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Two vicariant *Semispathidium* species from tropical Africa and central Europe: *S. fraterculum* nov. spec. and *S. pulchrum* nov. spec. (Ciliophora, Haptorida)

Wilhelm Foissner[a,*], Sebastian Hess[a], Khaled Al-Rasheid[b]

[a]*Universität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria*
[b]*King Saud University, Department of Zoology, Riyadh, Saudi Arabia*

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Abstract

Using standard methods, we describe two new *Semispathidium* species from semiterrestrial habitats of tropical Africa and central Europe. *Semispathidium fraterculum* nov. spec. and *S. pulchrum* nov. spec. differ from each other and from two Namibian (Southwest African) congeners by body size, the shape and location of the extrusomes; the shape of the macronucleus, the number of ciliary rows, the number of cilia within the rows, and the number of dikinetids comprising the dorsal brush rows. The globular resting cyst of *S. fraterculum* is unique in having countless granules on the inside of the external layer, forming sharp-angled rows. The distribution of these and two further, not yet described south African species indicates that the genus *Semispathidium* evolved in subtropical Africa. *Semispathidium fraterculum* and *S. pulchrum* are highly similar, both morphologically and ecologically, differing in mainly one important feature: the shape and location of the extrusomes used for prey capture. This suggests that they evolved from a common ancestor whose areal was divided by a vicariant event, causing an independent evolution of the split populations. We argue that this and other species pairs indicate that the vicariance speciation model is applicable to protists.
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Keywords: Biodiversity; *Enchelyodon*; Resting cysts; Soil; *Spathidium*; Vicariant speciation model

Introduction

Much skill, careful live observation, and protargol impregnation are required for the identification of haptorid ciliate genera and species because most relevant features are rather inconspicuous, usually recognizable only with high power oil immersion objectives and/or scanning electron microscopy (Foissner and Xu 2007; Foissner et al. 2002). This applies also to the two new species described here. At first glance, they appear as typical members of *Enchelyodon*, an "old" genus with distinct oral bulge, a dikinetidal circumoral kinety, and meridional ciliary rows (for a review, see Kahl 1930; modern descriptions, see Foissner et al. 2002, 2008b). However, on more detailed analysis, the ciliary rows do not extend meridionally but are curved anteriorly, as in *Spathidium* (Foissner and Xu 2007). Foissner et al. (2002) classified such "enchelyodonid spathidiids" in a new

*Corresponding author. Tel.: +43 662 8044 5615; fax: +43 662 8044 5698.
E-mail address: wilhelm.foissner@sbg.ac.at (W. Foissner).

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genus, *Semispathidium*. However, it is unknown whether *Semispathidium* belongs to the enchelyodonids or spathidiids.

Although being from quite different biogeographic regions, viz., tropical Africa and central Europe, the two new species are very similar, differing mainly in one feature: the shape and location of the extrusomes used for prey capture. This suggests that they evolved from a common ancestor, whose areal was divided, causing an independent evolution of the split populations. This kind of allopatric speciation has been termed "vicarism" (Lincoln et al. 1982) and was rarely taken into account in the discussion on protist distribution and biogeography (for reviews, see Foissner 2006, 2007). The two new species described here provide a good opportunity to investigate whether the vicariance model is applicable to protist speciation.

Materials and Methods

The origin of the material is provided in the individual species descriptions. Basically, both species are from ephemeral, semiterrestrial habitats, i. e., from floodplain mud and soil. The samples were air-dried for at least one month and then stored in plastic bags. Later, they were investigated with the non-flooded Petri dish method, as described by Foissner et al. (2002). Briefly, this simple method involves placing 20–500 g soil in a Petri dish (13–18 cm wide, 2–3 cm high) and saturating, but not flooding it, with distilled water. These cultures were analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28. The descriptions of the new species were based on material obtained from such cultures, i. e., no pure cultures could be established. Unfortunately, both species were very rare, leaving no material for a molecular analysis.

Morphological and presentation methods followed Foissner (1991) and Foissner et al. (2002). Terminology is according to Corliss (1979) and the refinements introduced by Foissner and Xu (2007).

Results

Description of *Semispathidium fraterculum* Foissner and Al-Rasheid nov. spec. (Figs 1–35, Table 1)

Diagnosis: Size about 170 × 30 μm in vivo. Cylindroidal with conspicuous, oblique oral bulge about about 15 × 5 μm in size. Macronucleus a moderately nodulated, tortuous strand. Two size-types of oral bulge extrusomes: type I filiform, slightly curved, about 20 × 0.3 μm in size; type II about 2.5 μm, oblong, form a ring in margin of oral bulge. On average 20 ciliary rows, three anteriorly modified to a heterostichad, isomorphic dorsal brush occupying an average of 19% of body length with up to 3 μm long bristles.

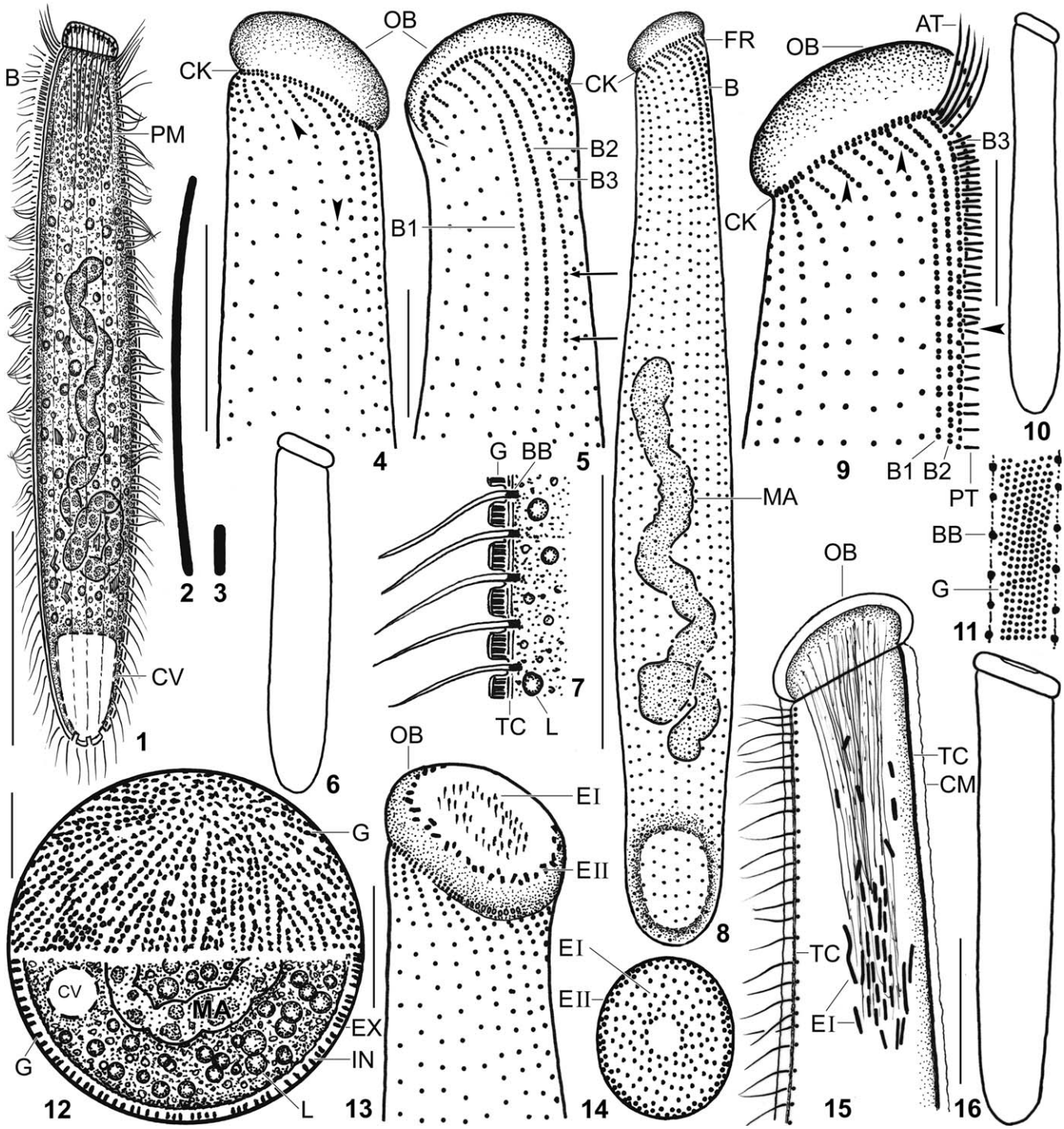
Type locality: Soil from the Chobe River floodplain, Kabolebole Peninsula, Botswana, southern Africa, E 17° 50′ S 25°.

