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Revision of the Family Spironemidae Doflein (Protista, Hemimastigophora), with Description of Two New Species, *Spironema terricola* N. Sp. and *Stereonema geiseri* N. G., N. Sp.

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ABSTRACT. Two new hemimastigophoran flagellates are described using light and electron microscopy, and the family Spironemidae is revised. *Spironema terricola* n. sp. occurs in soil from the Grand Canyon (southwest USA). It moves in a conspicuously euglenoid manner and differs from *S. multiciliatum* Klebs by its vermiform shape and shorter kineties. *Spironema terricola* is similar to Goodey's *Spironema multiciliatum* from soil in England. However, Goodey's vermiform species has a very elongate nucleus and is thus neither identical with *S. terricola,* which has a roundish nucleus, nor with Klebs' lanceolate *S. multiciliatum*; we consider it a new species, *Spironema goodeyi* n. sp. *Stereonema geiseri* n. g., n. sp. was discovered in the Aufwuchs (periphyton) of a river in Bavaria, Germany. The new genus differs from *Spironema* by its acontractility, and from *Hemimastix* by its shorter kineties and less plicate cortex. The fine structure of *Spironema* and *Stereonema* is very similar to that of *Hemimastix* Foissner et al., viz., the cortex is composed of two plates having diagonal symmetry and the flagellated basal bodies have associated a short and a long microtubular ribbon. All species have unique extrusomes of the same type. The main differences between the three genera and five species recognized are contractility, length of kineties, body size, shape of cell and nucleus, and particulars of the cortex and extrusomes. The phylogenetic relationships of the Hemimastigophora are still uncertain. However, the diagonal symmetry of the cortical plates and the pronounced euglenoid movement of *Spironema* spp. suggest a common ancestor with euglenids.

Supplementary key words. Euglenids, *Hemimastix,* heterotrophic flagellates, phylogeny, systematics.

IN 1988, Foissner et al. [8] established a new protistan phylum, the Hemimastigophora, for a puzzling heterotrophic flagellate, *Hemimastix amphikineta,* discovered in Gondwanian soils. *Hemimastix* was assigned to the family Spironemidae Doflein [5], although the original description of the type species, *Spironema multiciliatum* Klebs, 1893 [13], is incomplete. Almost 100 years after the discovery of *S. multiciliatum* we report on another species of *Spironema* found in a soil sample from the Grand Canyon of the southwestern United States. In addition we describe a related freshwater flagellate from Germany that represents a new genus. Light and electron microscopy confirm their similarity with *H. amphikineta* and provide the genera and species belonging to the Hemimastigophora with refined definitions. The entire taxon has been revised and comprises three genera and five species. Our findings suggest that hemimastigophorans are more widespread and diverse than hitherto recognized.

MATERIAL AND METHODS

Spironema terricola was discovered in a soil sample from the Grand Canyon. Raw cultures were set up with the non-flooded petri dish method [6]. *Stereonema geiseri* was discovered in the Aufwuchs (periphyton) of a meso-saprobic river (Amper) in Bavaria, Germany. In both species the raw material was studied because attempts to establish pure cultures failed. Light and electron microscopic methods used are described in [7, 8].

Revision of the Family Spironemidae Doflein, 1916

The general architecture of the Hemimastigophora was extensively studied in *H. amphikineta* [8]. *Spironema terricola* and *Stereonema geiseri* show the same overall organization, especially a striking similarity in the kinetal and mitochondrial fine structure. These features are thus only briefly described and summarized in the diagnoses for the phylum and family. Genus and species diagnoses and species descriptions contain the diagnostic features and other differing details, which are summarized and compared in Table 1. Further information is to be found in the explanations of the figures.

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Phylum Hemimastigophora Foissner, Blatterer & Foissner 1988

(Class Hemimastigea Foissner et al., 1988 and order Hemimastigida Foissner et al., 1988 have the same characteristics)

Cilia-like flagella arranged in slightly spiraled kineties shorter or as long as body (Fig. 1–12, 35–39, 56, 57). Single basal bodies with complex system of microtubular roots and posterior membranous sac (Fig. 1, 24, 25, 45, 58). Cortex consisting of plicate plates with diagonal (rotational) symmetry; supported by distinct, finely granular layer (epiplasm) in flagellated region and by microtubules either evenly spaced or in discrete groups (Fig. 30, 34, 49–53, 58–61). Mitochondrial cristae tubular to saccular (Fig. 1, 30–32, 50). Complex extrusomes (Fig. 25, 26, 46–48, 62–64; character probably only of value at ordinal level). Fission in free-swimming condition, symmetrogenic [8]. Incertae sedis within kingdom Protista Haeckel.

Remarks. No left and right side can be distinguished in the Hemimastigophora because of the special diagonal (rotational) symmetry of the cortical plates. The cortex is supported by microtubules and/or by a finely granular layer of moderate electron density (Fig. 25, 30–34, 49–53, 58–61). This layer was called epiplasm because of its similarity with the epiplasm of ciliates and euglenids [8]. In some species (*H. amphikineta, S. terricola*) the outer zone of the epiplasm appears as a heavy osmiophilic sheet. This sheet consists of amorphous material, i.e. lacks any tripartite structure indicative of a membrane (Fig. 34, 58).

The mitochondrial cristae are irregularly tubular to saccular (Fig. 28, 30–32, 49, 50). One or two tubules in each mitochondrion may be inflated to roundish caverns (Fig. 1; Fig. 20–26 in [8]). Such caverns are present in all species investigated and are therefore unlikely to be fixation artifacts. Disc-like (euglenoid) cristae have never been seen.

The Hemimastigophora lack paraxial or paraflagellar structures, flagellar hairs (mastigonemes) and plastids.

Family Spironemidae Doflein, 1916

Small to medium sized (about 10–60 μm), vermiform to ovoid, more or less distinctly flattened hemimastigids with more or less distinct anterior constriction producing head-like "capitulum" (Fig. 25). Two spiraled kineties in more (*Hemimastix*; Fig.

Table 1. Gross comparison of *Spironema terricola, Stereonema geiseri* and *Hemimastix amphikineta*.[a]

Character	*S. terricola*	*S. geiseri*	*H. amphikineta*
Size in vivo (μm)	42 × 3[b]	About 25 × 7	14 × 7[b]
Shape	Vermiform with long tail	Lanceolate with short tail	Ellipsoid, distinctly flattened
Capitulum	Conspicuous	Inconspicuous	Conspicuous
Kineties and epiplasm	In anterior ⅛ of body	In anterior half of body	Along whole body length
No. flagella/kinety	7.6[b]	About 12	12.3[b]
Size of nucleus (μm)	4 × 2.6[b]	About 4	3.3 × 2.9[b]
Euglenoid movement	Present	Absent	Absent
Biotope	Soil	Freshwater	Soil
Cortex			
epiplasm-bearing portion	Tapering	Tapering	± evenly broad
osmiophilic sheet	Present	Absent	Present
flagellar furrows	Indistinct	Indistinct	Distinct
flagellar ridges	Absent	Absent	Present
membranous sacs diametre (nm)	61	89	49
serrate structure	Absent	Present	Absent
Basal bodies			
diametre (nm)	183	184	223
length (nm)	236	179	189
Flagella			
diametre (nm)	312	332	299
axoneme diametre (nm)	198	205	191
Extrusomes			
tip	Cap-like	Bipartite bulbous	Cap-like
shaft centre	Thin	Thick	Thick
Endoplasmic reticulum	Conspicuous along nucleus	Inconspicuous	Conspicuous along whole body

[a] Detailed light microscopic morphometry for *S. terricola* and *H. amphikineta*, see Table 2; no detailed data are available for *S. geiseri*. See species descriptions for detailed morphometry of fine structural data. Data of *H. amphikineta* are from [8].

[b] Arithmetic means from protargol-impregnated specimens.

1, 58, 61) or less (*Spironema, Stereonema*; Fig. 59, 60) distinct furrows located at the sites where the cortical plates abut. Basal bodies conspicuously short (about as long as wide), each associated with membranous sac, short microtubular ribbon and long microtubular ribbon extending to or slightly beyond posterior kinetid, where it parallels and sometimes touches short ribbon; microtubular ribbons of same basal body connected by granular material (Fig. 1, 24, 45); flagellar basket consisting of 9 filamentous (non-microtubular) arms (transitional fibres) originating from proximal end of basal body (Fig. 1, 24, 30); transitional plate thick and concave, transitional cylinder inconspicuous (Fig. 30, 44, 51). Single nucleus with prominent central nucleolus (Fig. 29, 32, 50) persisting throughout division (Fig. 7–11 in [8]; very likely endomitosis). Contractile vacuole near posterior end of body (Fig. 2, 5, 15, 37, 40). Extrusomes bottle-like, consisting of cylindroid posterior compartment and rod-like anterior compartment (Fig. 62–64). Food uptake at anterior end; defined oral structures, however, not recognizable. Three genera (*Spironema, Hemimastix, Stereonema*) with a total of five species.

Type genus. *Spironema* Klebs, 1893.

Remarks. Whether hemimastigophoran protists with more than two kineties exist is not known. If so, they should be separated at family level.

The flagella arise from single pits in *Spironema* and *Stereonema* (indistinct furrow, Fig. 59, 60) or from a continuous groove in *Hemimastix* (distinct furrow, Fig. 58, 61).

The complex extrusomes are remarkably similar in all genera though differing in details (Fig. 62–64). This and the very similar fine structure of the kinetids and the mitochondria indicate a close relationship confirming the classification of *Hemimastix amphikineta* as a member of the Spironemidae.

Although defined oral structures are not recognizable, the Hemimastigophora feed on bacteria and/or flagellates. Food uptake has been observed at the anterior end [8, 13], but the exact location of ingestion is not known. Contrary to our earlier idea [8], we now regard food uptake in the centre of the capitulum as rather unlikely because of the continuous epiplasm. Ingestion in the anterior regions of the flagellar furrows, at sites which lack the epiplasm, is more likely and agrees with the observations by Klebs ([13], see below). Dictyosomes are always in the anterior cell portion and may provide food vacuole membranes (Fig. 55).

Genus *Spironema* Klebs, 1893

Diagnosis. Small to medium sized (about 15–50 μm) Spironemidae with kineties in indistinct furrows and terminating near or above mid-body. Posterior end with distinct tail. Cortex soft and slightly plicate; cells highly contractile, showing euglenoid movement.

Nomenclature. The paper by Klebs was probably available one year earlier than indicated on the title page of the journal, because both Goodey [9] and Skuja [20] cited it as 1892. In the absence of further information we recognize 1893 as the year of publication.

Type species. *Spironema multiciliatum* Klebs, 1893.

Remarks. Differs from *Stereonema* and *Hemimastix* mainly by its pronounced contractility. Such a difference is widely accepted to be of generic significance (e.g. *Euglena* and *Phacus* [14]). It is not clear from Klebs' description of *S. multiciliatum*

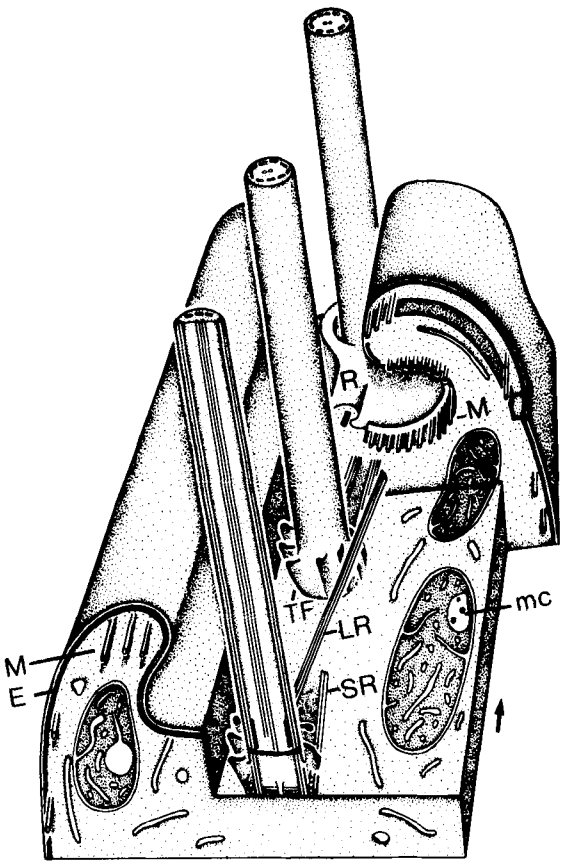

Fig. 1. "Cut-away" ultrastructural view of cortical and subcortical organelles of the hemimastigophoran flagellate, *Hemimastix amphikineta*. Arrow points toward posterior end of organism. From [8].

