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Descriptions of *Protospathidium serpens* (Kahl, 1930) and *P. fraterculum* n. sp. (Ciliophora, Haptoria), Two Species Based on Different Resting Cyst Morphology

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ABSTRACT. *Protospathidium serpens* (Kahl, 1930) is frequent in semiterrestrial and terrestrial habitats worldwide. Conventionally, all populations are considered as conspecific because they have very similar overall morphologies and morphometrics. We studied in detail not only the morphology of the vegetative cells but also the resting cysts using live observation, protargol impregnation, and scanning electron microscopy. These revealed a cryptic diversity and biogeographic pattern in details of the dorsal brush and cyst wall morphology. The cyst wall is spiny in the Austrian specimens, while smooth in the South African and Antarctic populations. Accordingly, *P. serpens* consists of at least two species: *P. serpens* (with spiny cyst wall) and *P. fraterculum* n. sp. (with smooth cyst wall); the latter is probably composed of two distinct taxa differing by the absence (South African)/presence (Antarctic) of a monokinetidal bristle tail in brush row 3, the number of dikinetids comprising brush row 1 (seven versus three), and the total number of brush dikinetids (29 versus 17). *Protospathidium serpens* is neotypified with the new population from Austria. The significance of resting cyst morphology is discussed with respect to alpha-taxonomy and overall ciliate diversity.

Key Words. Biodiversity, biogeography, neotypification, soil ciliates, taxonomy.

MORPHOLOGICAL taxonomy of protists is limited by the general scarcity of characters, as compared to multicellular organisms. However, the potential is often not fully used, even for rather simple features, such as resting cyst morphology. Usually resting cysts can be easily obtained by isolation and starvation of some specimens in a moist chamber. We tested this feature in several spathidiids (Haptoria, Spathidiida) and found a bewildering diversity of resting cyst morphology, not only in different genera and species but also in several populations of a single species, *Protospathidium serpens* (Kahl, 1930).

Protospathidium serpens is frequent, but rarely abundant, in semiterrestrial and terrestrial biotopes worldwide and in a great variety of habitats, including highly saline sites (Foissner 1981, 1996a, b, 1998, 1999, 2000; Foissner, Agatha, and Berger 2002; Kahl 1930; Petz and Foissner 1997; Xu and Foissner 2004). For instance, it occurred in six out of 73 sites investigated in Namibia, Southwest Africa (Foissner et al. 2002) and in three out of 20 sites studied in Germany (Foissner 2000). Morphologically, all populations showed such a pronounced similarity in the ''usual'' features that they were considered as conspecific. However, we shall show that three groups can be distinguished, if more sophisticated features are included in the analysis, such as details of the dorsal brush and resting cyst morphology.

Indeed, the main goal of the present paper is to overcome the prevailing view that resting cysts are simple spheres useless for α-taxonomy. Likely, this is the main reason for the scarcity of data, as compared, for instance, to those on the pattern and number of the oral and somatic ciliary rows.

MATERIALS AND METHODS

Protospathidium serpens (Kahl, 1930) Foissner, 1981 was found in a soil sample from the meadow surrounding the Henkerhaus in the town of Salzburg, Austria. It was reactivated from dried soil, that is, from the cystic stage with the non-flooded Petri dish method, as described in Foissner (1987a) and Foissner et al. (2002).

Protospathidium fraterculum, whose conjugation was investigated by Xu and Foissner (2004), was discovered in a soil sample (pH 6.5) from the dry bed of the Mlambane River in the Kruger National Park, Republic of South Africa. A semi-pure culture was established in Eau de Volvic enriched with some drops of percolate from the non-flooded Petri dish culture and a few crushed wheat grains to stimulate growth of bacteria and prey protozoa (*Chilomonas* sp., *Cyrtolophosis mucicola*, middle-sized colpodas, and hypotrichs). Spontaneous mass conjugation and encystment occurred in the declining culture.

For comparison, a Namibian population of the *P. serpens* complex was studied. This population was found at Namibian site 16, that is, in the non-flooded Petri dish culture of mud and soil from a dry roadside ditch in the southern escarpment of the Namib Desert (see Foissner et al. 2002 for detailed site description).

In the Salzburg specimens, encystment was induced by transferring some dozens of specimens onto a microscope slide with a concave deepening containing a drop of habitat water (= soil percolate from the non-flooded Petri dish culture). The preparation was stored in a moist chamber and checked every 24 h. About half of the starved specimens encysted within 48 h; the remaining died. The cysts were studied four days after setting up the preparation. In the Antarctic and the South African populations encystment occurred in the cultures (Foissner 1996a; Petz and Foissner 1997).

Cells were studied in vivo using a high-power oil immersion objective and differential interference contrast optics. The ciliary and nuclear patterns were revealed by protargol protocol A and scanning electron microscopy (SEM) as described in Foissner (1991). As concerns scanning electron microscopy, the old preparations of the Antarctic specimens were of poor quality. However, the tail of brush row 3 was clearly recognizable. Counts and measurements on prepared specimens were performed at a magnification of 1,000×. In vivo measurements were conducted at magnifications of 100–1,000×. Drawings of live cells were based on free-hand sketches, while those of impregnated specimens were made with the help of a drawing device.

Terminology. Terminology and classification are according to Corliss (1979), Foissner and Foissner (1988), Foissner et al. (2002), and Kahl (1930). However, the ''tail of brush row 3,'' a term coined by Foissner et al. (2002), needs some explanation. The dorsal brush of most haptorids is composed of three short rows of dikinetids. Rows 1 and 2 then continue posteriorly as ordinary somatic ciliary rows, while row 3 has a more or less long ''tail'' between the dikinetidal and ordinary somatic portion. This ''tail'' is clearly defined as consisting of minute, monokinetidal bristles (Fig. 43).

RESULTS

Description of the neotype of *Protospathidium serpens* (Kahl, 1930) Foissner, 1981 (Table 1 and Fig. 1–20, 23–39).

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Size 70–130 × 12–25 μm in vivo, usually about 100 × 18 μm, as calculated from 12 in vivo measurements, which fits the protargol-prepared cells when about 10% shrinkage is considered (Table 1); length:width ratio highly variable (4.5–9.2:1 in vivo and 3.5–9.8:1 in preparations), on average near 5.5:1 both in vivo and in prepared cells. Shape also highly variable, that is, narrowly spatulate (ellipsoidal) to cylindroidal (Fig. 1, 7–9), oblique anterior (oral) end about one-third shorter than widest trunk region usually in or above mid-body; posterior end rounded, in protargol preparations frequently more or less distinctly narrowed above contractile vacuole, providing cells with a bluntly fusiform appearance; indistinctly flattened laterally (Fig. 1, 7–11, 13, 16, 23–26). Macronucleus in middle third of body, usually a tortuous, more or less nodulated strand with ends frequently inflated, rarely very long and highly tortuous, while oblong and slightly curved in one out of > 50 specimens analysed (Fig. 11); contains many nucleoli up to 5 μm long. Two to seven, usually four micronuclei about 2 μm across in variable position adjacent to or in shallow macronuclear depressions; rarely rather distant from macronucleus (Fig. 1, 10, 11, 14, 16, 26). Contractile vacuole in posterior end, with several excretory pores in pole area. Extrusomes in two to three rough circles around indistinct central depression of oral bulge (Fig. 3, 33, 39), rod shaped and slightly curved or rod shaped and inconspicuously narrowed distally, about 2.5–3 × 0.3 μm in size; anterior end occasionally distinctly impregnated, appearing as a black dot (Fig. 1–3, 13, 14, 39). Cortex very flexible, contains about six rows of minute (~ 0.2 μm), loosely arranged granules embedded in a ~ 0.7-μm-thick, gelatinous layer between each two ciliary rows (Fig. 5 and 6). Cytoplasm colorless, usually studded with lipid droplets 0.5–5 μm across and small food vacuoles (about 5 μm) with prey remnants and bacterial spores. Swims rather rapidly by rotation about main body axis.

