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Received 9-1-94, 2-2-95; accepted 3-27-95.

J. Euk. Microbiol., 42(5), 1995, pp. 476–490

Morphology and Ecology of *Siroloxophyllum utriculariae* (Penard, 1922) N. G., N. Comb. (Ciliophora, Pleurostomatida) and an Improved Classification of Pleurostomatid Ciliates

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ABSTRACT. The morphology and infraciliature of *Siroloxophyllum utriculariae* (Penard, 1922) n. g., n. comb. were studied in live cells, with the scanning and transmission electron microscope, as well as in specimens impregnated with protargol and silver carbonate. The new genus, *Siroloxophyllum*, belongs to the Loxophyllidae and has a specific combination of characters, viz. an oral bulge surrounding almost the entire cell, three perioral kineties, a single brush kinety, and a single right dorsolateral kinety. The ecology and faunistics of *S. utriculariae* are reviewed. It is a rare and infrequent predator preferring clean freshwaters. The somatic monokinetid of *S. utriculariae* has typical haptorid ultrastructure, including two transverse microtubular ribbons. The oral bulge is patterned string-like with riffles containing the transverse microtubular ribbons originating from the oral kinetids. Perioral kineties 1 and 2 consist of dikinetids having one basal body each ciliated; the nonciliated basal body is associated with a nematodesmal and a transverse microtubular ribbon. Perioral kinety 3 consists of ciliated monokinetids having a fine structure similar to the somatic kinetids; they form triads with the dikinetids from perioral kinety 2. The classification of pleurostomatid ciliates is reviewed. Two suborders (Amphileptina, Litonotina) and three families (Amphileptidae, Litonotidae, Loxophyllidae n. fam.) are recognized and defined.

Supplementary key words. *Acineria*, Haptoria, infraciliature, *Litonotus*, *Loxophyllum*, *Opisthodon*, *Pseudoamphileptus*, taxonomy, ultrastructure.

THE pleurostomatid ciliates have attracted comparatively few ciliatologists, possibly because they are well circumscribed and their close relationship with haptorid ciliates has never been questioned. However, many new species have been described since the revision by Kahl [28], most by Vuxanovici [45,46] and Song & Wilbert [41]. Foissner's group studied the infraciliature of representatives of most known genera and provided improved diagnoses for *Amphileptus* [13], *Litonotus* [13], *Acineria* [1] and *Loxophyllum* [23]. Foissner [12] also estab-

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lished a new genus, *Pseudoamphileptus*, for *Amphileptus macrostoma* and rediscovered the long missed genus *Opisthodon* [13], already placed on the *nomina oblita* list by Corliss [5]. These data and some electron microscope studies [2, 37] provided a firm base for an improved classification of the group [21].

More recently, Lipscomb & Riordan [34] destroyed the homogeneity of the pleurostomatids by including typical haptorids like *Spathidium* and *Didinium*. This view is not supported by the present results which emphasize the structural and ontogenetic peculiarities of the pleurostomatids, setting them up clearly from the haptorids s. str.

MATERIALS AND METHODS

Organisms and preparations. The two populations of *S. utricullariae* studied were isolated from a slowly running stream in Berlin and from the sludge of a rapid gravity filter of the Bad Füssing waterworks near Munich [18]. Both populations could be cultured for some time on diluted lettuce medium enriched with dried yolk to provide bacterial food for their prey, mainly *Glaucoma scintillans* and *Cinetochilum margaritaceum*.

Cells were studied in vivo using a high-power oil immersion objective and differential interference contrast [14]. Protargol [16; protocol 1] and silver carbonate [15] were used to reveal the infraciliature. Preparations for scanning (SEM) and transmission (TEM) electron microscopy were performed as described previously [17, 32].

Counts and measurements on silvered specimens were performed at a magnification of ×1,000. In vivo measurements were conducted at a magnification of ×100–1,000. Although these provide only rough estimates it is worth giving such data as specimens usually shrink in preparations or contract during fixation. The standard deviation and coefficient of variation were calculated for morphometric data. Drawings of live specimens are based on free-hand sketches, those of impregnated cells were made with a camera lucida.

Terminology. Standard terminology as outlined in [5, 35] is applied for the light and electron microscope data. A few uncommon terms used in the light microscope description and the generic key are explained in the following paragraphs.

Dorsal brush. Row(s) of shortened, paired cilia near the anterior dorsal margin of the cell (Fig. 3, 7, 29). Usually, these rows are continuous with the anterior ends of one or several left lateral somatic kineties.

Dorsolateral kineties. Two kineties at the right (*Loxophyllum* [23]), or one kinety each at the right and left (*Siroloxophyllum*, Fig. 6, 19, 30) margin of the dorsal side; distinguished from regular somatic ciliary rows either by shortened cilia and/or in extending around the posterior end of the cell, forming a more or less distinct suture with the abutting posterior ends of the regular somatic kineties.

Oral bulge. A nonciliated eminence along the oral slit, often indistinct in pleurostomatids. Appears more or less distinctly string-like patterned in SEM-micrographs (Fig. 10, 29). The actual oral opening is defined as the bulge region which is accompanied by that portion of perioral kineties 1 and 2 which has paired basal bodies and nematodesmata.

Perioral kineties. Two or three kineties lining the oral bulge, usually continue posteriorly as somatic ciliary rows. Kineties 1 and 2 always composed of paired basal bodies, at least along oral opening (Fig. 7, 8, 11, 26). Perioral kinety 1 lines the left bulge wall, kineties 2 and 3 the right [13].

Spica. A suture formed by shortened ciliary rows in the midline of the anterior right body half; typically found in *Amphileptus* (Fig. 49).

RESULTS

Siroloxophyllum n. g.

Diagnosis. Loxophyllidae with oral bulge surrounding almost entire cell. Three perioral kineties extending from anterior end to mid-body. Single brush kinety near dorsal margin. Single right dorsolateral kinety.

Type species. *Amphileptus utricullariae* Penard, 1922.

Etymology. Composite from the Greek words *siro* (string), *loxos* (oblique) and *phyllum* (leaf). Neuter gender. Name refers to string-like appearance of oral bulge.

Type specimens. One holo (genus) type slide and one voucher slide of protargol impregnated *Siroloxophyllum*, Munich population, have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Accession numbers: 26, 27/1994. The slides also serve as neotypes for the species, *Amphileptus utricullariae* Penard, 1922 [36], because Song & Wilbert [41] made no mention of deposited neotype material.

Redescription of *Siroloxophyllum utricullariae* (Penard, 1922) n. comb.

Light and scanning electron microscopy. Morphometric data shown in Table 1 are repeated in this section only as needed for clarity. Many characters of *S. utricullariae* and of other pleurostomatids [13, 23] vary greatly, as indicated by the rather high coefficients of variation (most ≥ 15%).

Size highly variable within and between populations, in vivo 65–270 × 20–80 μm according to our observations and literature data [27, 28, 36, 41], usually about 140–200 × 30–60 μm; up to 30% contractile, fixed and stained specimens thus smaller due to contraction and shrinkage. Shape likewise highly variable, often, however, lanceolate with widest portion in or close underneath mid-body, anterior half usually more distinctly narrowed than posterior and slightly curved dorsally, but not snout-like as in *Loxophyllum*; anterior end narrowly rounded, posterior end broadly rounded to elongated. Field material flattened leaf-like (up to 4:1), with very flat and hyaline, about 7 μm wide fringe containing extrusomes. Right side flat to slightly concave, left more or less distinctly vaulted bearing 3–8 distinct crests in central third; crests 2–5 μm high and 2 μm wide, gradually flattened toward body ends, become inconspicuous and even disappear in well-fed specimens (Fig. 1, 4, 5, 14, 15, 21, 22).

Nuclear apparatus in or near centre of cell, stands out as bright blister against darker, granulated cytoplasm (Fig. 1). Usually two macronuclear nodules and one micronucleus; number constant in Berlin and Bonn population, highly variable in specimens from Munich with, however, a median of two as in the other populations (Table 1). Macronuclear nodules small as compared to size of cell, slightly ellipsoid, often close together (≤ 2 μm), micronucleus then not within but on cleft; nucleoli roundish, distributed throughout nodules (Fig. 7, 14, 15, 18). Micronucleus slightly ellipsoid, 2–3 × 2 μm, within or on cleft formed by macronuclear nodules (Fig. 1).

Two contractile vacuoles, each with numerous excretory pores on right surface (Fig. 24), in anterior and posterior third of cell, respectively; anterior vacuole near ventral side, posterior vacuole near dorsal side, thus forming highly characteristic diagonal pattern with nuclear apparatus in between (Fig. 1, 14, 21).

