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Life Cycle, Morphology, Ontogenesis, and Phylogeny of *Bromeliothrix metopoides* nov. gen., nov. spec., a Peculiar Ciliate (Protista, Colpodea) from Tank Bromeliads (Bromeliaceae)

Wilhelm FOISSNER

Universität Salzburg, FB Organismische Biologie, Salzburg, Austria

Summary. *Bromeliothrix metopoides* was discovered in tank bromeliads from Central and South America. Pure cultures could be established in various media stimulating growth of its food, i.e. bacteria and heterotrophic flagellates of the genus *Polytomella*. The new ciliate was investigated in the light- and scanning electron microscope, with various silver impregnation techniques, and with molecular methods, using the small-subunit rDNA. The morphology and its changes during the life cycle are documented by 167 figures and a detailed morphometry. *Bromeliothrix metopoides* is about 27–55 × 22–36 µm in size and has a complex life cycle with *Metopus*-shaped, bacteriophagous theronts and trophonts (microstomes) and obovate, flagellate-feeding macrostomes having a large, triangular oral apparatus. The thin-walled resting cysts of the theronts and trophonts are uniquely ellipsoidal, while the thick-walled cyst of the macrostome morph is globular. Reproduction occurs in freely motile condition either by binary fission or polytomy, producing a unique, motile "division chain" composed of four globular offspring, of which the central ones are connected by a curious, plug-like holdfast. Division is associated with a complete reorganization of the parental oral and somatic infraciliature. Stomatogenesis is merotelokinetal as in other members of the order Colpodida. The right polykinetid is generated by the rightmost postoral kinety, while the left polykinetid is produced by the two left postoral kineties and five left side kineties. The division in freely motile condition resembles the Exocolpodidae Foissner *et al.*, 2002, to which *Bromeliothrix* is tentatively assigned, differing from *Exocolpoda* mainly by the formation of a macrostome morph and a division chain. *Bromeliothrix* has a ciliary and silverline pattern typical for members of the family Colpodidae. This matches the molecular classification which, however, hardly reflects the outstanding division and life cycle, suggesting some decoupling of morphological and molecular evolution. The specific morphological and ontogenetic traits of *Bromeliothrix* are interpreted as adaptations to the highly competitive habitat, favouring *r*-selected life strategies. *Bromeliothrix metopoides* is widespread in various tank bromeliads and can be easily cultivated in a wide variety of limnetic and terrestrial media. Thus, it remains obscure why this ciliate is restricted to tank bromeliads, i.e. did not occur in about 2,000 soil and freshwater samples investigated globally, including some 100 samples from Central and South America.

Key words: Central America, division chain, life cycle, macrostome formation, merotelokinetal stomatogenesis, polytomic division, protist endemism, South America.

Address for correspondence: Wilhelm Foissner, Universität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria; E-mail: wilhelm.foissner@sbg.ac.at

INTRODUCTION

Since the turn of the past century, many specific organisms have been described from the little water bodies occurring in tank bromeliads (Picado 1913, Diesel and Schubart 2000). A significant proportion is endemic, showing spectacular adaptations, for instance, the bromeliad crab, *Metopaulias depressus*. It transports empty shells of snails into the tanks to achieve optimal pH and calcium conditions for the brood (Diesel and Schubart 2000). Although there are some ecological studies on protists from tank bromeliads, their taxonomy obtained little attention (for a brief review, see Foissner *et al*. 2003). Only recently, Foissner (2003b, c), Foissner *et al.* (2003, 2009), Fried and Foissner (2007), and Foissner and Wolf (2009) established the occurrence of a specific ciliate community in tank bromeliads. In the present study, I describe a further bromeliad ciliate, *Bromeliothrix metopoides* nov. gen., nov. spec. This species is a challenge to ecologists and biogeographers. Although it forms easily resting cysts for dispersal, is widespread in Central and South America, and thrives so readily in a wide variety of culture media that it becomes a "weed" in the laboratory, it seems to be restricted to tank bromeliads, i.e. I could not find it in about 2,000 soil and freshwater samples investigated globally, including rather many from Central and South America. Thus, *B. metopoides* is an extraordinary example of protist endemism, a subject of continued controversy (for a review, see Foissner 2008).

Although being small and colourless, *B. metopoides* is a flagship in a biogeographical sense because of the extraordinary division chain and the restriction to the bromeliad-bearing area, i.e. Central and South America. Thus, I shall describe it in great detail so that it can be identified unmistakably. Further, this ciliate provides a rare opportunity to investigate colpodid division in the scanning electron microscope because it divides in freely motile condition, while most relatives divide in cysts covered with a membrane (Foissner 1993; for an exception see, *Exocolpoda augustini* in Foissner *et al.* 2002).

MATERIALS AND METHODS

Bromeliothrix metopoides was discovered in August 1998, when Dr. T. Cordeiro was a guest in my laboratory and brought along some tank water from a small bromeliad growing on a tree in the Nature Reserve Mata do Buraquinho in the town of João Pessoa, Paraiba, Brazil, W 35° S 8° (Foissner and Cordeiro 2000). Later on, I found this species in over 70 bromeliad samples from Central America, the Carribean, and South America (see section on Occurrence and Ecology). However, if not stated otherwise, all figures and data are from the type population collected by T. Cordeiro.

Pure cultures of *B. metopoides* could be established in Eau de Volvic (French table water) enriched with some crushed wheat grains to promote growth of indigenous bacteria and heterotrophic flagellates, on which the ciliate feeds. In the cultures established, there were various small flagellates (≤ 15 µm; *Cercomonas*, *Ochromonas* etc.), a minute amoeba, and a middle-sized flagellate belonging to the genus *Polytomella* because it was heterotrophic and had four flagella at the base of a distinct papilla (Fig. 7d; Pavillard 1952). As far as I could observe, the macrostome morph of *B. metopoides* feeds only *Polytomella*, while theronts and trophonts possibly ingest some of the small flagellates, although their main food is various bacteria. The *Polytomella* has a size of about 40 × 32 µm (n = 11); has four contractile vacuoles; lacks an eyespot; and has the nucleus and other organelles in the anterior body half, while the posterior two thirds are occupied by a large vacuole; possibly, it is a novel species. *Bromeliothrix metopoides* grows rapidly (doubling time ≤ 5 h), is easily cultivated, and forms resting cysts throughout the life cycle. Thus, it is easily maintained.

Bromeliothrix metopoides was investigated with the methods described in Foissner (1991), i.e. *in vivo* using a high-power oil immersion objective and interference contrast; with various silver impregnation techniques (silver nitrate, silver carbonate, protargol), and by scanning electron microscopy (SEM). Deciliated specimens were obtained with the method described by Foissner (2003a). 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. Illustrations of live specimens were based on free-hand sketches and micrographs, while those of prepared cells were made with a drawing device.

The small-subunit rDNA of *B. metopoides* has been analysed by Foissner *et al*. (2003), where the species was named "new colpodid." Standard methods were used as described, e.g. by Weisse *et al*. (2008). Later, the phylogenetic analysis was refined by Dunthorn *et al*. (2008).

Terminology is according to the monographs of Corliss (1979) and Foissner (1993, 1996). In analogy to *Tetrahymena* (Corliss 1973), I apply the terms microstome (MI, small or ordinary mouthed theronts and trophonts) and macrostome (MA, large mouthed), and use both as an adjective (e.g. macrostome specimens) and as a noun (e.g. macrostomes).

Actually, three morphs of *B. metopoides* can be distinguished in silver preparations (Table 1): the theronts (TH), which are usually less than 30 µm long, *Metopus*-shaped and microstomous, thus having only bacteria in the food vacuoles; the trophonts (TR), which are usually 30–45 µm long, *Metopus*-shaped and ordinary-mouthed, thus having mainly bacteria but also some minute flagellates in the food vacuoles; and the large-mouthed macrostomes (MA), which are 45–55 µm long, obovate to broadly ellipsoidal, and have only *Polytomella* in the food vacuoles. While (TH) and (TR) are easily distinguished from the (MA) by body shape (*Metopus*-shaped vs. obovate), they are difficult to distinguish from each other. These and other differences are shown in Table 1, both as absolute values and as percentages.

RESULTS

Family Exocolpodidae Foissner *et al*., 2002

Diagnosis: Colpodida Puytorac *et al.*, 1974, as defined by Foissner (1993), dividing in freely motile (nonencysted) condition. Oral cavity in anterior body half.

Type genus: *Exocolpoda* Foissner *et al*., 2002.

Genus *Exocolpoda* Foissner *et al*., 2002

Diagnosis: Medium-sized, microphagous Exocolpodidae with small, conical vestibulum. Left wall of oral cavity overhangs right. Right oral polykinetid composed of few to many short, more or less disordered kineties.

Type species: *Colpoda augustini* Foissner, 1987.

Genus *Bromeliothrix* nov. gen.

Diagnosis: Small to medium-sized Exocolpodidae (?) with bacteriophagous theronts and trophonts having a small, conical vestibulum, and a predaceous macrostome morph with large, triangular oral apparatus. Left wall of oral cavity overhangs right. Right oral polykinetid composed of many short, slightly disordered kineties. Produces a "division chain" with four globular daughters, the central ones connected by a plug-like holdfast. Resting cysts of theronts (TH) and trophonts (TR) ellipsoidal and thin-walled, those of macrostome (MA) morph globular and thick-walled.

Type species: *Bromeliothrix metopoides* nov. spec.

Etymology: Composite of the habitat (bromeliads) and the Greek noun *thrix* (hair ~ ciliate s.l.), meaning "a ciliate living in the bromeliad family." Feminine gender.

Description of *Bromeliothrix metopoides* nov. spec.

Diagnosis: Theronts (TH) and trophonts (TR) *Metopus*-shaped, i.e. with conspicuous preoral dome; size about 27 × 22 µm and 38 × 30 µm *in vivo*, respectively; macrostomes (MA) obovate or bluntly reniform and about 55 × 36 µm. Nuclear apparatus in posterior body half, macronucleus and micronucleus broadly ellipsoidal. A single contractile vacuole in posterior end. Mucocysts ellipsoidal, mainly within kineties. Usually 12 somatic ciliary rows, those of right side spread fanlike anteriorly, producing conspicuous, barren areas between rows 1 and 2 and 2 and 3. Left side rows densely ciliated anteriorly, preoral dome thus with a ciliary tuft. Oral cavity small and conical in (TH) and (TR), large and triangular in (MA) occupying central third of right side of cell. Left oral polykinetid of (TH) and (TR) elongate rectangular and usually composed of 15 kineties, that of (MA) cuneate and composed of 12 kineties.

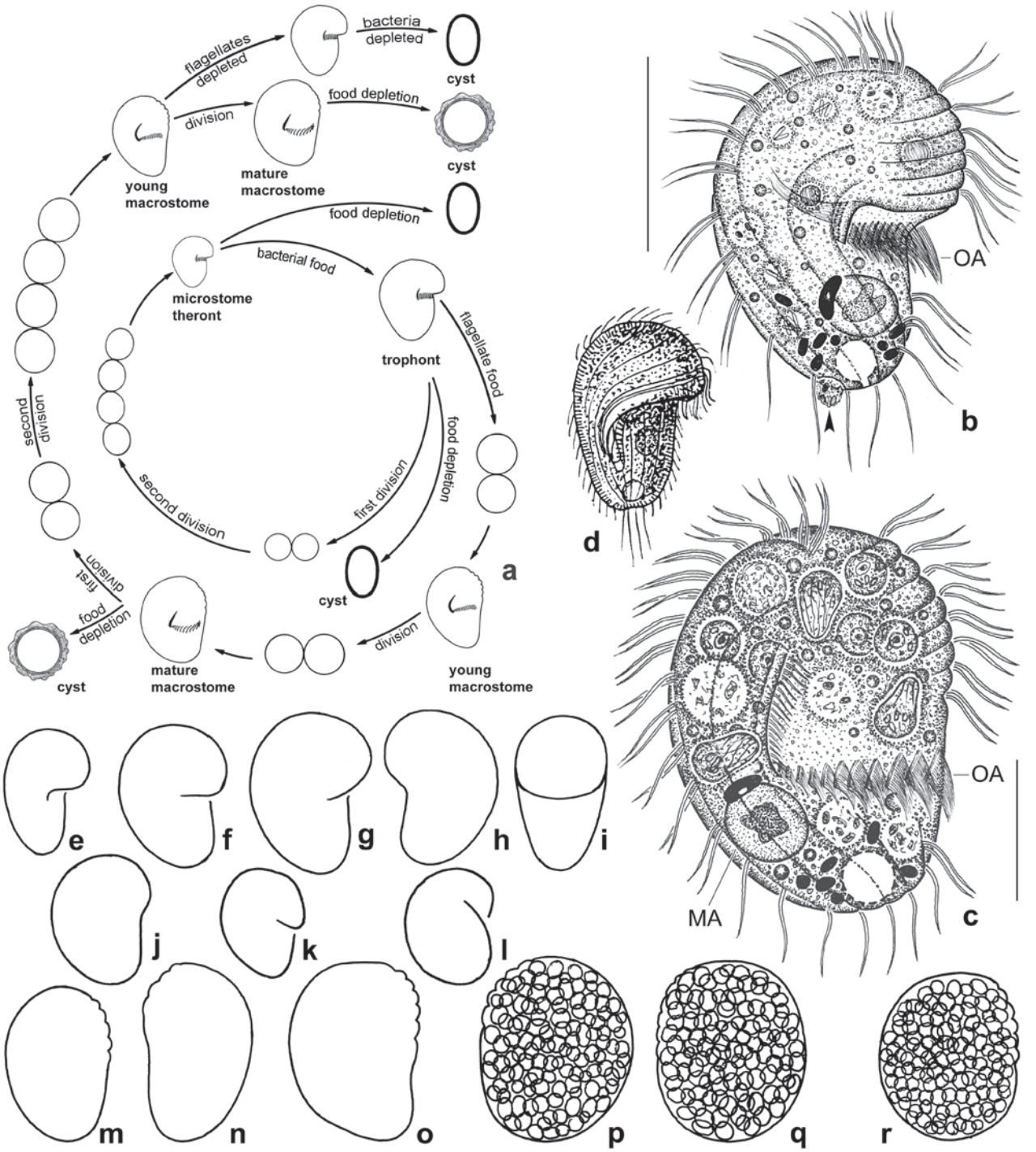
Type locality: A tank bromeliad from a tree of the Nature Reserve Mata do Buraquinho in the town of João Pessoa, Paraiba, Brazil, W 35° S 8°.

Type material: 4 holotype slides with silver nitrate, protargol, and silver carbonate-impregnated specimens, respectively, and 17 paratype slides with morphostatic and dividing cells from the type locality have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). The protargol slides appear poor at first glance because of the deeply impregnated mucocysts. However, more detailed analysis shows that the infraciliature is also well impregnated. Further, 4 silver nitrate-impregnated and 1 protargol-impregnated voucher slide from two Mexican populations have been deposited in the same collection; they have better quality than the slides from the type locality. All specimens depicted and further relevant cells are marked by black ink circles on the coverslip.

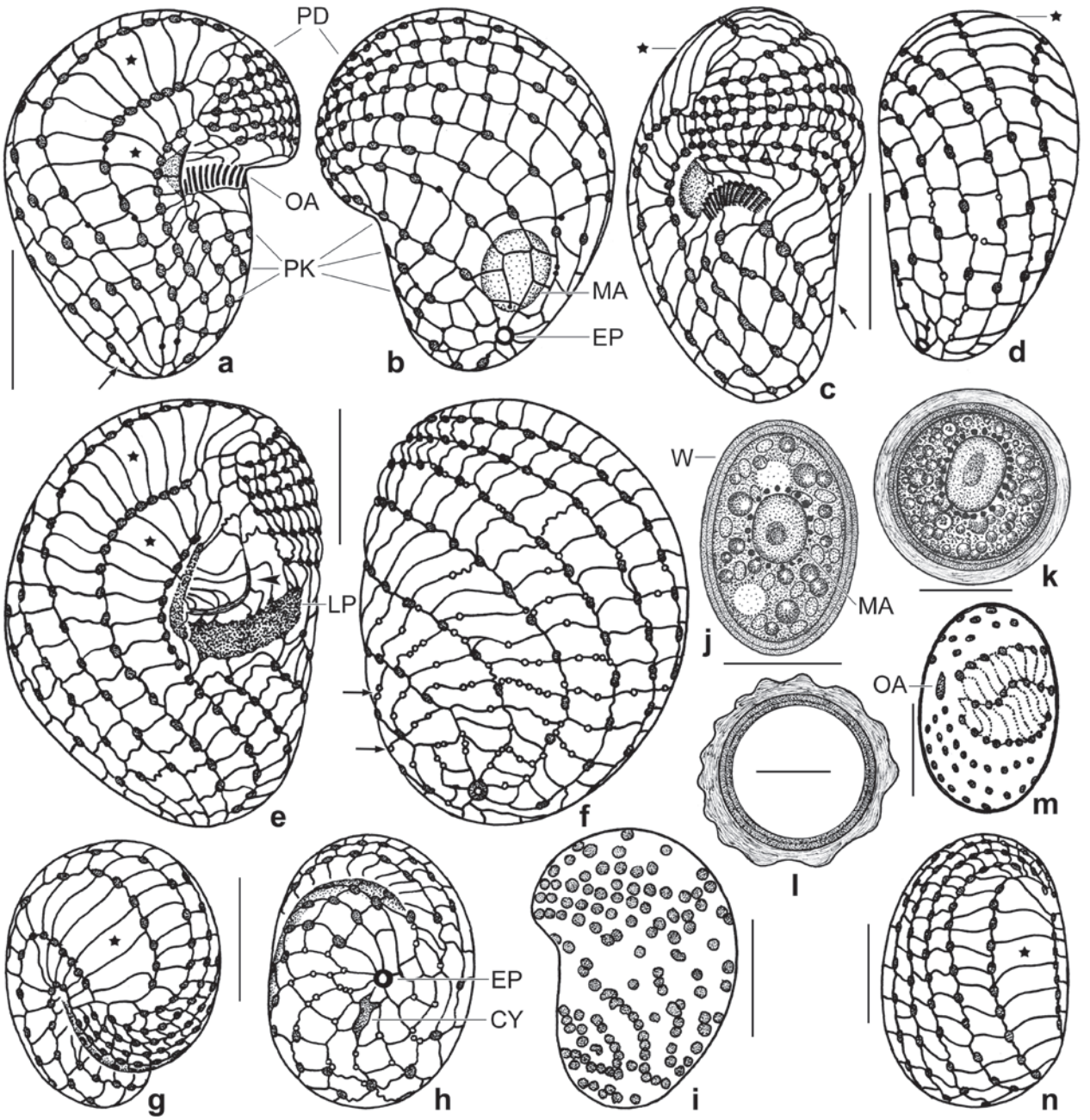
I designated four slides as holotype because each shows structures and specimens (micro- and macrostomes) indispensable for correct identification. Alternatively, one of the two protargol slides can be considered as the holotype, while the others could be hapantotypes. Possibly, the designation is not of relevance because the four slides are from cultures of a single population.

Etymology: Composite of the generic name *Metopus* and the Greek comparative suffix *oides* (similar to), meaning "a ciliate having a form similar to that of *Metopus*."

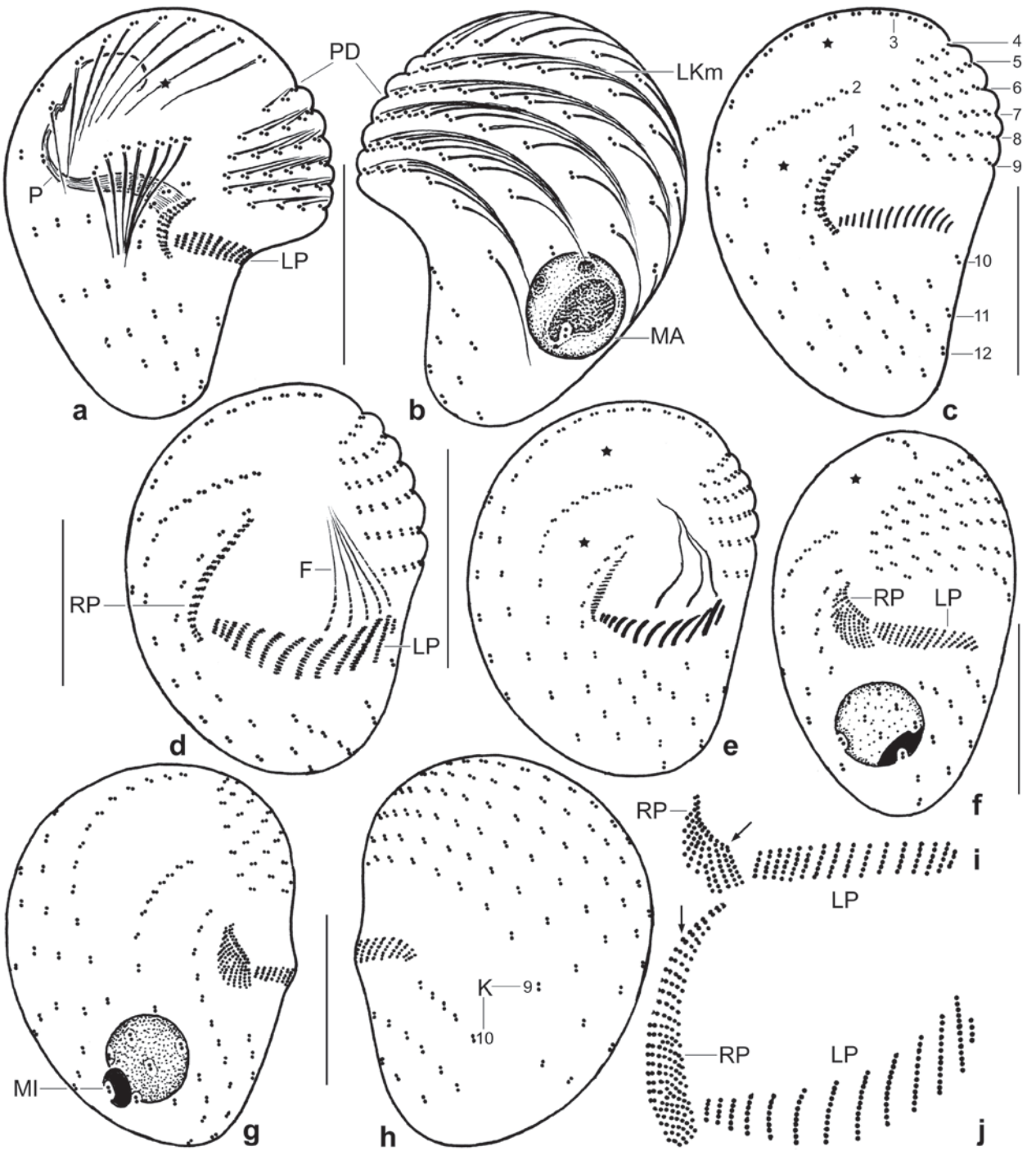
Life cycle (Fig. 1a): The life cycle of *B. metopoides* has been clarified with pure cultures fed either with environmental bacteria or with bacteria plus flagellates (see section on Material and Methods). In field samples, microstomous (TH) and (TR) are most common, while (MA) occur very rarely. When cultivated with bacteria, only (TH) and (TR) develop (Figs 1b, e–h, 4a, j), while (MA) are formed when the flagellate *Polytomella* is added. The development of the (MA) needs two generations: in the first one, the cell looses the *Metopus*-shape and the oral apparatus becomes larger (Figs 1n, 3c); the second generation produces mature macrostomes with a large oral apparatus and an obovate to broadly ellipsoidal or reniform body (Figs 1c, o–r, 5a, b, h, i, 7a, 9a–d). The analysis of the food vacuoles showed that (TH) feed on bacteria and (MA) on flagellates, while (TR) possibly take both, but prefer bacteria. The main



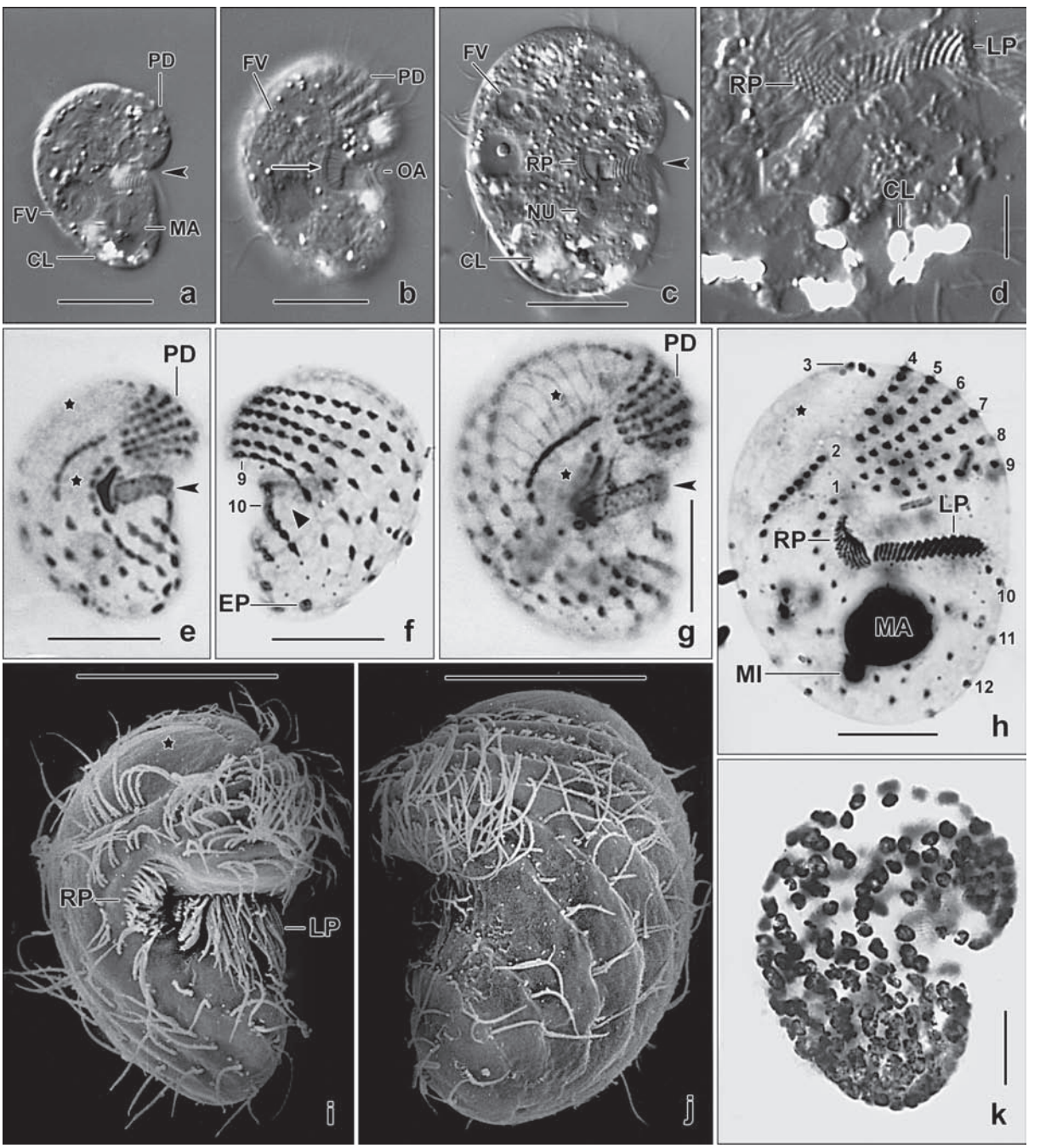
Figs 1a–r. *Bromeliothrix metopoides* (a–c, e–r) and *Metopus pullus* (d, from Kahl 1932) from life (e–r, redrawn from micrographs). **a** – *B. metopoides* has a rather complex life cycle, i.e. generates bacteriophagous, microstomous theronts and trophonts with ellipsoidal resting cysts and flagellate-feeding macrostomes with globular cysts. Further, *B. metopoides* divides in freely motile (non-encysted) condition, producing division chains composed of four offspring. For details, see text; **b** – right side view of a microstomous trophont, length 35 µm. Theronts and trophonts feed on bacteria and thus have small food vacuoles, each containing often only few bacterial rods. The arrowhead marks fecal mass just leaving the cell; **c** – right side view of a representative macrostome, length 55 µm. Macrostomes feed on flagellates and thus have large food vacuoles; **d** – the shape and size of certain *Metopus* species, e.g. the 80 µm long *M. pullus*, are highly similar to those of theronts and trophonts of *B. metopoides*. **e–h**, **j** – shape variability of theronts and trophonts; **i** – ventral view; **k**, **l** – oblique anterior and posterior polar views; **m–o** – shape variability of ordinary macrostomes; **p–r** – when packed with food, the macrostomes are almost globular. MA – macronucleus, OA – oral apparatus. Scale bars: 20 µm.



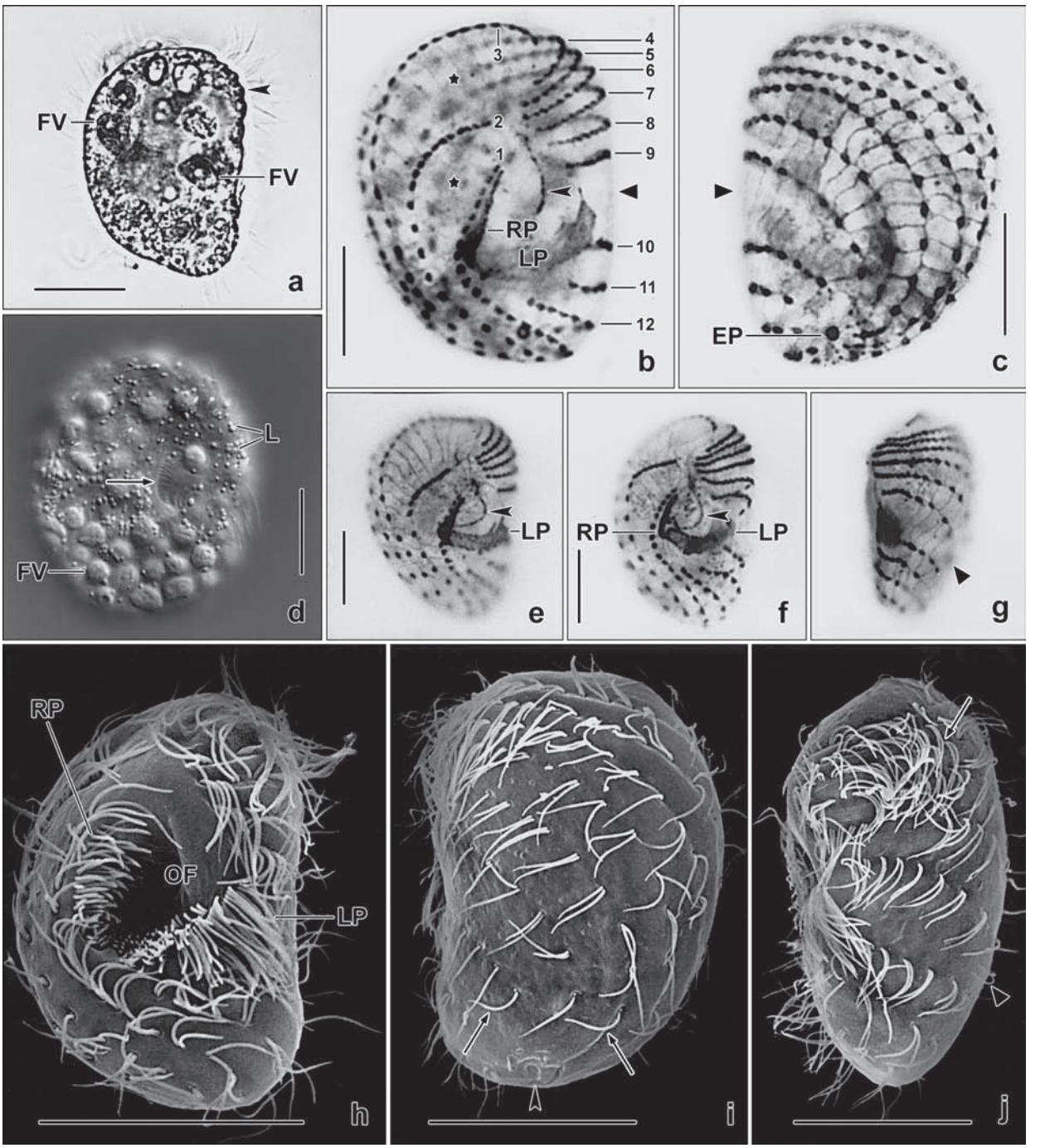
Figs 2a–n. *Bromeliothrix metopoides*, trophic (a–i) and cystic (j–n) specimens from life (j–l) and after silver nitrate (a–h, m, n) and protargol (i) impregnation, showing the ciliary and silverline pattern. The anterior widening of the body spreads ciliary rows 2 and 3, producing large barren areas (asterisks). **a**, **b** – right and left side view of trophonts, the left one representing a holotype, 40 µm. Note the *Metopus*-shaped body and the granule (arrow) remaining after the release of an extrusome; **c** – ventral view of a specimen with 13 ciliary rows, i.e. seven left side kineties. The arrow marks a cuneate, barren area between the last left side kinety and the leftmost postoral kinety; **d** – dorsal view, showing the flattened left side; **e**, **f** – right and left side view of macrostomes, the left one representing a holotype, length 58 µm. Note the large oral area traversed by a thick, curved silverline (arrowhead), marking the cytostome. Arrows denote minute silverline rings, marking attached extrusomes; **g**, **h** – anterior and posterior polar view of trophonts; **i** – protargol reveals the extrusomes, which are mainly within the kineties; **j** – the resting cysts of the theronts and trophonts are ellipsoidal and the wall consists of two layers. Young cysts are surrounded by a slime layer up to 10 µm thick (Fig. 7d, e), which is soon digested by bacteria and thus not shown; **k**, **l** – the resting cyst of the macrostomes is globular and the wall consists of three layers, which become more distinct when the cyst contents is removed (l); **m**, **n** – silver impregnation shows that the kinetids and the silverline pattern are maintained in young cysts. CY – cytoproct, EP – excretory pore, LP – left oral polykinetid, MA – macronucleus, OA – oral apparatus, PD – preoral dome, PK – postoral kineties, W – cyst wall. Scale bars: 10 µm (j–n), 15 µm (a–d, g–i), and 20 µm (e, f).



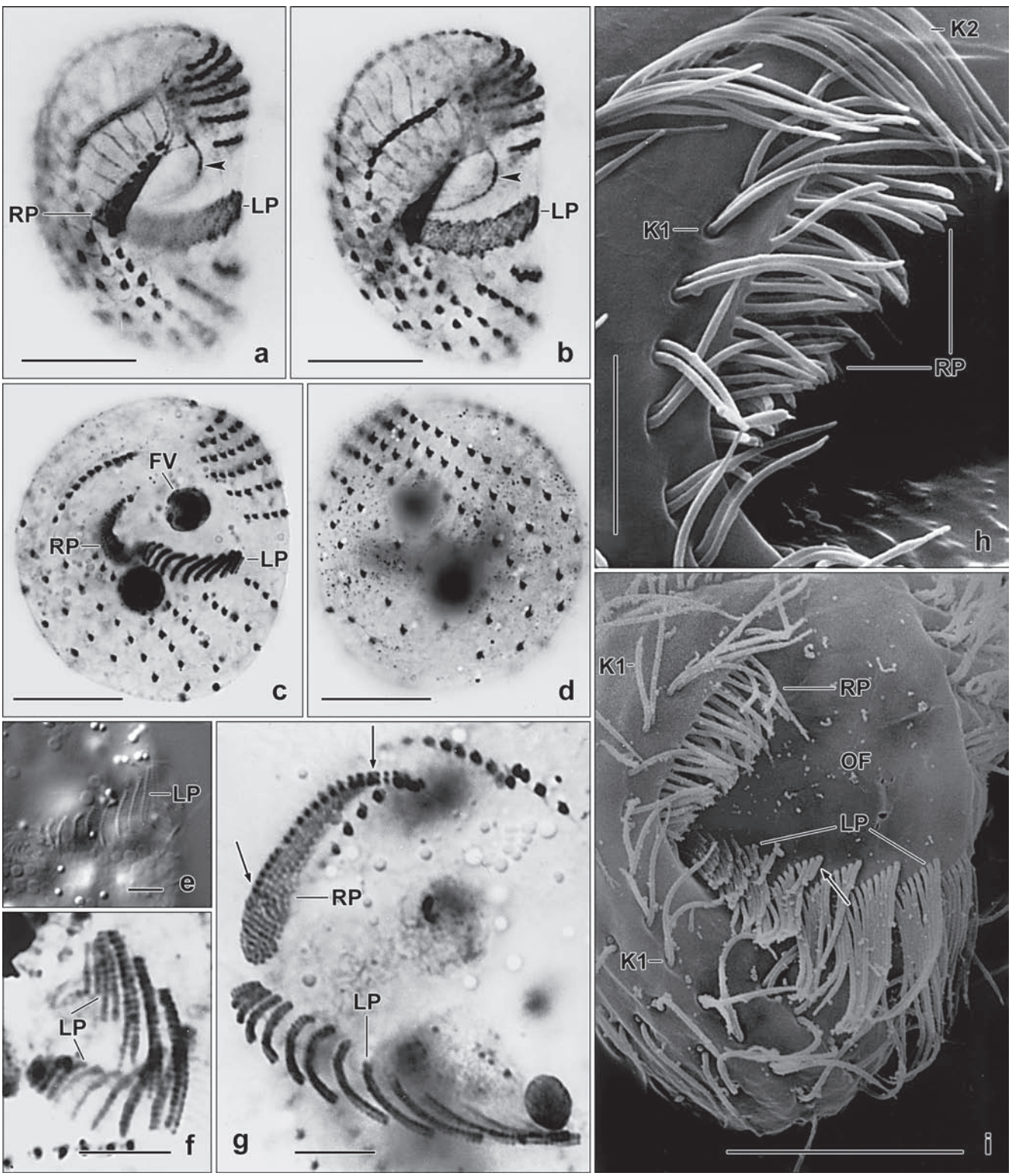
Figs 3a–j. *Bromeliothrix metopoides*, ciliary pattern after protargol (a–e) and silver carbonate (f–j) impregnation. The anterior widening of the body spreads ciliary rows 2 and 3, producing large barren areas (asterisks). **a**, **b** – right and left side view of a specimen developing to the trophont stage. Note the large, barren area between kineties 2 and 3 (asterisk), the conspicuous fibre system, and the long pharynx extending anteriorly; **c** – ventral view of a specimen developing to a macrostome. In this stage, the cells are obovate and the oral structures enlarge. Numerals denote ciliary rows; **d**, **e** – ventral views of mature macrostomes with bluntly reniform body and large oral structures. The right polykinetid appears narrower than it is (see Fig. j) because it is seen edge-on; **f** – ventral view showing the oral ciliary fields; **g**, **h** – right and left side view of ciliary pattern of a trophont. Note the large, barren area between kineties 9 and 10; **i**, **j** – oral ciliary pattern of a trophont/theront (i) and a macrostome (j). Redrawn and slightly schematized from micrographs. The arrows mark a row of dikinetids proximally. F – fibres, K – kinety, LKm – LKm fibre, LP – left oral polykinetid, MA – macronucleus, MI – micronucleus, P – pharynx, PD – preoral dome, RP – right oral polykinetid. Scale bars: 15 µm (a–c, f–h) and 20 µm (d, e).



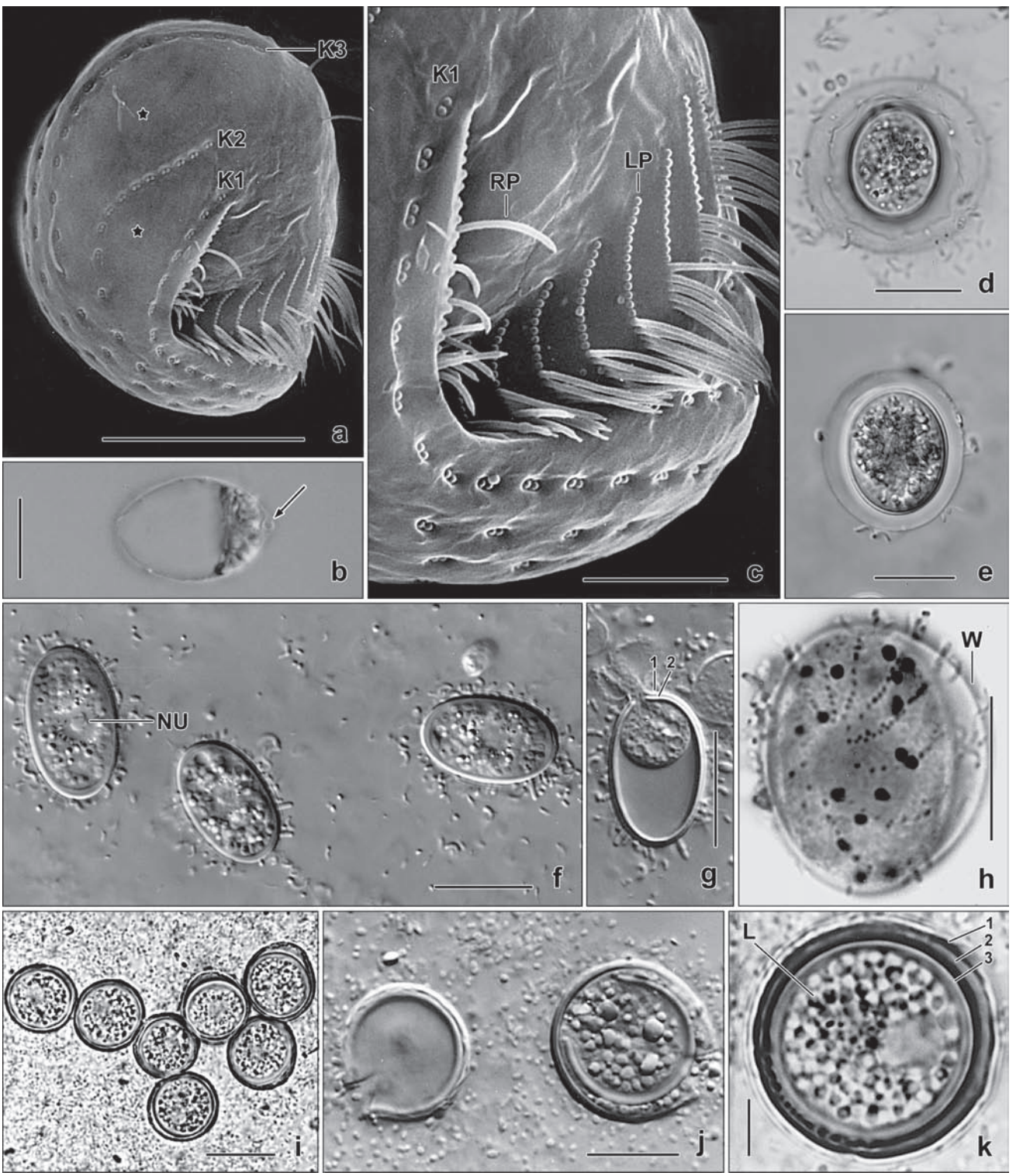
Figs 4a–k. *Bromeliothrix metopoides* from life (a–d), after silver nitrate (e–g), silver carbonate (h) and protargol (k) impregnation, and in the SEM (i, j). The anterior widening of the body spreads ciliary rows 2 and 3, producing large barren areas (asterisks). The arrowheads mark the left oral polykinetid. **a** – a freely motile theront, showing the *Metopus*-shaped body and the beard formed by the cilia of the left oral polykinetid (arrowhead); **b** – right side surface view of a young trophont, showing the right margin of the oral opening (arrow) and the deeply indented ciliary rows of the preoral dome; **c**, **d** – squashed specimens, showing various details and the beard formed by the cilia of the left polykinetid (arrowhead); **e**, **f**, **h**, **i**, **j** – right side (e, i), left side (f, j), and ventral (h) views of the ciliary pattern of theronts developing to trophonts. Note the densely spaced kinetids on the preoral dome, producing a rather conspicuous ciliary tuft (i, j), and the large, barren areas (asterisks) in the anterior right quadrant of the cells. The arrowhead marks the left polykinetid; numerals denote the ciliary rows; and the triangle (f) marks a barren area between kineties 9 and 10; **g** – right side view of a macrostome, showing the silverline pattern and the large barren areas (asterisk) between kineties 1 and 2 and 2 and 3 (see also next plate); **k** – extrusome pattern of right side of a *Metopus*-shaped trophont. The extrusomes, which are inflated due to the preparation procedures, are mainly within the kineties. CL – cytoplasmic crystals, EP – excretory pore, FV – food vacuoles, LP – left oral polykinetid, MA – macronucleus, MI – micronucleus, NU – nucleolus, OA – oral apparatus, PD – preoral dome, RP – right oral polykinetid. Scale bars: 10 μm (d, k), 15 μm (a–c, e, f, h–j), and 20 μm (g).



Figs 5a–j. *Bromeliothrix metopoides* from life (a, d), after Chatton-Lwoff silver nitrate impregnation (b, c, e–g), and in the SEM (h–j). **a** – a young macrostome not yet packed with food vacuoles, thus having an obovate and slightly reniform body. The preoral area is serrated by the ciliary rows (arrowhead); **b**, **c**, **e**, **f** – right side (b, e, f) and left side (c) views of macrostomes, showing the ciliary pattern and the colpodid silverline pattern (c); numerals denote kineties (b). The arrowheads mark a thick, strongly curved silverline representing the cytostome. Asterisks and triangles mark large, barren areas between kineties 1 and 2, 2 and 3, and 9 and 10; **d** – an almost globular macrostome studded with food vacuoles and lipid droplets. The arrow marks the right margin of the oral opening; **g**, **j** – ventral views, showing that even macrostome cells are flattened laterally, especially on the right side. Note the conspicuous preoral ciliary tuft (arrow). The triangles mark the barren area between kineties 9 and 10; **h**, **i** – right and left side view of macrostomes, showing the broadly reniform body, the large oral field, the dense ciliation of the oral polykinetids, and the somatic ciliature, which consists of ciliated dikinetids, except in the posterior region, where the anterior cilium is lacking (arrows). The arrowhead marks the excretory pore. EP – excretory pore of the contractile vacuole, FV – food vacuoles, L – lipid droplets, LP – left oral polykinetid, OF – oral field, RP – right oral polykinetid. Scale bars: 20 μm.



Figs 6a–i. *Bromeliothrix metopoides*, macrostomes from life (e), after silver nitrate (a, b) and silver carbonate (c, d, f, g) impregnation, and in the SEM (h, i). **a**, **b** – right side view of same specimen in two focal planes to show the ciliary pattern and the cytostome (arrowheads); **c**, **d** – right and left side view of ciliary pattern; **e–g** – oral structures (see text for explanation). Arrows mark a row of dikinetids at proximal end of right polykinetid; **h**, **i** – details of oral structures; arrow marks shortened cilia in the anterior region of the rows comprising the left polykinetid. The cilia of the right polykinetid decrease in length from anterior to posterior. FV – food vacuole, K1, 2 – kineties, LP – left polykinetid, OF – oral field, RP – right polykinetid. Scale bars: 5 µm (e–h), 10 µm (i), and 20 µm (a–d).



Figs 7a–k. *Bromeliothrix metopoides* (a, c–k) and *Polytomella* sp. (b) from life (b, d–g, i–k), after silver carbonate impregnation (h), and in the SEM (a, c). **a**, **c** – a deciliated macrostome, showing the large barren areas (asterisks) between kineties 1 and 2 and 2 and 3. The left polykinetid consists of monokinetal ciliary rows; **b** – the macrostomes feed on *Polytomella*; arrow marks the papilla, from which four flagella emerge; **d**, **e** – young cysts are covered by a thick slime layer; **f**, **g** – theronts and trophonts have ellipsoidal cysts with a bipartite wall (numerals 1, 2); **h** – the basal bodies are retained in young cysts; **i–k** – macrostomes have globular cysts with tripartite wall (numerals 1–3). K1, 2, 3 – kineties, L – lipid droplets, LP, RP – left and right polykinetid, NU – nucleolus, W – cyst wall. Scale bars: 5 µm (c, k), 15 µm (b, d–h, j), and 20 µm (a, i).

Table 1. Morphometric data on *Bromeliothrix metopoides*. Based on cultivated, mounted, silver-impregnated, randomly selected specimens from type locality. Measurements in µm. CHL – Chatton-Lwoff silver nitrate impregnation, CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of specimens investigated, OS – osmic acid, P – protargol impregnation, SC – silver carbonate impregnation, SD – standard deviation, SE – standard error of mean, $\bar{x}$ – arithmetic mean, % Increase – the increase in the mean value for macrostomes relative to trophonts and microstomes (theronts).

Characteristics	State	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n	% Increase
Body, length	Theronts [a, b]	CHL	25.6	26.0	1.7	0.4	6.7	23.0	29.0	21	–
	Trophonts [a, b]	CHL	36.4	37.0	4.1	0.9	11.3	30.0	47.0	21	42.2
	Macrostomes [b]	CHL	51.7	51.0	4.4	1.0	8.5	45.0	58.0	21	102.0
	Ther.+Trop. [c]	P	28.6	28.0	3.9	0.9	13.6	22.0	38.0	19	–
	Macrostomes [c]	P	43.3	46.0	8.3	2.0	19.1	30.0	52.0	17	51.4
	Ther.+Trop.	SC	30.2	30.0	2.3	0.5	7.6	25.0	35.0	21	–
	Ther.+Trop. [d]	OS	36.8	36.0	5.0	1.2	13.6	30.0	45.0	19	–
	Macrostomes [d]	OS	61.4	62.0	6.8	1.4	11.1	45.0	70.0	23	66.8
Body, width in lateral view	Theronts [a, b]	CHL	19.4	20.0	1.5	0.3	6.7	17.0	22.0	21	–
	Trophonts [a, b]	CHL	28.3	28.0	3.3	0.7	11.5	23.0	36.0	21	45.9
	Macrostomes [b]	CHL	38.3	37.0	4.2	0.9	11.0	32.0	46.0	21	97.4
	Ther.+Trop. [c]	P	23.1	22.0	3.8	0.9	16.4	18.0	31.0	19	–
	Macrostomes [c]	P	33.5	36.0	5.8	1.4	17.5	24.0	42.0	17	45.0
	Ther.+Trop.	SC	22.1	22.0	2.1	0.5	9.6	16.0	26.0	21	–
	Ther.+Trop. [d]	OS	27.6	27.0	4.6	1.1	16.7	20.0	35.0	19	–
	Macrostomes [d]	OS	45.1	45.0	6.0	1.2	13.2	33.0	55.0	23	63.4
Body length: width, ratio	Theronts [a, b]	CHL	1.3	1.3	0.1	0.1	5.5	1.2	1.5	21	–
	Trophonts [a, b]	CHL	1.3	1.3	0.1	0.1	6.6	1.2	1.5	21	0
	Macrostomes [b]	CHL	1.4	1.3	0.1	0.1	7.8	1.2	1.7	21	7.7
	Ther.+Trop. [c]	P	1.3	1.3	0.1	0.1	11.1	0.9	1.5	19	–
	Macrostomes [c]	P	1.3	1.3	0.1	0.1	5.8	1.2	1.4	17	0
	Ther.+Trop.	SC	1.4	1.4	0.1	0.1	7.2	1.2	1.6	21	–
	Ther.+Trop. [d]	OS	1.3	1.3	0.1	0.1	7.5	1.2	1.6	19	–
	Macrostomes [d]	OS	1.4	1.4	0.1	0.1	5.8	1.2	1.5	23	7.7
Anterior body end to anterior end of right oral polykinetid, distance	Theronts [a, b]	CHL	9.7	10.0	1.7	0.4	17.1	7.0	12.0	21	–
	Trophonts [a, b]	CHL	13.5	13.0	2.4	0.5	17.5	10.0	21.0	21	39.2
	Macrostomes [b]	CHL	17.9	18.0	2.8	0.6	15.4	12.0	22.0	21	84.5
	Ther.+Trop. [c]	P	11.7	12.0	2.0	0.5	17.1	9.0	17.0	19	–
	Macrostomes [c]	P	17.1	19.0	4.3	1.1	25.2	10.0	22.0	17	46.2
	Ther.+Trop.	SC	11.5	12.0	1.5	0.3	12.7	8.5	15.0	21	–
Anterior body end to posterior end of right oral polykinetid, distance	Theronts [a, b]	CHL	15.1	15.0	1.3	0.3	8.8	13.0	17.0	21	–
	Trophonts [a, b]	CHL	19.6	19.0	2.3	0.5	11.5	16.0	26.0	21	29.8
	Macrostomes [b]	CHL	30.9	31.0	3.4	0.8	11.1	25.0	36.0	21	104.6
	Ther.+Trop. [c]	P	17.8	18.0	2.4	0.6	13.5	14.0	25.0	19	–
	Macrostomes [c]	P	27.7	30.0	4.6	1.1	16.6	19.0	33.0	17	55.6
	Ther.+Trop.	SC	17.2	17.0	1.8	0.4	10.3	13.0	21.0	21	–
Anterior body end to macronucleus, distance	Theronts [a, b]	CHL	14.7	15.0	2.5	0.6	17.2	9.0	19.0	21	–
	Trophonts [a, b]	CHL	22.6	23.0	3.3	0.7	14.4	16.0	28.0	21	53.7
	Macrostomes [b]	CHL	32.7	35.0	4.4	1.0	13.4	23.0	40.0	21	122.4
	Ther.+Trop. [c]	P	18.1	17.0	3.3	0.8	18.4	14.0	26.0	19	–

Characteristics	State	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n	% Increase
	Macrostomes [c]	P	24.2	27.0	8.2	2.0	33.8	10.0	35.0	17	33.7
	Ther.+Trop.	SC	19.1	19.0	2.1	0.5	11.2	14.0	22.0	21	–
Macronucleus, length	Theronts [a, b]	CHL	6.6	6.0	1.3	0.3	20.2	5.0	10.0	21	–
	Trophonts [a, b]	CHL	7.9	8.0	1.4	0.3	17.7	6.0	11.0	21	19.7
	Macrostomes [b]	CHL	11.7	12.0	2.3	0.5	19.9	8.0	16.0	21	77.3
	Ther.+Trop. [c]	P	8.9	9.0	0.7	0.2	8.3	8.0	10.0	19	–
	Macrostomes [c]	P	12.7	13.0	2.9	0.7	23.1	8.0	18.0	17	42.7
	Ther.+Trop.	SC	7.9	7.5	1.0	0.2	12.8	6.0	10.0	21	–
Macronucleus, width	Theronts [a, b]	CHL	5.6	6.0	0.8	0.2	14.3	4.0	8.0	21	–
	Trophonts [a, b]	CHL	6.7	7.0	1.2	0.3	17.1	5.0	10.0	21	19.6
	Macrostomes [b]	CHL	8.9	9.0	1.1	0.2	12.5	7.0	10.0	21	58.9
	Ther.+Trop. [c]	P	7.3	7.0	0.8	0.2	11.1	6.0	9.0	19	–
	Macrostomes [c]	P	10.9	11.0	2.6	0.6	23.8	7.0	15.0	17	49.3
	Ther.+Trop.	SC	7.2	7.5	0.8	0.2	10.4	6.0	8.5	21	–
Micronucleus, length	Ther.+Trop. [c]	P	2.8	3.0	0.4	0.1	12.4	2.0	3.5	19	–
	Macrostomes [c]	P	2.8	2.5	0.4	0.1	14.4	2.5	3.5	17	0
	Ther.+Trop.	SC	3.8	3.5	0.5	0.1	13.7	3.0	5.0	21	–
Micronucleus, width	Ther.+Trop. [c]	P	2.0	2.0	0.3	0.1	14.6	1.5	2.5	19	–
	Macrostomes [c]	P	2.1	2.0	0.3	0.1	14.4	1.5	2.5	11	–
	Ther.+Trop.	SC	2.6	2.5	0.3	0.1	13.1	2.0	3.5	21	–
Ciliary rows right of oral apparatus, number	Theronts [a, b]	CHL	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21	–
	Trophonts [a, b]	CHL	3.1	3.0	–	–	–	3.0	4.0	21	0
	Macrostomes [b]	CHL	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21	0
	Ther.+Trop. [c]	P	3.0	3.0	0.0	0.0	0.0	3.0	3.0	19	–
	Macrostomes [c]	P	3.0	3.0	0.0	0.0	0.0	3.0	3.0	17	0
	Ther.+Trop.	SC	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21	–
Ciliary rows on left side, number	Theronts [a, b]	CHL	6.2	6.0	–	–	–	6.0	7.0	21	–
	Trophonts [a, b]	CHL	6.6	7.0	0.6	0.1	9.1	6.0	8.0	21	6.5
	Macrostomes [b]	CHL	6.7	7.0	0.6	0.1	8.4	6.0	8.0	21	8.1
	Ther.+Trop. [c]	P	6.0	6.0	0.0	0.0	0.0	6.0	6.0	19	–
	Macrostomes [c]	P	6.0	6.0	0.0	0.0	0.0	6.0	6.0	17	0
	Ther.+Trop.	SC	6.1	6.0	–	–	–	6.0	7.0	21	–
Postoral ciliary rows, number	Theronts [a, b]	CHL	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21	–
	Trophonts [a, b]	CHL	3.2	3.0	–	–	–	3.0	4.0	21	–
	Macrostomes [b]	CHL	3.2	3.0	–	–	–	3.0	4.0	21	–
	Ther.+Trop. [c]	P	3.0	3.0	0.0	0.0	0.0	3.0	3.0	19	–
	Macrostomes [c]	P	3.0	3.0	0.0	0.0	0.0	3.0	3.0	17	–
	Ther.+Trop.	SC	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21	–
Ciliary rows, total number	Theronts [a, b]	CHL	12.2	12.0	–	–	–	12.0	13.0	21	–
	Trophonts [a, b]	CHL	12.8	13.0	0.8	0.2	6.0	12.0	15.0	21	4.9
	Macrostomes [b]	CHL	13.0	13.0	0.5	0.1	3.8	12.0	14.0	21	10.6
	Ther.+Trop. [c]	P	12.0	12.0	0.0	0.0	0.0	12.0	12.0	19	–
	Macrostomes [c]	P	12.0	12.0	0.0	0.0	0.0	12.0	12.0	17	0
	Ther.+Trop.	SC	12.1	12.0	–	–	–	12.0	13.0	21	–

Characteristics	State	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n	% Increase
Dikinetids in kinety 1, number	Theronts [a, b]	CHL	9.2	9.0	1.5	0.3	16.0	6.0	13.0	21	–
	Trophonts [a, b]	CHL	11.8	12.0	1.9	0.4	16.1	9.0	16.0	21	28.3
	Macrostomes [b]	CHL	16.4	16.0	1.8	0.4	11.0	13.0	20.0	21	78.3
	Ther.+Trop. [c]	P	10.4	11.0	1.4	0.3	12.9	8.0	12.0	19	–
	Macrostomes [c]	P	14.0	14.0	1.9	0.5	13.4	11.0	18.8	14	34.6
	Ther.+Trop.	SC	10.2	10.0	1.5	0.3	14.1	9.0	14.0	21	–
Dikinetids in kinety 2, number	Theronts [a, b]	CHL	10.7	11.0	1.5	0.3	13.7	8.0	14.0	21	–
	Trophonts [a, b]	CHL	13.1	13.0	2.2	0.5	16.9	10.0	18.0	21	22.4
	Macrostomes [b]	CHL	18.0	18.0	1.5	0.3	8.1	15.0	20.0	21	68.2
	Ther.+Trop. [c]	P	13.4	14.0	1.2	0.3	8.7	11.0	15.0	19	–
	Macrostomes [c]	P	16.9	17.0	2.3	0.7	13.7	14.0	22.0	12	26.1
	Ther.+Trop.	SC	12.2	12.0	1.6	0.4	13.4	9.0	15.0	21	–
Dikinetids in kinety 3, number	Theronts [a, b]	CHL	12.0	12.0	1.3	0.3	10.9	9.0	15.0	21	–
	Trophonts [a, b]	CHL	13.7	14.0	2.0	0.4	14.6	11.0	18.0	21	14.2
	Macrostomes [b]	CHL	19.0	19.0	1.6	0.3	8.3	14.0	21.0	21	58.3
	Ther.+Trop. [c]	P	14.6	14.0	1.4	0.3	9.5	12.0	17.0	19	–
	Macrostomes [c]	P	19.5	19.0	1.5	0.4	7.4	18.0	23.0	12	33.6
	Ther.+Trop.	SC	12.7	12.0	1.7	0.4	13.1	10.0	16.0	21	–
Dikinetids in kinety 9 (or 10, if there was a total of 13), number	Theronts [a, b]	CHL	8.2	9.0	1.1	0.5	13.4	7.0	9.0	5	–
	Trophonts [a, b]	CHL	9.5	9.0	1.5	0.3	15.4	8.0	14.0	21	15.9
	Macrostomes [b]	CHL	11.6	12.0	2.1	0.5	18.4	8.0	18.0	21	41.5
	Ther.+Trop. [c]	P	11.2	12.0	1.4	0.3	12.8	9.0	13.0	19	–
	Macrostomes [c]	P	12.5	12.5	2.4	1.2	19.0	10.0	15.0	4	11.6
	Ther.+Trop.	SC	9.5	9.0	1.3	0.3	13.5	8.0	13.0	21	–
Right oral polykinetid, length	Theronts [a, b]	CHL	5.4	5.0	0.6	0.1	11.0	4.0	6.0	21	–
	Trophonts [a, b]	CHL	6.1	6.0	0.7	0.2	11.8	5.0	7.0	21	13
	Macrostomes [b]	CHL	13.2	13.0	1.6	0.4	12.2	11.0	16.0	21	44.4
	Ther.+Trop. [c]	P	6.4	6.0	0.8	0.2	13.1	5.0	8.0	19	–
	Macrostomes [c]	P	10.8	11.0	1.0	0.2	9.0	9.0	12.0	17	68.8
	Ther.+Trop.	SC	6.4	6.5	0.5	0.1	8.2	5.0	7.0	21	–
Right oral polykinetid, width	Theronts [a, b]	CHL	2.9	3.0	–	–	–	2.0	3.0	21	–
	Trophonts [a, b]	CHL	3.1	3.0	–	–	–	2.5	4.0	21	6.9
	Macrostomes [b]	CHL	3.7	4.0	0.6	0.1	16.2	3.0	5.0	21	27.6
	Ther.+Trop. [c]	P	2.3	2.0	–	–	–	2.0	3.0	19	–
	Macrostomes [c]	P	1.9	2.0	–	–	–	1.0	2.5	17	–17.4
	Ther.+Trop.	SC	3.0	3.0	–	–	–	2.5	3.5	21	–
Right oral polykinetid, number of kineties	Ther.+Trop. [c]	P	16.1	17.0	1.7	0.4	10.7	12.0	18.0	17	–
	Macrostomes [c]	P	21.9	21.0	1.9	0.5	8.7	19.0	26.0	17	36.0
	Ther.+Trop.	SC	12.8	13.0	1.8	0.4	14.0	10.0	15.0	21	–
Left oral polykinetid, length	Theronts [a, b]	CHL	7.8	8.0	0.8	0.2	10.4	6.0	9.0	21	–
	Trophonts [a, b]	CHL	8.9	9.0	1.1	0.2	12.5	7.0	11.0	21	14.1
	Macrostomes [b]	CHL	17.7	18.0	2.4	0.5	13.5	13.0	21.0	21	126.9
	Ther.+Trop. [c]	P	8.7	9.0	1.2	0.3	13.3	6.0	11.0	19	–
	Macrostomes [c]	P	15.8	16.0	1.9	0.5	12.1	10.0	20.0	17	81.6

Characteristics	State	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n	% Increase
	Ther.+Trop.	SC	10.3	10.0	1.2	0.3	11.5	8.5	13.0	21	–
Left oral polykinetid, width	Theronts [a, b, e]	CHL	2.2	2.0	–	–	–	2.0	2.5	21	–
	Trophonts [a, b, e]	CHL	2.8	3.0	–	–	–	2.0	3.5	21	27.3
	Macrostomes [b, e]	CHL	5.0	5.0	0.5	0.1	10.1	4.0	6.0	21	127.3
	Ther.+Trop. [c, e]	P	2.4	2.5	–	–	–	2.0	3.0	19	–
	Macrostomes [c, e]	P	4.9	5.0	0.6	0.1	11.3	4.0	6.0	17	104.2
	Ther.+Trop.[e]	SC	2.2	2.0	–	–	–	2.0	2.5	21	–
Left oral polykinetid, number of kineties	Ther.+Trop. [c]	P	15.0	15.0	2.2	0.5	14.5	10.0	19.0	19	–
	Macrostomes [c]	P	11.9	12.0	0.9	0.2	7.5	10.0	14.0	17	–20.7
	Ther.+Trop.	SC	16.2	16.0	0.9	0.2	5.8	14.0	18.0	21	–

[a] Theronts and trophonts are difficult to distinguish. I considered microstomous cells with a length of < 30 µm as theronts, those with > 30 µm as trophonts. Macrostomes are easily recognizable by the large oral apparatus.
[b] From same culture and slides.
[c] From a different culture than used for silver nitrate impregnation.
[d] Fixed with osmic acid (4%) and measured immediately.
[e] The widest site of the ribbon has been measured. The individual kineties are slightly longer because they are obliquely arranged.

morphs are generated by at least one transition division each and in both directions, i.e. (TH) develop to (TR) and (TR) to (MA) and *vice versa*. Thus, a lot of intermediate morphs occur in blooming cultures.

All morphs can divide and make resting cysts (Fig. 1a). Ellipsoidal, thin-walled cysts are produced by (TH) and (TR), while the (MA) make globular, thick-walled cysts. However, the latter are comparatively rare because (MA) usually divide into TH-like cells when the flagellate food is depleted. As far as I could observe, encysted cells did not excyst in the same culture dish, but air-dried cultures can be reactivated after years.

Description: For the sake of clarity, all morphs are described together. The three main morphs differ distinctly in average body size (Table 1), while individual specimens are sometimes not unequivocally assignable. Using some *in vivo* measurements and the data from Table 1, assuming 5% shrinkage in the Chatton-Lwoff silver nitrate preparations, the following *in vivo* sizes can be calculated: (TH) on average 27 × 22 µm, (TR) 38 × 30 µm, (MA) 55 × 36 µm. Thus, (TR) are larger than (TH) by about 40%, and (MA) are twice as large as (TH). However, rather different values were obtained, when I measured the size again during writing the paper. A drop of specimens from a flourishing culture was fixed with a drop of 4% osmic acid to obtain "live" sizes (Table 1): (TH) and (TR) 36.8 × 27.6 µm, (MA) 61.4 × 45.1 µm. Obviously, sizes are strongly influenced by culture and preparation conditions. Under special culture conditions, very small (~15 µm in old cultures) or very large (~80 µm in overfeed cultures) specimens may occur. Thus, the total length range of the Brazilian population is 15–80 µm!

The shape is basically very constant, but markedly different in (TH/TR) and (MA). The (TH) and (TR) are invariably *Metopus*-shaped (Fig. 1d), i.e. the anterior half has a conspicuous preoral dome, while the posterior portion gradually tapers and the rear end is rounded. This complex shape generates many forms, depending on the angles and sides viewed (Figs 1b, e–l, 2a–d, g-i, 3a, b, 4a, e, f, i–k). Although closely related to the colpodids s. str., according to the gene sequence (Fig. 8), the shapes are never *Colpoda*-like, i.e. narrowed anteriorly (for reviews, see Foissner 1993 and Foissner *et al*. 2002). The preoral dome causes a distinct groove on the ventral side and an angular indentation at left body margin, when the cell is viewed laterally. The bottom of the groove is occupied by the oral apparatus. The right and anterior body margin are distinctly curved. The ventral and dorsal view are obovate with the right side frequently more distinctly flattened than the left (Figs 1i, 2d, g, h).

The shapes of the (MA) are entirely different from those of the (TH) and (TR), but resemble those of several hausmanniellids, a colpodid family reviewed by Foissner (1993) and Foissner *et al*. (2002). Usually, the (MA) are broadly obovate, ellipsoidal, or bluntly reniform (Figs 1c, m–o, 2e, f, 3d, e, 4g, 5a–c, e–i, 6a, b, 7a, 9a–c, 12a), while overfed specimens become broadly ellipsoidal or even globular (Figs 1p–r, 9d). The left

body margin is flat or slightly concave, while the right, the anterior, and the posterior margin are distinctly convex. The right side is flattened, the left more or less convex and narrowed posteriorly; thus, ventral and dorsal views are obovate or almost cuneate (Fig. 5g, j).

The nuclear apparatus is in the posterior body half, but may be displaced anteriorly or to mid-body and dorsal side in overfed (MA). *In vivo*, it is difficult to recognize, both in (TH/TR) and in (MA), because it is comparatively hyaline and/or often covered by food vacuoles (Figs 1b, c, 2b, 3b, f, g, 4a, c, h; Table 1). The macronucleus is globular to ellipsoidal and distinctly enlarged in the (MA). In most specimens, the macronucleus contains a globular, lobate, or ribbon-like, granular nucleolus, which is more refractive than the very hyaline nucleoplasm and thus usually that part of the nucleus which is recognizable *in vivo* (Fig. 4a, c). The micronucleus is attached to the macronucleus and about 4 µm across and 2 µm thick *in vivo*, i.e. it is discoidal and more compact than the macronucleus; in silver preparations, it is more or less inflated or shrunken, depending on the method (Table 1).

The contractile vacuole is in the rear body end with the excretory pore in or near the pole centre (Figs 1b, c, 2b, d, f, h). Usually, it is surrounded by crystals (see below). The cytoproct is dorsal of the excretory pore and appears as an oblong, granular area in silver nitrate preparations (Fig. 2h). The fecal mass, which is globular and slimy, contains bacterial spores and crystal-like inclusions (Fig. 1b).

The cortex is of ordinary structure and more or less distinctly furrowed by the ciliary rows, especially in the preoral dome, where six to seven distinct indentations are recognizable in all morphs (Figs 1b, c, 2c, e, f, 3a–e, 4a, b, i, j, 5a, b, h, i). The cortex contains numerous extrusomes, possibly mucocysts; however, they do not stain and are not extruded when methyl green-pyronin is applied. Most extrusomes are within the ciliary rows, but some are scattered, especially in the posterior third of the body. The extrusomes are hardly recognizable *in vivo* because they are pale and only about 1 × 0.5 µm in size. However, they have a strong affinity to protargol and thus appear as brownish to blackish globules 1–2 µm across in the protargol slides, more or less hidding the ciliary pattern (Figs 2i, 4k). In silver nitrate preparations (Fig. 2b, d, f, h), they may appear either as minute rings (when attached) or as granules (when just extruded; Foissner 1977).

The cytoplasm is colourless and the inclusions are the same in all morphs, while their amount differs: (i) few (TH, TR) to many (MA) lipid droplets 0.5–5 µm across are scattered through the cell (Figs 1b, c, 5d); (ii) 10–20 crystals (?) surround the contractile vacuole, individual crystals ellipsoidal to indistinctly polygonal, 1–3 µm in size, moderately refractive in transmitted light, orange to orange-red under interference contrast (Figs 1b, c, 4a, c, d); (iii) few (TH, TR) to many (MA) food vacuoles 2–6 µm across in (TH) and (TR) and 8–30 µm in the (MA). The small food vacuoles of the (TH) and (TR) contain few bacteria, often only one or two (Figs 1b, 4a–c); rarely bacteria-sized starch grains or minute pieces of larger starch grains are ingested. The (MA) may have ingested up to 50 flagellates, forming globular and polymorphic food vacuoles because the flagellate cell wall is rather inflexible not rounding up when the organism dies (Figs 1c, 5a, d, 9d).

Bromeliothrix metopoides swims continuously, very rarely creeping on mud accumulations or on the bottom of the culture dish; most resting cysts produced during the growth phase are attached to the surface of the culture medium. In the field samples and in larger laboratory flasks, specimens are found in both, the "pelagial" and the loose bottom mud, where they swim between the particles. The (TH) and (TR) swim rapidly, whirling up and down and rotating about the main body axis. The (MA), which do not whirl, swim moderately rapid, rotating about the main body axis.

The somatic ciliary pattern is similar to that of small species of the genus *Colpoda* (for a review, see Foissner 1993), but with some specializations, possibly caused by the unique body shape, viz., fan-like spread kineties on the right side and a distinct ciliary tuft on the preoral dome. The arrangement and number of the ciliary rows is highly similar in all morphs, while the number of kinetids is higher in the (MA) by about 50% (Table 1). Kinety numbering is according to Foissner (1993): kinety 1 is the first right of the oral apparatus and the last is the rightmost postoral kinety (Figs 3c, h, 4h, 5b). There are 12–15, usually 12–13 ciliary rows (Figs 1b, c, 2a, b, e, f, 3a, b, d, e, 4e–j, 5b, c, e–i, 6a–d; Table 1): three on the right side, six to eight on the left, and three to four extend postorally. The anterior half of kinety 1 is close to the right mouth margin. Kineties 2 and 3 are spread fan-like anteriorly, producing two rather large, barren areas in the right anterior quadrant of the cell (Figs 2a, d, e, g, 3a, c–g, 4e, g, h, i, 5b, e, f, 6a, b, 7a). Kineties 4 to 9 commence on the preoral dome and curve posteriorly on the left side of the cell. They are very densely ciliated and close together in the dome area, producing a conspicuous ciliary tuft and a distinct

preoral suture with the three right side kineties. The postoral kineties are inconspicuous and without peculiarities; however, there is a conspicuous, cuneate cleft between the last left side kinety and the first postoral kinety, but a diagonal groove is absent, as in the small *Colpoda* species (Figs 2b, c, 3h, 4f, h, 5c, g). Kineties 2 to 12 are slightly or rather distinctly shortened posteriorly, where the anterior cilium of the dikinetids is lacking. Thus, the posterior quarter of the cell is very sparsely ciliated, especially in (TH) and (TR).

The somatic kinetids and their fibrillar associates are colpodid, i.e. the ciliature consists of dikinetids forming sigmoidal ciliary rows (Figs 1b, c, 3a–h, 4i, j, 5h–j, 6c, d, 9a, b). The cilia are about 8 µm long, and elongated caudal cilia are absent. The dikinetids are obliquely arranged, especially in the anterior third of kineties 1 and 2 and in the dome portion of the left side kineties; those in the anterior third of kineties 2 and 3 and those of the left side rows are associated with long fibres, forming conspicuous bundles (Fig. 3a, b); very likely, these bundles represent transverse microtubule ribbons originating from the posterior basal body of the dikinetids, forming the so-called LKm-fibres typical for the class Colpodea (Foissner 1993, Lynn 2008). Further, there are two short fibres, spread V-like, on both the right and left side of the dikinetids, as in other colpodids (Foissner 1993, 1994; Foissner and Kreutz 1998).

The oral apparatus is in the middle ventral third of the cell and inconspicuous in (TH) and (TR), while strikingly large in the (MA). The basic organization is the same in all morphs and as that known from genera of the families Colpodidae and Hausmanniellidae (for reviews, see Foissner 1993, Foissner *et al*. 2002). None the less, both morphs will be described separately because of considerable differences in details.

In the (TH) and (TR), the oral apparatus extends in the transverse furrow produced by the forehead (Figs 1b, 2a, c, 3a, f–i, 4a–e, h, i). Thus, it is difficult to observe *in vivo* and in preparations. The oral cavity is a small funnel whose right wall is occupied by the right polykinetid. When observed from the right side, the polykinetid appears as a narrow stripe of disordered kinetids (Figs 3a, 4a–c). However, the real shape and organization is best studied in squashed silver carbonate preparations which show that it is crescentic and consists of an average of 16 short, only slightly disordered kineties, each having a dikinetid proximally (Figs 3f, g, i, 4d, h, i). The left oral polykinetid is outside the oral funnel, except of its right end. It is an elongate rectangular to slightly sigmoidal, about 2.5 µm wide ribbon composed of an average of 15 equidistantly, narrowly spaced and slightly obliquely arranged kineties. The cilia are about 2 µm long at the proximal end of the ribbon, gradually increasing to about 7 µm at the distal end, forming a distinct beard at left body margin (Figs 1b, 4a, i), as in *Colpoda steinii* (Foissner 1993) and *Exocolpoda augustini* (Foissner *et al.* 2002). The pharyngeal fibres are distinct in protargol preparations (Fig. 3a). They form a long and thus conspicuous funnel surprisingly extending anteriorly, as in the marynid colpodids, i.e. not posteriorly as in species of the families Colpodidae and Hausmanniellidae (Foissner 1993, Foissner *et al*. 2002).

In the (MA), body shape is changed so strongly (see shape description above) that the oral apparatus, which is one to two times larger than in the (TH), becomes displaced onto the right side, extending to or even over body's midline (Figs 1c, 2e, f, 3d, e, 4g, 5a, b, d–f, h, i, 6a, b, 7a, 9a, b; Table 1). The oral cavity is a large, triangular area in the central third of the cell and deepens gradually from left to right. The cytostome, which is recognizable only in silver nitrate preparations, is not within a funnel, as in the (TH) and (TR), but outside, forming a strongly concave, thick silverline traversing the oral field (Figs 2e, 5b, e, f, 6a, b, 7a, c, 9b). The right oral polykinetid is as in (TH) and (TR), but the shape changed from crescentic to curved-obcuneate and the number of kineties increased from an average of 16 in the former to 21 in the latter (Figs 3j, 6c, g; Table 1). The polykinetid commences directly at the slightly thickened right mouth margin because vestibular kineties are absent (Fig. 6h, i). Thus, the cilia are well recognizable, showing another peculiarity: their length decreases from about 5 µm anteriorly to 2 µm posteriorly (Figs 1c, 6h, i, 9a, 12a). The left polykinetid forms a conspicuous ribbon traversing the cell in mid-body. The ribbon is slightly concave and distinctly cuneate, i.e. its width increases from about 2 µm proximally to an average of 5 µm distally, where the last kinety is usually shortened to three to six cilia (Figs 1c, 2e, 3c–e, j, 5d–f, h, 6a–c, e, g, i, 7a, c, 9a, b; Table 1). The kineties are widely spaced and their number decreases from an average of 15 in the (TH/TR) to 12 in the (MA). The longest kineties are composed of about 15 cilia, whose lengths decrease from about 8 µm distally to about 2 µm proximally. Further, the length of the cilia gradually decreases within the kineties of the proximal half of the polykinetid from about 6 µm posteriorly to 2–3 µm anteriorly (Figs 5h, 6i, 9a, 12a, d). Long fibres originate from the distal kineties and extend anteriorly

(Fig. 3d, e). Scanning electron micrographs show that the kineties are composed of a single row of cilia (Figs 6i, 7c), while silver preparations occasionally show the kineties to be composed of two granule rows (Fig. 6f, g). A detailed analysis showed that the second granule row, which is often lighter impregnated, occurs only when the kinety is viewed laterally exposing the nodes of the complex fibre system underneath the polykinetid. The nodes then look like basal bodies.

Resting cysts: As mentioned in the ecology section, *B. metopoides* produces resting cysts easily, both in growing and in old cultures. The cysts are made on the surface of the culture medium as well as on the bottom of the culture dishes and on mud accumulations. As cysts are formed also in young, growing cultures, albeit sparsely, food and medium depletion cannot be the sole encystment stimuli, but certainly they are main factors. The resting cysts are comparatively stable because *B. metopoides* can be cultivated from the dry mud found in the tanks after the dry seasons and from laboratory pure cultures air-dried for some years.

Bromeliothrix metopoides produces two kinds of resting cysts, viz., ellipsoidal, thin-walled cysts and, preferably in old cultures, globular, thick-walled ones. Although some transitions occur, the two cyst types are highly distinct because the thick-walled cysts are not only globular but also have three distinct wall layers; further, they become rather deeply brownish after some months. The origin of the two types is not entirely clear, but the following experiment and the cyst volume indicate that the ellipsoidal cysts are from microstomes (2,113 μm^3), while the globular cysts are from macrostomes (4,002 μm^3). The experiment ran as follows: about 30 (MA) were isolated and transferred to flagellate-free medium. After 12 h, about half of the (MA) have divided and produced about 60 (TH), which soon made ellipsoidal and rather hyaline cysts. The other half of the cells did not divide but formed refractive (due to many lipid droplets), globular cysts within three days. The following description is confined to features recognizable in the light microscope because a paper on cytochemistry and fine structure (TEM) is in preparation.

The ellipsoidal cysts have an average size of 21 × 14 µm (length: 21.2, M 21.0, SD 2.0, SE 0.2, CV 9.5, Min 18, Max 26, n 24; width: 13.8, M 14.0, SD 1.6, SE 0.3, CV 11.4, Min 12, Max 17, n 24). As with body size (see above), slightly higher values were obtained when I measured the cysts again in January 2010: length (22.5, M 22, SD 3.1, SE 0.9, CV 9.9, Min 18, Max 26, n 11), width (16.3, M 16, SD 1.2, SE 0.4, CV 7.3, Min 14, Max 18, n 11). Further, the cysts were less distinctly ellipsoidal (length: wide ratio 1.38 vs. 1.54). The wall is 1–1.5 µm thick and consists of two structureless, compact, smooth layers (Figs 2j, 7f, g): internal layer slightly thinner than external one, colourless; external layer colourless to yellowish, with thin slime cover in old cysts, while young cysts have an up to 10 µm thick, rather compact slime cover staining intensely with alcian blue (Fig. 7d, e). Obviously, this layer has usually decomposed in cysts older than a month. The cyst content is composed of the following structures: nuclear apparatus with central nucleolus of macronucleus as distinct as in morphostatic cells; few to many lipid droplets up to 2 µm across; many pale inclusions, possibly mitochondria, about 3 × 2 µm in size; some empty (?) vacuoles up to 3 µm across; and minute (≤ 1 µm) but highly refractive (lipid ?) granules covering the macronucleus (Figs 2j, 7f). The basal bodies and silverlines are maintained, at least in young cysts, while the oral apparatus is resorbed (Figs 2m, n, 7h).

The globular cysts are on average 20 µm across (19.7, M 20.0, SD 1.4, SE 0.2, CV 6.9, Min 17, Max 23, n 47). The wall is 2–3 µm thick and composed of three distinct layers (Figs 2k, l, 7i–k): internal layer as in the ellipsoidal cysts, i.e. bipartite, about 1–2 µm thick, opaque, structureless, colourless, and smooth; external layer 1–2 µm thick, compact, may be separated from the internal layer by an up to 5 µm wide space, light brown to orange, smooth in young cysts, more or less wrinkled in old ones, without distinct slime cover but distal margin slightly thickened. The cyst content is dominated by lipid droplets up to 2 µm across and the macronucleus covered with highly refractive (lipid ?) granules ≤ 1 µm in size (Fig. 7i–k).

Occurrence and ecology: *Bromeliothrix metopoides* is possibly the most widespread and common bromeliad-specific ciliate. I found it in Mexico, Central America (Costa Rica), the Dominican Republic, Jamaica, Ecuador, Venezuela, Peru, Brazil, and Chile. Further, *B. metopoides* is the sole bromeliad-specific ciliate, I found several times in bromeliads from Botanical Gardens, hotels, and flower shops, for instance, in the Botanical (Kew) Gardens of London and Kuala Lumpor (Malaysia). All these populations were highly similar morphologically and ontogenetically, producing macrostomes and division chains with four offspring.

In the laboratory, *B. metopoides* is a real weed easily infecting other cultures and growing on various media, such as tap water or Eau de Volvic enriched with crushed wheat kernels or parts of a mealworm; in bac-

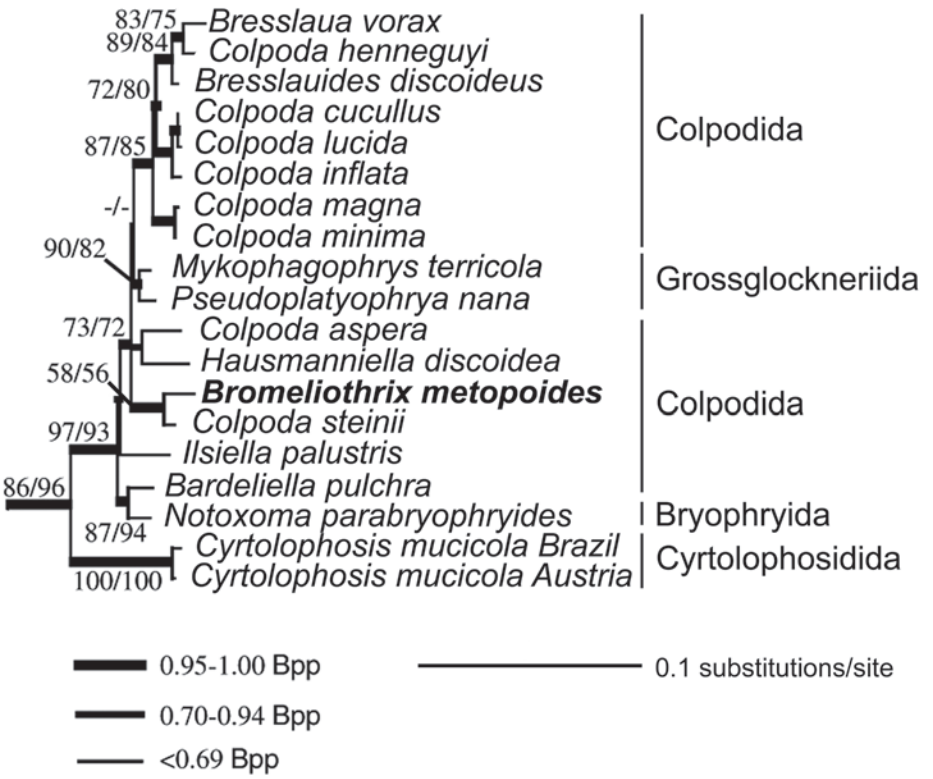


Fig. 8. Position of *B. metopoides* nov. gen., nov. spec. in the phylogenetic tree of the Colpodea, based on SSU rDNA gene sequences (from Dunthorn *et al*. 2008). The most likely Bayesian tree is shown. Bayesian posterior probability support is shown by differences in thickness of branches. Numerical bootstrap values are shown next to the branches as: Maximum parsimony (MP)/Maximum likelihood (ML). Values < 50% are shown as –/–.

terized yeast extract; in diluted salad and soil medium either alone or together with a huge variety of bacterivorous and predaceous ciliates and micrometazoans, such as *Blepharisma* spp., *Tetrahymena* spp., *Paramecium* spp., *Vorticella* spp., *Leptopharynx* spp., *Bursaria truncatella*, *Sterkiella* sp. (a still undescribed, predacous hypotrich from bromeliads), *Coniculostomum monilata*, *Condylostomides* sp., gastrotrichs, and rotifers. It also grows in soil, i.e. when added to non-flooded Petri dish cultures (Foissner *et al*. 2002) with litter and soil from northern coniferous forests and tropical rain forests. Actually, *B. metopoides* grows in all media containing bacteria and medium-sized flagellates, while small (*Colpoda steinii*, *Cyrtolophosis mucicola*) and middle-sized (*Colpoda maupasi*) ciliates are not ingested; likewise, cannibalism has never been observed. Occasional investigations of the food vacuoles of field specimens and of infected cultures of a variety of other ciliates suggest that *B. metopoides* feeds only on bacteria and middle-sized flagellates. The (MA) possibly needs a rather specific flagellate diet (*Polytomella*) because my recent experiments showed that another middle-sized, heterotrophic flagellate, *Chilomonas paramaecium* (?), is not ingested, by either the (TH/TR) or the (MA). On the contrary, (MA) developed when a small (15–20 µm) and a large (40 µm) *Polytomella* species were provided as food.

Detailed studies on the ecology of *B. metopoides* are in progress. The life cycle and the faunistic observations suggest that *B. metopoides* is an *r*-selected ciliate well adapted to the highly competitive bromeliad habitat: it grows in a great variety of media and habitats (see above), reproduces rapidly by polytomy (see ontogenesis), can switch from a bacteriophagous microstome morph to a predaceous macrostome morph, and easily produces resting cysts.

Phylogenetic analysis (Fig. 8)

The small-subunit rDNA sequence places *B. metopoides* in the family Colpodidae, regardless of the

number of species used for tree construction (Foissner *et al*. 2003, Dunthorn *et al*. 2008): it forms a poorly supported clade with two small colpodas, viz., *C. steinii* and *C. aspera*. This clade is sister to a clade, also poorly supported, containing middle-sized and large colpodas, such as *C. cucullus* and *C. magna*, as well as the mycophagous colpodids *Pseudoplatyophrya* and *Mycophagophrys* (Fig. 8).

Ontogenesis

Overview: The division of *Bromeliothrix* is homothetogenic and occurs in active (non-encysted) condition. The stomatogenesis is merotelokinetal, and the parental somatic and oral infraciliature is completely reorganized (Figs 1a, 12a–i, 15a–c, 17a, f). *Bromeliothrix* has a homomeric macronucleus which divides, like the micronucleus and the cell proper, in the ordinary way (Figs 9e–i, k, l, 10g–j, 13b, d, e, g, 16a–c, e–h). Thus, these processes will not be described in detail, but the reader is referred to textbooks and the monographs of Foissner (1993, 1996).

The ontogenesis of *Bromeliothrix* shows three major steps, which, however, partially overlap: reorganization of the parental somatic and oral infraciliature; division s. str., i.e. the production of daughter cells; and post-divisional shaping of body and ciliary pattern. Under good conditions, the whole process takes about 5 hours.

While predivisional reorganization is similar to that of other colpodids, ontogenesis is more complex due to the formation of three distinct morphs (Figs 1a–c, 4a, i, 5a, h): theronts, trophonts, and macrostomes. These morphs are generated by at least one transition division each and in both directions, i.e. theronts develop to trophonts and trophonts to macrostomes and *vice versa*. Thus, a lot of intermediate morphs occur whose distinction is sometimes difficult (Figs 1a, 3c, 18a, c–e).

Basically, *B. metopoides* can divide ordinarily, i.e. by binary fission producing two daughters (Figs 1a, 10g–j, 13a–i) or make a division chain by multiple (polytomic) division, producing four offspring that are connected by a special holdfast (Figs 1a, 16a–h, 17a–g). Both, (TR) and (MA) have this ability, but I could not find out the reasons that cause binary or multiple fission. Possibly, binary fission occurs mainly in the transition stages, e.g. when a (TH) develops to a (TR) and a (TR) to a (MA).

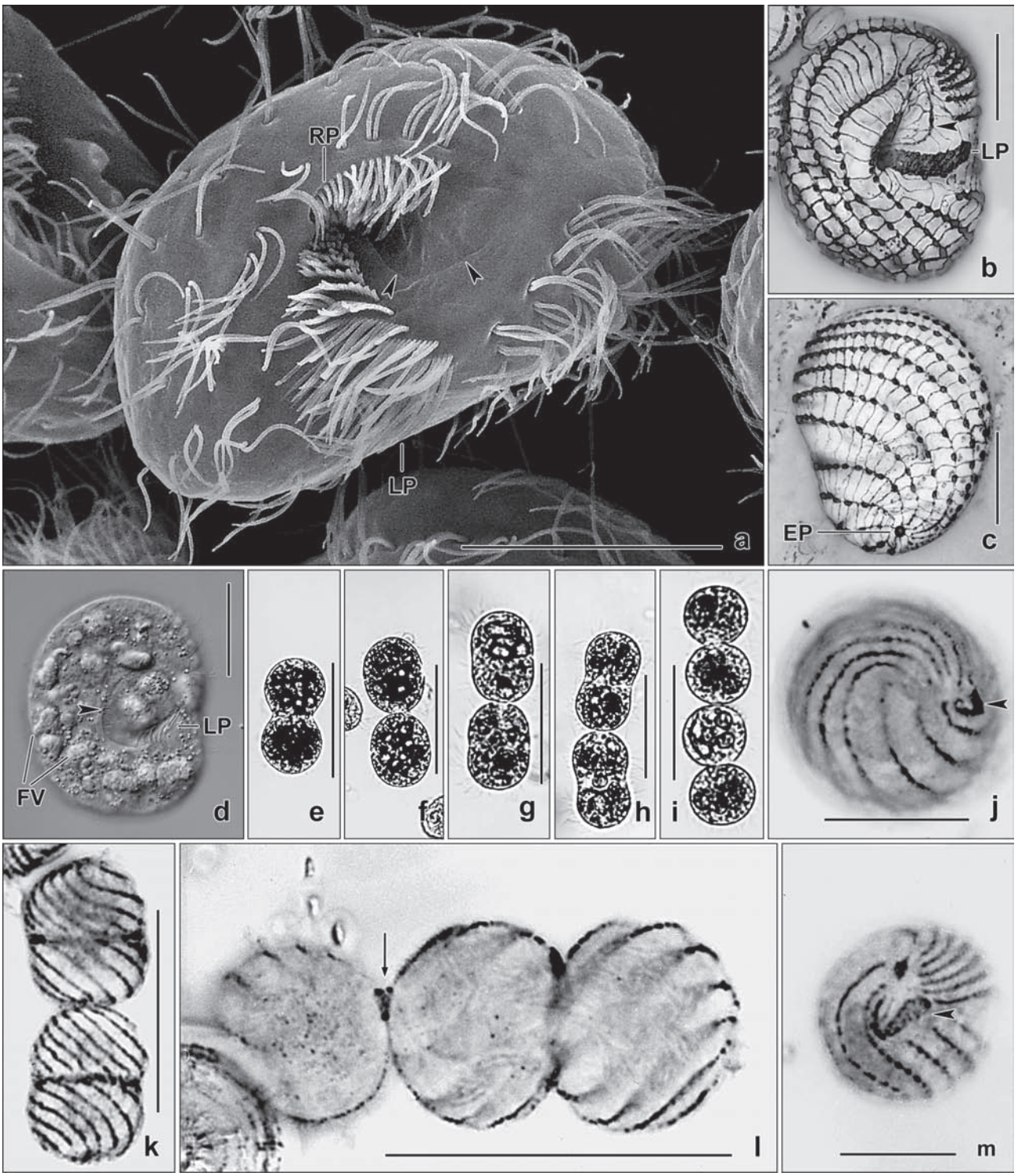
A further curiosity of all reorganizers and dividers is their ability to swim like morphostatic cells, although their shape changed completely, and a view in a well-growing culture is unforgettable due to the three morphs living side by side, especially the swimming division chains (Figs 1a, e–r, 9d–i, 17a). Considering that middle and late dividers have resorbed about two thirds of the parental cilia and the new cilia are still too short to be of use (Figs 15a–c, 17a, f, 18a, c), the seemingly unaffected movement is a challenge to motility researchers.

This overview shows that the ontogenesis of *B. metopoides* is rather complex, requiring detailed description and illustration, especially because *Bromeliothrix* offers the almost unique possibility to observe the process in the scanning electron microscope; most other genera and species of the order Colpodida divide in a membranous cyst, making SEM impossible (Foissner 1993, Foissner *et al*. 2002).

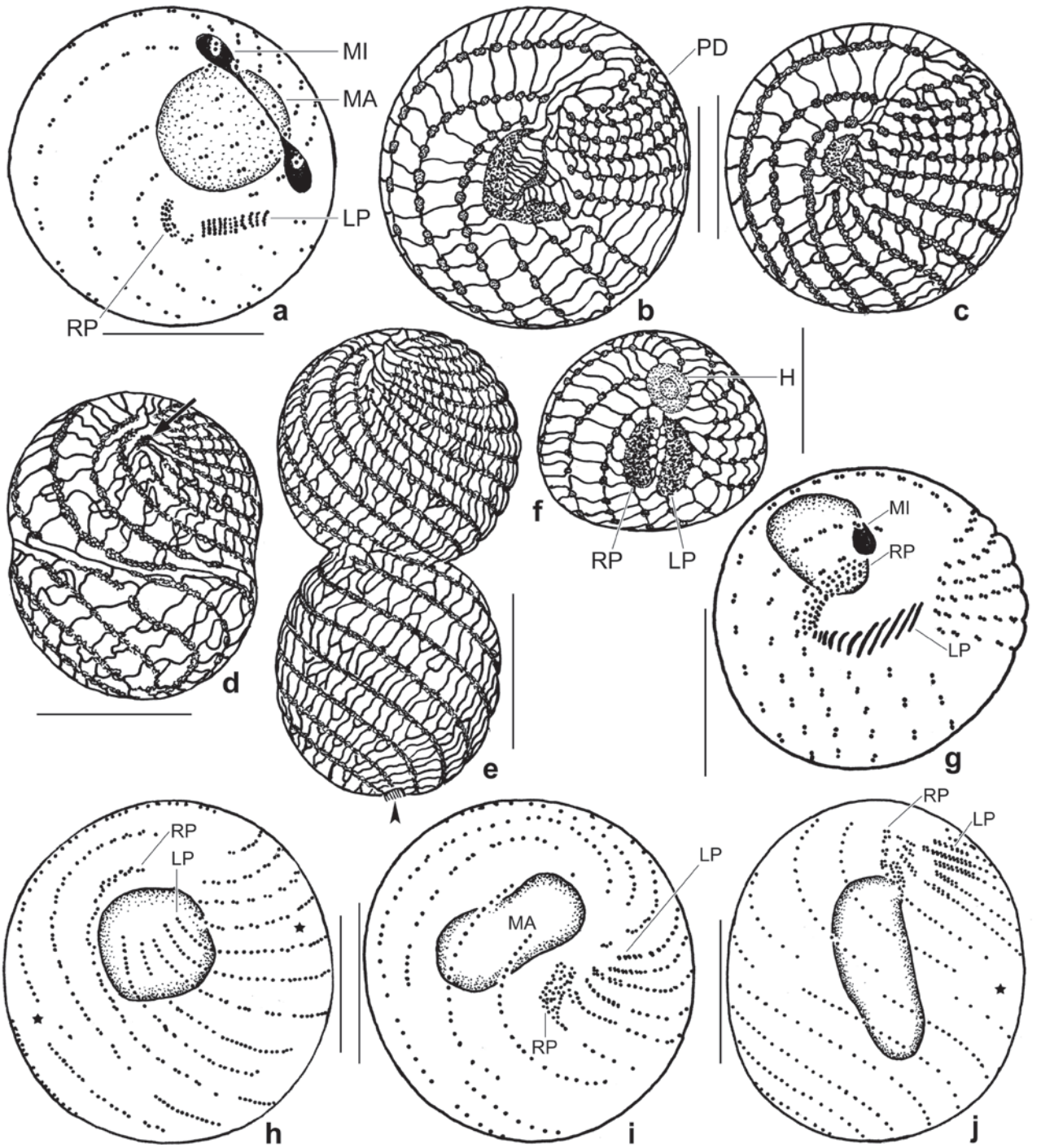
Predivisional reorganization (Figs 9j, m, 10a–c, g–j, 11a–e, 12b, c, e–i): At first glance, early reorganizers are difficult to distinguish from very late dividers and early post-dividers because both are globular and have a similar ciliary pattern. Fortunately, there is a reliable marker, namely, the oral polykinetids, which are arranged right-angled in the former (Figs 9m, 10a, b, g, 11a, 12b, e), while in parallel in the latter (Figs 13f, h, i, 17e, f, 18a–c).

The reorganization commences by rounding up the body, which becomes a perfect sphere with the oral apparatus in mid-body. This causes a strong spiralization of the somatic ciliary rows (Figs 9j, m, 10a–c, g, 11a, 12b). When the cell has rounded up, the oral area flattens and the resorption of the oral polykinetids commences. The cilia of both polykinetids decrease in length, those of the left one become clavate, while those of the right one keep their shape and become gradually shorter from distal to proximal, the last cilia resorbed being those associated with the row of dikinetids at the proximal margin of this ciliary field. In the left polykinetid, cilia resorption commences along the margins, i.e. the central cilia of the rows disappear last (Fig. 12a–f).

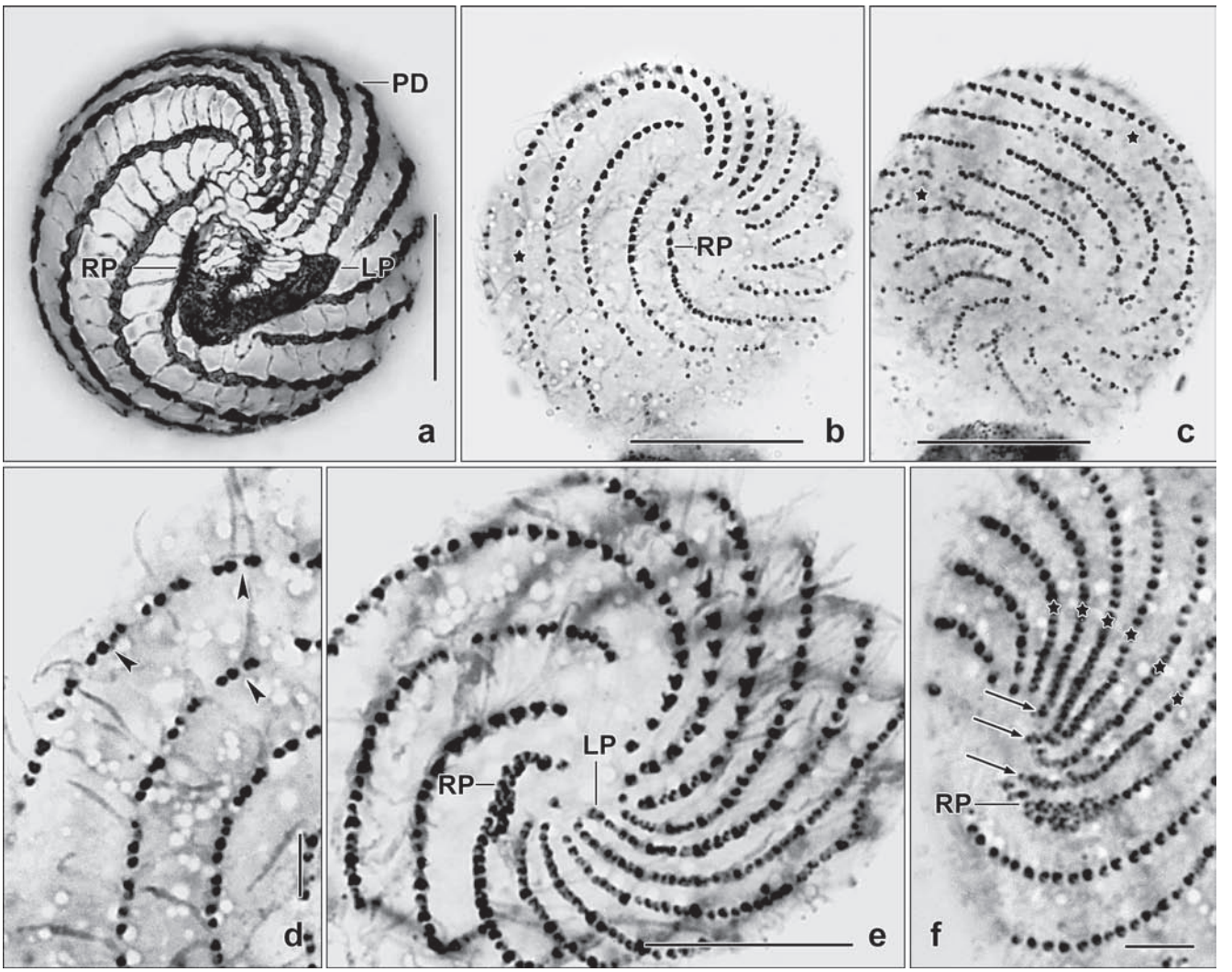
The resorption of the somatic ciliature commences when most or all of the oral apparatus has disappeared. First, the anterior cilium of the pairs is resorbed, while the posterior remains for a long time, as explained before. Thus, late reorganizers and dividers are monokinetal (Figs 12h, i, 15a). Parental cilia even remain within the oral anlagen, becoming conspicuously clavate when resorbed in middle and late dividers (Fig. 15g, h). Concomitantly with cilia resorption, an intense production of basal bodies commences and a very narrowly-meshed, polygonal silverline pattern appears within the kineties (Figs 10c, h, 11b–d, 12h, i). The further pro-



Figs 9a–m. *Bromeliothrix metopoides*, Brazilian (a, d–m) and Mexican (b, c) specimens from life (d–i), after silver nitrate impregnation (b, c, j–m), and in the SEM (a). **a** – right side overview of a young macrostome; arrowheads mark cytostome (cp. b); **b**, **c** – right and left side view of ciliary and silverline pattern of Mexican macrostomes, which are very similar to the Brazilian ones; arrowhead in (b) marks the cytostome; **d–m** – when mature (d), the macrostome rounds up and resorbs the oral apparatus (j, m; arrowheads). After the first division (e), each daughter divides again (f, g, k), finally producing a division chain with four globular offspring (h, i), of which the central ones are connected by a special holdfast impregnating with silver nitrate (l; arrow). The arrowhead in (d) marks the right mouth margin. EP – excretory pore, FV – food vacuoles, LP – left polykinetid, RP – right polykinetid. Scale bars: 20 µm (a–d, j, m) and 40 µm (e–i, k, l).

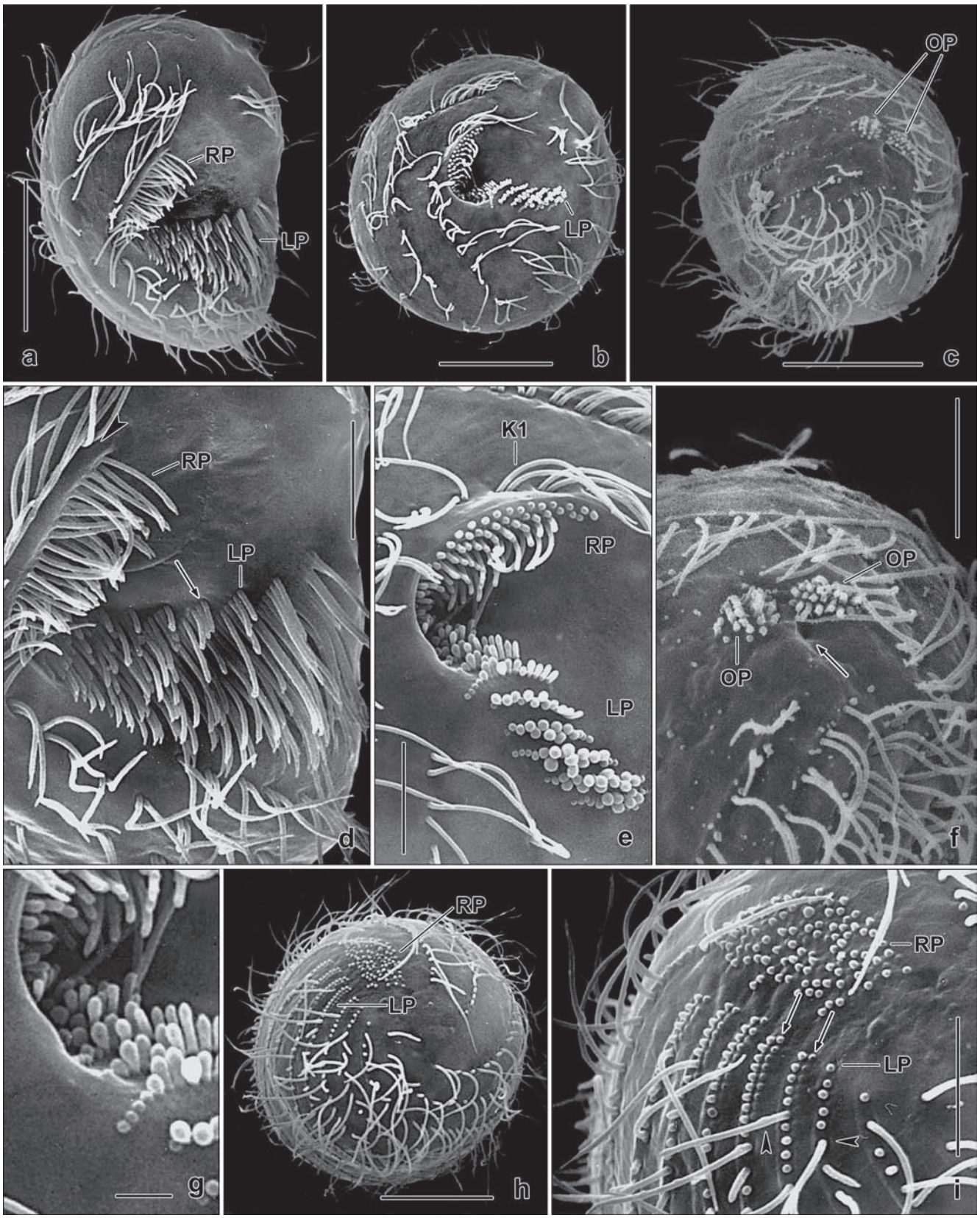


Figs 10a–j. *Bromeliothrix metopoides*, ciliary and silverline pattern of predivisional reorganizers and dividers after protargol (a, g–j) and silver nitrate (b–f) impregnation. **a**, **b** – trophont and macrostome reorganizers. The cells round up, the ciliary rows become distinctly sigmoidal, and the oral apparatus commences resorption; **c** – late macrostome reorganizer. The oral apparatus almost disappeared and the ciliary rows show a narrow-meshed silverline pattern; **d** – early macrostome divider, showing reorganization of the silverline pattern. The oral apparatus disappeared (arrow); **e** – anterior pair of a division chain, as shown by the holdfast (arrowhead). The ciliary rows are still in reorganization, while new oral structures are lacking; **f** – middle cell of a trophont division chain, showing the holdfast and the reorganized ciliary and silverline pattern; **g–j** – beginning of an ordinary macrostome division, producing two daughters. This series is continued in Fig. 13a–i. The asterisks mark the prospective division furrow. The cell rounds up (g) and resorbs (h) and rebuilds (i, j) the oral structures from the anterior end of the postoral and some left side kineties. H – holdfast, LP – left polykinetid, MA – macronucleus, MI – micronucleus, PD – preoral dome, RP – right polykinetid. Scale bars: 10 μm (f) and 15 μm (a–e, g–j).



Figs 11a–f. *Bromeliothrix metopoides*, predivisional reorganization of the oral and somatic ciliary pattern of macrostomes after silver nitrate (a) and silver carbonate (b–f) impregnation. **a** – the cell rounds up, causing a strong spiralization of the ciliary rows. The oral cavity flattens, exposing the polykinetids. **b**, **c**, **d** – the oral structures have been resorbed and the cells begin to divide (asterisks, marking the prospective division furrow), concomitantly with an intense proliferation of basal bodies, forming typical triplets and quadruplets (d). The rightmost postoral kinety commences proliferation of basal bodies for the right oral polykinetid; **e** – new oral ciliary fields originate at the anterior end of the rightmost postoral kinety (right polykinetid) and the left postoral and some left side kineties (left polykinetid); **f** – very early stomatogenesis, showing that the anterior portion (arrows) of the anlagen (asterisks) for the left oral polykinetid possibly migrates to the anlage for the right polykinetid. Scale bars: 10 μm (d, f) and 20 μm (a–c, e).

Figs 12a–i. *Bromeliothrix metopoides*, a morphostatic (a, d) and some reorganizing (b, c, e–i) macrostomes in the scanning electron microscope. **a**, **d** – ventral view of a macrostome. The arrow marks shortened cilia in the anterior half of the kineties comprising the left oral polykinetid. The cilia of the right polykinetid decrease in length from anterior to posterior; **b**, **c**, **e–g** – when division begins, the cell rounds up (b, c) and resorbs the oral structures (e, f). The arrow in (f) marks the minute cytostomial opening. The resorbing cilia of the left polykinetid are distinctly clavate (e, g). The cilia of the dikinetidal row of the right polykinetid are resorbed last. **h**, **i** – a late reorganizer, showing that the cell became monokinetidal, i.e. the anterior cilia of the somatic pairs have been resorbed, while the posterior cilia (arrowheads) keep the cell motile. New oral structures arise at the anterior end of the postoral and some left side kineties. The individual kineties of the left polykinetid originate gradually from right to left (arrows). The right oral polykinetid is a large anarchic field of basal bodies, which later becomes more regularly arranged. K1 – somatic kinety1, LP – left oral polykinetid, OP – remnants of oral polykinetids, PD – preoral dome, RP – right oral polykinetid. Scale bars: 1 μm (g), 5 μm (d–f, i), 10 μm (b, c) and 15 μm (a, h).


RP
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a
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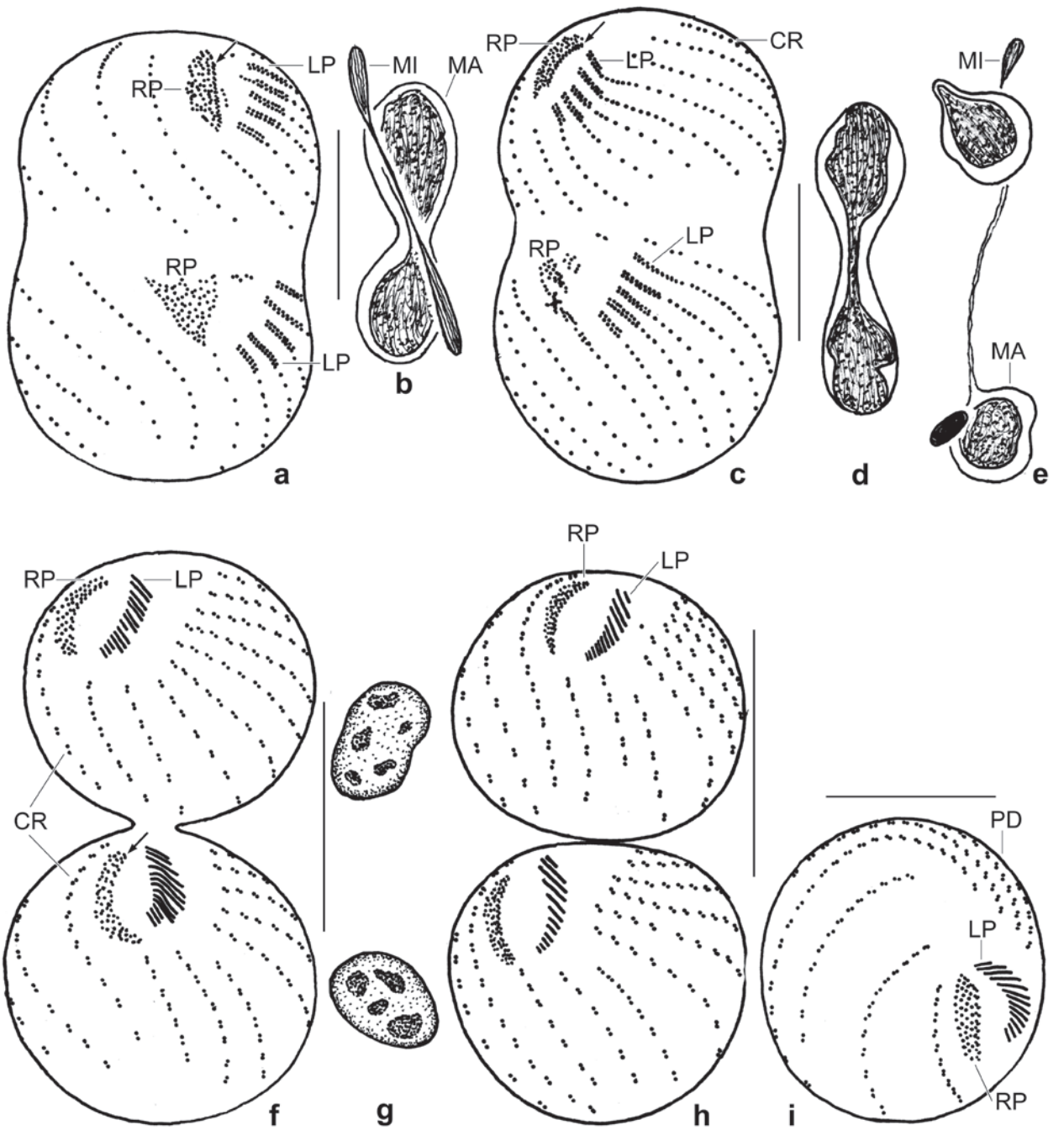
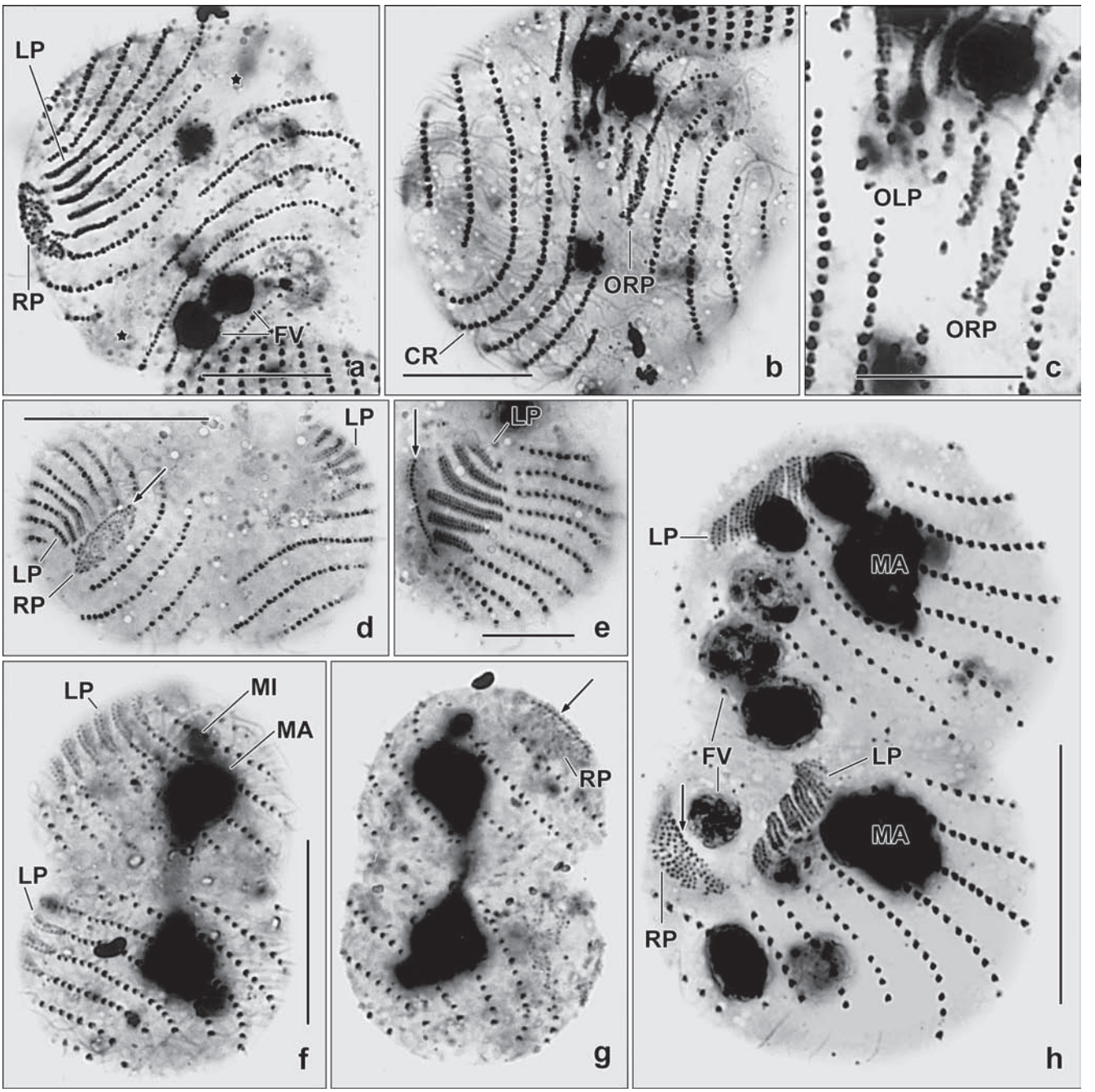
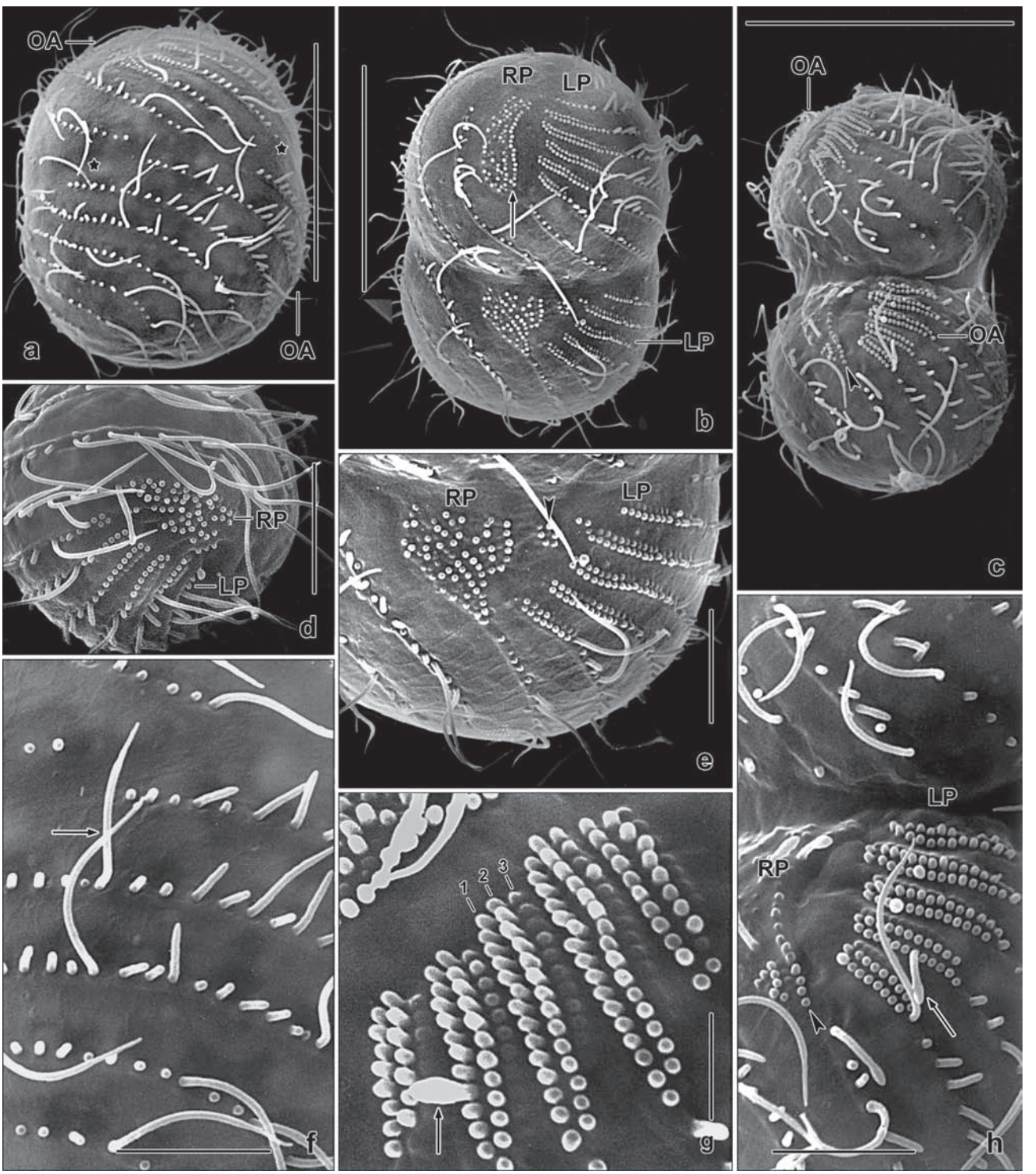


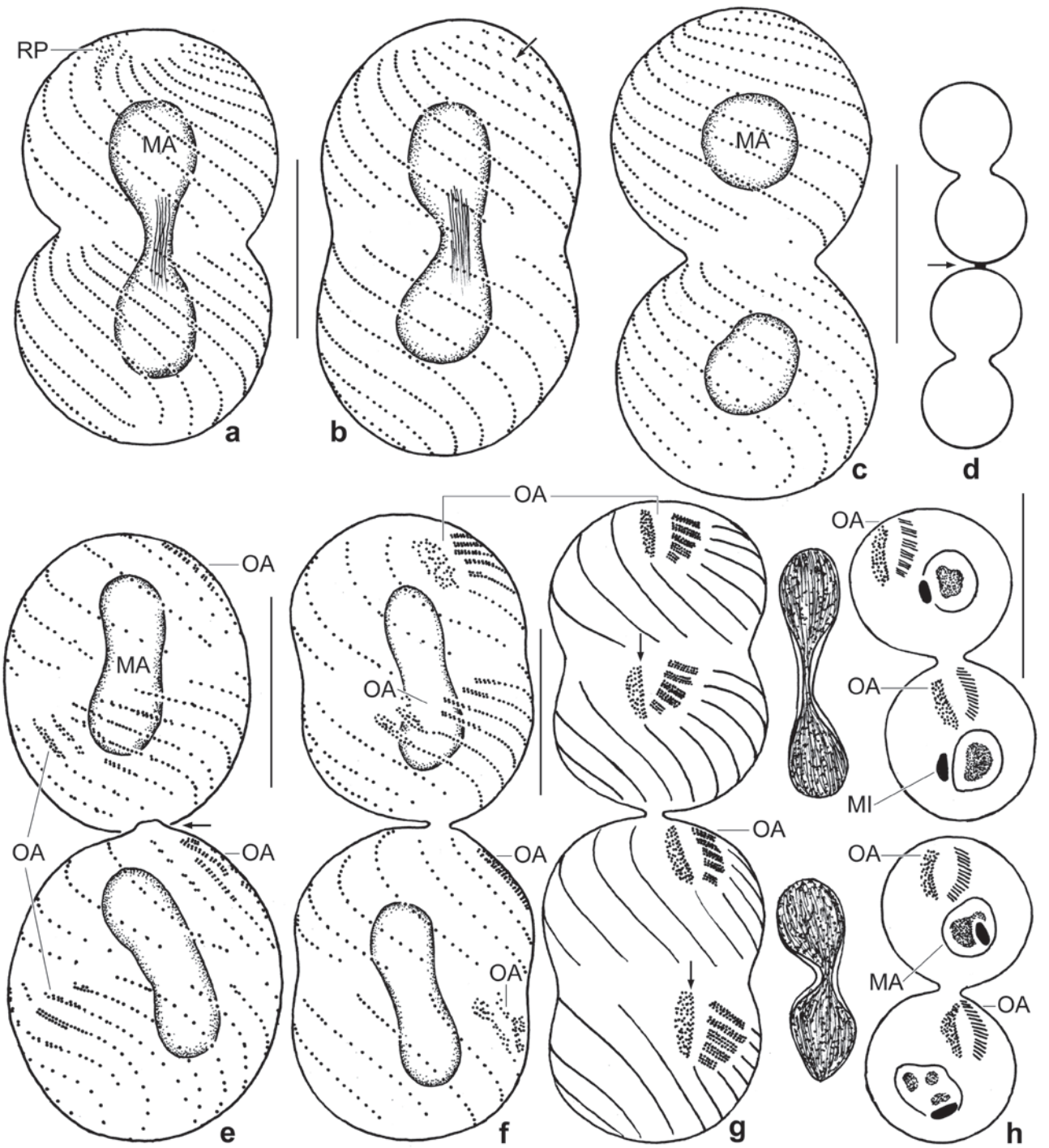
Figs 13a–i. *Bromeliothrix metopoides*, protargol preparations of middle and very late macrostome dividers producing two daughters (for the earlier stages, see Fig. 10g–j). **a–d** – ventral views of middle dividers, showing the origin of the new oral ciliary fields from the rightmost post-oral kinety (right polykinetid) and the left postoral and some left side kineties (left polykinetid), still connected with the anlagen. The anlage of the right polykinetid consists of an anarchic field of basal bodies and a row of dikinetids proximally, which assembles much later in the opisthe (f, h). The anlagen of the left polykinetid consist of well-ordered rows of basal bodies. The somatic ciliature still consists of narrowly spaced monokinetids. The macronucleus and the micronucleus divide in the usual way (b, d); **e**, **f** – a very late divider with fully separated micronuclei, while the macronuclei are still connected by a membranous strand (e). The somatic basal bodies assembled into dikinetids. The anlagen of the left oral polykinetid become three-rowed and commence length differentiation; and a row of dikinetids develops at the proximal margin of the right oral polykinetid of the opisthe (arrow); **g**, **h** – the daughters just separated. The left oral polykinetid assumes the cuneate shape typical for macrostomes; **i** – a macrostome post-divider with the new oral polykinetids arranged in parallel. CR – ciliary rows, LP – left polykinetid, MA – macronucleus, MI – micronucleus, PD – preoral dome, RP – right polykinetid. Scale bars: 15 µm (a, c, i) and 20 µm (f, h).