Somatic cilia 7–8 μm long in vivo, arranged in an average of 10 equidistant, bipolar, ordinarily spaced and ciliated (average ciliary distance 3 μm) rows with cilia more densely spaced anteriorly than posteriorly. Ciliary rows attached to oral kinetofragments, anterior portion slightly curved dorsally on right side, while abutting on oral kinetofragments at right angles on left and dorsal side; frequently some bare basal bodies within kineties (Table 1 and Fig. 1, 13, 14, 16–18, 23–32). Dorsal brush located slightly dorsolaterally, dikinetidal and three-rowed, inconspicuous because occupying merely about 15% of body length and bristles only up to 3 μm long in vivo; all rows have some ordinary cilia anteriorly and continue as ordinary ciliary rows posteriorly (Fig. 23–25, 30–32). Brush row 1 composed of two to six, usually only three dikinetids, the last pair bears a 1-μm-long anterior bristle and a 7-μm-long ordinary cilium posteriorly; anterior bristle of other dikinetids about 2 μm long and slightly inflated in mid, posterior bristle knob-like, that is, only about 0.5 μm long (Fig. 4, 12, 28, 30–32). Brush row 2, longest, composed of 8–17, on average 12 ordinarily spaced dikinetids (average distance 1.1 μm; Table 1); bristles similar to those of row 1 in anterior portion, while posterior bristles up to 1 μm long; length of bristles sharply decreases posteriorly (Fig. 4, 13, 29–32). Brush row 3 slightly shorter than row 2, composed of 5–11, on average seven, comparatively loosely spaced dikinetids (average dikinetidal distance 1.5 μm; Table 1); anterior and posterior bristle of dikinetids each up to 3 μm long and sometimes spread V-like, slightly curved, and inflated distally; monokinetidal bristle tail lacking or reduced to a single, 1.5-μm-long bristle anterior to an ordinary somatic cilium, as shown by many properly impregnated specimens and the scanning electron micrographs (Fig. 4, 14, 16, 18, 29–32, 35).

Oral bulge rather distinct in vivo, though shorter by about one-third than widest trunk region, because about 10 × 7 × 5 μm in size and knob-like separated from body proper; slanted by about 30–45°, surface flat to slightly convex, contains fibers spiraling to depressed temporary cytostome (s. l.) in bulge center (Table 1 and Fig. 1, 3, 7–11, 26–29, 32–35, 39). Circumoral kinety of similar shape as oral bulge, composed of dikinetidal kinetofragments obliquely attached to ciliary rows and separated from each other by gaps two to four dikinetids wide. Individual kinetofragments composed of an average of four dikinetids each associated with a cilium, a fiber spiraling to bulge center, and a 20–30-μm-long nematodesma contributing to the oral basket, which is rather distinct after protargol impregnation (Table 1 and Fig. 13, 14, 16–18, 26–35, 39).

Resting cyst. The four-day-old cyst is colorless, spherical, on average 17.6 μm across (M = 18, SD = 1.1, CV = 6.2, Min = 16, Max = 20, n = 12), and has an about 1.5-μm-thick, spiny wall. The spines, which are up to 3.5 μm long and flexible, likely originate from the inner wall; notably, the spines are distinct only in mature cysts. The cytoplasm is packed with the tortuous macronucleus and lipid droplets up to 3 μm across (Fig. 15, 36–38).

Occurrence and ecology. Kahl (1930) discovered *Protospathidium serpens* in a shallow road drain, a semiterrestrial habitat, in the surroundings of Hamburg, Germany. The neotype population is from a rather similar biotope, a meadow soil under a temporary pond in the town of Salzburg. All other populations, i.e. those mentioned in Foissner (1981, 1998, 1999, 2000) and Foissner et al. (2002), cannot be assigned properly because cysts were not investigated. This applies also to the Namibian population studied in the course of the present study (Fig. 41).

Description of *Protospathidium fraterculum* n. sp. (type population; Table 1 and Fig. 40, 44–58). Spontaneous mass conjugation and encystment occurred in the South African population. Thus, the protargol slides contained many exconjugants, encysting, and already encysted specimens, which were used to reconstruct the encystment process. Morphometry and description are based on cells with an ordinary body shape and macronuclear strand.

Size in vivo about 90–120 × 15–20 μm in vivo, while only 67 × 13 μm in the protargol preparations, indicating strong shrinkage by 37%; length:width ratio, in contrast, hardly changed, that is, near 6:1 in vivo and prepared cells. Shape also highly variable, that is, narrowly to very narrowly spatulate (ellipsoidal), oblique anterior (oral) end about one-third shorter than widest trunk region in or underneath mid-body; posterior end rounded; indistinctly flattened laterally (Fig. 44, 45, 51). Macronucleus in middle third of body, invariably a tortuous, more or less nodulated strand with ends frequently slightly inflated; contains many nucleoli about 1 μm across. Two to five, usually three micronuclei, about 2 μm across, in variable positions adjacent to or in shallow macronuclear depressions; rarely rather distant from macronucleus (Fig. 44 and 51). Contractile vacuole in posterior end, some excretory pores in pole area. Extrusomes studded in oral bulge and scattered in cytoplasm, rod-shaped and slightly curved with ends narrowly rounded, about 4 μm long in vivo (Fig. 44–47); oral extrusomes never impregnated with the protargol method used, while some cytoplasmic developmental stages frequently stained rather intensely. Cortex bright and very flexible, contains about five rows of minute (~ 0.5 μm), loosely arranged, highly refractive granules between ciliary rows (Fig. 49). Cytoplasm colorless, contains many lipid droplets, 0.5–5 μm across, and food vacuoles with heterotrophic flagellates (*Chilomonas* sp.) and small- to middle-sized ciliates (*Cyrtolophosis mucicola*, *Colpoda* sp., hypotrichs). Glides slowly and serpentinously on microscope slides and between soil particles, rotates about main body axis when swimming.

Somatic ciliature as described in *P. serpens* above, with minor morphometric differences shown in Table 1. Brush bristles of similar configuration in all rows, 2.5–3 μm long and slightly inflated distally. Brush row 1 shorter than rows 2 and 3, composed