E, epiplasm; LR, long microtubular ribbon; M, cortical microtubule; mc, mitochondrial cavern; R, flagellar ridge; SR, short microtubular ribbon; TF, transitional fibre.

whether the kineties extend to mid-body or base of tail. Considering the two other species assigned by us to this genus, it is reasonable to assume that the kineties of *S. multiciliatum* terminate near mid-body.

Spironema multiciliatum Klebs, 1893
(Fig. 2–4)

Diagnosis. In vivo 14–18 × 2–3 μm. Body lanceolate with tail about ⅓ of cell length. Capitulum inconspicuous. Kineties with 6–13 flagella each.

Type location. Ditch water in Germany. Exact location unknown.

Type material. Not available.

Description [13]; translated from German:

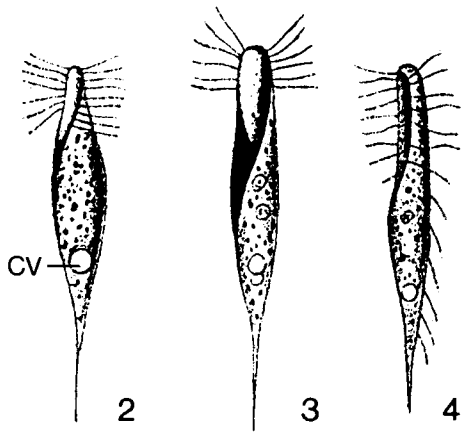

Fig. 2–4. *Spironema multiciliatum* from life, 14–18 × 2–3 μm (from [13]). 2. View from short axis. 3. View from broad axis. 4. Specimen with flagella up to posterior end.

CV, contractile vacuole.

The body shape is highly characteristic because of the long, acute tail, the two spiraled grooves which commence at the anterior end and extend up to the tail filament. The ciliature is very unusual. The edge of each spiral groove is provided with numerous small cilia which normally do not move uniformly but independently from each other. In the first specimens I observed cilia only in the anterior portion (Fig. 2, 3). Recently, I noticed specimens with cilia up to the posterior end or the beginning of the tail, respectively (Fig. 4). I may have overlooked them earlier.

The animal moves sluggishly back and forth, occasionally settling with the acute tail. The posterior region of the body including the tail is fairly stiff and changes hardly. In contrast, the anterior portion is highly metabolic, contracts and extends again, bends and curves to all sides. Small bodies are propelled towards the grooves by the movement of the cilia and small bacteria (small greenish bodies) probably sink into the cytoplasm beneath the anterior portions of the groove and become enclosed in vacuoles.

The contractile vacuole is in the posterior portion where the tail begins.

Unfortunately, the reproduction could not be observed.

Occurrence. As yet recorded only from Germany by Klebs [13] and Zölffel (pers. commun.; Fig. 68 in [8]).

Remarks. *Spironema multiciliatum* differs from *S. goodeyi* and *S. terricola* in size and shape of body, in the absence of a conspicuous capitulum, and in habitat. The nucleus has not been described by Klebs.

Spironema terricola n. sp.
(Fig. 5–34, 59, 62, 65; Table 1, 2)

Diagnosis. In vivo 30–50 × 3–4 μm. Body vermiform with very thin tail half as long as cell. Capitulum conspicuous. Kineties in anterior ⅛ of cell, each kinety with about 8 flagella. Nucleus roundish.

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Fig. 5–23. *Spironema terricola* n. sp. from life (5–9, 13–19) and after protargol impregnation (10–12, 20–23). **5, 13.** Settled specimens adhering to detritus with tail are often curved. Note conspicuous capitulum. **6.** Curved, slightly contracted specimen. **7.** Highly contracted specimen. **8, 9.** Contracting cells viewed from short axis and from broad axis. **10, 22.** Prepared specimens viewed from broad axis. Both kineties are recognizable (arrows). **11, 12, 20, 21, 23.** Prepared, slightly contracted cells viewed from short axis at different focus planes to reveal both kineties, which converge beneath capitulum. **14.** Gliding specimen. **15–19.** Commencing and advanced stages of euglenoid movement. Note extreme deformation of body. Bars and scale bar divisions = 10 μm.

C, capitulum; CV, contractile vacuole; D, detritus; EX, extrusome; FV, food vacuole; N, nucleus; T, tail.

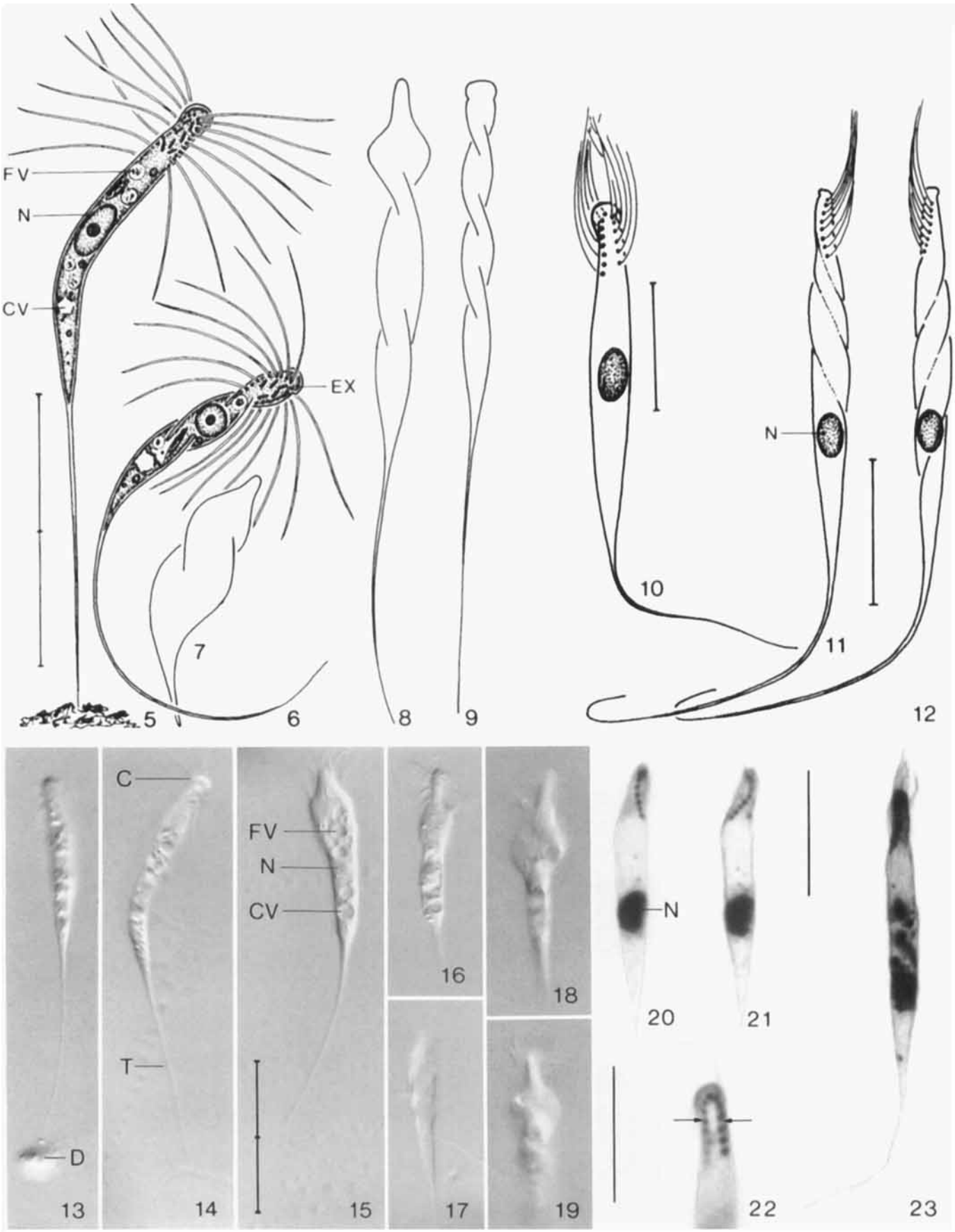
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Table 2. Morphometric characterization of *Spironema terricola* (St) and *Hemimastix amphikineta* (Ha).[a]

Character	Species	$\bar{x}$	SD	CV	Min	Max
Body, length	St	41.7	7.5	17.9	30	50
	Ha	13.7	1.5	10.9	12	17
Body, width	St	3.1	0.5	15.9	2.5	4
	Ha	6.6	0.9	13.6	5	8
Distance anterior end to posterior end of kineties	St	4.9	0.7	14.1	4	6
	Ha	kineties as long as body				
Distance anterior end to nucleus	St	12.7	3	23.9	9	17
	Ha	usually in posterior half				
Distance anterior end to contractile vacuole	St	25	4	16.3	20	30
	Ha	vacuole at posterior end				
Nucleus, length	St	4	0.8	20.4	3	5
	Ha	3.3	0.4	12.1	3	4
Nucleus, width	St	2.6	0.5	17.3	2	3
	Ha	2.9	0.4	13.8	2	3.5
Flagella, number per kinety	St	7.6	0.5	7	7	8
	Ha	—	—	—	—	—
Flagella, total number	St	15.1	1.1	7.3	14	16
	Ha	24.2	1.5	6.2	20	28

[a] All data are based on 7 (*S. terricola*) or 23 (*H. amphikineta*) randomly selected, protargol-impregnated and mounted nondividers. Data for *H. amphikineta* are from [8] where CV was incorrectly calculated. These values were recalculated on the basis of the original data. All measurements in μm.

CV, coefficient of variation in %; Max, maximum; Min, minimum; SD, standard deviation; $\bar{x}$, arithmetic mean.

Type location. Soil from the Grand Canyon, USA (upper entrance to Bright Angel Trail), 36° N, 112° E.

Type specimens. One slide of holotype specimens and three slides of paratype specimens (all protargol-impregnated) have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz, Austria. Accession numbers: 44, 45, 46, 47/1992.

Etymology. "Terricola" for "living in soil."

Description. Light microscopy (Fig. 5–23, 29; Table 1, 2): Cells often curved or distinctly bent at mid-body or near origin of tail (Fig. 5, 6, 14); non-rigid, may extremely deform; anteriorly more distinctly flattened than in mid-body; capitulum conspicuous in broad axis view (Fig. 9, 14, 22); tail unflattened, very thin and fragile, readily shortens under cover glass pressure and probably also during preparation (Fig. 5–15). Nucleus near centre of thickened portion of cell (Fig. 5, 6, 10, 15, 20). Contractile vacuole near base of tail, surrounded by bleb-like vesicles (Fig. 5, 6, 15, 33). About 30 tiny (0.8 × 0.3 μm), rod-like inclusions in anterior cell portion, corresponding to posterior compartment of extrusomes (Fig. 5, 6, 29). Flagella 10 μm long, rather rigid, beat asynchronously. Food vacuoles globular, in mid-body. Occasionally a spindle-like inclusion 3 × 1.5 μm in size (Fig. 5, 6, 15).

Usually slowly gliding on tail or trembling and moderately fast swimming while rotating about longitudinal axis. Anterior body portion revolves in settled specimens which use tail as anchoring device (Fig. 5, 13). Swimming and gliding cells show distinct euglenoid movement from time to time, even if seemingly undisturbed. Cell dilations commence near apex and progress towards tail (Fig. 15–19). Contracted cells conspicuously twisted up to beginning of tail (Fig. 7–9, 11, 12, 17–19). Movement soon ceases in squeezed cells.

