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NOTES ON THE TINTINNOINA

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

2. ON *PETALOTRICHA ENTZI* SP. NOV.

BY
CHARLES ATWOOD KOFOID

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

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

Since Haeckel's (1873) discovery of this species no one has seen it, although Cleve (1901) ransacked hundreds of samples from the surface waters of the tropical and semitropical Atlantic and Brandt (1906, 1907) and Laackmann (1910) have monographed the group with extensive collections from these regions.

It is the general experience that pelagic protozoa are cosmopolitan and are found widely and quite generally in large numbers. There are, to be sure, especially among the highly differentiated and minute dinoflagellates of the family *Dinophysidae,* not a few instances in which only a very few individuals of a species have ever been seen. This may be due to escape through the meshes of the silk net on the one hand and thus not necessarily to rarity in nature, or on the other to actual rarity which is not unknown in nature among highly specialized tropical species of plants and animals, as among orchids, birds of paradise, and species of cowries (*Cypraea*). A classic instance in recent literature of a persistently rare species is *Oenothera lamarckiana,* and other cases are not unknown among fresh-water rotifers. However, in the case of *Dictyocysta* the genus is not extremely specialized and the other species are all fairly abundant and of wide distribution in all warm or temperate seas. The occurrence of a rare species in this genus is therefore to be looked upon with suspicion and some other explanation than rarity in nature sought for absence of records of its reappearance.

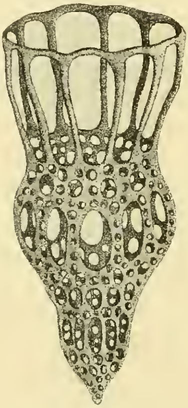
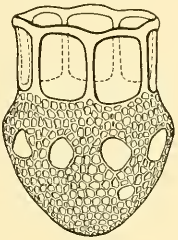


Fig 1. *Dictyocysta tiara* Haeckel. × 500 After Haeckel (1873, pl. 27, fig. 7).

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

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

When the formalin in sea water in which the plankton containing *Dictyocysta* is permitted to evaporate under the cover glass the lorica undergoes a peculiar shrinkage, which distorts it as in Haeckel's (1873) figure. A lorica of *D. lepida* which is thus distorted is shown in text-figure 2. The same symmetrical collapsing of the aboral region which appears in *D. tiara*, reducing it to a tapering form much narrower than in the uncontracted stage (fig. 3), occurs also in this lorica as dessication ensues. We conclude therefore that Haeckel's (1873) figure of *D. tiara* represents only a large lorica of *D. lepida* with ten instead of the usual eight fenestrae which had shrunken in the aboral region as the result of dessication, perhaps on the plankton net in the interval between one collection and another, or on the side of the container, or in the course of examination upon the slide. Haeckel's lorica measures 100μ in length, a size almost attained (95μ) by *D. lepida grandis* Brandt.



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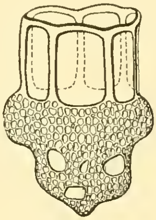

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Fig. 2. *Dictyocysta lepida* Ehrenberg var., on uncontracted lorica. × 500. The loricae shown in figures 2 and 3 belong to *D. templum* var. *b*. Brandt.

Fig. 3. *Dictyocysta lepida* Ehrenberg var. × 500. Original showing contracting aboral region due to shrinkage.

Our figures are made from one of the smaller (55μ), stouter varieties. Had they been made from a more slender one such as Brandt's *D. templum* var. *f.* or his var. *g. grandis* the resemblance to *D. tiara* would doubtless be more striking than in our figures.

2. ON *PETALOTRICHA ENTZI* SP. NOV.

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

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

Petalotricha entzi sp. nov.

Figures 4–6

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

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

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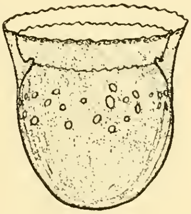

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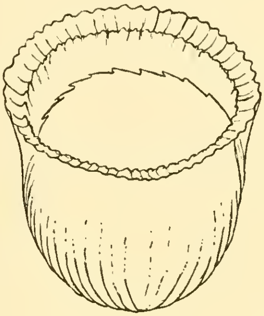

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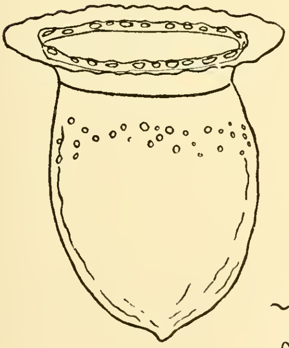

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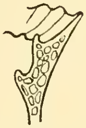

Fig. 4. *Petalotricha entzi* sp. nov. After Entz, Jr. (1905, figs. 30–32). Figure 4 is a full lateral view showing optical section of the wall, fig. 5 a tilted lorica showing the fluted upper surface of the oral shelf, and the distinctly serrate angle formed by the inwardly projecting nuchal ledge, and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

Fig. 7. *Petalotricha ampulla ampulla* (Fol) Kent, an unusually elongated lorica. Figure 8, the same, normal proportions. These two figures magnified about 333 diameters (based on Reichert obj. 8, comp. oc. 4, used by Entz).

striae pass from the depressions between the ridges on the oral shelf aborally on the inner sloping face of the collar for a short distance. The limits of the collar are visible in optical section or inner view only. It is 0.2 oral diameter in height and forms a cone of 30° contracting aborally.

