ACTA
PROTOZOOLOGICA

Acta Protozool. (2014) 53: 159–194
http://www.eko.uj.edu.pl/ap
doi:10.4467/16890027AP.14.015.1596

Five New Spathidiids (Ciliophora: Haptoria) from Caribbean Tank Bromeliads

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Abstract. There is a widespread belief that spathidiids have few morphological features. In contrast, we show a rich morphological diversity in five new species discovered in tank bromeliads from the Caribbean, using live observation; protargol impregnation; morphometry; scanning electron microscopy; and resting cyst morphology, demonstrating lepidosomes (organic scales) for the first time in spathidiid haptoria. *Arcuospathidium bromelicola* nov. spec. is very similar to the previously described *A. muscorum* but its resting cyst has conspicuous, pillar-shaped lepidosomes on the surface. *Protospathidium lepidosomatum* nov. spec. is very similar to the previously described *P. muscicola* but has outstanding, nipple-shaped (vs. conical) lepidosomes on the cyst surface. *Spathidium bromeliophilum* nov. spec., whose ontogenesis is highly similar to that of *S. turgitorum*, differs from similar species by the body length:width ratio, the number of ciliary rows, the shape of the oral bulge, and details of the ciliary pattern. *Spathidium bromelicola* nov. spec. is similar to *S. muscicola* (extrusomes bluntly fusiform and 4 µm long vs. rod-shaped and > 15 µm long) and *S. stammeri* (resting cyst wall smooth vs. spinous). *Spathidium wolfi* nov. spec. has an anterior and a posterior contractile vacuole. It differs from the supposed nearest relative, *S. faurefremieti*, by body size (on average 135 × 25 µm vs. 240 × 17 µm), the shape of the macronucleus (moniliform vs. a long, tortuous strand), and the total number of dorsal brush bristles (on average 47 vs. 72). The bent oral bulge of *Arcuospathidium bromelicola* and *Spathidium bromeliophilum* as well as the occurrence of lepidosomes on the cyst surface of *Arcuospathidium bromelicola* and *Protospathidium lepidosomatum* are discussed.

Key words: Biodiversity, Dominican Republic, Jamaica, lepidosomes, ontogenesis of *Spathidium bromeliophilum*, resting cysts.

INTRODUCTION

"The old genus *Spathidium*, erected by Dujardin in 1841, has not been revised in the last 60 years. It currently contains 100 morphologically very similar species. It would be difficult to justify squeezing more new ciliates into such a genus before rationalizing the existing complement, for it is likely that the genus currently supports a variety of synonyms and morphotypes" (Finlay *et al.* 1996). This ecologist's view, which is shared also by some taxonomists (Buitkamp 1977, Wenzel 1955), is refuted by the monograph of Foissner and Xu (2007). They showed not only a very low synonymy rate (< 10%) in spathidiids but they also added many

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new species. The present study shows that further species wait to be discovered.

This kind of increase in diversity is mainly due to refined methods, such as silver impregnation and scanning electron microscopy, and the exploitation of unconventional features, such as resting cyst morphology. Another source for increase is the exploration of new habitats, as shown in the present study. The five new species are from the water and mud of tank bromeliads, where we discovered more than 40 new taxa in a comparatively low number of samples (~ 200), indicating many more undescribed species (Dunthorn *et al.* 2012, Foissner *et al.* 2003).

MATERIALS AND METHODS

The origin of the material is provided in the individual species descriptions. Basically, it is from the tanks of various arboreal and terrestrial bromeliads from Jamaica and the Dominican Republic. Usually, several small samples from the tanks of a single bromeliad species were pooled. The Jamaican samples amounted to only 10–20 ml each. Thus, we added tap water to fill a 13 cm Petri dish. The solid material of the original sample was arranged in the dish centre by gently swirling the dish. Then, 3–6 slightly crushed wheat kernels were added around and in the central material. Such "raw cultures" were inspected twice a week. In case a promising species developed, 5 ml of the raw culture were transferred into a Petri dish containing tap water and some squashed wheat kernels to obtain a "semipure culture".

The morphological methods follow Foissner (1991) and Vd'ačný and Foissner (2012). Terminology is according to Corliss (1979) and the refinements made by Foissner and Xu (2007) and Vd'ačný and Foissner (2012).

RESULTS

Arcuospathidium bromelicola nov. spec. (Figs 1a–n, 2a–m, 3a–k; Table 1)

Diagnosis: Size about 85 × 30 µm *in vivo*; spatulate to narrowly spatulate. Macronuclear strand in middle body third, tortuous to horseshoe-shaped; micronucleus broadly ellipsoidal. Contractile vacuole in rear body end. Extrusomes rod-shaped with rounded ends, about 4–5 × 0.3 µm *in vivo*. On average 14 ciliary rows. Dorsal brush distinctly heterostichad, rows 1 and 2 of similar length, composed of an average of 10 and 13 dikinetids, respectively; row 3 shorter than longest row 2 by about 55%, composed of an average of 7 dikinetids. Oral bulge oblique, moderately convex, cuneate, on average 25 µm long *in vivo*, in 70% of specimens slightly to distinctly bent in proximal third. Resting cyst surface studded with 2–3 µm high pillars distally frayed.

Type locality: In tank bromeliads from the "Upper Cedar Valley", southern slope of the Blue Mountains, Jamaica, 18°2′N 76°34′W.

Type material: 1 holotype and 3 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: Composite of Bromeliaceae, the plants in whose leaf-tanks it occurs, and *cola* from the Latin verb *colere* (to dwell), referring to its habitat.

Description: Size 60–115 × 15–40 µm *in vivo*, usually about 85 × 30 µm, as calculated from live measurements and the morphometric data in Table 1, adding 15% for preparation shrinkage. Body length: width ratio *in vivo* 2.5:1, after protargol impregnation 3.3:1. Shape narrowly spatulate (theronts) to spatulate (trophonts), dorsal outline slightly sigmoidal, ventral convex with rather distinct concavity at proximal end of oral bulge, anterior end oblique, posterior rounded; laterally flattened up to 2:1 (Figs 1a, e, f, i, m, n, 2a, b, 3a, b). Macronucleus in middle third of body, a tortuous strand in about 71% of specimens (out of 21 cells), horseshoe-shaped in about 24%, and C-shaped in one specimen, contains many small and some large nucleoli; some post-conjugants with two macronuclear nodules (Fig. 3j). Micronucleus attached to macronucleus at various positions, ellipsoidal, in three specimens with a minute, hemispherical cap; often difficult to identify because of similarly sized and impregnated cytoplasmic inclusions (Figs 1a, e, m, n, 3a, b, f, i; Table 1). Contractile vacuole in rear body end, on average 3 excretory pores (Figs 1a, m, n, 2a; Table 1). Extrusomes packed in oral bulge and scattered in cytoplasm, rod-shaped with rounded ends, *in vivo* about 4–5 × 0.3 µm in size; cytoplasmic extrusomes sometimes impregnate with the protargol method used (Figs 1a, d, j, 2c, 3i; Table 1). Cortex very flexible, contains ordinarily spaced rows of granules between each two kineties; granules colourless and hyaline, ≤ 0.2 µm in size (Fig. 1b). Cytoplasm colourless, oral area hyaline, trunk usually opaque due to food vacuoles up to 30 µm across and few to many globular or slightly irregular lipid droplets up to 6 µm in size. Feeds on middle-sized ciliates, such as *Colpoda* sp. recognizable in two specimens, *Glaucomides bro-*

Table 1. Morphometric data on *Arcuospathidium bromelicola*.

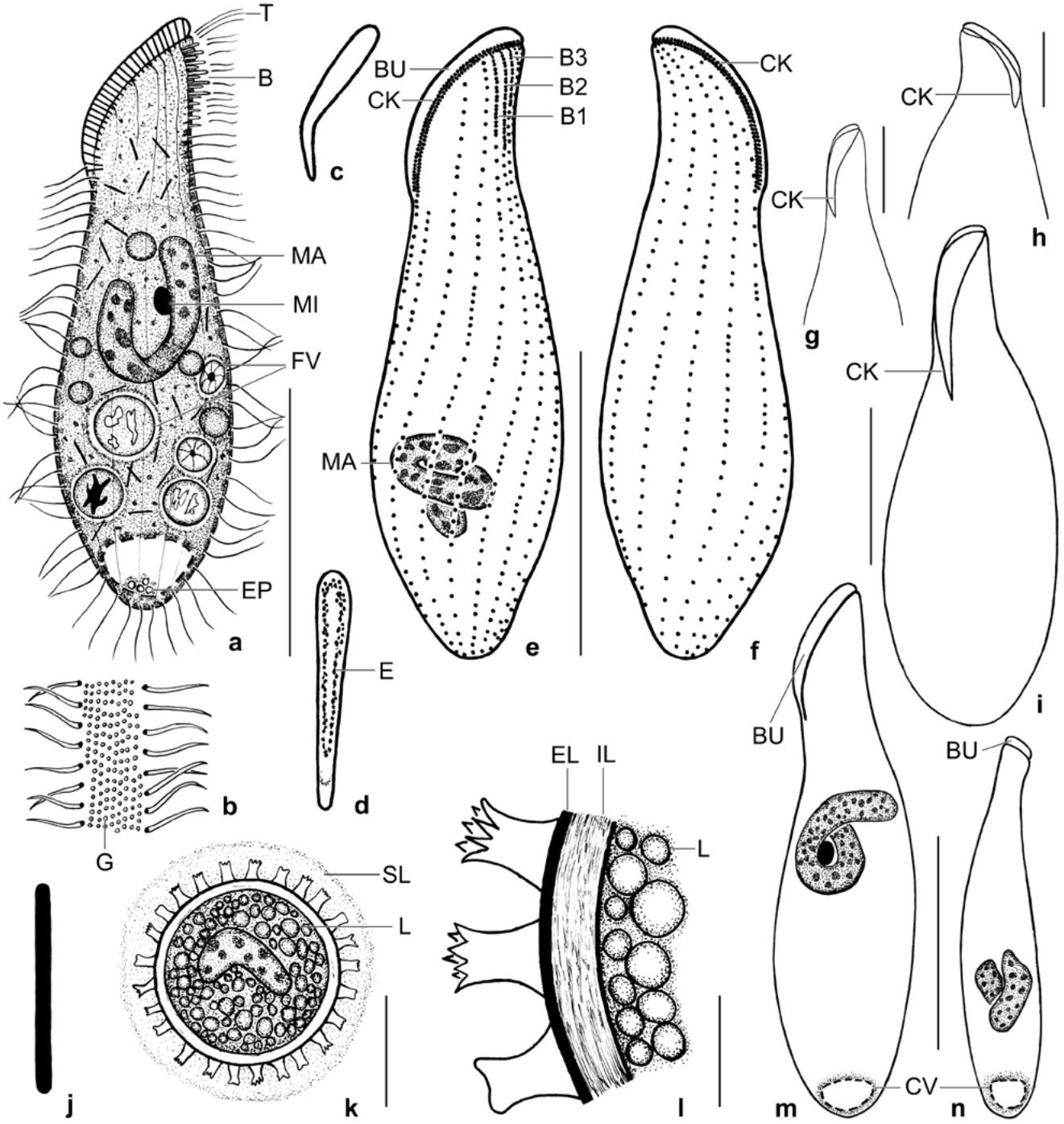
Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length *in vivo* (rough values), µm	78.2	80.0	–	–	–	60.0	100.0	11
Body, width *in vivo* (rough values), µm	31.4	30.0	–	–	–	25.0	40.0	11
Body length:width, ratio *in vivo* (rough values)	2.5	2.7	–	–	–	1.8	3.2	11
Body, length, µm	75.3	71.3	11.0	2.1	14.6	55.3	99.8	27
Body, width at proximal end of circumoral kinety, µm	12.4	12.0	1.8	0.4	14.8	9.1	16.5	23
Body, maximum postoral width, µm	23.8	22.8	5.0	1.0	20.9	14.3	35.3	27
Body length:width, ratio	3.3	3.4	0.7	0.1	21.0	1.8	4.4	27
Oral bulge, length, µm	23.1	22.8	2.9	0.7	12.6	17.1	30.2	21
Oral bulge, width, µm	5.5	5.7	0.6	0.1	10.4	4.6	6.3	19
Oral bulge, height, µm	3.0	2.9	0.7	0.1	23.0	1.7	4.0	21
Body length:oral bulge length, ratio	3.1	3.2	0.4	0.1	12.6	2.3	4.0	21
Body width:oral bulge length, ratio	1.1	0.9	0.3	0.1	26.3	0.8	1.7	19
Anterior body end to macronucleus, distance, µm	28.7	27.4	10.1	2.2	35.1	10.8	51.3	21
Macronucleus figure, length, µm	17.1	17.4	3.4	0.7	19.8	11.4	22.8	21
Macronucleus, length (extended and thus approximate), µm	41.2	41.0	–	–	–	34.8	51.3	19
Macronucleus, width, µm	5.0	5.1	0.7	0.2	14.3	4.0	6.3	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length, µm	3.5	3.4	0.4	0.1	11.2	2.9	4.0	11
Micronucleus, width, µm	2.3	2.3	0.5	0.2	22.2	1.7	2.9	11
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	11
Ciliary rows, number	13.7	14.0	1.2	0.2	8.5	12.0	16.0	22
Kinetids in a right side ciliary row, number	43.5	44.0	5.1	1.1	11.7	34.0	55.0	21
Circumoral kinety to last dikinetid of brush row 1, distance, µm	8.9	9.1	1.1	0.2	12.2	6.8	11.4	23
Circumoral kinety to last dikinetid of brush row 2, distance, µm	10.5	10.5	2.0	0.4	18.9	3.7	14.3	23
Circumoral kinety to last dikinetid of brush row 3, distance, µm	5.8	5.7	1.4	0.3	23.3	3.4	8.6	23
Dorsal brush row 1, number of dikinetids	10.0	10.0	2.0	0.4	19.7	7.0	15.0	23
Dorsal brush row 2, number of dikinetids	13.3	13.0	1.7	0.4	13.1	11.0	17.0	23
Dorsal brush row 3, number of dikinetids	6.7	7.0	1.4	0.3	20.8	4.0	10.0	23
Dorsal brush, number of rows[b]	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
Extrusome, length, µm	3.6	3.4	0.4	0.1	11.8	2.9	4.0	12
Extrusome, width, µm	0.3	0.2	–	–	–	0.2	0.5	12
Excretory pores, number	3.1	3.0	–	–	–	?	6.0	19
Resting cyst, diameter *in vivo*, µm	25.8	25.0	3.3	1.1	12.8	20.0	30.0	9

[a] Data based, if not mentioned otherwise, on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a raw culture. 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.

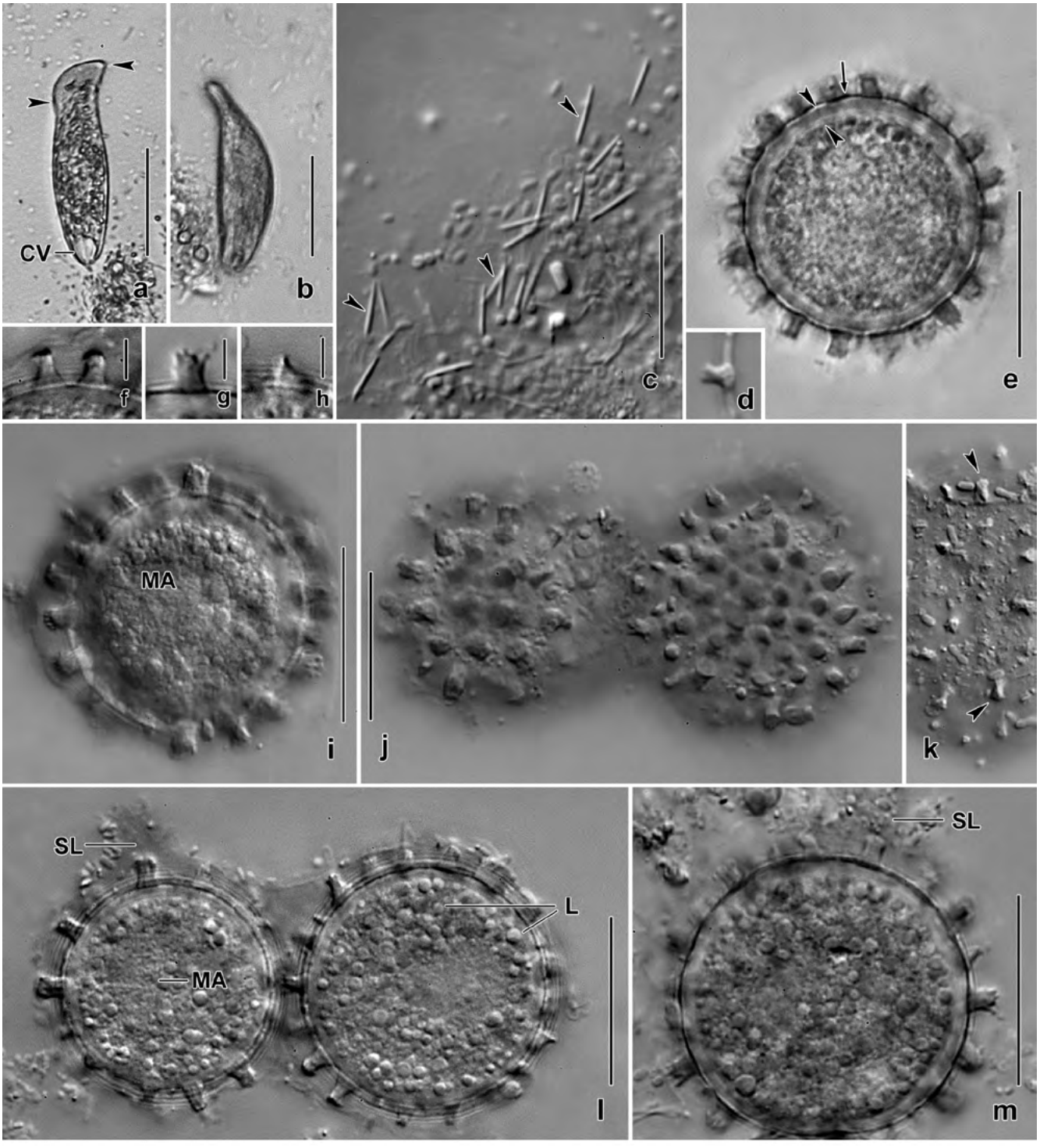
[b] One specimen with some supernumerary dikinetids, forming a fourth row left of row 3.

melicola, flagellates, and possibly starch grains from the wheat kernels added to the culture (Fig. 3k). Swims moderately fast or glides rapidly, most cells gather at margin of organic accumulations composed of bacteria, flagellates and *Glaucomides*.

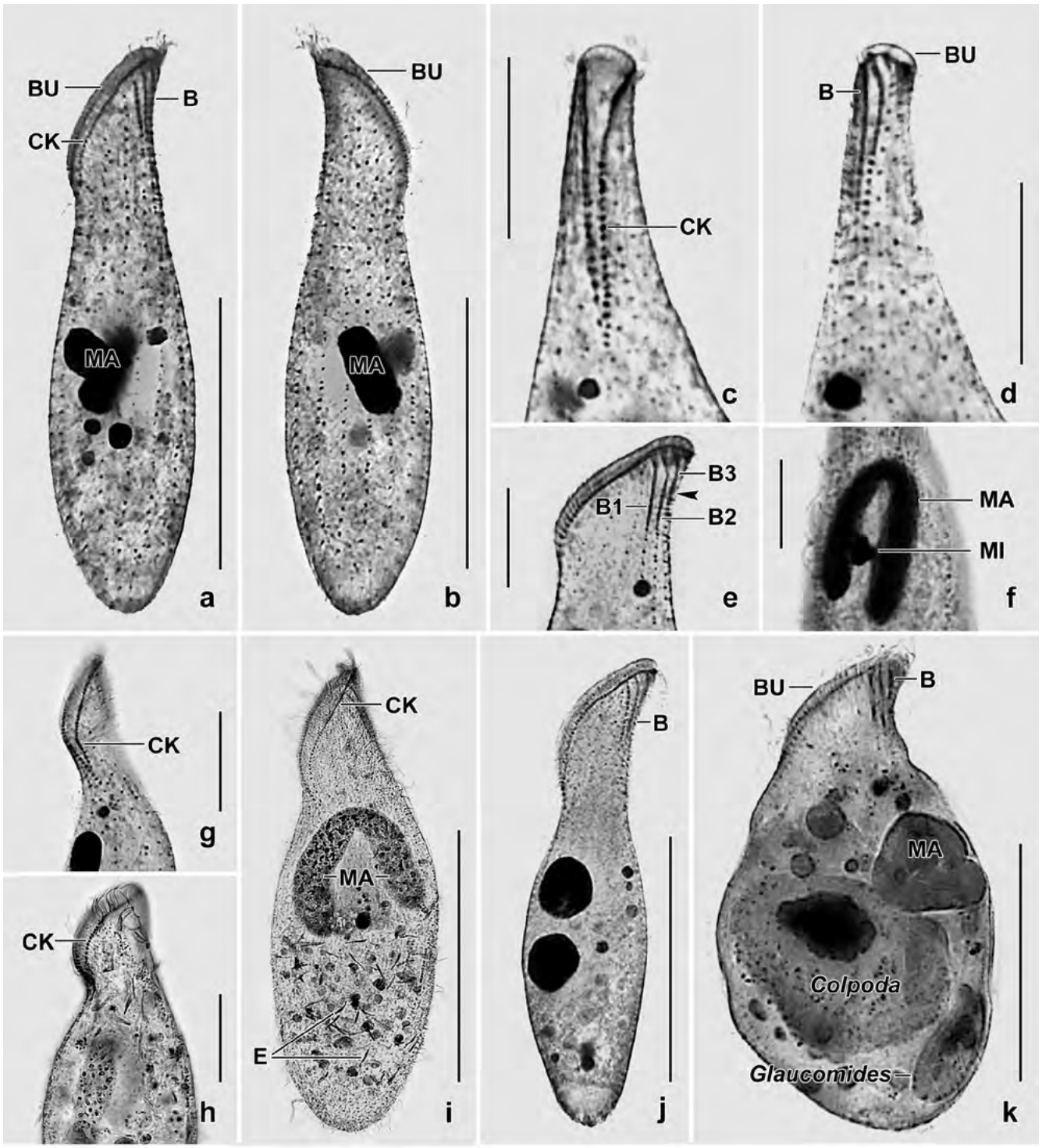
Cilia *in vivo* 9 µm long, arranged in an average of 14 meridional, ordinarily (theronts) to widely (trophonts) spaced rows abutting on circumoral kinety and composed of densely spaced cilia not condensed anteriorly; right side rows anteriorly curved dorsally, left side rows



Figs 1a–n. *Arcuospathidium bromelicola* from life (a–d, j–l) and after protargol impregnation (e–i, m, n). **a** – left side view of a representative specimen, length 85 µm; **b** – surface view, showing the minute (≤ 1 µm) cortical granules arranged in oblique rows; **c** – the oral bulge is distinctly curved in 70% of specimens; **d** – frontal view of a straight oral bulge studded with extrusomes; **e**, **f** – ciliary pattern of left and right side and macronucleus of holotype specimen, length 85 µm; note the *Arcuospathidium* ciliary pattern on the left side (e), i.e., the ciliary rows do not curve ventrally anteriorly; **g–i** – shape variability of oral bulge, respectively, circumoral kinety; **j** – mature extrusomes are rod-shaped and about 4 µm long; **k**, **l** – overview and detail of resting cyst in optical section. Note the conspicuous, pillar-shaped lepidosomes most frayed anteriorly; **m**, **n** – length comparison of trophont and theront. BU – oral bulge, B(1–3) – dorsal brush (rows), CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, EL – external layer, EP – excretory pore, FV – food vacuoles, G – cortical granulation, IL – internal layer, L – lipid droplet, MA – macronucleus, MI – micronucleus, SL – slime layer, T – anterior tail of dorsal brush rows. Scale bars: 2.5 µm (l), 15 µm (h, k), 20 µm (g, i), and 40 µm (a, e, f, m, n).



Figs 2a–m. *Arcuospathidium bromelicola* from life. **a**, **b** – left side and ventral view, showing body outline of a specimen with strongly convex oral bulge (arrowheads); **c** – oral bulge extrusomes (some marked by arrowheads) are rod shaped and about 4 × 0.3 µm in size; **d** – a pillar of a squashed cyst; **e** – bright field micrograph of a resting cyst in optical section, showing the thin external layer (arrow) and thick internal layer (opposed arrowheads); **f–h** – optical section of cyst pillars, showing the variability of the distal end; **i**, **j** – optical section and surface view, showing the narrowly spaced pillars; **k** – arrowheads mark overturned pillars in a squashed cyst; **l**, **m** – optical sections, showing the cysts filled with lipid droplets and surrounded by a narrow slime layer. CV – contractile vacuole, L – lipid droplets, MA – macronucleus, SL – slime layer. Scale bars: 2.5 µm (f–h), 10 µm (c), 15 µm (e, i, j, l, m), and 40 µm (a, b).



Figs 3a–k. *Arcuospathidium bromelicola* after protargol impregnation. **a**, **b** – left and right side view of a representative specimen; **c**, **d** – ventral and dorsal view of anterior body region, showing the cuneate, slightly curved circumoral kinety and the dorsal brush; **e** – left side view, showing the heterostichad dorsal brush with end of row 3 marked by an arrowhead; **f** – a horseshoe-shaped macronucleus accompanied by an ellipsoidal micronucleus with a minute, hemispherical cap; **g–i** – specimens with distinctly curved oral bulge, respectively, circumoral kinety; **j** – post-conjugant with two macronuclear nodules; **k** – a trophont with two large food vacuoles which contain ciliates and dislocate the macronucleus. BU – oral bulge, B(1–3) – dorsal brush (rows), CK – circumoral kinety, E – extrusomes, MA – macronucleus, MI – micronucleus. Scale bars: 10 µm (e, f), 15 µm (c, d, g, h), 30 µm (k), and 40 µm (a, b, i, j).

straight. Dorsal brush heterostichad and isomorphic, rows 1 and 2 composed of 7–15 and 11–17 dikinetids, respectively, both of almost same length with longest row 2 occupying only 14% of body length on average; bristles slightly inflated, anterior bristles about 2.5 µm long, posterior about 2 µm. Row 3 shorter than row 2 by 55%, composed of 4–10 dikinetids, followed by a short posterior tail composed of 3–5 monokinetidal bristles 2 µm long; all rows with a short anterior tail of ciliated monokinetids (Figs 1a, e, f, 3a, b, d, e, j, k; Table 1).

Oral bulge studded with extrusomes, about as long as widest trunk region, inclined to main body axis by about 40–60°, surface strongly convex in 24% of specimens (out of 21), moderately convex in 62%, and flat in 14%, cuneate in ventral view, in protargol preparations 23 × 6 × 3 µm in size; in 70% of specimens slightly to distinctly bent mainly in proximal third (Figs 1a, c, d, g–i, 2a, 3a–c, e, g–i; Table 1). Circumoral kinety of the same shape as oral bulge, composed of narrowly spaced dikinetids. Oral basket not impregnated with the protargol method used.

Resting cysts *in vivo* on average 26 µm across (Table 1); covered by a thin mucus layer containing bacteria and flagellates. Cyst wall light brown to honey-brown, about 2 µm thick, composed of a thin external and a thick internal layer both structureless in the light microscope. Cyst surface studded with 1–3 µm high, honey-brown pillars (lepidosomes?) distally frayed; pillars rarely spinous, can be overturned in squashed cysts, indicating that they are lepidosomes. Cyst contents close to wall, dominated by lipid droplets 1–3 µm across. Macronuclear strand shorter than in vegetative specimens (Figs 1k, l, 2d–m).

Occurrence and ecology: As yet found only at type locality; became moderately abundant in raw cultures.

Remarks: *Arcuospathidium bromelicola* is very similar to *A. muscorum muscorum* (for a review, see Foissner and Xu 2007), from which it differs by the structure of the cyst (wall with conspicuous pillars vs. smooth) and the shape of the oral bulge (proximal third often rather strongly curved vs. straight or slightly curved).

Protospathidium lepidosomatum nov. spec. (Figs 4a–o, 5a–k, 6a–g; Tables 2, 3)

Diagnosis: Size about 70 × 15 µm *in vivo*; narrowly spatulate. On average 9 scattered, broadly ellipsoidal macronuclear nodules and 5 globular micronuclei. Contractile vacuole in rear body end. Extrusomes rod-shaped with rounded ends, *in vivo* about 3 × 0.3 µm in size. On average 11 ciliary rows. Dorsal brush distinctly heterostichad, row 1 shorter than the longest row 2 by about 58%, composed of an average of 3 dikinetids; rows 2 and 3 of similar length, composed of an average of 10 and 7 dikinetids, respectively. Oral bulge oblique, flat, obovate, on average 9 µm long *in vivo*. Resting cyst surface studded with 1–2 µm high, nipple-shaped lepidosomes.

Type locality: In tank bromeliads from the "Upper Cedar Valley", southern slope of the Blue Mountains, Jamaica, 18°2′N 76°34′W.

Type material: 1 holotype and 2 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: Composite of Greek nouns *lepidotos* (scaled) and *soma* (body), referring to the lepidosomes on the cyst surface.

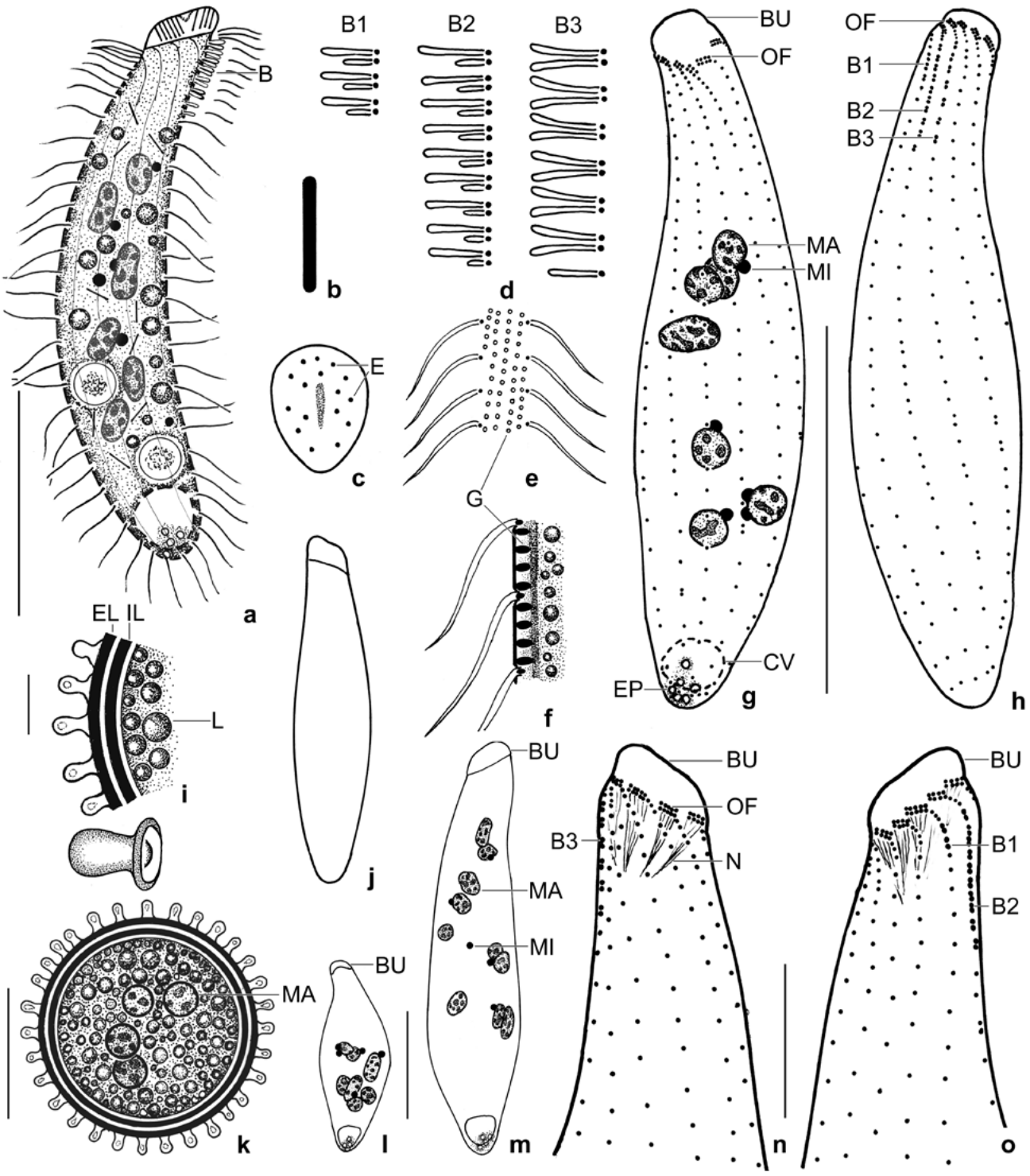
Description: Size and length:width ratio rather variable (CV ~ 15%) due to slender theronts and thick trophonts (Table 2); *in vivo* 50–80 × 10–20 µm, on average 70 × 15 µm; in protargol preparations 63 × 16 µm and 72 × 18 µm when adding 15% for preparation shrinkage. Usually narrowly, rarely very narrowly spatulate and slightly curved dorsally or strongly inflated by food vacuoles (Fig. 5k). Anterior end oblique, about 8 × 6 µm in protargol preparations, posterior end narrowly rounded to slightly acute (Figs 4a, g, h, j, l, m, 5a, b, g–i). Four to 14, on average nine globular to ellipsoidal macronuclear nodules, most in middle third of body; nucleoli 1–3 µm across (Figs 4a, g, l, m, 5a, d, g, k). Post-dividers with a nodulated macronuclear strand or a mixture of nodules and short strands (Fig. 5h, i), as expected in multinucleate species (Foissner *et al.* 2002); some post-conjugants with two macronuclear nodules. Two to eight, on average five micronuclei 1–2 µm across scattered among macronuclear nodules (Figs 4a, g, 5b). Contractile vacuole in rear body end, on average four excretory pores (Figs 4a, g, 5b). Extrusomes packed in oral bulge and scattered in cytoplasm, *in vivo* rod-shaped with rounded ends and about 2.5–3 × 0.3 µm in size; both oral bulge and cytoplasmic extrusomes sometimes impregnate with the protargol method used (Figs 4a–c, 5e; Table 2). Cortex very flexible, gelatinous, about 0.8 µm thick, cortical granules rather strongly refractive and arranged in about four rows between each two kineties, about 0.5 × 0.25 µm in size

Table 2. Morphometric data on *Protospathidium lepidosomatum*.

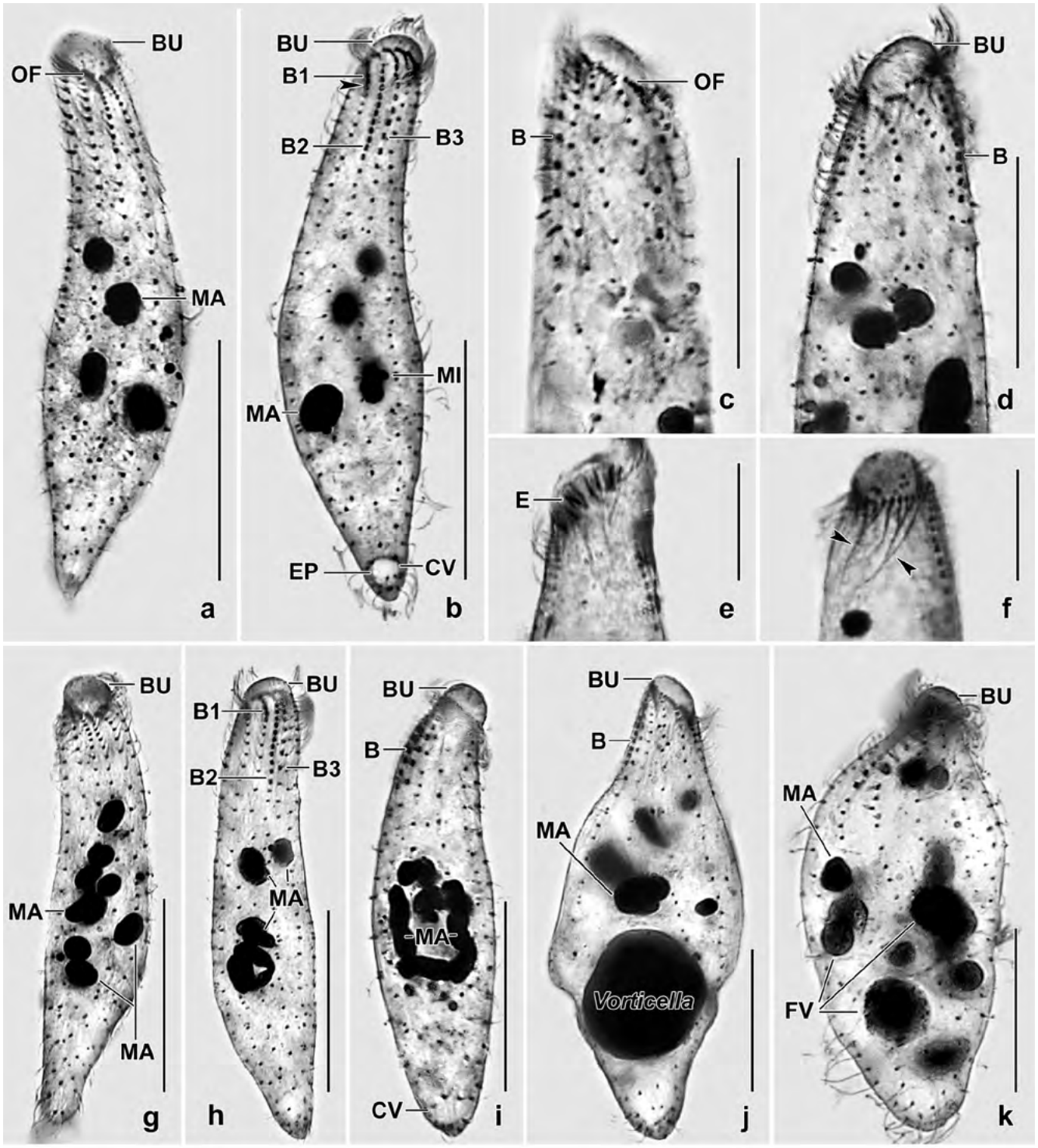
Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length *in vivo* (rough values), µm	66.8	70.0	–	–	–	50.0	80.0	11
Body, width *in vivo* (rough values), µm	15.0	15.0	–	–	–	10.0	20.0	11
Body length:width, ratio *in vivo*	4.7	4.7	–	–	–	2.5	7.5	11
Body, length, µm	63.9	62.7	10.5	1.9	16.4	38.8	84.4	29
Body, maximum postoral width, µm	16.2	16.0	2.8	0.4	14.1	13.1	22.8	29
Body length:width, ratio	4.0	4.0	0.6	0.1	14.5	2.5	5.2	29
Oral bulge, length (cells oriented ventrally), µm	7.4	7.4	0.6	0.2	8.1	6.3	8.6	13
Oral bulge, length (cells oriented laterally), µm	8.1	8.0	1.0	0.2	11.6	6.3	9.6	17
Oral bulge, width, µm	5.8	5.7	0.8	0.2	13.8	4.6	7.4	13
Oral bulge, height, µm	2.7	2.8	0.6	0.1	20.6	1.7	3.4	21
Body length:oral bulge length, ratio (cells oriented ventrally)	8.2	7.8	1.4	0.4	16.7	6.2	11.3	13
Body length:oral bulge length, ratio (cells oriented laterally)	8.2	8.4	1.2	0.3	14.9	5.3	10.3	17
Body width:oral bulge length, ratio (cells oriented ventrally)	2.2	2.2	0.3	0.1	14.2	1.8	2.8	13
Body length:oral bulge length, ratio (cells oriented laterally)	2.1	2.0	0.4	0.1	19.1	1.6	3.4	17
Anterior body end to first macronuclear nodule, distance, µm	18.5	18.2	4.3	0.9	23.2	11.4	27.4	21
Macronucleus figure, length, µm	27.5	28.5	9.3	2.0	33.6	14.8	45.6	21
Macronuclear nodules, length, µm	5.6	5.7	1.5	0.3	27.1	4.0	9.7	21
Macronuclear nodules, width, µm	3.6	3.4	0.5	0.1	14.6	2.9	4.6	21
Macronuclear nodules, number	9.1	9.0	2.8	0.6	31.4	4.0	14.0	21
Micronuclei, diameter, µm	1.6	1.7	0.2	0.1	15.1	1.1	2.0	21
Micronuclei, number	5.0	5.0	1.5	0.3	30.0	2.0	8.0	21
Ciliary rows, number	10.8	11.0	0.7	0.2	6.3	10.0	13.0	21
Kinetids in a right side ciliary row, number	24.3	23.0	4.4	1.0	18.1	17.0	33.0	21
Circumoral kinety to last dikinetid of brush row 1, distance, µm	5.1	4.6	1.3	0.3	26.2	3.4	8.6	21
Circumoral kinety to last dikinetid of brush row 2, distance, µm	12.1	12.5	1.7	0.4	14.1	7.4	14.3	21
Circumoral kinety to last dikinetid of brush row 3, distance, µm	11.0	11.4	1.7	0.4	15.5	6.8	13.1	21
Dorsal brush row 1, number of dikinetids	3.1	3.0	0.6	0.1	19.4	2.0	4.0	21
Dorsal brush row 2, number of dikinetids	10.0	10.0	1.1	0.2	10.5	9.0	12.0	21
Dorsal brush row 3, number of dikinetids	6.5	6.0	1.0	0.2	15.1	4.0	8.0	21
Dorsal brush, number of rows[b]	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Dikinetids composing a right side oral kinetofragment, number	4.1	4.0	0.6	0.1	13.8	3.0	6.0	21
Extrusome, length, µm	2.5	2.6	–	–	–	1.7	3.1	17
Excretory pores, number	3.9	3.0	1.4	0.3	35.2	?	8.0	23
Resting cyst, diameter with lepidosomes *in vivo*, µm	20.1	20.0	1.7	0.4	8.4	17.0	23.0	17
Resting cyst, diameter without lepidosomes *in vivo*, µm	15.6	15.5	–	–	–	13.0	19.0	17
Cyst wall thickness including lepidosomes *in vivo*, µm	2.4	2.5	0.4	0.1	17.5	1.5	3.0	17

[a] Data based, if not mentioned otherwise, on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a raw culture. 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.

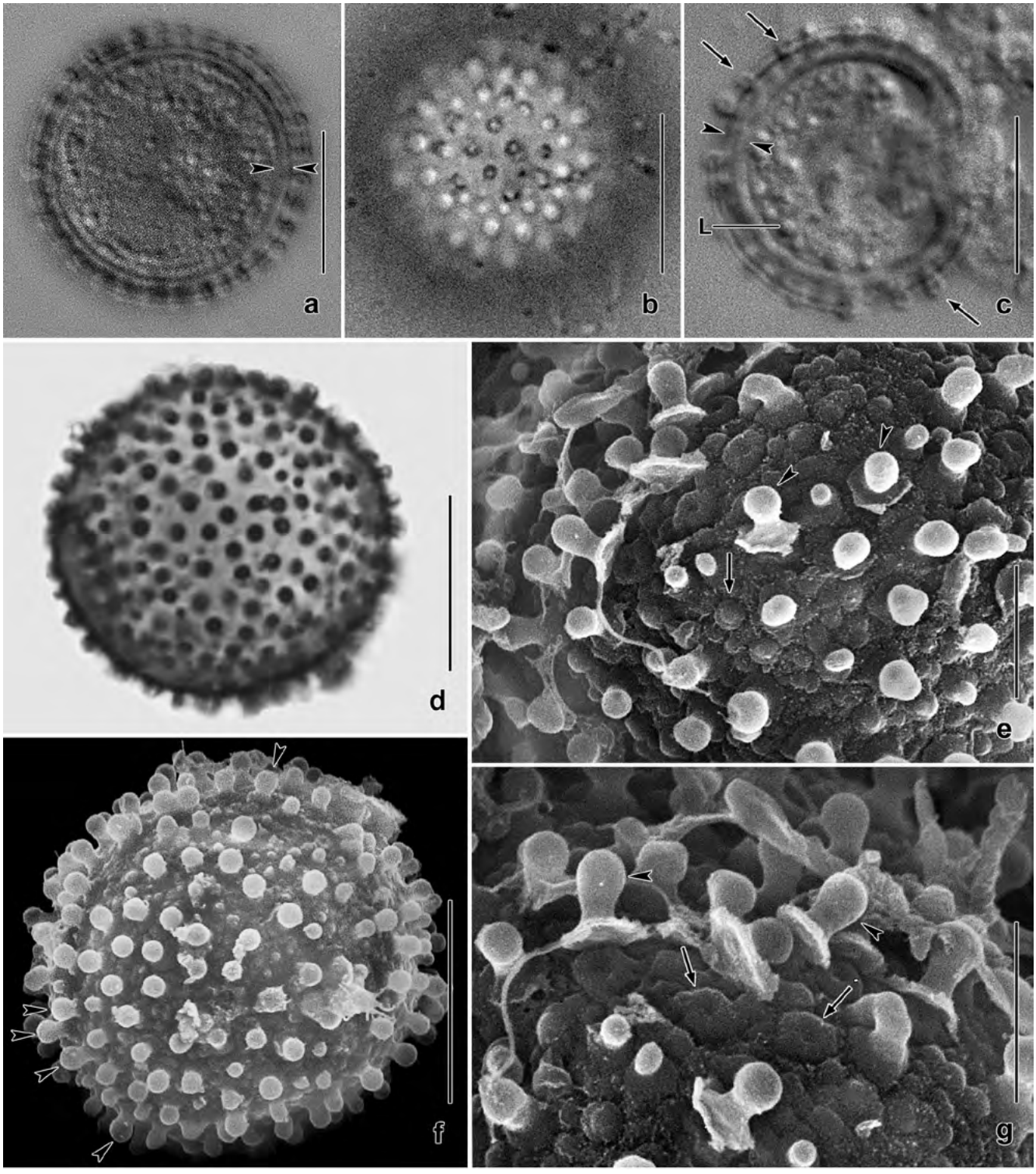
[b] Two specimens with some supernumerary dikinetids, forming a short fourth row.



Figs 4a–o. *Protospathidium lepidosomatum* from life (a–f, i–k), after protargol impregnation (g, h, l–o), and in the SEM (i). **a** – left side view of a representative specimen, length 70 μm; **b** – mature extrusome, 2.5 μm; **c** – frontal view of oral bulge; **d** – slightly schematized dorsal brush; **e**, **f** – surface view and optical section of cortex; **g**, **h** – ciliary pattern of ventral and dorsal side and nuclear apparatus of holotype specimen, length 75 μm; **i**, **k** – details and overview of resting cyst in optical section. Note the nipple-shaped lepidosomes; **j** – body outline; **l**, **m** – maximum size variability; **n**, **o** – right and left side view, showing the ciliary pattern. BU – oral bulge, B(1–3) – dorsal brush (rows), CV – contractile vacuole, E – extrusomes, EL – external layer, EP – excretory pores, G – cortical granules, IL – internal layer, L – lipid droplets, MA – macronuclear nodules, MI – micronuclei, N – nematodesma bundle, OF – oral kinetofragments. Scale bars: 2.5 μm (i), 10 μm (k, n, o), 30 μm (a, l, m), and 40 μm (g, h).



Figs 5a–k. *Protospathidium lepidosomatum* after protargol impregnation. **a**, **b** – ventral and dorsal view, showing the nuclear apparatus and the heterostichad dorsal brush with end of row 1 marked by an arrowhead; **c**, **d** – right and left side view of anterior body region, showing the disconnected oral kinetofragments; **e** – rarely, the oral bulge extrusomes impregnate; **f** – arrowheads indicate nematodesma bundles originating from the oral kinetofragments; **g–i** – variability of macronucleus; **j** – a specimen with a large food vacuole containing a *Vorticella*; **k** – an inflated specimen with some food vacuoles up to 10 µm across. BU – oral bulge, B(1–3) – dorsal brush (rows), CV – contractile vacuole, E – extrusomes, EP – excretory pore, FV – food vacuoles, MA – macronuclear nodules, MI – micronucleus, OF – oral kinetofragments. Scale bars: 10 µm (e, f), 20 µm (c, d, j, k), and 30 µm (a, b, g–i).



Figs 6a–g. *Protospathidium lepidosomatum* from life (a–c), after protargol impregnation (d), and in the scanning electron microscope (e–g). **a** – optical section showing the external and internal cyst wall (opposed arrowheads); **b**, **f** – surface views showing the nipple-shaped lepidosomes (arrowheads); **c** – a squashed cyst, showing the thick wall (opposed arrowheads) and lepidosomes with a less refractive centre (arrows); **d** – the lepidosomes impregnate with the protargol method used; **e**, **g** – high magnification of the nipple-shaped lepidosomes (arrowheads). When detached, minute convexities become recognisable (arrows). L – lipid droplet. Scale bars: 2.5 µm (e, g) and 10 µm (a–d, f).

(Figs 4e, f). Cytoplasm with rather many lipid droplets 1–3 µm in diameter and food vacuoles with unidentifiable contents up to 10 µm across; one specimen with a *Vorticella* sp. about 20 µm in size (Figs 5j, k). Swims rather rapidly.

Cilia *in vivo* 8 µm long, ordinarily spaced. On average 11 meridional, ordinarily spaced ciliary rows more densely ciliated anteriorly than posteriorly, arranged in typical *Protospathidium* pattern (Foissner and Xu 2007). Dorsal brush distinctly heterostichad and isomorphic, row 1 with 1.5 µm long slightly inflated bristles, composed of 2–4 dikinetids, shorter than row 2 by about 60%. Rows 2 and 3 with 2 µm long slightly inflated bristles, composed of 9–12 and 4–8 dikinetids, respectively, of almost the same length with the longest row 2 occupying only 16% of body length on average. Row 3 with widely spaced dikinetids and a short posterior tail composed of 2–3 monokinetidal bristles; all rows with a short anterior tail of ciliated monokinetids (Figs 4a, d, g, h, n, o, 5a–d, g–i; Table 2).

Oral bulge about half as long as widest trunk region, oblique by about 25–45°, surface flat to slightly convex, obovate in ventral view, in protargol preparations on average 8 × 6 × 3 µm in size; studded with extrusomes (Figs 4a, c, j, n, 5e, j; Table 2). Circumoral kinety broadly elliptical, composed of dikinetidal kinetofragments attached obliquely to the respective ciliary rows and separated from each other by gaps 1–3 dikinetids wide; kinetofragments composed of 3–6 dikinetids each associated with an 8 µm long cilium and a short nematodesma. Oral basket composed of cuneate, about 10–15 µm long nematodesma bundles originating from oral kinetofragments (Figs 4g, h, n, o, 5a–d, f, g).

Resting cysts very refractive due to the lepidosomes and the thick wall, *in vivo* 15–25 µm across, on average 20 µm with lepidosomes included (Table 2). Cyst wall yellowish, about 2–2.5 µm thick including lepidosomes, composed of an external and an internal layer both structureless in the light microscope. Cyst surface studded with 1–2 µm high, usually nipple-shaped lepidosomes attached to minute wall convexities and having a less refractive centre of varying size; impregnate with protargol. Cyst contents close to wall, composed of lipid droplets 1–3 µm across and globular macronuclear nodules faintly impregnated with protargol (Figs 4i, k, 6a–g).

Occurrence and ecology: Discovered in tank bromeliads from Jamaica, became moderately abundant in raw cultures. Surprisingly, later we found this species with its highly characteristic lepidosomes in a non-flooded Petri dish culture with leaf litter and surface soil from a park at the margin of the town of Salzburg, Austria.

Remarks: *Protospathidium lepidosomatum* is very similar to *P. muscicola* (for a review, see Foissner and Xu 2007), from which it differs by the nipple-shaped (vs. conical) lepidosomes covering the cyst wall. All other important features are near or within the variability of *P. muscicola* (Table 3).

Table 3. Comparison of *Protospathidium lepidosomatum* with populations of *P. muscicola* from Benin (Dragesco and Dragesco-Kernéis 1979, 1986), Austria (Berger *et al.* 1984), Venezuela and Botswana (Foissner and Xu 2007).

Species	*P. lepidosomatum*	*P. muscicola*			
Characteristics[a]	Jamaica	Benin	Austria	Venezuela	Botswana
Body length:width, ratio	2.5–5.2 (4.0)	~ 5[b]	3.6–10.2 (7.0)	3.7–6.8 (5.1)	no data
Ciliary rows, number	10–13 (10.8)	10–12	7–10 (8.8)	9–12 (10.5)	9–12
Macronuclear nodules, number	4–14 (9.1)	10–20	15–30	9–21 (14.1)	no data
Dorsal brush row 1, number of dikinetids	2–4 (3.1)	2[b]	3–7 (4.6)	2–6 (4.1)	2–8[b]
Dorsal brush row 2, number of dikinetids	9–12 (10.0)	9[b]	7–14 (11.7)	9–19 (14.9)	11–14[b]
Dorsal brush row 3, number of dikinetids	4–8 (6.5)	6[b]	7–11 (8.8)	6–14 (9.7)	7–9[b]
Extrusome shape	oblong	no data	no data	very narrowly ovate	oblong
Extrusome, size *in vivo*, µm	2.5–3 × 0.3	no data	no data	3 × 0.5	2.5–3 × 0.3–0.4

[a] Data based, if not mentioned otherwise, on mounted, protargol-impregnated and randomly selected specimens. First values minimum and maximum; arithmetic means in parentheses.

[b] According to the related illustrations.

***Spathidium bromeliophilum* nov. spec. (Figs 7a–r, 8a–g, 9a–h, 10a–e, 11a–j, 12a–f, 13a–g, 14a–c, 15a–f; Tables 4, 5)**

Diagnosis: Size about 135 × 35 µm *in vivo*. Narrowly spatulate with oblique, slightly dumbbell-shaped to cuneate oral bulge about as long as widest trunk region and screwed like a propeller blade (∞-shaped); ventral portion of bulge and circumoral kinety more or less bent laterally in 80% of specimens. On average 30 ellipsoidal, scattered macronuclear nodules and several globular micronuclei. Extrusomes bluntly fusiform and asymmetrical, about 5 µm long. Two size-types of cortical granules. On average 17 ciliary rows, 3 anteriorly differentiated to an isostichad dorsal brush occupying 22% of body length: shortest row 1 composed of 17 dikinetids, rows 2 and 3 of similar length but composed of 23 and 17 dikinetids on average, respectively; monokinetidal tail of brush row 3 extends to mid-body. Type III resting cyst.

Type locality: In tank bromeliads from the "Upper Cedar Valley", southern slope of the Blue Mountains, Jamaica, 18º2′N 76º34′W.

Type material: 1 holotype and 5 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: Composite of Bromeliaceae, the plant family in whose leaf-tanks it occurs, and *philum*, from the Greek adjective *philos* (loving).

Description: Size and length:width ratio highly variable *in vivo* and in protargol preparations, depending on culture age and conditions: 90–180 × 20–50 µm *in vivo*, usually about 135 × 35 µm (n = 7); length:width

Table 4. Morphometric data on *Spathidium bromeliophilum*.

Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length, µm	125.1	124.0	12.9	2.7	10.3	108.0	156.0	23
Body, width, µm	30.8	28.0	5.5	1.1	17.8	26.0	44.0	23
Body length:width, ratio	4.2	4.4	0.9	0.2	21.7	2.6	5.6	23
Oral bulge, length, µm	32.8	33.0	2.9	0.6	9.0	28.0	37.0	23
Oral bulge (circumoral kinety), width, µm	8.6	9.0	1.2	0.3	14.2	6.0	11.0	21
Oral bulge, height, µm	2.7	2.8	0.4	0.1	13.9	2.0	3.3	21
Body length:length of oral bulge, ratio	3.8	3.8	0.4	0.1	9.6	3.1	4.7	23
Oral bulge length:body width, ratio	1.1	1.1	0.2	0.0	18.0	0.7	1.4	23
Circumoral kinety to last dikinetid of dorsal brush row 1, distance, µm	21.8	23.0	3.2	0.7	14.6	16.0	27.0	23
Circumoral kinety to last dikinetid of dorsal brush row 2, distance, µm	27.1	27.0	3.3	0.7	12.3	21.0	32.0	23
Circumoral kinety to last dikinetid of dorsal brush row 3, distance, µm	26.2	26.0	3.8	0.8	14.6	19.0	34.0	23
Anterior body end to first macronuclear nodule, distance, µm	32.7	32.0	5.3	1.1	16.1	22.0	42.0	23
Macronuclear nodules, length, µm	6.2	6.0	1.1	0.2	17.6	5.0	8.0	23
Macronuclear nodules, width, µm	3.8	4.0	0.9	0.2	24.7	3.0	7.0	23
Macronuclear nodules, number	30.5	30.0	7.0	1.5	23.0	17.0	41.0	21
Somatic ciliary rows, number	17.1	17.0	1.4	0.3	8.3	15.0	21.0	23
Ciliated kinetids in a right side kinety, number	54.0	51.0	8.7	1.9	16.1	44.0	72.0	21
Dorsal brush rows, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	23
Dikinetids in dorsal brush row 1, number	16.8	18.0	2.4	0.5	14.5	13.0	21.0	21
Dikinetids in dorsal brush row 2, number	23.3	22.0	3.7	0.8	16.0	18.0	30.0	21
Dikinetids in dorsal brush row 3, number	16.9	17.0	2.1	0.5	12.5	14.0	22.0	21
Resting cysts, diameter *in vivo*, µm	41.4	42.0	3.0	0.8	7.2	38.0	47.0	15

[a] Data based, if not mentioned otherwise, on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a semipure culture. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of specimens investigated, SD – standard deviation, SE – standard error of arithmetic mean, $\bar{x}$ – arithmetic mean.

ratio 2.4–6.8:1, frequently 4:1; acontractile but very flexible (Table 4). Shape narrowly spatulate, mid-body circular in cross-section, hyaline anterior region laterally flattened and more or less set off from trunk by the slightly narrowed neck. Anterior end (oral bulge) ordinarily oblique, posterior portion slightly tapering and evenly rounded (Figs 7a, f–j, m, n, 8a–c, 9a). On average 30 macronuclear nodules scattered throughout cytoplasm; individual nodules globular to ellipsoidal, about 6 × 4 µm in size in protargol preparations. Micronuclei 1.5–2 µm across, scattered between macronuclear nodules, exact number difficult to determine due to similar-sized and impregnated lipid droplets (Figs 7a, n, 8a–c, 15f; Table 4). Contractile vacuole in posterior body end, with several excretory pores in pole area. Extrusomes bluntly fusiform and asymmetrical, about 5 × 0.5–0.6 µm long *in vivo*, studded in oral bulge and scattered in cytoplasm; anterior third usually lightly impregnated with the protargol method used (Figs 7a, d, 9c, g, 13b, e). Cortex very flexible, contains about 10 rows of granules in two size-types (0.2 and 0.4 µm) between each two kineties (Figs 7e, 9h). Cytoplasm colourless, more or less packed with lipid droplets up to 10 µm across and food vacuoles with granular contents, except for flattened and hyaline neck region (Fig. 7a). Feeds on medium-sized ciliates (*Colpoda* spp., *Vorticella* sp.), flagellates, and naked amoebae, the latter being engulfed within about 30 seconds, whereby the oral bulge centre opens and the prey glides into a bursiform sac soon becoming a globular food vacuole (Figs 7k, l). Wriggles in slimy bacterial and protozoan masses, showing great flexibility.

Cilia about 8 µm long *in vivo*, ordinarily spaced (~ 2.5 µm), arranged in an average of 17 ordinarily spaced, equidistant rows (~ 5.7 µm in protargol preparations); those of right side attached to circumoral kinety in acute angles and with 2–7 closely spaced cilia in anterior region, those of left side abutting at nearly right angles (Figs 7a, m, n, 8a–c, e–g, 9a, b, 13a, b, e). Dorsal brush three-rowed, rarely a fourth row occurs, comparatively short because occupying on average only 22% of body length; isostichad, i.e., all rows of similar length; inconspicuous because bristles only up to 2.5 µm long and as thin as ordinary somatic cilia; all rows with an anterior tail of 4–8 densely spaced monokinetids; tail rarely lacking. Shortest brush row 1 about 22 µm long and composed of 17 dikinetids, rows 2 and 3 of very similar length (27 µm vs. 26 µm on average) but different in number and spacing of dikinetids, i.e., 23 ordinarily spaced (0.7–1.5 µm) and 17 widely spaced (≥ 1.5 µm) dikinetids, respectively; row 3 continues to mid-body with a monokinetidal tail of 2–2.5 µm long bristles (Figs 7a, m, q, 8b–d, f, g; Table 4).

Oral bulge slightly longer than body width on average, inclined about 45º to ventral side, flat to indistinctly convex with dorsal end slightly higher than ventral, slightly cuneate to dumbbell-shaped with bluntly pointed ventral end; ventral half more or less curved laterally in 80% of specimens, straight in the rest; bright because packed with extrusomes; obliquely striated due to microtubule bundles originating from circumoral kinety; without temporary cytostome (Figs 7a–c, f–j, r, 8a, b, e, f, 9a, b, 13a, b, e; Table 4). Bulge base surrounded by an ∞-shaped circumoral kinety composed of closely spaced dikinetids giving rise to nematodesma bundles forming an ordinary oral basket (Figs 7n, r, 8a–c, e–g, 9b, 13a, b, e).

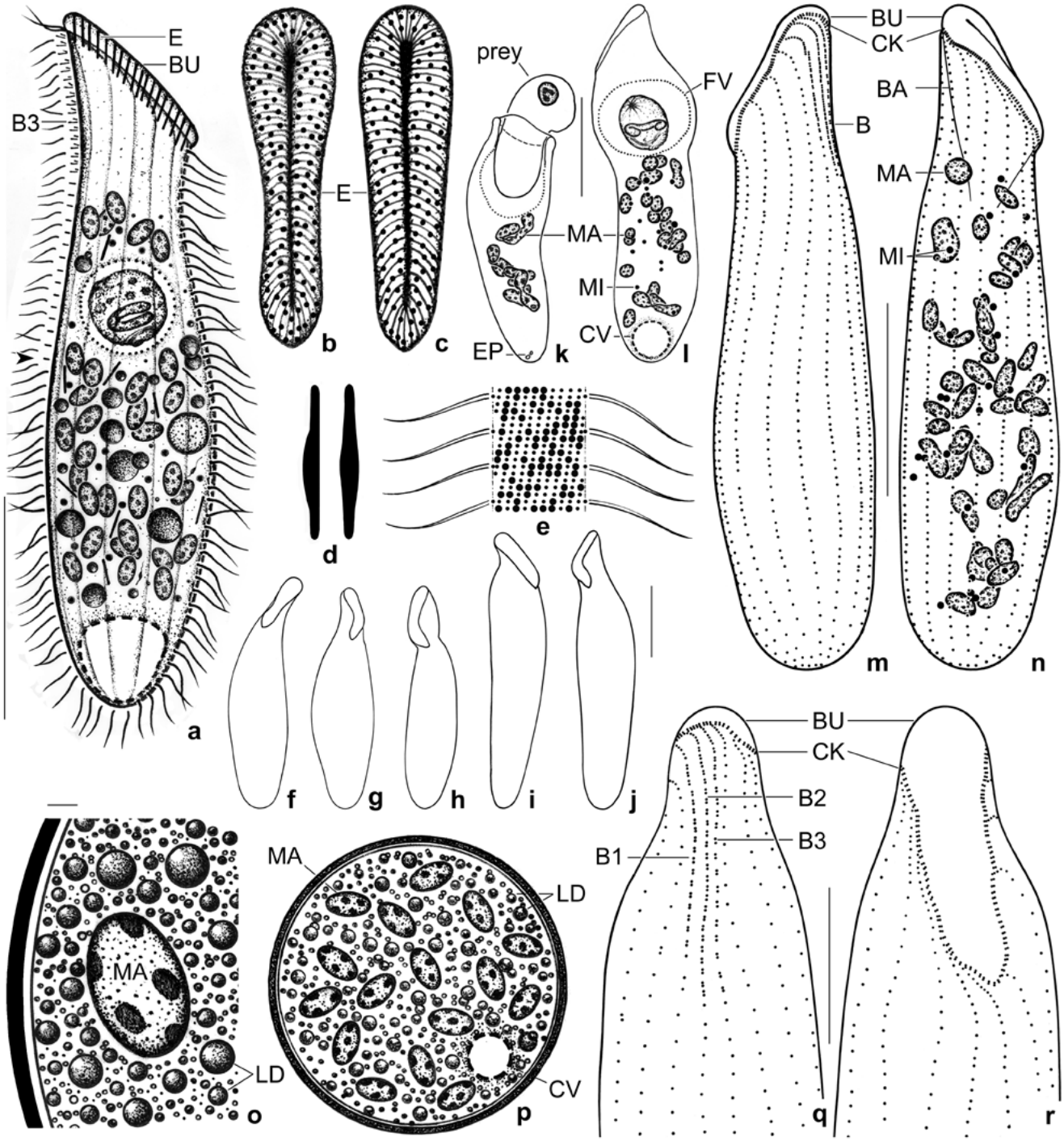
Starvation induces formation of type III resting cysts (Foissner and Xu 2007). Mature cysts on average 42 µm across *in vivo* (Table 4). Cyst wall yellowish, smooth, about 1 µm thick, separated from cytoplasm by a ~ 0.5 µm thick, hyaline sheet (endocyst?) not recognizable in squashed cysts (Figs 7o, 9e, f). Cyst plasm packed with four large (postconjugants?) or many smaller macronuclear nodules about 6 × 4 µm in size, granules ≤ 1 µm across, and lipid droplets 1–2 µm in size (Fig. 7p). Contractile vacuole still visible after one week, becomes active when cyst is slightly pressed by coverslip. Several degenerated cysts with clumped cytoplasm and contractile vacuole observed in two encystment trials (Fig. 9d).

Ontogenesis (Figs 8c, 10a–e, 11a–j, 12a–f, 13c, f, g, 14a–c, 15a–f; Table 5)

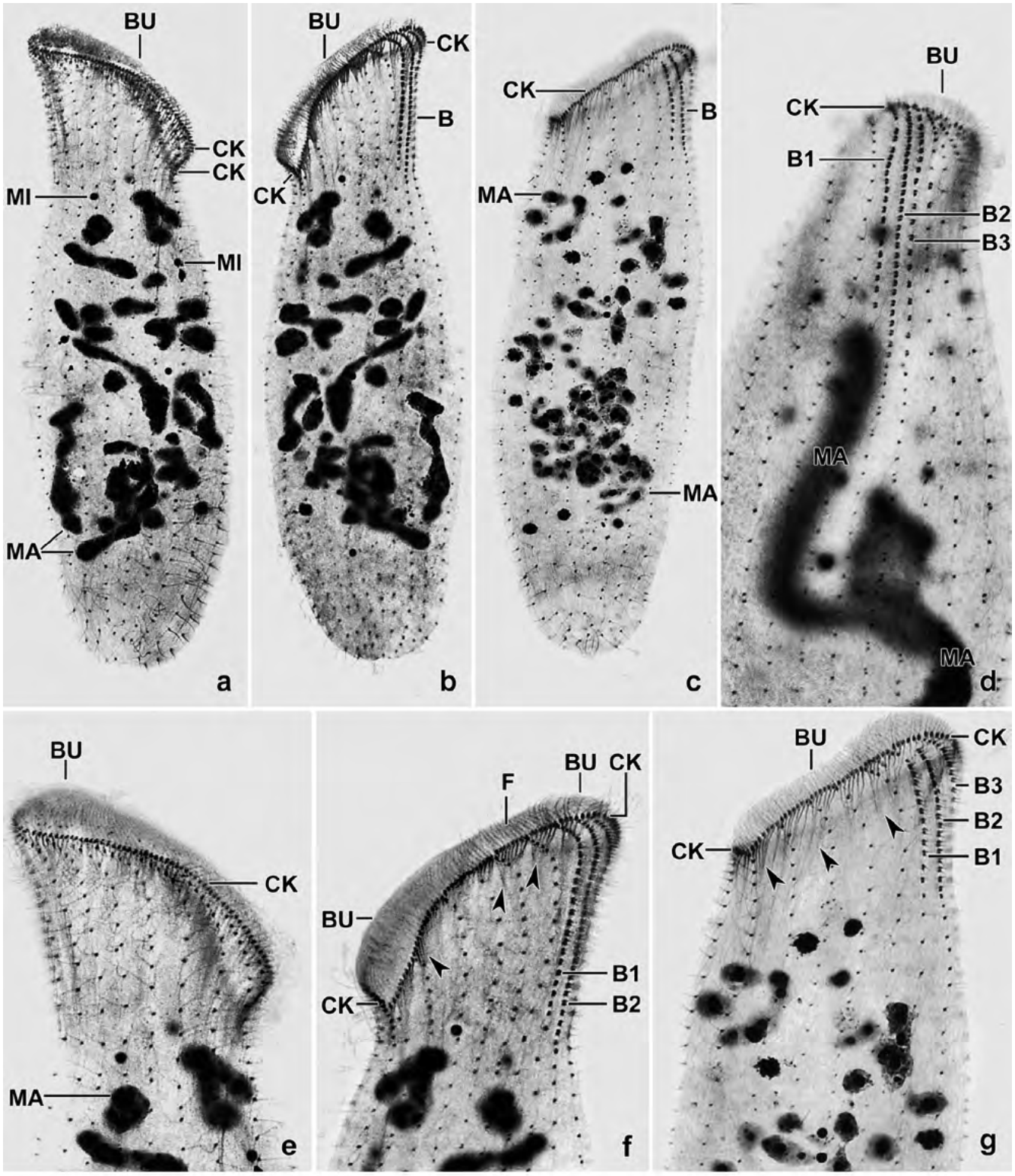
Essentially identical to that of *Spathidium turgitorum* described in detail by Foissner *et al*. (2002), i.e., it is homothetogenic, holotelokinetal, and occurs in nonencysted (freely motile) condition. Thus, we refer the reader to Table 5 and the many figures. However, one process should be highlighted, viz., the origin of the macronuclear nodules, which develop in post-dividers via a three-dimensional netting of the long macronuclear strand present in the dividers (Figs 11a–j, 15e, f).

Occurrence and ecology: As yet found only at type locality. A semipure culture could be established.

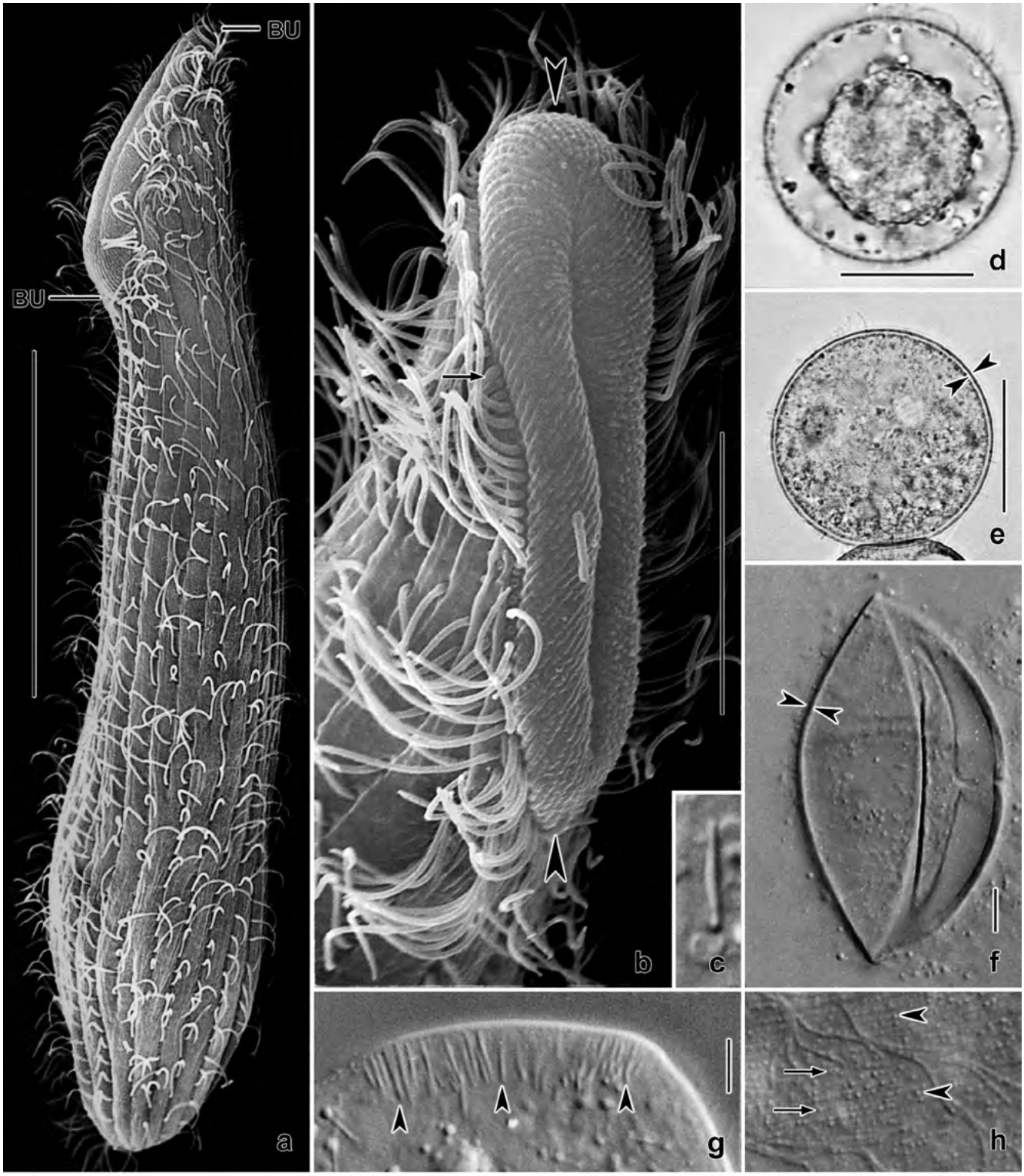
Remarks: Two species can be confused with *S. bromeliophilum*. *Spathidium anguilla*, as redescribed by Foissner (1984), is much more slender than *S. bromeliophilum* (~ 8:1 vs. ~ 4:1) and has only 11 (vs. 17) ciliary



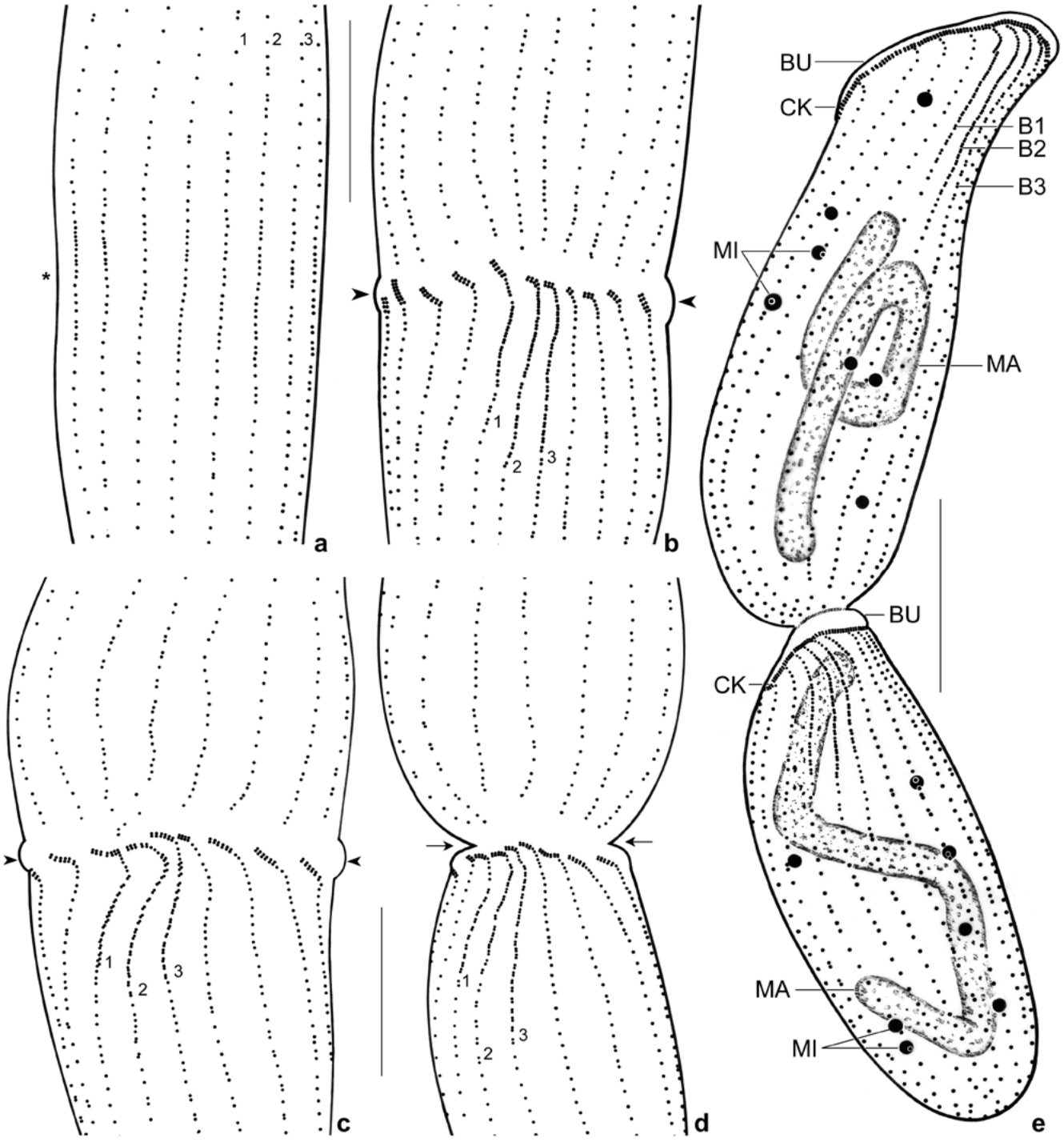
Figs 7a–r. *Spathidium bromeliophilum* from life (a–e, o–p) and after protargol impregnation (f–n, q, r). **a** – right side view of a representative specimen, length 135 µm. Arrowhead marks end of bristle tail of brush row 3; **b**, **c** – the ∞-shaped oral bulge, which is slightly dumbbell-shaped and/or cuneate, is studded with extrusomes. The oblique fibre bundles originate from the circumoral dikinetids; **d** – two views of the same extrusome, length 5 µm; **e** – surface view, showing the two size-types of cortical granules, diameter 0.2 µm and 0.4 µm; **f–j** – variability of shape of body and oral bulge whose ventral third is often curved laterally; **k**, **l** – a specimen engulfing a *Colpoda* (k) and another that has just engulfed a *Vorticella*; **m**, **n** – ciliary pattern of right and left side and nuclear apparatus of holotype specimen, length 138 µm; **o**, **p** – detail and overview of a resting cyst with inactive contractile vacuole, diameter 42 µm; **q**, **r** – ciliary pattern of anterior dorsal and ventral side, showing the isostichad dorsal brush and the wide spacing of the dikinetids in row 3. B(1–3) – dorsal brush (rows), BA – oral basket, BU – oral bulge, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, EP – excretory pores, FV – food vacuole, LD – lipid droplets, MA – macronuclear nodules, MI – micronuclei. Scale bars: 40 µm (a, f–n), 20 µm (q–r) and 2 µm (o).



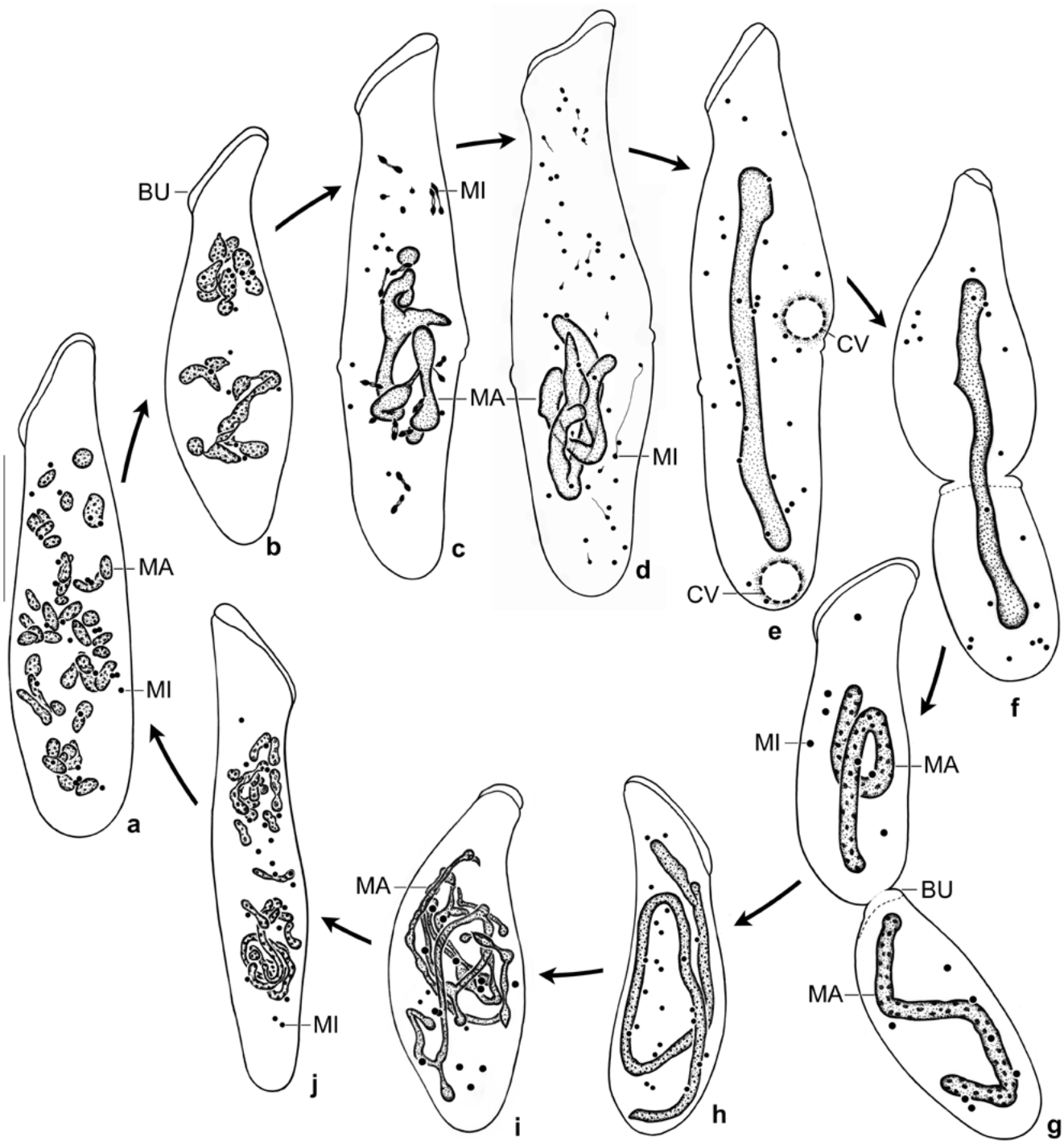
Figs 8a–g. *Spathidium bromeliophilum* after protargol impregnation. **a**, **b** – right and left side view of the same specimen; **c** – left side view of a specimen with many small macronuclear nodules; **d** – anterior dorsal view of the proter from a late divider, showing the isostichad dorsal brush; **e–g** – anterior right (e) and left (f, g) side views of the specimens shown in Figs (a–c). Arrowheads (f, g) mark nematodesma bundles forming the oral basket. B(1–3) – dorsal brush (rows), BU – oral bulge, CK – circumoral kinety, F – oral bulge fibres, MA – macronucleus, MI – micronuclei. (Without scale bars because from ± squashed specimens.)



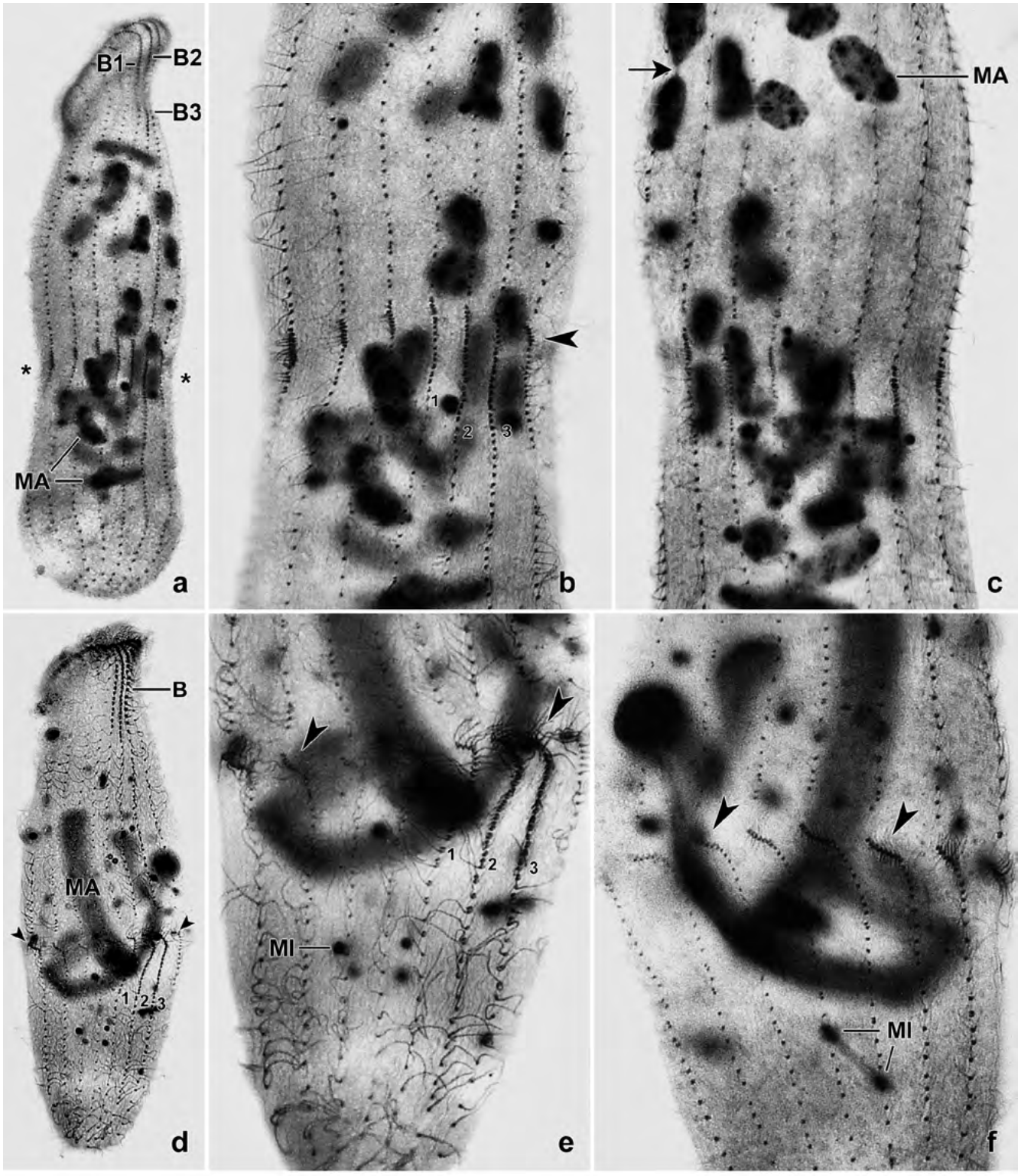
Figs 9a–h. *Spathidium bromeliophilum* in the SEM (a, b) and from life (c–h). **a** – a slender specimen; **b** – ventrolateral view, showing the cuneate oral bulge (arrowheads) and circumoral cilia (arrow); **c**, **g** – the extrusomes are about 5 µm long and are slightly asymmetrical (c); **d–f** – type III resting cysts, about 40 µm across. Mature cysts (e) show a thin (~ 1 µm) and smooth wall (opposed arrowheads), separated from the cytoplasm by a ~ 0.5 µm thick hyaline sheet (endocyst?) not recognizable in squashed cysts (f). When degenerated, the cytoplasm detaches from the wall (d); **h** – surface view showing the two-size types of cortical granules, diameter 0.2 µm and 0.4 µm (arrows and arrowheads). BU – oral bulge. Scale bars: 40 µm (a), 25 µm (d, e), 10 µm (b) and 5 µm (f, g).



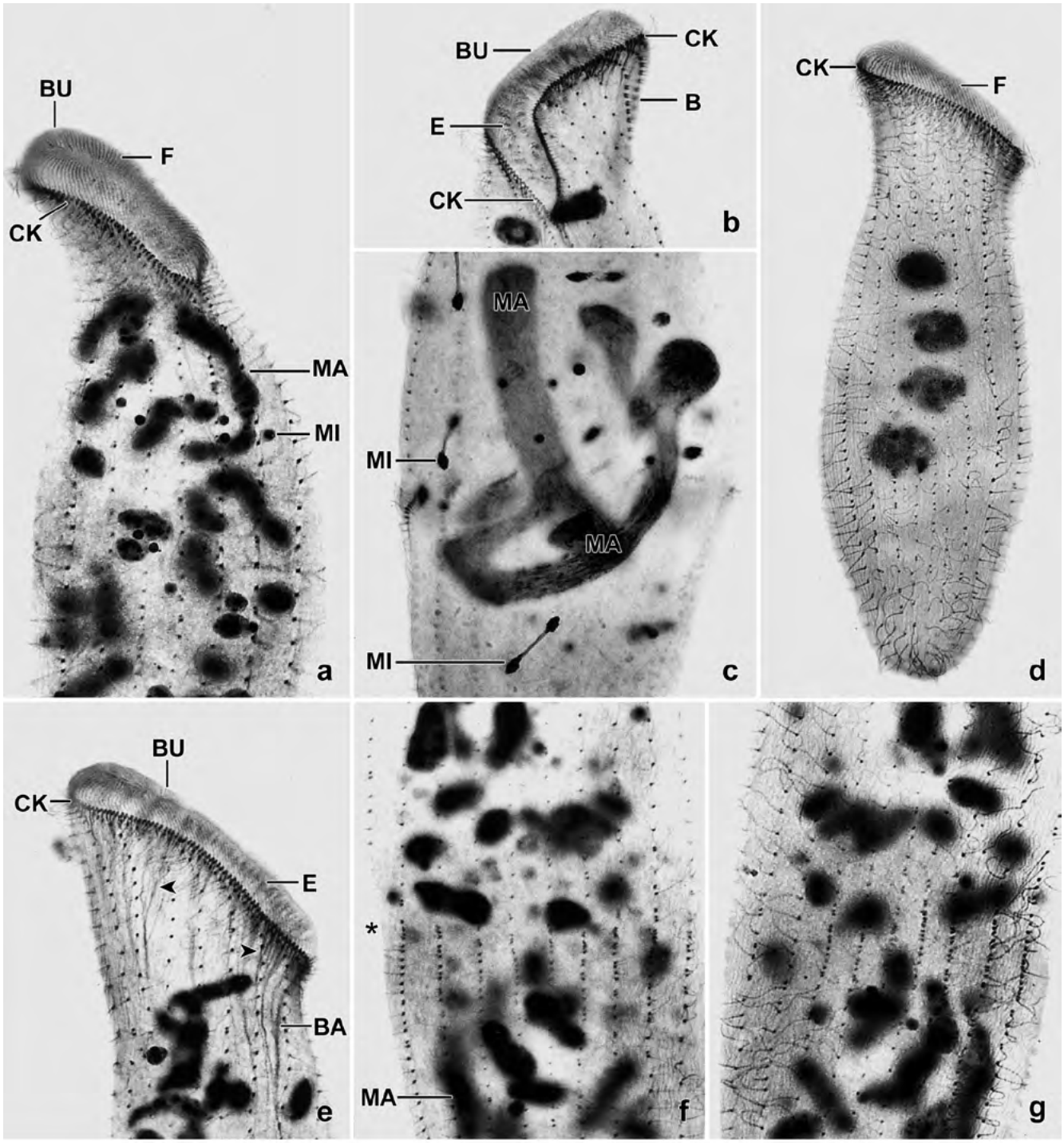
Figs 10a–e. *Spathidium bromeliophilum*, ontogenesis of ciliary pattern after protargol impregnation. Corresponding body and nuclear changes are shown in Figs 11a–j. **a** – very early divider, showing basal body production in the prospective division zone. Asterisk denotes a slight indentation in fission area. Nuclear apparatus as shown in Figs 11a, b; **b** – early divider where the developing dorsal brush rows (1–3) and the circumoral kinety fragments (placed between arrowheads) of the opisthe are already recognizable. Note blebs in fission area left of the circumoral kinety fragments (arrowheads). Nuclear apparatus as shown in Fig. 11c; **c** – middle stage, showing blebs (arrowheads) in prospective fission area. Nuclear apparatus as shown in Figs 11d, e; **d** – late divider with a single macronuclear strand (Fig. 11f) and conspicuous division furrow (arrows); **e** – very late divider with proter and opisthe about to separate, length 170 µm (Fig. 11g). B(1–3) – dorsal brush rows, BU – oral bulge, CK – circumoral kinety, MA – macronucleus, MI – micronuclei. Scale bars: 20 µm (a–d) and 30 µm (e).



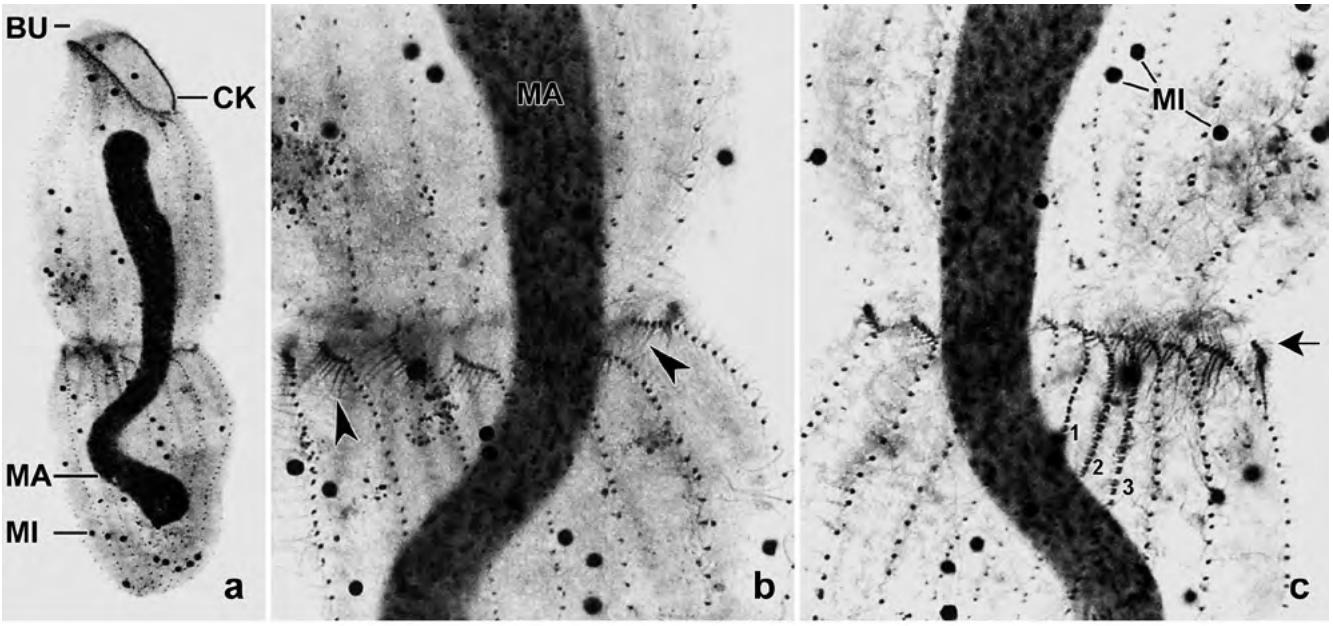
Figs 11a–j. *Spathidium bromeliophilum*, body and nuclear changes in dividers (a–g) and post-dividers (h–j) after protargol impregnation; drawn to scale. For the ciliary pattern, see Figs 10a–e, 12a–f, 15a–d. **a** – a morphostatic specimen with many macronuclear nodules scattered throughout the cytoplasm; **b** – early divider with most macronuclear nodules fused; **c**, **d** – middle dividers in which the macronuclear nodules fused to an irregular mass. Note micronuclear division and the slightly inflated fission area; **e**, **f** – middle-late and late stage, showing elongation of body and macronucleus, and division furrow. The opisthe is considerably narrower than the proter (f), a rare feature (Table 5); **g** – very late divider, showing proter and opisthe about to separate while the macronucleus has already divided; **h–j** – post-dividers develop a three-dimensional macronuclear reticulum (h, i) that breaks into many nodules during the maturation of the cell (j, a). BU – oral bulge, CV – contractile vacuole, MA – macronuclear nodules, MI – micronuclei. Scale bar: 40 μm.



Figs 12a–f. *Spathidium bromeliophilum*, dividers after protargol impregnation. **a–c** – overview and details of an early divider. Note body indentation (asterisks), basal bodies production in the division zone of all kineties (arrowhead), and beginning fusion of macronuclear nodules (arrow in c); **d–f** – a middle divider, showing an inflated fission area (small arrowheads), the developing dorsal brush (1–3), the oral kinetofragments (large arrowheads), and the macronuclear strand that developed by fusion of the macronuclear nodules. B(1–3) – dorsal brush rows, MA – macronucleus, MI – micronuclei. (Without scale bars because from ± squashed specimens.)



Figs 13a–g. *Spathidium bromeliophilum* after protargol impregnation. **a**, **b**, **e** – anterior body portion of three specimens, showing the oral bulge whose ventral third is curved laterally in 80% of the specimens (b) and straight in the rest (a, e). Note the oblique microtubule bundles originating from the dikinetids of the circumoral kinety (a) and forming the oral basket (e, arrowheads); **c** – view of the inflated fission area of a middle divider, showing micronuclear fission and the macronuclear strand that developed by fusion of the macronuclear nodules; **d** – post-conjugant, showing four macronuclear nodules and the slightly convex oral bulge whose dorsal end is higher than the ventral end; **f**, **g** – an early divider, showing a slight body indentation (asterisk) and basal body production in the prospective division zone of most kineties. B – dorsal brush, BA – oral basket, BU – oral bulge, CK – circumoral kinety, E – extrusomes, F – oral bulge fibres (microtubule bundles), MA – macronucleus, MI – micronuclei. (Without scale bars because from ± squashed specimens.)



Figs 14a–c. *Spathidium bromeliophilum*, late divider after protargol impregnation. **a**–**c** – overview and details of the fission area, showing the ventral (b) and dorsal (c) side. Arrowheads (b) mark the growing nematodesma bundles (oral basket fibres) originating from the dikinetids of the circumoral kinety fragments. Arrow (c) denotes a bleb left of the circumoral kinety fragments of the opisthe. 1, 2, 3 – dorsal brush rows, BU – oral bulge, CK – circumoral kinety, MA – macronucleus, MI – micronuclei. (Without scale bars because from ± squashed specimens.)

rows on average. The second species, *Arcuospathidium multinucleatum* (reviewed in Foissner and Xu 2007), has a more cuneate and longer oral bulge (ratio of oral bulge length:body width 1.5–2.4:1 vs. 1.1:1), a temporary cytostome (lacking in *S. bromeliophilum*), and an *Arcuospathidium* (vs. *Spathidium*) ciliary pattern. The last character is not very distinct but sustained by three ontogenetic features (Table 5): the body is distinctly inflated (vs. not inflated in *Arcuospathidium*) in fission area of mid-dividers, the oral kinetofragments are separate (vs. aligned) in very late dividers, and the circumoral kinety develops in the simple (vs. complex) manner. There are four other multinucleate species that are similar to *S. bromeliophilum* and which have been compared by Foissner *et al.* (2008).

Main differences between *S. bromeliophilum* and *S. alqasabi* (averages, protargol): body length (125 µm vs. 171 µm), ratio body length:length of oral bulge (3.8 vs. 5.7), length of dorsal brush row 2 (27 µm vs. 42 µm), number of dikinetids in dorsal brush row 2 (23 vs. 32), temporary cytostome (absent vs. present).

