

Two new soil ciliates (Protozoa, Ciliophora) from Namibia: *Diplites telmatobius* nov. gen., nov. spec. and *Apobryophyllum etoschense* nov. spec.

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Summary

Two new soil ciliates from terrestrial habitats of Namibia (Southwest Africa) are described using live observation, silver impregnation, and morphometry. *Diplites telmatobius* nov. gen., nov. spec., which was discovered in the mud of rock-pools, belongs to the Acropisthiidae and has two dorsal brush rows and two kinds of extrusomes: oral extrusomes clavate, body extrusomes in rows and rod-shaped. *Apobryophyllum etoschense* nov. spec., which was found in soil from the margin of the Etosha Pan, has the following characteristics: Size about 130 x 30 µm; spatulous. Macronucleus reniform. Two types of extrusomes in oral bulge and somatic cortex, where they form minute bundles arranged to about 10 distinct, longitudinal rows. 16 somatic ciliary rows on average, those of left side anteriorly differentiated to complex brush.

Introduction

AFRICAN SOILS contain a very rich ciliate community. 507 species, of which 240 were undescribed, were found in 92 samples (Foissner, 1997a). The present paper reports on two species from Namibia, which belong to the haptorid gymnostomes. In this group, which contains only rapacious species, the extrusomes, which are used to capture the prey, are important generic and species characteristics (Foissner, 1984; Foissner & Foissner, 1988; Kahl, 1927). Their size, shape, and location must be studied in live specimens because they usually become distorted in silver preparations or do not stain at all.

Material and Methods

Samples were collected in February and March 1994 in Namibia (Southwest Africa) and air-dried for at least four weeks. The ciliates were reactivated from the resting cysts by the non-flooded Petri dish method, as described in Foissner (1987). Briefly, this simple method involves placing 10 - 50 g terrestrial material in a Petri dish (10 - 15 cm in diameter) and saturating but not flooding it with distilled water. Such cultures were analyzed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, and 28. The descriptions of the new species are based on material obtained from such cultures, that is, no clone cultures were set up.

Methods follow those used in my previous papers (e.g., Foissner, 1991, 1996) and thus need not to be

detailed here. Briefly, live specimens were studied with bright field and interference contrast, and preparations were made with protargol (protocol A in Foissner 1991) and silver carbonate, as described in Foissner (1991).

Diplites nov. gen.

Diagnosis: Acropisthiidae Foissner & Foissner, 1988 with two dorsal brush rows and two kinds of extrusomes: oral extrusomes clavate, body extrusomes rod-shaped and in rows.

Type species: *Diplites telmatobius* nov. spec.

Etymology: Composite of the Greek words "di" (two) and "hoplites" (soldier), meaning "a ciliate with two kinds of extrusomes". Masculine.

Comparison with related genera: *Diplites* has an apical cytostome, two brush rows, extrusomes within the oral bulge, and nematodesmal bundles originating from the oral dikinetids and from oralized somatic monokinetids (Fig. 2). Accordingly, it belongs to the family Acropisthiidae, as defined by Foissner & Foissner (1988) and Foissner (1996).

The family Acropisthiidae contains three genera, which are distinguished mainly by the shape of the extrusomes (Foissner & Foissner, 1988): nail-like in *Fuscheria*, graver-like in *Actinorhabdos*, rod-like in *Acropisthium*. *Diplites telmatobius*, in contrast, has clavate oral extrusomes and rows of rod-shaped body extrusomes, an extraordinary feature, absent in the other genera.

Diplites telmatobius resembles some species of the genus *Lagynophrya*, suggesting that it might be classified within this genus. However, *Lagynophrya* has fine, rod-shaped extrusomes (Grain, 1970; Kahl, 1927, Wilbert, 1986), three brosse rows (Dingfelder, 1962; Grain, 1970; Wilbert, 1986), and lacks oralized somatic kinetids (Grain, 1970). Thus, it belongs to a different order, the Spathidiida Foissner & Foissner, 1988. Unfortunately, the type of *Lagynophrya*, *L. mutans* Kahl, 1927, has not yet been reinvestigated with modern techniques. Accordingly, synonymy of *Diplites* with *Lagynophrya* cannot be excluded, although this is unlikely considering the literature mentioned above.

***Diplites telmatobius* nov. spec.** (Figs. 1 - 14, Table 1)

Diagnosis: Size in vivo about $55 \times 20 \mu\text{m}$; elongate-bursiform. Macronucleus usually ellipsoidal (3:1). Oral extrusomes about $2 \times 0.8 \mu\text{m}$, body extrusomes about $3 \times 0.2 \mu\text{m}$. On average 16 somatic kineties, 7 dikinetids in brush kinety 1, and 3 dikinetids in brush kinety 2.

Type location: Rock-pools from the river in the Aushchlucht near Bullsport, Namibia (about $16^{\circ}20'\text{E}$, 24°S).

Type slides: Two slides (1 holotype and 1 paratype) with protargol-impregnated specimens have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. The slides contain several specimens, with relevant cells marked by a black ink circle on the cover glass.

Etymology: Composite of the Greek words "telma, telmatos" (puddle) and "bios" (live), referring to the occurrence in small, astatic habitats.

Description: Size in vivo $45 - 65 \times 15 - 25 \mu\text{m}$, usually about $55 \times 20 \mu\text{m}$, as calculated from measurements of live specimens and values shown in Table 1, assuming a shrinkage of 10 - 15% due to the preparation procedures. Shape fairly constant, elongate-bursiform, anterior end conical due to projecting oral bulge, posterior more or less distinctly broadened and evenly rounded, slightly asymmetrical, that is, dorsally more convex than ventrally; slightly flattened or unflattened laterally. Macronucleus usually in posterior body half, contains numerous small nucleoli, shape highly variable, in 60% (out of 25 specimens investigated) rod-like, ellipsoidal or dumb-bell shaped (Figs. 3, 11, 14), in 20% reniform (Fig. 1), in the others, although having a normal infraciliature, fragmented to 3 - 4 globules, possibly due to postconjugational reorganization processes. Usually 1, occasionally 2, micronucleus attached to macronucleus. Contractile vacuole in posterior body end, with several excretory pores in centre of posterior pole. Somatic extrusomes in rows as long as body, rod-shaped, about $3 \times 0.2 \mu\text{m}$, that is, very fine and thus easily overlooked in live cells (Figs. 1, 5), do not stain with protargol; thus their exact location (within or between ciliary rows) could not be determined. Cortex flexible, distinctly furrowed along ciliary rows in vivo and in protargol impregnated specimens, contains some loosely arranged, bright granules (mucocysts?) $0.2 - 0.5 \mu\text{m}$ across (Fig. 7). Cytoplasm colourless, contains some $1 - 2 \mu\text{m}$ sized fat globules and food vacuoles up to $15 \mu\text{m}$ across

filled with indeterminable residues and almost intact specimens of *Drepanomonas revoluta*. Movement without peculiarities.