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

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

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

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

Transmitted September 28, 1915.

Zoological Laboratory,
University of California.

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Vol. 16, No. 7, pp. 89-94, plate 12
December 11, 1915

6. BINARY AND MULTIPLE FISSION IN *HEXAMITUS*

BY

OLIVE SWEZY

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7. ON A NEW TRICHOMONAD FLAGELLATE, *TRICHOMITUS PARVUS*, FROM THE INTESTINE OF AMPHIBIANS

BY

OLIVE SWEZY

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BINARY AND MULTIPLE FISSION IN *HEXAMITUS*

BY

OLIVE SWEZY

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

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

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

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

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

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

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

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

MATERIAL AND TECHNIQUE

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

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

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

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

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

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

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

Hexamitus ovatus sp. nov.

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

Morphology

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

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

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

The chromatin consists of one large, elongated, centrally located mass, apparently continuous at the anterior end with the blepharoplast. This chromatin mass is often curved or club-shaped and is nearly as long as the nucleus. A very definite nuclear membrane is present which, especially prior to division, usually takes a heavy stain with iron haematoxylin (pl. 9, fig. 1).

The cytoplasm is granular and vacuolated, with no definite periplast and no distinction between ectoplasm and endoplasm. *Intra-vitam* staining with neutral red shows, with *H. ovatus* as well as *H. intestinalis*, the presence of a few, usually three or four, deeply staining granules in the cytoplasm of the posterior third of the body. In the living protoplasm a number of more or less highly refractive bodies or granules are visible, scattered through the cytoplasm. In spite of the lack of a structually differentiated periplast, the body is notably uniform in outline, exhibiting few or no amoeboid tendencies.

BINARY FISSION

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

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

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

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

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

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

Multiple Fission

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

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

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

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

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

Hexamitus batrachorum sp. nov.

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

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

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

MORPHOLOGY

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

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

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

FISSION

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

Hexamitus intestinalis Dujardin

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

MORPHOLOGY

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

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

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

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

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

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

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

Binary Fission

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

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

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

Discussion

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

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

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

SUMMARY

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

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

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

Transmitted September 29, 1915.

Zoological Laboratory,
University of California.

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EXPLANATION OF PLATE 9

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

Binary fission in *Hexamitus ovatus*, sp. nov. from *Diemyctylus torosus*. × 2583.

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

Fig. 2. The same, axostyles twisted.

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

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

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

Fig. 6. Prophase with spireme formation.

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

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

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

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

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

Fig. 12. The same stage. Chromosomes distinct.


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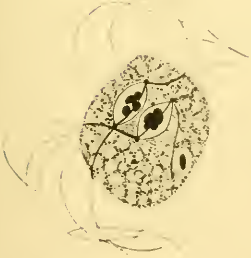
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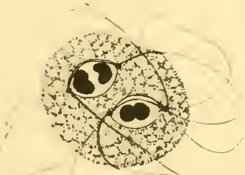
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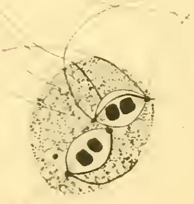
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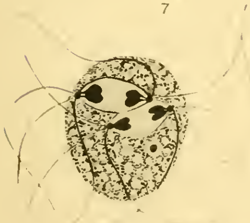
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EXPLANATION OF PLATE 10

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

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

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

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

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

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

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

Fig. 19. The same, with four individuals.

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

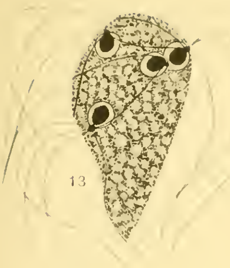
Figs. 21–24. *Hexamitus batrachorum* sp. nov. from *Rana pipiens.*

Fig. 21. Trophozoite showing characteristic nuclear appearance.

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

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

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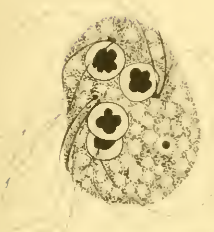
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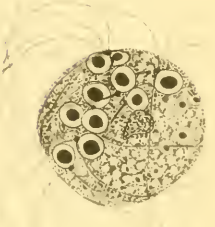
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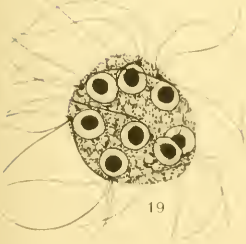
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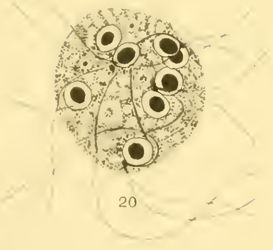
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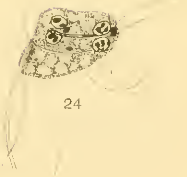
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EXPLANATION OF PLATE 11

Figs. 25–41. *Hexamitus intestinalis* from *Diemyctylus torosus*. × 2583

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

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

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

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

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

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

Fig. 31. Prophase with spindle formation.