Electron microscopy (Fig. 24–28, 30–34): For general organization see characterization of phylum and family and Table 1. Cortical plates slightly plicate (Fig. 30, 31, 59). Epiplasm consists of heavily staining osmiophilic sheet (outer layer) and thicker, lighter staining inner layer (Fig. 34); continuous in capitulum, gradually reduced toward ends of kineties, therefore not evenly broad as in *Hemimastix* but tapering (Fig. 25, 30, 31, 59, 65). Flagella-free portion of cortex without epiplasm, i.e. solely supported by microtubules (Fig. 27, 32, 33, 59). Cortical microtubules along short axis continuous over capitulum (loop-like), i.e. originate and end in tail. Cortical microtubules along broad axis originate from both sides of capitulum, viz. slightly beneath cell's apex and from cell membrane near flagellar pits; also extend to tail (Fig. 25, 27, 65; compare *Stereonema geiseri*: Fig. 48, 53).

Mean diameter of flagella 312 nm (range 250–350 nm, n = 5), axonemes 198 nm (175–214 nm, n = 5), basal bodies 183 nm (176–190, n = 5), membranous sacs 61 nm (60–62 nm, n = 6); mean length of basal bodies 236 nm (227–250, n = 4).

Extrusomes about 4 × 0.23 μm in size, barely recognizable in light microscope (Fig. 29), anchored in epiplasm along kineties and in capitulum. Anterior extrusome compartment ("nail") with thin granular centre, tip cup-like, posterior end ("nail head") with three granule-like thickenings. Posterior bottle-like compartment with two bulbous dilations at anterior end and electron lucent zone separating nail head from content of bottle (Fig. 24–26, 30, 31, 34, 62).

Food vacuoles contain electron-dense, amorphous masses and membrane residues (Fig. 28). Numerous globular vesicles, about

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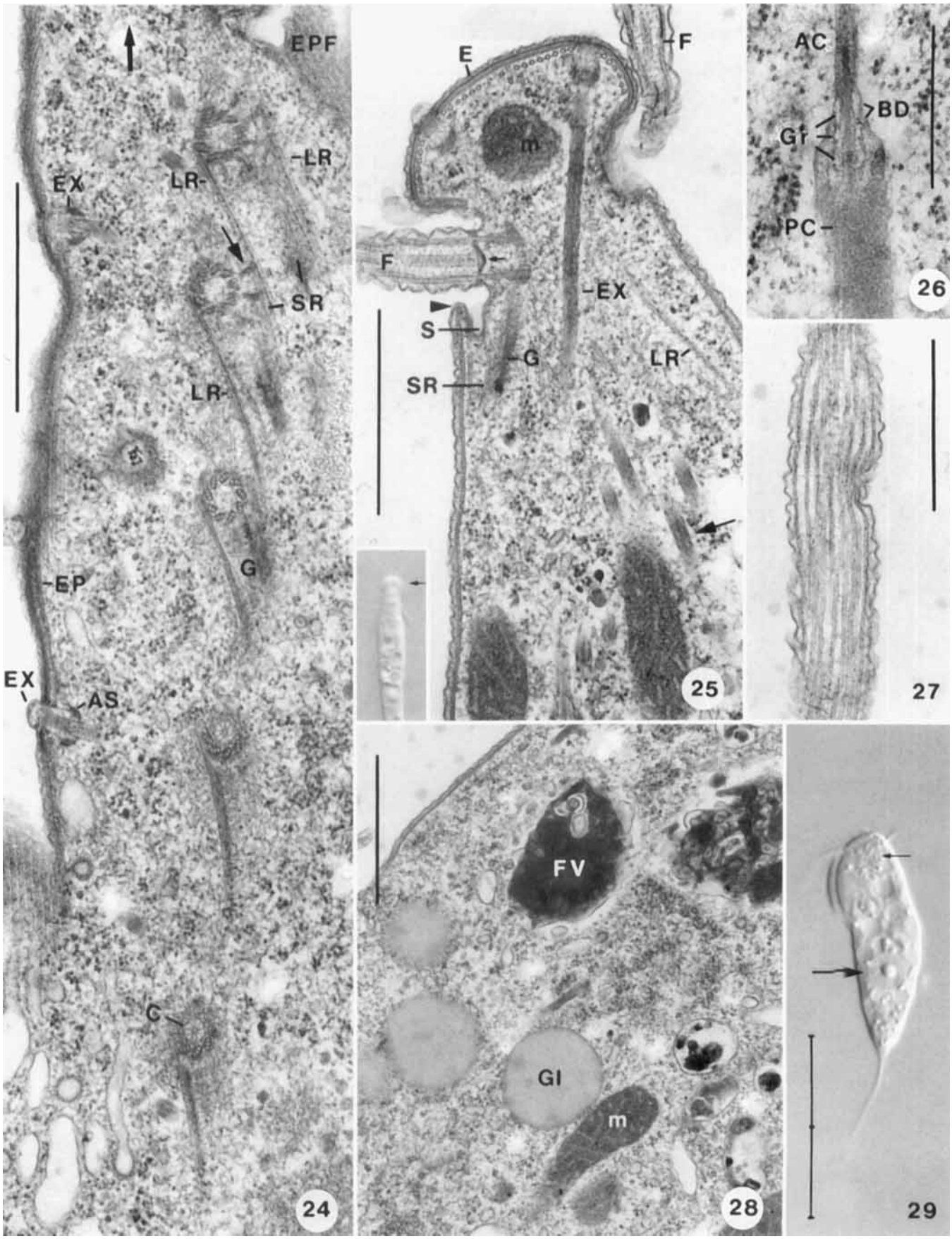
Fig. **24–29**. *Spironema terricola* n. sp. (Fig. 24–28, TEM micrographs; Fig. 29 and inset in Fig. 25, interference contrast light micrograph). **24**. Kinety seen from outside of cell. The long microtubular ribbons originate near the epiplasm-bearing portion of the cortical plates and extend posteriorly. They may contact the transitional fibres (thin arrow) and the short microtubular ribbon of the next kinetid. The short and long microtubular ribbon of the same basal body are connected by granular material. Note increasing distance between basal bodies from anterior (thick arrow) to posterior end of cell. Extrusomes are anchored in the epiplasm along the kinety. Bar = 1 μm. **25**. Longitudinal section of anterior region showing conspicuous capitulum (cp. inset, arrow) containing extrusomes. The two kineties commence beneath the capitulum, which is underlain by epiplasm. Note concave transitional plate of flagellum and a cortical microtubule (arrowhead) originating at the cell membrane near the membranous sac, which extends posteriorly from the flagellar pit and parallels the microtubular ribbons of the associated kinetid. In appropriate sections, the anterior compartment (shaft) of the extrusome looks like three parallel microtubules (arrow). Bar = 1 μm. **26**. The extrusomes have two bulbous dilations and three granular thickenings where the anterior compartment inserts into the posterior compartment. Details of the extrusome tip are shown in Fig. 24 and in the schematic Fig. 62. Bar = 500 nm. **27**. The tail consists of converging cortical microtubules. Bar = 750 nm. **28**. Mid-body region with food vacuoles, homogeneous globules and mitochondria. Bar = 1 μm. **29**. Squeezed cell showing thicker, posterior compartment of extrusomes (small arrow) and nucleus with prominent nucleolus (large arrow). Scale bar division = 10 μm.

AC, anterior (nail-like) compartment of extrusome; AS, anchoring structure of extrusome; BD, bulbous dilation of posterior compartment of extrusome; C, cartwheel structure; E, epiplasm; EP, epiplasm-bearing portion of cortical plate; EPF, epiplasm-free region of cortical plate; EX, extrusome; F, flagellum; FV, food vacuole; G, granular material; Gl, homogeneous globular vesicle; Gr, granular thickenings of anterior compartment of extrusome; LR, long microtubular ribbon; m, mitochondrion; PC, posterior (bottle-like) compartment of extrusome; S, membranous sac; SR, short microtubular ribbon.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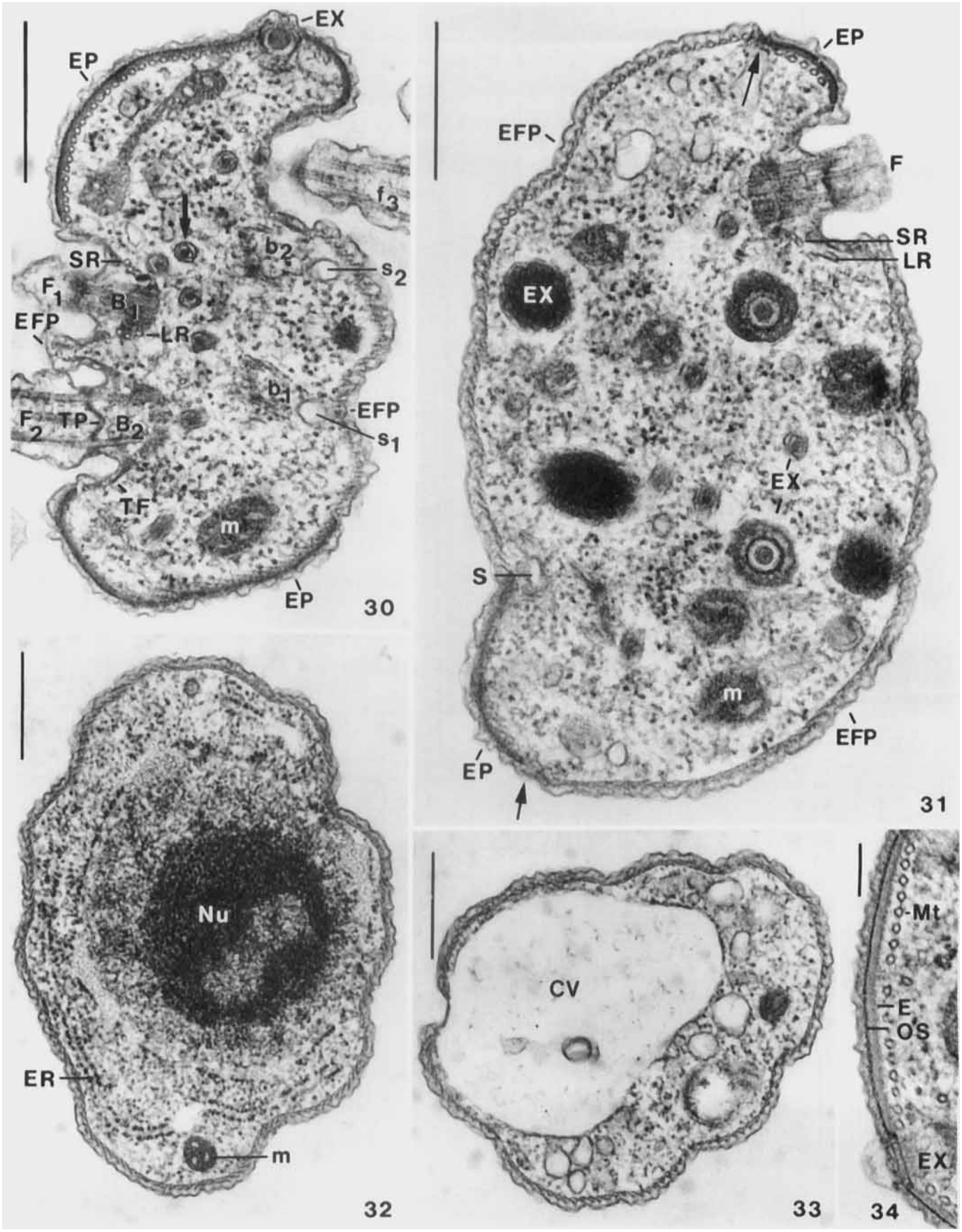
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800 nm in diametre, with homogeneous content of medium electron density scattered throughout cytoplasm (Fig. 28). Cisterna of rough endoplasmic reticulum conspicuous along nucleus (Fig. 32).

Occurrence. As yet found only at type location, viz. in a pine forest at the edge of the Grand Canyon. *Spironema terricola* occurred in soil of grass patches scattered in a thick (up to 10 cm) layer of litter on red soil. The soil appeared virtually dry when collected and was air-dried for three weeks. It is thus reasonable to assume that *S. terricola* emerged from resting cysts.