Cilia about $8 \mu\text{m}$ long, rather widely spaced, arranged in longitudinal, equidistant rows commencing close underneath circumoral kinety. Postciliary microtubule ribbons 4 - 5 kinetids long, form conspicuous fibre right of ciliary row (Fig. 14). Closely spaced basal bodies (dikinetids?) occur at irregular intervals in all somatic kineties, possibly produced by just divided basal bodies, as indicated by the lack of cilia in the anterior kinetosomes. Dorsal brush in shallow furrow recognizable in vivo and appropriately oriented impregnated cells (Figs. 1, 5), composed of densely spaced dikinetids having about $2 \mu\text{m}$ long cilia (Figs. 1, 3, 5, 6, 13).

Oral bulge in centre of anterior pole, broadly conical (50% of specimens) or cylindrical with a conical cape, in vivo about $5 \times 2 - 3 \mu\text{m}$, forms minute but rather distinct dome due to the compact extrusomes contained. Extrusomes clavate, slightly curved, about $2 \times 0.8 \mu\text{m}$, attached to bulge cortex with thinner anterior portion, occasionally slightly impregnated with protargol (Fig. 5). Circumoral kinety at base of oral bulge, composed by rather widely spaced dikinetids having only the left (posterior) basal body ciliated. Oral basket very inconspicuous in live cells, composed of fine nematodesmata originating from unciliated basal bodies of circumoral dikinetids and 3 - 4 basal bodies at anterior end of all somatic kineties (oralized somatic monokinetids, see Foissner & Foissner, 1988); nematodesmata extend to midline of cell, forming small bundles rather distinctly impregnated with protargol (Figs. 2, 4, 8, 9, 10, 12).

Occurrence and ecology: As yet found only at type location, that is, in dry mud from rock-pools in the centre and at the bank of a temporary mountain river. Thus, it is impossible to decide whether *D. telmatobius* prefers limnetic or terrestrial habitats.

Comparison with related species: *Diplites* is monotypic. However, the type species highly resembles some *Lagenophrya* species, especially *L. armata*, discovered by Kahl (1935) in mosses from Wisconsin, USA. This species is similar in size (length $60 - 80 \mu\text{m}$) and has, like *D. telmatobius*, an ellipsoidal macronucleus and minute ($2 - 3 \mu\text{m}$), sturdy oral extrusomes. However, Kahl (1935) definitely states: "*L. armata* has 1 row of long and 2 rows of short dorsal bristles". Thus, it cannot be identical with *D. telmatobius*, which has only two minute brush rows. Furthermore, Kahl (1935) does not mention body extrusomes, although he studied

L. armata with oil immersion, as evident from his detailed observations on the brush; therefore, body extrusomes of the kind found in *D. telmatobius* are very likely lacking in *L. armata*.

The general body plan and the infraciliature of *D. telmatobius* are virtually identical with those of *Fuscheria* spp. and *Actinorhabdos trichocystiferus* Foissner, 1984. Thus, these species are easily confused both in live and silver slides. In fact, they can be reliably distinguished only by the extrusomes, a rather sophisticated character, which must be determined from live specimens because the extrusomes usually do not stain with protargol and/or change their shape due to the preparation procedures.

***Apobryophyllum etoschense* nov. spec.** (Figs. 15 - 38, Table 2)

Diagnosis: Size in vivo about 130 x 30 µm; spatulous. Macronucleus reniform. Two types of extrusomes (type 1 fusiform and 5 µm long, type 2 rod-shaped and 3 µm long) in oral bulge and somatic cortex, where they form minute bundles arranged to about 10 distinct, longitudinal rows. 16 somatic kineties on average, those of left side anteriorly differentiated to complex brush.

Type location: Soil from margin (*Sporobolus* grass girdle) of Etosha Pan, Namibia (about 16°E, 19°S).

Type slides: Two slides (1 holotype and 1 paratype) with protargol-impregnated specimens have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. The slides contain several specimens with relevant cells marked by a black ink circle on the cover glass.

Etymology: Named after the site found.

Description: Size in vivo 100 - 160 x 25 - 45 µm, usually about 130 x 30 µm, as calculated from measurements of live specimens and values shown in Table 2, assuming a shrinkage of 10 - 15% due to the preparation procedures; length:width ratio 3:1 - 6:1, on average 4.3:1 (Table 2). Spatulous to broadly knife-shaped, that is, anterior ventral half distinctly curved and gradually narrowing to form bluntly pointed, slightly projecting dorsal anterior end; blade flattened leaf-like and thus hyaline, slightly broadened; handle evenly rounded posteriorly and unflattened in mid-portion, cells thus fusiform in ventral and dorsal view (Figs. 15, 18, 19, 22, 28, 31). Macronucleus subequatorial in posterior body half, reniform in 75%, dumb-bell shaped in 10%, spirialized in 10%, and irregular in 5% of specimens (n=20). Several micronuclei attached to macronucleus, in vivo about 3 µm

across, difficult to separate from globular fat inclusions in protargol preparations. Contractile vacuole in posterior end, about 4 excretory pores in midline of right posterior surface (Figs. 15, 19). Two types of extrusomes (type 1 slightly fusiform and about 5 µm long, type 2 rod-shaped and about 3 µm long; both impregnate with silver carbonate and protargol, but shape and size differences become indistinct) in oral bulge and somatic cortex, where they form about 10 distinct, longitudinal rows each composed of small extrusome bundles contained in minute warts (Figs. 15, 16, 23, 24, 32, 33, 37, 38); extruded extrusomes about 20 µm long and of typical toxicyst structure (Fig. 17). Cortex flexible, contains about six rows of colourless granules (about 1 x 0.3 µm) each between two ciliary rows (Figs. 29, 30). Cytoplasm usually packed with colourless globular and irregular fat inclusions 1 - 7 µm across, which often impregnate with protargol making cells very opaque. Feeds on ciliates, e.g., *Colpoda inflata*. Glides slowly on slide surface and soil particles.