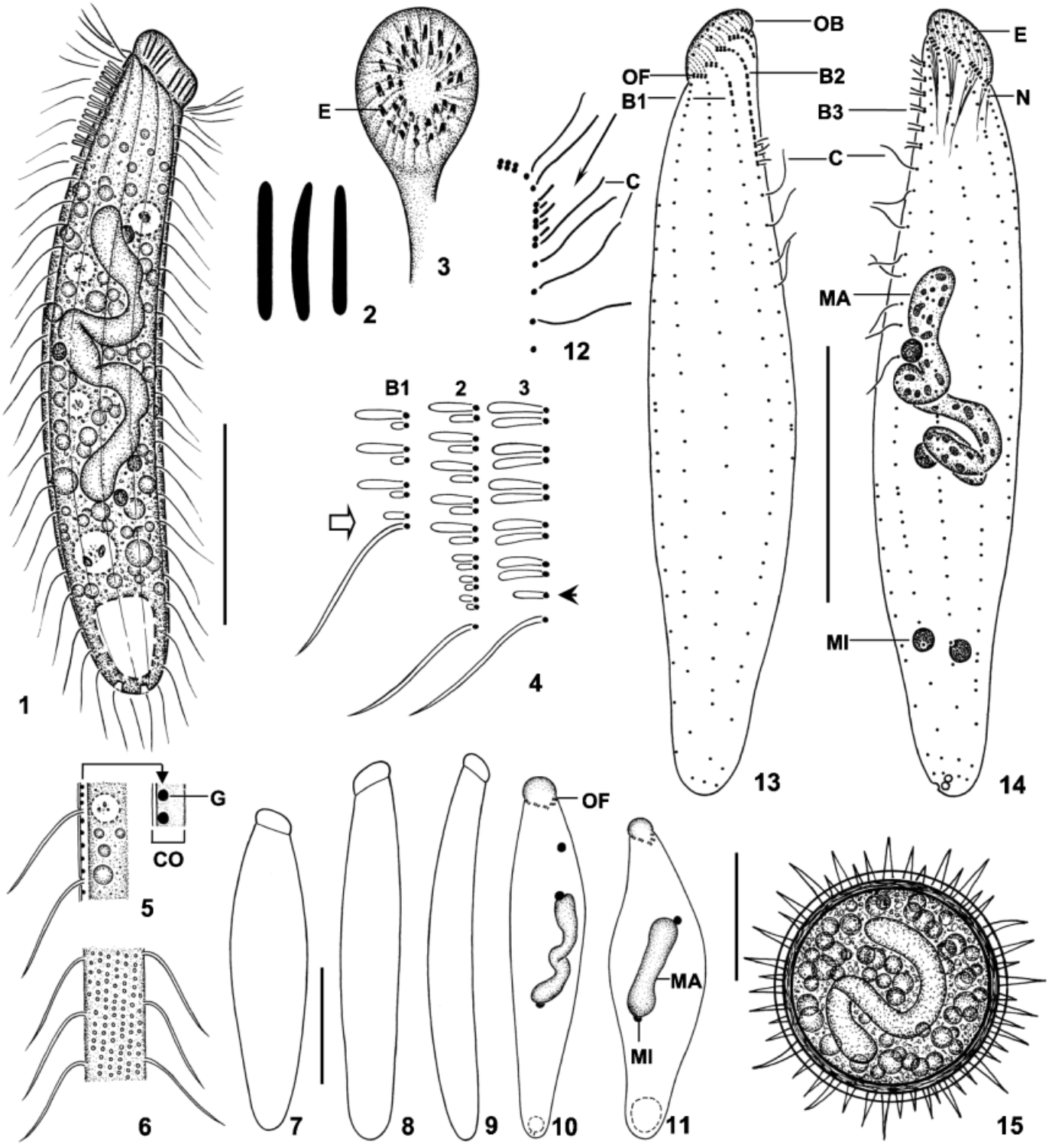

Fig. **1–15.** *Protospathidium serpens*, Austrian neotype specimens from life (1–9, 15) and after protargol impregnation (10–14). **1.** Right side view of a representative specimen. **2.** Basically, extrusomes are rod-shaped and about 2.5–3 × 0.3 μm in size. **3.** Frontal view of oral bulge studded with extrusomes arranged in two to three rough circles around the indistinct central bulge depression. **4.** Dorsal brush. The last dikinetid of row 1 bears a 1-μm-long bristle (blank arrow) and a 7-μm-long ordinary cilium. A monokinetidal bristle tail is lacking in row 3; rarely, a single bristle is recognizable (arrowhead). **5, 6.** Optical section and surface view showing cortical granules (G) embedded in an about 0.7-μm-thick, jelly layer. **7–11.** Variability of body shape and nuclear pattern. **12–14.** Ciliary pattern of left and right side and nuclear apparatus of main neotype specimen. **15.** The resting cyst has an ~ 1.5-μm-thick, spiny wall. B1–3, dorsal brush rows; C, somatic cilia; CO, cortex; E, extrusomes; G, cortical granules; MA, macronucleus; MI, micronuclei; N, nematodesmata; OB, oral bulge; OF, oral kinetofragments. Scale bars = 30 μm for 1, 7–11 (drawn to scale), 13 and 14, and 10 μm for 15.

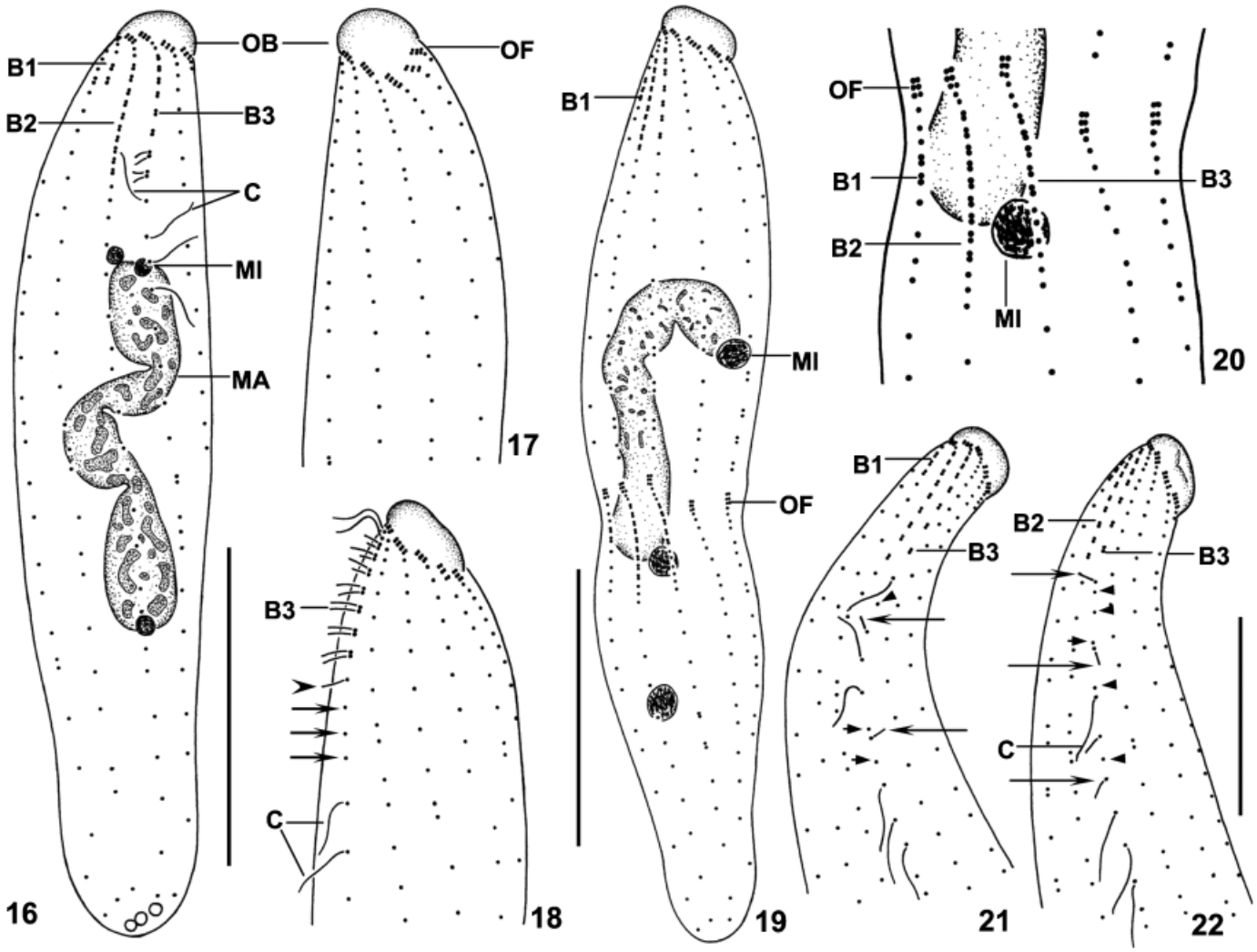

Fig. **16–22.** *Protospathidium serpens*, Austrian neotype specimens after protargol impregnation. **16, 17.** Dorsal and ventral view of same specimen. Note lack of a monokinetidal bristle tail at posterior end of brush row 3. **18.** Right side anterior portion of a specimen with brush row 3 followed by a single bristle (arrowhead) and three bare basal bodies posteriorly (short arrows). **19, 20.** An early divider, as indicated by the condensing macronucleus and the inflated micronuclei. The three brush rows (1–3) of the opisthe are not generated concomitantly, but row 1 is formed later that rows 2 and 3. B1–3, dorsal brush rows; C, somatic cilia; MA, macronucleus; MI, micronuclei; OB, oral bulge; OF, oral kinetofragments. Scale bars = 30 μm. **21, 22.** *Protospathidium fraterculum* n. sp., new drawings of anterior portion of two protargol-impregnated specimens from the Antarctic population investigated by Foissner (1996a). The monokinetidal tail of brush row 3 extends to mid-body and is composed of bristles (arrows) mixed with ordinary cilia and bare basal bodies (arrowheads). B1–3, dorsal brush rows; C, somatic cilia. Scale bar = 20 μm.

of five to nine, on average seven dikinetids; row 2, longest, composed of 10–16, on average 13 dikinetids, more densely packed (average dikinetidal distance 0.8 μm; Table 1) than those of rows 1 and 3; row 3 slightly shorter than row 2, composed of 7–13, on average nine, about 1-μm spaced dikinetids, lacks a monokinetidal bristle tail, as recognizable in some properly impregnated specimens (Fig. 48, 50–53).