Remarks. See *S. goodeyi.*

Spironema goodeyi n. sp.
(Fig. 35–37)

Diagnosis. In vivo 20–50 × 4 μm. Body vermiform with short tail about ¼ of cell length. Capitulum conspicuous. Kineties in anterior ⅛ of cell, each kinety with 3–9 flagella. Nucleus distinctly elongate.

Type location. Agricultural soil from Rothamsted, Broadbalk (England).

Type material. Goodey made hematoxylin slides but did not mention where they have been deposited. Probably, they are at the University of Birmingham or at the Rothamsted Research Station.

Description. From [9]:

> This highly interesting organism occurred in one culture made from Broadbalk 1865 soil. It appeared both on the surface and at the bottom of the culture. My attention was first attracted to it by reason of its great length and its peculiar method of locomotion. It moved slowly in a very hesitating jerky manner for the most part, but would suddenly exhibit rapid and violent spiral twists commencing at its anterior end and travelling down the body, at which times it was propelled at a reasonably fast pace. It was obvious that the organellae causing the slow jerky motion were situated at or towards the anterior end, though they could not be distinguished under a low power of the microscope. Towards the posterior end a contractile vacuole could be seen in diastole and systole.
>
> I was able to obtain film preparations which, when fixed and stained, revealed the structure of the organism very clearly. The body is extremely long in comparison with the width, and is dorsoventrally flattened. It measures anything from 20–50 μm in length, and averages about 4 μm in width. The middle region is generally the widest part of the body. The anterior end is either rounded or has a lateral knob-like portion on either side. The posterior end is drawn out into a long and exceedingly fine tapering tail, and the contractile vacuole occurs just where the body begins to narrow down.

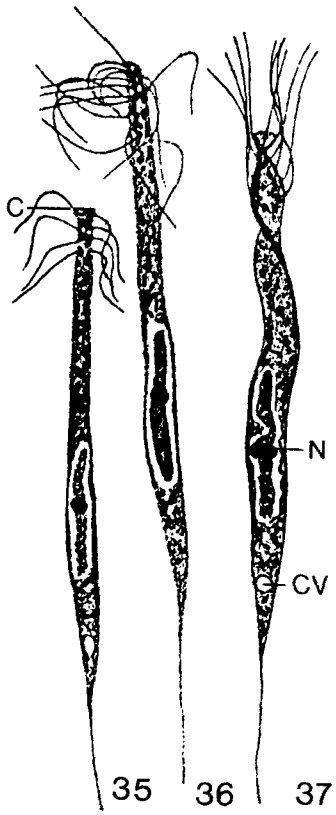

Fig. **35–37**. *Spironema goodeyi* n. sp., hematoxylin stain, 20–50 × 4 μm (from [9]). **35, 36**. Extended specimens. Note conspicuous capitulum in left cell and elongate nucleus. **37**. Contracting specimen. C, capitulum; CV, contractile vacuole; N, nucleus.

> The flagella are numerous and comparatively short. They vary in number from seven to eighteen, and the smaller the organism the fewer the flagella. They are situated in most cases in two lateral rows towards the anterior end of the body, one row being dorsally and the other ventrally placed. I have carefully noted the disposition and number of the flagella, and find that they are not equally distributed on either side, but exhibit a considerable amount of variation in this respect. Klebs, on his Pl. xvi, fig. 9c (Fig. 4), shows a row of flagella extending backwards on one side as far as the beginning of the tail. I have not found anything like this in my organisms.

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Fig. **30–34**. *Spironema terricola* n. sp. (TEM micrographs). **30**. Slightly oblique section (observer looks toward anterior end of cell) beneath capitulum showing converging kineties (cp. 59, 65). The basal bodies of the kinety at the left side of the micrograph are longitudinally sectioned (B_1, B_2), those of the kinety at the right side are obliquely sectioned (b_1, b_2), showing the membranous sacs (s_1, s_2); the basal body of the third flagellum (f_3) is out of section. The long microtubular ribbon originates from the proximal end of the basal body, the short microtubular ribbon originates more distally and is connected with the basal body via dense material which is part of the transitional fibres (cp. 24). The cortical plate at the left upper corner is completely underlain with epiplasm except for the small area between the flagella; the epiplasm-free portion of the plate at the right lower corner (more posteriorly sectioned) is enlarged. Note thin granular centre of anterior extrusome compartment (arrow). **31**. Cross section at level of last (posterior) flagella. Arrows indicate transitions between epiplasm-bearing and epiplasm-free portions of cortical plates. The epiplasm-bearing portion is considerably narrowed in this region of the cell (cp. 30). **32**. Transverse section at nucleus level. The epiplasm is absent and the cortex is supported solely by microtubules. Note large, plate-like cistern of rough endoplasmic reticulum. **33**. Transverse section at level of contractile vacuole which is surrounded by bleb-like vesicles. **34**. Longitudinal section of cortex showing osmiophilic outer sheet of epiplasm and extrusome tip. Bars = 500 nm (30–33), 125 nm (34).

B, b, basal bodies of kineties at left and right side of micrograph, respectively; CV, contractile vacuole; E, epiplasm; EFP, epiplasm-free portion of cortical plate; EP, epiplasm-bearing portion of cortical plate; ER, endoplasmic reticulum; EX, extrusome; f, F, flagella at right and left side of micrograph, respectively; LR, long microtubular ribbon; m, mitochondrion; Mt, microtubule; Nu, nucleolus; OS, osmiophilic sheet; S, membranous sac; SR, short microtubular ribbon; TF, transitional fibre; TP, transitional plate.

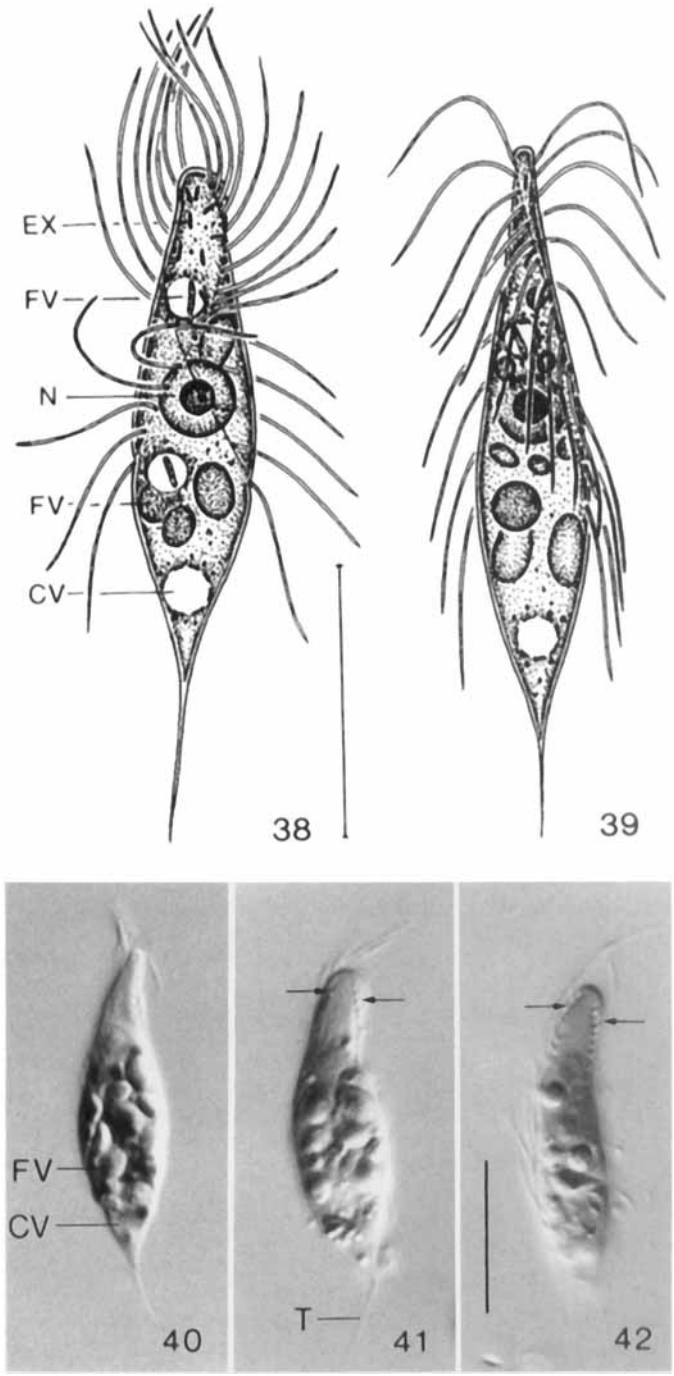


Fig. **38–42**. *Stereonema geiseri* n. g., n. sp. from life. **38**, **41**, **42**. Broad axis views showing kineties converging beneath capitulum (arrows in Fig. 41, 42). **39**, **40**. Small axis views. Note abundant refractile inclusions (food vacuoles?). The short tail may bend stiffly at base (Fig. 41). Bars = 10 μm.

CV, contractile vacuole; EX, extrusome; FV, food vacuole; N = nucleus; T, tail.

A few of the organisms were fixed just as they were twisting spirally, and one of these is shown in fig. 48 (Fig. 37). It will be seen from this that the edges of the body are curved, and that the flagella have their origin close to the edges. Each flagellum arises from a small basal granule or blepharoplast distinctly seen in the stained material. I cannot say whether there is a mouth, and although I watched the creatures in life for a long time, I never saw them take in food. There are numerous large granular bodies, however, in the cytoplasm in many of the forms which appear to be ingested bacteria, and because of this, I am of the opinion that a mouth is present. I believe it is situated towards the anterior end, for I have made out, in some cases, a somewhat lighter area here which might be considered as the mouth. In the greater part of the body the cytoplasm is very finely granular and evenly distributed, but towards the posterior end, in the region of the contractile vacuole, it is frequently much vacuolated.

The nucleus is a very interesting structure. It is, in most cases, of considerable length, and is situated about half-way down the body. It consists of a long narrow rod of granular material, frequently one-quarter to one-third of the body in length. Towards the middle of it is placed a circular karyosome of deeply staining chromatin. The extra-karyosomic portion of the nucleus appears to be very little different from the general cytoplasm in staining reactions, and is separated from the latter on all sides by a very narrow clear space. There does not appear to be any nuclear membrane. At all events, I have not made out anything comparable with the nuclear membrane of other flagellates and amoebae. From the appearance of the stained examples, it seems that all the chromatin is concentrated in the deeply staining karyosome, there being only small scattered granules in the rest of the nucleus.

Occurrence. As yet recorded only from type location.

Remarks. Goodey identified this organism as *S. multiciliatum* Klebs. However, differences in size and shape (very elongate, conspicuous capitulum) and in biotope (soil) warrant species status. *Spironema goodeyi* is very similar to *S. terricola,* but the shape of the nucleus is clearly different; since this character is widely used for taxa separation, we recognize the organism as a new species. *Spironema terricola* probably has a longer tail than *S. goodeyi.* However, this is not certain, because Goodey's drawings are from fixed specimens whose tails may have been shrunk. Both species differ from *S. multiciliatum* in body shape and size and in length of kineties, i.e. by the proportion of the flagellated and non-flagellated area.

Genus *Stereonema* n. g.

Diagnosis. Medium-sized (20–30 μm) Spironemidae with kineties in indistinct furrows and terminating near mid-body. Posterior end with distinct tail. Cortex soft and slightly plicate; cells deformable but acontractile.

Type species. *Stereonema geiseri* n. sp.

Etymology. *Stereonema* is of Greek derivation. "Stereo" means stiff, and "nema" means stick; *Stereonema* therefore denotes a "stiff stick."

Remarks. *Stereonema* differs from *Spironema* by the inability to perform euglenoid movement. Like in *Hemimastix,* even extreme stimuli (strong cover glass pressure, heat) do not induce metaboly. It is, however, clearly separate from *Hemimastix* by the shorter kineties, the reduced epiplasm and the simpler organization of the flagellar furrows.