Cilia about 10 µm long, loosely spaced, especially in anterior third, arranged in equidistant meridional rows distinctly separate from circumoral kinety (Figs. 15, 19, 20). Dorsal brush at anterior end of left lateral kineties, about as long as blade of knife (1/3 body length, Table 2), complex, that is, composed of cilia of different length (anterior cilia of dikinetids 1 - 2 µm, posterior 3 - 4 µm long) and shape (clavate or rod-shaped), as shown in Figures 25 - 28, 34 - 36: leftmost two rows at dorsal margin of cell, of "usual" haptorid structure, last row extends with single, rod-shaped bristles to almost posterior body end; in rows 1 - 5, dikinetidal bristles irregularly alternate with normal somatic cilia; anterior basal body of dikinetids smaller than posterior (Fig. 26).

Oral bulge 2 - 3 µm thick throughout and thus inconspicuous in live specimens (Fig. 22), hardly distinct from body proper in posterior half, extends from anterior dorsal to posterior dorsal end, where it forms a small but distinct notch (Figs. 15, 19, 20, 31, 32), filled with extrusomes (Figs. 15, 16, 33, 38). Circumoral kinety at base of oral bulge, composed of dikinetids having only one (anterior?) basal body ciliated; dikinetids associated with fine nematodesmata and more narrowly spaced in anterior than posterior half of kinety (Figs. 19, 20, 21, 25).

Occurrence and ecology: As yet found only at type location, that is, a slightly saline, reddish to greyish soil in the *Sporobolus* - *Suaeda* girdle surrounding the Pan.

Generic classification and comparison with related species: *A. etoschense* matches the genus diagnosis rather well: "Spathidiidae with oral bulge extending to and around posterior end of organism. Dorsal brush on anterior left side of cell, left brush kinetics regular and dikinetidal, right brush kinetics fragmented and very likely monokinetidal (Foissner, 1998)". However, the structure of the dorsal brush is obviously slightly different. Whether this is of generic or subgeneric significance must await the discovery of further, related species.

No species has been found in the literature which might be identical with *A. etoschense*. It is easily distinguished from the sole congener, *A. terricola* Foissner, 1998, by the macronucleus (reniform vs. filiform) and, in silver slides, by the structure of the dorsal brush. The particular arrangement of the extrusomes and the brush structure are highly reminiscent of *Prorodon armatides*, a freshwater species, which has a small, anteriorly located oral opening (Foissner, 1997). Live specimens of *A. etoschense* are easily confused with medium-sized and large *Arcuospathidium* species, for instance, *A. lionotiforme*, which have a similar shape and nuclear apparatus. The best in vivo character for separating these species is the arrangement of the extrusomes, which are restricted to the oral bulge in *Arcuospathidium* and distributed in a peripheral girdle in *Apobryophyllum*.

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TABLE 1. Morphometric data from *Diplites telmatobius*.

Character ^a	$\bar{x}$	M	SD	CV	Min	Max	n
Body, length	51.2	53	5.5	10.6	41	59	19
Body, width	21.3	20	3.5	16.3	16	28	19
Oral bulge, width	4.8	5	-	-	4	5	19
Oral bulge, height	2.3	3	-	-	2	3	19
Anterior somatic end to macronucleus, distance	22.4	23	4.7	21.0	15	35	19
Macronucleus, length	19.3	19	3.2	16.7	13	26	19
Macronucleus, width	7.3	7	1.0	13.7	6	10	19
Micronucleus, length	2.8	3	-	-	2	4	19
Micronucleus, width	2.3	2	-	-	2	4	19
Brush row 1, length ^b	6.3	6	1.0	15.8	5	9	19
Brush row 2, length ^b	3.4	3	0.6	17.7	2	4	19
Somatic kineties, number	15.9	16	0.5	3.3	15	17	19
Ciliated basal bodies in a ventral somatic kinety, number	19.2	18	4.2	21.6	12	26	19
Macronuclei, number	1.0	1	0.0	0.0	1	1	19
Micronuclei, number	1.2	1	-	-	1	2	19
Brush kineties, number	2.0	2	0.0	0.0	2	2	19
Dikinetids in brush row 1, number	6.7	7	1.0	15.5	5	9	19
Dikinetids in brush row 2, number	3.3	3	-	-	2	4	19

^a Data based on protargol-impregnated and mounted specimens from field. Measurements in μm .

CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, $\bar{x}$ arithmetic mean.

^b Distance from circumoral kinety to proximal end of row.

