchial) lamellae, etc. In the crowded cavities of the appendages they appear to swarm over the surfaces of the appendicular musculature, the endothelium of the body wall, and between the sinewy or chitinous terminal pieces (tendons) at the joints.

About 10 per cent of the adult amphipods were heavily infected, approximately 10 per cent were lightly infected, and the remaining 80 per cent were devoid of astome representatives. Since no statistical data were kept on this point the incidence is estimated rather than calculated. The incidence was decidedly lower in immature hosts. During the month of August, when our preparations of the conjugating forms were being made, approximately 20 per cent of the heavily infected amphipods contained conjugants in great numbers.

The arthropods were macerated in a minimum of seawater or normal saline solution and smeared onto albuminized cover-slips. In order to clearly distinguish the number and arrangement of the ciliary lines and the nuclei at the same time special treatment was necessary. Accordingly, the morphological studies are based on smears fixed in BOUIN'S or FLEMMING'S (strong) mixtures, stained with HEIDENHAIN'S haematoxylin, and differentiated in cold ferric alum or, more frequently, in 10 per cent hydrogen peroxide (KIDDER, 1933). The standard technical methods used in this study were, for the most part, similar to those reported in our previous study on the ectocommensal ciliates of the Orchestiidae (KIDDER and SUMMERS, 1935).

A. Systematic position, morphology, and division of *Anoplophrya orchestii* sp. nov.

STEIN (1852) was the first to record an astome from an amphipod, *Gammarus pulex*. He named this ciliate *Opalina branchiarum*. KENT (1880—1882), as well as later authors, placed this organism in the genus *Anoplophrya* which STEIN had created somewhat later (1860), calling it *A. branchiarum* STEIN. Then BALBIANI (1885) described *A. circulans* from the fresh-water isopod, *Asellus aquaticus*. The following year SCHNEIDER (1886) proposed the name *Hyalina* for this isopod parasite but as indicated by CÉPÈDE (1910), this name was preoccupied.

BUTSCHLI (1889) believed that *Anoplophrya circulans* BALB. and *A.* (*Opalina*) *branchiarum* ST. were identical. It is clear from the title of COLLIN'S (1909) study of the conjugation of *A. branchiarum* from *Gammarus* that he too considered *A. branchiarum* identical with *A. circulans*. BRUMPT (1913) commented upon the fact that the structure and the phenomena relating to conjugation in the paratites of *Asellus* and *Gammarus* are nearly identical. He suggested that the discrepancy in size between the conjugating pairs from the two different hosts may possibly be due to their physiological differences.

CÉPÈDE (1910) proposed a new genus, *Collinia*, for the astomatous parasites of the blood-vascular system of fresh-water Arthrostraca (Amphipoda and Isopoda). Three species were rocognized: *Collinia branchiarum* ST. from *Gammarus pulex*, *C. circulans* BALB. from *Asellus aquaticus*, and *C. neoniphargi* CÉPÈDE from *Neoniphargus moniezi*. His generic diagnosis was as follows: lines of cilia few in number; number of contractile vacuoles variable, according to the size of the somewhat polymorphic individuals. CÉPÈDE based his classification partly upon host-parasite relationships. On the other hand, CHEISSIN (1930) called attention to the morphological similarities between the astomatous parasites of divers sites of infection, e. g., gut and body cavity. With the organization of the ciliates in mind rather than their different sites of infection he placed the coelozoic Astomata from Arthrostraca again in the genus *Anoplophrya*, thus making *Collinia* a synonym of *Anoplophrya*. We are inclined to follow CHEISSIN rather than CÉPÈDE as regards the systematic position of this relatively homogeneous group of parasites.

It is clear from our studies that there are striking similarities between the coelozoic forms from *Orchestia* and those previously

described for *Asellus*. However, we have examined Astomata from our common fresh-water isopod, *Asellus communis*, and found that these are definitely not the same as those from *Orchestia*. It is probable that a critical comparison of the Astomata from *Asellus*, *Gammarus*, and *Orchestia* will indicate that the host-parasite specificity is relatively strong.

Our composite figures of the trophozoites are based upon the picture presented by living material as well as that especially prepared for ciliary structures (*supra vide*).

General Description. The body shape of *Anoplophrya orchestii* varies somewhat with the size of the individual. In average representatives it is fusiform, tapering from a wide, broadly rounded posterior end to an evenly rounded point anteriorly. Large individuals are often sub-spherical with obliquely truncated ends (Pl. 9 Figs. 3, 4); smaller specimens may be fusiform, pear-shaped, or globose (Pl. 9 Figs. 1, 2, 5, 6).

The ciliary lines are meridionally arranged and unequally spaced. They are more numerous and closely set on one surface, fewer and farther apart on the opposite. For convenience these are designated as the dorsal and ventral surfaces respectively. There is a decided tendency for the rows to spiral, although frequently in the small fusiform specimens they pass directly from pole to pole. Usually there is a gap between the rows on the ventral surface where cilia are lacking, forming a "naked area" (Pl. 9 Figs. 8, 9). While the width of the naked area often depends upon the size and shape of the organism this may not always obtain since this character may differ considerably between two individuals otherwise alike. Rarely, in small representatives, a second but less conspicuous naked area occurs on the dorsal surface, dividing the rows of cilia into two sub-equal groups (Pl. 9 Figs. 11, 12). The rows are frequently more numerous on the right side of the naked area. In individuals known to be ex-conjugants, and others of similar size, there are often three or four equidistant and closely set rows in this position (Pl. 9 Figs. 2, 12).

Although the number of rows and their exact disposition fluctuates in cells of identical size the number of lines appears to be a general function of cell size. In 120 individuals the number of ciliary lines varied between the limits of seven and forty-five, with a mean of twenty-two. Individuals with ten, sixteen, and thirty-six rows occurred most frequently.

In our material the gamonts and pre-conjugants are preponderant in a group consisting of individuals between the limits of 8—20 μ in length and having 8—10 ciliary lines; ex-conjugants and reorganizing forms comprise the major part of an intermediate class, those between 20—30 μ in length, with 12—16 rows; a third group embraces the larger trophozoites of 35—65 μ in length, with 20—45 rows. Additional data regarding the size relations are given in Table 1.

Table 1.
Measurements of Greatest Body Length.

Types of Individuals	No. of Individuals	Mean	Mode	Extremes
Conjugants (largest member of a pair)	75	12.8 μ	14 μ	9—19 μ
Reorganizing forms (ex-conj.)	96	20.2 μ	20 μ	16—25 μ
Trophozoites	117	22.8 μ	20 μ	6—59 μ
Mixed trophozoites and exconj. (No pairs)	308	29.0 μ	22 μ	9—68 μ

The contractile vacuoles, like other structures in *Anoplophrya orchestii*, are subject to marked fluctuation not only in number but also in position. There are two series of contractile vacuoles in the larger trophozoites (Pl. 9 Figs. 3, 4), one on each side of the body, with an average of about five members in each. Trophic organisms approximately 30—35 μ in length or smaller normally possess a single contractile vacuole in the mid-line near the posterior end of the body (Pl. 9 Figs. 1, 2, 5, 6). This is also true of the conjugants and ex-conjugants. It should be noted that when several vacuoles are present it is rarely possible to find one in the terminal position. While it may be assumed that additional vacuoles arise in response to an increase in the protoplasmic volume we have no explanation for the differences in position.