Stereonema geiseri n. sp.
(Fig. 38–55, 60, 63, 65; Table 1)

Diagnosis. In vivo 20–30 × 5–8 μm. Body lanceolate with tail about ¼ of cell length. Capitulum inconspicuous. Kineties with about 12 flagella each. Nucleus roundish.

Type location. River Amper about 2 km downriver from the sewage plant at Geiselbullach, east of Fürstenfeldbruck, Bavaria, Germany, 48° 13′ N, 11° 21′ E.

Type specimens. Fig. 38–42. No permanent slides available.

Dedication. This new species is dedicated to the Bavarian entomologist Dr. Remigius Geiser in appreciation of his help in nomenclatural problems.

Description. Light microscopy (Fig. 38–42, Table 1): Shape rather constant, in broad axis view distinctly lanceolate, in short

axis view fusiform; anterior end more distinctly flattened than posterior half; tail straight or obliquely bent. Nucleus near or slightly above centre of thickened portion of body. Contractile vacuole near base of tail, surrounded by bleb-like vesicles (Fig. 38–40). Several tiny (0.8 × 0.3 μm), rod-like inclusions in anterior cell portion, corresponding to posterior compartment of extrusomes (Fig. 38). Flagella 6 μm long, rather rigid, beat asynchronously. Food vacuoles roundish, contain single bacteria. Many colourless, refractile, ellipsoid inclusions 0.5–2 μm in size scattered throughout cytoplasm; very likely, these are condensed food vacuoles (Fig. 38–43). Movement very slow, trembling, cannot swim.

Electron microscopy (Fig. 43–55, 60, 63, 65): For general organization see characterization of phylum and family and Table 1. Cortical plates as in *Spironema terricola,* i.e. slightly plicate, but tapering epiplasm without osmiophilic sheet; some regions with serrate structures (striation) between microtubules and epiplasm or cell membrane, respectively (Fig. 45, 48–54).

Mean diametre of flagella 332 nm (range 300–364 nm, n = 7), axonemes 205 nm (185–223 nm, n = 7), basal bodies 184 nm (180–200, n = 5), membranous sacs 89 nm (77–117 nm, n = 5); mean length of basal bodies 179 nm (175–182, n = 5).

Extrusomes about 4 × 0.23 μm in size, barely recognizable in light microscope (Fig. 38), anchored in epiplasm along kineties and in capitulum. Anterior extrusome compartment ("nail") with thick granular centre, tip bipartite by two bulbous dilations, posterior end club-shaped, i.e. much simpler than in *S. terricola* and *H. amphikineta,* and not separated by electron-lucent zone from granular content of posterior extrusome compartment. Anterior end of posterior extrusome compartment with single bulbous dilation as in *H. amphikineta* (Fig. 46, 47, 63). Food vacuoles with bacteria, membrane residues and electron-dense masses, the latter probably corresponding to the conspicuous ellipsoid inclusions seen in the light microscope (Fig. 40, 43, 50).

Occurrence and ecology. Occasionally found in the Aufwuchs (periphyton) of meso-saprobic rivers in Germany and Austria (Amper near Munich, Ager near Linz).

Genus *Hemimastix* Foissner, Blatterer & Foissner, 1988

Diagnosis. Small (less than 20 μm), conspicuously flattened Spironemidae with kineties in distinct furrows and extending to posterior end. Cortex rigid, extremely plicate and with distinct flagellar ridge; cells ametabolic and acontractile.

Type species. *Hemimastix amphikineta* Foissner et al., 1988.

Remarks. Differs from *Spironema* and *Stereonema* by the highly plicate, rigid cortex, the continuous flagellar groove ("distinct furrow") and the flagellar ridge, which is a bulge around and between the flagellar bases (Fig. 1, 58, 61). It can be clearly distinguished from *Spironema* by the absence of euglenoid movement.

Hemimastix amphikineta Foissner, Blatterer & Foissner, 1988
(Fig. 1, 56–58, 61, 64; Table 1, 2)

Diagnosis. In vivo 14–20 × 7–10 μm. Body ellipsoid, about 2:1 flattened. Capitulum conspicuous. Kineties with about 12 flagella each. Nucleus roundish.

Type location. Upper soil layer (0–3 cm) of a bushland in Brisbane-Waters National Park, near Sydney, East Australia, E 151°, S 34°.

Type specimens. One slide of holotype specimens and three slides of paratype specimens (all protargol-impregnated) have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz, Austria. Accession numbers: 86, 87, 88, 89/1989.

Description. Light microscopy (Fig. 56, 57; Table 1, 2): Shape remarkably constant, ellipsoidal both from short and broad axis view; posterior end slightly tapered, anterior end transverse truncate by distinct capitulum. Nucleus subequatorial. Contractile vacuole at posterior end, surrounded by bleb-like vesicles. Extrusomes not recognizable in light microscope. Flagella 5–9 μm long, rigid, beat asynchronously. Food vacuoles 1–5 μm in diametre, contain remnants of bacteria and heterotrophic flagellates. Cytoplasm with refractile granules (0.3–1.5 μm) often

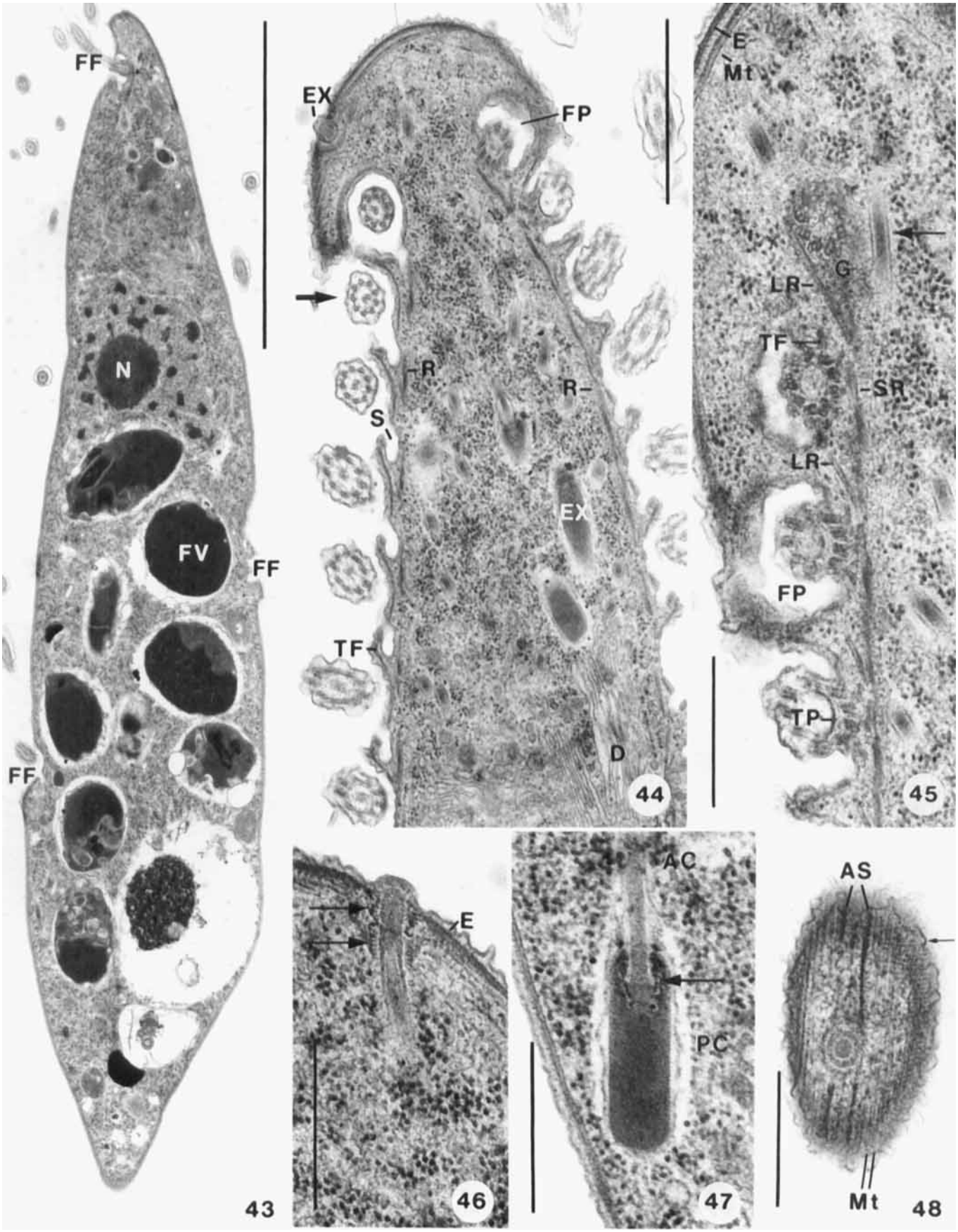
→

Fig. **43–48**. *Stereonema geiseri* n. g., n. sp. (TEM micrographs). **43**. Tangential section of thickened body portion. Note nucleus with prominent nucleolus and many inclusions (food vacuoles?). Bar = 5 μm. **44**. Anterior end sectioned along kineties. The membranous sacs extend posteriorly and are supported by the microtubular ribbons of the basal bodies. The arrow denotes a flagellum where the delicate transitional cylinder can be seen. Bar = 1 μm. **45**. Slightly tangential section of kinety seen from outside of cell. Granular material extends between microtubular ribbons. Note anterior extrusome compartment (shaft; arrow) with thick, granular centre. Bar = 500 nm. **46**. Longitudinal section of bipartite, bulbous extrusome tip (arrows). Bar = 500 nm. **47**. Bottle-like posterior extrusome compartment containing club-shaped end of anterior compartment (arrow). Bar = 500 nm. **48**. Transverse section of extrusome tip at level of anchoring structure. Note fine striations (arrow) perpendicular or slightly oblique to cortical microtubules. Bar = 500 nm.

AC, anterior compartment of extrusome; AS, anchoring structure of extrusome; D, dictyosome; E, epiplasm; EX, extrusome; F, flagellum; FF, flagellar furrow; FV, food vacuole; FP, flagellar pit; G, granular material; LR, long microtubular ribbon; Mt, microtubule; N, nucleus; PC, posterior compartment of extrusome; R, microtubular ribbons of basal bodies; S, membranous sac; SR, short microtubular ribbon; TF, transitional fibre; TP, transitional plate.

Fig. **49–55**. *Stereonema geiseri* n. g., n. sp. (TEM micrographs). **49**. Transverse section of anterior end at level of fifth flagellum. Arrows indicate transitions between epiplasm-bearing and epiplasm-free regions of cortical plates. Note short basal body and anterior extrusome compartment (shaft) with thick granular centre. Bar = 1 μm. **50, 51**. Sections at nucleus level where the epiplasm is confined to a narrow region adjacent to the flagellar furrows. Thick arrows indicate transitions between epiplasm-bearing parts and epiplasm-free regions. Note transitional plate (arrowhead) and transitional cylinder (thin arrow). Bars = 2 μm and 500 nm. **52**. A serrate structure is sometimes found between the epiplasm and the cortical microtubules and between the epiplasm and the cell membrane; it corresponds to the delicate striation seen in tangential sections (Fig. 48). Note absence of osmiophilic sheet in epiplasm. Bar = 250 nm. **53**. Transverse section of capitulum which is inconspicuous and underlain by epiplasm. Note discontinuous cortical microtubules (arrowhead) which originate beneath cell apex and are perpendicular to the continuous cortical microtubules paralleling the short axis of the cell (cp. Fig. 65). Bar = 500 nm. **54**. Transverse section of tail showing converging cortical microtubules. Bar = 250 nm. **55**. Dicytosome. Bar = 500 nm.