Extrusomes (toxicysts) 6–8 μm long, thin (diameter about 0.4 μm) and slightly curved, both ends evenly rounded (Fig. 2, 17, 18, 33); anchored in single line, and possibly in pairs (Fig. 10), to oral bulge, some scattered in cytoplasm, never aggregated to warts as in some *Loxophyllum* species; form conspicuous layer in marginal fringe of cell, lacking only in anterior dorsal area

Table 1. Morphometric data of *Siroloxophyllum utriculariae* populations.[a]

Character	Population[b]	x̄	M	SD	SD_x	CV	Min	Max	n
Body, length	Berlin	133.0	135.0	23.8	3.6	17.9	65	180	44
	Munich	146.2	140.0	20.8	3.7	14.2	100	194	29
	Bonn	199.6	?	28.9	8.3	14.5	165	264	12
Body, maximum width	Berlin	31.0	29.5	7.1	1.1	22.9	16	45	44
	Munich	54.6	55.0	10.8	2.0	19.8	30	75	29
	Bonn	53.8	?	5.4	1.7	10.1	44	62	12
Macronuclear nodule, length	Berlin	10.7	11.0	1.5	0.2	14.0	8	13	40
	Munich	13.9	14.0	1.5	0.3	10.9	11	18	31
	Bonn	14.8	?	2.5	0.7	16.9	13	17	14
Macronuclear nodule, width	Berlin	7.8	7.5	1.1	0.2	14.1	6	11	40
	Munich	10.8	10.5	2.2	0.4	20.0	7	16	31
Micronucleus, largest diameter	Berlin	2.4	2.2	0.5	0.1	19.4	1.5	4	32
	Munich	2.9	3.0	1.0	0.2	35.4	2	6	29
Right lateral somatic kineties, number (incl. right dorsolateral kinety)	Berlin	12.0	12.0	2.6	0.6	22.0	11	14	18
	Munich	16.3	16.0	1.9	0.4	11.9	12	20	30
	Bonn	16.2	?	?	?	?	15	19	11
Left lateral somatic kineties, number (incl. dorsal brush row and left dorsolateral kinety)	Berlin	6.7	7.0	0.7	0.1	10.6	5	8	36
	Munich	6.2	6.0	1.2	0.2	19.5	4	9	29
	Bonn	6.0	6.0	0.0	0.0	0.0	6	6	11
Macronuclear nodules, number	Berlin	2.0	2.0	0.0	0.0	0.0	2	2	41
	Munich	2.1	2.0	0.5	0.1	26.1	1	4	30
	Bonn	2.0	2.0	0.0	0.0	0.0	2	2	20
Micronuclei, number	Berlin	1.0	1.0	0.0	0.0	0.0	1	1	41
	Munich	1.0	1.0	—	—	—	1	2	30
	Bonn	1.05	?	—	—	—	1	2	20
Contractile vacuoles, number	Berlin	2.0	2.0	0.0	0.0	0.0	2	2	22
	Munich	2.0	2.0	0.0	0.0	0.0	2	2	20

[a] Data based on randomly selected, protargol-impregnated and mounted specimens from exponentially growing cultures (Berlin and Munich populations) and field material (Bonn population). Measurements in μm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of observations; SD, standard deviation; SD_x, standard deviation of the mean; x̄, arithmetic mean.

[b] Data of Bonn population from [41]; very likely incorrect, at least partially, because the length of the figured, protargol impregnated specimens is smaller (142 μm) than the minimum value (165 μm) provided! Similar discrepancies exist with the number of somatic kineties.

where oral bulge is absent (Fig. 1, 16, 18, 19); stain heavily with silver carbonate (Fig. 16), but not with protargol.

Cortex thin, highly flexible, without special granules, e.g. mucocysts. Cytoplasm colourless, in central region of cell more or less densely filled, depending on food supply, with brightly shining fat globules and food vacuoles; no special cytoplasmic crystals. Feeds on small and medium sized ciliates (*Glaucoma scintillans, Cinetochilum margaritaceum, Colpidium colpoda*) and probably also on bacteria and/or detritus. Moves slowly, glides with densely ciliated right side on flat substrates or crawls elegantly on and between detritus aggregations showing great flexibility and deformation of body.

General plan of somatic and oral infraciliature as in other members of order [13]. In an old culture, most specimens had greatly reduced numbers of kineties, viz. 4–7 on right and 3–4 on left side, while body size was not markedly reduced.

Right side densely ciliated, kineties with cilia about 7 μm long successively shortened along anterior half of perioral kinety 3 and in posterior region of cell, where some abut to right dorsolateral kinety and third perioral kinety. Right dorsolateral kinety very near to dorsal margin of cell, bears regular somatic cilia and fibrillar associates, commences at anterior end of cell and curves around its posterior end (Fig. 6, 11, 21). Left side more sparsely ciliated than right, its ciliature consists of somatic kineties, a brush kinety, and a dorsolateral kinety (Fig. 3, 7, 8, 12, 22, 28–30). Somatic kineties in central third of cell on top of cortical crests, distinctly shortened anteriorly and posteriorly, cilia reduced to 1–2 μm long stumps and about twice as widely spaced as on right side. Brush kinety in anterior third of body between leftmost somatic ciliary row and left dorsolateral kinety, about 4 μm apart from dorsal margin of cell, consists of 30–50 very closely spaced dikinetids having 1–3 μm long cilia in anterior third of cell and of closely spaced, nonciliated monokinetids in posterior portion ("tail"), which extends left of a flat cortical crest (cp. Fig. 7, 28); anterior portion of brush on bottom of depression formed by anterior end of oral bulge, often fragmented, right fragments sometimes connected with crest kineties; cilia of dikinetids cylindroid to slightly inflated distally, anterior cilium usually slightly longer than posterior, length of cilia decreases from anterior to posterior (Fig. 28, 29); dikinetidal axis usually parallel to main body axis, rarely oblique or almost transverse, especially if anterior portion is fragmented. Left dorsolateral kinety very near dorsal margin of cell, extends along its whole length and is thus continuous with perioral kinety 1 at both ends, bears about 2 μm long cilia and is thus easily distinguished from the almost adjacent right dorsolateral kinety which has regular (long) somatic cilia (Fig. 7, 13, 30).

Oral bulge surrounding almost entire cell, leaving blank only small area at anterior end of dorsal side (Fig. 1, 3, 12, 13, 20, 29), about 2 μm high and thus difficult to recognize in the light microscope (Fig. 15, 18, 20). Anterior end of bulge curved to left surface of cell, producing inconspicuous crest right of which brush kinety commences. Bulge surface patterned string-like, with small hemispherical structures between riffles, possibly tips of toxicysts (Fig. 10). Nematodesmata very fine, originate from barren basal bodies of perioral dikinetids (see TEM section), recognizable only up to mid-body, indicating that functional mouth is much shorter than oral bulge. Perioral kinety 1 at left margin of oral bulge, merges into left dorsolateral kinety anteriorly and posteriorly, anterior half composed of regularly spaced

dikinetids, posterior portion made of equidistantly spaced monokinetids; anterior basal body of dikinetids lighter stained than posterior one, bears about 2 μm long, cylindroid ciliary stump (Fig. 3, 8, 12, 13, 27, 29, 30); dikinetids orientated obliquely to kinety axis, i.e. parallel to main body axis, without kinetodesmal fibres in silver carbonate impregnated specimens (Fig. 26). Perioral kinety 2 at right margin of oral bulge, composed of tightly spaced dikinetids, at least in anterior half, as indicated by distribution of nematodesmata; anterior or posterior basal body of dikinetids with regular, about 7 μm long cilium; dikinetids orientated obliquely or almost transversely to main body axis, without kinetodesmal fibres in silver carbonate stains (Fig. 6, 11, 17, 19, 26). Perioral kinety 3 right of and very close to kinety 2, ends indistinctly separate from right dorsolateral kinety, composed of monokinetids throughout; kinetids with normal long cilia and conspicuous kinetodesmal fibres orientated more laterally than those of somatic kinetids, at least in anterior half (Fig. 6, 11, 17, 19, 24).

Transmission electron microscopy. The fine structural investigations are not very detailed because they were not the main objective of the study. Thus, the description will be brief, emphasizing some new findings.

The somatic kinetids of *S. utriculariae* have typical haptorid pattern, including two transverse microtubular ribbons (Fig. 31). The first transverse ribbon extends obliquely anteriad and is longer than the second ribbon, which extends transversely (radially). Both ribbons originate near triplets 3–5. The postciliary microtubules are very long and form distinct stripes recognizable in protargol stains (Fig. 9, 31).

The string-like pattern of the oral bulge is conspicuous also in ultrathin sections (Fig. 32). The riffles contain the transverse microtubular ribbons originating from the nonciliated basal bodies of the perioral dikinetids (Fig. 32–35). There is no permanent oral opening.

Perioral kinety 1 is composed of oblique dikinetids. The anterior basal body bears a short cilium and inconspicuous postciliary and transverse microtubular ribbons; the posterior basal body is not ciliated and associated with a conspicuous nematodesma and a transverse microtubule lamella extending into the oral bulge (Fig. 7, 33–35). Perioral kinety 2 consists of dikinetids as kinety 1. Its structure could not be unequivocally clarified. One basal body of the dikinetids, possibly the anterior, is nonciliated and associated with a nematodesma and a long transverse microtubule ribbon extending into the oral bulge; the other basal body bears a normal long cilium (Fig. 25) and is possibly associated with a postciliary and/or transverse microtubule ribbon (Fig. 32–35). The kinetids of perioral kinety 3 are ciliated and look like somatic kinetids, except of the kinetodesmal fibres which extend more obliquely (Fig. 6, 17, 24, 25, 32). They form typical triads with the dikinetids of perioral kinety 2 (Fig. 32), as described by Bohatier & Njine [2] in *Litonotus*.

Divisional morphogenesis. Stomatogenesis and cell division of *S. utriculariae* proceed as described by Fryd-Versavel et al. [24] in *Amphileptus pleurosigma*. We thus provide only a summary of our observations. Proliferation of basal bodies occurs intrakinetally in all kineties, migrating kinetofragments do not occur, and the parental infraciliature is apparently retained unchanged. The most conspicuous event is the appearance of paired brush cilia close underneath the prospective division furrow (Fig. 23). These cilia very likely grow out from the nonciliated monokinetids found in the rear ("tail") of the parental brush kinety (Fig. 7). The new tails produced in the proter and opisthe are also barren. Thus, the brush kinety shows a nonciliated preequatorial and posterior portion throughout the entire division process (Fig. 23). How the dikinetids for the opisthe are produced, i.e. by rearrangement of tail monokinetids or by addition of new basal bodies to existing monokinetids, could not be ascertained and needs TEM investigation of dividing specimens.

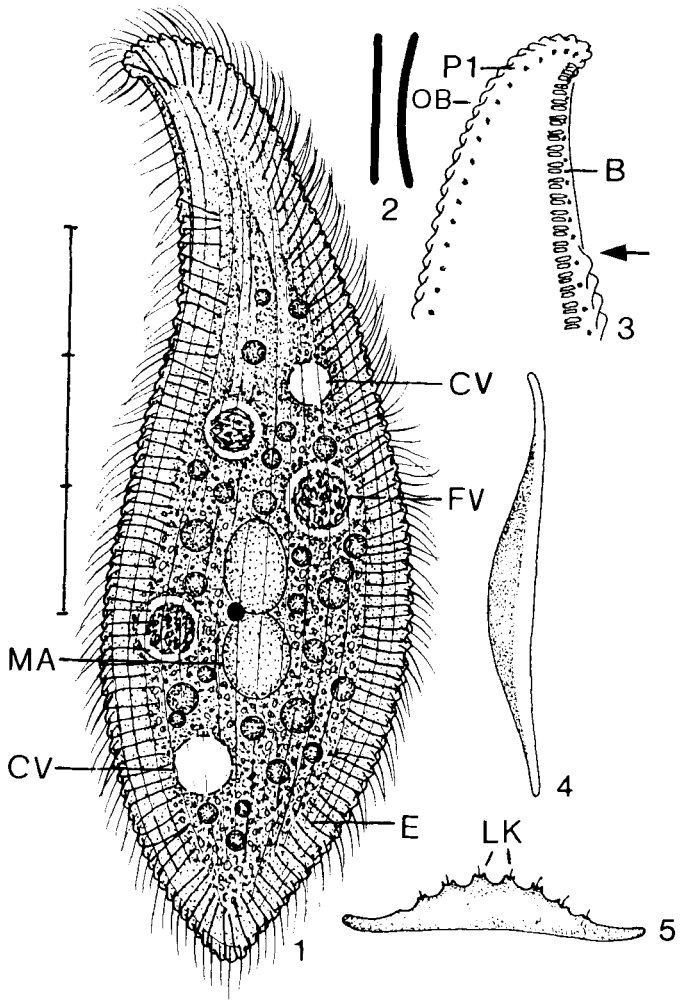

Fig. 1–5. *Siroloxophyllum utriculariae* from life (Munich population). 1. Right lateral view of typical specimen. Scale bar division = 20 μm. 2. Extrusomes are 6–8 μm long and curved. 3. Anterior end of left side. The oral bulge (arrow) ends close to the top of the cell, leaving blank only a small area at the anterior dorsal margin. 4, 5. Dorsal and transverse view showing flattening of cell. The left surface is distinctly furrowed and bears shortened cilia. B, dorsal brush; CV, contractile vacuoles; E, extrusomes; FV, food vacuole; LK, left lateral somatic kineties; MA, macronuclear nodule; OB, oral bulge; P1, perioral kinety 1.

The macronuclear nodules fuse and the micronucleus divides during the early stages of stomatogenesis, i.e. before the division furrow is recognizable. After the division furrow has appeared, the roundish macronuclear mass divides into two nodules which migrate into the proter and opisthe, respectively, where they divide again to produce the interphase pattern.

Ecology. This section is a compilation of the faunistic and ecological literature available on *S. utriculariae*. Few records are known, most are from running and stagnant freshwaters; those from mosses and soils in Germany [48] and New Zealand [43, 44] are very likely misidentifications, because the species died in our cultures without forming permanent (resting) cysts, indicating that it cannot live in soil. Furthermore, we have never found it in the more than 1,000 soil and moss samples inves-

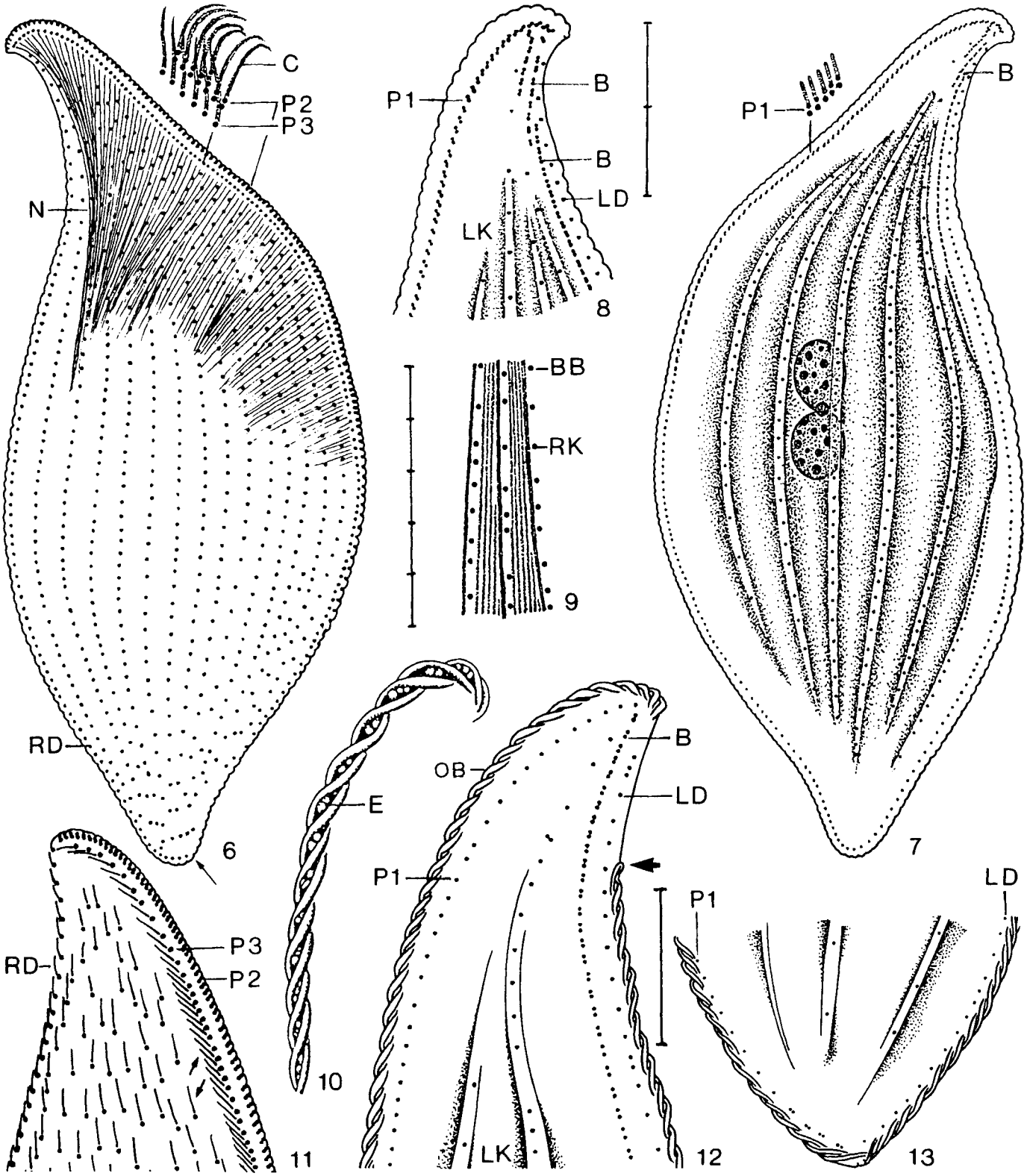

Fig. **6–13**. *Siroloxophyllum utricullariae*. 6–9. protargol impregnation. 10, 12, 13. drawn from scanning electron micrographs. 11. silver carbonate impregnation. (6–10, 12, 13 from Munich population; 11 from Berlin population). **6–8**. Infraciliature of right and left side of specimen with fragmented dorsal brush (enlarged detail, **8**). Arrow marks right dorsolateral kinety curving around posterior end. Perioral kinety 1 has ciliary stumps originating from the anterior basal body of the oral dikinetids, perioral kineties 2 and 3 have normal cilia. **9**. Long ribbons of postciliary microtubules extend between somatic ciliary rows. **10**. Surface view of anterior end of oral bulge. **11**. Infraciliature in anterior region of right side. Note different orientation of kinetodesmal fibres (arrows) in perioral kinety 3 and somatic kinetids; perioral kinety 2 lacks kinetodesmal fibres. **12**, **13**. Ciliary pattern in left anterior and posterior region. Perioral kinety 1 and left dorsolateral ciliary row are continuous at posterior end. The oral bulge surrounds almost the entire cell, leaving blank only a small area at the anterior dorsal margin (arrow). B, dorsal brush; BB, basal body; C, cilium; E, extrusomes; LD, left dorsolateral kineties; LK, left lateral somatic kinety; N, nematodesmata; OB, oral bulge; P1, 2, 3, perioral kineties; RD, right dorsolateral kinety; RK, right lateral somatic kineties. Bar division = 10 μm.

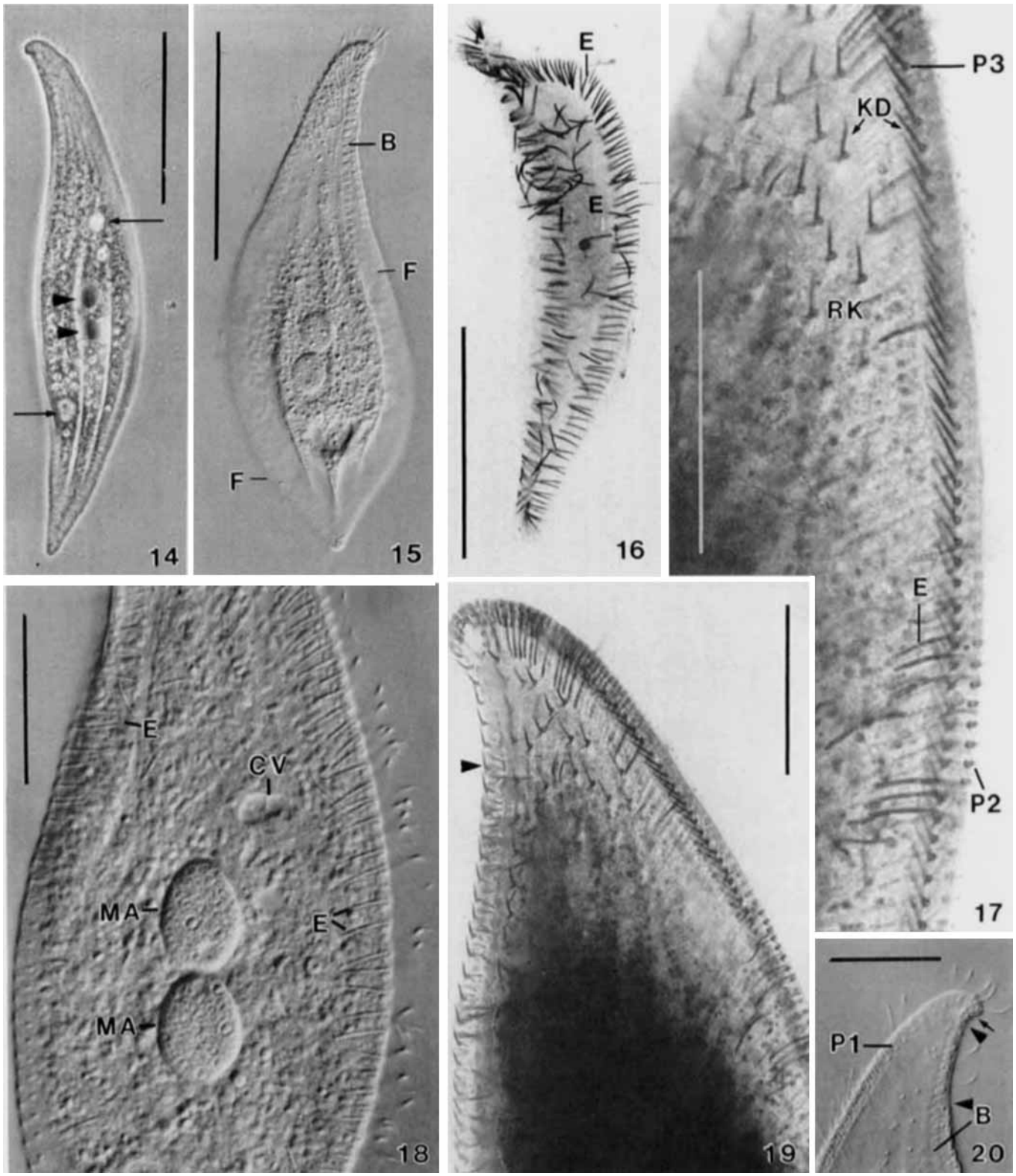

Fig. **14–20**. *Siroloxophyllum utricullariae*. **14**. from life. 15, 18, 20. fixed as for scanning electron microscopy. 16, 17, 19. silver carbonate impregnation (14, 16, 17, 19 from Berlin population; 15, 18, 20 from Munich population). **14**, **15**, **16**, **18**. Right and left lateral views. Extrusomes are concentrated in hyaline fringe surrounding cell. Arrows mark contractile vacuoles, arrowheads indicate macronuclear nodules. **17**, **19**. Oral and somatic infraciliature. Kinetodesmal fibres of kinetids of perioral kinety 3 are more laterally directed than those of somatic kinetids. Arrowhead marks right dorsolateral kinety. **20**. The anterior end of the oral bulge (arrow) is curved to the right at the left side of the cell. Arrowheads mark small region between ends of oral bulge (cp. Fig. 29). B, dorsal brush; CV, contractile vacuole; E, extrusomes; F, fringe; KD, kinetodesmal fibres; MA, macronuclear nodules; P1, 2, 3, perioral kineties; RK, right lateral somatic kineties. Bars in 14–16 = 60 μm. Bars in 17–20 = 20 μm.

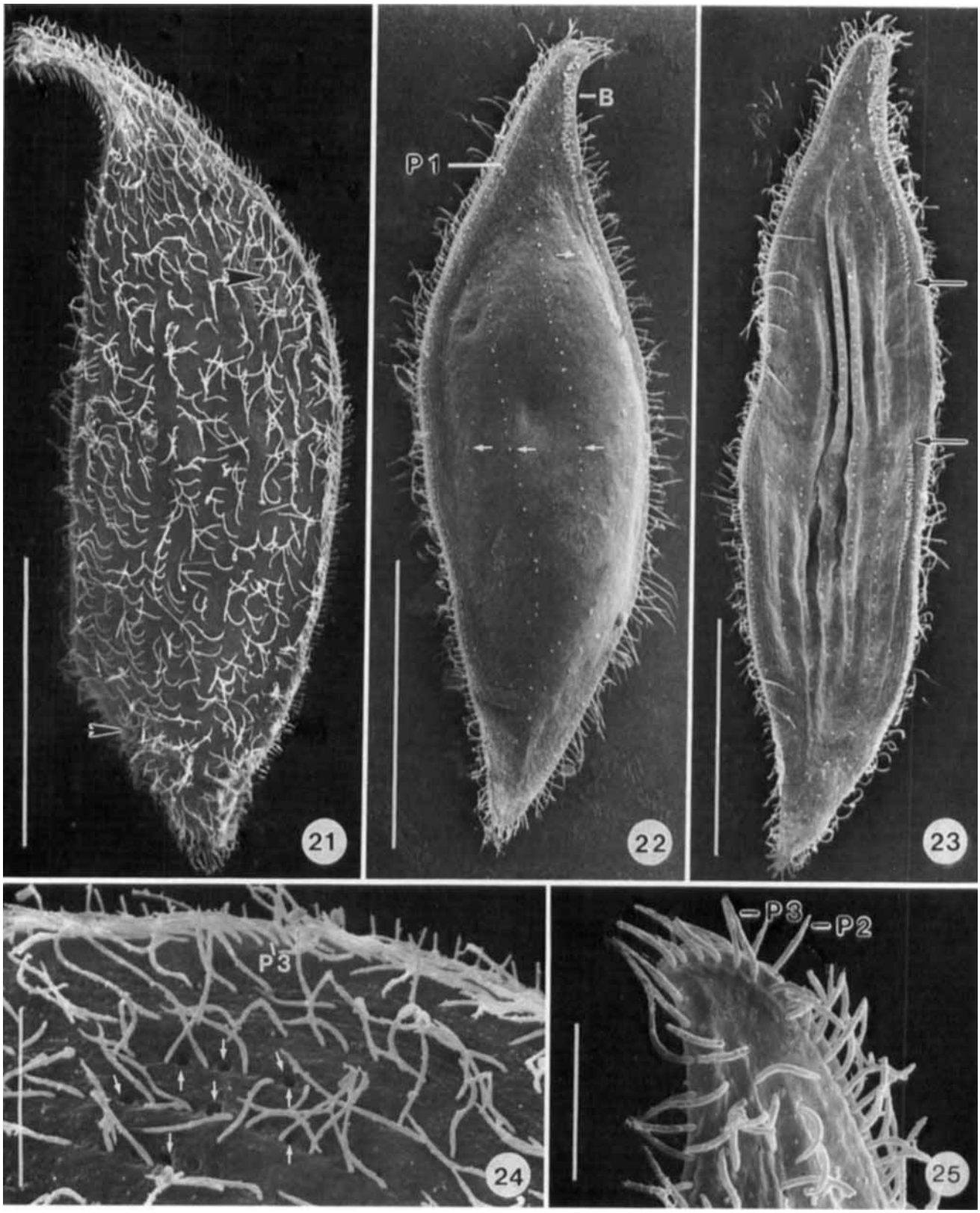

Fig. **21–25**. *Siroloxophyllum utriculariae*, SEM micrographs (21–24, Berlin population; 25, Munich population). **21**, **22**. Right and left lateral view of morphostatic cells. 22 shows a well-fed specimen lacking furrows on left side (cp. 23, 28). Arrowheads mark excretory pores of contractile vacuoles (cp. 24); arrows indicate left lateral somatic kineties having very short cilia. **23**. Middle divider. Arrows mark posterior (proter) and anterior (opisthe) end of dorsal brush, respectively. **24**. Each contractile vacuole has many excretory pores (arrows). **25**. Right anterior end showing that perioral kineties 2 and 3 are ciliated. B, dorsal brush; P1, 2, 3, perioral kineties. Bars in 21–23 = 50 μm. Bars in 24, 25 = 10 μm.

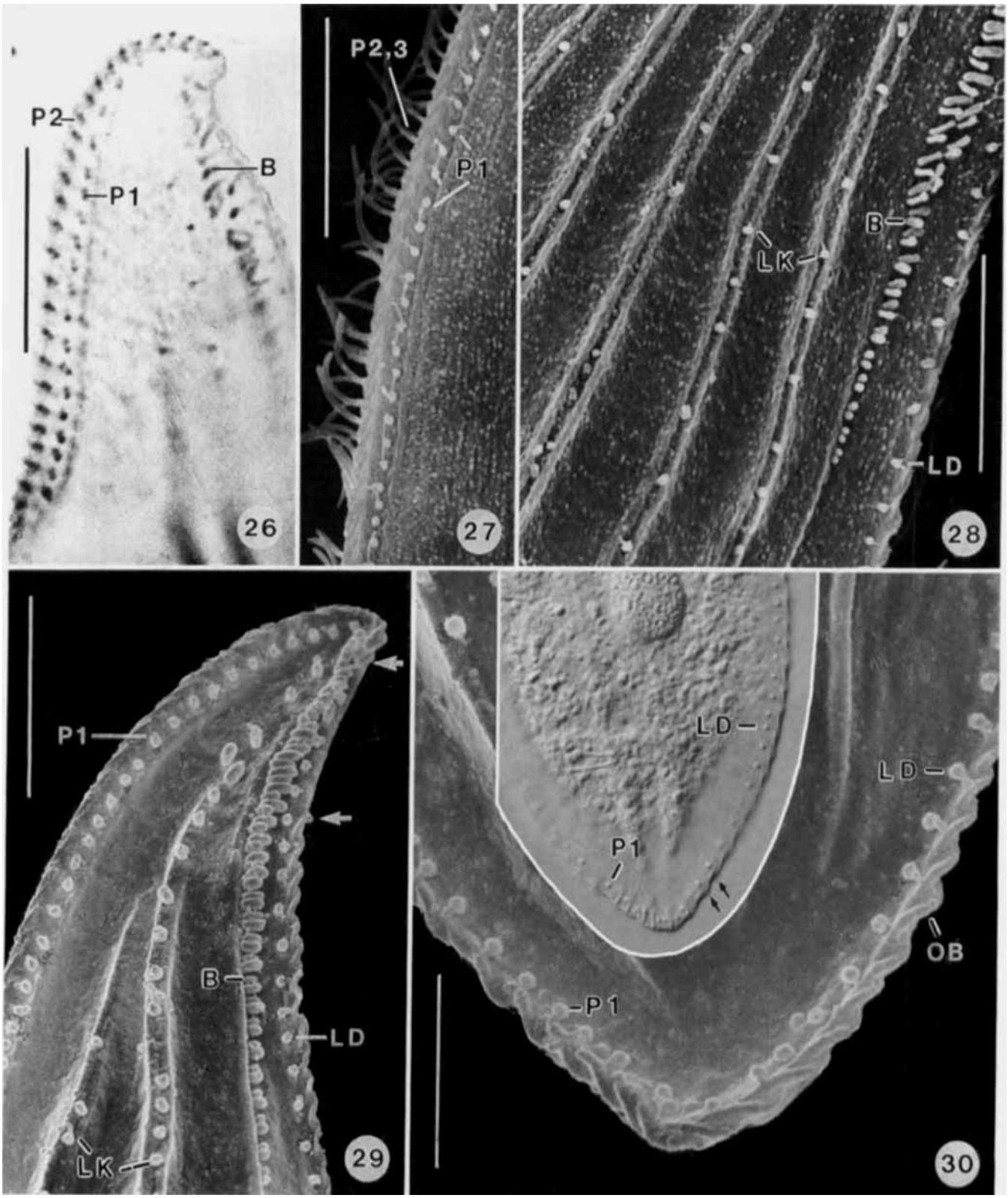

Fig. **26–30**. *Siroloxophyllum utricullariae*. 26. silver carbonate impregnation. 27–30. SEM micrographs. Inset in 30 is light micrograph of fixed specimen. (26–28 from Berlin population; 29–30 from Munich population). **26**, **27**. Oral infraciliature. Perioral kineties 1 and 2 consist of dikinetids having only anterior or posterior basal bodies ciliated (cp. 6, 7). **28**, **29**. Left anterior ends of strongly furrowed specimens. The length of the dorsal brush cilia decreases gradually from anterior to posterior and no ciliary stumps are recognizable in the furrow extending posteriorly of the brush kinety although basal bodies are present (cp. Fig. 7). The oral bulge surrounds almost the entire cell, leaving only a small area at the anterior dorsal side blank (arrows). **30**. Left posterior end in the light (inset) and scanning electron microscope. Perioral kinety 1 and left dorsolateral kinety are continuous and the oral bulge has a distinct string-like pattern recognizable even in the light microscope (arrows). B, dorsal brush; LD, left dorsolateral kinety; LK, left lateral somatic kineties; OB, oral bulge; P1, 2, 3, perioral kineties. Bars in 26–29 = 10 μm. Bar in 30 = 5 μm.

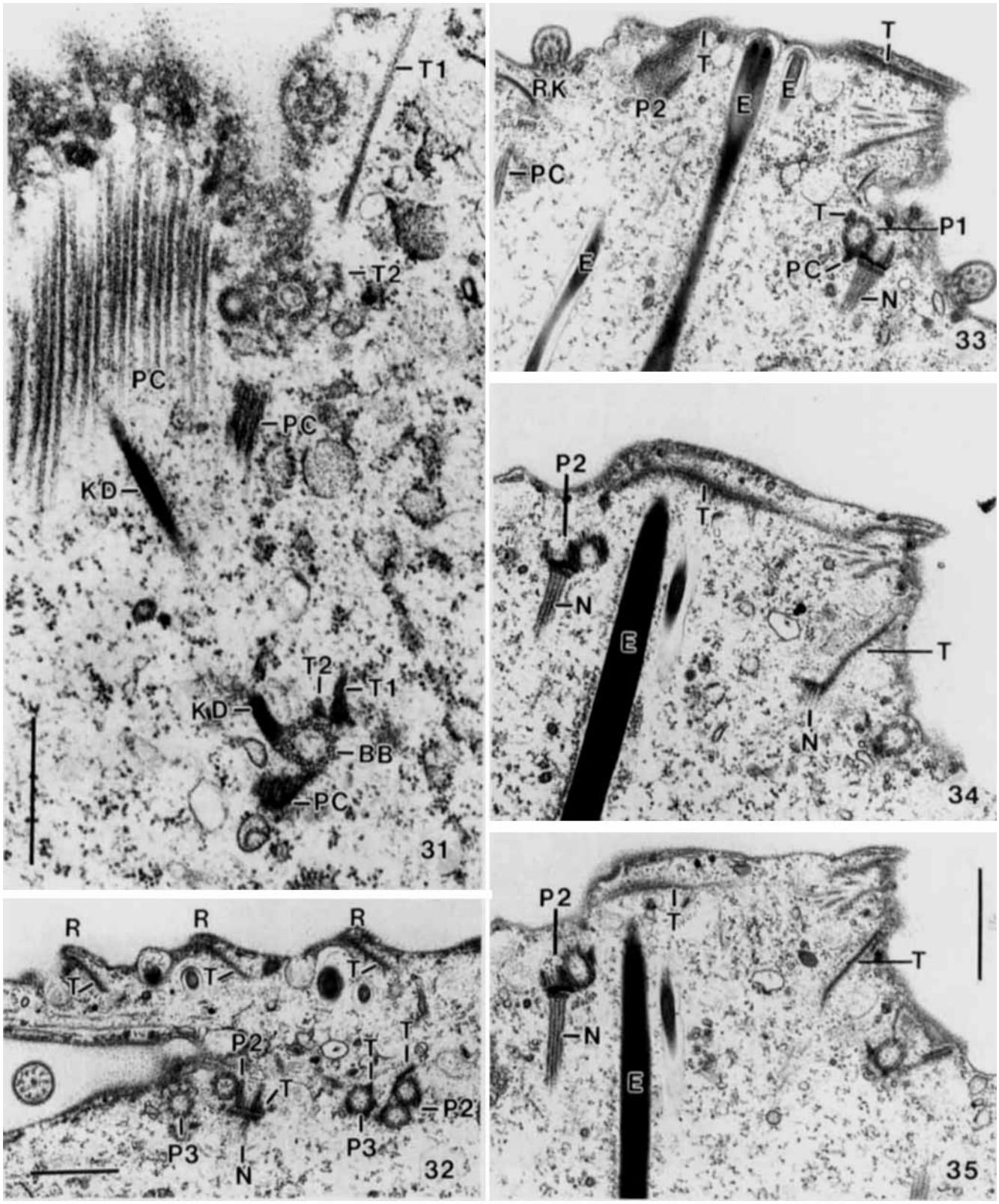

Fig. 31–35. *Siroloxophyllum utriculariae*, TEM micrographs from Berlin population. **31**. Tangential section of right side showing ultrastructure of somatic cortex and monokinetids. **32**. Oblique longitudinal section showing riffles of oral bulge and triads formed by kinetids of perioral kineties 2 and 3. **33–35**. Oblique serial section of oral area showing details of kinetids from perioral kineties 1 and 2. BB, basal body; E, extrusome (toxicyst); KD, kinetodesmal fibre; N, nematodesma; PC, postciliary microtubular ribbon; P1, 2, 3, perioral kineties; R, riffles of oral bulge; RK, right lateral somatic kinety; T, transverse microtubular ribbons of oral kineties; T1, T2, transverse microtubule ribbons of somatic monokinetids. Bars = 1 μm.

tigated during the last decade. Thus, all reliable records are from freshwaters of central and eastern Europe and Mexico [40]. It seems that *S. utriculariae* is a rare species, usually occurring with low abundance.

Penard [36] and Kahl [27, 28] found *S. utriculariae* between *Utricularia* weed in Geneva (Switzerland) and Hamburg (Germany), respectively. Several records [7, 11] are available from the Danube river, where *S. utriculariae* lives in the periphyton of stones, and from oligosaprobic and mesosaprobic rivers, brooks and ponds in Germany [3, 22], Bulgaria [6, 7] and Mexico [40]. Detcheva [6] provides the following abiotic parameters from a single record in a beta-mesosaprobic river in Bulgaria: pH 7.7, 8 mg/L O_2 (94% saturation), 3.6 mg/L biological oxygen demand (5 days), 12.6 mg/L chemical oxygen demand, 118 mg/L Ca^{2+}, 24 mg/L Mg^{2+}, 0.25 mg/L NH_4^+-N, 1.9 mg/L NO_3^--N, 0.06 mg/L NO_2^--N, 0.2 mg/L Fe^{2+}, 0.2 mg/L Mn^{2+}, 0.06 mg/L phenols. We found *S. utriculariae* infrequently and with low individual numbers in beta-mesosaprobic to alpha-mesosaprobic rivers near Munich, Germany ([22] and Fig. 36). It occurred more regularly and abundantly in the sludge of rapid gravity filters of some waterworks in this region; the abundance variations observed could be not correlated with specific biotic and process parameters [18].

Siroloxophyllum utriculariae glides slowly and elegantly in the periphyton of natural and artificial substrates. Like other members of the group it is a predator. However, detailed observations from natural populations are not available. In cultures it feeds on small to medium-sized ciliates, like *Glaucoma scintillans* and *Colpidium colpoda*, which are apparently quickly digested because the cells are usually rather hyaline and rarely contain identifiable prey residues; bacteria and/or organic detritus are probably also ingested. Biomass of 10^6 medium-sized (150 × 50 × 20 μm) cells about 90 mg [23]. Sládeček et al. [39] and Wegl [47] consider *S. utriculariae* as an excellent indicator of beta-mesosaprobic conditions and provide the following valency spectrum: beta-mesosaprobic; oligosaprobity (o) = 1, betamesosaprobity (b) = 8, alpha-mesosaprobity (a) = 1, indication weight (I) = 4, saprobity index (SI) = 2.0. However, the data available indicate that the oligosaprobic proportion should be increased in the valency; but this needs further investigations [23].

