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# The Apertospathulidae, a New Family of Haptorid Ciliates (Protozoa, Ciliophora)

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ABSTRACT. We studied the morphology of three rare haptorid ciliates, using live observation and silver impregnation: *Apertospathula verruculifera* n. sp., *Longispatha elegans* n. gen., n. sp., and *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb. Simple ethanol fixation (50–70%, v/v) is recommended to reveal the ciliary pattern of ''difficult'' ciliates, such as *R. porculus*, by protargol impregnation. The three genera investigated have a distinct feature in common, viz., a lasso-shaped oral bulge and circumoral kinety, where the right half is slightly to distinctly longer than the left and the circumoral kinety is open ventrally. Thus, they are united in a new spathidiid family, the Apertospathulidae n. fam., which probably evolved from a *Bryophyllum*-like ancestor by partial reduction of the oral bulge and circumoral kinety. *Apertospathula verruculifera* has a wart-like process, the palpus dorsalis, at the anterior end of the dorsal brush. The right branch of the circumoral kinety is only slightly longer than the left one. *Longispatha elegans* has a straight oral bulge and circumoral kinety, the right branch of which extends to the posterior end of the body while the left branch ends in the anterior third of the body. *Rhinothrix porculus*, a curious ciliate with a snout-like dorsal elongation of the oral bulge, the palpus oralis, has a highly characteristic ciliary pattern: the oral pattern is as in *Longispatha*, but the bulge and circumoral kinety extend spirally to the posterior end of the body while the somatic kineties course meridionally. This is achieved by inserting some shortened kineties in the curves of the oral bulge.

**Key Words.** *Bryophyllum*, *Legendrea*, *Longispatha dioplites* (Foissner, Agatha, and Berger, 2002) n. comb., *Perispira*, *Rhinothrix antennata* (Kahl, 1926) n. comb., *Rhinothrix barbatula* (Penard, 1922) n. comb., soil protozoa, *Spathidium*, *Sphagnum* protozoa, Venezuela.

PENARD (1922) and Kahl (1926, 1930a, b) discovered many haptorid ciliates, but later the interest declined (Corliss 1979). More recently, research on haptorids was revived and many new taxa were described (Foissner 1984, 2003a,b; Foissner and Foissner 1985, 1988; Foissner, Agatha, and Berger 2002; Foissner, Berger, and Schaumburg 1999; Lipscomb and Riordan 1990; Lynn and Small 2002; Song and Wilbert 1989). Most of the new species and genera were discovered in terrestrial habitats, which certainly contain many more undescribed species. The same is true for *Sphagnum* bogs (Kreutz & Foissner, unpubl. data), which have attracted few ciliate researchers, except for Penard (1922), Kahl (1930a, b), and Grolière (1977). They described many new ciliates from such biotopes in Central Europe, while global diversity of *Sphagnum* bog ciliates is completely unexplored. The ease with which new haptorid and other new ciliates can be found in terrestrial and *Sphagnum* habitats suggests that they contain hundreds or even thousands of undescribed species globally.

Unfortunately, classification of the haptorids is based entirely on morphology because gene sequence data are very sparse. Nevertheless, it is important to describe species still in the traditional way, to demonstrate the fascinating diversity originating from a comparatively simple basal organization. The present study was initiated by the rediscovery of *Spathidium porculus* (Penard, 1922) Kahl, 1930, a curious species with a snout-like dorsal process. Silver impregnation revealed a highly distinct somatic and oral ciliary pattern facilitating the classification of some problematic taxa, viz., the genus *Apertospathula* Foissner, Agatha, and Berger, 2002 and three curious species discovered in Namibia and Venezuela.

## MATERIALS AND METHODS

*Apertospathula verruculifera* and *Longispatha elegans* were discovered in surface soil from Venezuela, using the non-flooded Petri dish method (Foissner 1987). Briefly, this simple technique involves placing 50–500 g of litter and soil in a Petri dish, 10–15 cm in diameter, and slightly over-saturating but not flooding the sample with distilled water. Such cultures were analyzed for ciliates by inspecting about 2 ml of the percolate on days 2, 7, 14, 21, and 28. *Rhinothrix porculus* was found in a mud sample from a *Sphagnum* bog in Germany. See the Occurrence and ecology section in the descriptions of individual species for details on sites.

Natural material as described above was used for all investigations because several culture attempts failed. Living cells were studied using a high-power oil immersion objective and differential interference contrast. Silver impregnation and scanning electron microscopy (SEM) were performed as described by Foissner (1991). However, all attempts to reveal the ciliary pattern (infraciliature) of *R. porculus* with protargol and silver carbonate failed. This is a problem in several haptorids, especially in those having a thick cortex and compact cortical granules. After many experiments, we discovered that such species can be mastered by using simple alcohol fixation with a final concentration of 50–70%; for instance, 20-ml samples are added to 30-ml 80% ethanol. The fixation is weak and thus not applicable to fragile ciliates that, however, usually do not pose problems with ordinary protargol methods. Further experiments showed that simple alcohol fixation often provides excellent preparations in ciliates that are difficult to impregnate, such as the large heterotrich *Condylostoma* and the oral structures of *Paramecium*.

Counts and measurements on silvered specimens were performed at a magnification of 1,000×. In vivo measurements were conducted at magnifications of 40–1,000×. Drawings of live specimens were based on free-hand sketches and micrographs; those of impregnated cells were made with a drawing device. Terminology is according to Corliss (1979) and Foissner et al. (2002).

Type material of the populations studied is deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. All are protargol-impregnated, and important cells are marked by a black ink circle on the coverslip.

## RESULTS

***Apertospathula verruculifera* n. sp. (Table 1 and Fig. 1–21).** Size 40–60 × 18–30 µm in vivo, usually about 50 × 25 µm, as calculated from some in vivo measurements and the preparations; length:width ratio near 2:1 both in vivo and in prepared specimens (Table 1 and Fig. 1, 20). Obovate, bluntly fusiform, or clavate after systole of contractile vacuole, dorsal side usually more

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Table **1.** Morphometric data on *Apertospathula verruculifera* (AV) and *Longispatha elegans* (LE).

| Characteristics[a] | spp | $\bar{x}$ | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|---|
| Body, length | AV | 40.4 | 40.0 | 4.3 | 0.9 | 10.6 | 32.0 | 48.0 | 21 |
| | LE | 99.3 | 96.0 | 7.8 | 2.0 | 7.9 | 87.0 | 113.0 | 15 |
| Body, width | AV | 19.8 | 20.0 | 2.6 | 0.6 | 13.3 | 15.0 | 24.0 | 21 |
| | LE | 15.8 | 16.0 | 3.1 | 0.8 | 19.8 | 12.0 | 23.0 | 15 |
| Body length:width, ratio | AV | 2.1 | 2.0 | 0.2 | 0.1 | 10.7 | 1.7 | 2.5 | 21 |
| | LE | 6.5 | 6.4 | 1.4 | 0.4 | 21.5 | 4.1 | 8.9 | 15 |
| Oral bulge (circumoral kinety), maximum length | AV | 7.1 | 7.0 | 1.0 | 0.3 | 14.4 | 6.0 | 9.0 | 16 |
| Oral bulge, dorsal height | AV | 2.1 | 2.0 | – | – | – | 1.5 | 3.0 | 19 |
| Oral bulge length:body width, ratio | AV | 0.4 | 0.3 | 0.1 | 0.1 | 17.6 | 0.3 | 0.5 | 16 |
| Circumoral kinety to last dikinetid of brush row 1, distance | AV | 5.2 | 5.0 | 0.8 | 0.2 | 14.5 | 4.0 | 6.0 | 11 |
| | LE | 11.7 | 12.0 | 1.3 | 0.3 | 10.9 | 10.0 | 15.0 | 15 |
| Circumoral kinety to last dikinetid of brush row 2, distance | AV | 6.0 | 6.0 | 1.2 | 0.4 | 19.7 | 4.0 | 8.0 | 11 |
| | LE | 13.1 | 13.0 | 1.7 | 0.4 | 12.8 | 10.0 | 16.0 | 15 |
| Circumoral kinety to last dikinetid of brush row 3, distance | AV | 3.6 | 4.0 | – | – | – | 3.0 | 4.0 | 10 |
| | LE | 6.9 | 7.0 | 1.2 | 0.3 | 16.8 | 5.0 | 10.0 | 15 |
| Anterior body end to last left side circumoral kinetid, distance | LE | 12.1 | 13.0 | 2.7 | 0.7 | 22.4 | 7.0 | 17.0 | 15 |
| Anterior body end to macronucleus, distance | AV | 11.9 | 12.0 | 4.1 | 0.9 | 34.8 | 6.0 | 23.0 | 21 |
| | LE | 47.5 | 49.0 | 9.7 | 2.5 | 20.3 | 27.0 | 65.0 | 15 |
| Macronucleus, length | AV | 7.6 | 8.0 | 2.1 | 0.5 | 27.9 | 4.0 | 11.0 | 21 |
| | LE | 17.6 | 18.0 | 3.2 | 0.8 | 17.9 | 13.0 | 26.0 | 15 |
| Macronucleus, width in mid | AV | 4.8 | 5.0 | 0.7 | 0.1 | 14.1 | 4.0 | 6.0 | 21 |
| | LE | 5.3 | 5.0 | 0.7 | 0.2 | 13.6 | 4.0 | 6.0 | 15 |
| Macronucleus, number | AV | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 21 |
| Micronucleus, length | LE | 2.9 | 3.0 | – | – | – | 2.5 | 3.5 | 14 |
| Micronucleus, width | LE | 2.6 | 2.5 | – | – | – | 2.0 | 3.0 | 14 |
| Somatic kineties, number | AV | 10.7 | 11.0 | 0.7 | 0.2 | 7.0 | 10.0 | 12.0 | 19 |
| | LE | 7.3 | 7.5 | 0.3 | 0.2 | 10.6 | 7.0 | 9.0 | 12 |
| Kinetids in a right side kinety, number | AV | 12.5 | 12.0 | 3.4 | 0.8 | 27.1 | 8.0 | 21.0 | 17 |
| | LE | 12.2 | 12.0 | 2.1 | 0.6 | 17.2 | 10.0 | 15.0 | 11 |
| Dorsal brush rows, number | AV | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 15 |
| | LE | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 15 |
| Dikinetids in brush row 1, number[b] | AV | 5.8 | 6.0 | 0.7 | 0.2 | 12.6 | 5.0 | 7.0 | 13 |
| | LE | 7.7 | 8.0 | – | – | – | 7.0 | 8.0 | 15 |
| Dikinetids in brush row 2, number[b] | AV | 6.9 | 7.0 | 1.1 | 0.3 | 16.1 | 5.0 | 8.0 | 13 |
| | LE | 8.3 | 9.0 | 0.8 | 0.2 | 9.8 | 7.0 | 9.0 | 15 |
| Dikinetids in brush row 3, number[b] | AV | 3.6 | 4.0 | 0.7 | 0.2 | 18.0 | 3.0 | 5.0 | 13 |
| | LE | 4.4 | 4.0 | – | – | – | 4.0 | 5.0 | 15 |
| Circumoral dikinetids, number | LE | 42.8 | 42.0 | 4.8 | 1.3 | 11.3 | 38.0 | 57.0 | 15 |

[a]Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from non-flooded Petri dish cultures. Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean, spp, species, $\bar{x}$, arithmetic mean.

[b]Partially based on specimens from SEM preparations.

distinctly convex than ventral; anterior (oral) body end broadly rounded with wart-like, oblique oral bulge appearing as a minute triangular process; gradually narrowed posteriorly with rear end occasionally bluntly pointed and/or wrinkled after systole of contractile vacuole. Palpus dorsalis between anterior ends of brush rows 1 and 2, about 2–3 × 1–2 µm in size, well recognizable in vivo and some prepared cells (Fig. 1, 4–11, 13, 17, 18, 20). Macronucleus in middle body third, globular to ellipsoidal, on average 7.6 × 4.8 µm in protargol preparations, composed of two nodules in 2 out of 52 specimens analyzed; many granular inclusions about 1 µm across. Micronucleus rarely unequivocally identifiable due to many cytoplasmic inclusions (Table 1 and Fig. 1, 4, 13–15, 17). Contractile vacuole in rear end, likely with one excretory pore in pole center. Extrusomes studded in oral bulge, oblong, indistinct in vivo because pale and only about 2 × 0.3 µm in size (Fig. 1–3); do not impregnate with the method used. Cortex thin and flexible, conspicuously furrowed in anterior body third (Fig. 11, 19); cortex bolsters filled with ordinary cytoplasmic inclusions, do not contain extrusomes. Cortical granules minute (< 0.5 µm) and pale, difficult to recognize. Cytoplasm colorless, middle body third usually dark at low magnification because packed with lipid droplets 1–5 µm across and (methanogenic?) 2–3-µm-long bacteria intensely impregnating with protargol (Fig. 1, 4, 13). Underneath oral bulge refractive granules appearing as a dark spot at low magnification (Fig. 1). Food not known, likely small ciliates considering the numerous lipid droplets (Fig. 17–20). Swims moderately rapidly, rotating about main body axis.

Cilia about 12 µm long in vivo, distances of cilia within rows gradually increase from about 1 µm anteriorly to 5 µm posteriorly, arranged in an average of 11 meridional, widely (~ 7 µm) and equidistantly spaced kineties clearly separate from circumoral kinety and only indistinctly curved dorsally at anterior end. Dorsal brush three-rowed, very inconspicuous because extending only 15% of body length and bristles merely up to 1 µm long in vivo. Bristles clavate and comparatively thick, posterior bristle of pairs dot-like because < 0.4 µm long. Brush rows 1 and 2 of similar length, each composed of six to seven dikinetids on average; row 3 about half as long as row 2, composed of an average of only four dikinetids followed by a short bristle tail of three to four monokinetids (Table 1 and Fig. 1, 10, 13, 16, 18, 21).

Oral bulge indistinct because occupying only 40% of body width; gradually merging into body proper ventrally; and merely 2–3 µm high in dorsal region, appearing as a wart-like eminence in vivo; obovate in frontal view, bulge halves separated by rather

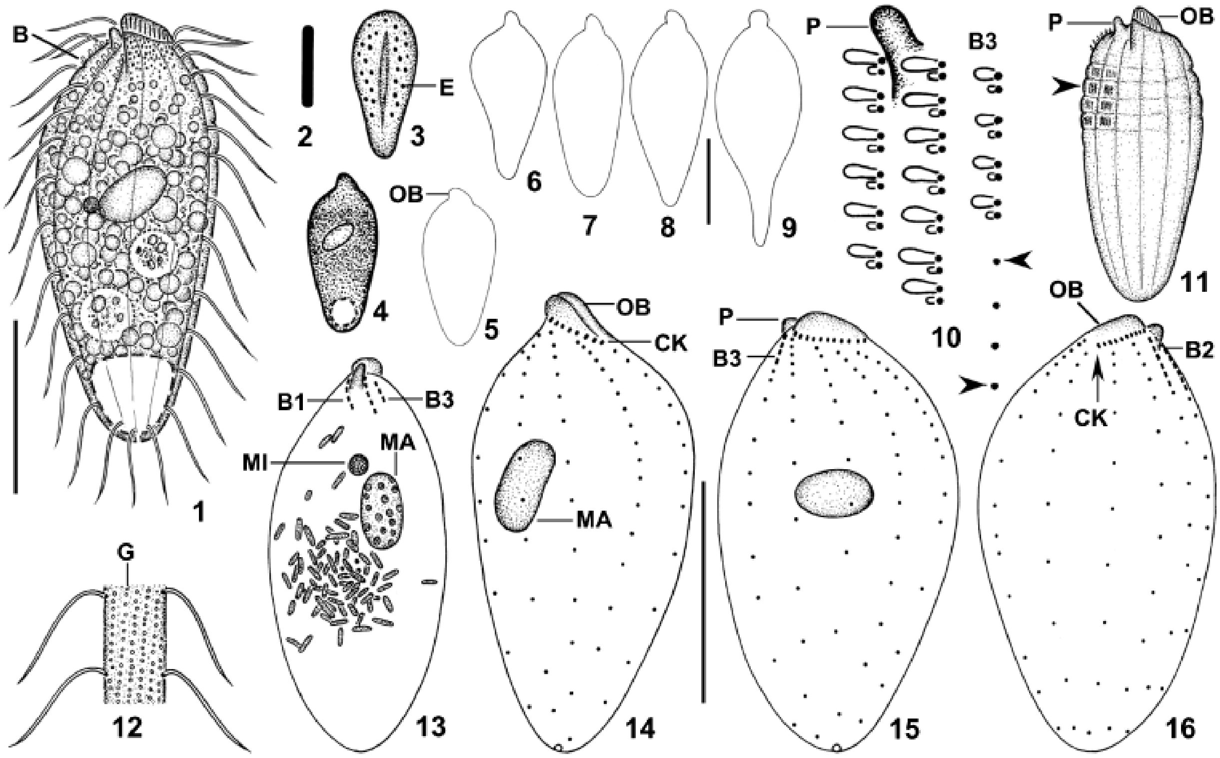


Fig. **1–16.** *Apertospathula verruculifera* n. sp. from life (1–12) and after protargol impregnation (13–16). **1, 11.** Dorsolateral views of representative specimens, length about 50 µm. Arrowhead in Fig. 11 marks cortex bolsters. **2.** Oral bulge extrusome, length 2 µm. **3.** Frontal view of oral bulge. **4.** Specimens are dark at low magnification due to countless, refractive lipid droplets. **5–9.** Shape and size variability. **10.** Dorsal brush and palpus dorsalis, bristles up to 1 µm long in vivo. Arrowheads denote bristle tail of brush row 3. **12.** Surface view showing cortical granulation. **13.** Dorsal view showing body center packed with bacterial rods. Note the short dorsal brush rows. **14.** Ventrolateral view of ciliary pattern. **15, 16.** Ciliary pattern of right and left side and macronucleus of holotype specimen, length 40 µm. Note the shortened left branch of the circumoral kinety (arrow) not abutting on the right branch. B1–3, dorsal brush (rows); CK, circumoral kinety; E, extrusomes; G, cortical granules; MA, macronucleus; MI, micronucleus; OB, oral bulge; P, palpus dorsalis. Scale bars = 20 µm, drawn to scale for Fig. 4–9 and 13–16.

distinct furrow (Table 1 and Fig. 1, 3–9, 14–17, 21). Circumoral kinety of similar shape as oral bulge, ventral opening distinct in protargol and SEM preparations, composed of about 25 dikinetids, each associated with a cilium and a nematodesma. Oral basket rods fine and rarely impregnated, oral basket thus inconspicuous (Table 1 and Fig. 1, 14–16, 21).

**Occurrence and ecology.** *Apertospathula verruculifera* was moderately abundant in a non-flooded Petri dish culture set up with mud and soil from the surface of a flat, dry coastal puddle in the Maracay National Park, north coast of Venezuela, W68° N10°. The bottom was covered with halophytes and crusts of cyanobacteria. The sample, which was collected in 1996 and studied in April 1997, had pH 7.3 (in water) and was highly saline (> 30‰). Later, we found *A. verruculifera* in a similar habitat of the Dominican Republic, viz., in saline soil from the margin of a mangrove forest. Thus, *A. verruculifera* possibly prefers ephemerally flooded coastal puddles and/or soils.

***Longispatha elegans* n. gen., n. sp. (Table 1 and Fig. 22–36).** Size 80–120 × 15–25 µm in vivo, usually about 100 × 18 µm; length:width ratio near 6:1 both in vivo and in protargol preparations; flattened up to 2:1 laterally, especially in hyaline oral region. Narrowly to very narrowly spatulate to ellipsoidal, anterior third strongly oblique with right oral bulge half sail-like projecting subapically; very flexible but not contractile (Table 1 and Fig. 24, 25, 35). Nuclear apparatus in mid-body, stands out as a whitish structure from strongly granulated cytoplasm. Macronucleus oblong with ends often slightly inflated; chromatin granules globular, moderately large. Micronucleus attached to macronucleus, globular to broadly ellipsoidal, about 3 × 2.6 µm in protargol preparations (Table 1 and Fig. 24, 36). Contractile vacuole in rear end, excretory pore(s) not impregnated. Cortex flexible and bright, thin, cortical granules not recognizable. Extrusomes studded in widened dorsal region of oral bulge and right bulge half to form a row extending to rear body end, highly refractive and thus appearing as bright dots when bulge is viewed frontally; lacking in left bulge half. Individual extrusomes narrowly ovate and about 3–4 × 0.8 µm in size, do not impregnate with the protargol method used (Fig. 24, 26–28). However, protargol-impregnated cells contain 2-µm-long rods and 3-µm-long, narrowly ovate to broadly fusiform structures, likely developing extrusomes. Cytoplasm colorless, postorally packed with lipid droplets 1–5 µm across and globular food inclusions, likely heterotrophic flagellates and or small naked amebae. Movement conspicuous because very slowly gliding on microscope slide.

Cilia about 12 µm long in vivo, ca 10 µm apart and thus very loosely spaced, arranged in an average of seven widely (~ 7 µm) and equidistantly spaced kineties distinctly separate from circumoral kinety and hardly curved dorsally at anterior end (Table 1 and Fig. 24, 35, 36). Three dorsal rows anteriorly differentiated to short (about 17% of body length) brush composed of paired, up to 6-µm-long bristles having some specialities (Fig. 23, 24, 29, 30, 32, 34–36): row 1 exactly as in *L. dioplites*, that is, composed of an average of eight dikinetids, of which the anterior bristle intensely impregnates with protargol (Fig. 30); row

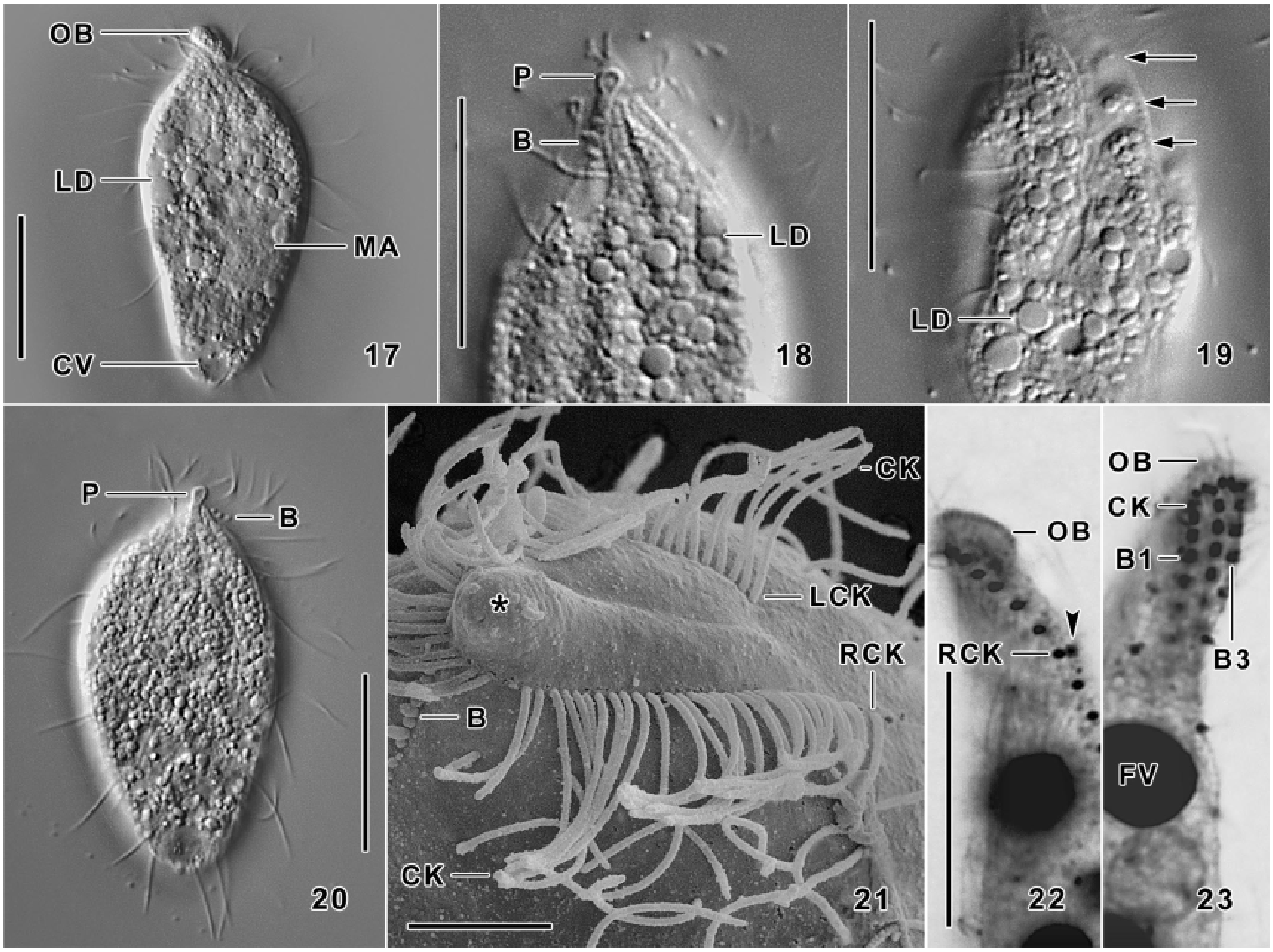


Fig. **17–23.** Micrographs of *Apertospathula verruculifera* n. sp. (17–21) and *Longispatha elegans* n. gen., n. sp. (22, 23). **17–21.** *Apertospathula verruculifera* n. sp. from life (17–20) and in the scanning electron microscope (21). **17.** Ventrolateral view of a slightly squeezed specimen studded with lipid droplets. Note the short oral bulge (OB). **18, 20.** Dorsal views showing the palpus dorsalis (P) and several of the minute dorsal brush bristles (B). Note the rather loosely spaced but 12-µm-long cilia. **19.** Ventral view showing cortex bolsters (arrows) and lipid droplets. **21.** Frontolateral view showing the broad gap between the ends of the left branch of the circumoral kinety (LCK) and its right branch (RCK) , that is, the main feature of the genus. Note the wart-like dorsal end of the oral bulge (asterisk). This specimen has about 70 circumoral dikinetids and thus probably belongs to another, similar species. **22, 23.** *Longispatha elegans* n. gen., n. sp., ciliary pattern of anterior body region after protargol impregnation. The arrowhead denotes the last oral dikinetid of the short left branch of the circumoral kinety. B1–3, dorsal brush (rows); CK, (cilia of the) circumoral kinety; CV, contractile vacuole; FV, food inclusion; LCK, left branch of circumoral kinety; LD, lipid droplets; MA, macronucleus; OB, oral bulge; P, palpus dorsalis; RCK, right branch of circumoral kinety. Scale bars = 20 µm (17–20, 22, 23) and 5 µm (21).

2 also composed of an average of eight dikinetids, posterior bristle of two to three middle dikinetids clavate and elongated to 6 µm; row 3 consists of an average of four dikinetids with up to 2-µm-long bristles and has a short tail of monokinetids whose bristles slightly decrease in length from about 2 µm anteriorly to 1.5 µm posteriorly.

Oral bulge hyaline, right bulge half sail-like protruding subapically, left half very short occupying only 10% of body length, dorsal region head-like inflated, ventral region narrowed, frontal view thus narrowly cuneate. Circumoral kinety composed of an average of 43 dikinetids, open ventrally with a short left branch and a long right one extending to near the posterior end; kinetids widely spaced, except in the dorsal region of kinety, each associated with an ~ 12-µm-long cilium and a short, fine nematodesma contributing to the inconspicuous oral basket, which was faintly impregnated in one specimen (Table 1 and Fig. 22–26, 28, 31–36).

**Occurrence and ecology.** As yet found only at type locality, that is, field soil in the surroundings of the village of El Sapo, about 50 km north of Pto. Ayachuco, Venezuela, W75° N6°. Likely, the area is sometimes flooded by the Orinoco River, as indicated by the very fine, gray soil. The field (Mahada) in which *L. elegans* was discovered was in the tenth year of use and planted with bananas. Very low numbers of *L. elegans* occurred in the non-flooded Petri dish culture set up with a mixture of banana and grass litter and surface soil.

**Redescription of *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb. (Table 2 and Fig. 37–53, 66–91).** Size 80–130 × 25–40 µm, usually about 100 × 30 µm in vivo, according to some in vivo measurements and the morphometric data (Table 2).

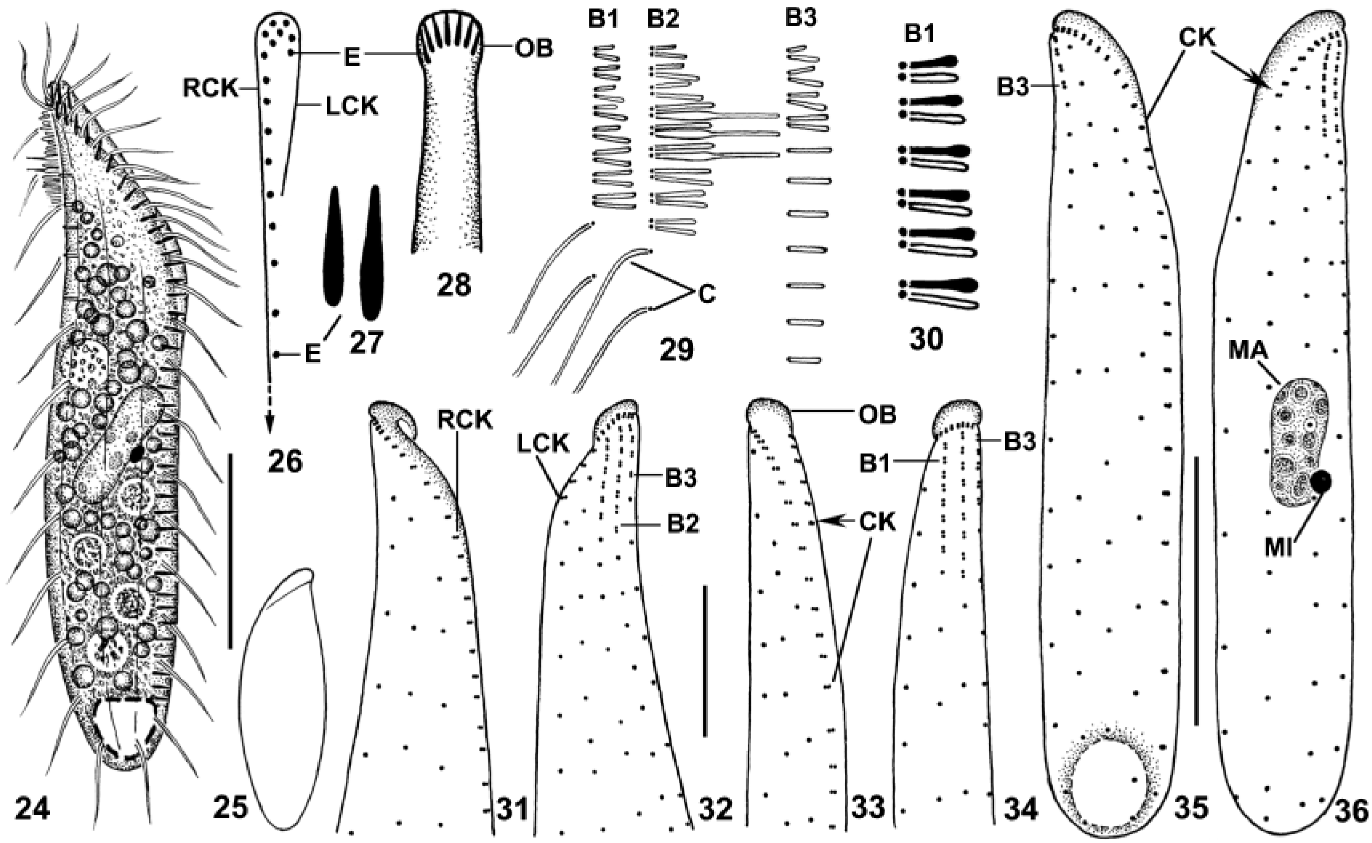


Fig. **24–36.** *Longispatha elegans* n. gen., n. sp. from life (24–29) and after protargol impregnation (30–36). **24.** Right side view of a representative specimen with many lipid droplets, length about 110 µm. **25.** Blunt shape variant. **26.** Frontal view of oral bulge. The right branch of the oral bulge contains a row of extrusomes and extends to near the posterior end of the body, while the left is much shorter. **27.** Extrusomes, length 3–4 µm. **28.** Dorsal view of anterior body region. **29.** Dorsal brush, bristles drawn to scale, somatic cilia (C) not shown in full length. Note the 6-µm-long bristles in row 2 and the bristle tail of row 3. **30.** Dorsal brush row 1. The anterior bristle of the pairs impregnates darkly. **31, 32.** Ventrolateral and dorsolateral view of ciliary pattern in anterior body portion. **33, 34.** Ciliary pattern of ventral and dorsal side in anterior body portion. **35, 36.** Ciliary pattern of right and left side and nuclear apparatus of holotype specimen, length 93 µm. The right branch of the circumoral kinety (CK) extends to the posterior end, while the left is much shorter (arrow). This pattern is the main feature of the new genus *Longispatha*. B1–3, dorsal brush rows; C, ordinary somatic cilia; CK, circumoral kinety; E, extrusomes; LCK, left branch of circumoral kinety; MA, macronucleus; MI, micronucleus; OB, oral bulge; RCK, right branch of circumoral kinety. Scale bars = 30 µm (24, 35, 36) and 10 µm (31–34 drawn to scale).

Length:width ratio moderately variable, that is, about 2.5–4.5:1 in vivo, usually 3–4:1 both in vivo and in protargol preparations, where specimens tend to become stouter; very flexible but not contractile and only slightly flattened laterally, mainly in narrowed posterior half. Shape dominated by the dorsally projecting oral bulge, forming a distinct process (nose or snout), for which we suggest the term palpus oralis; usually more or less narrowly obovate (3–4:1), especially when seen ventrally and dorsally, while narrowly ellipsoidal or oblong in lateral view (Fig. 37, 38, 46–50, 66, 69, 71–73), becoming cylindroidal (4–5:1) in starved specimens. Macronucleus in or near mid-body, oblong ($\sim$ 3:1), rarely globular or reniform; chromatin granules in vivo up to 3 µm across and distinct. Micronucleus in minute depression of mid-macronucleus, conspicuous in vivo because about $6 \times 4$ µm in size, broadly ellipsoidal to almost hemispherical (Fig. 37, 38, 50, 67, 71, 73, 75, 89–91). Contractile vacuole in posterior end, large, forms from several smaller vacuoles; some excretory pores in pole area (Fig. 37, 49, 50, 67, 71, 73). Cytopyge in posterior pole area, fecal mass globular and compact, migrates through the contractile vacuole (Fig. 72). Cortex very flexible, of ordinary appearance, contains about eight rows of narrowly spaced, minute ($\sim$ 0.5 µm), colorless granules between two kineties (Fig. 53); cortex and granules impregnate heavily in ordinarily fixed specimens obscuring the ciliary pattern, but remain unstained in alcohol-fixed cells and silver carbonate preparations.

Extrusome apparatus highly developed, composed of three types of specifically arranged and located toxicysts; inconspicuous in vivo because individual extrusomes thin and/or short and/or hidden by cytoplasmic inclusions; very conspicuous but more or less distorted in silver carbonate preparations; impregnate faintly with protargol, except for some deeply stained cytoplasmic developmental stages. Type I (body) toxicysts within or slightly left of ciliary rows, about as widely spaced as cilia, extend transversely into body proper, basically rod-shaped with narrowed ends, distinctly curved when attached to cortex and becoming straight when detached, in vivo about $7 \times 0.5$ µm in size, circa 15 µm long when exploded; often acicular in silver carbonate preparations (Fig. 40, 50, 53, 75, 77, 79, 82, 85, 86, 88). Type II and III toxicysts associated with oral bulge. Type II extrusomes about $50 \times 0.5$ µm in size, rod-shaped and slightly curved, attached to inner margin of anterior loop of circumoral kinety, extend obliquely backwards; in vivo distinct in flattened cells and very conspicuous, but often wrinkled in silver carbonate preparations; developing type II extrusomes form some small bundles with about five rods each in cytoplasm (Fig. 39, 44, 50, 52, 81, 83). Type III oral bulge extrusomes oblong and about $1.5 \times 0.3$ µm in

Table **2.** Morphometric data on *Rhinothrix porculus*.

| Characteristics[a] | $\bar{x}$ | M | SD | SE | CV | Min | Max | n |
|---|---|---|---|---|---|---|---|---|
| Body (trunk), length | 93.1 | 93.0 | 9.3 | 2.0 | 10.0 | 77.0 | 114.0 | 21 |
| Body, lateral width (ventral to dorsal) | 29.6 | 29.0 | 2.3 | 0.5 | 7.9 | 25.0 | 33.0 | 21 |
| Body, width in ventral or dorsal view | 26.7 | 27.0 | 3.0 | 0.7 | 11.1 | 22.0 | 33.0 | 17 |
| Body length:width, ratio | 3.2 | 3.1 | 0.3 | 0.1 | 9.7 | 2.5 | 3.7 | 21 |
| Palpus oralis, length | 8.0 | 8.0 | 1.0 | 0.2 | 12.1 | 7.0 | 10.0 | 21 |
| Palpus oralis, width | 3.6 | 3.5 | 0.5 | 0.1 | 12.7 | 3.0 | 4.0 | 21 |
| Oral bulge (circumoral kinety), maximum width | 7.6 | 8.0 | 0.9 | 0.3 | 12.0 | 6.0 | 9.0 | 8 |
| Oral bulge (excluding palpus), height | 2.7 | 3.0 | 0.4 | 0.1 | 15.0 | 2.0 | 3.0 | 21 |
| Circumoral kinety to last dikinetid of brush row 1, distance | 17.4 | 18.0 | 1.6 | 0.4 | 9.0 | 15.0 | 21.0 | 19 |
| Circumoral kinety to last dikinetid of brush row 2, distance | 19.2 | 20.0 | 1.6 | 0.4 | 8.4 | 16.0 | 22.0 | 19 |
| Circumoral kinety to last dikinetid of brush row 3, distance | 12.2 | 12.0 | 0.7 | 0.2 | 5.8 | 11.0 | 13.0 | 19 |
| Anterior body end to last left side circumoral kinetid, distance | 25.4 | 25.0 | 3.6 | 0.8 | 14.2 | 18.0 | 33.0 | 21 |
| Anterior body end to macronucleus, distance | 38.1 | 36.0 | 6.7 | 1.5 | 17.6 | 27.0 | 51.0 | 21 |
| Macronucleus figure, length | 25.8 | 25.0 | 4.1 | 0.9 | 16.1 | 20.0 | 35.0 | 21 |
| Macronucleus, maximum width | 8.4 | 9.0 | 1.3 | 0.3 | 15.8 | 6.0 | 11.0 | 21 |
| Macronucleus, number | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 21 |
| Micronucleus, across | 4.7 | 5.0 | 0.5 | 0.1 | 9.8 | 4.0 | 5.0 | 21 |
| Micronucleus, number | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 21 |
| Somatic kineties, number | 20.9 | 21.0 | 1.0 | 0.2 | 4.8 | 19.0 | 23.0 | 21 |
| Kinetids in a right side full kinety, number | 28.7 | 29.0 | 6.8 | 1.5 | 23.6 | 21.0 | 45.0 | 21 |
| Kinetids in the left-most short kinety, number | 9.6 | 10.0 | 2.7 | 0.7 | 28.6 | 5.0 | 14.0 | 15 |
| Kinetids in the right-most short kinety, number | 12.6 | 13.0 | 3.5 | 1.0 | 27.5 | 7.0 | 18.0 | 11 |
| Dorsal brush rows, number | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 21 |
| Dikinetids in brush row 1, number | 14.9 | 15.0 | 1.5 | 0.4 | 10.3 | 13.0 | 17.0 | 19 |
| Dikinetids in brush row 2, number | 18.6 | 19.0 | 1.7 | 0.4 | 9.0 | 16.0 | 21.0 | 19 |
| Dikinetids in brush row 3, number | 12.2 | 12.0 | 1.2 | 0.3 | 10.1 | 10.0 | 14.0 | 19 |
| Bristles in monokinetidal tail of brush row 3, number | 14.4 | 14.0 | 2.4 | 0.7 | 16.8 | 12.0 | 19.0 | 11 |

[a]Data based on alcohol-fixed, mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from *Sphagnum* mud. Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean; $\bar{x}$, arithmetic mean.

size, arranged in many oblique rows, each consisting of 5–10 toxicysts; extrusome rows in both halves of oral bulge, including palpus oralis, form ladder-like pattern extending to posterior end underneath left branch of circumoral kinety; conspicuous in silver carbonate preparations imaging course of oral bulge, fairly distinct also in vivo because individual extrusomes rather refractive; scattered and numerous in cytoplasm, where they are about 2 µm long and often flask-shaped (Fig. 41–43, 51, 76, 79, 80, 84–88).

Cytoplasm colorless, contains some lipid droplets up to 10 µm across and numerous highly refractive inclusions up to 6 × 4 µm in size in anterior, rarely also in posterior half of body (Fig. 37, 38, 45, 71–78, 81). Specimens thus dark under low ($\leq 100\times$) bright-field magnification and details of oral apparatus and dorsal brush difficult to analyze. Inclusions globular to ellipsoidal, rarely ring-shaped, the larger ones often with a central cleft, do not dissolve when specimens are squashed but disappear in starving cells. Food vacuoles up to 15 µm across, in some specimens contain oral baskets of microthoracid ciliates or euglenids, suggesting that the refractive inclusions described above are paramylon grains; one specimen contained a small desmid. Swims slowly to rather rapidly by rotation about main body axis; highly flexible under slight coverslip pressure.

Unfortunately, no dividers were contained in the protargol slides, likely because the population was disturbed by four days of transport from Constance to Salzburg. However, some conjugants were observed later.

Cilia about 10 µm long in vivo, slightly condensed in anterior portion of rows, arranged in an average of 21 meridional, widely (~ 5 µm) and equidistantly spaced kineties forming an indistinct *Spathidium* pattern, that is, anterior portion of right side kineties slightly curved dorsally, while left side kineties are straight or slightly curved ventrally. Some shortened kineties in the curves formed by the sigmoidal oral bulge, that is, anterior and posterior to mid-body (Table 2 and Fig. 37, 38, 66–70, 85, 89–91). Dorsal brush exactly on dorsal side of cell, three-rowed and isostichad, composed of about 1-µm spaced dikinetids and one or two, rarely three, ordinary cilia at anterior end of each row; of ordinary distinctness, longest row 2 extends 21% of body length on average, bristles about as thick as ordinary cilia and decreasing in length from 4–5 µm anteriorly to 1–2 µm posteriorly. Brush row 1 slightly shorter than row 2, composed of an average of 15 dikinetids; longest row 2 composed of 19 dikinetids on average; row 3 shorter by about 30% than rows 1 and 2, composed of an average of 12 dikinetids followed by an average of 14 monokinetidal bristles, 2–3 µm long, forming a tail extending to mid-body (Table 2 and Fig. 38, 66, 70).

Oral bulge, except for palpus oralis, inconspicuous in vivo when viewed laterally because merely 3–4 µm high and clearly recognizable only in anterior quarter of cell; conspicuous when viewed ventrally because sigmoidally curved, extending spirally the whole length of body, and cuneate with width decreasing from 7–10 µm anteriorly to about 4 µm posteriorly; inflated anterior portion with distinct central cleft dividing bulge in a right half extending to the posterior end and a left half restricted to the anterior third of the body. Palpus oralis conspicuous because 5–12 µm long in vivo and distinctly projecting from dorsal anterior end of cell; cylindroidal and slightly contractile, belongs to the oral bulge because studded with type III extrusomes (Table 2 and Fig. 37, 38, 51, 66–74, 79, 84, 91). Circumoral kinety of same shape as oral bulge, that is, lasso-like and widely open ventrally (~ 4 µm); right branch extends full body length, left branch restricted to anterior 37% of body on average and not continuing posteriorly as ordinary somatic kinety; composed of dikinetids very narrowly spaced in anterior third, where faintly impregnated nematodesmata originate from the right and left branch of the

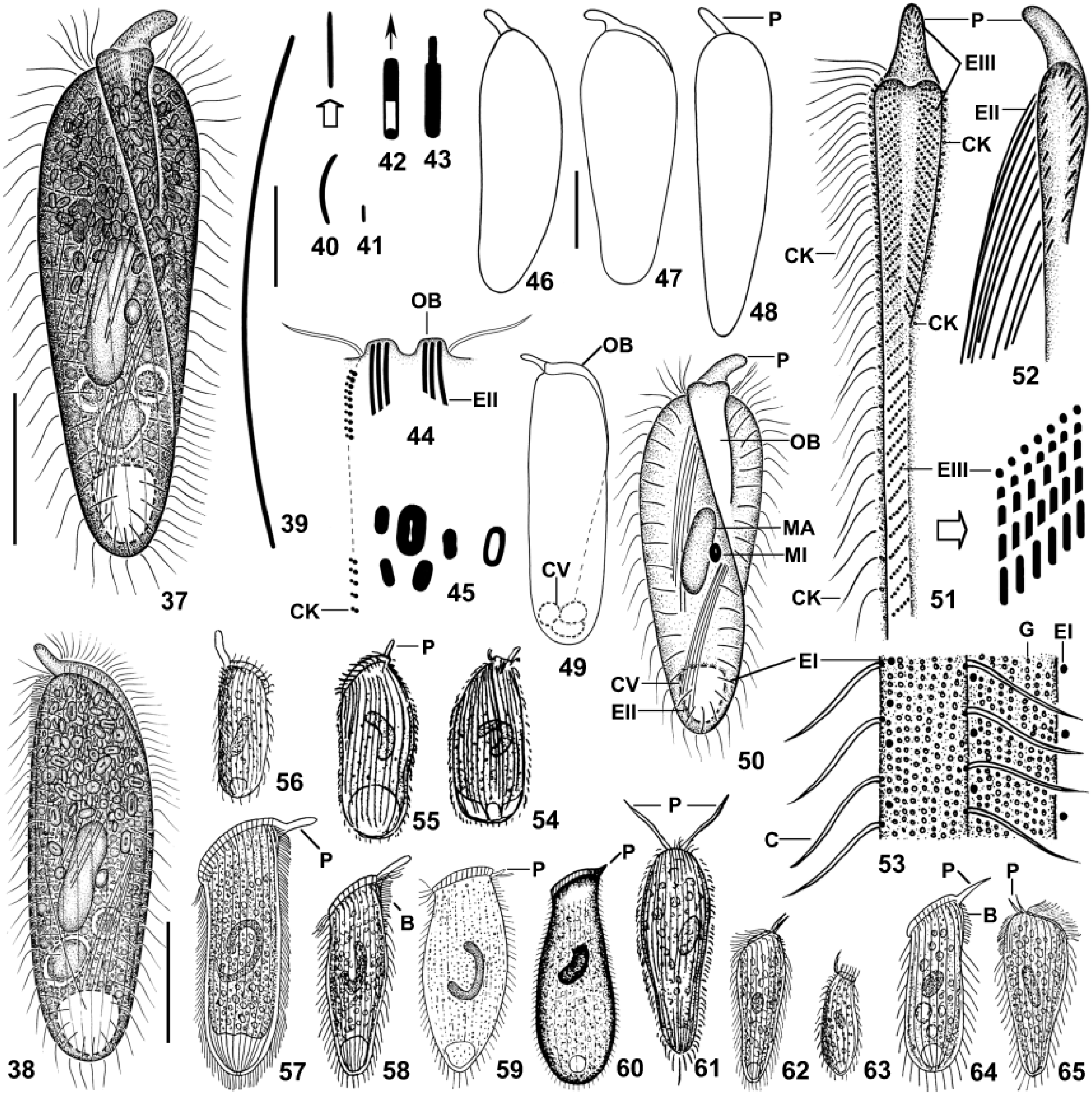


Fig. **37–65.** Drawings of *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb., *Rhinothrix barbatula* (Penard, 1922) n. comb., and *Rhinothrix antennata* (Kahl, 1926) n. comb. **37–60.** *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb. from life (37–42, 44–60) and after silver carbonate impregnation (43). (37–53) from this study, (54, 55) from Penard (1922), (56) from Kahl (1926), (57) from Kahl (1930a), (58) from Kahl (1930b), (59) from Majejkajte (1977), and (60) from Lokot (1987). **37, 38, 50.** Ventral and right side views of representative specimens, length about 100 µm. Usually, cells are dark due to refractive inclusions. **39.** Type II oral bulge extrusome, length about 50 µm. **40.** Type I (body) extrusomes attached and detached from cortex, length about 7 µm. **41.** Type III oral bulge extrusome, length 1.5 µm. **42.** Exploding type III extrusome. **43.** Flask-shaped type III extrusome from cytoplasm, length 2 µm. **44.** Optical section of oral bulge near dorsal end. Circumoral kinety (CK) from silver carbonate-impregnated specimens. **45.** Refractive cytoplasmic inclusions, length 2–6 µm. **46–49.** Dorsal (46, 48) and right side (47, 49) views showing shape and size variability. **51.** Ventral view of anterior half of oral bulge; right part of bulge when seen obliquely to show the oblong type III extrusomes arranged in many short, oblique rows. Note that the oral palpus also contains type III extrusomes and thus is an elongation of the oral bulge. **52.** Ventral view of anterior third of oral bulge showing the location of the 50-µm-long type II extrusomes not illustrated in full length. **53.** Surface view showing cortical granulation and refractive ''pearls'' caused by the type I (body) extrusomes. **54, 55.** Left side and dorsal view, length 75–125 µm. **56–58.** Right and left side views, length 60–150 µm. **59, 60.** Original figures or poor redrawings of specimens shown in 55–58, length not given. **61, 62, 65.** *Rhinothrix barbatula* (Penard, 1922) n. comb. (61) from Penard (1922), (62) from Kahl (1930b), and (65) from Kahl (1930a), lengths 95, 120, and 120 µm, respectively. This species has two oral palps. **63, 64.** *Rhinothrix antennata* (Kahl, 1926) n. comb. (63) from Kahl (1926) and (64) from Kahl (1930a), lengths 40–90 µm. The acute oral palp

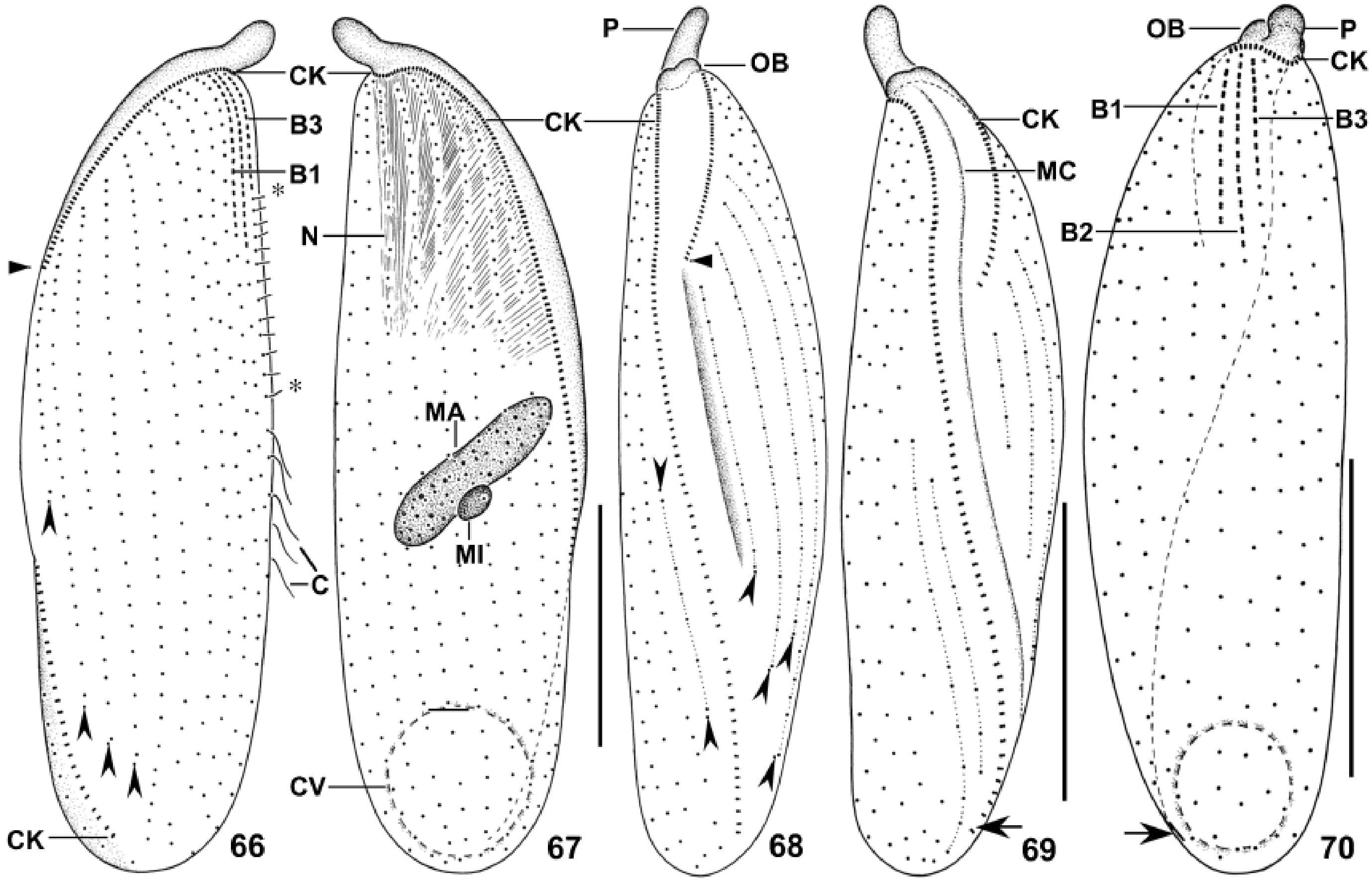


Fig. **66–70.** *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb., somatic and oral ciliary pattern after protargol impregnation. The circumoral kinety (CK) is sigmoidal and extends spirally to the posterior end (arrows, 69, 70). The somatic kineties, in contrast, extend meridionally. This is achieved by the insertion of some shortened kineties (arrowheads) in the curves of the circumoral kinety. Basically, the circumoral kinety (CK) extends the whole length of the body, but the left branch is much shorter (last kinetid marked by triangles) causing its lasso-like shape. **66, 67.** Left and right side view of a representative specimen. Asterisks mark bristle tail of brush row 3. **68, 69.** Ventral views. **70.** Dorsal view showing the inconspicuous, isostichad dorsal brush composed of dikinetids (B1–3). The hatched line marks the course of the circumoral kinety on the opposite side. B1–3, dorsal brush rows; C, somatic cilia; CK, circumoral kinety; CV, contractile vacuole; MA, macronucleus; MC, mouth cleft; MI, micronucleus; N, nematodesmata (oral basket rods); OB, oral bulge; P, palpus oralis. Scale bars = 30 μm, 66–68 drawn to scale.

circumoral kinety, forming a rather conspicuous oral basket (Table 2 and Fig. 38, 51, 66–69, 85, 89–91).

**Occurrence and ecology.** We found *R. porculus* in the acidic (pH ~ 5) mud of the effluent brook of the Simmelried mire near the village of Hegne, that is, in the surroundings of the town of Constance, Germany, about 250 km NE of Geneva, the supposed type locality. One of us (M. K.) has investigated this small (diam. < 1 km), eutrophic mire for some years, but found *R. porculus* only at two sites, viz., in the effluent mud described above and in an about 70-cm-wide puddle filled with *Sphagnum* mud to a depth of over 2 m. When collected in early October 2004, no *R. porculus* was recognized in the sample. However, after 1 week standing at room temperature ( ~ 21 °C), the species became rather abundant, that is, there were 30–50 specimens/ml. In the sampling jar, *R. porculus* inhabited the about 5-mm-thick mud layer, together with various microaerobic (i.e. *Spirostomum*, *Pseudoblepharisma*) and anaerobic (i.e. *Metopus* spp.) ciliates and a small *Oscillatoria* mat. When isolated on a slide, *R. porculus* survived for some hours but then died, suggesting that it is microaerobic.

There are few records of *R. porculus*, and all are from Europe and Asia; only three are substantiated by figures. Basically, misidentification of *R. porculus* is unlikely due to the highly characteristic palpus oralis (Fig. 37, 38, 54–58). However, there are probably similar, not yet described species/genera, such as *Apertospathula verruculifera*, where the palpus is associated with the dorsal brush (Fig. 1). Unfortunately, Penard (1922) did not provide information on where he discovered *R. porculus* (Fig. 54, 55); likely it was a moorland puddle in the surroundings of Geneva, Switzerland. Kahl (1926, 1930a, b) found it several times in sapropelic mud in the surroundings of Hamburg, Germany (Fig. 56–58). He mentioned that the species is common but never abundant and often contains rhodobacteria, which, however, could come from prey ciliates. Majejkajte (1977) reported *R. porculus* from the European part of Russia and illustrated the species (Fig. 59), but did not give the source; possibly, it is a poor redrawing of Penard's or Kahl's illustrations. The same is true for the report of Lokot (1987) from freshwater of the Baikal area, Russia (Fig. 60).

distinguishes this species from *R. porculus* shown in 54–58. B, dorsal brush; C, cilia; CK, circumoral kinety; CV, contractile vacuole; EI–III, extrusome types; G, cortical granules; MA, macronucleus; MI, micronucleus; OB, oral bulge; P, palpus oralis. Scale bars = 30 μm (37, 38, 46–49 drawn to scale) and 10 μm (39–41 drawn to scale).

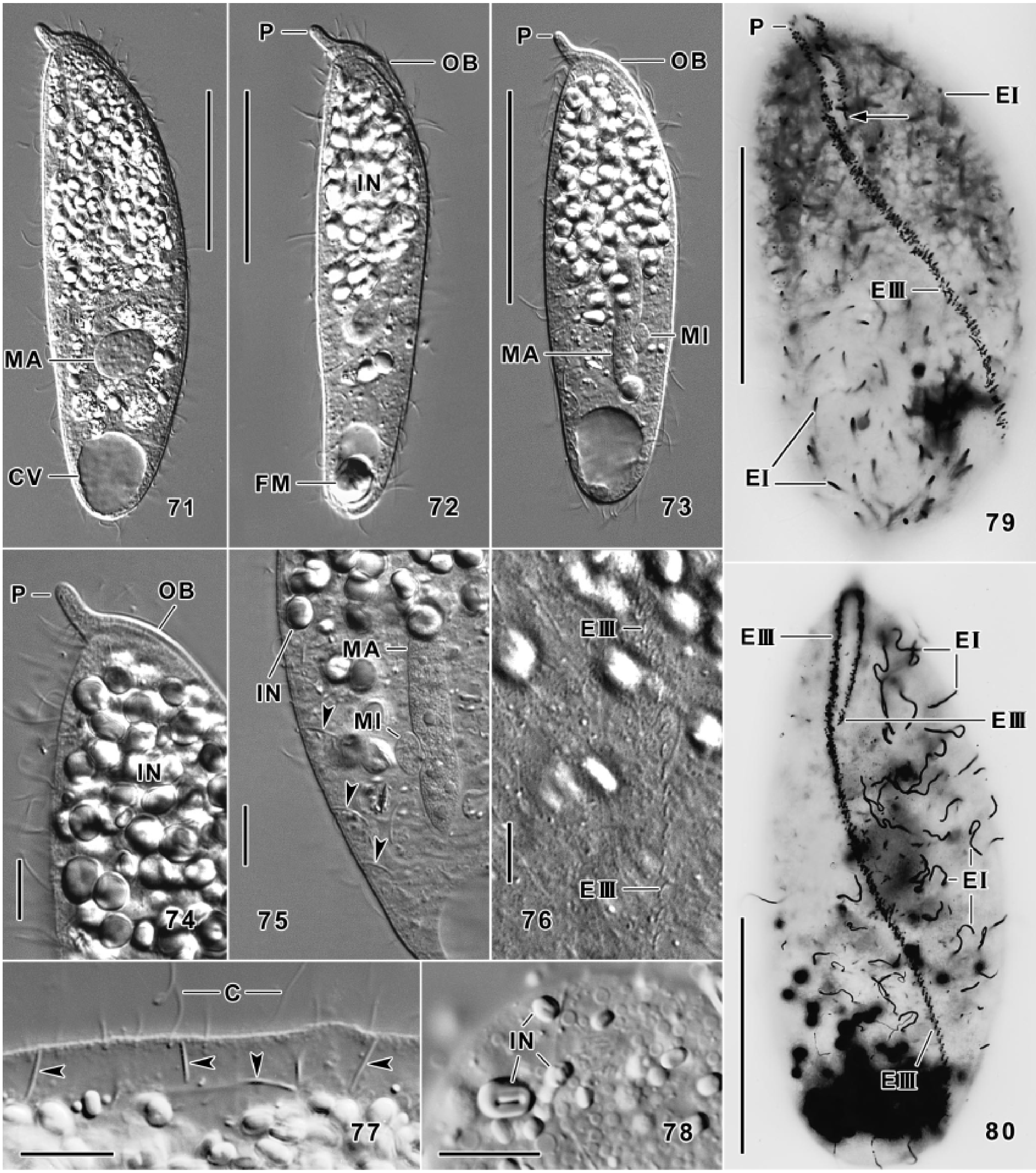


Fig. **71–80.** *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb., general organization from life (71–78) and after silver carbonate impregnation (79, 80). **71–74.** Right side overviews of freely motile specimens showing shape variability, the conspicuous oral palp (P), the bright inclusions (IN) in anterior half of the body, and the indistinctness of the oral bulge (OB) and the extrusomes. **75, 77.** Optical sections showing the nuclear apparatus and the about 7-μm-long body extrusomes (arrowheads). **76.** Ventral view of middle body portion showing the short rows formed by the type III oral bulge extrusomes. **78.** Bright cytoplasmic inclusions. **79, 80.** Ventral views showing the body extrusomes (EI) and the spiral organization of the oral apparatus, whose lasso-like shape is imaged by the minute, oblique rows formed by the type III extrusomes. C, somatic cilia; CV, contractile vacuole; EI-III, extrusome types; FM, fecal mass; IN, bright inclusions; MA, macronucleus; MI, micronucleus; OB, oral bulge; P, palpus oralis. Scale bars = 40 μm (71–73, 79, 80) and 10 μm (74–78).