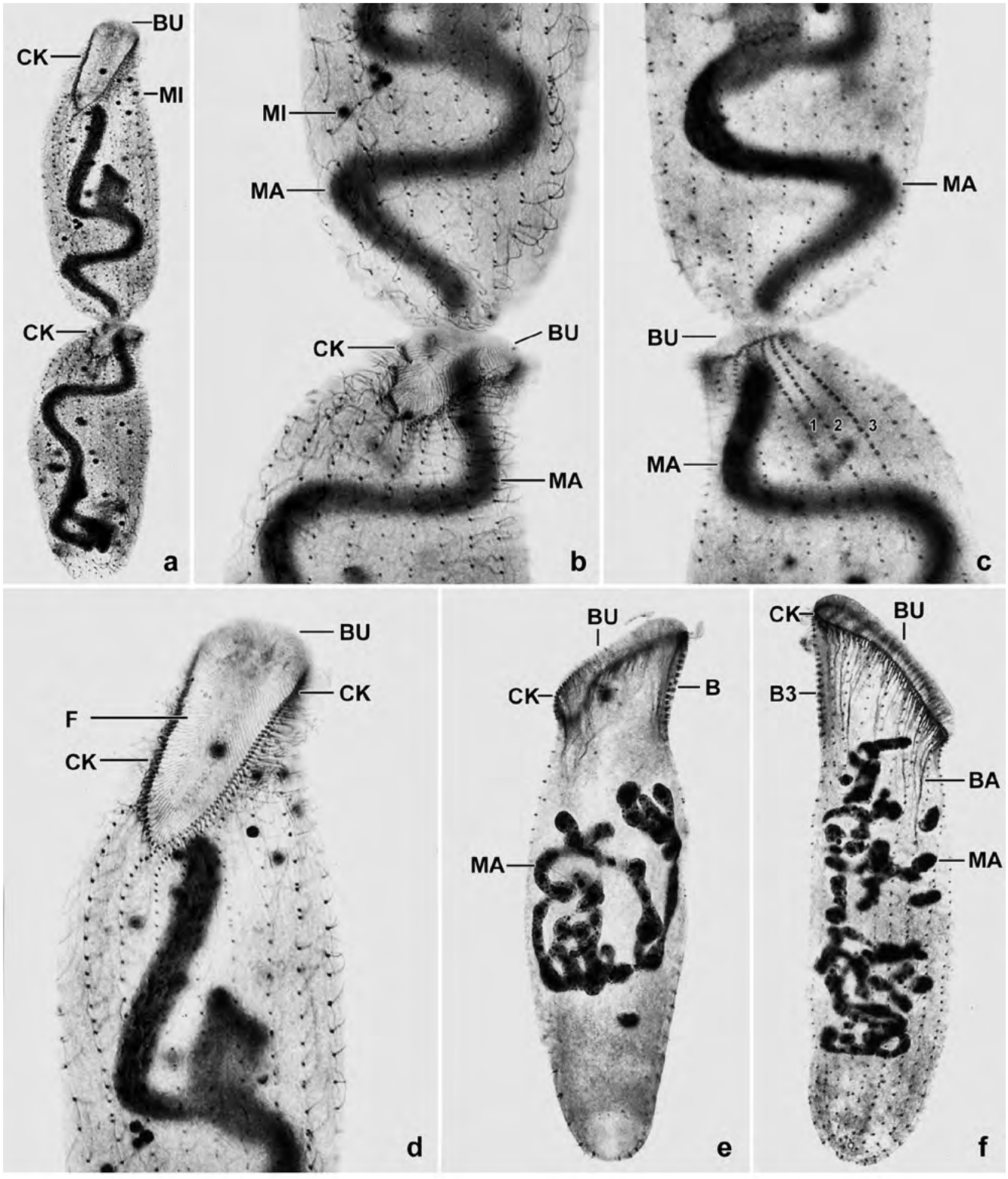
Main differences between *S. bromeliophilum* and *S. fraterculum* (averages, protargol): length of dorsal brush kinety 1 (22 µm vs. 34 µm), length of dorsal brush kinety 2 (27 µm vs. 38 µm), length of dorsal brush kinety 3 (26 µm vs. 36 µm), number of dikinetids in brush row 1 (17 vs. 27), in row 2 (23 vs. 40), and in row 3 (17 vs. 27), number of macronuclear nodules (30 vs. 67), number of ciliary rows (17 vs. 28).

Main differences between *S. bromeliophilum* and *S. foissneri* (averages, protargol): dorsal brush isostichad vs. heterostichad, length of brush row 1 (22 µm vs. 32 µm), of row 2 (27 µm vs. 37 µm), and row 3 (26 µm vs. 11 µm), dikinetids in brush row 1 (17 vs. 30), in row 2 (23 vs. 35), and in row 3 (17 vs. 10), number of ciliary rows (17 vs. 23).

Main differences between *S. bromeliophilum* and *S. seppelti* (averages, protargol): dorsal brush isostichad vs. heterostichad, number of dikinetids in brush row 3 (17 vs. 8), length of brush row 3 (26 µm vs. 10 µm), number of macronuclear nodules (30 vs. > 100), temporary cytostome (absent vs. present).

Spathidium bromelicola nov. spec. (Figs 16a–k, 17a–i, 18a–j; Table 6)

Diagnosis: Size about 190 × 30 µm *in vivo*. Narrowly to very narrowly spatulate with steep to very steep, narrowly oblong oral bulge pointed ventrally and about



Figs 15a–f. *Spathidium bromeliophilum* after protargol impregnation. **a–d** – overview and details of a very late divider, showing the elliptical oral field of the opisthe whose circumoral kinety fragments have not yet aligned ventrally; **e**, **f** – post-dividers, showing the three-dimensional macronuclear reticulum (e) that breaks into many nodules (f). B(1–3) – dorsal brush (rows), BA – oral basket, BU – oral bulge, CK – circumoral kinety, F – oral bulge fibres, MA – macronucleus, MI – micronuclei. (Without scale bars because from ± squashed specimens.)

Table 5. Ontogenetic comparison of spathidiids (modified from Foissner and Xu 2007).

Characteristics | Species	*Arcuospathidium cultriforme scalpriforme*	*Arcuospathidium muscorum*	*Cultellothrix coemeterii*	*Protospathidium serpens*	*Spathidium turgitorum*	*Spathidium bromeliophilum* nov. spec.	*Homalozoon vermiculare*
Body becomes longer and more slender in early dividers	yes (distinctly)	?	yes	no (only stouter)	no	yes (only longer)	? (contractile)
Body distinctly inflated in fission area during middle stages	no	no	yes	yes	yes	yes	no
Proter longer than opisthe	yes (ratio 1.5:1)	yes	yes	yes	yes	yes	yes
Proter distinctly wider than opisthe in late dividers	no	no	no	no	no	yes	no
Early dividers with indentation in fission area	yes	?	yes	yes	no?	yes	yes
Blebs recognizable in fission area	yes	?	no	no	yes	yes	yes
Macronucleus distinctly longer in early dividers	no	no	yes	does not apply	does not apply	does not apply	does not apply
Micronucleus greatly (≥ 3 times) enlarges in early middle dividers	no	no	yes	no	no	no	no
Dorsal brush row 2 produced distinctly earlier than rows 1 and 3	yes	?	no	no	no	no	no
Left side oral kinetofragments curve rightwards earlier than right ones	yes	yes	yes	no	no	no	?
Oral kinetofragments straight or slightly/distinctly curved	distinctly curved	distinctly curved	slightly curved	straight	slightly curved	slightly curved	distinctly curved
Individual oral kinetofragments separate or loosely aligned in very late dividers	aligned	aligned	aligned	separate	separate	separate	separate
Circumoral kinety develops in simple or complex manner [a]	complex	complex	simple	simple	simple	simple	simple
Shaping of oral bulge and circumoral ciliature completed in late dividers or in post-dividers	late post-dividers	late post-dividers	late post-dividers	very late dividers	post-dividers	post-dividers	post-dividers
Division axis distinctly oblique?	yes	no	no	no	no	no	no
Anterior portion of opisthe's kineties strongly curved in mid-dividers	yes	yes	no	no	no	no	no
Shape of fission area in late dividers	clavate	clavate	roundish	roundish	roundish	elliptical	roundish

[a] Simple = by alignment of kinetofragments one after another; complex = by shifting and overlapping individual oral kinetofragments.

1.5 times as long as widest trunk region. Macronucleus long and tortuous; multimicronucleate. Extrusomes narrowly ovate to bluntly fusiform, about 4 × 0.8 µm in size. On average 20 ciliary rows, ventral and first left side row widely spaced anteriorly, producing an obtriangular, bare area in most specimens. Dorsal brush three-rowed, isostichad, occupies about 23% of body length; 25 widely spaced dikinetids in row 3. Type IV resting cysts.

Type locality: Tanks of bromeliads from the Botanical Garden on the Pico Isabel de Torres, north coast of the Dominican Republic, N19°45′ W70°42′.

Type material: 1 holotype and 2 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: A noun in apposition composed of the plant family name Bromeliaceae and the adjectivally used Latin verb *cola* (dwelling in bromeliads).

Description: Size moderately variable, 150–250 × 25–40 µm *in vivo*, usually near 190 × 30 µm, as calculated from some *in vivo* measurements and the morphometric data, adding 15% for preparation shrinkage (Table 6); length:width ratio 5.4–8.8:1 in protargol preparations, on average near 6.5:1 both *in vivo* and prepared cells (Table 6). Narrowly to very narrowly spatulate, rarely almost cylindroidal, only slightly widened in mid-body and thus almost parallel-sided; anterior (oral) end steeply to very steeply slanted, neck usually indistinct, posterior end ordinarily rounded;

Table 6. Morphometric data on *Spathidium bromelicola*.

Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length, µm	178.1	175.0	19.5	4.3	11.0	150.0	228.0	21
Body, width, µm	27.1	26.0	4.6	1.0	17.1	20.0	36.0	21
Body length:width, ratio	6.7	6.7	1.1	0.2	15.9	5.4	8.8	21
Oral bulge, length, µm	39.8	39.0	4.4	1.0	11.0	33.0	52.0	21
Oral bulge (circumoral kinety), width, µm	5.4	5.0	–	–	–	5.0	6.0	7
Oral bulge, height, µm	3.8	4.0	–	–	–	3.0	4.0	21
Oral bulge length:body width, ratio	1.5	1.4	0.2	0.1	16.0	1.2	1.9	21
Circumoral kinety to last dikinetid of brush row 1, distance, µm	36.3	35.0	4.4	1.0	12.1	30.0	47.0	21
Circumoral kinety to last dikinetid of brush row 2, distance, µm	40.4	40.0	5.4	1.2	13.3	32.0	56.0	21
Circumoral kinety to last dikinetid of brush row 3, distance, µm	35.8	36.0	4.4	1.0	12.4	26.0	47.0	21
Anterior body end to macronucleus, distance, µm	53.3	55.0	11.0	2.4	20.6	34.0	76.0	21
Macronucleus figure, length, µm	91.0	94.0	19.9	4.3	21.9	55.0	132.0	21
Macronucleus, length (extended and thus approximate), µm	223.8	240.0	–	–	–	150.0	300.0	21
Macronucleus, width in mid, µm	4.8	5.0	0.7	0.2	14.7	4.0	6.0	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronuclei, across, µm	3.3	3.0	0.5	0.1	16.5	3.0	5.0	21
Micronuclei, number	11.6	11.0	2.3	0.5	19.7	9.0	16.0	21
Ciliary rows, number	19.6	20.0	1.3	0.3	6.5	17.0	21.0	21
Kinetids in a right side kinety, number	94.0	90.0	21.8	4.8	23.2	63.0	155.0	21
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	32.3	31.0	4.7	1.0	14.4	25.0	42.0	21
Dikinetids in brush row 2, number	39.0	38.0	5.6	1.2	14.3	33.0	53.0	21
Dikinetids in brush row 3, number	25.4	25.0	3.7	0.8	14.4	21.0	33.0	21

[a] Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a non-flooded Petri dish culture. 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.

flattened only in hyaline oral region (Figs 16a–c, g, 17a, 18a). Macronucleus in central quarters of cell, frequently slightly nodulated and with strongly coiled ends, tortuous and longer than body when extended; contains many nucleoli up to 3 µm across. On average 11 globular micronuclei near and attached to macronucleus (Figs 16a, g, h, 17a, 18a; Table 6). Contractile vacuole in rear body end, some excretory pores scattered in pole area. Extrusomes studded in oral bulge and scattered in cytoplasm, narrowly ovate to bluntly fusiform and 3.5–4.5 × 0.7–1 µm in size (Figs 16a, d–f, 18i, j); do not impregnate with the protargol method used, not even the ends. Cortex very flexible and rather conspicuous due to highly refractive, densely spaced granules, forming about seven rows between each two kineties; individual granules about 0.5 µm across, colourless and compact. Cytoplasm colourless, contains few to many lipid droplets and, usually mainly in posterior half, many food vacuoles with remnants of heterotrophic flagellates. Glides rapidly on microscope slides and between mud particles, showing pronounced flexibility.

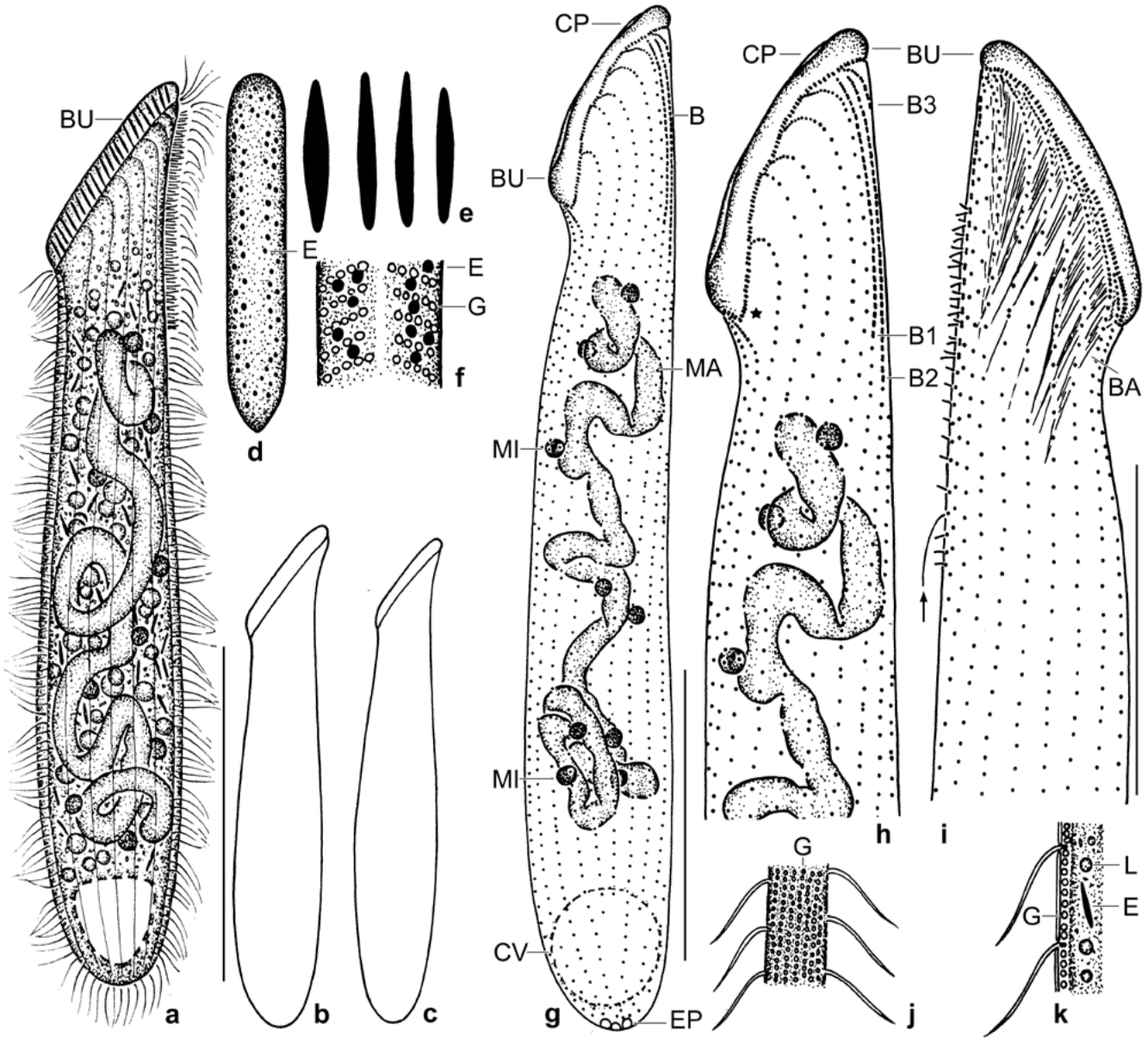
Somatic cilia about 9 µm long *in vivo*, arranged in an average of 20 ordinarily spaced, mostly bipolar, densely ciliated rows abutting on circumoral kinety in typical *Spathidium* pattern. Right side ciliary rows frequently attached to the individual circumoral kinetofragments; leftmost ventral and first left side ciliary row widely spaced anteriorly, producing a conspicuous, obtriangular, bare area in two thirds of 23 specimens analysed (Figs 16a, g–i, 17a, d, 18a, d–f; Table 6); the remainders have an additional ciliary row at this site. Dorsal brush almost exactly on dorsal side and almost perfectly isostichad, occupies an average of 23% of body length, inconspicuous because bristles only up to 3 µm long *in vivo*; all rows commence with some ordinary cilia anteriorly and continue as somatic kineties posteriorly (Figs 16a, g–i, 17a, c, e, 18a, e; Table 6). Brush row 1 composed of an average of 32 dikinetids, anterior bristle of dikinetids slightly clavate and about 3 µm long, posterior bristle rod-shaped and about 1.5 µm long. Brush row 2 slightly longer than rows 1 and 3, composed of an average of 39 rather narrowly spaced dikinetids associated with bristles similar to those described for row 1. Brush row 3 composed of an average of 25 comparatively widely spaced dikinetids, anterior bristle of dikinetids rod-shaped and about 2 µm long, posterior slightly clavate and about 2.5 µm long; posterior tail extends to second body third, conspicuous because heteromorphic, that is, composed of about 2 µm long bristles irregularly alternating with ordinary cilia (Figs 16i, 17a).

Oral bulge occupies anterior body end slanted by 50 to 80°, on average 1.5 times as long as widest trunk region; of ordinary distinctness, that is, about 4 µm high *in vivo* and with flat to moderately convex surface; oblong to narrowly oblong with more or less pointed ventral end; cytopharyngeal entrance marked by minute concavity near dorsal bulge end. Circumoral kinety of similar shape as oral bulge, usually continuous, composed of narrowly spaced dikinetids each associated with a cilium, a nematodesma, and a faintly impregnated fibre extending to oral bulge midline. Nematodesmata about 40 µm long, bundled and thus forming a fairly distinct oral basket (Figs 16a–d, g–i, 17a, d, e, 18a, d–h; Table 6).

Of 10 specimens isolated, eight commenced resting cyst formation after four days. Young cysts look like type I cysts, that is, have a ~ 0.5 µm thick, smooth wall touching the encysted cell. Two week-old cysts look different, representing a distinct type (see Foissner and Xu 2007 and Figs 17b, 18b, c). They have two ~ 1 µm thick, smooth walls separated by a wide space, containing few to many lipid droplets highly similar to those found in the encysted cell. External wall on average 44 µm across (40–48 µm, n 8), with reddish shimmer and many circular diffraction lines. Internal wall 30 µm across (25–34 µm), with bluish shimmer in the bright field microscope, attached to encysted cell packed with lipid droplets 1–8 µm across and a bright blister, likely marking the contractile vacuole.