It is obvious that a systematic grouping based upon the number and position of the contractile vacuoles is precarious. This criterion has been used, in part, for the separation of the subfamilies Anoplophryinae Cépède and Perezellinae (Cépède) Cheissin (see Cheissin, 1930). It is also relatively certain that the polymorphism exhibited by these organisms, as regards size, number and arrangement of ciliary rows, and number and position of the contractile vacuoles is an expression of the developmental phases to be discussed later.

The macronucleus is voluminous in relation to the cell as a whole, usually moreso in large than in small individuals. In shape

it corresponds roughly to the outline of the cell containing it. Notable exceptions to this are the relatively young ex-conjugants where the macronucleus is characteristically spindle-shaped or pyriform. The chromatin is dispersed as irregularly shaped granules, somewhat small and uniform in size. When basic stains are sufficiently extracted from the nucleus a core of one or more endosomal bodies of varying sizes may be seen (Pl. 9 Figs. 18, 19). These are not rendered with the FEULGEN fuchsin-sulfurous acid mixture following acid hydrolysis but they do react with certain plasma dyes in techniques such as the BORREL method, indicating possibly, a deficiency in some of the nucleic acid constituents.

A single spherical micronucleus occurs near the mid-region, juxtaposition with the macronucleus of resting organisms.

In addition to *Anoplophrya orchestii*, *Orchestia agilis* harbors another large but rare form which we cannot describe fully for lack of material. From all our preparations we have recovered only seven or eight of these forms and have had but a glimpse of it in the living state. They are extremely delicate, cytolysing within a few moments after liberation from the host. Although fixed specimens show the arrangement of the cilia very well the nuclei appear to have disintegrated in all of them. This is not surprising in view of the fact that in the common species the nucleus becomes a large watery vesicle before the cell body cytolyzes.

The puzzling arrangement of the ciliary lines in one specimen is accurately represented in Pl. 9 Fig. 7. Counting these in the equatorial plane at the posterior third of the body there are 10 plus 16 rows on one surface and 8 plus 26 on the other. The relationship between the rows on the two surfaces is not clear however. The only living specimens observed were very flat and definitely twisted. In this organism there are two naked areas arranged as shown in the figure.

Cytology of division. The division rate in *Anoplophrya orchestii* is relatively high although not uniform throughout the size range, being slightly higher in large organisms if one is to judge by the frequency of division figures encountered. There is also a noticeable tendency for the divisions to be unequal which, together with the greater division rate, indicates that the small gamonts may be derived from the larger trophozoites. In this connection we are at a loss to account for the origin of variations in the number of rows of cilia. As far as can be observed in all

types of preparations for ciliary structures, including the silver precipitation methods, the ciliary make-up of both resulting daughter organisms is identical. Variations may take place simultaneously in both daughters during division or, less likely, during the interdivisional period. Reorganization of the motor organellae immediately subsequent to division has not been encountered.

While the macronuclear volume varies with the size of the cell that of the micronucleus remains almost constant. In the initial prophase activity the micronucleus enlarges to about three times its original size. The chromatin material becomes a loose aggregate of numerous, small, almost uniform chromomeres dispersed in a matrix of faintly staining ground substance. At this time there is a narrow zone of hyaline substance between the granular mass and the nuclear membrane. The number of prophase chromomeres approximates the number of bodies later projected into the metaphase. The prophase activity apparently does not include a spireme formation.

The mitotic spindle arises entirely within the nuclear membrane from the region of the clustered chromatin granules. It is not possible to see whether it arises directly from the granules or from the lightly staining intergranular substance. At any rate, columns of achromatic material arise at opposite ends of the central aggregate and grow towards the two definitive poles. Frequently the material constituting a developing column is revealed as a fasciculus of non-focalized fibrillae.

In the metaphase the chromosomal bodies are compactly arranged in the equatorial plane (Pl. 9 Fig. 13). Despite the wealth of material we are unable to give an accurate estimate of the chromosome number. They are very small, closely packed, and relatively numerous.

By the time daughter chromosomes begin to separate the spindle is usually completely formed and focalized. It extends through the nucleus in the long axis from points at opposite ends of the nuclear membrane. As the chromosomes migrate towards the poles the length of the spindle increases while its breadth at the equator decreases. The duration of the anaphase is greater than any of the other division phases. The most frequent configuration of the micronucleus in large trophozoites is as shown in Pl. 9 Fig. 14. This condition seems to be a characteristic feature of the larger individuals, one which suggests the long-lived kinetic phases in some of the Opalinidae. When the chromosomes approach their

respective poles a definite central spindle component may be seen. In passing we can only point out that this may represent interzonal fibers, continuous fibers, or both, as have been described for Metazoa.

The central spindle substance disappears as the daughter nuclei separate. For a short time the only visible connection between them is the flared nuclear membrane (Pl. 9 Figs. 15, 16). The daughter nuclei then pass to opposite ends of the cell (Pl. 9 Figs. 17, 18).

Visible changes in the macronucleus preparatory to division are slight. About the only activity shown is a perceptible contraction as the micronucleus enters the metaphase. In alum differentiated haematoxylin preparations after BOUIN'S fixation the previously mentioned core of endosomal bodies are well shown. When the macronucleus constricts and divides the endosomes are passively distributed although those which occupy an equatorial position within the nucleus sometimes constrict and divide (Pl. 9 Figs. 18, 19). The attenuated macronucleus and the cell body pull apart almost simultaneously.

B. Conjugation and Reorganization.

The conjugants comprise a group of small ellipsoidal or pyriform individuals whose length varies between the limits of approximately 9—19 μ (see Table 1). They are further characterized by a relatively small number of ciliary lines (approximately 8—10), and a single terminally situated contractile vacuole. In the initial stages of conjugation the macronucleus is ovoid or slightly fusiform in shape (Pl. 10 Fig. 20).

In the formation of pairs the gamonts tend to fuse with ventral surfaces (i. e., the naked areas) apposed although this relation is not invariable. Before amphimixis the angle between the conjugants is approximately 90 degrees. Following fertilization it widens until the organisms come to lie almost in a straight line.

The first indication of micronuclear participation occurs immediately following the union of gamonts. The volume of clear ground substance increases considerably so that a thick cortical layer of nuclear sap separates the membrane from the enclosed chromatin endosome. Coincident with the swelling of the vesicle the endosomal mass undergoes a decrement in its staining affinities and "loosens" to form a crescent-shaped aggregate of flocculent chromatin. In this state of flux the chromatin aggregate may assume an eccentric

position within the vesicle (Pl. 10 Fig. 20). The flocculent material subsequently condenses into a number of discrete chromomeres which appear to be suspended in achromatic medium (Pl. 10 Fig. 21).

From the midst of the clumped chromomeres, or even before these merge from the flocculent state, a column of achromatic fibrillae arises and extends across the long axis of the nucleus towards the position later occupied by one pole of the spindle (Pl. 10 Fig. 22). In the organism represented by Pl. 10 Fig. 20 these fibrillae are well defined. The chromatin masses in this pair are slightly more compact than usual at this time, also the fibrillar column seems to be prematurely focalized in one member of the pair. The origin of the first maturation division figure in *Anoplophrya orchestii* strongly suggests the parachute formation of many hypotrichs such as *Uroleptus mobilis* (Calkins, 1919), *Euplotes patella* (Turner, 1930), etc. The bouquet arrangement of the chromatin threads in the prophase of *A. branchiarum* as described by Collin (1909) and Brumpt (1913) has no exact parallel in this species. Also these authors found few and elongate chromosomes in the first maturation division.