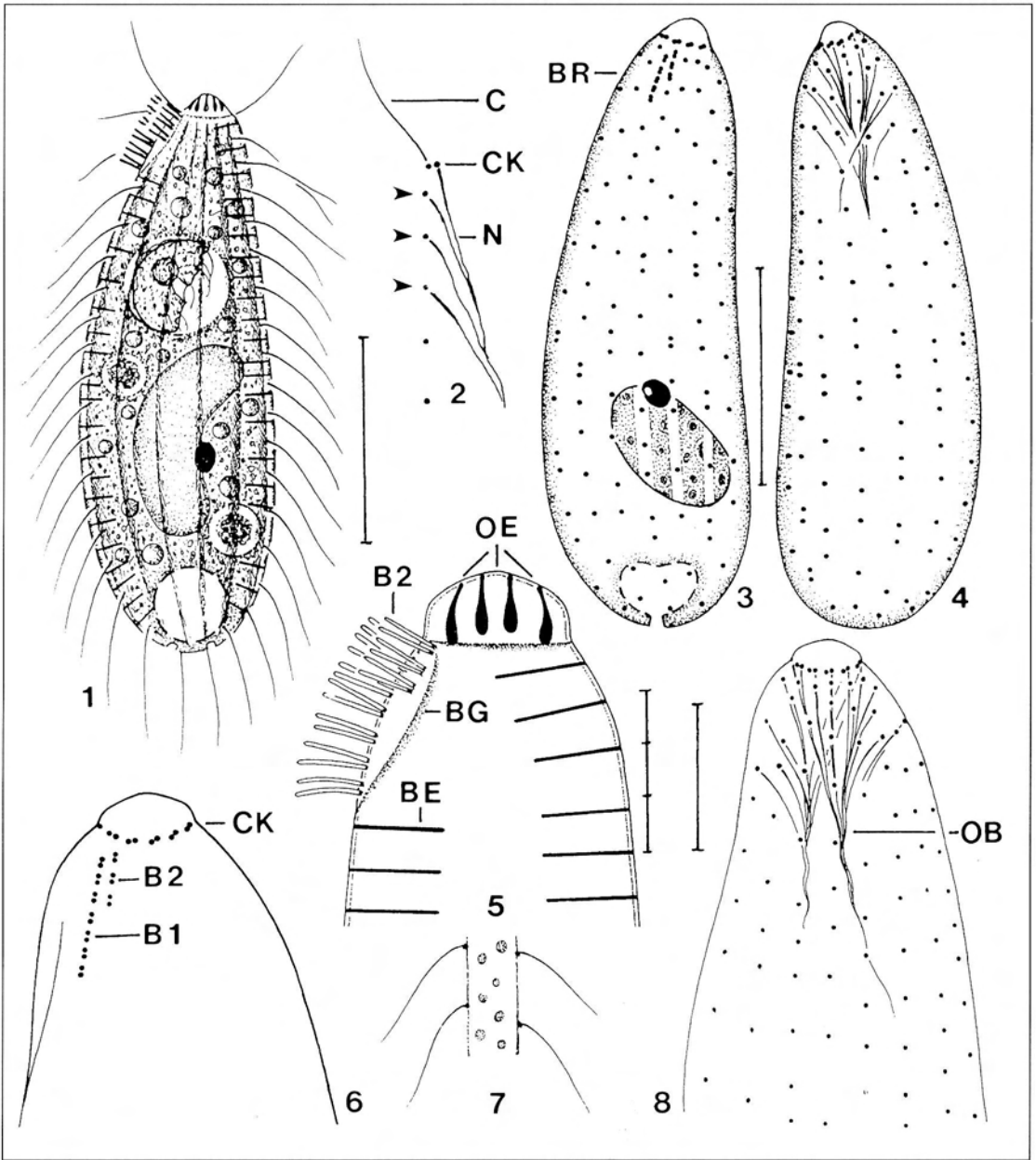
TABLE 2. Morphometric data from *Apobryophyllum etaschense*

Character ^a	$\bar{x}$	M	SD	CV	Min	Max	n
Body, length	115.9	110	20.7	17.9	86	148	15
Body, width	27.4	27	4.2	15.4	22	36	15
Anterior end to last brush dikinetid, distance	36.0	35	5.6	15.5	25	47	15
Macronucleus, length ^b	30.8	29	7.6	24.6	19	43	15
Macronucleus, width	8.2	8	0.9	11.5	7	10	15
Somatic kineties, number (including brush rows)	15.7	16	1.8	11.4	13	20	15
Dorsal brush, number of rows	6.8	7	0.6	8.2	6	8	15

^a Data based on protargol-impregnated and mounted specimens from field. Measurements in μm .

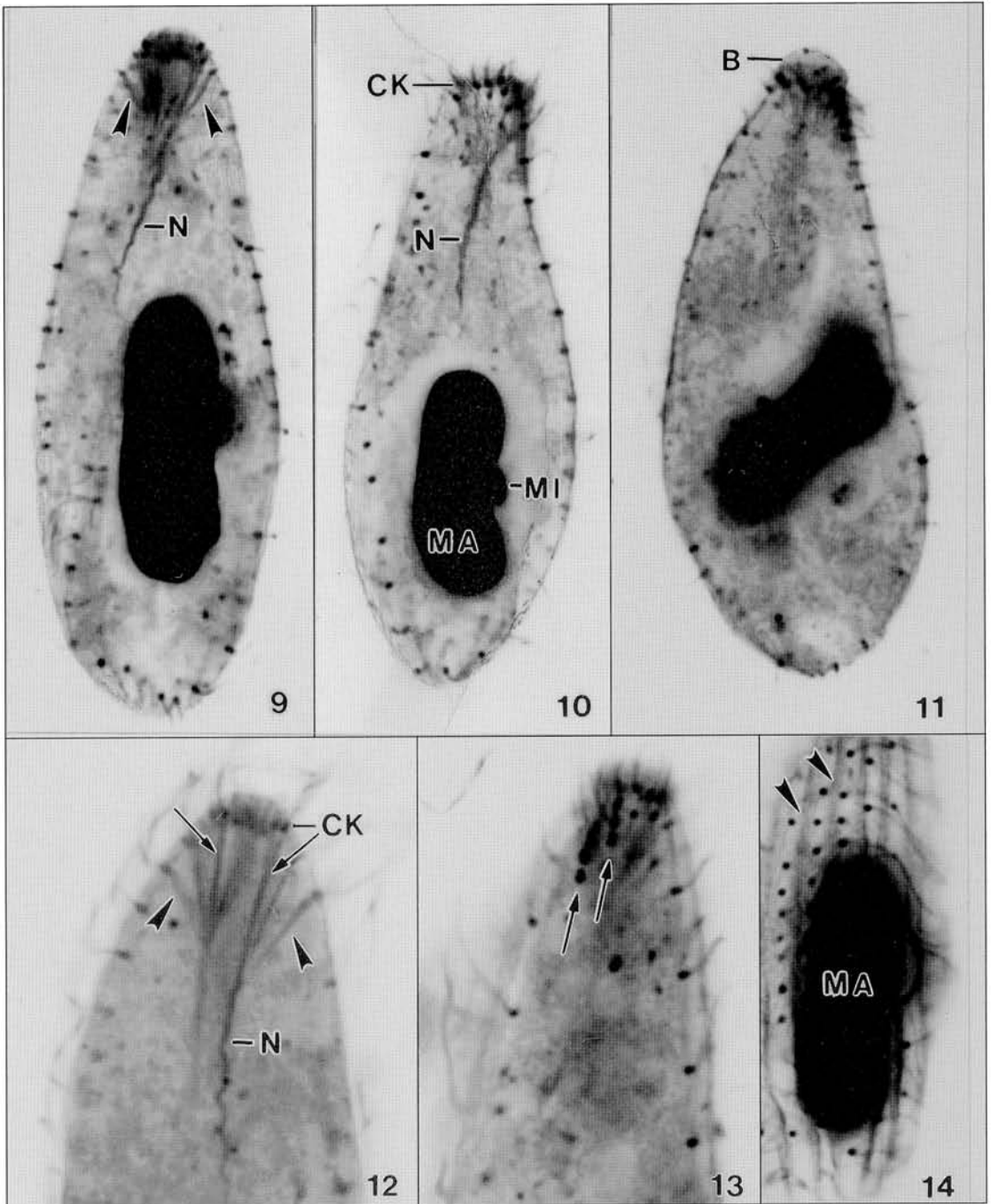
CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, $\bar{x}$ arithmetic mean.

^b Measured as length of chord.



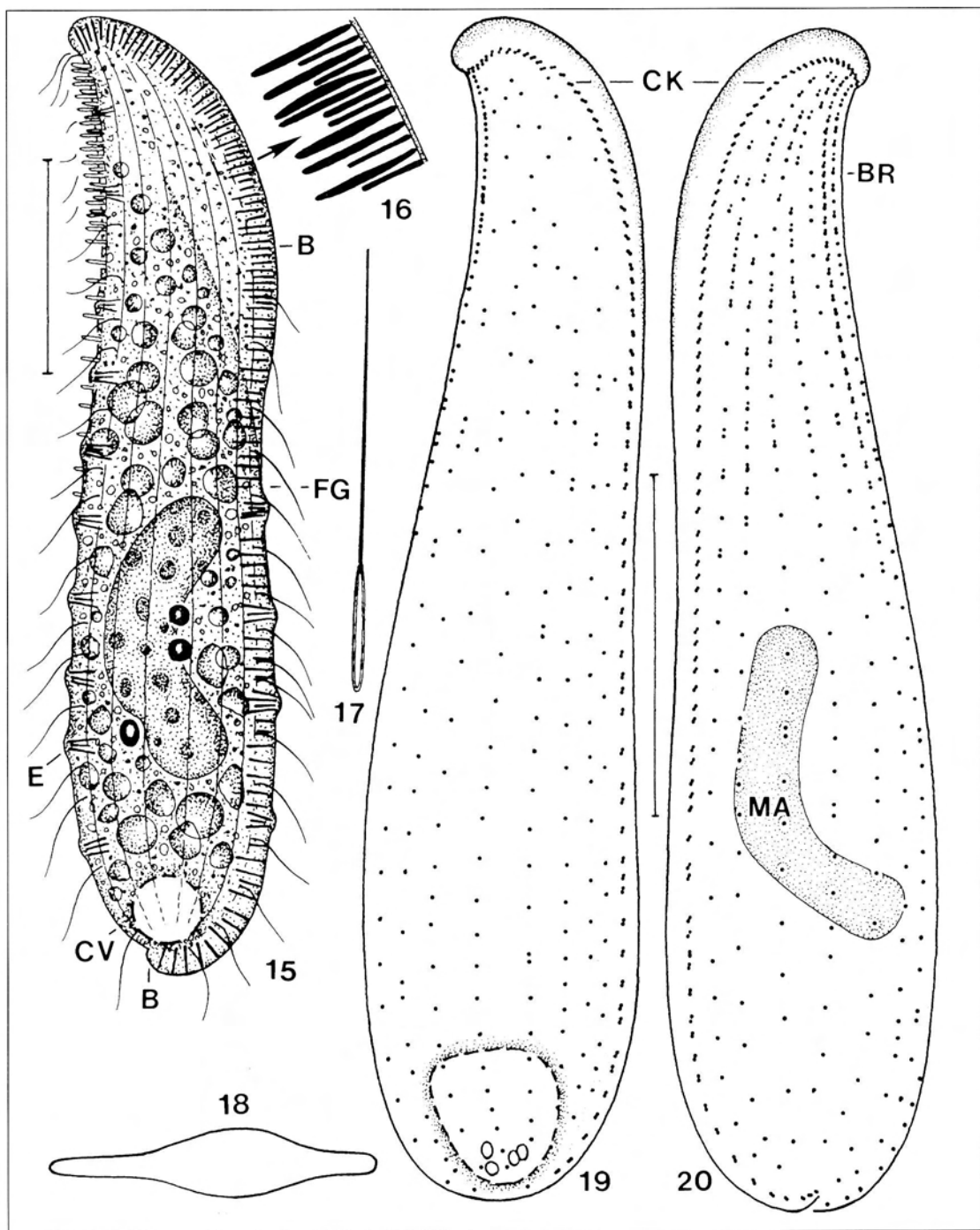
FIGS. 1 - 8.

Diplites telmatobius from life (1, 5, 7) and after protargol impregnation (2 - 4, 6, 8). 1: Right lateral view of a representative specimen with a just ingested *Drepanomonas revoluta*. 2, 8: Ventral anterior body portion showing oral basket composed of nematodesmata originating from the unciliated left basal body of the circumoral dikinetids and from ciliated, oralized somatic monokinetids (arrowheads) at the anterior end of the somatic kineties. 3, 4: Infraciliature of dorsal and ventral side. 5, 6: Anterior body portion showing main genus and species characters, that is, brosse, claviform oral extrusomes, BG - brush groove, BR - dorsal brush, B1, 2 - brush rows, C - cilium, CK - circumoral kinety, N - nematodesmata, OB - oral basket, OE - oral extrusomes, PC - postciliary microtubule ribbons. Scale bar division 2 μ m (Fig. 5), 10 μ m (Fig. 8), 20 μ m (Figs. 1, 3, 4).



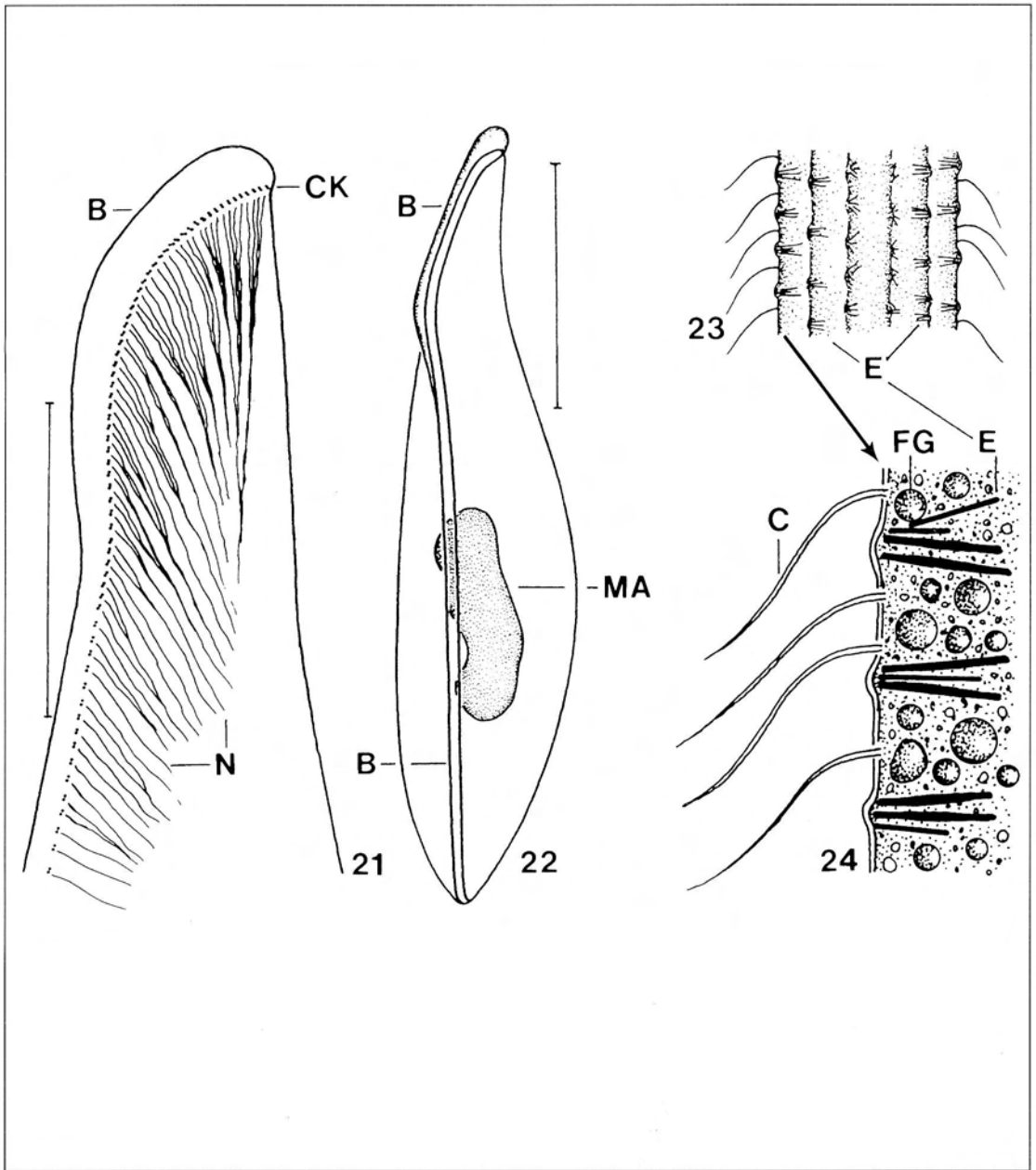
FIGS. 9 - 14.

Diplites telmatobius, somatic and oral infraciliature after protargol impregnation. 9 - 12: General body plan and oralized somatic monokinetids, from which nematodesmata originate (arrowheads). Arrows mark nematodesmata originating from circumoral dikinetids. 13: Dorsal anterior portion showing very short dorsal brush rows (arrows). 14: Surface view showing a conspicuous postciliary microtubule ribbon (arrowheads) right of each ciliary row. B - oral bulge, CK - circumoral kinety, MA - macronucleus, MI - micronucleus, N - nematodesmata.



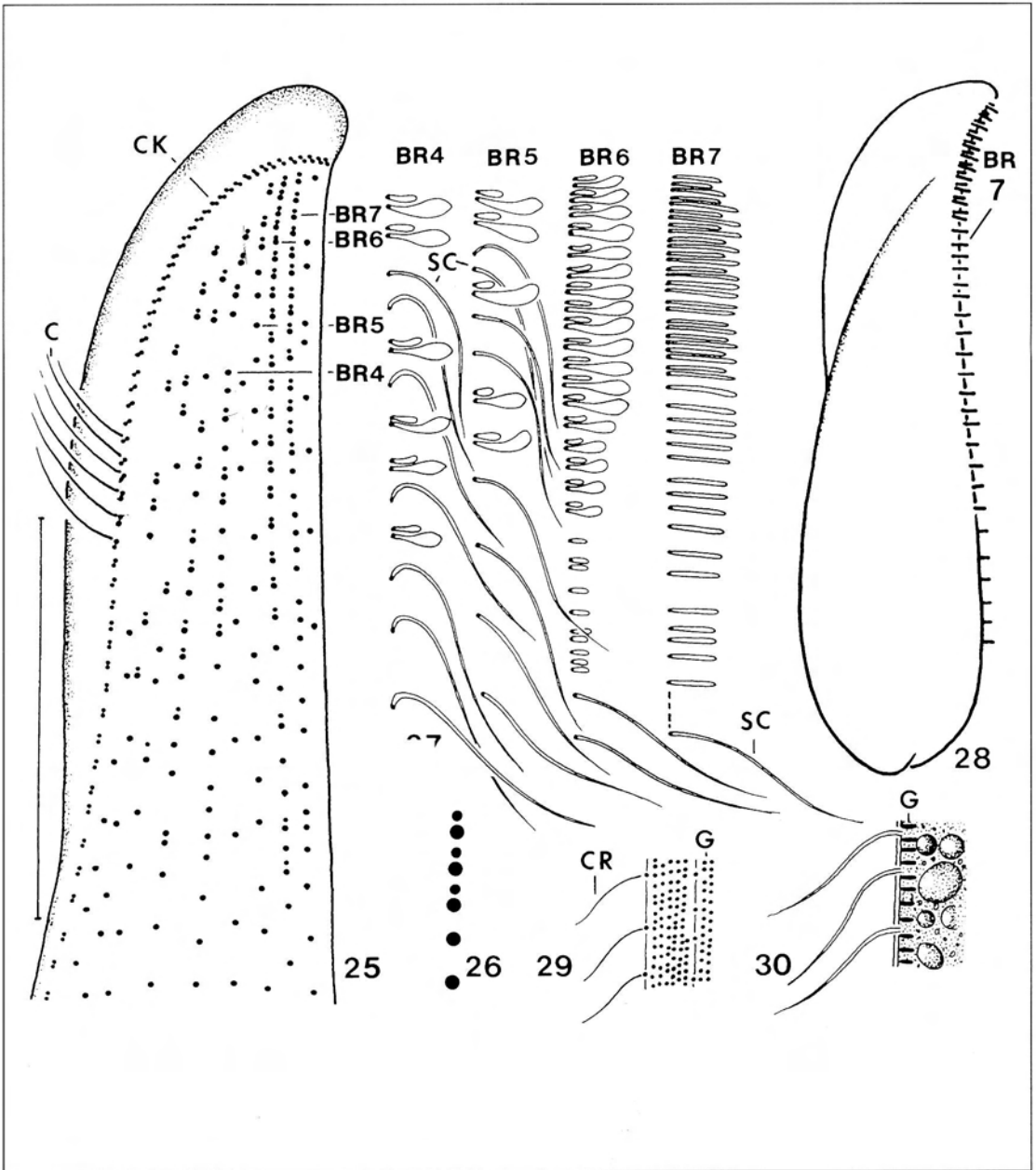
FIGS. 15 - 20.

Apobryophyllum etoschense from life (15 - 18) and after protargol impregnation (19, 20). 15: Right lateral view of a representative specimen with many fat globules. 16: Extrusomes (5 and 3 μ m respectively) in oral bulge. 17: Exploded extrusome, 20 μ m. 18: Dorsal view of a well-nourished specimen (anterior end left). 19, 20: Infraciliature of right and left side (cp. Figs. 25 - 28). B - oral bulge, BR - dorsal brush, CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, FG - fat globules, MA - macronucleus. Scale bars 30 μ m.



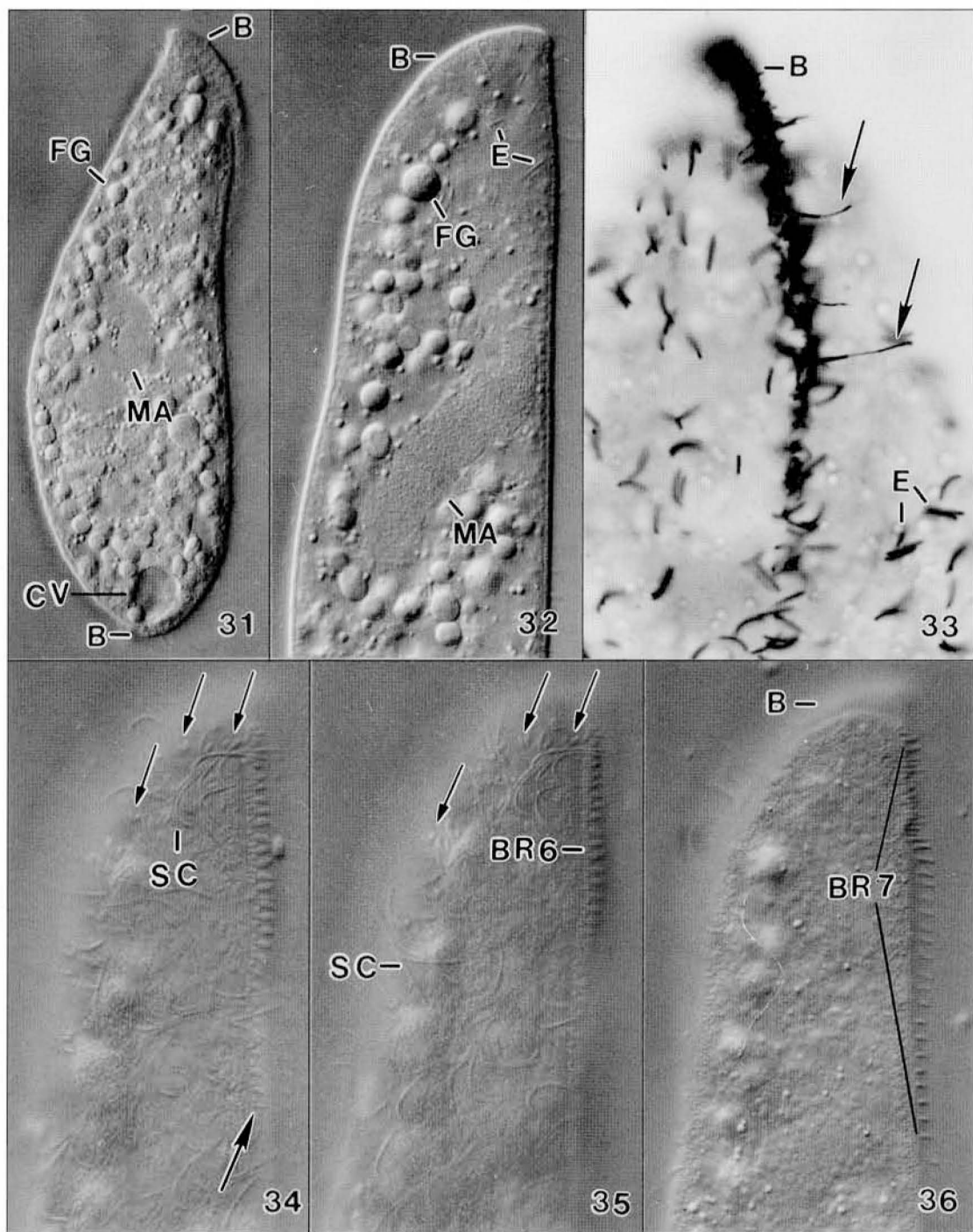
FIGS. 21 - 24.

Apobryophyllum etoschense from life (23, 24) and after protargol impregnation (21, 22). **21**: Oral infraciliature in left anterior portion of cell. **22**: Ventral view showing general organization. **23, 24**: Surface view and optical section of dorsal cell margin in mid-body, showing that *A. etoschense* has about 10 rows of bundled body extrusomes (cp. Figures 37, 38). B - oral bulge, C - cilium, CK - circumoral kinety, E - extrusomes, FG - fat globule, MA - macronucleus, N - nematodesmata. Scale bars 30 μ m.



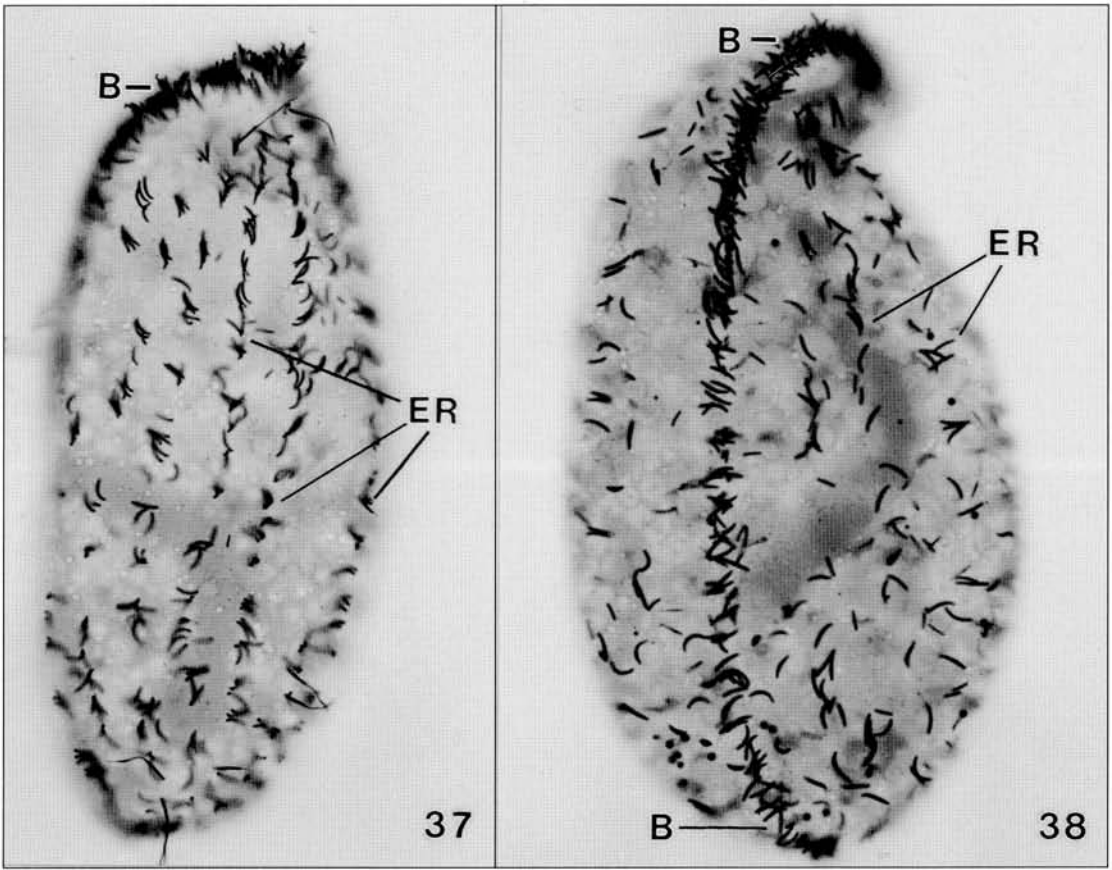
FIGS. 25 - 30.

Apobryophyllum etoschense from life (27 - 30) and after protargol impregnation (25, 26). **25, 27, 28:** The anterior left portion of the cell is occupied by the dorsal brush, whose cilia are highly differentiated (cp. Figures 34 - 36). Note, especially, normal somatic cilia between brush dikinetids in the right brush rows. Cilia drawn to scale, largest brush cilia about 3 μ m, normal somatic cilia about 10 μ m long. **26:** The brush is composed of dikinetids, whose anterior basal body is smaller than the posterior. **29, 30:** Surface view and optical section of cortex. B4 - 7 - dorsal brush rows, C - cilia, CK - circumoral kinety, CR - ciliary row, G - cortical granules, SC - normal somatic cilia. Scale bar 30 μ m.



FIGS. 31 - 36.

Apobryophyllum etoschense from life (31, 32, 34-36) and after silver carbonate impregnation (33). **31**: Right lateral view of a slightly squeezed specimen. **32, 34, 35, 36**: Anterior body half of same specimen at different focus levels to show the extrusomes and the complicated dorsal brush. Large arrow marks posterior end of brush row 2; small arrows denote isolated brush dikinetids in rows 1 - 5. **33**: Ventral anterior portion. The oral bulge contains many extrusomes, some of which are exploded (arrows). B - oral bulge, B6, 7 - dorsal brush rows, CV - contractile vacuole, E - extrusomes, FG - fat globules, MA - macronucleus, SC - somatic cilia.



FIGS. 37, 38.

Apobryophyllum etoschense, left side and ventral view after silver carbonate impregnation. *Apobryophyllum etoschense* not only has extrusomes in the oral bulge (B) but also in the somatic cortex, where they form about 10 rows (ER), each composed of small extrusome bundles (cp. Figures 1, 23, 24).

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