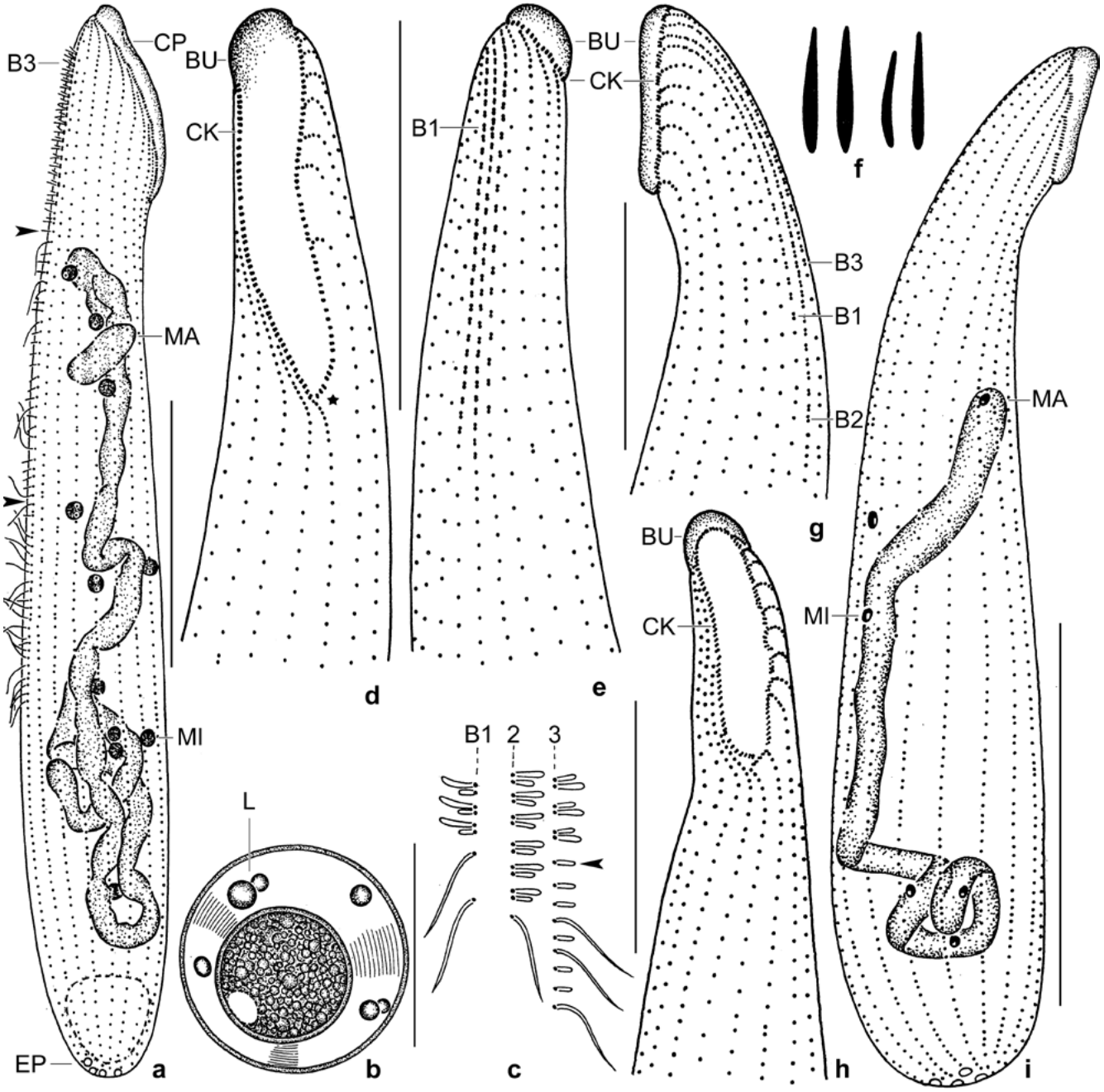
Occurrence and ecology: As yet found only at type locality, where it was rare in the non-flooded Petri dish culture. Whether or not *S. bromelicola* is specific to bromeliad tanks needs further investigations.

The type locality is on the Caribbean island Hispaniola, which is divided politically in Haiti (east) and the Dominican Republic (west). The Pico Isabel de Torres is an about 800 m high mountain in the surroundings of the town of Puerto Plata on the north coast of the Dominican Republic. On the peak of the mountain there is a Botanical Garden with many small and middle-sized bromeliads on the trees of some forested areas. The mud and soil comprising the sample was collected from dry tanks of several bromeliad species and specimens and was used to set up a non-flooded Petri dish culture, as described in Foissner *et al.* (2002). The rewetted sample had pH 6.4 and a surprisingly high salinity of 8‰ (refractometer method).

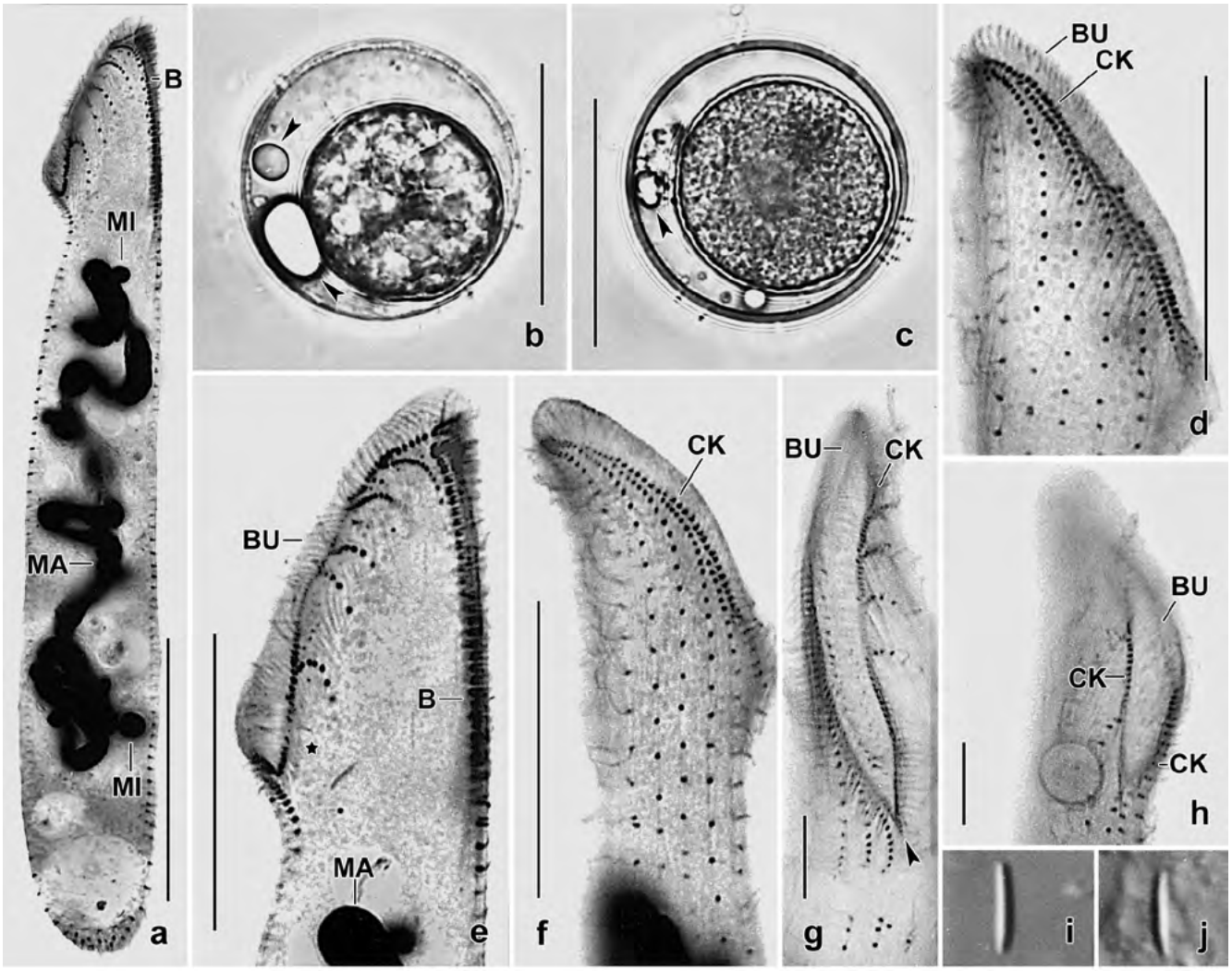


Figs 16a–k. *Spathidium bromelicola* from life (a–f, j, k) and after protargol impregnation (g–i). **a** – left side view of a representative specimen, length 190 µm; **b**, **c** – shape variants; **d**, **f** – frontal view of oral bulge, showing the arrangement of the cortical granules and the extrusomes; **e** – oral bulge extrusomes, length 3.5–4.5 µm; **g–i** – left and right side view of holotype specimen, length 175 µm. The arrow marks the heteromorphic tail of brush row 3. The asterisk denotes the obtriangular space between the last ventral and the first left side ciliary row; **j**, **k** – surface view and optical section of cortex. B(1–3) – dorsal brush (rows), BA – oral basket, BU – oral bulge, CP – cytopharyngeal entrance, CV – contractile vacuole, E – extrusomes, EP – excretory pores, G – cortical granules, L – lipid droplet, MA – macronucleus, MI – micronuclei. Scale bars: 40 µm (h, i), 50 µm (g), and 70 µm (a).

Remarks: The most similar species is possibly *S. aciculare* Foissner *et al.*, 2002 (Figs 17f–i). However, it differs from *S. bromelicola* in the shape of the oral bulge (elongate elliptical vs. elongate cuneate) and the dorsal brush (heterostichad vs. isostichad). Take care not to confuse *S. bromelicola* with *S. muscicola* (extrusomes bluntly fusiform and about 4 µm long vs. rod-shaped and > 15 µm long), and, especially, with *S. stammeri*, which is very similar, except for the extrusomes (bluntly fusiform and about 4 µm long vs. rod-



Figs 17a–i. *Spathidium bromelicola* (a–e) and *Spathidium aciculare* (f–i, from Foissner *et al.* 2002) from life (b, c, f) and after protargol impregnation (a, d, e, g–i). **a** – right side view of a 200 µm long specimen with impregnated cilia, showing the heteromorphic tail of brush row 3 (arrowheads); **b** – resting cyst with inactive contractile vacuole, diameter 45 µm; **c** – posterior portion of dorsal brush, longest bristles 3 µm. The arrowhead denotes the heteromorphic, monokinetidal tail of brush row 3; **d**, **e** – ventral and dorsal views, showing the slightly cuneate oral bulge and the isostichad dorsal brush with dikinetids more widely spaced in row 3 than in rows 1 and 2. The asterisk marks the widened area between the last ventral and the first left side ciliary row; **f** – *Spathidium aciculare*, oral bulge extrusomes from two Australian populations, length 7–8 µm; **g**, **h** – *Spathidium aciculare*, left side and frontal view of anterior body region, showing the heterostichad dorsal brush and the elongate elliptical circumoral kinety (oral bulge); **i** – *Spathidium aciculare*, right side overview. B(1–3) – dorsal brush rows, BU – oral bulge, CK – circumoral kinety, CP – cytopharyngeal entrance, EP – excretory pores, L – lipid droplets, MA – macronucleus, MI – micronuclei. Scale bars: 30 µm (g, h), 40 µm (b, d, e), and 50 µm (a, i).



Figs 18a–j. *Spathidium bromelicola* from life (b, c, i, j) and after protargol impregnation (a, d–h). **a** – left side overview; **b**, **c** – resting cysts with large lipid droplets (arrowheads) between internal and external wall; **d**, **f** – right side views of oral body portion; **e** – left side view of oral body portion, showing the *Spathidium* ciliary pattern and the enlarged area (asterisk) between last ventral and first left side ciliary row; **g**, **h** – frontal views of the narrow oral bulge and its acute ventral end (g, arrowhead); **i**, **j** – oral bulge extrusomes, length about 4 µm. B – dorsal brush, BU – oral bulge, CK – circumoral kinety, MA – macronucleus, MI – micronuclei. Scale bars: 10 µm (g, h), 30 µm (d–f), 40 µm (b, c), and 50 µm (a).

shaped and 8–12 µm long) and the spiny (vs. smooth) resting cyst (Foissner and Xu 2007, Wenzel 1959).

Spathidium wolfi nov. spec. (Figs 19a–l, 20a–f, 21a–c, 22a–k; Table 7)

Diagnosis: Size about 135 × 25 µm *in vivo*. Very narrowly spatulate to bluntly fusiform with oblique, cuneate oral bulge half as long as widest trunk region. Macronucleus moniliform, composed of an average of 8 ellipsoidal nodules; multimicronucleate. Two contractile vacuoles, one dorsally at margin of first and second body third, the other in rear end. On average 15 ciliary rows, 3 of them differentiated to isostichad, short dorsal brush occupying 19% of body length; 14 widely spaced dikinetids in row 3.

Type locality: Tanks of terrestrial bromeliads at the foot of Mt. Diablo, northwest of Spanish Town, Jamaica, N18° W77°.

Type material: 1 holotype and 3 paratype slides with protargol-impregnated specimens have been de-

Table 7. Morphometric data on *Spathidium wolfi*.

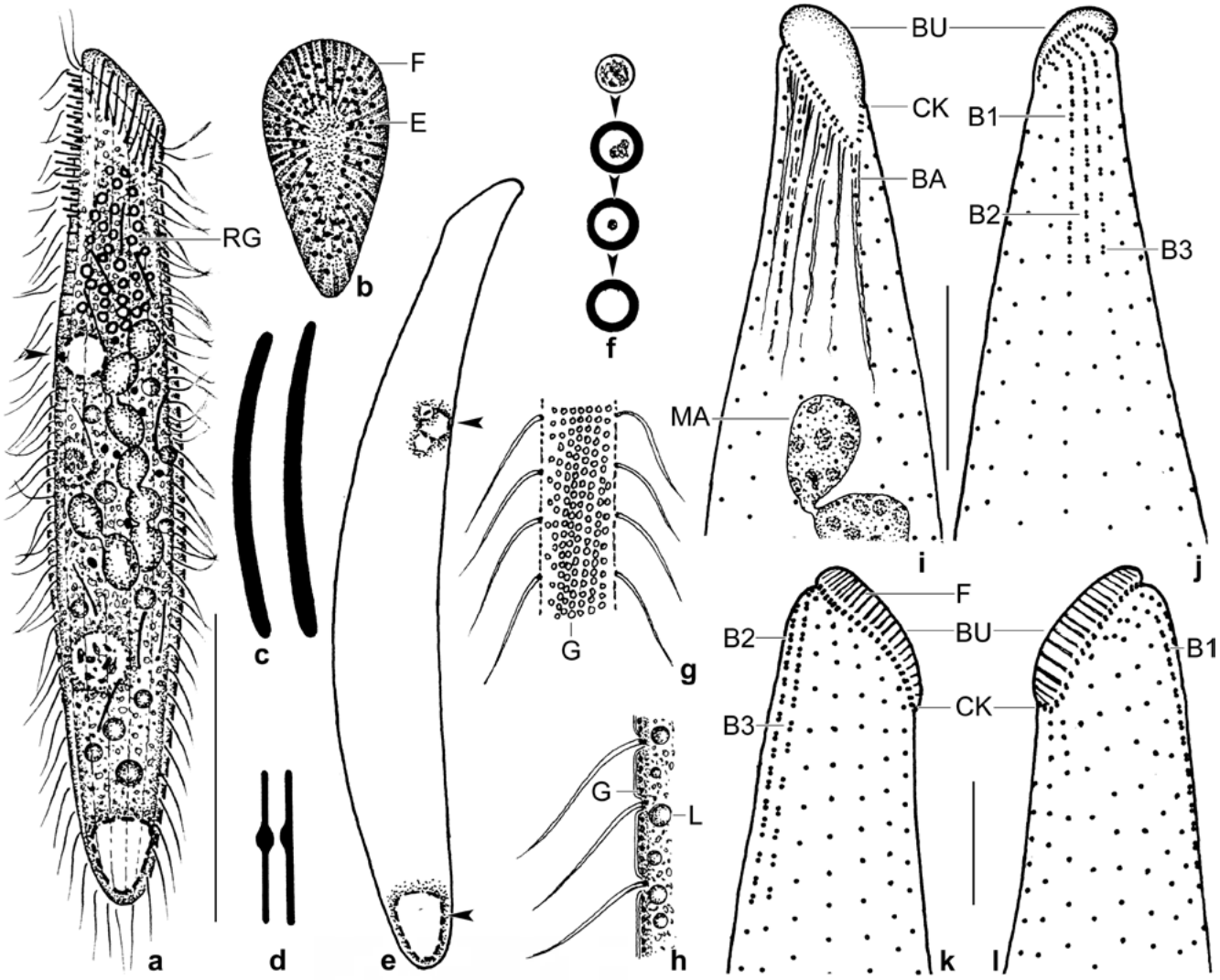
Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length, µm	118.9	117.0	10.8	2.4	9.1	100.0	145.0	21
Body, width, µm	29.9	30.0	4.9	1.1	16.4	21.0	44.0	21
Body length:width, ratio	4.1	3.9	0.9	0.2	22.3	2.5	6.0	21
Oral bulge, length, µm	14.6	14.0	1.4	0.3	9.8	12.0	17.0	21
Body width:oral bulge length, ratio	2.1	2.1	0.3	0.1	15.3	1.4	2.9	21
Oral bulge, height, µm	3.2	3.0	–	–	–	2.0	4.0	21
Oral bulge, width, µm	5.7	6.0	0.7	0.2	12.6	5.0	7.0	21
Anterior end to macronucleus, distance, µm	39.7	39.0	7.7	1.7	19.5	29.0	58.0	21
Anterior body end to first excretory pore, distance, µm	41.3	41.0	3.0	0.7	7.3	36.0	47.0	21
Circumoral kinety to end of brush row 1, distance, µm	20.3	21.0	2.3	0.5	11.4	16.0	24.0	21
Circumoral kinety to end of brush row 2, distance, µm	22.5	23.0	2.6	0.6	11.7	17.0	27.0	21
Circumoral kinety to end of brush row 3, distance, µm	19.4	19.0	2.8	0.6	14.4	15.0	26.0	21
Macronucleus figure, length, µm	38.4	35.0	10.1	2.2	26.2	27.0	68.0	21
Macronucleus, length extended, µm	65.7	60.0	–	–	–	45.0	105.0	21
Macronucleus, width in mid, µm	5.1	5.0	0.4	0.1	7.6	4.0	6.0	21
Macronuclear nodules, number	8.1	8.0	1.4	0.3	17.1	5.0	11.0	21
Micronuclei, largest diameter, µm	2.3	2.2	–	–	–	2.0	3.0	21
Micronuclei, number	8.6	8.0	2.8	0.6	31.9	6.0	17.0	21
Ciliary rows, number (including brush rows)	15.0	15.0	1.1	0.2	7.5	13.0	17.0	21
Ciliated kinetids in a right side kinety, number	41.2	40.0	8.8	1.9	21.3	23.0	65.0	21
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	15.5	16.0	2.3	0.5	15.1	12.0	22.0	21
Dikinetids in brush row 2, number	18.1	19.0	2.7	0.6	14.8	12.0	22.0	21
Dikinetids in brush row 3, number	14.1	14.0	1.9	0.4	13.3	11.0	19.0	21
Excretory pores for anterior contractile vacuole, number	2.6	3.0	–	–	–	2.0	3.0	21
Excretory pores for posterior contractile vacuole, number	2.9	3.0	–	–	–	2.0	4.0	21

[a] Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a raw culture. 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.

posited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip.