Oral bulge rather distinct in vivo, though only 3 μm high and narrower by about one-third than widest trunk region, because knob-like separated from body proper; slanted by about 30–45°, in frontal view obovate and about 7 × 5 μm in protargol-impregnated specimens; surface flat and with fine fibers spiraling to the depressed bulge center, where the temporary cytostome (s. l.) is recognizable as a minute cone (Table 1 and Fig. 44–46, 50–53). Circumoral kinety of similar shape as oral bulge, composed of dikinetidal kinetofragments obliquely attached to ciliary rows and separated from each other by gaps one to three dikinetids wide. Individual kinetofragments composed of an average of four dikinetids each associated with a cilium, a fine fiber converging in bulge center, and a 20–30-μm-long nematodesma contributing to the oral basket, which is recognizable only after protargol impregnation (Table 1 and Fig. 50–53).

Encystment and resting cysts (Table 1 and Fig. 40, 54–58). When encystment commences, the cells become shorter and stouter, as well as more transparent than vegetative specimens because only a few small (~ 1 μm) lipid droplets remain in the cytoplasm. No changes are recognizable in the infraciliature (Fig. 54). Next, the cells become ellipsoidal and the infraciliature is gradually resorbed, commencing with the dorsal brush and circumoral kinetofragments, while the ciliary rows are still recognizable (Fig. 55). The next stage is characterized by production of a hyaline, about 0.5-μm-thick cyst wall (Fig. 56). The cell is now spherical and has lost the oral apparatus, while some somatic kinetids are still recognizable within rough rows. During the last

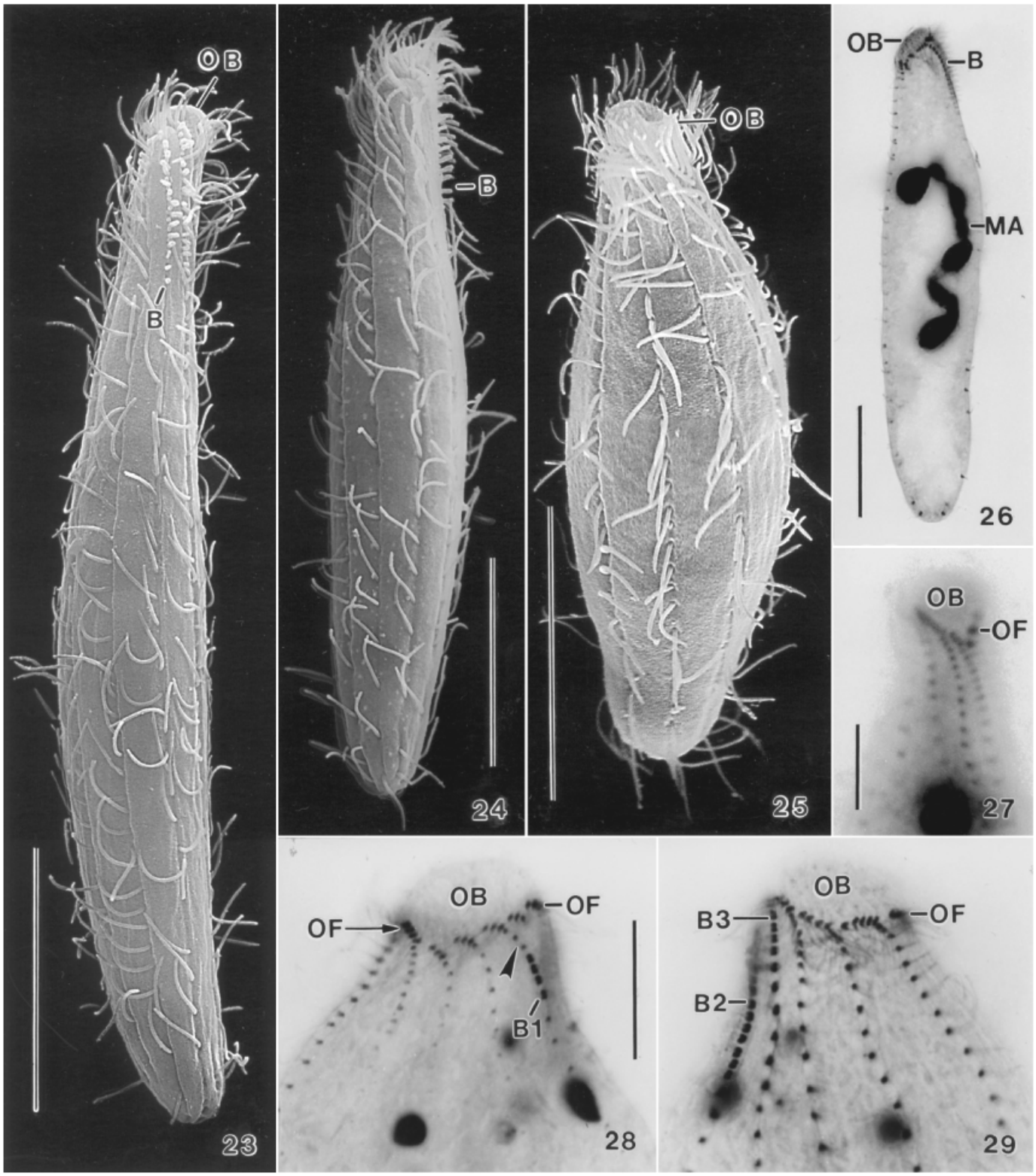

Fig. **23–29.** *Protospathidium serpens*, Austrian neotype specimens in the scanning electron microscope (23–25) and after protargol impregnation (26–29). **23–25.** Dorsal, left side, and ventrolateral overviews showing variability in body shape and size. **26.** Left side overview showing the irregularly nodulated macronucleus. **27.** Ventral view of oral portion of a specimen from a permanent slide. Note the obovate oral bulge and the separated circumoral kinetofragments. **28, 29.** Ventrolateral and dorsolateral view of a specimen flattened by coverslip pressure. The arrow marks a circumoral kinetofragment showing the dikinetidal composition. The arrowhead denotes the anterior tail of three ordinary cilia of the short brush row 1. B(1–3), dorsal brush (rows); MA, macronucleus; OB, oral bulge; OF, oral kinetofragments. Scale bars = 20 μm for 23–36, and 10 μm for 27–29.