Type material: One holotype slide and six paratype slides with protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip.

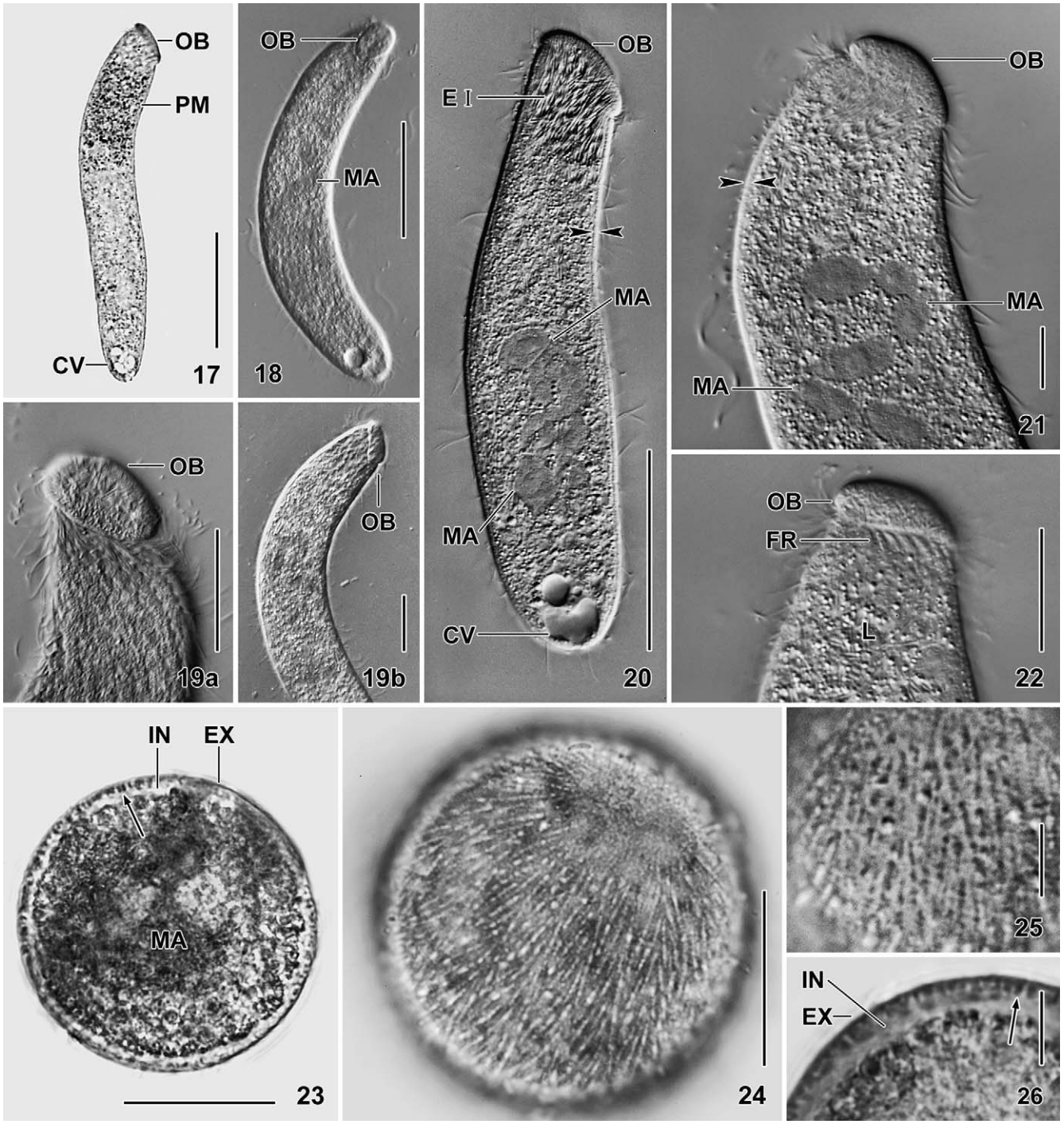
Etymology: The Latin *fraterculum* (diminutive of frater = brother) refers to the supposed vicariant relationship to *S. pulchrum*.

Description: As concerns body size and shape, see also first chapter of Discussion. Size 90–220 × 25–40 μm in vivo, usually near 170 × 30 μm, as calculated from some in vivo measurements and the morphometric data (Table 1); about 20% contractile under moderate coverslip pressure. Shape elongate ellipsoid to cylindroidal, length: width ratio 4–8:1 more or less narrowing anteriorly and posteriorly, anterior end slightly inclined ventrally, posterior rounded, not flattened laterally (Figs 1, 8, 17, 18, 20, 27, 33); rarely indistinctly funnel-shaped (Fig. 16) or slightly dumbbell-shaped (Fig. 10). Very flexible and thus difficult to immobilize for detailed investigation. Macronucleus on average between first and mid of last body third, a more or less tortuous strand with an average length of 117 μm, less distinctly nodular in protargol preparations than in vivo, where the knotty outline is conspicuous and assists in identification (Fig. 21). Nucleoli scattered, globular to irregular, numerous (Figs 1, 8, 20, 21, 27, 33). Micronuclei not unequivocally identified, possibly numerous (≥10) and only 1–2 μm across. Contractile vacuole in rear end, with several excretory pores (Figs 1, 8, 17, 20).