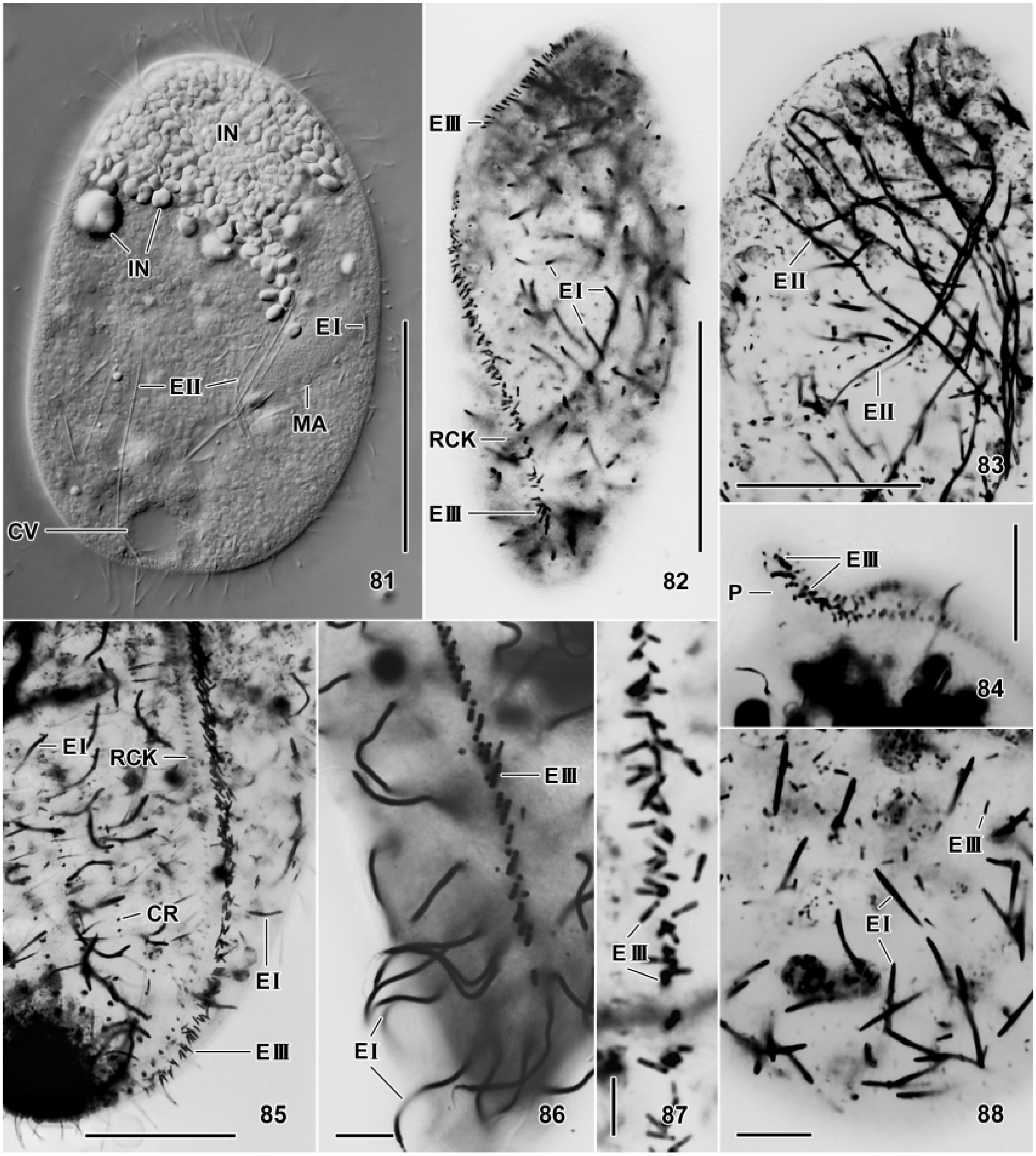


Fig. **81–88.** *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb., extrusome apparatus from life (81) and after silver carbonate impregnation (82–88). *Rhinothrix porculus* has three types of rod-shaped extrusomes recognizable in vivo and in silver carbonate preparations. Type I extrusomes are attached to the somatic cortex and about 7 μm long; when exploding, they become acicular (85, 88) and contorted when fully exploded (86). Type II extrusomes are about 50 μm long and restricted to the loop-like part of the oral bulge (81, 83). Type III extrusomes are only 1.5 μm long and form many short rows imaging the course of the oral bulge (82, 84–88). **81, 83.** Squashed specimens showing type II extrusomes and bright inclusions. **82.** Left side overview. **84.** The oral palp contains type III extrusomes. **85–87.** Stripe formed by type III extrusomes in posterior half of body. **88.** Exploding type I extrusomes are acicular. CR, ciliary rows; CV, contractile vacuole; EI–III, extrusome types; IN, bright inclusions; MA, macronucleus; P, palpus oralis; RCK, right branch of circumoral kinety. Scale bars = 40 μm (81, 82), 20 μm (83, 85), 10 μm (84, 86, 88), and 4 μm (87).

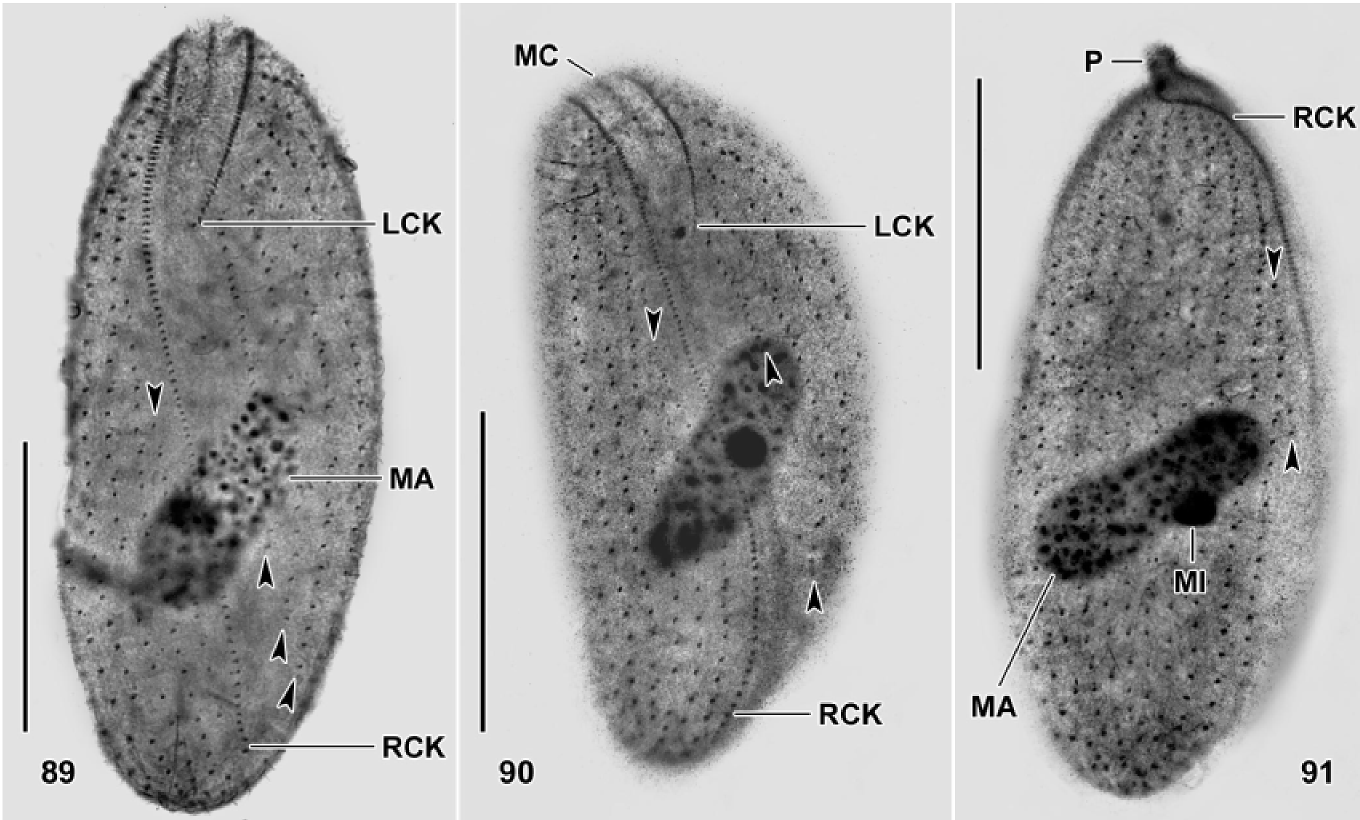


Fig. **89–91.** *Rhinothrix porculus* (Penard, 1922) n. gen., n. comb., somatic and oral ciliary pattern after protargol impregnation. The left branch of the circumoral kinety (LCK) is distinctly shortened, while the right branch of the circumoral kinety (RCK) makes a sigmoidal curve, extending spirally to the posterior body end. The somatic kineties, in contrast, extend meridionally. This pattern is achieved by the insertion of some shortened kineties (arrowheads) in the curves of the circumoral kinety. **89, 90.** Ventral views. **91.** Right side view showing the oral palp and the nuclear apparatus. LCK, left branch of circumoral kinety; MA, macronucleus; MC, mouth cleft; MI, micronucleus; P, palpus oralis; RCK, right branch of circumoral kinety. Scale bars = 40 µm.

Further reports (all without illustrations): bottom mud of the Elbe River in Hamburg, Germany (Roy 1938), especially in organically enriched mud above the bottom and in organically enriched bottom sand (Bartsch and Hartwig 1984). Grabacka (1971) found *R. porculus* in the mud of 7 out of 10 fish-rearing ponds in Poland, both in fertilized and unfertilized ponds. Chorik and Vikol (1973) observed *R. porculus* in the bottom mud of cooling plants in Moldavia and classified it as an α-mesosaprobic indicator species (Vikol and Choric 1994). Oleksiv (1985) and Babko and Kovalchuk (1992) reported *R. porculus* from some ponds in the Ukraine. Alekperov (1988) found *R. porculus* in 9 out of 12 freshwater sites from Azerbaidjan and calculated a biomass of 0.0005 g for $10^6$ individuals (Alekperov, Asadullayeva, and Zaidov 1996). However, this biomass is much too low and likely caused by a calculation error ($10^6$ specimens of *Cyclidium glaucoma* have a biomass of about 1 mg).

## DISCUSSION

**The new family Apertospathulidae.** The genera *Apertospathula*, *Longispatha*, and *Rhinothrix* are classified into a new family, the Apertospathulidae, characterized by a loop-like oral bulge and circumoral kinety, a unique feature not found in any of the described haptorid and spathidiid families. The Apertospathulidae fits into the subclass Haptoria and the order Spathidiida, as defined by Corliss (1979) and Foissner and Foissner (1988). Briefly, the Spathidiida have a somatic ciliature with dorsal brush and a toxicyst-containing oral bulge surrounded by a dikinetidal circumoral kinety, which is associated with nematodesmata forming the oral basket. Further, *Rhinothrix* and *Apertospathula* were originally classified in the family Spathidiidae (Foissner et al. 2002; Kahl 1930a, b). Indeed, *Apertospathula* resembles small *Spathidium* species, except for the ventrally opened circumoral kinety. In contrast, *Longispatha* and *Rhinothrix* resemble the spathidiid families Bryophyllidae and Perispiridae because the oral bulge and the circumoral kinety basically extend the whole length of the body (Foissner and Lei 2004; Wirnsberger, Foissner, and Adam 1984). However, the Bryophyllidae and Perispiridae have a complete oral bulge and circumoral kinety, while the left half of the oral bulge and circumoral kinety is distinctly shortened in the Apertospathulidae, which thus possibly evolved from a *Bryophyllum*-like ancestor. This appears comprehensible in *Longispatha* and *Rhinothrix*, while *Apertospathula* spp. look similar to *Spathidium* spp. Thus, we cannot exclude the fact that *Apertospathula* evolved from a different ancestor and the family Apertospathulidae is biphyletic.