Fig. 32. Prophase with equatorial plate.

Fig. 33. Metaphase of division.

Fig. 34. Anaphase of division.

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

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

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

Fig. 38. Somatella with six pairs of nuclei.

Fig. 39. Somatella with three pairs of nuclei.

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

Fig. 41. The same, showing the presence of two large vacuoles between the nuclei.


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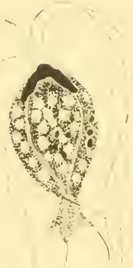
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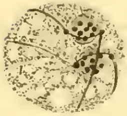
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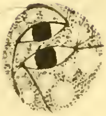
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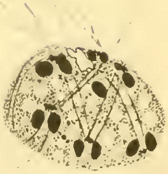
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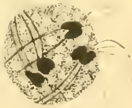
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ON A NEW TRICHOMONAD FLAGELLATE, *TRICHOMITUS PARVUS*, FROM THE INTESTINE OF AMPHIBIANS

BY
OLIVE SWEZY

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

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

MORPHOLOGY

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

The comparatively large nucleus is situated at the anterior end, immediately behind the blepharoplast and touching it (pl. 12, fig. 1). It is globular in shape and in staining with iron haematoxylin always, or nearly always, shows a darkly staining border, the nuclear membrane. Part of the chromatin is sometimes massed around the periphery in blocks, usually about five in number, with a central karyosome (pl. 12, fig. 11). In many other cases nearly all of the chromatin is massed in a single central karyosome (pl. 12, fig. 2).

Lying against the anterior surface of the nuclear membrane is the rather large blepharoplast from which the motor apparatus arises. This consists of three equal anterior flagella, about 2.5 times the length of the body (pl. 12, fig. 4) in length, together with an undulating membrane which passes backwards on the surface to the posterior end of the body and terminates in a trailing flagellum. The membrane is well developed, often having a width of 2 or 3μ, and is bordered along both edges by deeply staining lines, the outer or chromatic margin is very slender, and the one at the line of attachment to the body, the chromatic basal rod, or parabasal body, is slightly thicker and more conspicuous by reason of its deeper stain. The membrane is without granulations or apparent structure. The outer margin greatly exceeds the inner one in length and is thrown into 5–10 ripples or folds (pl. 12, fig. 2).

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

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

Mitosis

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

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

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

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

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

Throughout mitosis and even after the completion of the division of the nucleus and the separation of the two daughter nuclei the blepharoplasts remain connected by a darkly staining line, the paradesmose (pl. 12, fig. 9). It lies outside the nuclear membrane and is apparently attached only to the blepharoplasts. This line gradually loses its staining reactions and disappears.

Each chromosome is connected with the blepharoplast by a slender fibril (pl. 12, fig. 9), apparently the remains of a central spindle. These fibrils later disappear and the chromatin becomes distributed in the usual manner of the resting nucleus of the trophozoite (pl. 12, fig. 11).

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

MULTIPLE FISSION

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

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

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

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

Trichomitus gen. nov.

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

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

Transmitted September 30, 1915.

Zoological Laboratory,
University of California.

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EXPLANATION OF PLATE 12

Trichomitus parvus gen. nov., sp. nov.

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

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

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

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

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

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

Fig. 6. Slightly later stage of the same.

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

Fig. 8. End of prophase with chromatin massed at the center of nucleus. "Amphiaster" stage.

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

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

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

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

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

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

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

Fig. 16. Three-nucleated somatella.

Fig. 17. Final stage in plasmotomy.

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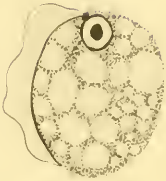

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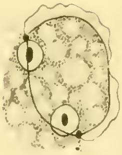

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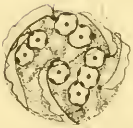

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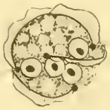

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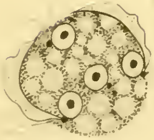

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BY

IRWIN C. SCHUMACHER

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CONTENTS

INTRODUCTION

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

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

Acknowledgments

This work was carried on at the University of California, at Berkeley, under the direction of Professor C. A. Kofoid. Whatever

there be in it of merit or value is due chiefly to his kind and helpful suggestions and to the interest he always showed therein. My acknowledgments and thanks are also due Mrs. Purington for her valuable help with my drawings and to Mr. E. B. Talt of Albany for his kindness and thoughtfulness in aiding me to obtain the material for this work.

TECHNIQUE

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

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

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

In my study of the ciliates from the caecum of the horse I have identified the following forms previously reported from Italy or Germany: *Cycloposthium bipalmatum* (Fiorentini) Bundle, *Paraisotricha colpoidea* Fiorentini, *Didesmis ovalis* Fiorentini, and *Didesmis quadrata* Fiorentini

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

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

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

MORPHOLOGY

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

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

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

In size this *Blepharocorys* stands about midway in the series of the ciliates from the caecum of the horse. The following table gives the dimensions of several members of this species: