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Hysterocineta eiseniae n. sp., an endoparasitic ciliate from the earthworm *Eisenia lönnbergi*.

By

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With plate 14.

Introduction.

Hysterocineta DIESING, 1866, (syn. *Ladopsis* CHEISSIN, 1928) and *Ptychostomum* STEIN, 1860, (syn. *Lada* VEJDOVSKY, 1882) are closely related holotrichous ciliates that inhabit the posterior intestinal region of certain oligochetes and molluscs. From JAROCKI'S (1934) critical review of the literature on the little-known genus *Hysterocineta* it is found that only three species have been described. These are *H. paludinarum* (STEIN, 1861) DIESING, 1866, (syn. *Ladopsis bithyniae* NIKOLAJEWA, 1929) from the snails *Bithynia tentaculata* and *B. leachi; H. benedictiae* (CHEISSIN, 1928) JAROCKI, 1934, from several genera of Baikal snails; and *H. bacteriophila* (MIYASHITA, 1933) JAROCKI, 1934, from the freshwater oligochete *Criodrilus* sp. Several times as many species of *Ptychostomum* have been described. STUDITSKY (1930) has enumerated the diagnostic characters and the hosts of ten species described up to that date, and four species to my knowledge have since been added.

CHEISSIN (1932) established the family Ptychostomidae to include *Ptychostomum* and *Hysterocineta*, but, as JAROCKI has pointed out, DIESING much earlier (1866, p. 508) created the family Hysterocinetinea (= Hysterocinetidae) to receive these two genera. Thus it is evident that the name Ptychostomidae is merely a synonym of Hysterocinetidae, with *Hysterocineta* as type genus by nomenclatural implication. The name Ladidae CEPEDE, 1910, is also invalid, in that *Lada* VEJDOVSKY is a synonym of *Ptychostomum*, as ROSSOLIMO (1925) demonstrated, and is furthermore a homonym of *Lada* WRZESNIOWSKI, 1879, an amphipod, as HEIDENREICH (1935) pointed out.

Hysterocineta and *Ptychostomum* are remarkable in that they possess anteriorly on the ventral surface a circular or horseshoe-shaped sucker for attachment and posteriorly and terminally a mouth and cytopharynx which are much reduced in size. (By anterior is meant merely the end that bears the sucker and is directed forward in swimming; by ventral is meant the surface on which the sucker is located. Thus these terms are purely descriptive and from the standpoint of comparative morphology may be entirely inaccurate.) The most dependable criterion for differentiating the two genera is the character of the nuclei. In *Ptychostomum* the macronucleus is bean-shaped or broadly ellipsoidal; its longer axis is always at right angles to the longitudinal axis of the animal. In *Hysterocineta* it is an elongated ellipsoid with its long axis parallel to that of the animal. In *Ptychostomum* the micronucleus is found just anterior to the macronucleus, often in a depression in the latter, whereas in *Hysterocineta* it is found always at the side of the macronucleus.

Both genera show a characteristic zone of food vacuoles which is restricted to the endoplasm of the posterior half of the body. It is generally agreed that nutrition in these forms is in part saprozoic, and that we are dealing here with types that are now in the process of acquiring the saprozoic habit and the astomatous condition.

While the affinities of *Hysterocineta* and *Ptychostomum* with the Ancistrumidae and other members of the suborder Thigmotricha on the one hand and with the Hymenostomata on the other have been discussed at some length by ROSSOLIMO (1925, 1926), KIJENSKIJ (1925), MIYASHITA (1928) and CHEISSIN (1932), there is no general agreement regarding their systematic position. (The classification just referred to is that of KAHL, 1934). Until their affinities with the Thigmotricha can be more clearly demonstrated, I am disposed to follow CHEISSIN and to place them in a special family, the Hysterocinetidae, of the suborder Hymenostomata, order Holotricha.

The present paper is the first record of the occurrence of a species of *Hysterocineta* in a terrestrial oligochete — the earthworm *Eisenia lönnbergi*. I am designating this species as *H. eiseniae* n. sp.

Material; Methods; Incidence of Infection.

Reference may be made to BEERS (1938) for a description of the method of removing the ciliates from their host and for a brief discussion of certain points in which the host departs from Smith's description (1917) of *Eisenia lönnbergi.*

More than 450 adult specimens of *Eisenia lönnbergi* from the environs of Chapel Hill were examined for *Hysterocineta.* Approximately 80 % of them were infected. Some worms contained only from 10 to 20 ciliates, but many — at least a third of them — contained from 50 to 300 specimens, so that in general the infection of a host may be described as heavy. The localization of *Hysterocineta* in the intestine of *Eisenia* is very striking. If the intestine is thought of as consisting of four parts, fully 90 % of the specimens of *Hysterocineta* are found in the third quarter. Approximately 60% of the worms were also infected with the astomatous ciliate *Metaradiophrya asymmetrica,* which has already been described (Beers, 1938), though *Metaradiophrya* occupies a more anterior position in the intestine than *Hysterocineta.*

Specimens of *Eisenia* were examined during every month of the year in a search for seasonal variations in the incidence of infection and for cyclical changes in the life history of *Hysterocineta.* These examinations yielded nothing of special interest; the hosts were consistently infected throughout the year with active, free-swimming individuals, some of which were occasionally found to be dividing. Neither conjugation nor encystment was observed.

Hysterocineta eiseniae n. sp.; Description; Comparisons with Other Species.

General morphology. The cell-body of *Hysterocineta eiseniae* is elongate and slender (Pl. 14 Fig. 1). The anterior end is bluntly pointed, but it lacks precise bilateral symmetry in that it is bent slightly to the right. The posterior end is rounded or truncate. The right margin of the animal is slightly convex, the left slightly concave, in agreement with Diesing's original generic diagnosis. The dorsal surface is slightly convex; the ventral, correspondingly concave (Pl. 14 Fig. 2). The body is distinctly flattened dorso-ventrally, and therefore is somewhat ribbon-like (Pl. 14 Figs. 2 and 6). It is highly transparent and flexible, though non-contractile.

Considerable variation exists in size and body proportions in different individuals. Full-grown specimens usually measure 190—210μ in length, 35—40μ in width and 10μ in thickness (i. e., dorso-ventrally). There are other less numerous specimens that measure 150—170μ by 50—55μ. Evidently there are long, slender races and short, wide races, as well as intermediates. In some hosts the short, wide specimens predominate, but in general the slender ones are the commoner. Similar variations in form occur in *Itychostomum tanishi* from the snail *Viviparus japonicus*, according to MIYASHITA (1928).

At the anterior end is the sucker (S, Pl. 14 Fig. 1). At the posterior end and slightly removed toward the left side is the short, tubular cytopharynx (CP). A superficial, transparent ectoplasmic layer containing the basal granules of the cilia may be distinguished, though none too clearly, from the inner more granular endoplasm (Pl. 14 Figs. 5—7). In the endoplasm there are many refractile granules measuring about 0.5 μ in diameter (Pl. 14 Fig. 1). They are blackened with osmic acid and seem to be of a lipoid nature.

Nuclei. The macronucleus is a slender, cylindrical or ellipsoidal body which is found either at the center of the cell or displaced slightly into the posterior half (Pl. 14 Figs. 1 and 2). Its long axis is always parallel to that of the cell-body. In specimens of average size it measures 45—50 μ by 8 μ. In many, though not in all, individuals the macronucleus shows a slight depression anteriorly in its left side (Pl. 14 Fig. 1). Near this depression — invariably on the left side and near the anterior end of the macronucleus — is found the single, fusiform micronucleus, which measures 4—5 μ in length and 2—3 μ in width.

Contractile vacuole. In *H. eiseniae* there is a single contractile vacuole which is well removed toward the posterior end of the body (Pl. 14 Fig. 1). In intestinal contents diluted with distilled water — the usual medium in which specimens were examined — it pulsates at 15-second intervals. At diastole its large diameter (15 μ) causes the dorsal and ventral surfaces of the cell-body to protrude locally (Pl. 14 Fig. 2). The point of discharge is in the mid-dorsal line (Pl. 14 Fig. 7), as in other members of the genus. Near the contractile vacuole there are sometimes found several smaller accessory vacuoles (Pl. 14 Fig. 1). At times the contractile vacuole forms by the coalescence of some of these smaller vacuoles, again it forms independently of them. Similar accessory vacuoles are figured by MIYASHITA (1933) in *H. bacteriophila*.