Cr, chromatine; E, epiplasm; EFP, epiplasm-free portion of cortical plate; EP, epiplasm-bearing portion of cortical plate; EX, extrusome; F, flagellum; FF, flagellar furrow; FV, food vacuole; LR, long microtubular ribbon; m, mitochondrion; Mt, microtubule; Nu, nucleolus; S, membranous sac; SR, short microtubular ribbon; TF, transitional fibre; TP, transitional plate.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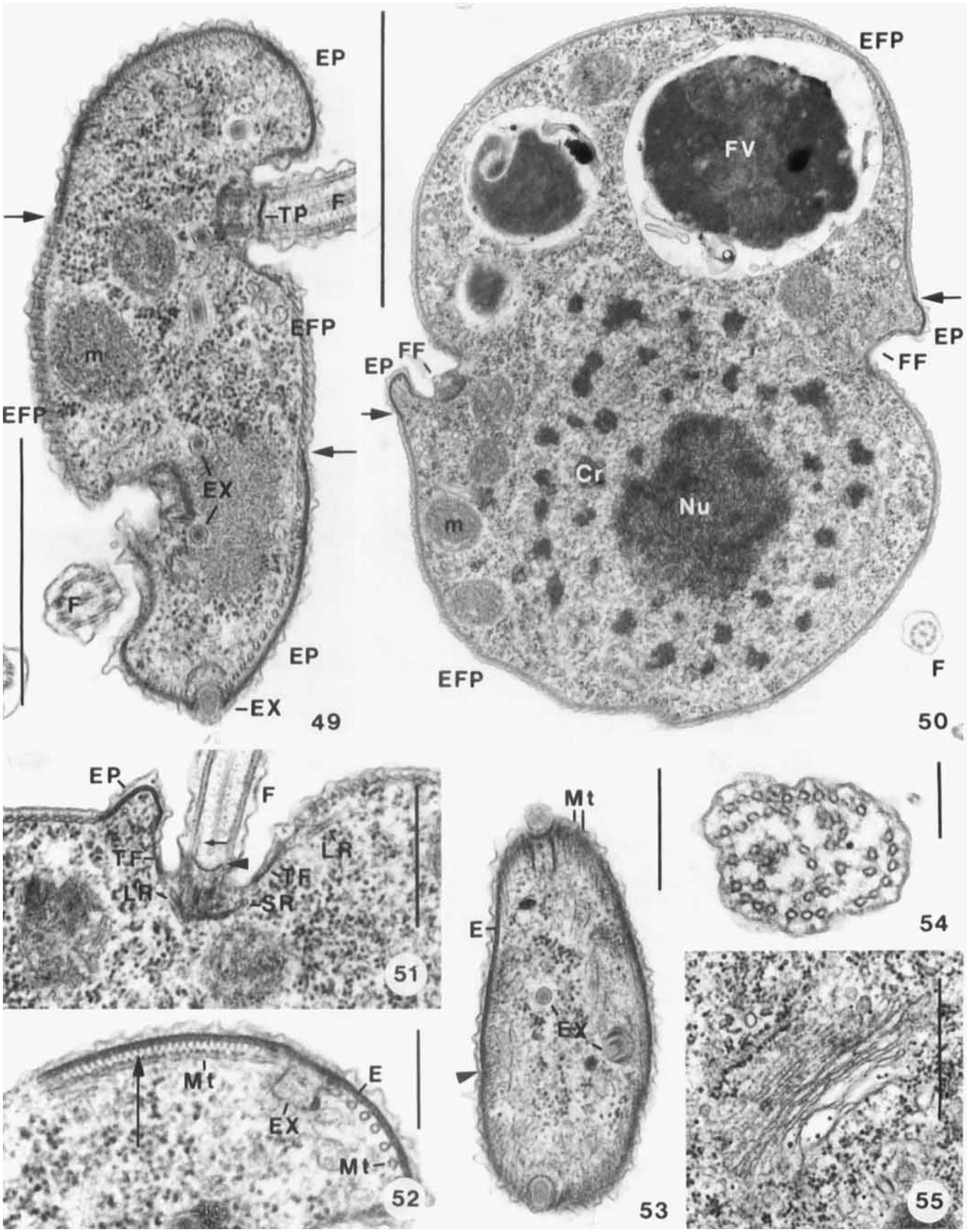
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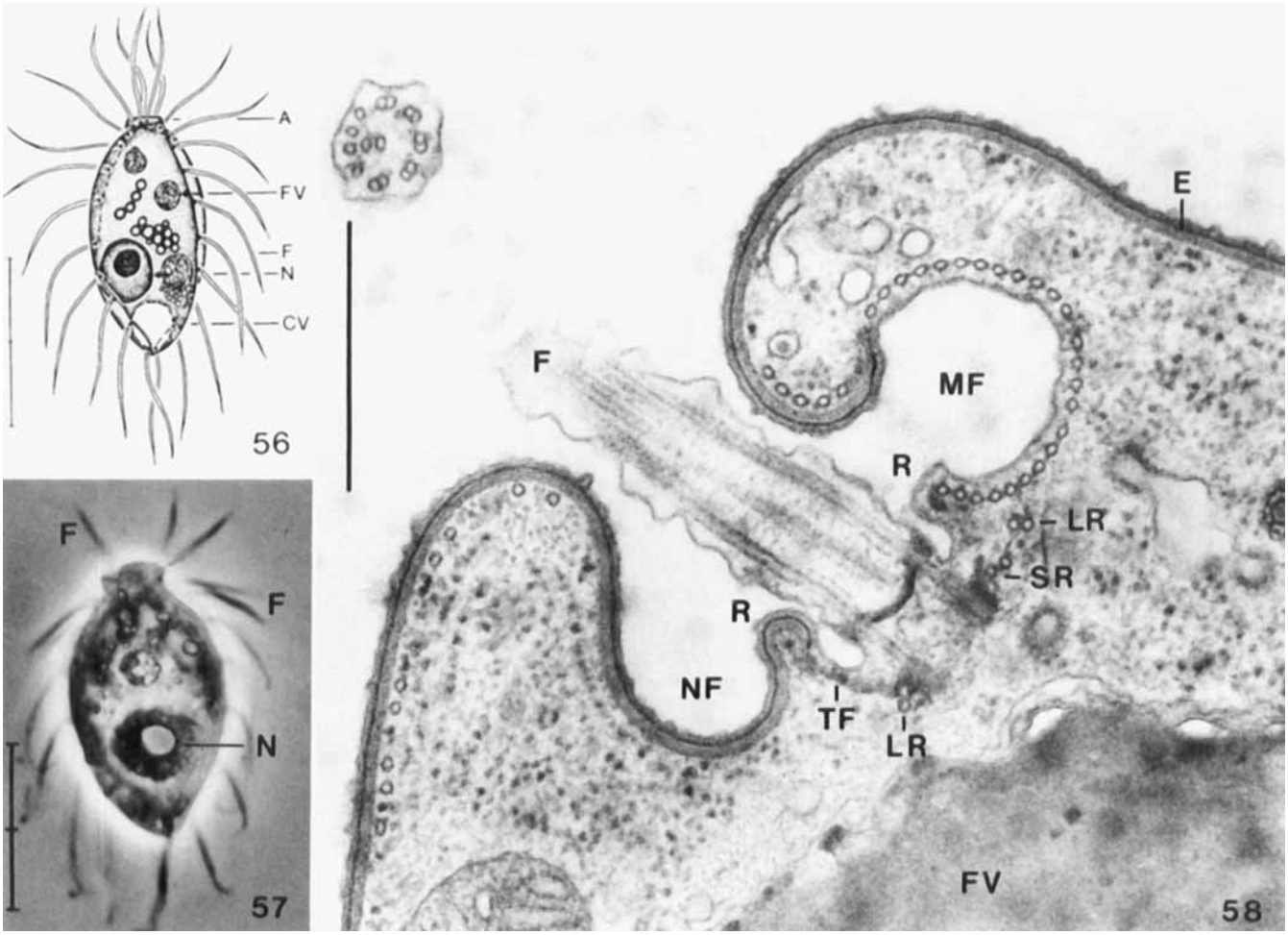

Fig. 56–58. *Hemimastix amphikineta* (from [8]). **56, 57.** Broad axis views of live and silver-carbonate-impregnated specimens. Scale bar divisions = 5 μm. **58.** TEM micrograph of transverse section showing bipartite flagellar furrow which consists of the epiplasm-bearing naked furrow and the epiplasm-free microtubule furrow. Note flagellar ridge and highly plicate cortex. Bar = 500 nm.

A, truncated anterior end (capitulum); CV, contractile vacuole; F, flagellum; FV, food vacuole; LR, long microtubular ribbon; M, mitochondrion; MF, microtubule furrow; N, nucleus; NF, naked (microtubule-free) furrow; R, flagellar ridge; SR, short microtubular ribbon; TF, transitional fibre.

linearly arranged. Movement trembling, creeping among detritus, cannot swim.

Electron microscopy (Fig. 1, 58, 61, 64). See [8] and characterization of phylum and family for detailed description. Cortex highly plicate, flagellar furrow bipartite, i.e. consisting of a microtubule and a naked furrow (Fig. 58). Epiplasm with electron-dense sheet, continuous nearly up to posterior end; epiplasm-free region confined to microtubule furrow. Distinct ridge between and around flagella (Fig. 1, 58, 61).

Extrusomes anchored in and near capitulum. Anterior compartment with thick granular centre, tip cup-like, posterior end ("nail head") with three granule-like thickenings. Posterior extrusome compartment with single anterior bulbous dilation (Fig. 64).

Occurrence and ecology. Discovered in several terrestrial biotopes from Australia and Chile (sandy and humic soils, bark of trees). Later we found it in soils and bromeliad mud from Costa Rica, in a soil sample from Gough Island, Antarctica, and in the bark of an Ohia tree in Hawaii. *Hemimastix amphikineta* is as yet found only south of the tropic, i.e. occurring mainly in the southern hemisphere like some other protists [6]. Zölffel (pers. commun.) claims to have found it in a forest litter in Berlin, Germany. However, he may have confused the Berlin samples with a sample we sent him from the type location.

All these soil samples were air-dried for at least 20 days. Some of them, e.g. the bark of an Ohia tree in Hawaii appeared completely dry when collected. It is thus reasonable to conclude that *H. amphikineta* emerged from resting cysts.

DISCUSSION

Family Spironemidae. The Spironemidae were established by Doflein [5] for a unique, multiflagellate freshwater organism, *Spironema multiciliatum* Klebs. Recently, *Hemimastix amphikineta* Foissner et al. from soil has been assigned to this family [8]. *Spironema multiciliatum* has not yet been reinvestigated. However, the description by Klebs [13] is rather detailed (see above), leaving no doubt that *S. multiciliatum, S. terricola* and *Stereonema geiseri* are closely related organisms (Fig. 2–6, 38, 39). The fine structure of the new species is very similar to that of *H. amphikineta,* which confirms our former classification of *Hemimastix* in the Spironemidae (see Table 1 for detailed comparison and Fig. 59–64).