The sigmoidal oral apparatus of *Rhinothrix* resembles that of *Perispira*, another aberrant spathidiid genus (Wirnsberger et al. 1984). However, *Perispira* has not only a complete oral bulge and circumoral kinety but also spiralized somatic kineties, while those of *Rhinothrix* extend meridionally. This is achieved by the insertion of some shortened kineties in the curves of the bulge (Fig. 66–69), and suggests a different evolutionary history of the two genera.

**The new genera *Longispatha* and *Rhinothrix*.** *Longispatha* and *Rhinothrix* are very distinct genera differing from each other by the somatic ciliary pattern (without versus with shortened ciliary rows along the ventral portion of the oral bulge), the course of the oral bulge (meridional versus spiral), and the palpus oralis (absent versus present). As concerns *Longispatha*, there is a single species with the same organization in the literature, viz., *Apertospathula dioplites*. This species, which will be transferred to *Longispatha* in the diagnostic section, was described by Foissner et al. (2002), who mentioned that it likely represents a distinct genus.

*Rhinothrix porculus* was classified into *Legendrea* by Penard (1922) and into *Spathidium* by Kahl (1930a, b). Today, *Legendrea* is confined to species with extrusomes in tentacle-like body processes (Curds 1982; Kahl 1930a, b), and *Spathidium* comprises species with uniform somatic ciliature and complete oral bulge and circumoral kinety (Foissner 1984; Foissner et al. 2002). Thus, *Legendrea porculus*, which has shortened ciliary rows, lasso-like oral structures, and a palpus oralis, requires the new genus *Rhinothrix*. Two other species have distinct dorsal processes, viz., *Holophrya barbatula* and *Spathidium antennatum* (Kahl 1926, 1930a, b; Penard 1922). Thus, they will be transferred to *Rhinothrix* in the diagnostic section. Unfortunately, both species are poorly known, and we cannot exclude the fact that their processes are not formed by the oral bulge as in *R. porculus*, but by the dorsal brush cortex as in *Apertospathula verruculifera* (Fig. 61–65).

**The new species *Apertospathula verruculifera* and *Longispatha elegans*.** *Apertospathula verruculifera* differs from the congeners and *Spathidium* species sensu lato by the following combination of features: obovate body; ellipsoidal macronucleus; minute, oblong extrusomes; the structure of the dorsal brush, especially the palpus dorsalis; and the cortex bolsters (Foissner et al. 2002; Kahl 1930b).

*Longispatha elegans* differs from the single congener, *L. dioplites* (Foissner, Agatha, and Berger 2002), by body shape ($\sim$ 6.5:1 versus 3.7:1) and the extrusomes (one versus two types). There is no *Spathidium* species sensu lato in the literature that could be identical with *L. elegans*.

**Identification of *Rhinothrix porculus*.** Our live observations (Fig. 37–53, 71–78) differ markedly from those of Penard (Fig. 54, 55) and Kahl (Fig. 56–58), suggesting that the Simmelried population could be a different species or even genus. Neither Penard (1922) nor Kahl (1926, 1930a, b) mentioned that the rather distinct oral bulge extends spirally to the posterior end, although this is easily seen when the specimens slowly rotate about the main body axis. Likewise, they overlooked (?) the highly differentiated extrusome pattern, although Kahl (1930b) described 6–8-μm-long, rather thick oral bulge extrusomes and Penard (1922) mentioned minute pearls in the ciliary rows, possibly marking the somatic extrusomes present in our population. Further, neither Penard nor Kahl mentioned the conspicuous accumulation of bright inclusions in the anterior body half. It is difficult to believe that both Penard (1922) and Kahl (1926, 1930a, b) overlooked all these distinct features. Our identification is thus based on body size and shape, the conspicuous palpus oralis, and the assumption that Penard and Kahl studied the species superficially because of the highly characteristic oral palp. Further studies are necessary to validate or falsify our identification. Considering the high diversity of *Spathidium*-like ciliates, we would be not surprised to find a species matching the descriptions of Penard and Kahl. Thus, we do not neotypify *Spathidium porculus* with our population.

## Diagnoses

The diagnoses are adapted to fit a forthcoming monograph of the spathidiids (Foissner and Xu, manuscript submitted).

### Subclass Haptoria Corliss, 1979
### Order Spathidiida Foissner and Foissner, 1988
### Apertospathulidae n. fam.

**Diagnosis.** Spathidiida with lasso-shaped oral bulge and circumoral kinety open ventrally.

**Type genus.** *Apertospathula* Foissner, Agatha, and Berger, 2002.

### *Apertospathula* Foissner, Agatha, and Berger, 2002

**Improved diagnosis.** Indistinctly spatulate, flexible Apertospathulidae with brush located dorsally or slightly dorsolaterally; individual brush rows without or with very short anterior tail of ordinary cilia. Mouth and circumoral kinety (oral bulge) straight and distinctly shorter than body proper; right branch of circumoral kinety slightly longer than left.

**Type species (by original designation).** *Apertospathula inermis* Foissner, Agatha, and Berger, 2002.

**Etymology.** Composite of the Latin nouns *apertum* (open field) and *spatha* (spatula), and the diminutive suffix *ula*, referring to the ventrally opened circumoral kinety and the similarity to small species of the genus *Spathidium*. Feminine gender.

### *Apertospathula verruculifera* n. sp.

**Diagnosis.** Size about 50 × 25 μm in vivo. Obovate to clavate with oral bulge occupying about one-third of trunk width; palpus dorsalis between anterior ends of brush rows 1 and 2. Macronucleus broadly ellipsoidal. Extrusomes oblong, about 2 μm long. On average 11 ciliary rows and ca 25 circumoral dikinetids. Dorsal brush three-rowed with bristles up to 1 μm long; on average six dikinetids in row 1, seven in row 2, and four in row 3.

**Type locality.** Saline mud and soil from flooded grassland in the Maracay National Park, north coast of Venezuela, W 68° N10°30′.

**Etymology.** The Latin adjective *verruculifera* (bearing a wart) is composed of the noun *verruca* (wart) and the verb fero (*bearing*), referring to a main feature of the species, viz., the minute palp between dorsal brush rows 1 and 2.

### *Longispatha* n. gen.

**Diagnosis.** Indistinctly spatulate, flexible Apertospathulidae with brush located dorsally; individual brush rows without anterior tail of ordinary cilia. Mouth (oral bulge) meridionally extending to near posterior body end. Right branch of circumoral kinety about as long as body, left branch distinctly shorter extending in anterior half of body.

**Type species.** *Longispatha elegans* n. sp.

**Etymology.** Composite of the Latin adjective *longa* (long) and the Latin noun *spatha* (spatula), referring to the long, spatula-like oral bulge. Feminine gender.

**Species assignable.** *Longispatha dioplites* (Foissner, Agatha, and Berger, 2002) n. comb. Basionym: *Apertospathula dioplites* Foissner, Agatha, and Berger, 2002.

### *Longispatha elegans* n. sp.

**Diagnosis.** Size about 100 × 18 μm in vivo. Narrowly to very narrowly spatulate, right oral bulge half projects sail-like in anterior third. Macronucleus oblong. Extrusomes narrowly ovate, about 4 × 0.8 μm in size. On average, 7 somatic kineties and 43 circumoral dikinetids. Dorsal brush three-rowed, rows 1 and 2 each composed of eight dikinetids, row 3 of four dikinetids on average.

**Type locality.** Field (Mahada) soil in the surroundings of the village of El Sapo and the Orinoco River, that is, about 50 km north of Pto. Ayachuco, Venezuela, W75° N6°.

**Etymology.** The Latin adjective *elegans* refers to the elegant appearance of the species.

*Rhinothrix* n. gen.

**Diagnosis.** Indistinctly spatulate, flexible Apertospathulidae with brush located dorsally; individual brush rows with short anterior tail of ordinary cilia. Some shortened ciliary rows along the right and left side of right branch of circumoral kinety covering the bare, triangular fields caused by the spiral course of the oral bulge. Mouth (oral bulge) spirally extending to or near the posterior end, dorsal bulge end elongated to a short process (palpus oralis). Right branch of circumoral kinety about as long as body, left branch distinctly shorter extending in anterior half of body.

**Type species.** *Rhinothrix porculus* (Penard, 1922) n. comb. Basionym: *Legendrea porculus* Penard, 1922. Combination: *Spathidium porculus* (Penard, 1922) Kahl, 1930.

**Etymology.** Composite of the Greek nouns *rhino* (nose) and *thrix* (hair, meaning cilia senus lato), meaning a ciliate with a nose-like process. Feminine gender.

**Species assignable.** *Rhinothrix antennata* (Kahl, 1926) n. comb. Basionym: *Spathidium antennatum* Kahl, 1926. *Rhinothrix barbatula* (Penard, 1922) n. comb. Basionym: *Holophrya barbatula* Penard, 1922. Combination: *Spathidium barbatula* (Penard, 1922) Kahl, 1930.

*Rhinothrix porculus* (Penard, 1922) n. comb.

**Improved diagnosis.** Size about $100 \times 30\,\mu m$ in vivo. Obovate to oblong with about 10-μm-long, cylindroidal palpus oralis. Macronucleus oblong; micronucleus broadly ellipsoidal. Three types of basically rod-shaped extrusomes: type I along ciliary rows, curved, about $7 \times 0.5\,\mu m$; type II associated with anterior loop of circumoral kinety, about $50 \times 0.5\,\mu m$; type III forms oblique rows in oral bulge and oral palp, oblong, about $1.5 \times 0.3\,\mu m$. On average, 21 meridionally arranged ciliary rows. Dorsal brush with up to 5-μm-long bristles, row 1 composed of 15 dikinetids, row 2 of 19, and row 3 of 12 dikinetids on average. Left branch of circumoral kinety extends in anterior quarter of cell.

**Type locality.** Not given; likely a moorland puddle in the surroundings of Geneva, Switzerland, E6° N46°.

**Etymology.** Not given in the original description. The Latin noun *porculus* (pig) probably refers to the pig snout-like palpus oralis and a sapropelic habitat.

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