Mouth apparatus. Along the posterior margin of the body there is a shallow transverse peristomial groove (PG) occupying the distance between A and B of Fig. 8. This groove is bounded by a ventral lip and a slightly longer dorsal lip (VL and DL, Pl. 14 Figs. 2 and 8). Each lip is supplied with two or three rows of long, posteriorly directed cilia (PC), not all of which are shown in Fig 8.

These cilia appear to be essentially non-motile. Toward the left side of the body the peristomial groove deepens and leads into the cytostome (CS, Pl. 14 Fig. 8), which, in turn, leads into the short, tubular cytopharynx (CP). In the peristomial groove there is a well-developed undulating membrane (UM) which is continued into the cytopharynx, along the wall of which it makes $1\,^1/_2$ clockwise turns (animal viewed from the oral surface). In quiet specimens mounted in diluted intestinal contents the action of the undulating membrane may be seen to continue for at least an hour. The cytopharynx is 8—10 μ long and about 3 μ in diameter. The peristomial cilia are 10—15 μ long. In structure the mouth apparatus conforms faithfully to the general Hysterocinetid plan.

In so far as I can ascertain the mouth is entirely non-functional in *H. eiseniae*. Although I have examined carefully many hundreds of living specimens, I have never seen in the cytoplasm a single structure that resembles a food vacuole. Indeed, the vacuolated zone of endoplasm lying posterior to the macronucleus and occupied by great numbers of conspicuous food inclusions in *paludinarum* (NIKOLAJEWA, 1929, Plate 1) and *benedictiae* (CHEISSIN, 1932, Plate 1, Fig. 4), and by a smaller number in *bacteriophila* (MIYASHITA, 1933, Fig. 3), is entirely absent in *eiseniae*.

While specimens of *eiseniae* remain alive and active for many hours in diluted intestinal contents, I have never been able to induce them to ingest Chinese ink particles. On the other hand, *benedictiae* begins to ingest ink particles within a minute under these conditions, though *paludinarum* ingests them only after prolonged immersion in the suspension, according to CHEISSIN (1932).

Adherent organelle. The sucker has roughly the shape of an inverted V and occupies the lateral margins of the antero-ventral surface (Pl. 14 Figs. 1 and 3). The left half is slightly longer and wider than the right, as in *benedictiae*. The posterior border of each half is evenly rounded, and each half narrows anteriorly. The left half is 25—30 μ long; the right, 21—26 μ. The maximum width of each half is about 8 μ.

A cross section through the sucker (Pl. 14 Fig. 5) shows that it is a shallow groove devoid of cilia, though cilia are present in the area of the ventral surface that is embraced by the halves of the sucker.

Two systems of superficial fibers, a transverse and a longitudinal, are present in the ventral surface of the sucker. Both systems show up well in the living animal when dark-field illumination is employed, and both stain intensely with iron haematoxylin (Pl. 14 Fig. 3). The transverse fibers appear in each half of the sucker as 25—30 delicate striations of the surface; the longitudinal ones, of which there are only 7—9, appear as coarser, more conspicuous markings. Careful focusing shows that the longitudinal fibers are slightly dorsal to the transverse ones.

This type of fibrillar system differs conspicuously from that of *benedictiae*, in which Cheissin (1932) describes the arrangement as follows: There is a single system of supporting fibers located superficially in the sucker. (These fibers correspond to the transverse fibers of *eiseniae*, though the posterior ones run even more obliquely.) There is also a bundle of myonemes which take their origin in the endoplasm dorsally and insert into the anterior end of the sucker.

I have been unable to locate a corresponding system of myonemes in *eiseniae*, and I am compelled to look upon both fibrillar systems as having a supporting function.

The sucker in *eiseniae* appears to be only weakly functional. Specimens which attach to the bottom of a depression slide are easily dislodged, and most of the specimens in the intestine are found to be moving freely in the lumen.

Ciliation. The ciliation is dense and uniform over the general body surface. The cilia, which have a length of 6—7 μ, are arranged in from 80—100 longitudinal, parallel rows with intervals of about 1 μ between the rows. As stated already, the cilia extend anteriorly between the halves of the sucker (Pl. 14 Figs. 3 and 5), so that of the entire body surface only the area of the sucker itself is unprovided with them.

Appropriate treatment with silver nitrate (Klein's method) reveals a silver line system that differentiates with remarkable distinctness. A portion of the body thus treated, together with the posterior tip of the sucker, is shown in Fig. 4. There are longitudinal fibrils and transverse connecting fibrils; these together mark out a system of minute rectangles. Each basal granule rests on the anterior surface of a cross fibril, midway between the two adjacent longitudinal fibrils. This arrangement prevails over the entire body surface, except for the regions of the sucker and the peristome. In *benedictiae* and *bacteriophila* the basal granules of each ciliary row are connected by a longitudinal fiber and cross fibers occur at frequent but irregular intervals, so that the pattern lacks the geometrical uniformity found in *eiseniae*.

Division. Division in *H. eiseniae* (Pl. 14 Figs. 9—13) is by transverse unequal binary fission, as in other species of the genus and in *Ptychostomum.* The posterior daughter cell in the early stages of division is distinctly smaller than the anterior (Pl. 14 Figs. 9—11). It remains attached to the anterior individual until its sucker is fully developed (Pl. 14 Fig. 12), and it grows in the meantime, so that when the two cells separate the posterior one is nearly as large as the anterior (Pl. 14 Fig. 13). The sucker at division is retained by the anterior individual.

Comparisons. The accompanying Table 1, based on original observations on *H. eiseniae* and on the published descriptions of the remaining species (none of which I have had opportunity to examine), will serve to make clear the differences that exist among the four species of *Hysterocineta.* In connection with Table 1 it must be remembered that, in addition to the ciliary organelles mentioned under the heading „Peristome“, there is present in each species an undulating membrane which is continued into the cytopharynx.

In conclusion, it seems desirable to enumerate the distinguishing features of the Family Hysterocinetidae (Suborder Hymenostomata) and its two genera, in that Cheissin's descriptions in Russian are somewhat unavailable and Diesing's diagnoses are misleading, since he mistook the sucker for a mouth and the mouth for a cytopyge.

Family Hysterocinetidae Diesing. Endoparasitic ciliates found in the posterior intestinal region of certain oligochetes and gastropods. Length, 100—200 μ. Body dorso-ventrally flattened; elongate-elliptical or oval in outline. Ciliation, dense and uniform. Anteriorly on the ventral surface there is for attachment a non-ciliated sucker in the form of an oval, a horseshoe or an inverted V; sucker provided with skeletal and in some cases with contractile fibers. Mouth apparatus much reduced. Peristomial groove and cytostome, posterior and terminal; peristome transverse. Cytostome leads into a short, tubular cytopharynx which is directed anteriorly; occupying a portion or all of the peristomial groove is an undulating membrane which continues through the cytostome and along the wall of the pharynx, usually as a right-wound spiral. Endoplasm usually divisible into two zones about equal in size — a non-vacuolated anterior one and a posterior one occupied by food vacuoles. Macronucleus and micronucleus at center of body. Single contractile vacuole opening dorsally in posterior half of body. Reproduction by transverse, unequal, binary fission (posterior daughter cell smaller than anterior). Encystment unknown; conjugation rare. Two genera.

Table 1.

Comparative data on the four known species of Hysterocineta.

	H. paludinarum (Stein)	*H. benedictiae* (Cheissin)	*H. bacteriophila* (Miyashita)	*H. eiseniae* Mihi
Host and locality	Snails *Bithynia tentaculata* and *B. leachi.* Prague; Peterhof (U.S.S.R.).	Snails of genera *Benedictia*, *Kobeltocochlea* and *Baikalia.* Lake Baikal.	Freshwater oligochete *Criodrilus* sp. Japan.	Earthworm *Eisenia lönnbergi.* Chapel Hill, North Carolina, U.S.A.
Length; width	97–210 μ; 48—100 μ.	200—350 μ; 40—100 μ.	70—130 μ; 30—45 μ.	190—210 μ; 35—40 μ.
Macronucleus	Ovoidal; length, 15–40 μ; diameter, 10—30 μ.	Ovoidal; size, $^{1}/_{5}$ to $^{1}/_{4}$ of body dimensions.	Ellipsoidal; length, 30—50 μ; diameter, 10—15 μ.	Elongate-ellipsoidal; length, 45—50 μ; diameter 8 μ.
Micronucleus	Fusiform; length, 1—2 μ.	Fusiform; length, 1—3 μ.	Spherical; diameter 3—4 μ.	Fusiform; 4—5 μ by 2—3 μ.
Shape and structure of sucker	Inverted V; length, 30—45 μ; rows of mucoid granules in ectoplasm.	Horseshoe; with transverse skeletal fibers and a bundle of myonemes.	Ellipse; length, 50 μ; cilia in middle; transv. skeletal fibers in anterior half.	Inverted V; length, 25—30 μ; many transv. and few longitudinal skeletal fibers.
Position of contractile vac.	Slightly posterior to macronucleus.	Slightly posterior to macronucleus.	Midway between macron. and post. end of body.	Very near posterior end of body.
Peristome	With membranelles dorsally.	Long cilia on dorsal and ventral margins.	A dorsal membranelle; also long cilia dors. and vent.	Long cilia on dorsal and ventral margins.
Cytopharynx	With longitudinal fibers that unite anteriorly and end freely in endoplasm.	Fibers unobserved.	Fibers unmentioned.	Fibers absent.
Endoplasmic vacuoles; ingestion	Many food vacs. in post. half; mouth functional; ingests carbon particles.	Food vacs. in left post. quarter; mouth functional; ingests carbon particles.	Small number of food vacs. in post. half; mouth functional.	Vacuoles absent; mouth evidently non-functional; carbon particles rejected.
Endoplasmic inclusions	Fat droplets and rod-shaped mitochondria.	Fat droplets and mitochondria.	Rod-shaped bodies regarded as symbiotic bacteria.	Refractile granules, diam. 0.5 μ; probably lipoid.

1. Genus *Ptychostomum* Stein. Macronucleus ellipsoidal or bean-shaped, with its long axis at right angles to the long axis of the cell-body. Micronucleus spherical, conspicuous, anterior to macronucleus, often in a depression in the latter.

2. Genus *Hysterocineta* Diesing. Macronucleus ellipsoidal, with its long axis parallel to that of the cell-body. Micronucleus small and inconspicuous, usually fusiform, located at left side of macronucleus.

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Explanation of plate.

Plate 14.

Hysterocineta eiseniae n. sp.

Fig. 1. Free-hand sketch of living specimen. Dorsal view, to show typical shape. × 380.

Fig. 2. Free-hand sketch of living specimen. Viewed from the right side, to show thinness of body. × 380.

Fig. 3. Anterior end, ventral view, showing transverse and longitudinal fibers of the sucker and basal granules of the cilia in the region of the sucker. Schaudinn's fluid; iron haematoxylin. Camera lucida. × 750.

Fig. 4. Silver line system of a part of ventral surface. Each basal granule is attached to the anterior border of a cross-fibril, midway between the adjacent longitudinal fibrils. The silver markings on the sucker, which bears no cilia, are of no significance. Klein's method. Camera lucida. × 2000.

Fig. 5. Cross-section through the posterior part of the sucker. Bouin's fluid; iron hae. Camera lucida. × 750.

Fig. 6. Cross-section at the level of the micronucleus. Schaudinn; Mann's eosin-methyl blue. Camera lucida. × 750.

Fig. 7. Cross-section through the contractile vacuole, showing point of discharge in the mid-dorsal line. Schaudinn; iron hae. Camera lucida. × 750.

Fig. 8. Posterior end, ventral view, diagrammatic, to show in particular the peristomial groove, cytostome, cytopharynx and undulating membrane. × 800.