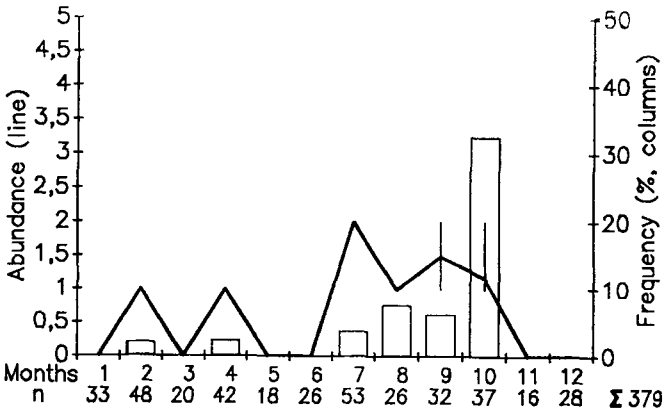

Fig. **36**. Frequency and rated abundance [semi-logarithmic scale: 1 (rare), 2, 3, 5, 7, 9 (very numerous)] of *S. utriculariae* in 379 samples collected during 1987–1991 in beta- to alphamesosaprobic Bavarian streams.

DISCUSSION

***Siroloxophyllum* as a new genus.** Kahl [28] transferred *Amphileptus utriculariae* [36] to *Loxophyllum*. This was accepted by Song & Wilbert [41], who reinvestigated the species using protargol impregnation (Fig. 43–48). Our investigations show that *A. utriculariae* belongs neither to *Amphileptus* nor *Litonotus* (because it lacks a median suture and has a right dorsolateral kinety) nor to *Loxophyllum*, whose left anterior end is occupied by a conspicuous field of paired brush cilia [23] which was overlooked by Song & Wilbert [41].

The most conspicuous character of *Siroloxophyllum* is the string-like patterned oral bulge surrounding almost the entire cell, leaving blank only a small area at the anterior dorsal end (Fig. 3, 12, 29). This feature is not easily recognized in living and protargol impregnated cells. However, if one is aware of its existence, it can be seen well under interference contrast (Fig. 20). Recent SEM observations showed that the oral bulge of very likely all pleurostomatid ciliates is patterned string-like [23]. The distinctiveness of the pattern varies; usually it is most conspicuous in suboptimally prepared specimens. Thus, the patterned oral bulge of *Siroloxophyllum* is not unique, but it is exceptional in surrounding almost the entire cell. It is not known whether *S. utriculariae* can open the whole bulge during feeding or—like other members of the family [23]—only that portion which is accompanied by the paired basal bodies of perioral kineties 1 and 2. Likewise, the mechanism which unlocks the bulge between perioral kineties 1 and 2 is obscure. Possibly, the transverse microtubular ribbons of the oral kinetids are involved.

An even more difficult character is the dorsolateral kineties. A left dorsolateral kinety is very likely present in all pleurostomatids (although often not designated or recognized as such), possibly with the exception of *Loxophyllum meleagris*, and located between the dorsal brush kinety and the rightmost somatic ciliary row or the right dorsolateral kinety [19, 23]. The left dorsolateral kinety, which was considered as regular left lateral somatic ciliary row by most previous authors, differs clearly from the left lateral kineties by being continuous with the monokinetidal tail of perioral kinety 1 (Fig. 7, 30); from the rightmost somatic ciliary rows of the right side and from the right dorsolateral kinety it differs by the short, stump-like cilia (Fig. 28, 30). Right dorsolateral kineties are present only in *Loxophyllum*, which has two [23], and in *Siroloxophyllum*, which possesses only one (Fig. 6, 19). The right dorsolateral kinety(ies) differs from the right lateral ciliary rows by surrounding the posterior end of the cell, forming a more or less distinct suture with the abutting ends of the regular somatic kineties.

The structure and/or location of the dorsal brush of *Siroloxophyllum* differ distinctly from *Loxophyllum*, *Pseudoamphileptus* and *Opisthodon*, but are similar to *Litonotus*, *Acineria* and *Amphileptus* (Fig. 49).

Thus, none of the four characters given in the genus diagnosis is unique to *Siroloxophyllum*, i.e. it is only the specific combination of the characters which separates the new genus from its relatives.

Species assignable to *Siroloxophyllum*. A reinvestigation of the protargol impregnated type slides of *Loxophyllum australe* [19] showed that it has the main characteristics of *S. utriculariae*. Thus, it has to be transferred to this genus: *Siroloxophyllum australe* (Foissner & O'Donoghue, 1990) nov. comb. The two species differ mainly in the number of macronuclear nodules, usually two in *S. utriculariae* and four in *S. australe*. The number of right end left lateral somatic kineties is slightly higher in *S. utriculariae* than in *S. australe*. Very likely, other species will be added, e.g. *Loxophyllum carinatum* Vuxanovici and *L. semilunare* Vuxanovici (both redescribed in [41], but seemingly without dorsolateral kineties and thus not definitely assignable).

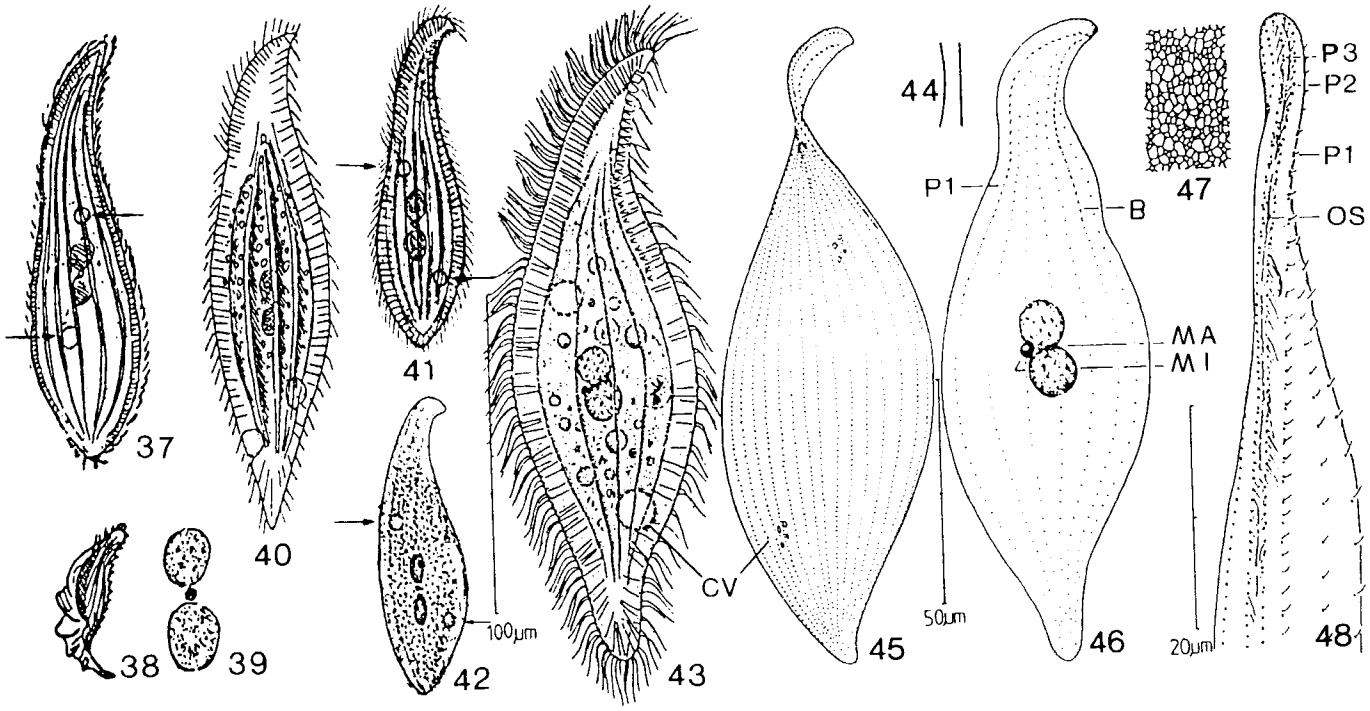

Fig. 37–48. Published drawings of *S. uricularíae*. **37–39**. Left lateral and dorsal view and nuclear apparatus from life, length 65–120 µm. Arrows mark contractile vacuoles. From [36]. **40**. Left lateral view from life, length 100 µm. From [27]. **41**. Left lateral view from life, length 150 µm. Arrows mark contractile vacuoles. From [28]. **42**. Left lateral view from life, size not indicated. Arrows mark contractile vacuoles. From [40]. **43–48**. Left (43, 46), right (45) and ventral (48) views from life (43) and after protargol impregnation (45, 46, 48); extrusomes (44) from life, silverline system (47) after dry silver nitrate impregnation. From [41]. B, dorsal brush; CV, contractile vacuoles; MA, macronucleus; MI, micronucleus; OS, oral slit (mouth entrance); P1, 2, 3, perioral kineties.

Comparison of descriptions of *S. uricularíae*. Our observations basically agree with those mentioned in the original description [36] and the two redescriptions [28, 41]. Thus, we do not doubt the identification and conspecifity of all populations. However, some differences should be noted. Penard [36] drew the anterior contractile vacuole near the dorsal margin and the posterior vacuole near the ventral side (Fig. 1, 21), whereas Kahl [28] and Song & Wilbert [41] definitely stated an opposite location (Fig. 41, 43, 45), which agrees with our observations (Fig. 1, 14, 18, 21). Thus, it may be assumed that Penard's indication is a simple mistake, all the more so as he did not definitely describe the location of the vacuoles. Another difference concerns the extrusomes which, according to Penard [36], are elongated in the strongly flattened and slightly protruding oral area (Fig. 37), whereas Kahl [28] and Song & Wilbert [41] found them to be of the same length over the whole perimeter of the cell (Fig. 41, 43), which matches our observations (Fig. 1, 16). Although the shape and size of the extrusomes are important species characteristics in gymnostomatid ciliates [13, 23], this difference cannot be weightened heavily because Penard [36] never used oil immersion objectives and thus very likely could not ascertain the real length of the extrusomes in the thicker, opaque parts of the cell.