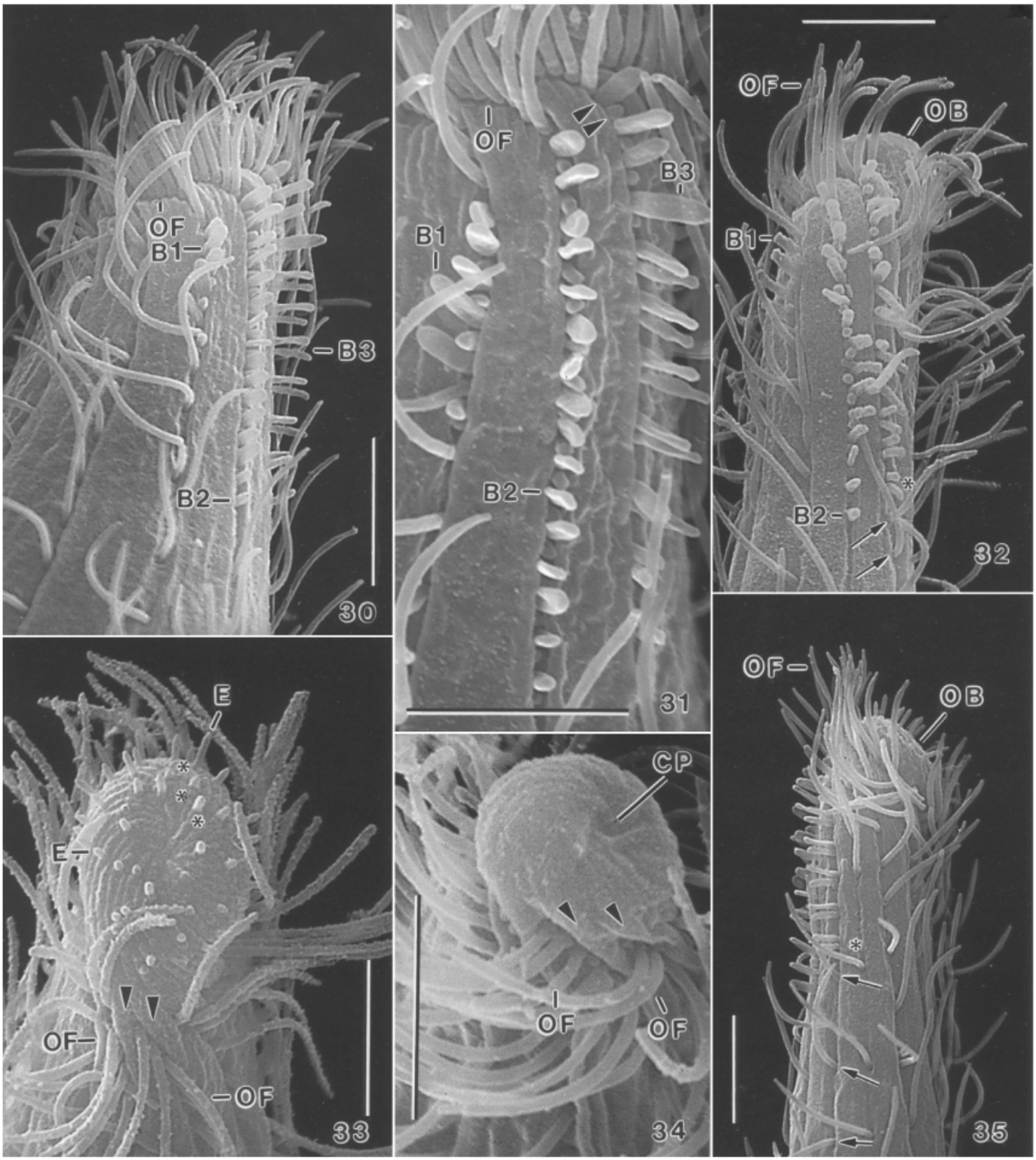

Fig. **30–35.** *Protospathidium serpens*, Austrian neotype specimens in the scanning electron microscope. **30–32, 35.** Dorsal and dorsolateral views showing dorsal brush area. Most brush bristles are inflated, but shrunken as indicated by their wrinkled appearance. Asterisks and arrows in 32 and 35 mark the last bristle pair of brush row 3 and the first post-brush cilium, showing that brush row 3 of *P. serpens* lacks a monokinetidal bristle tail, in contrast to the Antarctic population of *P. fraterculum*. The posterior bristle of the apical pairs of brush row 3 (arrowheads) is strongly shortened. **33, 34.** Ventral and ventrolateral views of oral area, showing the obovate oral bulge containing three rough circles of extrusomes (asterisks) and the deepened cytopharyngeal entrance in bulge center. The circumoral kinetofragments are separated from each other by conical ridges (arrowheads) produced by the oblique clefts from which the fragments originate. B1–3, dorsal brush rows; CP, cytopharyngeal opening; E, extrusomes; OB, oral bulge; OF, oral kinetofragments. Scale bars = 5 μm.

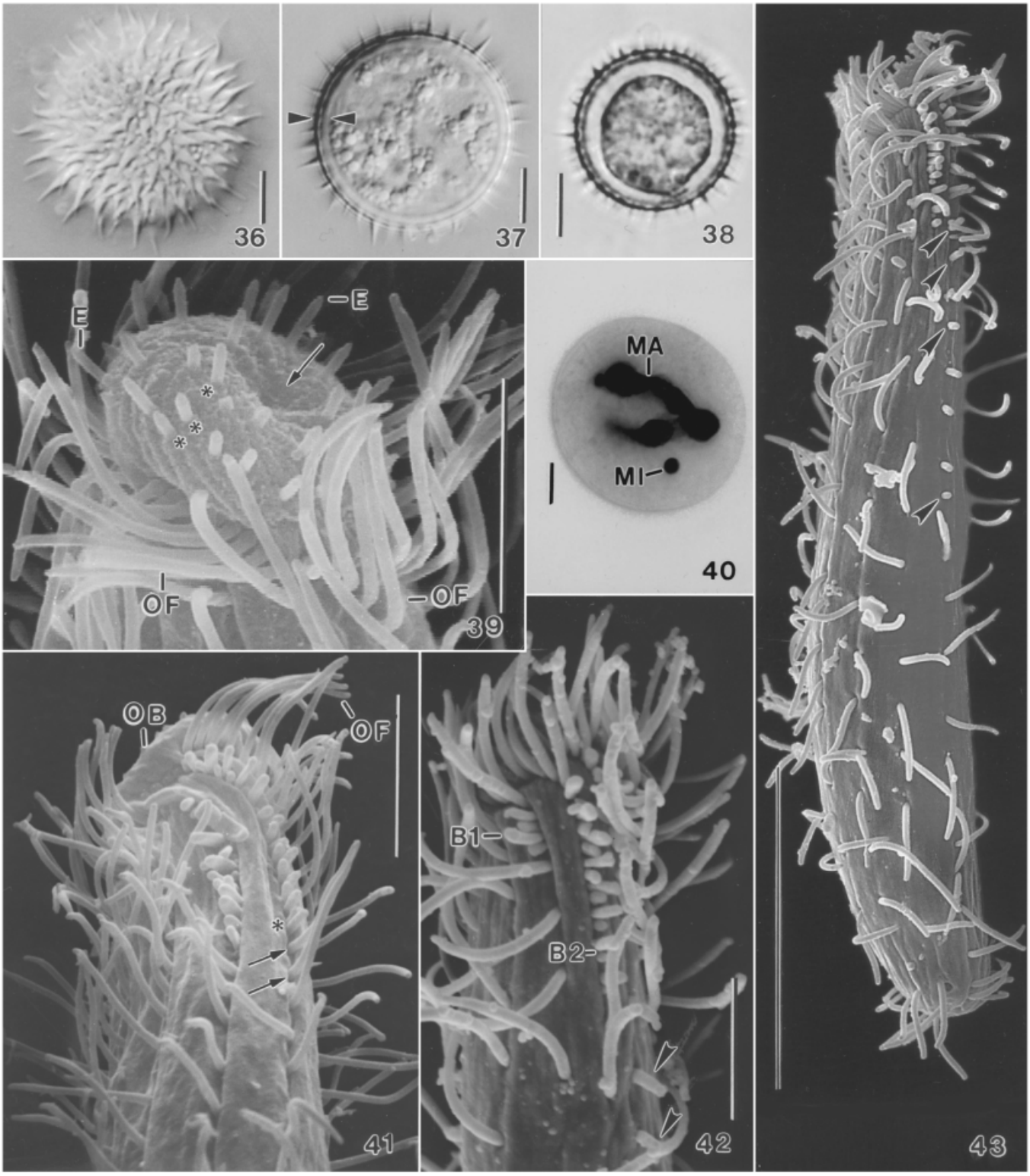

Fig. **36–43.** *Protospathidium serpens* (36–39, 41) and *P. fraterculum* (40, 42, 43) in vivo (36–38), after protargol impregnation (40), and in the SEM (39, 41–43). **36–38.** Surface view and optical sections of resting cysts from Austrian neotype specimens. The spiny wall (arrowheads and squashed cyst shown in 38) consists of two layers separated by a narrow, bright zone. **39.** Oral bulge of an Austrian neotype specimen showing the cytopharyngeal entrance (arrow) and the three extrusome circles (asterisks). **40.** Smooth resting cyst of a South African specimen. **41–43.** Dorsal brush of a Namibian and two sub-Antarctic (Marion Island) specimens, which have associated with brush row 3 a monokinetidal bristle tail (arrowheads) absent in the Namibian specimen (41, arrows, last bristle pair marked by asterisk) and in *P. serpens* (Fig. 32, 35). B1, 2, dorsal brush rows; E, extrusomes; MA, macronucleus; MI, micronucleus; OB, oral bulge; OF, cilia of oral kinetofragments. Scale bars = 5 μm (36–42) and 20 μm (43).