In the further development of the spindle the cluster of chromomeres assumes a central position in the nucleus, becoming dispersed in the region of the definitive equatorial plane (Pl. 10 Fig. 22). By this time the spindle extends into the antipodal region. With the completion of the achromatic figure the dispersed chromomeres assume the metaphase pattern. This first maturation spindle is larger and better defined than those of the succeeding maturation or post-gamic divisions.

Each chromomere of the prophase appears to represent a chromosome of the metaphase. Because of their small size we are unable to reach a definite conclusion regarding the number of chromosomes. In the early anaphase they separate as shown in Pl. 10 Fig. 23, where approximately one-half of the total number passes to each pole. At the late anaphase the chromosomes collect into a compact, homogeneous, densely staining cone in contact with the nuclear membrane at each pole (Pl. 10 Fig. 24). Frequently interconnecting strands of chromatic material occur. In stages later than this the central part of the elongating vesicle becomes clear. The relatively heavy intradesmose which has been figured for the corresponding stage in *Anoplophrya branchiarum* (Collin, 1909; Brumpt, 1913) is lacking not only in the first division but in subsequent divisions. The polar cones round up and separate from the vesicular part of the original nucleus, leaving a large ellipsoidal vesicle in the mid-region (Pl. 10

Fig. 25). As far as can be determined the clear vesicle gradually diminishes in size and disappears. This is probably comparable to the "résidu fusorial" of *Hoplitophrya* (*Anoplophrya*) *brasili* (COLLIN, 1912). The first maturation results in two daughter nuclei, each surrounded by a clear vesicle (Pl. 10 Fig. 26).

Individuals with nuclei undergoing the second maturation division are relatively rare in our preparations. The earliest phases which we have recorded are as illustrated in Pl. 10 Fig. 27. These spindles are small and, in contrast to those of the first division, appear to be more or less synchronously formed. There appears to be approximately as many chromosomes here as in the corresponding stage of the preceding division. Four micronuclear products are formed in each conjugant. They are about equal in size and homogeneity to those produced by the first division.

In almost every case only one of the four nuclei thus formed is destined to enter the third division (Pl. 10 Fig. 28). The remaining three undergo no further differentiation, persisting in *status quo* for a time before atrophic alterations begin. That which enters the prophase of the third division swells to double its interphase volume, its enlarged chromatin mass breaks up into fragments which form the chromosomes of the ensuing metaphase (Pl. 10 Fig. 29). The chromosomes of this division appear to consist of short linear aggregates of minute and roughly irregular granules. They are arranged as a compact group in the equatorial region of a very delicate spindle. We have been unable to differentiate between them or to observe the manner by which they divide. While these progressive changes are occurring in the activated nucleus, usually during the anaphase stage (Pl. 10 Fig. 30), it passes to an antero-median position adjacent to the point of union of the two conjugants.

The achromatic figure of the third anaphase disappears early, leaving the two clumped chromosomal aggregates at opposite ends of the unconstricted micronuclear membrane (Pl. 10 Fig. 31). The cytoplasms of the gamonts remain spearate until the anaphase of this division. The cell walls in contact with one another then dissolve and the cytoplasms of the two conjugants merge, forming a "protoplasmic bridge" (Pl. 10 Fig. 30). The division figures from each conjugant are projected into the zone of juncture where they come to lie close together and more or less parallel. The two spindles, one from each gamont, are oriented so that their equatorial regions lie approximately in the plane of the old septum. Constriction of the micronuclear membranes results in the formation of the pro-

nuclei. Reciprocal exchange is thus effected directly, as in *Chilodonella (Chilodon) uncinatus* (Mac Dougal, 1925), without the formation of the long attenuated or flared connectives which have been described for so many other ciliate types.

We have observed several anomalous cases in which the third maturation spindle of one gamont is carried bodily into the other. Instances such as these, as well as others to be described subsequently, may account for some of the unusual reorganization sequences. This is also further evidence that the syncytial amalgamation of the cytoplasms is so complete that various plastids may be carried from one organism to the other.

Immediately after the interchange and just before syngamy the juxtaposed gamete nuclei become slightly chromophobic. At first the nuclear material occurs as a flocculent mass but when swelling begins in preparation for the fertilization process the flocculent chromatin is dispersed to the periphery of the vesicles as indistinct, irregular granules or strands (Pl. 10 Fig. 32).

The exact method by which fertilization occurs can only be surmised. Pl. 10 Fig. 32 indicates that the union is effected by the confluence of the two enlarged pronuclei. The chromatin from complementary gametes is concentrated at apposite ends of the synkaryon (Pl. 10 Fig. 33). The individuality of the gametic contributions is lost, however, as the prophase threads arise (Pl. 10 Fig. 34). The threads arise from opposite ends of the zygote nucleus and intermingle in the mid-region as meridionally disposed strands. These strands contract into the chromosomes of the initial amphinuclear division (Pl. 10 Fig. 35). This division is the only one in which we have observed thread-like formations prior to the elaboration of chromosomes. In all others the chromosomes seem to be formed by the fragmentation of a central mass of chromatin. The regressing by-products of the second maturation division may or may not be evident at this time. If present, they are usually scattered about variously in the cytoplasm. They may degenerate completely by the middle of the third maturation division or they may persist as well defined bodies until after the metagamic divisions.

Following syngamy the amphinucleus leaves the apical region and passes towards the mid-region of the cell. The chromosomes of the first amphinuclear division are compact rod-like bodies as indicated in Pl. 10 Fig. 36. The large amphinuclear vesicle is clear and relatively homogeneous. A definite spindle is not apparent at this time although as the daughter chromosome groups separate and

pass towards their respective poles (Pl. 10 Fig. 37) a number of short, fine strands of deeply staining material project centrally from them. The two lightly staining nuclei produced by this division appear to be identical in structure (Pl. 10 Fig. 38). The fused telophase chromosome group within each vesicle persists as a loose-textured framework which soon breaks up into discrete prophase granules for the ensuing division. The granules coalesce into a compact disc-like mass of chromatin in the equatorial plane of the dividing nucleus (Pl. 10 Fig. 39, 40). The disc is so homogeneous that definite chromosomes, as individual entities, cannot be differentiated. The clumping of the chromatin in this fashion is characteristic of this particular division. The vesicles are relatively small and very clear. Spindle fibers cannot be seen at any time during this kinetic period.

The chromatin disc divides equatorially and the daughter halves assume the shape of blunt conical masses at each pole of the lengthening vesicle. Except for their smaller size the anaphase nuclei of this division are similar to those of the first. The division products, four in each conjugant, seem to be identical for a brief interval following their formation. They become small densely staining endosomal nuclei, equivalent in size to the interphase micronucleus of the trophozoites.

The macronucleus of the early conjugant begins to increase in length as the micronucleus approaches the metaphase of the first maturation division. The gradual elongation continues until by the end of maturation it extends well into the forward end of the cell in preparation for an exchange of macronuclear parts (Pl. 10 Figs. 30—33) which takes place soon after fertilization. The actual crossing-over of the macronuclei begins with the initiation of the fertilization process. We find this to be very helpful in identifying organisms in or near the fertilization stage.