Song & Wilbert [41] redescribed *S. uricularíae* very briefly, but provided some elegant drawings (Fig. 43–48) which, however, do not give any indication of dorsolateral kineties. We suppose that Song & Wilbert overlooked them because their description contains also other unfortunate mistakes. They figure all oral kineties as being composed of dikinetids and the oral slit between perioral kineties 2 and 3 (Fig. 48). Both observations are clearly disproved by our data (Fig. 8, 11, 32–35) and literature evidence [2, 13, 23].

Ultrastructure. The fine structure of the somatic kinetids of *S. uricularíae* is very similar, if not identical, to that of haptorids like *Spathidium* [49] and *Enchelydium* [20]. The second transverse microtubular ribbon was apparently overlooked in previous descriptions of pleurostomatids, but can be recognized in published micrographs of *Loxophyllum meleagris* (Fig. 16, 17 in [37]). *Siroloxophyllum uricularíae* is thus a ditransversal ciliate in the sense of Leipe & Hausmann [32].

The interpretation of the oral structures is more difficult. As concerns perioral kinety 1, our results agree with previous descriptions [2, 37], while the structure of perioral kineties 2 and 3 appears different in several respects. Whether these differences are genus specific or caused by interpretation problems needs further investigations. At least some data in Bohatier & Njine's [2] paper appear doubtful, for instance that perioral kineties 2 and 3 lack cilia. In *S. uricularíae* they are ciliated (Fig. 25) and form the typical mane recognized earlier by Kahl [28] in many pleurostomatids. A second problem is posed by the kinetodesmal fibre, which is, according to Bohatier & Njine [2], associated with the posterior basal body of the dikinetids of perioral kinety 2. Our data show that it originates from the monokinetids of perioral kinety 3 (Fig. 17, 19), which is more likely since the haptorid oral dikinetids generally lack a kinetodesma [34]. Perioral kinety 3 is very likely a specialized somatic kinety, analogous (because it apparently lacks nematodesmata) to the oralized somatic kinetids found in several haptorids [21]. A third problem concerns the species investigated by Bohatier & Njine [2]. Their figures doubtlessly show a *Litonotus* species, as in-

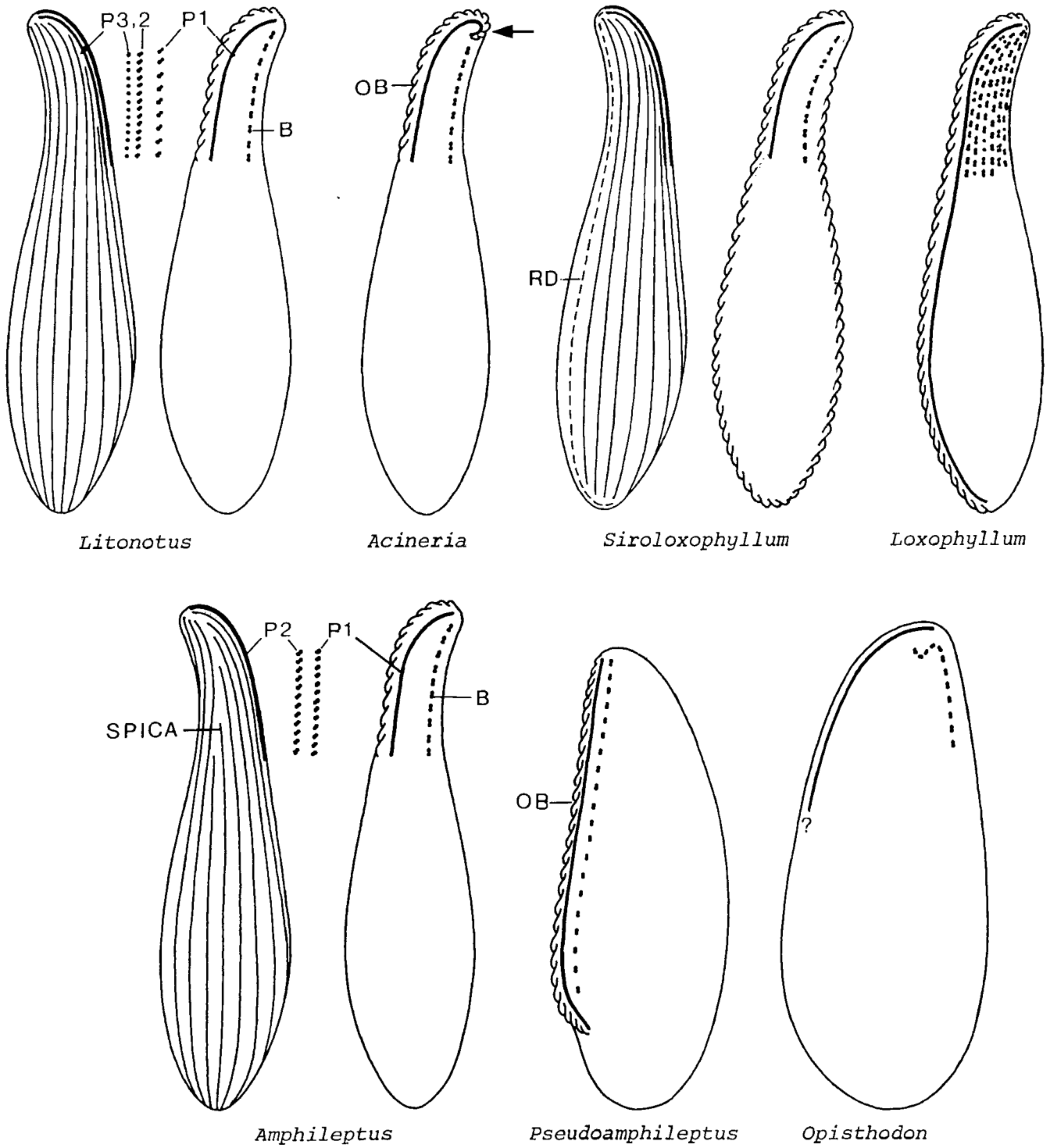

Fig. **49**. Genus distinction in the order Amphileptida by the arrangement of the right lateral ciliary rows (with spica in *Amphileptus, Pseudoamphileptus, Opisthodon*), the number of perioral kineties (three in *Litonotus, Acineria, Siroloxophyllum, Loxophyllum*; two in others), the presence (*Siroloxophyllum, Loxophyllum*)/absence of right dorsolateral kineties, the shape of the anterior body end (curved in *Acineria*, arrow), the dorsal brush (large field in *Loxophyllum*, pocketed in *Opisthodon*, very near oral bulge in *Pseudoamphileptus*, single row in others) and the length of the oral bulge (extending to posterior end in *Loxophyllum* and *Pseudoamphileptus*, surrounding cell in *Siroloxophyllum*, extending to mid-body in others). Note that *Pseudoamphileptus* and *Opisthodon* are still insufficiently defined, i.e. need redescription based on better impregnated specimens. *Heminotus* is excluded because its infraciliature is not known. B, dorsal brush; OB, oral bulge; P1, 2, 3, perioral kineties; RD, right dorsolateral kinety.

dicated by the triads formed by the kinetids of perioral kineties 2 and 3, but very likely not *L. quadrinucleatus* which lacks, according to Dragesco & Njine [8], perioral kinety 3 but has a conspicuous spica, indicating that it belongs to the genus *Amphileptus* [13]. Unfortunately, Bohatier & Njine [2] did not mention the source of their material.

Systematic relationships of *Siroloxophyllum* and classification of pleurostomatid ciliates. Traditionally, all pleurostomatid genera are lumped in a single family, Amphileptidae Bütschli [4, 5, 28]. However, Foissner & Foissner [21] split the pleurostomes into two suborders, viz. Amphileptina and Litonotina and recognized two families, viz. Amphileptidae and Litonotidae. More recently, Lipscomb & Riordan [34] suggested a very different classification based on cladistic methods, using, however, many unproven character states. They assigned to the pleurostomes not only lacrymariids and didiniids but also classical haptorids like *Spathidium, Bryophyllum* and *Homalozoon*. We believe that this was an unsuccessful upset, simply because the distinct asymmetry of the pleurostomatid oral and somatic ciliature is hardly found in any classical haptorid, with the notable exception of *Homalozoon,* a highly thigmotactic and specialized predator. Furthermore, Lipscomb & Riordan [34] did not take into account the different types of stomatogenesis occurring in pleurostomes s. str. (monotelokinetal) and haptorids s. str. (holotelokinetal; see [33] for definition of terms and literature). Obviously, their classification neglects two main features and is thus very likely artificial.

The classification suggested here thus follows Foissner & Foissner [21] and includes the data mentioned in their publication and in the present study.

Order Pleurostomatida Schewiakoff, 1896

Oral area flattened along ventral margin of laterally compressed body, surrounded by toxicysts; rhabdos made of three microtubular components: transverse ribbons originating from the oral dikinetids and in suborder Litonotina also from somatic monokinetids, nematodesmal bundles originating exclusively from oral dikinetids, and bulge microtubules; somatic ciliature with distinct left-right differentiation, including dorsal brush and, in some genera, one or two dorsolateral kineties; free-living and parasitic on other ciliates (mainly peritrichs), often large, lengthy voracious carnivores; widely distributed in freshwater, marine, and interstitial habitats. Type: Amphileptina Jankowski, 1967 [26].

Suborder Amphileptina Jankowski, 1967

Cytostome surrounded by a right and a left perioral kinety composed of dikinetids; right somatic ciliature with spica. Type: Amphileptidae Bütschli, 1889 [4].