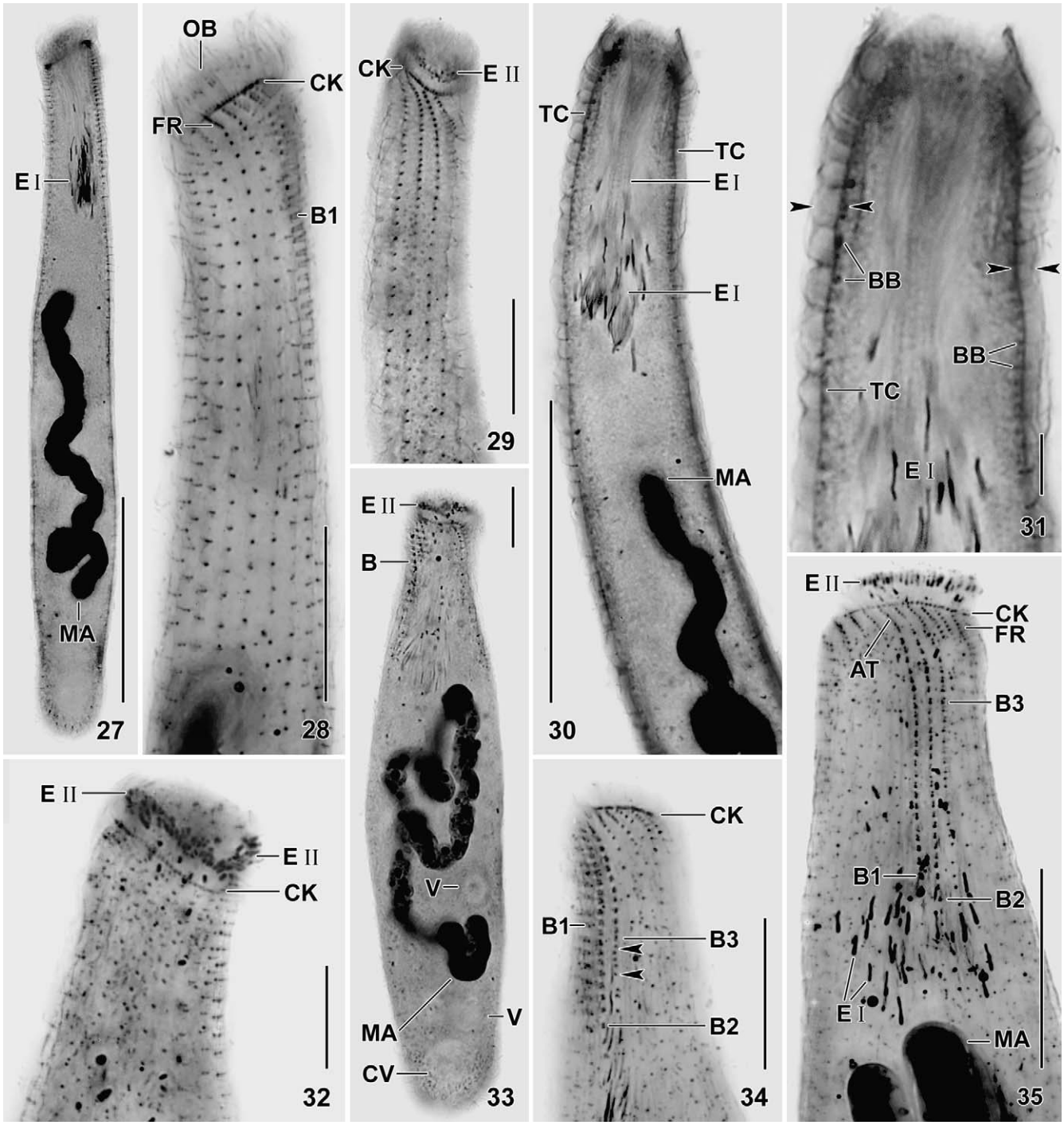
Two size-types of extrusomes, very likely toxicysts (Figs 1–3, 13–15, 27, 30, 32, 33, 35). Type I attached to main area of oral bulge, except of centre and margin; numerous, filiform and slightly curved, 15–20 μm long but merely about 0.3 μm thick, and thus distinct only at high magnification (>400x); in protargol preparations about 50 μm long (Table 1), even in the smallest individuals, indicating partial explosion during fixation; not impregnated with the protargol method used, except of a 2–8 μm long, deeply impregnating proximal portion, possibly containing the toxin; developing toxicysts fusiform, sometimes many attached to oral bulge (Fig. 20), not impregnated with the protargol method used. Type II extrusomes only about 2.5 μm long and thus difficult to recognize in vivo but, fortunately, deeply impregnating with protargol; scattered in margin of oral bulge, forming a rather thick ring



Figs 1–16. *Semispathidium fraterculum* n. sp. from life (1–3, 6, 7, 10–12, 14, 16) and after protargol impregnation (4, 5, 8, 9, 13,15). **1.** Right side view of a representative specimen, length 170 μm. **2, 3.** Type I and type II extrusomes, length 20 μm and 2.5 μm; drawn to scale. **4.** Right side view of a specimen with shortened ciliary rows (arrowheads). **5.** Dorsal brush in anterior body region. Arrows delimit narrowly spaced tail bristles of brush row 3. **6, 10, 16.** Shape variants. **7, 11.** Optical section and surface view showing the thick cortex containing densely arranged granules. **8, 9.** Left side view of holotype specimen, showing ciliary pattern and macronucleus, length 170 μm. Arrowheads mark kinetofragment-like ciliary condensations at anterior end of ciliary rows. **12.** Resting cyst in surface view (upper half) and optical section (lower half). On the inner surface of the external cyst layer are many minute rods, forming sharp-angled patterns (upper half). **13, 14.** Oblique and frontal view of oral bulge, showing the arrangement of the type I and type II extrusomes. Note slightly concave bulge centre. **15.** Optical section showing the about 2 μm thick cortex limited by the tela corticalis. AT – anterior tail of brush rows, BB – basal bodies, B (1–3) – dorsal brush (rows), CK – circumoral kinety, CM – cell membrane, CV – contractile vacuole, EI, II – extrusome types, EX – external layer, FR – kinetofragments, G – (cortical) granules, IN – internal layer, L – lipid droplets, MA – macronucleus, OB – oral bulge, PM – pharyngeal mass, PT – posterior tail of brush row 3, TC – tela corticalis. Scale bars 10 μm (Figs 5, 9, 12, 13, 15), 20 μm (Fig. 4), and 50 μm (Figs 1, 8).



Figs 17–26. *Semispathidium fraterculum* n. sp., trophic (17–22) and cystic (23–26) specimens from life under bright field (17, 23) and interference contrast (18–22, 24–26). **17, 19b.** Right side views of freely motile specimens. Note the refractive pharyngeal mass (17, PM) and the 4–5 μm high, discoidal oral bulge. **18**, **19a**. Ventral view of very slightly pressed (by coverslip) specimens, showing the broadly elliptical oral bulge. **20–22.** Moderately pressed specimens, showing, inter alia, the nodulated macronucleus, the anteriorly curved ciliary rows (22, FR), and the about 2 μm thick cortex delimited by opposed arrowheads. Most of the type I extrusomes are not fully developed, i. e., have a fusiform blister in mid (20). The oral bulge is completely deformed by the coverslip pressure, becoming strongly convex and obovate (cp. Figs 17, 19b). **23.** Optical section of a resting cyst. The cyst wall is about 2 μm thick and consists of a membranoid external layer and a hyaline internal layer. The arrow marks minute rods on the inner surface of the external layer (see also Fig. 26). **24–26.** In surface view, the cyst shows a curious, sharp-angled pattern (24, 25) made by 0.8×0.4 μm-sized granules (arrow). CV – contractile vacuole, EX – external cyst layer, EI – type I extrusomes, FR – kinetofragments, IN – internal cyst layer, MA – macronucleus, OB – oral bulge, PM – pharyngeal mass. Scale bars 5 μm (Figs 25, 26), 10 μm (Fig. 21), 20 μm (Figs 19, 22, 23, 24), and 50 μm (Figs 17, 18, 20).



Figs 27–35. *Semispathidium fraterculum* n. sp. after protargol impregnation. **27.** Optical section of holotype specimen, showing the cylindroidal body shape, the slightly nodulated macronucleus, and the partially exploded type I extrusomes having a deeply impregnated proximal portion. **28.** Left side ciliary pattern of holotype specimen. Note the obliquely truncated anterior body end and the condensed basal bodies (cilia) in the curved anterior portion of the ciliary rows. **29, 32.** Oblique ventral and right side view, showing the ciliary pattern and the minute type II extrusomes, which form a ring in the margin of the oral bulge. **30, 31.** Optical section showing the thick cortex (opposed arrowheads) and the basal bodies of the cilia underneath the deeply impregnated tela corticalis. **33.** A flask-shaped specimen with tortuous macronucleus. **34, 35.** Ciliary pattern of dorsal side in oral body portion. The dorsal brush consists of three rows of dikinetids: rows 1 and 2 have a similar length, while row 3 is distinctly shorter, but continues with a monokinetidal tail having very narrowly spaced bristles anteriorly (arrowheads). Note the comparatively long, ordinarily ciliated anterior tail of the brush rows (cp. Fig. 9). BB – basal bodies, B (1–3) – dorsal brush (rows), CK – circumoral kinety, EI, II – extrusome types, FR – kinetofragments, MA – macronucleus, OB – oral bulge, TC – tela corticalis, V – (food?) vacuoles. Scale bars 5 μm (Fig. 31), 10 μm (Fig. 32), 20 μm (Figs 28, 29, 33–35), and 50 μm (Figs 27, 30).