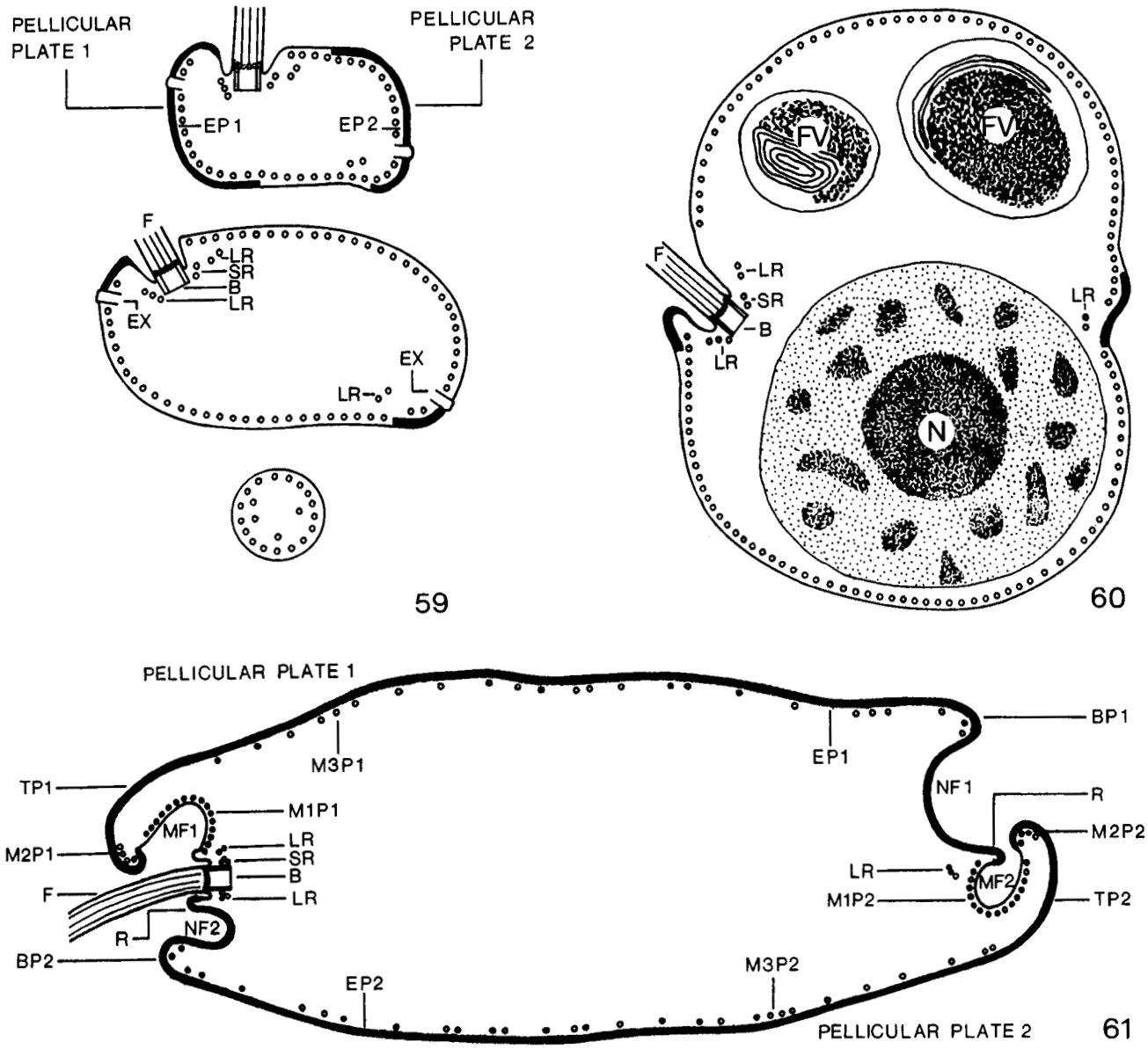

Fig. **59–61**. Schematic transverse sections of hemimastigophoran flagellates (cp. Fig. 30–33, 49, 50, 58), drawn approximately to scale. **59**. *Spironema terricola* n. sp. **60**. *Stereonema geiseri* n. g., n. sp. **61**. *Hemimastix amphikineta* (from [8]). Transverse sections of *Spironema terricola* (Fig. 59) are at levels of anterior and posterior flagella and of tail. *Stereonema geiseri* (Fig. 60) is sectioned in the nuclear region. Sections in other regions resemble those of *Spironema terricola*. *Hemimastix amphikineta* (Fig. 61) is sectioned in mid-body. Sections in other regions look alike along whole body except extreme anterior and posterior end. Each species has two cortical (pellicular) plates separated by two kineties (the one on the right is out of section). The cortical plates and the microtubular associates of the flagella have a characteristic diagonal (inversely mirror-image) or rotational symmetry. The cortical plates are underlain with epiplasm in the capitulum (not shown) and along the kineties. The epiplasm is therefore absent in the posterior, flagella-free regions of *Spironema* (Fig. 59) and *Stereonema* (Fig. 60). *Hemimastix* (Fig. 61) has both flagella and epiplasm along the whole body length and the epiplasm-free regions are confined to the small, evenly broad microtubule furrows. A flagellar ridge is present only in *Hemimastix* (Fig. 61).

B, basal body; BP1, blunt end of cortical (pellicular) plate 1; BP2, blunt end of cortical plate 2; EP1, epiplasm of cortical plate 1; EP2, epiplasm of cortical plate 2; EX, extrusome; F, flagellum; FV, food vacuole; LR, long microtubular ribbon; M1P1, microtubule group 1 of cortical plate 1; M2P1, microtubule group 2 of cortical plate 1; M3P1, microtubule group 3 of cortical plate 1; M1P2, microtubule group 1 of cortical plate 2; M2P2, microtubule group 2 of cortical plate 2; M3P2, microtubule group 3 of cortical plate 2; MF1, cortical furrow of plate 1 ("microtubule furrow 1"); MF2, cortical furrow of plate 2 ("microtubule furrow 2"); N, nucleus; NF1, cortical furrow of plate 1 ("naked furrow 1"); NF2, cortical furrow of plate 2 ("naked furrow 2"); R, ridge between and around the flagella; SR, short microtubular ribbon; TP1, thin end of cortical plate 1; TP2, thin end of cortical plate 2.

The vermiform shape and the contractility of *Spironema goodeyi* and *Spironema terricola* could be adaptations to the soil environment. *Hemimastix* could have evolved from a *Spironema terricola*-like organism by reducing the tail, causing the kineties to become as long as the body. This eventually results in a very small and flat organism having no need for euglenoid movement to exploit the habitat, viz. the tiny soil pores. The same evolutionary trends (elongation as in *S. terricola* versus

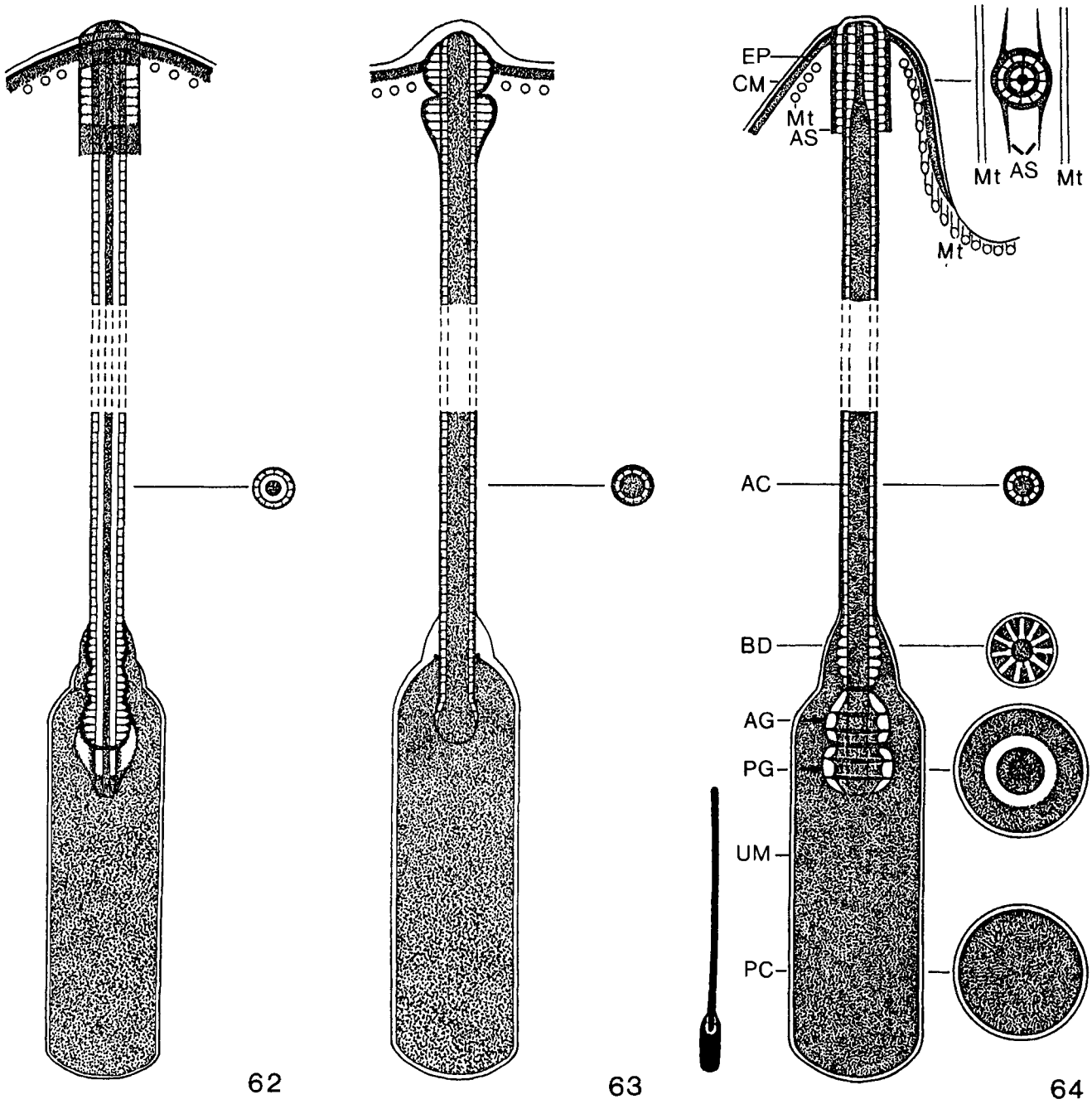

Fig. 62–64. Schematic drawings of extrusomes from longitudinal and transverse sections. **62.** *Spironema terricola.* **63.** *Stereonema geiseri.* **64.** *Hemimastix amphikineta* (from [8]). Main differences concern distal and proximal end of anterior, nail-like compartment and diametre of its granular centre. A complete organelle is shown in lower left corner of Fig. 64.

AC, anterior (nail-like) compartment; AG, anterior granule of nail-like compartment; AS, anchoring structure; BD, bulbous dilation; CM, cell membrane; EP, epiplasm; Mt, cortical microtubules; PC, posterior compartment; PG, posterior granule of anterior, nail-like compartment; UM, unit membrane enclosing whole extrusome.

size reduction as in *Hemimastix*) are found in soil ciliates [6]. Other, not yet known constraints may be responsible for the third apomorphy of *Hemimastix* (in addition to size reduction and loss of euglenoid movement), i.e. the highly plicate cortex (Fig. 58, 61).

Phylogenetic relationships of the Hemimastigophora. Foissner et al. [8] discussed extensively possible relationships of the Hemimastigophora with other protistan phyla. Thus, we focus our discussion on a few points mainly emerging from the new data presented in this paper.

Doubtless, the Spironemidae possess a unique combination of characters not found in other protistan phyla. Some affinities exist to the Ciliophora (microtubular associates of basal bodies = infraciliature) and to *Stephanopogon* (kineties composed of single, short basal bodies connected, however, by microfibrillar traces; furthermore, mitochondrial cristae are disc-like [15, 18]). Foissner et al. [8] suggested a relationship with the Euglenida mainly because of the diagonal (rotational) symmetry of the cortex (compare [16, 19]). In fact, it is easy to model a *Hemimastix* by combining two euglenid cortical strips (Fig. 66). The

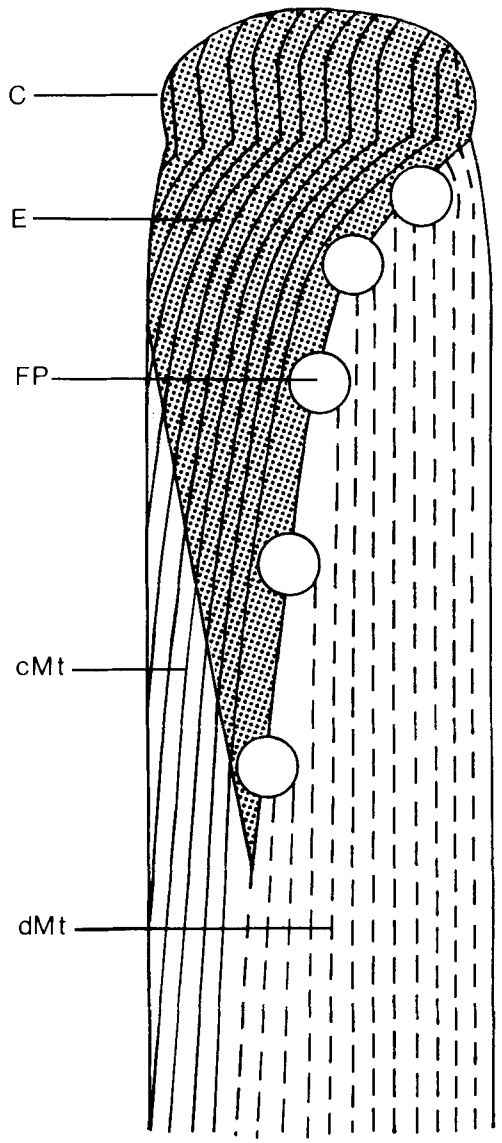

Fig. 65. Schematic drawing of the cortex of *Spironema* and *Stereonema*, viewed obliquely from short axis. The epiplasm and the cortical microtubules along the short axis (continuous microtubules) extend over the capitulum. The cortical microtubules along the broad axis (discontinuous microtubules) originate from the capitulum and from the cell membrane near the flagellar pits. The epiplasm tapers towards the ends of the kineties.