In the interval between fertilization and the beginning of the first metagamic division the forward end of the elongating macronucleus of each conjugant passes across the protoplasmic bridge into the cytoplasm of the alternate member of the pair (Pl. 10 Figs. 34, 36). Elongation continues until a reciprocal exchange of macronuclear parts is effected (Pl. 10 Figs. 38—40). Each conjugant retains approximately one-half of its original macronucleus and receives approximately one-half of that belonging to the other conjugant (Pl. 10 Fig. 41). Generally the exchange of macronuclear parts is complete by the middle of the second division. This macronuclei tend to break up into elongate fragments in preparations fixed in

fluids containing mercuric bi-chloride and acetic acid. This tendency is not particularly evident in specimens fixed otherwise (e. g., BOUIN's) hence may be regarded as a fixation phenomenon.

While macronuclear crossing-over is in progress the conjugants tend to straighten out in anticipation of the separation which soon follows. As a rule the crossed macronuclei pull apart and the conjugants separate sometime during the second amphinuclear division (Pl. 10 Fig. 41) although it may occur as early as the anaphase of the first metagamic mitosis or be delayed until differentiation of the four amphinuclear derivatives begins.

The regularity with which macronuclear crossing-over occurs is noteworthy. The process was in some way amiss in but one or two specimens out of several thousand. One interesting but very infrequent variation is the occurrence of triple conjugants (Pl. 10 Fig. 42). The distribution of the macronuclei in the specimen illustrated is a further manifestation of the orderliness of the crossing-over process. In two other triple specimens the distribution of macronuclear halves was 3—2—1. The distribution of the amphinuclear derivatives in the triple specimen shown is irregular. Presumably micronuclear material from each individual is represented in the six nuclei in two of the three conjugants. The possibility of an unusual interchange of amphinuclei or their derivatives between conjugants may contribute to the formation of ex-conjugants with the abnormal numbers of amphinuclear derivatives to be deseribed below.

The prevalence of ex-conjugants with undifferentiated amphinuclear derivatives (Pl. 10 Fig. 43) indicates that the period between the last metagamic division and macronuclear differentiation is relatively long. At the close of the indifferent period the distinctive behavior of one of the four nuclei identifies it as that which will become the rudiment of the new macronucleus. The first manifestation of differentiation is the initiation of a prolonged growth period accompanied by a gradual decline in the staining capacity of the chromatin (Pl. 10 Fig. 44). The swelling of its component parts is differential: at first the growth of the chromatin substance does not keep apace with the increase in the volume of the clear ground substance. The ultimate size of the vesicle is approximately as indicated by Figs. 44—46 Pl. 10. Coincident with the rapid swelling of the vesicle the enclosed chromatin material is transformed into a sphere of oxyphilic substance, homogeneously granular in texture, and usually eccentric in position. Within the enlarging granular

sphere fine, discrete chromatin particles begin to appear, increasing in number and size until the oxyphilic material is almost completely displaced. The reconstitution of the chromatin material in the growing anlage is usually finished before the old macronuclear parts begin to diminish in size. At this stage the chromatin mass occupies about one-third of the volume of the vesicle. A slowing up of the growth activity of the macronuclear anlage at this stage of activity is indicated by the great frequency with which it occurs.

From the beginning of differentiation the macronuclear anlage almost invariably occupies a position in the posterior part of the organism. It remains thus until the old macronuclear parts are completely resorbed.

Apparently any two of the four normally expected derivatives of the amphinucleus become the definitive micro- and macronuclei while the remaining two are resorbed after the anlage differentiates.

Although the post-gamic divisions are isochronous the ex-conjugants have varying numbers of nuclei. The nuclear makeup of 110 randomly selected ex-conjugants is shown in the tabular summary below.

Number of undifferentiated micronuclei	Number of macronuclear anlagen	Number of individuals
1	1	18
2	0	4
2	1	28
2	2	1
3	0	4
3	1	13
4	0	6
5	0	11
5	1	10
6	0	1
6	1	7
7	0	3
7	1	1
8	0	2
9	0	1

Ex-conjugants with four or less than four nuclei, including the macronuclear anlagen when differentiated, occur more frequently than those with a greater number, the ratio being about 73—37.

Ex-conjugants falling into the first category follow the normal expectancy. The fact that there are many organisms with two micronuclei and one anlage suggests that the non-persisting nuclei are resorbed at different times, resulting ultimately in organisms

with one micronucleus and a single macronuclear anlage. The premature separation of ex-conjugants before the end of the first amphinuclear division results in two undifferentiated amphinuclear products, which in the succeeding division give rise to four.

Those organisms containing from 5—9 nuclei may arise by one or more of the following methods:

1. Odd distributions may be effected by the transferrence of micronuclei or amphinuclear derivatives from one conjugant to the other, apparently at any time after the protoplasms of the conjugants merge. Best evidence of this is afforded by frequent cases where three second amphinuclear division spindles occur in the central part of one organism and one in the other (Pl. 10 Fig. 40). Uneven distributions of the interphase nuclei produced by this division also occur. The persisting maturation by-products which are also present in some instances often can be distinguished by their pale appearance and small size. Possible transferrence of micronuclear products during the third maturation division is suggested by Pl. 10 Fig. 30. Corroborating this notion, we have noted in very rare specimens, late conjugants and ex-conjugants without nuclei; pairs in which one individual contains supernumerary nuclei and the other contains none. The distribution of the nuclei in triple individuals (Pl. 10 Fig. 42) and the occurrence of ex-conjugants with two anlage (Pl. 10 Fig. 53) are also significant.

2. In some cases the supernumerary nuclei are the persisting maturation by-products. As a rule these disappear during the first metagamic mitosis (Pl. 10 Figs. 36, 37) but we occasionally find one or more of these intact during the late phases of the second amphinuclear division. If nuclei are not carried over from one organism to the other the maximum number of micronuclei in each organism following the two metagamic divisions would be seven, the four products of the two metagamic divisions plus the three maturation by-products. That is of course provided the maturation by-products do not undergo a subsequent division, which they apparently do not.

3. A third possibility is that one or more of the derivatives of the second amphinuclear division undergo a third division, as noted by Brumpt (1913). Of this we cannot be certain, but the micronuclei-like bodies of a few ex-conjugants appear as elongate spindle-shaped bodies. It is conceivable that those nuclei which play no further part in the history of the organism divide amitoti-

cally before degenerating. In organisms with from 7—9 nuclei the latter are sometimes grouped in pairs.

The regression of the old macronuclear parts begins after the resorbtion of all the amphinuclear products except those which become the functional nuclei of the reorganized individual. The macronuclear resorbtion, once initiated, is relatively rapid. Atrophic changes are not striking. In general resorbtion occurs without the loss of the smooth outline or extreme fragmentation, although exconjugants with more than two pieces are by no means rare (Pl. 10 Figs. 51, 52). The rounding up of the old macronuclear halves and the dispersion of fine particles into the cytoplasm as described for *Anoplophrya branchiarum* (COLLIN, 1909; BRUMPT, 1913) are not characteristic of *A. orchestii*.

Coincident with the onset of macronuclear resorbtion a variable number of small clear vesicles begin to appear in the cytoplasm in the environs of the old macronuclear halves (Pl. 10 Fig. 45). They seem to arise spontaneously in the cytoplasm, for precursory structures are not evident. The time and place of origin is indicative of a correlation with macronuclear resorbtion.