Table 1. Morphometric data on *Semispathidium fraterculum* (SF) and *S. pulchrum* (SP).

Characteristics [a]	Species	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	SF	153.9	152.5	20.5	4.8	13.3	125.0	195.0	18
	SP	136.7	139.0	27.7	6.2	20.3	88.0	185.0	20
Body, width in mid-body	SF	27.3	27.0	3.9	0.9	14.4	22.0	38.0	18
	SP	20.2	20.0	3.9	0.9	19.2	12.0	29.0	20
Body length: width, ratio	SF	5.8	6.0	1.2	0.3	20.1	4.0	7.6	18
	SP	8.5	8.5	1.3	0.3	14.9	6.3	10.3	20
Oral bulge, width	SF	14.3	14.0	2.0	0.5	14.0	10.0	18.0	18
	SP	12.9	13.4	2.3	0.5	18.0	8.0	16.0	20
Oral bulge, height	SF	5.6	5.0	1.2	0.3	20.7	4.0	9.0	18
	SP	3.5	3.2	0.6	0.1	16.6	3.0	5.0	20
Anterior body end to macronucleus, distance	SF	49.8	49.5	17.1	4.4	34.4	25.0	91.0	18
	SP	45.6	41.5	13.4	3.0	29.3	28.0	76.0	20
Macronucleus figure, length	SF	71.0	67.5	14.5	3.4	20.4	46.0	107.0	18
	SP	60.1	58.8	17.9	4.0	29.8	31.0	104.0	20
Macronucleus, length (spread and thus approximate)	SF	116.9	120.0	20.5	4.8	17.5	85.0	150.0	18
	SP	90.0	89.0	17.0	3.8	18.9	60.0	120.0	20
Macronucleus, width	SF	5.6	5.3	0.7	0.2	12.4	5.0	7.0	18
	SP	9.7	9.8	2.3	0.5	23.7	6.0	16.0	20
Micronuclei, length	SP	2.1	2.0	–	–	–	1.6	2.4	20
Micronuclei, width	SP	2.0	2.0	–	–	–	1.6	2.4	20
Circumoral kinety to end of brush row 1, distance	SF	28.4	30.0	4.5	1.3	15.8	21.0	38.0	12
	SP	35.1	33.6	8.5	2.0	24.3	25.0	52.0	19
Circumoral kinety to end of brush row 2, distance	SF	28.7	29.0	3.9	1.1	13.5	19.0	32.0	13
	SP	34.0	33.0	8.8	2.0	25.8	21.0	53.0	19
Circumoral kinety to end of brush row 3, distance[b]	SF	17.5	17.0	4.6	1.3	26.3	12.0	30.0	12
	SP	15.8	16.0	2.8	0.6	17.5	11.0	22.4	19
Dikinetids in brush row 1, number	SF	19.0	17.5	4.3	1.2	22.4	13.0	27.0	12
	SP	17.6	16.0	3.9	0.9	22.2	12.0	25.0	19
Dikinetids in brush row 2, number	SF	21.3	21.0	4.1	1.2	19.4	16.0	29.0	12
	SP	18.2	19.0	4.1	0.9	22.4	11.0	26.0	19
Dikinetids in brush row 3, number	SF	12.8	12.0	2.9	0.8	22.5	8.0	19.0	12
	SP	9.5	9.0	2.1	0.5	21.7	6.0	14.0	19
Ciliary rows, number	SF	20.4	20.0	1.5	0.4	7.4	19.0	25.0	15
	SP	20.8	21.0	1.6	0.4	7.7	17.0	24.0	20
Ciliated kinetids in a ventral kinety, number	SF	68.2	67.5	15.1	4.8	22.2	47.0	90.0	10
	SP	32.1	32.0	8.2	1.9	25.6	20.0	50.0	18
Anterior kinetofragments, length	SF	2.5	2.0	–	–	–	2.0	4.0	16
Anterior kinetofragments, number of cilia	SF	5.4	5.5	1.6	0.4	28.5	3.0	8.0	16
External oral basket, length	SP	14.4	15.0	2.6	0.6	17.8	9.0	19.8	20
Circumoral dikinetids, number	SP	52.4	52.5	7.5	1.7	14.4	40.0	65.0	20
Type I extrusome bundle, length	SF	48.8	50.0	4.3	1.0	8.9	40.0	55.0	18

[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, $\bar{x}$– arithmetic mean.

[b]Distance between circumoral kinety and last dikinetid of row.

(Figs 13, 14, 32, 33, 35); in one specimen many scattered throughout cytoplasm.

Cortex very flexible, conspicuous both in vivo and in protargol preparations because about 1.5 µm thick and separated from cytoplasm by a bright tela corticalis deeply impregnating with protargol (Figs 1, 7, 15, 20, 21, 30, 31); tela corticalis about 0.5 µm thick and absent from oral bulge. Cortical granules about 0.8 × 0.2 µm in size, pale and thus inconspicuous, but so densely arranged that a plate-like layer is formed; not impregnated with protargol (Figs 7, 11). Cytoplasm colourless, rather hyaline because lacking food vacuoles, contains some small, empty appearing vacuoles (Fig. 33); some crystals, possibly from prey; and rather many lipid droplets 1–2 µm across; subapically an accumulation of bright (lipid?) granules 0.5–1 µm across, similar to the pharyngeal mass of *Homalozoon vermiculare*, but less conspicuous. Food vacuoles not recognizable in vivo

and in protargol preparations, indicating lysis of prey outside of cell. Swims rapidly rotating about main body axis, very flexible wriggling between soil particles and curving around obstactles (Figs 17, 18). Dividers and conjugants not contained in the material investigated.

Somatic cilia about 10 μm long in vivo, their basal bodies deep in the cortex, i.e., underneath the tela corticalis (Figs 7, 30, 31), arranged in an average of 20 equidistant, ordinarily spaced and ciliated, meridional rows distinctly curved anteriorly and abutting on circumoral kinety; some rows shortened anteriorly or posteriorly (Fig. 4). Cilia densely spaced in oral area, especially in curved anterior portion of dorsal and lateral rows, forming more or less distinct kinetofragments, each consisting of four to eight cilia (Figs 1, 4, 5, 8, 9, 13, 28, 29, 32, 34, 35; Table 1). Dorsal brush composed of three rows with ordinarily spaced dikinetids, isomorphic and heterostichad, inconspicuous because extending only about 19 % of body length and bristles merely up to 3 μm long and as thick as ordinary cilia; all rows with distinct anterior tail composed of curved anterior portion of kineties and thus comprising four to eight monokinetids with ordinary cilia (Fig. 9). Rows 1 and 2 of similar length and composed on an average of 19 and 21 dikinetids, respectively. Row 3 distinctly shortened and composed of an average of 13 dikinetids followed by 5–10 narrowly spaced monokinetids, some of which form indistinct pairs; tail extending above mid-body with 2 μm long, rather widely spaced bristles (Figs 1, 5, 9, 28, 33–35; Table 1).

Oral bulge occupying oblique anterior body end, conspicuous because rather distinctly set off from body proper and about 5 μm high both in vivo and protargol preparations; surface in vivo basically flat but slightly screwed like a propeller blade and dorsally up to twice as high as ventrally (Fig. 32); usually distinctly convex in protargol preparations, indicating insufficient fixation; broadly elliptical to discoidal, becoming elliptical to obovate and convex under even slight coverslip pressure; contains distal portion of type I extrusomes and, around the margin, the minute type II toxicysts, as described above (Figs 1, 4–6, 8–10, 13–22, 27–29, 32, 33, 35). Circumoral kinety at base of oral bulge, slightly screwed, composed of narrowly spaced dikinetids associated with fine, short nematodesmata recognizable in only two out of 18 prepared specimens and thus not shown in the figures (Figs 4, 5, 8, 9, 27–29, 32, 34, 35).

Resting cyst: Encystment initiated by transferring specimens into a concave slide stored in a wet chamber for seven days. Cysts in vivo colourless, 40–50 μm across, on average 43.4 μm (n = 10). Wall about 2 μm thick, consists of an about 0.5 μm thick, membrane-like external layer and a bright, soft internal layer 1.5 μm thick. Inside of external layer covered with granules arranged in longitudinal rows forming curious, sharp-angled patterns (Figs 12, 23–26); granules about 0.8×0.4 μm in size, stain red with methyl green-pyronin, resemble the cortical granules of trophic cells (Fig. 26). Macronucleus unchanged, contractile vacuole still contracting from time to time. Cytoplasm densely granulated, contains many (lipid?) droplets 0.5–3 μm across (Figs 12, 23).

Occurrence and ecology: As yet found only at type locality, i.e., in the upper 5 cm soil layer from the Chobe River floodplain. Thus, it remains unknown whether *S. fraterculum* prefers terrestrial or limnetic habitats. In the non-flooded Petri dish culture, the abundance was low, indicating that it is a limnetic species.

Description of *Semispathidium pulchrum* Foissner, Hess and Al-Rasheid nov. spec. (Figs 36–62, Table 1)

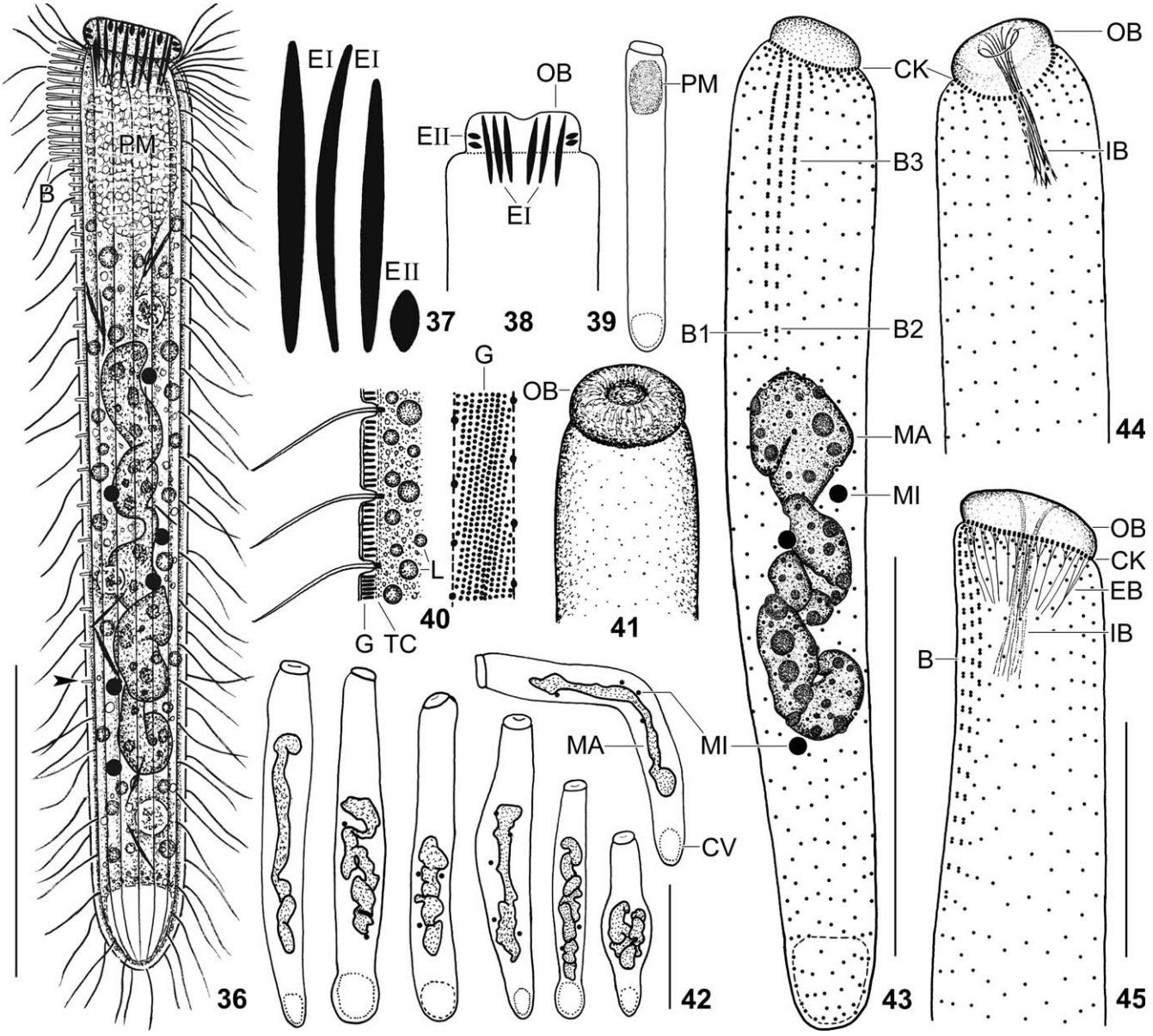
Diagnosis: Size about 150×20 μm in vivo. Cylindroidal with conspicuous, oblique oral bulge about 15×5 μm in size. Macronucleus a moderately nodulated, tortuous strand; several micronuclei. Two types of oral bulge extrusomes: type I very narrowly fusiform, about 9×0.8 μm in size, occupy main area of oral bulge; type II broadly fusiform, about 2×0.9 μm in size, form two rows in lateral lower half of oral bulge. On average 21 ciliary rows, three anteriorly modified to a heterostichad, isomorphic dorsal brush occupying an average of 25% of body length with up to 5 μm long bristles.

Type locality: Upper mud and soil layer of a shallow, ephemeral meadow puddle in the surroundings of the former Henkerhaus (house of the hangman) near the centre of the town of Salzburg, Austria, E 13° 02′ N 47° 47′.

Type material: One holotype slide and six paratype slides with protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip.

Etymology: The Latin adjective *pulchra* (beautiful) refers to the nice, crown-like oral bulge.