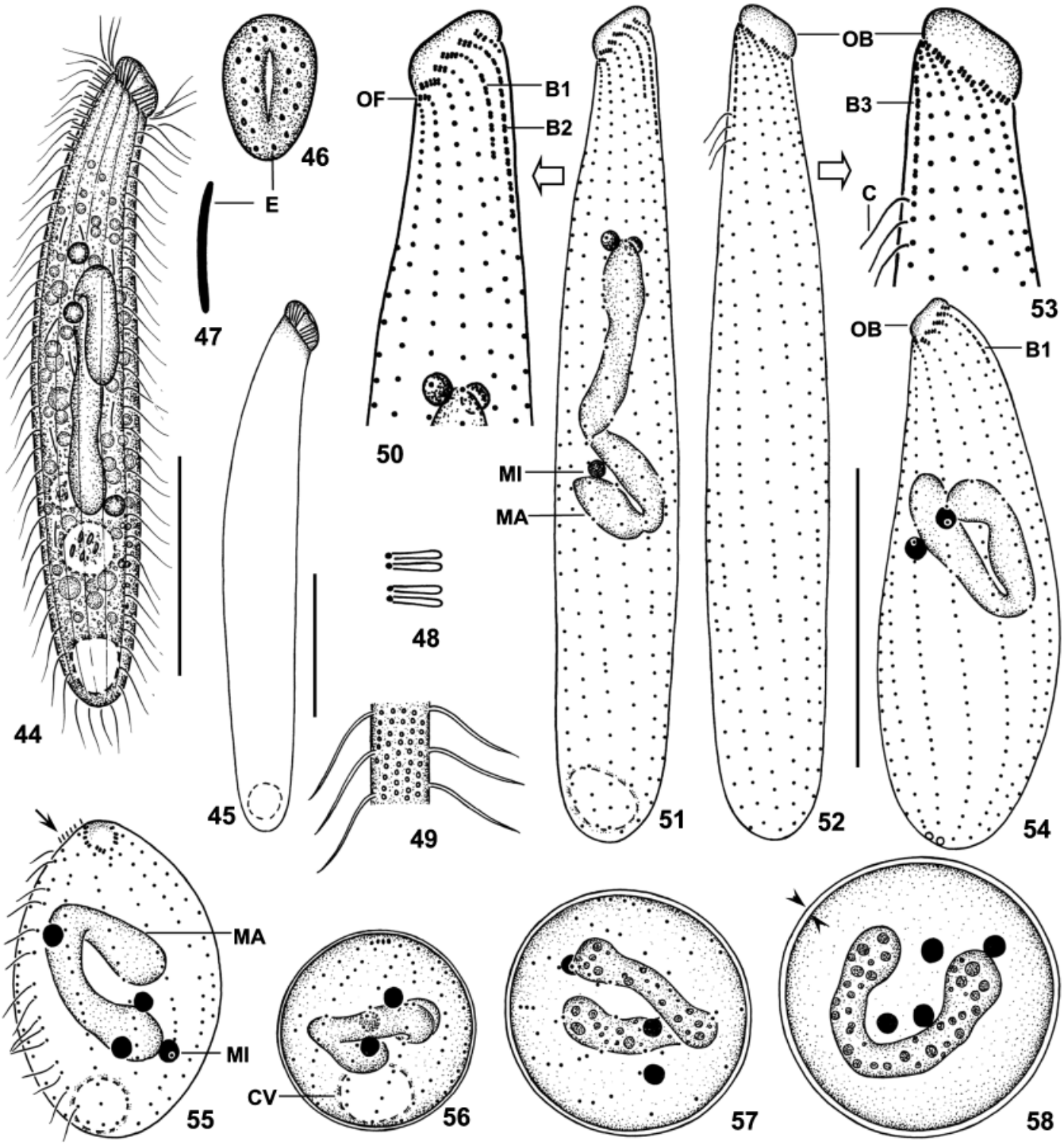

Fig. **44–58.** *Protospathidium fraterculum*, South African-type population from life (44–49) and after protargol impregnation (50–58). **44.** Right side view of a representative specimen. **45.** A long and slender specimen. **46.** Frontal view of oral bulge studded with extrusomes. **47.** Extrusomes are rod-shaped, slightly curved, and about 4 μm long. **48.** Dorsal brush bristles. **49.** Surface view showing cortical granulation. **50–53.** Ciliary pattern of left and right side and nuclear apparatus of holotype specimen. Brush row 1 is composed of an average of seven dikinetids and row 3 lacks a monokinetidal bristle tail. **54–58.** Encystment (see text for details). The smooth wall of the mature cyst (58) is the sole feature distinguishing *P. fraterculum* from *P. serpens*, which has a spiny cyst wall. Arrow indicates the highly reduced dorsal brush bristles. Opposed arrowheads mark the smooth, about 1-μm-thick wall of the resting cyst. B1–3, dorsal brush rows; C, somatic cilia; CV, contractile vacuole; E, extrusomes; MA, macronucleus; MI, micronuclei; OB, oral bulge; OF, oral kinetofragments. Scale bars = 30 μm; 51, 52, 54–58 drawn to scale.

stages, the cyst wall thickens to about 1 μm and the infraciliature disappears completely, although this should be verified by transmission electron microscopy (Fig. 57, 58).

Mature resting cysts are 20–28 μm across, usually near 23 μm (Table 1 and Fig. 58). The wall is about 1 μm thick and more or less distinctly wrinkled in protargol preparations, likely due

Table **1.** Morphometric data on *Protospathidium serpens* from Austria (upper line) and *P. fraterculum* from South Africa (lower line).

Characteristics[a]	$\bar{x}$	M	SD	CV	Min	Max
Body, length	90.3	92.0	7.2	7.9	77.0	105.0
	68.9	67.0	7.6	11.0	58.0	83.0
Body, width	18.4	19.0	3.5	18.8	10.0	24.0
	13.2	13.5	2.7	20.4	8.0	19.0
Body length:width, ratio	5.1	4.9	1.3	25.2	3.5	9.8
	5.4	5.4	1.0	18.7	3.7	7.3
Oral bulge, length	8.4	8.0	1.1	13.6	7.0	11.0
	6.8	7.0	0.9	13.3	5.0	8.0
Oral bulge, height	3.8	4.0	0.6	15.0	3.0	5.0
	2.6	2.5	0.4	13.3	2.0	3.0
Oral bulge length:body width, ratio	0.5	0.5	0.1	21.3	0.3	0.8
	0.5	0.5	0.1	19.5	0.3	0.8
Circumoral kinety to last dikinetid of brush row 1, distance	5.7	6.0	0.9	16.3	4.0	8.0
	6.9	7.0	1.1	15.8	5.0	9.0
Circumoral kinety to last dikinetid of brush row 2, distance	14.0	14.0	1.9	13.5	10.0	19.0
	10.3	10.0	1.6	15.4	7.0	13.0
Circumoral kinety to last dikinetid of brush row 3, distance	11.7	12.0	1.8	15.2	8.0	15.0
	9.3	9.0	1.5	15.7	6.0	12.0
Anterior body end to macronucleus, distance	27.6	26.0	8.7	31.4	12.0	54.0
	21.8	20.0	5.9	26.9	15.0	36.0
Macronucleus figure, length	32.9	33.0	7.3	22.2	16.0	48.0
	26.7	26.0	6.0	22.5	14.0	40.0
Macronucleus, length (spread)	46.2	45.0	6.4	13.9	32.0	60.0
	40.5	40.0	6.1	15.0	27.0	55.0
Macronucleus, width (middle, maximum)	4.4	4.0	0.8	18.1	3.0	6.0
	3.7	4.0	0.6	17.6	3.0	5.0
Macronucleus, number	1.0	1.0	0.0	0.0	1.0	1.0
	1.0	1.0	0.0	0.0	1.0	1.0
Micronuclei, across	1.9	2.0	0.3	18.2	1.5	3.0
	2.3	2.3	0.3	13.1	2.0	3.0
Micronuclei, number	4.2	4.0	1.2	28.2	2.0	7.0
	2.8	3.0	0.8	29.3	2.0	5.0
Somatic kineties, number	10.2	10.0	1.1	10.8	9.0	12.0
	12.3	12.0	0.6	5.1	11.0	14.0
Basal bodies in a right side kinety, number	31.0	31.0	6.0	19.2	21.0	42.0
	41.1	40.5	6.7	16.2	32.0	56.0
Dorsal brush rows, number	3.0	3.0	0.0	0.0	3.0	3.0
	3.0	3.0	0.0	0.0	3.0	3.0
Dikinetids in brush row 1, number	3.4	3.0	1.0	28.3	2.0	6.0
	6.8	7.0	1.0	14.4	5.0	9.0
Dikinetids in brush row 2, number	12.4	12.0	2.0	16.5	8.0	17.0
	13.0	12.0	2.0	15.2	10.0	16.0
Dikinetids in brush row 3, number	7.5	7.0	1.4	18.2	5.0	11.0
	9.5	9.0	1.6	16.6	7.0	13.0
Circumoral dikinetids in a right side kinetofragment, number	3.8	4.0	0.7	19.2	3.0	5.0
	3.6	4.0	0.7	18.1	3.0	5.0
Resting cysts of *P. fraterculum*						
from South Africa, across (n = 11)	23.4	23.0	3.2	13.6	20.0	28.0
Macronucleus, length (spread)	30.8	30.0	3.8	12.4	25.0	36.0
Macronucleus, width (middle)	4.2	4.0	0.6	14.4	3.0	5.0
Micronuclei, across	2.3	2.0	0.4	18.0	2.0	3.0
Micronuclei, number	2.8	3.0	0.6	21.4	2.0	4.0

[a]Data on *P. serpens* are based on 22 mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a non-flooded Petri dish culture. Data on *P. fraterculum* are based on the same preparation method, but 22 specimens were selected as explained in the description. Measurements in μm.