In HEIDENHAIN'S haematoxylin preparations fixed in BOUIN'S or SCHAUDINN'S fluids and moderately extracted with ferric alum or picric acid the visicles appear as light gray vacuoles with membranes of slightly darker tone. With further differentiation the gray gives way to a light yellow tint. They also react with the blue-green counterstain of the BORREL mixture. The number of cytoplasmic vesicles is at first small but with the continued resorbtion of the macronuclei they increase in number and volume. The ultimate number of vesicles varies, the central part of one organism may be literally packed with them whereas another at approximately the same stage of reorganization may contain relatively few. When closely packed they sometimes appear to constitute an amorphous yellow mass (Pl. 10 Fig. 46).

Inclusions in the form of minute densely staining granules appear within each of the "yellow vesicles", usually one within each vesicle (Pl. 10 Figs. 46, 47). The enclosed granules in turn grow until they are about one-fourth the size of the vesicle, at which time they frequently assume an eccentric position as shown in Pl. 10 Fig. 48. In the largest of these granules a dark cortex and a light medullary portion can be seen. The intra-vesicular bodies accept the same stains as the vesicles although to a greater

degree. Neither the granules nor the spheres respond to the FEULGEN test for nucleic acid.

Following the rise of the granules within the vesicles, granules of similar size and structure begin to appear free in the cytoplasm, becoming very numerous throughout the cell towards the completion of macronuclear resorbtion. Some of the intra-vesicular and the cytoplasmic bodies are coexistent for a time. The former decrease as the latter increase in number until finally the extra-vesicular types alone are present. This inverse relationship suggests that one type is converted into the other. Albeit the eccentric position of the granules within the vesicles invites the conclusion that they are subsequently extruded, diligent search has not confirmed this assumption.

The cytoplasmic granules have a transitory existence. Following the disappearance of the old macronuclei the staining capacity of the granules wanes until they finally fade from view.

In the majority of ex-conjugants the yellow spheres persist until the cytoplasmic bodies disappear and the new macronucleus moves into a central position (Pl. 10 Fig. 49), after which they too suffer a similar fate. The succession of cytoplasmic inclusions just described appears to be purely a reorganization phenomenon for none of the inclusions can be identified as permanent components of the cytoplasmic architecture.

The elaboration of the transient cytoplasmic elements is accompanied by a perceptible acceleration of growth activity in the macronuclear anlage. The clear cortical portion of the early anlage is obliterated by the accumulating mass of deeply staining chromatin granules (Pl. 10 Fig. 47). At about this time the new macronucleus moves forward into a central position where it assumes a characteristic fusiform shape (Pl. 10 Figs. 48, 49). The chromatin within the new macronucleus is arranged as spherical granules, uniform in size and regularly spaced. This feature of the nucleus, as opposed to that of the vegetative organisms, together with its fusiform shape, is often helpful in identifying recently reorganized individuals after other criteria of reorganization have disappeared.

It was stated earlier that the ex-conjugants are on the average larger than the gamonts. The increase in size takes place during the maturation divisions and to some extent after fertilization. Those individuals completing the reorganization process possess from 10—16 ciliary lines, an increase of 2—8 over the original number.

Discussion.

One of the most interesting questions that has arisen from our work on *Anoplophrya orchestii* is that of the variation in number and arrangement of the ciliary lines. In most forms the number of ciliary lines may be taken as a fairly safe diagnostic characteristic for specific determination, even when cell size may not. Here, however, it is obvious that neither of these criteria are of specific value. As was indicated above when a critical examination of a large number of ciliates was made all gradations in size between the extremes of 6 μ and 68 μ were found. Also the number of ciliary lines varied in almost perfect gradations between 9 rows in the smaller ciliates to 45 rows in the larger specimens. We are now convinced that variations in the size of the cell and the number of ciliary rows are correlated with developmental stages. This was the general conclusion reached by BALBIANI (1885) and by SCHNEIDER (1886) in regard to *Anoplophrya circulans* from the body cavity of *Asellus aquaticus* but strangely enough they found that the number of ciliary lines remains constant throughout the range in size.

BALBIANI thought that the size variations were due to seasonal changes whereas SCHNEIDER, who found ciliates of all sizes during conjugation, ascribed the variations to cyclical changes. In working on the astomes from *Gammarus* and *Asellus*, COLLIN (1909) and BRUMPT (1913) did not comment on the number of lines of cilia in organisms of different sizes but both agree with SCHNEIDER that the largest ciliates represent the trophozoites while the small forms are the pre- and post-conjugants.

Our observations lead us to agree with the above authors as to the probable expression of developmental stages in size variations but we must go beyond them in regard to the variations found in the ciliary pattern.

As to the *modus operandi* of ciliary line variation we can at presend only conjecture. From a careful study of our material with this point in view we are inclined to the belief that any variation which takes place does so in the inter-divisional period. Certainly we have observed too many perfectly clear preparations of ciliates just prior to plasmotomy to have missed all cases of differential addition or loss of lines if such a thing occurred at the time of division. Apparently the whole ciliary reorganization occurs in the cell prior to or during the very early stages of divisional activity or the process is delayed until the daughter ciliates separate. Sup-

porting this view is the fact that there appears to be a decided increase in the number of ciliary lines in the ex-conjugants before any of the cell divisions take place. Most of the ex-conjugants in late stages of reorganization possess approximately 16 rows while the number seen in individuals of a conjugating pair is 8 to 10. In this connection it is interesting to note the case of *Cyathodinium piriforme* as reported by LUCAS (1932). This species undergoes a process of reorganization without necessarily dividing. In doing so it changes its shape and completely loses its locomotor organelles. The cilia are reformed *in toto* from an "endocellular ciliature anlage".

In view of the fact that a great many species of ciliates grow smaller during the time when gamonts are together in the act of conjugation it is of interest to note the exact reverse in the case of *Anoplophrya orchestii*. As the later stages of conjugation are reached there is a slight but noticeable increase in size in both members of a pair. There appears to be a slight clearing of the cytoplasm but this is variable. We may assume that the diminution in size in the case of stomatous forms (*Uroleptus* CALKINS, 1919; *Diophrys*, unpublished observations of one of the authors, F.M.S.; etc.) results from the lack of food as in these forms the mouths are closed by their position of attachment. But in the astomatous ciliates it is probable that the feeding process continues (by absorption) during conjugational activity. In support of this assumption it should be noted that in some stomatous ciliates the mouths are functional during conjugation and the gamonts do not decrease in size. This is especially true of *Kidderia* (*Concophthirius*) *mytili* (KIDDER, 1933).

The perfectly regular occurrence of the exchange of macronuclear halves during conjugation is rather puzzling in view of the fact that the old macronucleus plays no apparent part in the reorganized cell or the future generations. This peculiar behavior of the old macronucleus has been reported in all species of the genus *Anoplophrya* where conjugation has been observed (*A. circulans* SCHNEIDER, 1886, BRUMPT, 1913; *A. branchiarum* COLLIN, 1909). It does not occur, however, in *Hoplitophrya* (*Anoplophrya*) *brasili* according to COLLIN (1912). Recently MACDOUGALL (1935) has described macronuclear exchange during conjugation in the freshwater stomatous ciliate *Chilodonella* sp. which exactly parallels the situation observed in *A. orchestii*. The significance of such an exchange is problematical. It may possibly represent a reminiscence of a more primitive protozoan condition before the separation of the trophic nuclear

materials from the germinal materials or it is conceivable that there may be some genetic significance attached to the process. SONNEBORN and LYNCH (1934) believe that some of the delayed action of character changes resulting from hybridization of *Paramecium* may possibly be due to the persistence of old macronuclear parts. They point out, as argument for their belief, the incorporation of old macronuclear fragments into the new macronuclear architecture that has been reported by IKEDA and OZAKI (1918) in *Boveria labialis* and by TURNER (1930) in *Euplotes patella*. It would be impossible at the present time to test the worth of the above assumption as it concerns *Anoplophrya*, so for the time being we will be forced to regard the phenomenon as an interesting cytological observation.