C, capitulum; cMt, continuous cortical microtubules; dMt, discontinuous cortical microtubules; E, epiplasm; FP, flagellar pit.

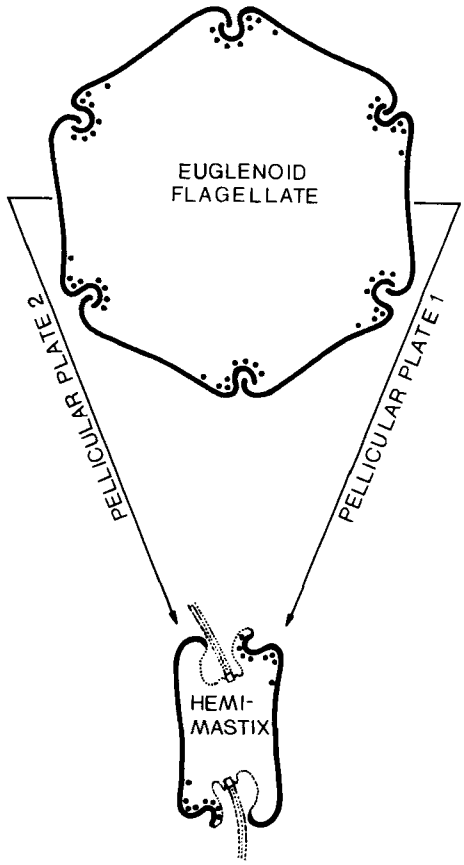

Fig. 66. A *Hemimastix*-like organism can be modeled by taking two opposite cortical (pellicular) plates and their associated microtubules (black dots) of a euglenoid flagellate and inserting two kineties between them. From [8].

idea of a relationship between euglenid and hemimastigophoran flagellates is strongly supported by our observations on *Spironema terricola*. The movement of this organism is impressive and very similar to that found, e.g. in *Euglena viridis* and *Peranema trichophorum* (Fig. 15–19). Euglenoid movement was already emphasized by Klebs in *Spironema multiciliatum* [13] and the resulting twisting of the body was mentioned and adequately illustrated in *Spironema goodeyi* (Fig. 37). The euglenid movement ("metaboly") is due to sliding of cortical strips relative to each other [21]. In euglenids, metaboly is most pronounced in genera with many strips and less distinct or absent in species with few pellicular strips [14, 22]. Although the precise mechanism of metaboly in the Hemimastigophora remains to be elucidated, our observations strongly suggest that the number of strips per se is not a causative agent for euglenid movement and should therefore not be used to separate euglenids from other taxa, like *Hemimastix*, as recently suggested [22]. Metaboly, but not the typical euglenid movement, is reported to occur in some kinetoplastids (Vickerman, pers. commun. and [24] for references), which are regarded as relatives or ancestors of euglenids by many specialists [1, 4, 12, 22–25]. Their cell membrane (especially that of the trypanosomes) and that of *Stephanopogon* is supported by a monolayer of microtubules [2,15, 18, 24]. This is similar to the microtubule layer beneath the epiplasm-free regions of the Hemimastigophora, especially of *Stereonema* and *Spironema* (Fig. 59–61). A noteworthy difference is, however, that the cortical microtubules of the Hemimastigophora do not originate near flagellar roots or basal bodies as in euglenids and kinetoplastids [2, 26], but from organelle-free regions of the cytoplasm (tail) or near the cortex (Fig. 65). Some bodonids and some euglenids possess extremely long extrusomes similar to the anterior compartment of the extrusomes of the Spironemidae ([1] and references therein; Fig. 62–64).

Taken together, the Hemimastigophora share more characters with the euglenid-kinetoplastid assemblage than with any other protistan taxon. The most important synapomorphy is the diagonal symmetry of the cortical plates. It is highly unlikely that such a complex character, resulting in a special kind of movement ("euglenoid metaboly"), evolved twice. If we accept Hennig's [10] phylogenetic concept, this complex synapomorphy is sufficient to establish a sister group relationship between euglenids and hemimastigophorans. The ancestral euglenid is proposed to be a phagotrophic flagellate with two basal bodies and

a rigid cortex consisting of a few strips [23]. Apparently, the Hemimastigophora fit most of these characters.

Recently, Cavalier-Smith [3] separated *Hemimastix* widely from the Euglenozoa because of its tubular mitochondrial cristae. In our opinion, this character is much weaker (see, for instance, the kinetoplastids and *Jacoba,* which have both tubular and disc-like cristae [17, 24] and O'Kelly [The genus *Jakoba,* a remarkable flagellate: structure, reproduction, and possible phylogenetic significance. *J. Euk. Microbiol.,* **40**:7A.]) than the unique cortical architecture relating euglenid and hemimastigophoran flagellates. For the moment, however, we keep separate the Hemimastigophora from the Euglenozoa mainly because of the kineties with their distinct "infraciliature," the different shape of the mitochondrial cristae, the lack of flagellar hairs and paraxial rods and the absence of a well-marked flagellar pocket. These differences indicate a long-lasting, separate evolution of the Hemimastigophora.

Another classification was suggested by Karpov [11]. He assigns *Hemimastix* to the order Apusomonadida Karpov & Mylnikov, mainly because he interprets the osmiophilic sheet in the epiplasm of *Hemimastix* as an additional membrane like that found in *Apusomonas.* This is, however, a misinterpretation, since this sheet has no tripartite structure (Fig. 34). Furthermore, it is absent in *Stereonema geiseri,* indicating that it is indeed a component of the epiplasm in *Hemimastix* and *Spironema.* In our opinion, *Apusomonas* is very different from the hemimastigophoran flagellates, since it has no cortical plates (and thus no diagonal symmetry), only two highly specialized flagella, and a very different system of flagellar roots.

KEY TO GENERA AND SPECIES

1. With euglenoid movement ..... *Spironema*
   a. Body lanceolate, up to 20 μm long; capitulum inconspicuous; freshwater ..... *S. multiciliatum*
   a'. Body vermiform, 20–50 μm long; capitulum conspicuous; soil ..... *b*
   b. Nucleus elongate ..... *S. goodeyi*
   b'. Nucleus roundish ..... *S. terricola*

1'. Without euglenoid movement 2

2. Kineties as long as body; cortex rigid; soil ..... single species, *Hemimastix amphikineta*

2'. Kineties terminating in mid-body; cortex soft; freshwater ..... single species, *Stereonema geiseri*

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LITERATURE CITED

1. Brugerolle, G. 1985. Des trichocystes chez les bodonidés, un caractère phylogénétique supplémentaire entre Kinetoplastida et Euglenida. *Protistologica,* **21**:339–348.

2. Brugerolle, G., Lom, J., Nohýnkovà, E. & Joyon, L. 1979. Comparison et évolution des structures cellulaires chez plusieurs espèces de Bodonidés et Cryptobiidés appartenant aux genres *Bodo, Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica,* **15**:197–221.

3. Cavalier-Smith, T. 1991. Cell diversification in heterotrophic flagellates. *In:* Patterson, D. J. & Larsen, J. (ed.), The Biology of Free-living Heterotrophic Flagellates. Clarendon Press, Oxford. Pp. 113–131.

4. Corliss, J. 1982. Numbers of species comprising the phyletic groups assignable to the kingdom Protista. *J. Protozool.,* **29**:499.

5. Doflein, F. 1916. Lehrbuch der Protozoenkunde, 4. Aufl., Fischer, Jena.

6. Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progr. Protistol.,* **2**:69–212.

7. Foissner, W. 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Europ. J. Protistol.,* **27**:313–330.

8. Foissner, W., Blatterer, H. & Foissner, I. 1988. The Hemimastigophora (*Hemimastix amphikineta* nov. gen., nov. spec.), a new protistan phylum from Gondwanian soils. *Europ. J. Protistol.,* **23**:361–383.

9. Goodey, T. 1916. Observations on the cytology of flagellates and amoebae obtained from old stored soil. *Proc. Zool. Soc. Lond.,* 309–330.

10. Hennig, W. 1982. Phylogenetische Systematik. Parey, Berlin.

11. Karpov, S. A. 1990. System of Protista. Omsk.

12. Kivic, P. A. & Walne, P. L. 1984. An evaluation of a possible phylogenetic relationship between the Euglenophyta and Kinetoplastida. *Origins of Life,* **13**:269–288.

13. Klebs, G. 1893. Flagellatenstudien. Teil I. *Z. Wiss. Zool.,* **55**: 265–351.

14. Leedale, G. F. 1967. Euglenoid flagellates. Prentice-Hall, Inc., Englewood Cliffs, NJ.

15. Lipscomb, D. L. & Corliss, J. O. 1982. *Stephanopogon,* a phylogenetically important "ciliate", shown by ultrastructural studies to be a flagellate. *Science,* **215**:303–304.

16. Mignot, J.-P. 1966. Structure et ultrastructure de quelques euglénomonadines. *Protistologica,* **2**:51–117.

17. Patterson, D. J. 1990. *Jakoba libera* (Ruinen, 1938), a heterotrophic flagellate from deep oceanic sediments. *J. Mar. Biol. Ass. U.K.,* **70**:381–393.

18. Patterson, D. J. & Brugerolle, G. 1988. The ultrastructural identity of *Stephanopogon apogon* and the relatedness of the genus to other kinds of protists. *Europ. J. Protistol.,* **23**:279–290.

19. Pochmann, A. 1953. Struktur, Wachstum und Teilung der Körperhülle bei den Eugleninen. *Planta,* **42**:478–548.

20. Skuja, H. 1956. Taxonomische und biologische Studien über das Phytoplankton schwedischer Binnengewässer. *Nova Acta R. Soc. Scient. Upsal. Ser. IV,* **16**:1–404.

21. Suzaki, T. & Williamson, R. E. 1986. Cell surface displacement during euglenoid movement and its computer simulation. *Cell Motil. Cytosk.,* **6**:89–95.

22. Triemer, R. E. & Farmer, M. A. 1991a. An ultrastructural comparison of the mitotic apparatus, feeding apparatus, flagellar apparatus and cytoskeleton in euglenoids and kinetoplastids. *Protoplasma,* **164**: 91–104.

23. Triemer, R. E. & Farmer, M. A. 1991b. The ultrastructural organization of the heterotrophic euglenids and its evolutionary implications. *In:* Patterson, D. J. & Larsen, J. (ed.), The Biology of Free-living Heterotrophic Flagellates. Clarendon Press, Oxford. Pp. 185–204.

24. Vickerman, K. 1989. Phylum Zoomastigina. Class Kinetoplastida. *In:* Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. J. (ed.), Handbook of Protoctista. Jones and Bartlett Publishers, Boston. Pp. 215–238.

25. Walne, P. N. & Kivic, P. A. 1989. Phylum Euglenida. *In:* Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. J. (ed.), Handbook of Protoctista. Jones and Bartlett Publishers, Boston. Pp. 270–287.

26. Willey, R. L. & Wibel, R. G. 1987. Flagellar roots and the reservoir cytoskeleton of *Colacium libellae* (Euglenophyceae). *J. Phycol.,* **23**:283–288.

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