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

to some shrinkage. Spines or other distinct structures on the cyst surface are absent, as proved by interference contrast microscopy of protargol-impregnated cysts (Fig. 40). The macronucleus is a semicircular strand slightly shorter than in the morphostatic cells. There are two to four, on average three micronuclei, each about 2 μm across, as in the vegetative specimens.

Protargol-impregnated cysts did not significantly shrink by the preparation procedures due to their stable wall. Thus, they appear very voluminous ($\sim 7{,}000\,\mu m^3$) as compared to the impregnated cells, which shrank by at least one-third (see above) and thus have a volume of only $\sim 6{,}000\,\mu m^3$. When the in vivo size ($\sim 100 \times 18\,\mu m$) is taken, the cell volume increases to $\sim 17{,}000\,\mu m^3$, that is, double the cyst volume, as usual.

Observations on *Protospathidium fraterculum* (Antarctic populations; Table 2 and Fig. 21, 22, 42, 43). To resolve some details, we re-investigated the Antarctic populations studied by Foissner (1996a, b). Unfortunately, no cysts were found on the protargol slides. The following additional details were noted: (1)

Table **2.** Comparison of main features of *Protospathidium serpens* and *P. fraterculum*.

Characteristics	*Protospathidium serpens*			*P. serpens* (neotype)	*P. fraterculum*			
Sources	Kahl (1930)	Foissner (1981)	Foissner & Xu (unpubl. data)	This paper (Table 1)	This paper (Table 1)	Foissner (1996a)	Petz and Foissner (1997)	
Locality	Germany	Austria	Namibia	Austria	South Africa	Antarctica	Antarctica Pop 1	Pop 2
Body, length (μm; in vivo)	70–90	70–90	~ 120	70–130	90–120	70–100	43–117 (68)	?
Body, width (μm; in vivo)	?	10–15	~ 20	12–25	15–20	12–18	8–25 (13)	?
Body, length:width (in vivo)	6.7	6.8	6	5.5	6	4.3*	5.3	4.8*
Oral bulge, length (μm)*	?	~ 8	?	7–11 (8.4)*	5–8 (6.8)*	6–9 (7.6)*	6–10 (9)*	5–11 (7.9)*
Macronucleus nodulated	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Extrusomes rod-shaped	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ciliary rows, number	~ 10	7–9 (8.3)*	7–12 (9)*	9–12 (10.2)*	11–14 (12.3)*	11–13 (11.6)*	11–16 (13)*	10–12 (11.1)*
Brush rows, number	?	3	2–3 (2.6)*	3*	3*	3–4 (3.5)*	3–4 (3.1)*	3–4 (3)*
Dikinetids in brush row 1, number	?	~ 2[a]	2–9 (6.1)*	2–6 (3.4)*	5–9 (6.8)*	2–4 (3)*	1–5 (3.4)*	1–5 (2.9)*
Dikinetids in brush row 2, number	?	6–8[a]	8–20 (14.6)*	8–17 (12.4)*	10–16 (13)*	7–12 (8.5)*	6–12 (9.2)*	4–10 (6.9)*
Dikinetids in brush row 3, number	?	4–6[a]	6–10 (7.6)*	5-11 (7.5)*	7–13 (9.5)*	4–7 (5.2)*	4–9 (6.6)*	3–7 (4.5)*
Brush dikinetids, total number	?	ca 15[b]	ca 28*	ca 22*	ca 29*	ca 17*	ca. 19*	ca. 14*
Brush row 3 with a distinct tail of monokinetidal bristles	?	?	No	No	No	Yes	Yes	Yes
Resting cysts spiny or smooth	?	?	?	Spiny	Smooth	Smooth	Smooth	Smooth

Data marked with (*) are from protargol preparations; those in brackets are means.
[a]According to description.
[b]Counted from the two specimens figured.

the anterior end of the extrusomes is occasionally impregnated, as in the Salzburg neotype specimens of *P. serpens*; (2) brush row 3 ends in a monokinetidal tail, which extends to mid-body, and is either heteromorphic (composed of bristles mixed with ordinary cilia and bare basal bodies; Fig. 21 and 22 from Signey Island specimens) or isomorphic (composed only of bristles; Fig. 42 and 43 from Marion Island specimens); and (3) the dikinetids are very loosely spaced in row 3 (average distance 1.8 μm), as in the neotype of *P. serpens*.

Occurrence and ecology. This species occurs in soil from South Africa and Antarctica. Foissner (1996a) described it as *P. serpens* from a *Deschampsia antarctica* grass sward of Signey Island, and Petz and Foissner (1997) found it in algal ornithogenic soil from Whitney Point, Clark Peninsula (population I), and in soil from Beall Island, Windmill Islands (population II). These three populations have a smooth cyst wall, as definitely stated by the authors and shown by Fig. 20 in Foissner (1996a). The population recorded by Foissner (1996b) from the sub-Antarctic Marion Island probably belongs also to *P. fraterculum* because brush row 3 has a distinct tail of short bristles (Fig. 42, 43, 59), which are, however, not mixed with ordinary cilia as in the Signey Island population, indicating some local differentiation. Unfortunately, cysts were not studied.

DISCUSSION

A cryptic diversity: resting cyst morphology as a taxonomic feature. The knowledge about ciliate resting cysts is rather limited, both morphologically and functionally (Corliss and Esser 1974; Gutiérrez et al. 2003). For instance, reliable ultrastructural investigations are available from less than 30 species (Gutiérrez et al. 2003). Light microscopical data are abundant, but have rarely played a significant role in species discrimination, though cysts have been used for decades in the taxonomy of, for instance, naked amebae (Page 1976; Page and Siemensma 1991) and dinoflagellates, in which fossil taxonomy has been based entirely on cyst features (Taylor 1987).

In ciliates, Beers (1952) is an early exception. He distinguished *Bursaria ovata* from *B. truncatella* by, inter alia, the absence of bridges connecting ectocyst and endocyst. Although recent electron microscopical investigations showed bridges in *B. ovata* too, they are less distinct than those of *B. truncatella* (Sergejeva et al. 1995). More recently, Foissner (1993) provided excellent examples in the genus *Colpoda*: *C. cucullus*, *C. flavicans*, and *C. lucida* are rather similar in most conventional features, while the resting cysts are highly different. Likewise, resting cyst features are very useful for distinguishing species within several oxytrichid genera, for instance, the wrinkled cyst of *Stylonychia mytilus* and the spiny cyst of *S. pustulata* (see Berger 1999 for a comprehensive review). Several of the many similar *Podophrya* species differ in cyst morphology (with or without stalk and/or apical plug) and number of cyst ribs (Foissner et al. 2002).

These taxa are examples where cyst wall features strengthen the species status suggested by conventional characteristics. For instance, the *Colpoda* species mentioned above also differ in the location of the contractile vacuole, the extrusomes, and details of the ciliary pattern (Foissner 1993). Thus, they do not pose a basic problem. In contrast, the split of *Protospathidium serpens*, which is based solely on resting cyst morphology, requires evidences that this feature is significant at the species level. We have three lines of argumentation. First, cyst wall features are as distinct and stable as the other main characteristics of a species, such as the ciliary and nuclear pattern (Berger 1999; Foissner 1993; Gutiérrez et al. 2003). Second, the examples discussed above (i.e. *Colpoda*

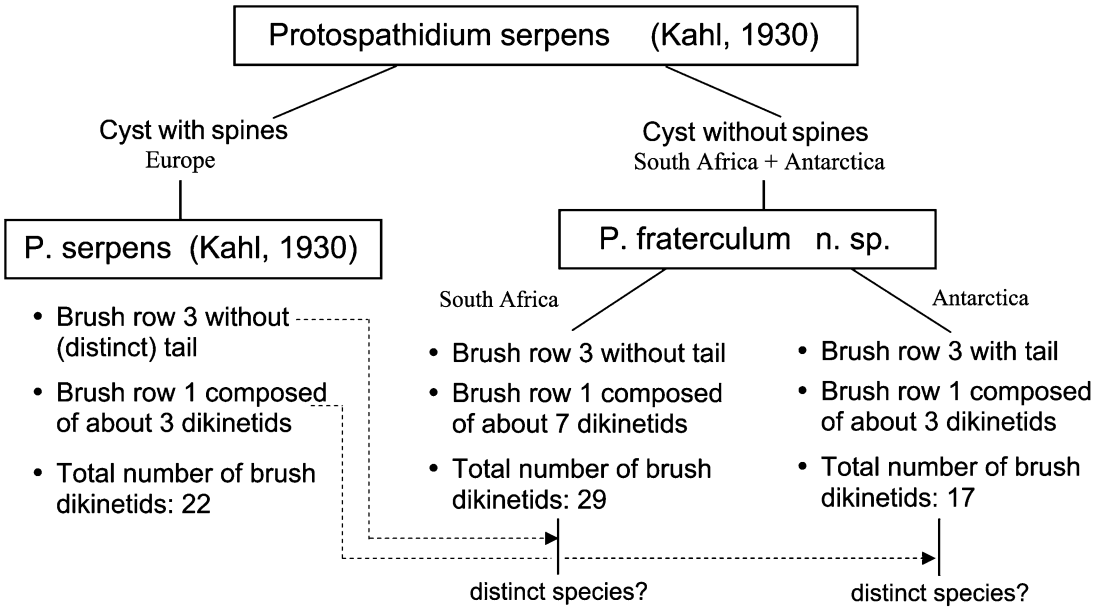

Fig. **59.** Taxonomy and biogeography of *Protospathidium serpens* and *P. fraterculum* based on resting cyst morphology and details of the dorsal brush.

spp., *Stylonychia* spp.) show that different cyst morphology is often associated with differences in other important features that define ''good species.'' Third, *P. serpens* is not an exception in showing cryptic differences in cyst morphology. There are two other well-documented cases. The first is found in the genus *Nassula*, where Foissner et al. (2002) noted that *Nassula tuberculata*, *Nassula granata*, and *Nassula terricola* are almost indistinguishable by conventional morphological characteristics, while their resting cysts are conspicuously different: tubercular in *N. tuberculata*, smooth in *N. granata*, and with distinct ridges in *N. terricola*. An even more striking example is the common *Halteria grandinella* which, like *P. serpens*, consists of two morphologically indistinguishable species either with a smooth or a spiny cyst wall (Foissner, manuscript in prep.); they also differ significantly in gene sequences (Katz & Foissner, manuscript in prep.).

All these data were not obtained by a systematic search but by chance. This suggests a high cryptic cyst diversity and thus probably a gross underestimation of the total number of species of ciliates, which is now very controversially discussed (Coleman 2002; Foissner 2002, 2004; Foissner et al. 2002; Lachance 2004). It should be mentioned that cyst wall morphology is also useful at higher taxonomic levels (Gutiérrez et al. 2003; Foissner, unpubl. data), though the overall appearance is sometimes very similar across the phylum. For instance, flask-shaped resting cysts with an apical escape opening occur in most heterotrichs and oligotrichs (Reid and John 1983), in peniculine hymenostomes (Foissner 1987b), and even in *Fuscheria*, a haptorid gymnostome (Foissner 1994). However, wall details are different, suggesting convergent evolution.

Separation of *Protospathidium serpens* and *P. fraterculum*. Accepting resting cyst morphology as a discriminator for species, we conclude that *P. serpens* must be split into two species: *P. serpens* (Kahl, 1930) and *P. fraterculum* n. sp. We put together the available data in Table 2 and used them for the scheme shown in Fig. 59. This compilation suggests a distinct biogeographic pattern in cyst morphology (spines present only in European specimens), while features of the trophic cells overlap rather distinctly. Nonetheless, the African and the Antarctic populations differ to an extent probably sufficient to consider both as distinct taxa. However, our suggestion to classify them as subspecies was abandoned by the Editor (not the reviewers!). Thus, we leave them unranked.

Neotypification. The identity of *P. serpens* is threatened due to the discovery of a ''morphological brother,'' differing only in resting cyst morphology. No original type material is available from *P. serpens* and its cyst. Thus, the species needs neotypification (Foissner 2002; Foissner et al. 2002).

Foissner (1981, 1996a) and Petz and Foissner (1997) have deposited protargol slides from the populations they studied in the Biology Center of the Upper Austrian Museum. However, the slides from Foissner (1981) contain few specimens, and those of Foissner (1996a) and Petz and Foissner (1997) are from a different biogeographic realm (Antarctica) and represent a different species: *P. fraterculum*. Accordingly, the Salzburg population investigated in the present study must serve as a neotype of *P. serpens*. This population is excellently prepared and is from the same biogeographic region as the specimens of Kahl (1930), who discovered *P. serpens* in Hamburg, Germany. These are prerequisites for successful neotypification, as discussed in Foissner (2002).

Diagnoses

Protospathidium serpens (Kahl, 1930) Foissner, 1981

1930 *Spathidium serpens* Kahl, Tierwelt Dtl., 18:158

1981 *Protospathidium serpens* nov. comb. (Kahl, 1930–35)—Foissner, *Zool. Jb. Syst.*, **108**:274 (description of an Austrian population)

Diagnosis of neotype. Size about 100 × 18 μm in vivo. Narrowly to very narrowly spatulate with oblique, obovate oral bulge shorter by about one-third than widest trunk region. Macronucleus a tortuous, more or less nodulated strand; multimicronucleate. Extrusomes rod-shaped and about 2.5–3 × 0.3 μm in size. On average 10 ciliary rows, three anteriorly differentiated to inconspicuous dorsal brush occupying ca 15% of body length and containing a total of 22 dikinetids: on average three dikinetids in row 1, 12 in row 2, and seven in row 3; row 3 without distinct monokinetidal bristle tail. Right side oral kinetofragments usually composed of 4 dikinetids. Resting cyst with spines.

Type and neotype localities. The type locality is a shallow road drain in the surroundings of Hamburg, Germany, E10° N53°30′. The neotype locality is soil under a temporary pond in the town of Salzburg, Austria, E13°40′ N47°47′.

Neotype specimens. Three neotype slides with protargol-impregnated specimens were deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. All specimens illustrated and some other well-impregnated cells were individually marked by a black ink circle on the coverslip.

Protospathidium fraterculum n. sp.

1996 *Protospathidium serpens* (Kahl, 1930) Foissner, 1981—Foissner, *Acta Protozool*., **35**:106 (detailed description of an Antarctic population)

1997 *Protospathidium serpens* (Kahl, 1930) Foissner, 1981—Petz & Foissner, *Polar Rec*., **33**:308 (detailed morphometry and brief description of two Antarctic populations)

2004 *Protospathidium serpens* (Kahl, 1930) Foissner, 1981—Xu & Foissner, *J. Eukaryot. Microbiol*., **51**:606 (detailed study on conjugation)

Diagnosis. Size about 90 × 15 μm in vivo. Narrowly to very narrowly spatulate with oblique, obovate oral bulge shorter by about one-third than widest trunk region. Macronucleus a tortuous, more or less nodulated strand; multimicronucleate. Extrusomes rod-shaped and about 3–5 μm long. On average 11–13 ciliary rows, three anteriorly differentiated to inconspicuous dorsal brush occupying about 11–15% of body length and containing a total of 17–29 dikinetids: on average three to seven dikinetids in row 1, 8–13 in row 2, and five to nine in row 3; row 3 with or without distinct monokinetidal bristle tail. Right side oral kinetofragments usually composed of four dikinetids. Resting cyst smooth.

Type locality. Soil from the dry bed of the Mlambane River in the Kruger National Park, Republic of South Africa, E31°40′ S25°50′.

Type specimens. We deposited one holotype slide and seven paratype slides with protargol-impregnated specimens in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz, Austria. The slides also contain the conjugants described by Xu and Foissner (2004). All specimens illustrated and some other well-impregnated cells were individually marked by a black ink circle on the coverslip.

Etymology. The Latin *fraterculum* (diminutive of *frater* = brother) refers to the high similarity of the vegetative cells to *P. serpens*.

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