The method of pronuclear crossing during conjugation is almost identical with that of *Chilodonella* (*Chilodon*) *uncinatus* (MACDOUGALL, 1925). Unlike the majority of forms, this stage (telophase of the third maturation division) is of relatively long duration, so we have had the opportunity to examine a great many cases of pronuclear exchange. It occurs just after the breakdown of the separating membranes between the two conjugants to form the protoplasmic bridge. In some cases the bridge at this time is very wide and the protoplasms of the two gamonts are intimately mixed while in others the protoplasmic fusion has progressed but to a limited degree. The exchange of pronuclei just precedes the start of the crossing-over of the macronuclear halves. As was stated above the protoplasmic currents in the region of the bridge may carry both pronuclei of one conjugant into the cytoplasm of the other member, resulting in irregularities of nuclear number during ex-conjugant reorganization. This situation is not the rule, however, as it seems to be in the case of *Metopus sigmoides* (NOLAND, 1927).

We have been entirely unable to determine the number of chromosomes on any of the division or maturation spindles, of which we have literally thousands. The minute size of the chromosomes and their closely packed condition defy analysis. We are certain, however, that *Anoplophrya orchestii* has a greater number than that given by COLLIN (1909) for *A. branchiarum* (six) or by BRUMPT (1913) for *A. circulans* (six to eight). This fact, together with the differences in the degeneration of the macronuclei, convince us that there is little chance that the ciliates from *Orchestia*, as described here, and those from *Asellus* and *Gammarus* are the same species.

The elaboration and general location of the peculiar yellow vesicles and the more deeply staining granules in the cytoplasm

of reorganizing ex-conjugants lead to the conclusion that they are somehow correlated with the degeneration of the old macronuclear halves, possibly representing a transient form of some of the products the macronuclear break-down. BRUMPT (1913) observed numerous cytoplasmic granules in the ex-conjugants of *Anoplophrya circulans* which he calls "granulations cyanophiles post-conjugales" because they retain the blue of the Mann stain. He believes that these granules represent reserve materials which allow the ciliates to exist without the advent of encystment. The granules of *A. circulans* seem to be comparable to the dark granules of *A. orchestii*. In the cytoplasm of the latter they lose their staining capacity and fade from view. We have found no evidence that they persist as permanent cell components as do the "mitochondria" of *Uroleptus halseyi*, formed in a somewhat similar manner (CALKINS, 1930).

The occurrence of the anomalies mentioned above in relation to the reorganizing ex-conjugants seems to have little or no effect on the nuclear number of succeeding generations. Either the ex-conjugants with abnormal numbers of nuclei are inviable or, more probable, the normal number is restored in some fashion. In either case the result would be the same for we have never found cases of abnormal numbers of nuclei in typical trophozoites (always one macro- and one micronucleus). The nuclear apparatus seems to be very stable in this highly polymorphic species.

Summary.

1. The astomatous ciliates which inhabit the body cavity of the amphipod *Orchestia agilis* belong to a single highly polymorphic species, *Anoplophrya orchestii* sp. nov.

2. The polymorphism is exhibited both in size and in number and position of ciliary lines. The size varies between 6 μ and 68 μ, while the number of ciliary lines varies between 7 and 45. In general the larger the ciliate the more numerous the ciliary lines.

3. Division is described as occurring very frequently in ciliates of all sizes. The micronucleus divides mitotically with numerous small chromosomes on a well defined spindle. The macronucleus divides by constriction.

4. Conjugation occurs between small individuals (9 μ to 19 μ). During conjugation there is a slight increase in the size of the gamonts.

5. There are three maturation divisions resulting in two pronuclei. The telophase of the third maturation division takes place in the region of the protoplasmic bridge and the exchange of pronuclei occurs during this division. The amphinucleus is formed by the confluence of the two complementary pronuclei.

6. The macronuclei of the gamonts elongate and a reciprocal exchange of macronuclear halves occurs just after the pronuclear exchange.

7. The conjugants separate and reorganize.

8. During ex-conjugant reorganization the amphinucleus divides twice, giving rise to four products. One product enlarges and becomes the macronuclear anlage, two degenerate, and the remaining one becomes the definitive micronucleus.

9. The old macronuclear halves shrink at the same time that two types of inclusions appear in the cytoplasm. One type is large and vesicular while the other is in the nature of deeply staining granules. These inclusions are interpreted as being products of the old macronuclear degeneration. Both types of inclusions lose their staining capacities and fade from view.

10. Numerous anomalies are reported including irregularities in nuclear number of ex-conjugants as well as the occurrence of triple conjugants. No significance is attached to these anomalies however.

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Explanation of Plates.

Plates 9 and 10.

All figures, except Pl. 1, fig. 7, are of *Anoplophrya orchestii*. Abbreviations for methods: B. Bouin's; F. Flemming's; H. Heidenhain's haematoxylin; A. 2 per cent iron alum (destain); S. 10 per cent hydrogen peroxide (destain); P. Picric acid (destain).

Plate 9.

Fig. 1. Small trophozoite as seen from dorsal side. F., H., S. × 1500.

Fig. 2. Same type seen from ventral surface. Note the naked area bounded on the left by the closely set rows of cilia. F., H., S. × 1500.

Fig. 3. Large trophozoite. Dorsal view. Note the numerous contractile vacuoles and the many rows of cilia. F., H., S. × 750.

Fig. 4. Same as figure 3. Ventral view. F., H., S. × 750.

Fig. 5. Small pear-shaped pre-conjugant. Ventral view. F., H., S. × 1500.

Fig. 6. Dorsal view of pre-conjugant. F., H., S. × 1500.

Fig. 7. Ciliary pattern of *Anoplophrya* Sp. from *Orchestia agilis*. F., H., S. × 1123.

Fig. 8. Cross section of a large trophozoite through the middle of the cell. F., H., A. × 750.

Fig. 9. Cross section through the middle of a small organism similar to that represented in figure 1. F., H., A. × 1500.

Fig. 10. Cross section through the middle of a pear-shaped pre-conjugant. F., H., A. × 1500.

Fig. 11. Cross section through one of a conjugating pair. The micronucleus is in the prophase of the first maturation division. B., H., A. × 1500.

Fig. 12. Cross section of an exconjugant showing the two halves of the old macronuclei and two products (of four) of the amphinuclear divisions. B., H., A. × 1500.

Fig. 13—19. Division.

Fig. 13. Metaphase. B., H., P. × 1500.

Fig. 14. Anaphase. B., H., P. × 1500.

Fig. 15. Early telophase showing the flared condition of the nuclear membrane between the daughter halves. B., H., P. × 1500.

Fig. 16. Slightly later stage than figure 15. B., H., P. × 1500.

Fig. 17. Late telophase. B., H., P. × 1500.

Fig. 18. Micronucleus in telophase. The macronucleus is beginning to constrict. Note the deeply staining endosomal bodies. B., H., A. × 1500.

Fig. 19. Late stage in the division of the cell. B., H., A. × 1500.

Plate 10.