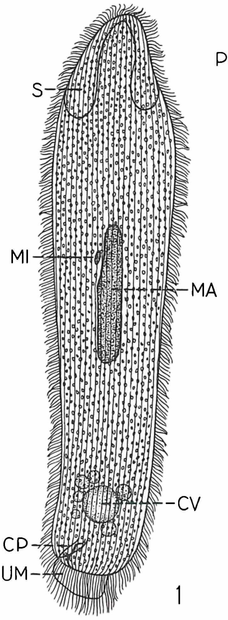
Figs. 9—11. Early division stages. Division is transverse, but unequal in that the posterior daughter cell is smaller than the anterior. Bouin; Delafield's hae. and eosin. Camera lucida. × 280.

Fig. 12. Division, later stage. Sucker differentiating in the posterior individual. Schaudinn; iron hae. and eosin. Camera lucida. × 280.

Fig. 13. Division, final stage, just before the separation of the two daughter cells. The posterior individual remains attached to the anterior until it is fully developed and is nearly as large as the anterior. Bouin; iron hae. Camera lucida. × 280.

Abbreviations.

BG, Basal granule
CP, Cytopharynx
CS, Cytostome
CV, Contractile vacuole
DL, Dorsal lip of peristome
MA, Macronucleus
MI, Micronucleus
PC, Peristomial cilia
PG, Peristomial groove
S, Sucker
UM, Undulating membrane
VL, Ventral lip of peristome



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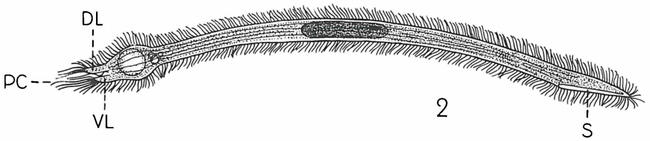

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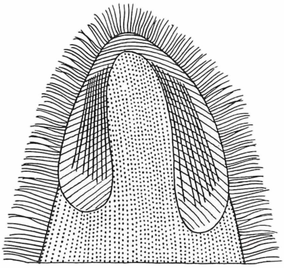

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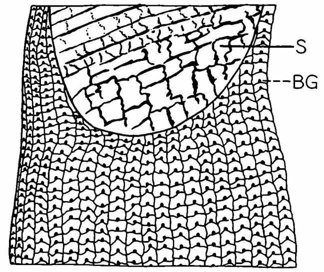

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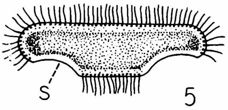

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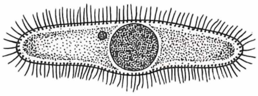

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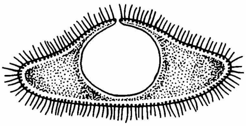

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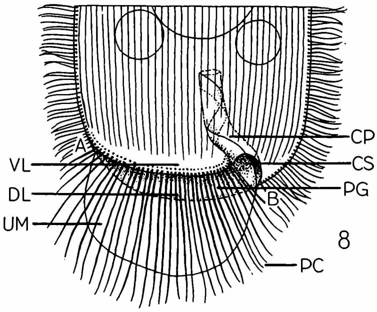

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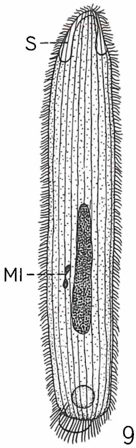

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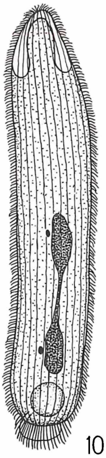

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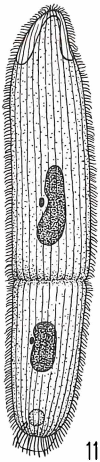

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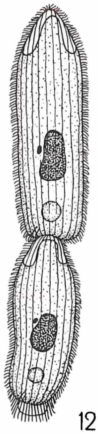

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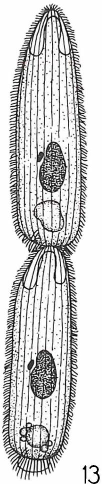

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Beers.

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