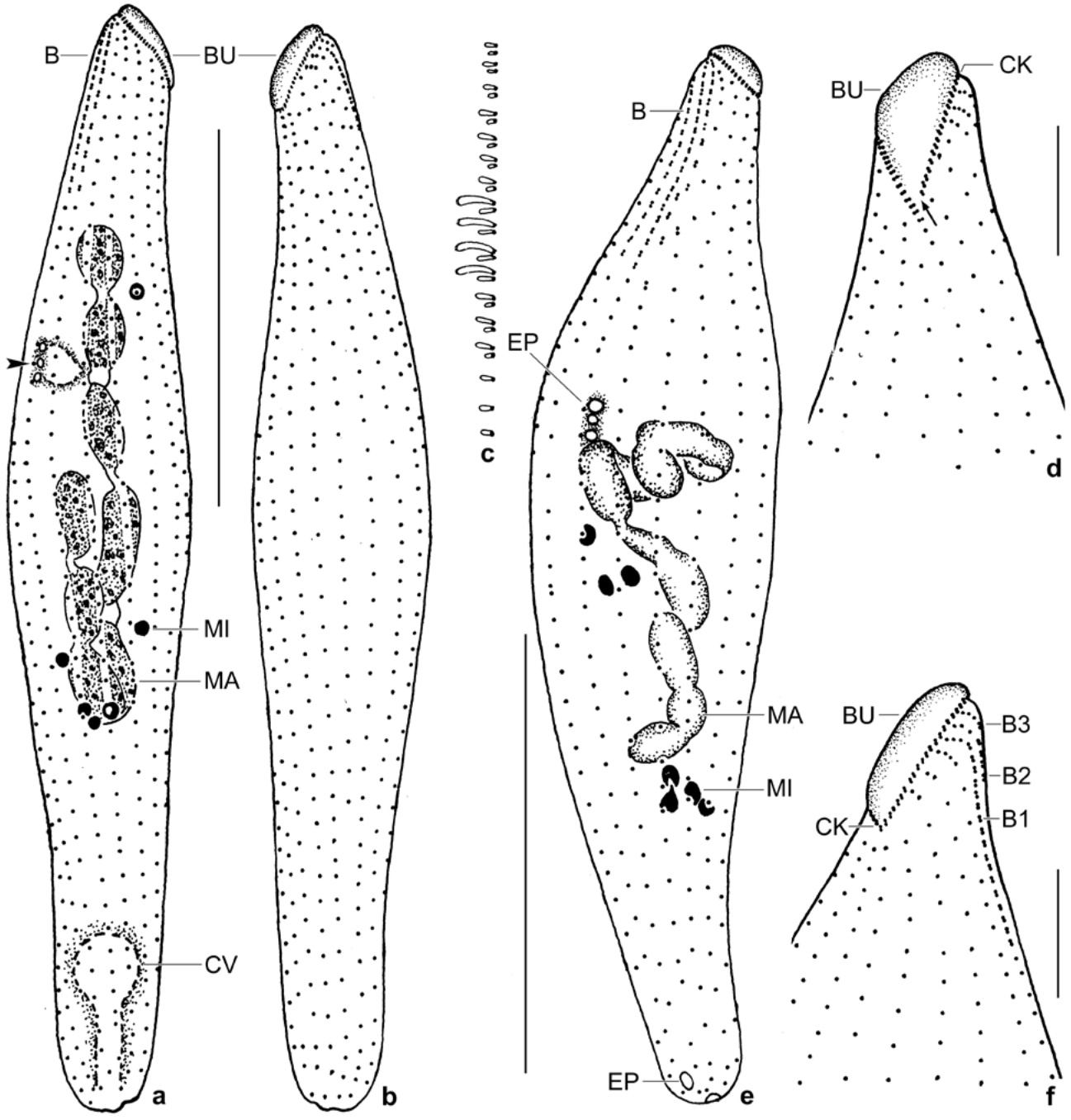
Dedication: The senior author dedicates this new species to Dr. rer. nat. habil. Klaus W. Wolf, Electron Microscopy Unit at the University of the West Indies, Kingston, Jamaica, who facilitated collection on the island.

Description: Size 100–160 × 18–35 µm *in vivo*, usually near 135 × 25 µm. Narrowly to very narrowly spatulate to bluntly fusiform, especially in preparations containing many specimens with inflated middle body third; length:width ratio thus rather different *in vivo* (~ 5.5:1) and preparations (~ 4:1). Neck indistinct, widest usually in middle body third, body ends up to 2:1 flattened. Anterior (oral) end oblique, posterior narrowly rounded (Figs 19a, e, 20a, b, e, 22a–d; Table 7). Macronucleus in middle body third, moniliform assuming various figures ranging from cylindroidal to circular, composed of an average of eight nodules connected by narrow bridges; individual nodules globular to elongate ellipsoidal, contain many small nucleoli. Micronuclei near and attached to macronucleus, inconspicuous, that is, about 2 µm across in protargol preparations (Figs 19a, 20a, e, 21b, 22a–d; Table 7). Two contractile vacu-

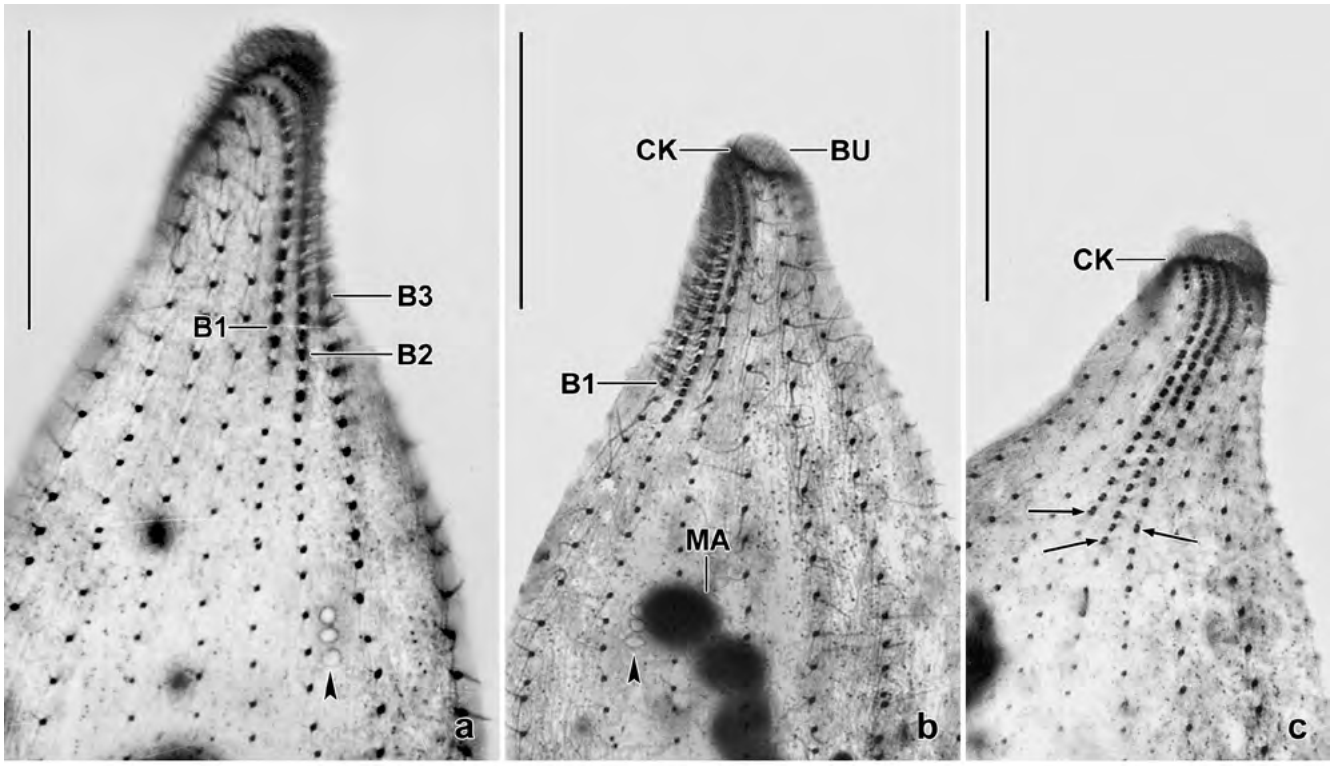


Figs 19a–l. *Spathidium wolfi* from life (a–h) and after protargol impregnation (i–l). **a** – right side view of a representative specimen, length 140 µm. The arrowhead marks the anterior contractile vacuole; **b** – frontal view of oral bulge; **c** – oral bulge extrusomes, length 10 µm; **d** – developing extrusomes in the cytoplasm; **e** – slender shape variant, showing the two contractile vacuoles (arrowheads); **f** – development of the "Ringgranula" (cp. Fig. 19a); **g**, **h** – surface view and optical section, showing the cortical granulation; **i**, **j** – ventral and dorsal view of anterior body region of a paratype specimen, showing the isostichad dorsal brush and the cuneate oral bulge; **k**, **l** – right and left side view of anterior body region of the holotype specimen (cp. Figs 20a, b). B(1–3) – dorsal brush rows, BA – oral basket, BU – oral bulge, CK – circumoral kinety, E – extrusomes, F – oral bulge fibres, G – cortical granules, L – lipid droplet, MA – macronucleus, RG – "Ringgranula". Scale bars: 15 µm (i–l) and 50 µm (a).

oles, each with adventive blisters during diastole (Figs 19a, e, 20a, e, 21a, b, 22b, c; Table 7): one dorsally at margin of first and second body third, the other in rear end, as usual. Anterior vacuole with an average of three serially arranged excretory pores invariably located between kineties differentiated to dorsal brush rows 2 and 3 anteriorly. Extrusomes studded in oral bulge and scattered in cytoplasm, where intensely impregnated, fusiform developmental stages occur (Fig. 19d); oral bulge extrusomes rod-shaped to indistinctly acicular and slightly curved, about 10 × 0.5 µm in size (Figs 19a–c); do not impregnate with the protargol method used. Cortex flexible, jelly-like, and about 1 µm thick, contains about six rows of colourless, very narrowly spaced granules between each two kineties; individual granules about 0.2 µm across, frequently impregnate



Figs 20a–f. *Spathidium wolfi* from life (c) and after protargol impregnation (a, b, d–f). **a**, **b** – ciliary pattern of right and left side and nuclear apparatus of holotype specimen; for oral details, see Figs 19k, l. The arrowhead marks the excretory pores of the anterior contractile vacuole, i.e., the main character of this species; **c** – supposed structure of dorsal brush row 3; **d** – ventral view of a paratype specimen with open circumoral kinety (arrow) and thus resembling *Apertospathula*; **e** – dorsolateral view of a paratype specimen, showing the ciliary pattern and the excretory pores of the anterior and posterior contractile vacuole; **f** – ventrolateral view of oral body region, showing the *Spathidium* ciliary pattern. B(1–3) – dorsal brush (rows), BU – oral bulge, CK – circumoral kinety, CV – contractile vacuole, EP – excretory pores, MA – macronucleus, MI – micronuclei. Scale bars: 10 µm (d, f) and 50 µm (a, b, e).

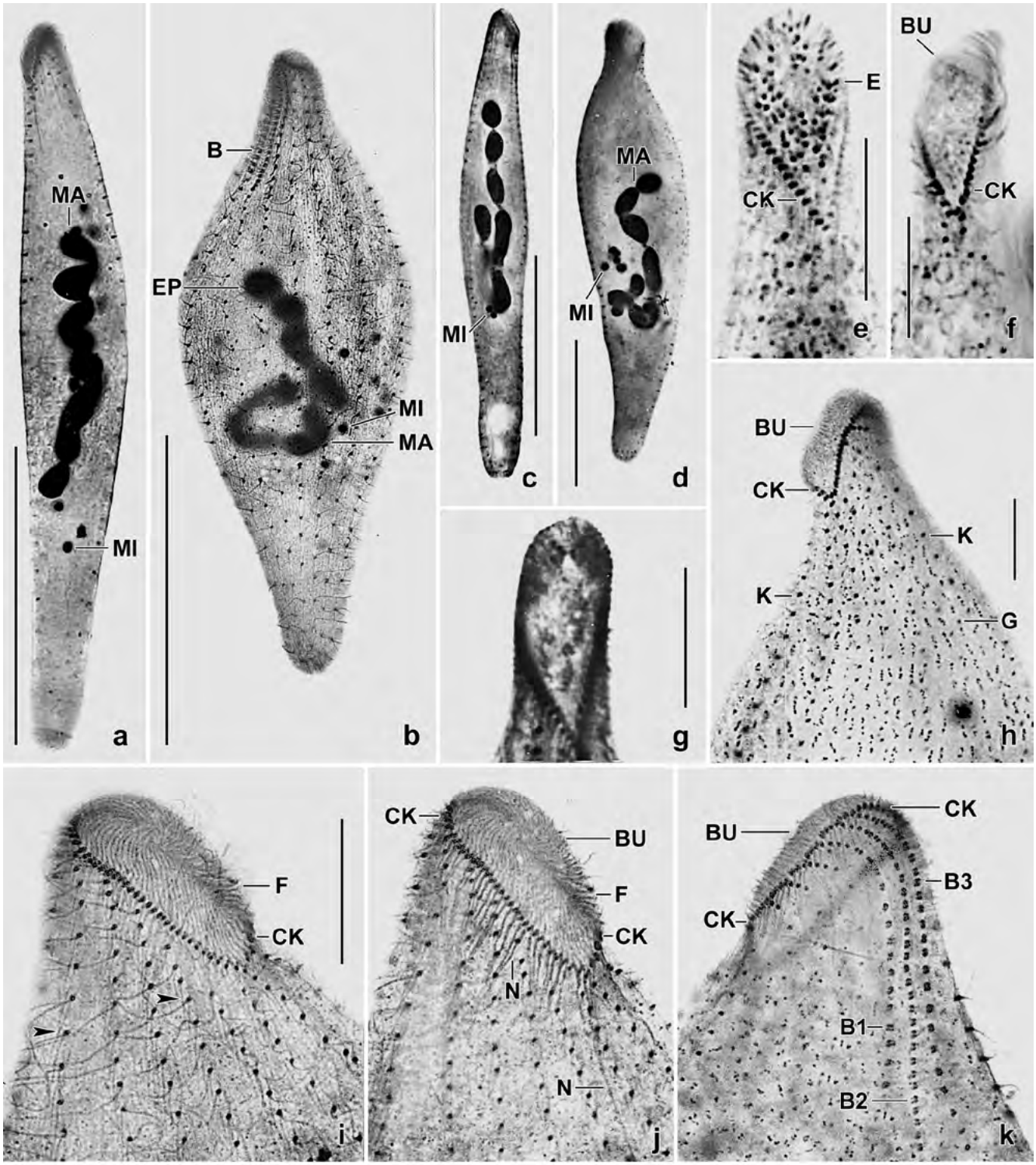


Figs 21a–c. *Spathidium wolfi*, dorsal views of anterior body region after protargol impregnation, showing the isostichad dorsal brush (c, arrows) and the excretory pores (arrowheads) of the anterior contractile vacuole. B(1–3) – dorsal brush rows, BU – oral bulge, CK – circumoral kinety, MA – macronucleus. Scale bars: 20 µm.

with protargol obscuring ciliary pattern (Figs 19g, h, 22h). Cytoplasm usually dark postorally due to many highly refractive lipid droplets up to 2 µm across and/or circular structures ("Ringgranula") 1.5–2 µm across. Ringgranula develop from globular precursors and do not dissolve when freed from cell; do not impregnate or dissolve with the protargol method used (Figs 19a, f). Food not known. Movement without peculiarities.

Cilia about 10 µm long *in vivo*, arranged in an average of 15 equidistant, usually bipolar, ordinarily spaced and ciliated rows abutting on circumoral kinety in fairly indistinct *Spathidium* pattern because ventral half kineties lack polymerized kinetids anteriorly and do not abut on circumoral kinety (Figs 19k, l, 20a, b, d–f, 22k; Table 7). Dorsal brush of ordinary structure and distinctness, occupies about 19% of body length, bristle details difficult to recognize *in vivo* due to the highly refractive cytoplasmic inclusions. Brush rows isostichad, each with distinct anterior tail and likely structured as shown in Fig. 20c, that is, with some 5–6 µm long bristles in posterior half; row 1 composed of an average of 16 dikinetids, row 2 of 18, and row 3 of 14 (Figs 19a, i–k, 20a, e, f, 21a–c, 22b, k; Table 7).

Oral bulge occupies anterior body end slanted by 45° to 60°, on average half as long as widest trunk region in inflated protargol-impregnated specimens (see above), while about as long as trunk width *in vivo*; of ordinary distinctness in lateral view, that is, about 4 µm high and with flat to slightly convex surface, while conspicuously obovate to cuneate in frontal view (Figs 19b, 20d, 22e–g), a rare shape in *Spathidium* s. str.; contains rather thick fibres originating from circumoral dikinetids; cytopharyngeal entrance not recognizable. Circumoral kinety of same shape as oral bulge, continuous but occasionally with a minute gap ventrally, similar as in *Apertospathula* (Fig. 20d); composed of about 40–80 dikinetids each associated with a cilium, an oral basket rod, and a distinct fibre extending into oral bulge cortex, as described above. Nematodesmata fairly distinct, form small bundles slightly longer than dorsal brush; oral basket thus rather distinct in appropriately impregnated specimens (Figs 19a, i–l, 20a, b, d, f, 22e–k; Table 7).



Figs 22a–k. *Spathidium wolfi* after protargol impregnation. **a** – a slender specimen; **b** – overview of a specimen inflated by the preparation; **c**, **d** – overviews showing the moniliform macronucleus; **e–g** – ventral views, showing the obovate (or broadly cuneate) circumoral kinety; **h** – ventrolateral view, showing kineties and cortical granules; **i–k** – anterior body portion at three focal planes. (i) Right side surface view, showing fibres in oral bulge and somatic cortex (arrowheads). (j) When focused slightly deeper, the nematodesmata become visible. (k) When focused to the left side, the dorsal brush and the *Spathidium* ciliary pattern become recognizable. B(1–3) – dorsal brush (rows), BU – oral bulge, CK – circumoral kinety, E – extrusomes, EP – excretory pores, F – fibres, G – cortical granules, K – kineties, MA – macronucleus, MI – micronuclei, N – nematodesmata. Scale bars: 10 µm (e–k) and 50 µm (a–d).

Occurrence and ecology: As yet found at type locality and in a granitic rockpool (Laja) near to the town of Pto. Ayachuco, Venezuela, where a single specimen is contained in the type slides of *Apertospathula lajacola*, indicating wide distribution in Central and South America. Further studies are required to determine the extent the species is specific to bromeliad tanks. Numbers were very low in the fresh sample, but after addition of a squashed wheat grain they multiplied rapidly for some days; however, efforts to establish pure cultures failed.

Remarks: Within the genus, *S. wolfi* and *S. faurefremieti* Foissner (2003) form a distinct subgroup characterized by two contractile vacuoles and a cuneate oral bulge. If further such species are discovered, they should be separated at subgeneric rank, at least. Actually, *S. wolfi* looks like a small *S. faurefremieti* but differs in body size (135 × 25 µm vs. 240 × 17 µm), macronucleus (moniliform vs. a long, tortuous strand), and the total number of brush bristles (about 47 vs. 72).

DISCUSSION

The bent oral bulge

When observed ventrally, the spathidiid oral bulge and circumoral kinety are straight or slightly bent the entire length, e.g., in *Arcuospathidium vermiforme*, *A. multinucleatum*, and *Cultellothrix tortisticha* (reviewed in Foissner and Xu 2007). This contrasts *Arcuospathidium bromelicola* (Fig. 3g) and *Spathidium bromeliophilum* (Fig. 13b) which have a more or less distinct bend in the ventral third of the oral bulge and circumoral kinety. We do not know the significance of this curious morphology but it might be related to the habitat because other bromeliad ciliates frequently have peculiar adaptations, e.g., *Bromeliothrix metopoides* which produces a motile division chain (Foissner 2010). Unfortunately, the feature is rather variable and thus of restricted value for species identification.

Lepidosomes in spathidiids

Lepidosomes are epicortical, organic structures of definite shape and are produced individually and intracellularly by trophic and/or cystic ciliates (for a review, see Foissner *et al.* 2005). In a more general sense, lepidosomes belong to the "scales", as defined by Preisig *et al.* (1994). The function of the lepidosomes is not known.

Lepidosome-producing ciliates are rare, except for the trachelophyllid haptorids where they are present in all species (Foissner *et al.* 2002). Thus, it was a great surprise to find lepidosomes on the cyst surface of a typical spathidiid: *Protospathidium lepidosomatum* (Figs 6a–g). On the other hand, spathidiids and trachelophyllids are rather closely related in 18S rRNA gene phylogenies (Vďačný *et al.*, manuscript in preparation).

Spines on the cyst surface have been reported by Foissner and Xu (2007) in *Protospathidium serpens* and *P. muscicola*, and pillars occur on the cyst surface of *Arcuospathidium bromelicola* (Figs 2f–m). However, further studies are necessary to prove their nature because, for instance, a spinous cyst surface can be caused either by lepidosomes or by outgrowths of the cyst wall s. str. (Foissner *et al.* 2007).

Frequency of spathidiids in tanks of bromeliads

Spathidiids are predators and thus usually rare in all habitats, including tank bromeliads (Dunthorn *et al.* 2012): of the five species found as yet and described here, only two were recorded during a campaign with 40 samples from 13 bromeliad species. This were *Spathidium bromeliophilum* (one time in tanks of *Hohenbergia urbaniana*) and *Arcuospathidium bromelicola* (one time each in tanks of *Achmea peniculigera* and *Hohenbergia inermis*).

Acknowledgements. Supported by the Austrian Science Fund (FWF project P-20360-B17). The technical assistance of Johannes Rattey M.Sc., Dr. Barbara Harl, Robert Schörghofer, and Andreas Zankl is greatly acknowledged. Thanks go also to Dr. Elisabeth Geiser, Salzburg, who collected some of the Jamaican samples.

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Received on 17th April, 2013; revised on 6th June, 2013; accepted on 15th June, 2013