Conjugation and reorganization of *Anoplophrya orchestii*. All figures are from material fixed in BOUIN'S fluid and stained with HEIDENHAIN'S haematoxylin. Differentiation was carried out in saturated aqueous Picric acid except in the cases of figures 46—49 where 2 per cent iron alum was employed. All figures. × 1500.

Fig. 20. Prophase of first maturation division.

Fig. 21. Chromatin in the form of small granules within the swollen nucleus.

Fig. 22. The left conjugant possesses a first maturation nucleus in a slightly later stage than that of the right. The spindle fibers make their appearance from the chromatin mass.

Fig. 23. Early anaphase of first muturation division. This individual possessed unusually large spindles.

Fig. 24. Anaphase of first maturation division.

Fig. 25. Telophase of first maturation division at the left, anaphase in the right hand individual.

Fig. 26. Products of the first maturation division.

Fig. 27. Second maturation division.

Fig. 28. Four products of the second maturation division in each gamont, three of which are small, compact bodies which will degenerate. The fourth represents the prophase of the third maturation division.

Fig. 29. Third maturation spindles. The protoplasmic bridge has completely formed and the macronuclei have elongated.

Fig. 30. Anaphase of third maturation division. In this pair both spindles have been carried into the protoplasm of the left gamont.

Fig. 31. Pronuclear crossing. This figure is typical and seems to be of fairly long duration.

Fig. 32. Fertilization. The two pronuclei on the left have fused to form the amphinucleus while those on the right are slightly later. Note the loose arrangement of the chromatin.

Fig. 33. Amphinuclei. The chromatin of the two pronuclei does not mix at this stage.

Fig. 34. Prophase of the first amphinuclear division. The macronuclei have started to cross.

Fig. 35. Later stage in first amphinuclear division.

Fig. 36. Metaphase of first amphinuclear division.

Fig. 37. Anaphase of first amphinuclear division.

Fig. 38. Products of the first amphinuclear division. The gamonts have straightened out and the macronuclei have nearly exchanged halves.

Fig. 39. Metaphase of the second amphinuclear division.

Fig. 40. About the same stage as figure 39. There are three second amphinuclear spindles in the right gamond and one in the left.

Fig. 41. Separation of conjugants. The two macronuclei have divided. In this case the amphinuclear divisions were slightly delayed so that two products only are seen in each gamont.

Fig. 42. Triple conjugantion.

Fig. 43. Exconjugant, just after separation from its mate. Four undifferentiated amphinuclear products and two macronuclear halves.

Fig. 44. Differentiation of macronuclear anlage.

Fig. 45. Degeneration of the old macronuclear halves. The two extra amphinuclear products have disappeared and the vesicular "yellow" spheres are just making their appearance.

Fig. 46. Later stage in the degeneration of the old macronuclear halves. Within the vesicular spheres the black granules are present.

Fig. 47. Growth of the new macronucleus.

Fig. 48. Later stage of the growth and elongation of the new macronucleus.

Fig. 49. Disappearance of the black granules and the gradual fading out of the vesicular spheres.

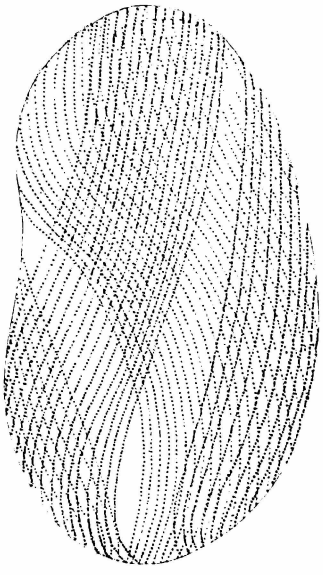
Figs. 50—53. Abnormalities during exconjugant reorganization.

Fig. 50. One macronuclear anlage and six small nuclei, three of which probably represent products of the maturation divisions late in degenerating.

Fig. 51. One anlage and seven small nuclei.

Fig. 52. One anlage and five small nuclei.

Fig. 53. Two macronuclear anlagen and two small nuclei.



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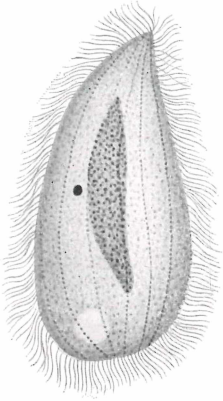

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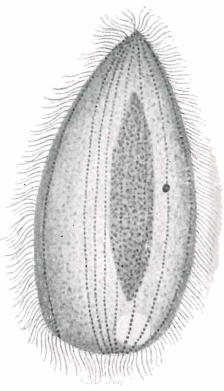

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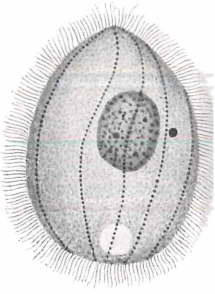

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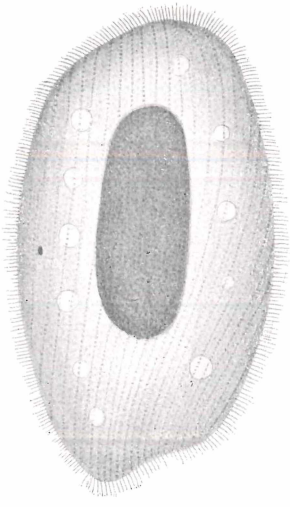

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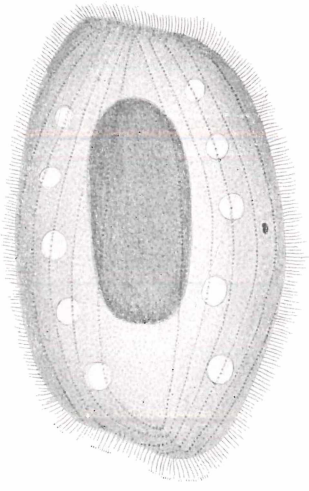

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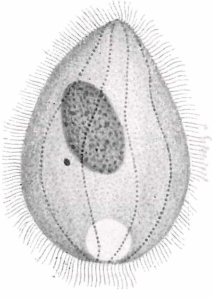

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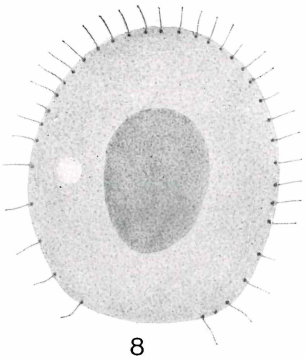

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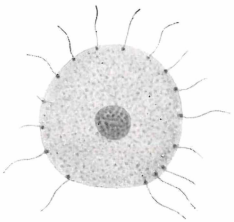

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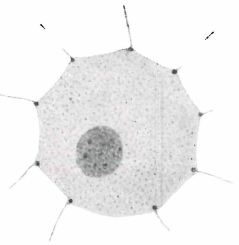

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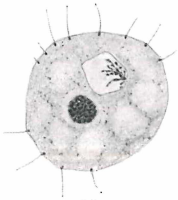

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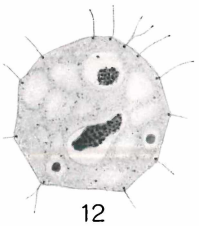

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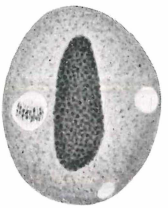