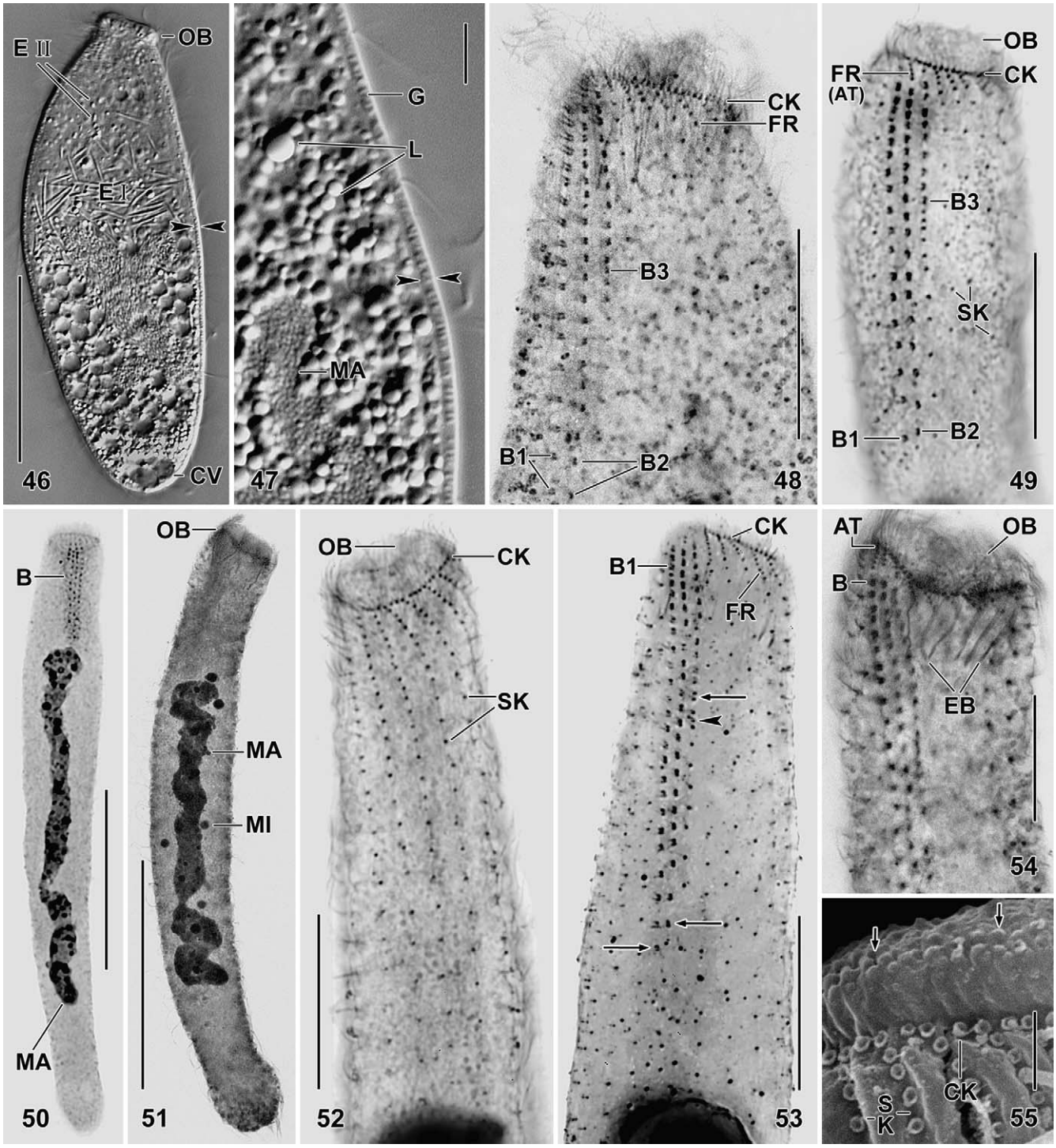
Description: As concerns body size and shape, see also first chapter of Discussion. Size 90–200 × 15–30 μm in vivo, usually near 150×20 μm, as calculated from some in vivo measurements and the morphometric data, assuming 15% preparation shrinkage (Table 1); up to 30% contractile under moderate coverslip pressure. Shape elongate ellipsoid to cylindroidal, length: width ratio 5–10:1, slightly narrowing anteriorly and posteriorly, anterior end slightly inclined, posterior rounded, not flattened laterally (Figs 39, 42, 51, 56, 58; Table 1); rarely slightly to distinctly funnel-shaped (Figs 36, 43, 50). Very flexible and thus difficult to immobilize by coverslip pressure. Macronucleus usually in third and fourth fifth of cell, cylindroidal and more or less



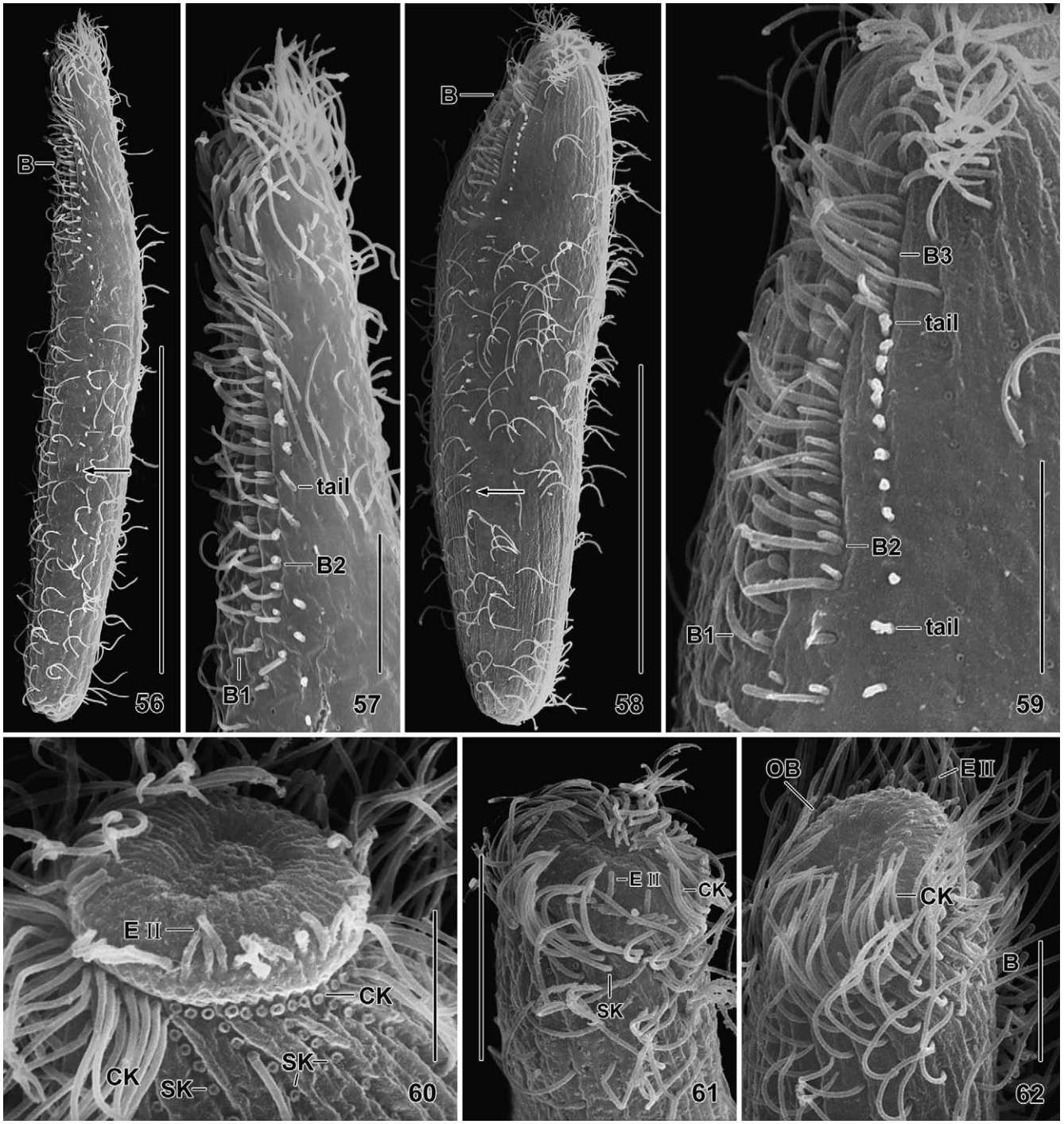
Figs 36–45. *Semispathidium pulchrum* n. sp. from life (36–41) and after protargol impregnation (42–45). **36.** Right side view of a representative specimen, length 150 μm. The arrowhead marks the end of the monokinetidal bristle tail of brush row 3. Note the fairly conspicuous pharyngeal mass (PM). **37.** Type I and type II extrusomes drawn to scale, length 9 μm and 2 μm. **38.** Semi-schematic view of extrusome arrangement. **39.** A cylindroidal specimen with fairly distinct pharyngeal mass. **40.** Optical section and surface view, showing the about 1 μm thick cortex containing very densely arranged granules, possibly mucocysts. **41.** The crown-like oral bulge is an important feature of *S. pulchrum* and *S. fraterculum* (cp. Figs 60–62), but difficult to observe. **42.** Variability of shape and size of body and macronucleus, drawn to scale. **43, 44.** Dorsal and ventral view of holotype specimen, showing the ciliary pattern, the nuclear apparatus, and the internal oral basket, length 140 μm. **45.** Right side view of anterior body portion, showing the dorsal brush as well as the inconspicuous external and the comparatively conspicuous internal oral basket. B (1–3) – dorsal brush (rows), CK – circumoral kinety, CV – contractile vacuole, EI, II – extrusome types, EB – external oral basket, G – cortical granules, IB – internal oral basket, L – lipid droplets, MA – macronucleus, MI – micronuclei, OB – oral bulge, PM – pharyngeal mass, TC – tela corticalis. Scale bars 20 μm (Fig. 45) and 50 μm (Figs 36, 42–44).

tortuous and nodulated, on average about 90 μm long, rarely twisted to a globular mass. Nucleoli scattered, globular to irregular, numerous, 0.5–4 μm in size. Several micronuclei adjacent to macronucleus, about 2 μm across and thus often difficult to distinguish from cytoplasmic inclusions (Figs 36, 42, 43, 47, 50, 51). Contractile vacuole in rear end, possibly with several excretory pores in pole area (Figs 36, 42, 43, 46).

Two types of fusiform extrusomes attached to oral bulge and scattered in cytoplasm, not or very faintly impregnated with the protargol method used (Figs 37, 38, 46, 55, 60, 61). Type I extrusomes attached to main



Figs 46–55. *Semispathidium pulchrum* n. sp. from life (46, 47), after protargol impregnation (49–54), and in the scanning electron microscope (55). **46, 47.** Squashed specimens showing the about 2 μm thick cortex (opposed arrowheads), the distinct oral bulge, and the two extrusome types. **48, 49.** Dorsal brush of a specimen each from Upper Austria and from the type locality in Salzburg, with labels marking the end of the brush rows. Fig. 48 is from a squashed, broadened specimen. **50, 51.** Variability in shape of body and macronucleus. The cell shown in Fig. 50 was originally very likely slightly conical but become narrowed anteriorly due to the preparation procedures. **52, 53.** Ventral and dorsal view of ciliary pattern. Arrows mark ends of dikinetidal portion of dorsal brush rows; row 3 continues with a monokinetidal tail having the anteriormost bristles very narrowly spaced (arrowhead). **54.** Anterior body portion, showing the external oral basket and the anterior tail of the brush rows. **55.** A deciliated specimen showing the two circles of type II extrusomes in the margin of the oral bulge (arrows). AT – anterior tail of brush rows, B (1–3) – dorsal brush (rows), CK – circumoral kinety, CV – contractile vacuole, EB – external oral basket, EI, II – extrusome types, FR – kinetofragments, G – cortical granules, L – lipid droplets, MA – macronucleus, MI – micronucleus, OB – oral bulge, SK – somatic kineties. Scale bars 2 μm (Fig. 55), 5 μm (Fig. 47), 10 μm (Fig. 54), 20 μm (Figs 48, 49, 52, 53), and 50 μm (46, 50, 51).



Figs 56–62. *Semispathidium pulchrum* n. sp. in the scanning electron microscope. **56–59.** Overviews and dorsal brush details of a cylindroidal and an elongate ellipsoidal specimen. Both cells are partially deciliated. The dorsal brush consists of three rows of dikinetidal bristles. Brush rows 1 and 2 have a similar length and the anterior bristle of the dikinetids is much shorter than the posterior one. Brush row 3 consists of a short dikinetidal portion followed by a long monokinetidal tail with ends marked by arrows; the row then continues with ordinary somatic cilia. In contrast to rows 1 and 2, the dikinetidal bristles of row 3 have the same length. **60–62.** Various views on the oral bulge, which has a more or less distinct central concavity and occupies the slightly oblique anterior end of the cell (61, 62). The margin of the bulge contains two rings of type II extrusomes (see also Fig. 55), some of which just release the bulge. A partially deciliated cell (60) shows that the circumoral kinety consists of a ring of narrowly spaced cilia; each ciliated basal body is accompanied by an unciliated one, which is not recognizable. The ciliary rows are curved anteriorly and abut on the circumoral kinety. B (1–3) – dorsal brush (rows), CK – cilia of circumoral kinety, EII – type II extrusomes, OB – oral bulge, SK – somatic kineties (ciliary rows). Scale bars 5 μm (Figs 60, 62), 10 μm (Figs 57, 59, 61), and 50 μm (Figs 56, 58).

portion of oral bulge, except of central area and margin, very narrowly fusiform and flattened on one side, in vivo conspicuous because strongly refractive and 8–10 × 0.6–0.8 μm in size. Type II extrusomes form two rough circles in lateral lower half of oral bulge, broadly fusiform and compact, only about 2 × 0.8–1 μm in size and thus easily overlooked; become minute rods when extruded (Figs 55, 60, 61).

Cortex very flexible, in vivo conspicuous because about 2 μm thick, sharply separated from cytoplasm by a tela corticalis, and containing countless rather refractive granules (Figs 36, 40, 46, 47); in protargol preparations less distinct than that of *S. fraterculum* because thinner and tela corticalis only faintly impregnated. Tela corticalis about 0.3 μm thick and absent from oral bulge. Cortical granules about 0.8 × 0.2 μm in size, moderately refractive and so densely arranged that a plate-like layer is formed (Figs 40, 47); not impregnated with protargol. Cytoplasm colourless, usually rather hyaline because lacking food vacuoles, contains bright, empty-looking vacuoles up to 5 μm across and lipid droplets 0.5–5 μm in size (Figs 46, 47); subapically an accumulation of rather refractive (lipid?) granules about 1 μm across, similar to the pharyngeal mass of *Homalozoon vermiculare*, but less conspicuous. Some specimens studded with lipid droplets (Fig. 47) and rather hyaline vacuoles, both only up to 5 μm across, indicating lysis of prey outside of cell. Swims rather rapidly by rotation about main body axis and shows great flexibility when wallowing in the mud. Dividers and conjugants not contained in the material investigated.

Somatic cilia about 10 μm long in vivo, their basal bodies rather deep in the cortex, i.e., underneath tela corticalis (Fig. 40), arranged in an average of 21 equidistant, ordinarily spaced, meridional rows distinctly curved anteriorly and abutting on circumoral kinety; cilia within rows ordinarily to loosely spaced, except of up to five densely spaced cilia in curved anterior portion, producing more or less distinct kinetofragments, especially in dorsal half of cell (Figs 36, 43–45, 48, 49, 52, 53, 56, 58, 60–62; Table 1). Dorsal brush composed of three rows with widely spaced dikinetids, isomorphic and heterostichad, in vivo moderately conspicuous because extending about 25% of body length and bristles up to 5 μm long; all rows with distinct anterior tail composed of curved anterior portion of kineties and thus comprising two to five monokinetids with ordinary cilia. Rows 1 and 2 of almost same length, each composed of about 18 dikinetids with anterior bristle of dikinetids about 2 μm long and posterior 5 μm. Row 3 about half as long as rows 1 and 2, composed of nine dikinetids an average and a long monokinetidal tail extending an average of 70% of body length; upper three to six tail bristles more narrowly spaced than those following; dikinetidal bristles both about 5 μm long in vivo, tail bristles 2 μm (Figs 36, 43, 45, 48–50, 53, 54, 56–59; Table 1).

Oral bulge occupies slightly oblique anterior body end, in vivo conspicuous because distinctly set off from body proper and about 15 × 5 μm in size; discoidal and flat with concave central area, thus forming a minute, nice crown (Figs 36, 41, 43–46, 49, 52, 60–62; Table 1); contains distal portion of type I extrusomes and, around the margin, the minute type II toxicysts, as described above (Figs 36, 38, 60, 61). Circumoral kinety at base of oral bulge, circular, composed of an average of 52 very narrowly spaced dikinetids, of which only the outer basal body is ciliated (Fig. 60). Nematodesmata inconspicuous because fine and only about 10 μm long, originate from circumoral dikinetids, form the slightly conical external oral basket (Figs 45, 54); internal basket slightly dumbell-shaped, composed of numerous, up to 20 μm long fibres originating also from the circumoral dikinetids (Figs 43–45, 48, 49, 52–55, 60–62; Table 2).

Table 2. Comparison of four *Semispathidium* species.

Characteristics	*S. enchelyodontides*[c]	*S. armatum*[c]	*S. fraterculum*	*S. pulchrum*
Size (μm)[b]	142 × 22	224 × 41	154 × 27	137 × 20
Macronucleus shape[b]	21 nodules	elongate ellipsoidal	nodulated strand	nodulated strand
Type I extrusomes, shape[a]	filiform	clavate	filiform	very narrowly fusiform
Type II extrusomes, shape[a]	oblong	lacking	oblong	broadly fusiform
Ciliary rows, number[b]	15	21	20	21
Ciliated kinetids in a ventral kinety, number[b]	45	91	68	32
Dikinetids, number in				
brush row 1[b]	11	25	19	20
brush row 2[b]	17	40	21	18
brush row 3[b]	9	14	13	9

[a]From live.
[b]From protargol preparations (Foissner's method).
[c]From Foissner et al. (2002).

Occurrence and ecology: As yet found at type locality and in Upper Austria, i. e., in the surroundings of the town of Kefermarkt. Both records are from ephemeral habitats, i.e., from mud and soil collected from the ground of ephemeral meadow pools, indicating that *S. pulchrum* is a limnetic species.

Discussion

Size and shape of *Semispathidium*

On average, both species were smaller and more frequently slightly funnel-shaped in vivo than in protargol preparations, where most (*S. pulchrum*) or all (*S. fraterculum*) specimens were elongate ellipsoid or cylindroidal. We believe that only part of these differences was caused by preparation artifacts. Possibly, the main reason was the culture (non-flooded Petri dish) method applied. When collecting specimens for live observation, the Petri dish was only slightly tilted to obtain a few drops of soil eluate. Very likely, these drops came mainly from the surface area, where soil and mud were loose and the soil pores thus large. When collecting material for protargol preparation, the Petri dish was strongly tilted several times to get as much soil eluate and specimens as possible. Thus, this sample contained much material from the deeper layers of the culture, where the soil was more compact and the pores thus narrower. Under these conditions, most specimens might have become longer, narrower, and cylindroidal.

Comparison with congeners

The genus *Semispathidium* was established by Foissner et al. (2002) for two Namibian (Southwest Africa) haptorids with circular oral bulge and spathidiid somatic ciliary pattern, i. e., with ciliary rows anteriorly curved dorsally on the right side of the body, while ventrally on its left side. The two species described here match this pattern perfectly and are distinct from the species described by Foissner et al. (2002). The seven main features separating the four species are compiled in Table 2.

A notion is required on the curious location of the type II extrusomes of *S. pulchrum* in the lower lateral half of the oral bulge (Figs 38, 55, 60). This is unique among the haptorids, suggesting a peculiar mode of hunting. The location resembles the family Pleuroplitidae Foissner, 1996, where the extrusomes form an extracytostomal bundle underneath the oral bulge on ventral side of cell.

Another remarkable difference occurs in the number of cilia in the individual rows: 68 in *S. fraterculum*, 32 in *S. pulchrum* (Tables 1, 2). Possibly, the special arrangement of the extrusomes increases preying success in *S. pulchrum,* i.e., allows to reduce swimming activity/speed. Such tendency is recognizable also in *S. armatum* (only one extrusome type; 91 cilia, 76 when "normalized" to the size of *S. enchelyodontides*) and *S. enchelyodontides* (two extrusome types, 45 cilia; Table 2).

Are *Semispathidium fraterculum* and *S. pulchrum* geographic vicariants?

Haptorid ciliates are predators, using toxicysts to immobilize and kill the prey, usually other ciliates or larger flagellates. The toxicysts are thus possibly their most important survival means, although some genera and species lost them secondarily (Foissner and Xu 2007; Oertel et al. 2008). The size, shape and arrangement of the toxicysts are highly divers (for an example, see Foissner and Xu 2007), possibly determining the prey spectrum. This applies also to *S. fraterculum* and *S. pulchrum* which, indeed, differ mainly in these features (Figs 2, 3, 13, 14, 37, 38, 55; Table 2). Other important characters, such as the shape of the macronucleus, the number of the ciliary rows, and the structure of the dorsal brush, are virtually identical (Table 2). This and the ecological similarity – both live in ephemeral habitats – are most parsimonious explained when assuming an ancestral population separated by a vicariance event, e. g., geographical isolation due to continental split.

The vicariance speciation model proposes that the components of major biota evolve together and are subject to parallel effects of geographical and climatic fluctuations, and that modern biogeographical distributions result from the division of ancestral biotas by the formation of natural barriers (Lincoln et al. 1982). Actually, vicarism can be considered as a special case of allopatric speciation and is the first important phase in the evolution of species. The spatial isolation stimulates genetic drift and race formation. Only when reproductive barriers have evolved, a sympatric occurrence of species with a common ancestor can occur (Sitte et al. 1991). There are several kinds of vicariance, e. g., geographical, ecological, and chronological (Sedlag and Weinert 1987). Typical examples of geographical vicariance are *Bison bison* (occurs in North America) and *B. bonasus* (Eurasia) as well as *Fagus* (northern hemisphere) and *Nothofagus* (southern hemisphere). See Bährmann et al. (1988), Sitte et al. (1991) and Heads (2005) for a more detailed discussion.

Although reliable distribution data on ciliates are rare (Chao et al. 2006) and 80% of their diversity seems to be undescribed (Foissner et al. 2008a), there is evidence for vicarism, i. e., for the existence of closely related and ecologically equivalent (vicars) taxa that replace each other geographically. Foissner (2007) demonstrated this

for species of the genera *Kuehneltiella* and *Apofrontonia*, but he could not determine where these genera evolved. In the case of *Semispathidium*, this seems possible: of the four species described, three are from subtropical Africa and one is from Central Europe. Two further, yet undescribed species occur in the Republic of South Africa and in Botswana. Thus, *Semispathidium* very likely evolved and radiated in southern Africa.

Much more difficult to fix is the vicariance event because genetic and fossil data are lacking and, as explained above, our knowledge on ciliate diversity and distribution is very limited. Possibly, the European *Semispathidium* evolved after the break of Pangaea (cp. Foissner 2006). This break very likely caused changes in the prey available, which might explain why *S. fraterculum* and *S. pulchrum* differ just by the toxicysts (see also above).

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