Remarks: This suborder is monotypic, i.e. includes only the family Amphileptidae Bütschli with the characteristics given for the suborder. The genera *Amphileptus* [10, 13; type by virtual tautonymy], *Opisthodon* [13, 42] and *Pseudoamphileptus* [12] belong to this family. *Hemiophrys* [50] is a junior synonym of *Amphileptus* [13]. *Amphileptus carchesii* Stein very likely needs a separate genus, because it has a heavily ciliated groove which secretes a loop-like structure anchoring the ciliate to the prey [23]. However, the separation should await a detailed study of its infraciliature.

Suborder Litonotina Foissner & Foissner, 1988

Cytostome surrounded by a right and left perioral kinety composed of dikinetids, right kinety accompanied by (oralized ?) somatic monokinetids forming a distinct 3rd perioral kinety whose transverse microtubular ribbons contribute to the rhabdos; right lateral ciliature with or without dorsolateral kineties, ciliary rows successively shortened along perioral and dorsolateral kineties. Type: Litonotidae Kent, 1882 [31].

Remarks: This suborder includes the families Litonotidae Kent and Loxophyllidae n. fam., differing mainly by the absence/presence of right lateral dorsolateral kineties.

Family Litonotidae Kent, 1882 [31]: Litonotina without right dorsolateral kineties. Type: *Litonotus* Wrześniowski, 1870 [50].

Remarks: This family includes the genera *Litonotus* [13, 50], *Acineria* [1, 9] and, possibly, *Heminotus* [29] whose infraciliature has been not yet described.

Family Loxophyllidae n. fam.: Litonotina with dorsolateral kineties. Type: *Loxophyllum* Dujardin, 1841 [9].

Remarks: Jankowski also mentioned a new family "Loxophyllidae" without, however, providing any characterization or type genus (Jankowski, A. W. 1975. A conspectus of the new system of subphylum Ciliophora Doflein, 1901. Abstract. *In*: Balashov, U. S. (ed.), Account of Scientific Sessions on Results of Scientific Work, Year 1974: Abstracts of Reports. Akad. Nauk SSSR, Zool. Inst. Leningrad. Pp. 26–27 [in Russian]). Thus, the name is illegitimate, i.e. not in accordance with the rules of nomenclature. According to Grain [25], Dujardin [9] also founded a family Loxophyllidae. However, this is not confirmed by an inspection of the original literature. Dujardin [9] included *Loxophyllum* and other pleurostomes in his new family Paramecidae.

The family includes two genera, viz. *Loxophyllum* [9, 23] and *Siroloxophyllum* n. g. *Loxophyllum* was formerly [21] assigned to the Amphileptina because the data available suggested that it lacks perioral kinety 3. This was disproved by a reinvestigation [23]. Very likely, some of the many marine and interstitial *Loxophyllum* species are not congeneric. Unfortunately, their infraciliature is not known and any separation would be premature.

Key to pleurostomatid genera. The following key uses data from the present study and the literature [1, 12, 13, 19, 23, 28]. No reliable information is known from *Heminotus,* which is thus excluded. For definition of specific morphological terms see Foissner [13], material and method section and Fig. 49. Differences between some genera are clearly recognizable only after protargol impregnation. Likewise, the proper generic classification of most species requires protargol impregnation or at least careful examination of living specimens with interference contrast.

1. Two perioral kineties. Right side somatic kineties shortened in midline of cell, forming more or less distinct suture (spica) in anterior half of ciliate. Right dorsolateral kineties absent ... 2
   Three perioral kineties. Right side somatic kineties shortened anteriorly and abutting to perioral kinety 3. With or without right dorsolateral kineties ... 4
2. Left anterior end smooth ... 3
   Left anterior end with small cavity containing anterior end of dorsal brush kinety ... *Opisthodon*
3. Oral bulge indistinct. Dikinetidal portion of perioral kineties extends to mid-body ... *Amphileptus*
   Oral bulge distinct. Dikinetidal portion of perioral kineties extends near posterior end of cell ... *Pseudoamphileptus*
4. With right dorsolateral kineties. Single brush kinety or dense field of short, paired cilia in anterior region of cell ... 5
   Without right dorsolateral kineties. Single brush kinety ... 6
5. Oral bulge extends along ventral side. Two right dorsolateral kineties. Many brush kineties continuous with anterior end of left lateral kineties ... *Loxophyllum*
   Oral bulge surrounds almost entire cell. Single dorsolateral kinety. Single brush kinety at dorsolateral margin of cell ... *Siroloxophyllum*

6. Oral bulge and perioral kineties terminate at anterior end of cell ..... *Litonotus*
Oral bulge and perioral kineties distinctly curved to left anterior surface of cell ..... *Acineria*

ACKNOWLEDGMENTS

Supported by the Bayrisches LAWW and the Austrian FWF (Project PO 8924-BIO). The technical assistance of Dr. Andreas Unterweger, Mag. Margit Palzenberger, Andreas Zankl and Mag. Eric Strobl is greatly acknowledged.

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Received 11-23-94; accepted 3-29-95

J. Euk. Microbiol., 42(5), 1995, pp. 490–505

Ultrastructure of the Parabasalid Protist *Holomastigotoides*

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ABSTRACT. The ultrastructure of two species of *Holomastigotoides* is presented. The basic unit of organization of these large cells is the flagellar band. Each flagellar band consists of a row of flagellar basal bodies linked by three fiber systems. The number of flagellar bands is species dependent. The flagellar bands originate at the cell apex and are arranged in parallel spirals of increasing gyre, thus defining the conical shape of the cell. In the cell apex a striated root called a parabasal fiber is juxtaposed with the basal bodies of each flagellar band. Linear extensions of two parabasal fibers function as the spindle poles for the persistent extra-nuclear spindle. The nucleus is in close contact with the spindle poles and spindle microtubules. Parallel sheets of microtubules which constitute axostyles are nucleated along the underside of the parabasal fibers. The axostyles extend away from the cell apex, with many reaching the basal region of the cell. Some of the axostyles follow the spiral pattern of the flagellar bands. Numerous Golgi bodies are spaced regularly along the flagellar bands. Together the parabasal fiber, axostyles and Golgi bodies associated with a flagellar band are termed a parabasal complex.

Supplementary key words. Basal bodies, cytoskeleton, fibers, mitotic spindle, parabasal complex, parabasalid, spindle poles, TEM.

HOLOMASTIGOTOIDES spp. are parabasalid protista found in the hindgut of four genera of primitive termites, *Prorhinotermes, Coptotermes, Heterotermes,* and *Psammotermes,* where they aid in digestion of wood [2, 3, 14, 18, 23]. Like all parabasalids, they have parabasal complexes, each of which is comprised of a Golgi complex and a striated root called a parabasal fiber, associated with flagellar basal bodies. The parabasal complex is the primary distinguishing characteristic of members of the Class (or Phylum, depending on classification scheme) Parabasalia of zooflagellate protists [1, 9, 10, 16]. Regardless of the hierarchical level of the taxon, the Parabasalia are considered a monophyletic group based on the conserved presence of parabasal complex, hydrogenosomes (organelles in which anaerobic metabolism occurs [19]), and axostyles, and the absence of mitochondria. There are two parabasalid orders: the Trichomonadida and Hypermastigida. Hypermastigotes tend to be larger and have more extensive flagellated regions than trichomonads. The flagellated regions consist of rows of flagella often arranged along bands that cover most of the cell surface or in large apical clusters [1, 9].

In the hypermastigote *H. tusitala* the extended flagellated regions consist of five bands of flagella which originate at the anterior apex of the cell and spiral posteriorly through approximately 5.5 gyres of increasing diameter, thus defining the conical shape of each cell [4, 5]. The smaller *H. diversa,* also found in *P. simplex,* has 8 flagellar bands, but fewer gyres. Previous light microscopy descriptions of *H. tusitala* and *H. diversa,* the focus of our work presented here, demonstrated the presence of two to four large chromosomes which are condensed during most of the cell cycle, an extra-nuclear, persistent mitotic spindle, linear spindle poles attached to two flagellar bands, and kinetochores at the nuclear envelope [4–8]. Limited transmission electron microscopic observations have been made on several other species of *Holomastigotoides* [11, 15, 22]. Gibbons and Grimstone [11] described and compared the ultrastructure of flagellar basal bodies of *Holomastigotoides* sp. with those of two other hypermastigote genera, *Pseudotrichonympha* and *Trichonympha.* The two latter genera have longitudinal rows of flagella (12,000–14,000 flagella in *T. campanula*) rather than the spiral arrangement of flagellar rows of *H.* spp. The hypermastigotes, including *H. hemigymnum,* were the subject of a comparative structural analysis of the mitotic apparatus and cell division [15]. Our aim in the present work is to provide a comprehensive ultrastructural description of two species of *Holomastigotoides* found in the termite *P. simplex,* with special regard to cell polarity and transitions in the cytoskeleton and parabasal complex.

MATERIALS AND METHODS

The hindgut was removed from *P. simplex* workers and the contents were placed immediately in fixative consisting of 5% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) in 0.025 M HEPES (Sigma Chemical Co., St. Louis, MO), pH 7.0 for 2 h on ice. After washing three times for 5 min, the fixed cell suspension was pelleted and embedded in a drop of 4% agarose (Sigma). The hardened agarose pellet was diced into small pieces prior to further handling. The cells were post-fixed for 1 h on ice in 1% osmium tetroxide (Electron Microscopy Sciences, Fort Washington, PA), 0.8% potassium ferrous cyanide (Fisher Scientific Co., Fair Lawn, NJ) in 0.2 M potassium phosphate buffer, pH 7.0. After washing with deionized water for 5 min, the cells were treated with 0.15% tannic acid (Fisher Scientific Co.) in potassium phosphate buffer for 5 min at room temperature. The cells were washed three times with H_2O for 5 min and then en bloc stained with 2% uranyl acetate (Fisher Scientific Co.) in 25% ethanol for 1 h. Following dehydration through an ethanol series, the cells were infiltrated gradually with Quetol 651 embedding resin (Ted Pella Inc., Redding, CA) and embedded in a thin layer of resin in LUX petri dishes or in BEEM capsules (Electron Microscopy Sciences). After polymerization of the resin, selected cells were

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