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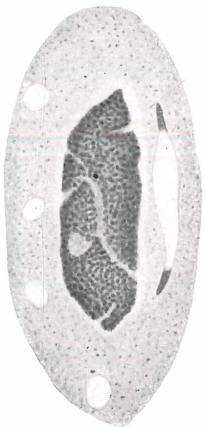

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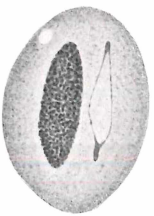

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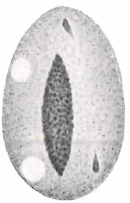

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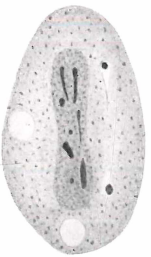

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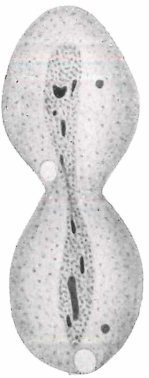

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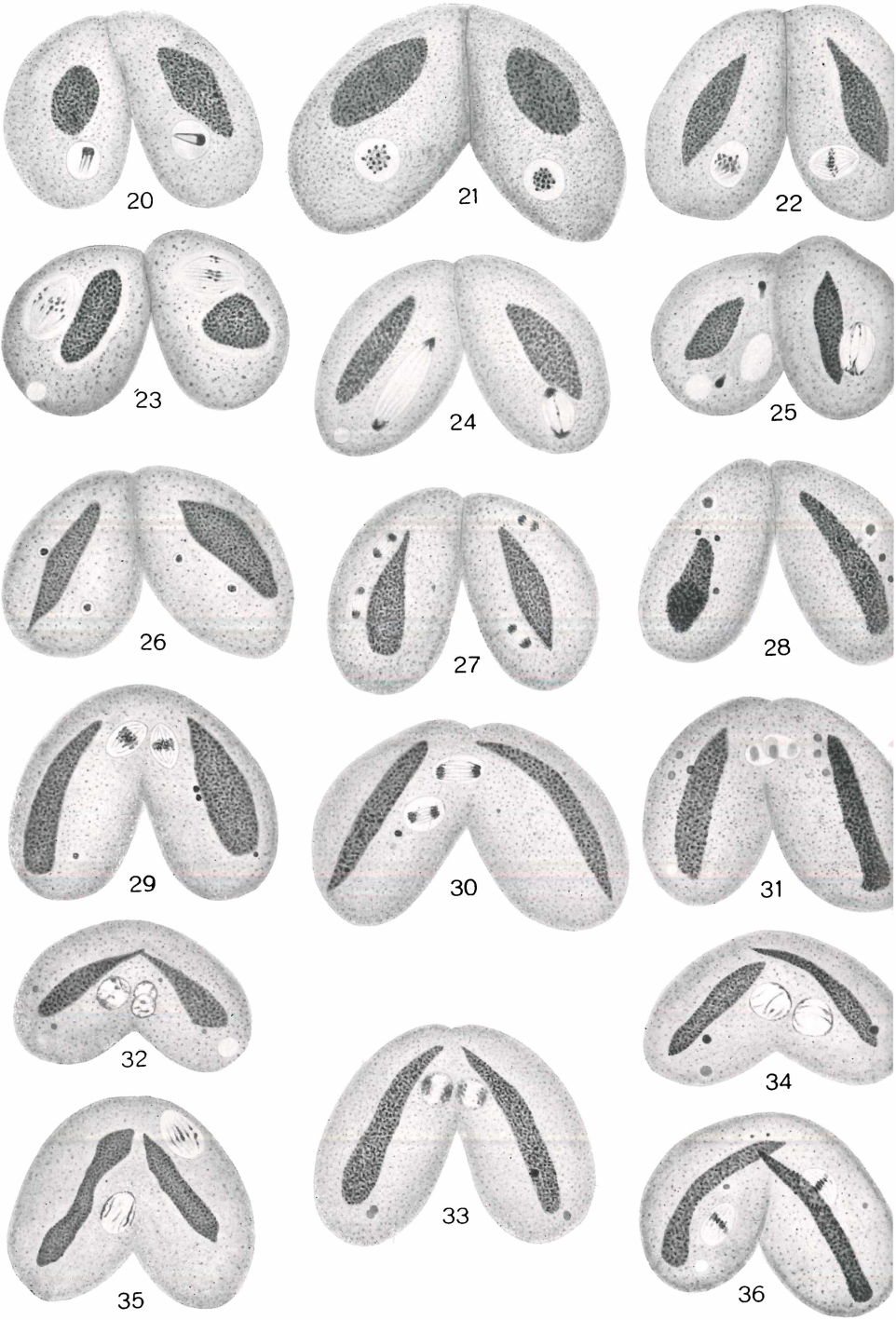
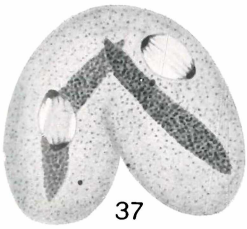
FISCHER IN JENA



Summers u. Kidder.



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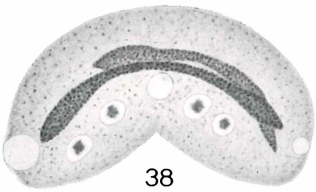

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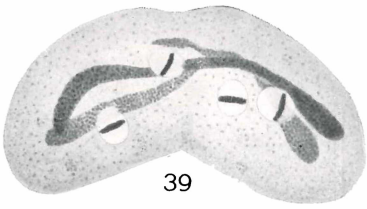

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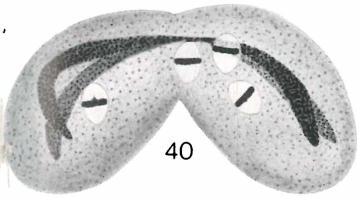

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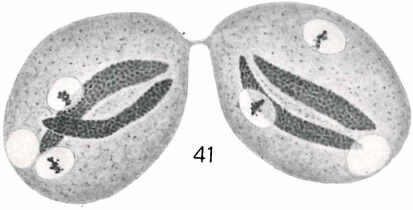

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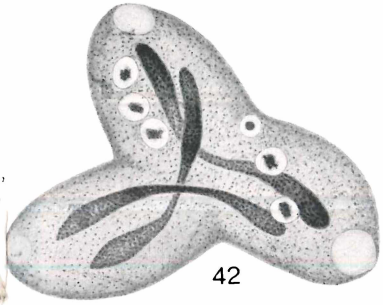

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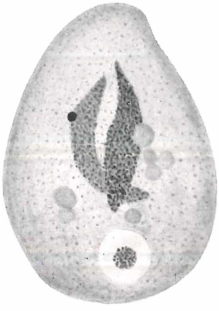

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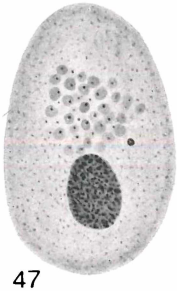

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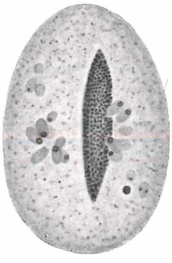

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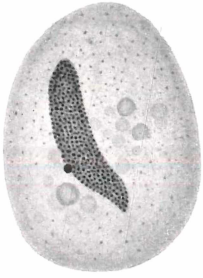

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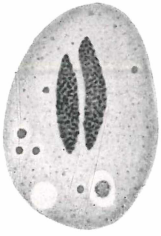

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GUST. FISCHER IN JENA.