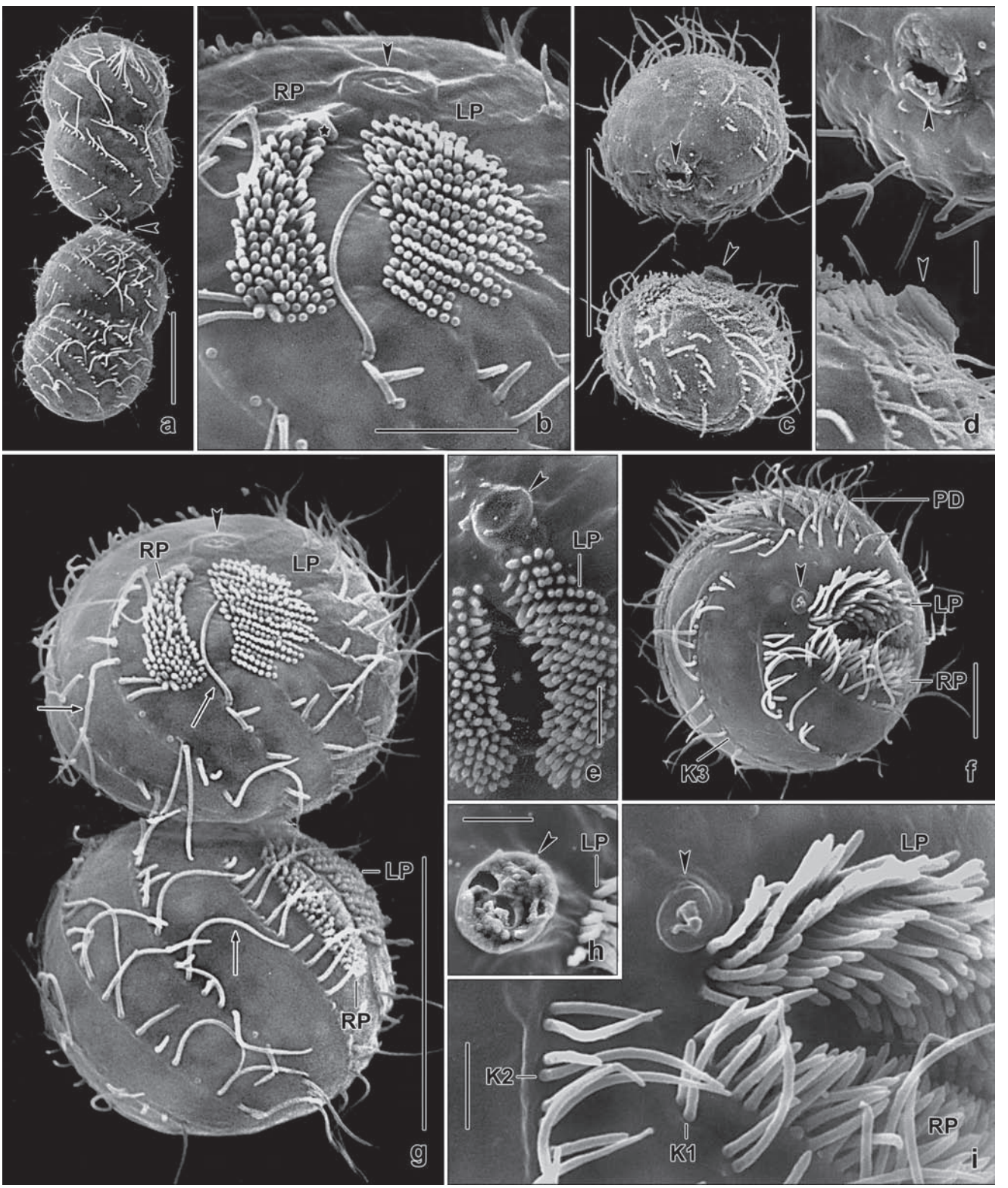
Figs 14a–h. *Bromeliothrix metopoides*, silver carbonate preparations of middle and late macrostome dividers producing two daughters (for the earlier stages, see Figs 10g–j and 11a–e). **a–c** – ventral and dorsal view of an early mid-divider, showing that the oral ciliary fields develop considerably later in the opisthe, where the anlagen are narrow anarchic fields of basal bodies (b, c), while ordered basal body rows occur in the anlagen of the left polykinetid of the proter (a). The asterisks indicate the prospective division furrow, where the ciliary rows spilt. There is intense proliferation of basal bodies in the somatic ciliary rows; **d** – a middle divider with an almost fully developed right polykinetid having a row of dikinetids (arrow) at the proximal margin. In the opisthe, this row has not yet assembled (here out of focus, but see Fig. 13c); **e–g** – late mid-dividers in frontal (e) and left and right side (f, g) view, showing the dividing nuclear apparatus; the strongly sigmoidal ciliary rows whose basal bodies have not yet assembled into dikinetids; and the anlagen of the left oral polykinetid, where a third row of basal bodies is developing (e). The arrows mark a row of dikinetids at the proximal margin of the right polykinetid; **h** – a late divider, where the somatic basal bodies organized into dikinetids and the anlagen of the left oral polykinetid consist of three rows of basal bodies each. Note that now a row of dikinetids assembled at the proximal margin of the right oral polykinetid of the opisthe (arrow). The cell still contains several large food vacuoles with flagellates. CR – ciliary rows, FV – food vacuoles, LP – left polykinetid, MA – macronucleus, MI – micronucleus, OLP – opisthe left polykinetid, ORP – opisthe right polykinetid, RP – right polykinetid. Scale bars: 10 μm (c, e), 15 μm (a, b), and 20 μm (d, f–h).



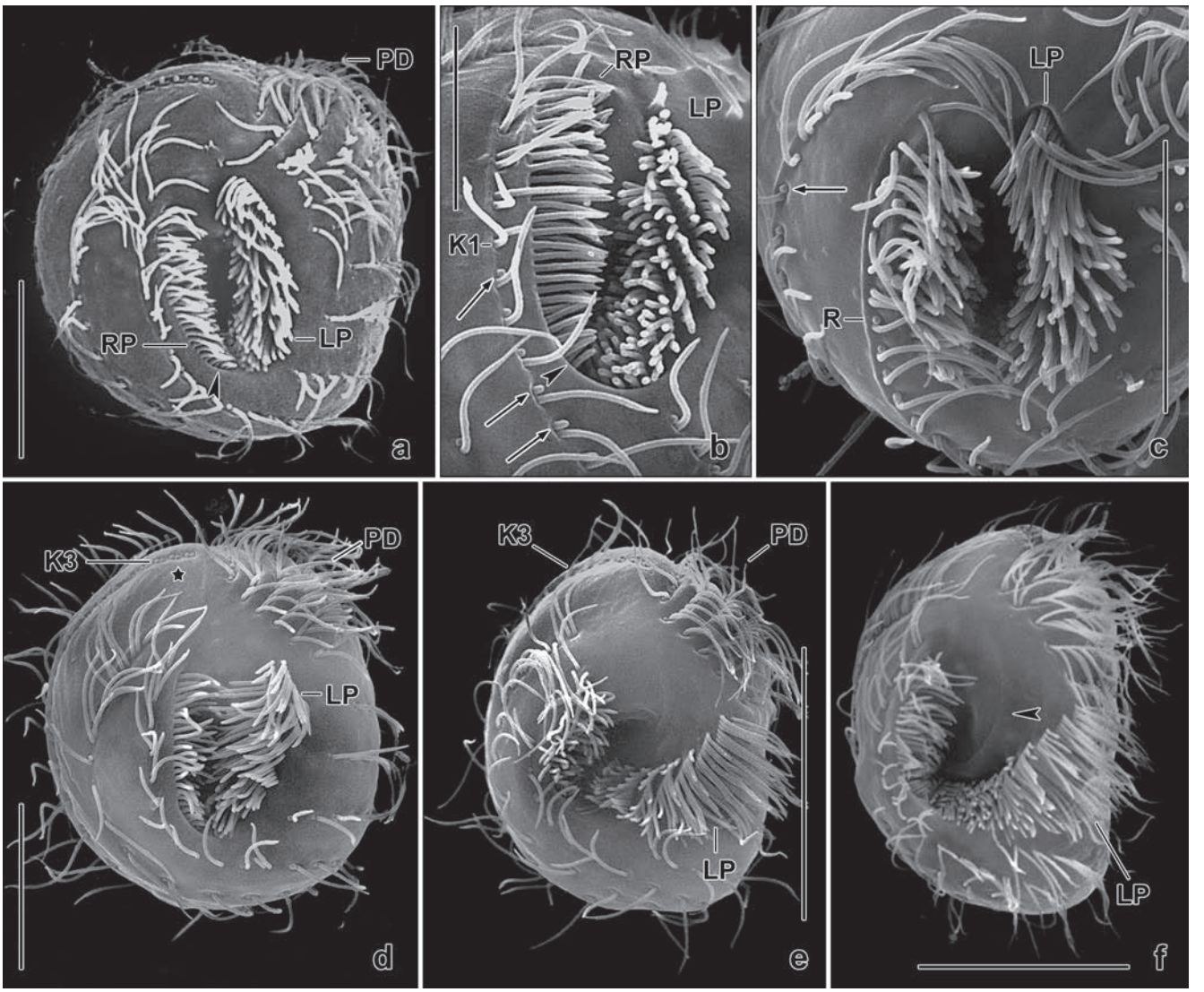
Figs 15a–h. *Bromeliothrix metopoides*, SEM micrographs of early and middle macrostome dividers producing two daughters (for earlier stages, see Fig. 12a–h). **a**, **f** – early divider with prospective division furrow marked by asterisks. New cilia emerge, while parental ones are resorbed (arrow); **b**, **d**, **e** – an early (d) and a middle (b, e) divider, showing the origin of the right oral polykinetid from the rightmost postoral kinety and of the left polykinetid from the anterior end of the two left postoral and some left side kineties. In the proter, a row of kinetids is forming at the proximal margin of the right polykinetid (b; arrow). The arrowhead marks an accumulation of basal bodies of unknown origin; **c**, **g**, **h** – late middle divider, showing the emergence of a third row of cilia in the anlagen of the left oral polykinetid (g; numerals). Arrows in (g, h) mark clavate cilia within the anlagen. Note a row of ordered cilia at the proximal margin of the right polykinetid of the opisthe (c, h; arrowheads). LP – left oral polykinetid, OA – oral apparatus, RP – right oral polykinetid. Scale bars: 2 μm (g), 5 μm (d, e, f, h) and 20 μm (a–c).



Figs 16a–h. *Bromeliothrix metopoides*, protargol impregnation of dividers producing four offspring, i.e. making a division chain. **a**, **b** – right and left side view of an early macrostome mid-divider without oral anlagen, showing that it will make a division chain; the small anarchic field (a; RP) is very likely a remnant from the predivisional reorganization of the right polykinetid. The somatic dikinetids disappeared due to the intense production of basal bodies, except in the dome area (b; arrow); **c** – a macrostome mid-divider without oral anlagen, showing that it will form a division chain (for details, see text); **d** – scheme of a division chain with the holdfast marked by arrow; **e**, **f** – late macrostome dividers with a holdfast (arrow) connecting the anterior and posterior cell, which commence the second division, producing a division chain with four offspring. Note the anlagen for four oral apparatuses; **g** – semi-diagrammatic view of a very late macrostome divider with nuclear division (right) and oral structures almost finished, i.e. having three-rowed anlagen in the left polykinetid and a row of dikinetids at the proximal margin of the right polykinetid; **h** – very late trophont divider with the oral apparatuses arranged in line. LP – left polykinetid, MA – macronucleus, MI – micronucleus, OA – oral apparatuses, RP – right polykinetid. Scale bars: 20 μm.



Figs 17a–i. *Bromeliothrix metopoides*, SEM micrographs of late dividers producing division chains with four offspring (a); figure (g) shows the posterior pair of a division chain, as proven by the holdfast (arrowhead). The arrowheads in (a–i) mark the holdfast, a unique organelle connecting the dividing pairs. Note the short, growing cilia in (f, g, i), possibly still mixed with some long, parental cilia (arrows). The asterisk in (b) marks a row with faster growing cilia, i.e. the dikinetal row recognizable in silver stains at the proximal margin of the right polykinetid (Fig. 16g). K1, 2, 3 – kineties, LP, RP – left and right polykinetid. PD – preoral dome. Scale bars: 2 µm (d, e, h, i), 5 µm (b, f) and 15 µm (a, c, g).



Figs 18 a–f. *Bromeliothrix metopoides*, SEM micrographs of macrostome post-dividers. **a–c** – in early post-dividers, the oral polykinetids are arranged in parallel and slightly invaginated so that the buccal vertex becomes recognizable (arrowheads). The right polykinetid is fully developed, while the cilia of the left polykinetid are still rather short. The ridges at the right margin of the ciliary rows will disappear soon (Fig d–f). The arrows mark growing anterior cilia of the somatic pairs; **d** – a middle, still globular post-divider with the ciliary rows basically arranged as in morphostatic cells, i.e. the barren areas become recognizable (asterisk). The oral polykinetids spread, V-like, because the left polykinetid moves like the hand of a clock; **e** – late post-dividers are broadly ellipsoidal and have a concave left polykinetid. The oral cavity grows; **f** – a young macrostome post-divider, showing the typical, bluntly reniform body shape (cp. Fig. 5a). The arrowhead marks the cytostome (cp. Figs 2e, 9b). K1, 3 – somatic kineties, LP, RP – left and right oral polykinetid, PD – preoral dome, R – ridge. Scale bars: 5 µm (b), 10 µm (a, c, d) and 15 µm (d, e).

cesses will be described in the chapters on "Genesis of the somatic ciliature" and "Post-divisional shaping of body and ciliature."

Binary fission (Figs 10d, h–j, 11b–f, 12h, i, 13a, c, e–g, i, 14a–h, 15a–h, 16h, 17b, f, g, 18a–d): Binary fission produces two daughters, the proter and opisthe. In *B. metopoides*, two oral apparatuses have to be generated because the parental apparatus has been resorbed. The oral apparatuses are not in line, but the opisthe apparatus is slightly shifted rightwards due to the spiral course of the ciliary rows. To simplify the matter, I shall describe mainly the genesis of the proter oral apparatus and supplement the description where the opisthe behaves different. Further, the late and very late division

events will be described mainly from specimens forming a division chain because the events are the same as in binary dividers.

Genesis of the oral apparatus: When the resorption of the parental oral apparatus has been completed, the ciliary rows split in mid-body and the anterior end of the rightmost postoral kinety commences with the proliferation of basal bodies, which form a narrow, curved, anarchic streak (Figs 10h, 11b, e, 14a–c). Concomitantly, an intense basal body proliferation commences at the anterior end of the two left postoral kineties and four or five left side kineties. Soon, these basal bodies form two ordered rows each, generating the anlage for the left oral polykinetid (Figs 10i, j, 11e, f, 12h, i).

I put a lot of effort into clarifying whether or not the left side anlagen contribute to the right side anlage, as widely assumed (see Discussion). In the silver preparations, some contribution cannot be excluded because there are frequently some basal bodies or minute kinetofragments between the right and left side anlage (Figs 10j, 11f). This contrasts with the SEM observations, where such basal bodies and fragments are usually absent (Figs 12h, i, 15b, d, e). As I have seen this in four SEM-prepared dividers, I tend to interpret the silver observations as an artifact, concluding that the right and left anlage of the oral apparatus develop independently in *B. metopoides*.

Mid-dividers are bluntly dumbbell-shaped and have a conspicuous anlage for the right oral polykinetid, consisting of an elliptical or obtriangular field of irregularly arranged basal bodies in the proter and opisthe, respectively (Figs 13a, c, 15b, e). The different shape of the proter and opisthe primordium has been seen in 12 specimens, but it is a transient phenomenon for which I do not have an explanation. Further, a row of dikinetids has been formed at the proximal margin of the proter`s anlage; in the opisthe, this row develops only in late dividers, showing a rather pronounced temporal asynchrony of proter and opisthe development (Figs 13f, 14h, 15c, h). The anlage for the left polykinetid consists of five, rarely six parallel rows of basal bodies at the anterior end of the two left postoral kineties and of four to five left side kineties (Figs 13a, c, 14d, f, g, 15b).

Late dividers are distinctly dumbbell-shaped and show three major processes (Figs 13f, 14h, 15c, g, h, 17b, g): the right oral anlage becomes crescentic and, in the opisthe, develops a row of dikinetids proximally, where the cilia sprout up earlier or faster than in the anarchic field; a third row of basal bodies is added to the left anlagen streaks, where the cilia begin to grow; and the somatic ciliature becomes dikinetal (see below). The left side anlagen streaks develop from posterior to anterior, but the gradient is not very pronounced (Fig. 15e, g, h).

In very late dividers the processes described above proceed. Specifically, the left side anlagen unite as a ribbon within which the kineties are appropriately sized so that the ribbon becomes obcuneate in the macrostomes. The cilia of the anlagen now form conspicuous bundles, but have not yet reached their final length (Figs 16h, 17f, g). The further development is described in the chapter on "post-divisional shaping of body and ciliature."

Genesis of the somatic ciliature: Concomitantly with oral ontogenesis, the somatic reorganization proceeds (Figs 10d, h–j, 11c, d, 14a–c, 15a, f): Y-shaped silverlines develop; cilia sprout up from the mature basal bodies; and new basal bodies are generated, forming typical triplets and quadruplets with the parental ones. During the next stage, when the division furrow develops, the basal bodies distribute equidistantly within the kineties, making mid-dividers monokinetidal (Figs 13a, c, 14d–g, 15a–c). Further, a minute ridge develops right of the ciliary rows (Fig. 15b–e) and the silverlines assume their ordinary pattern. When body and nuclear division have been almost completed, the basal bodies become dikinetal (Figs 13e–g, 14h), but the anterior cilium of the dikinetids sprout up only in the post-dividers, while the further developed posterior cilia and parental cilia keep the cell motile (Fig. 18a–d). I could not clarify how the dikinetidal state is achieved, i.e. by a union of existing basal bodies or by the production of new basal bodies in front of existing ones; some crude counts indicate that both modes could be involved. When fission is complete, the daughters are globular and the basic ciliary pattern becomes recognizable, viz., three widely spaced right side kineties and the densely ciliated dome kineties (Figs 13i, 18a). The further development is described in the chapter on "post-divisional shaping of body and ciliature."

Multiple (polytomic) fission (Figs 9h, i, 10e, 16a–g, 17a–i): As mentioned in the "overview" section, multiple fission (formation of a division chain), is possibly typical for mature (TR) and (MA). The bond between the chain pairs is rather fragile. Thus, they often become separated by the preparation procedures. None the less, they are rather easily distinguished from binary dividers because oral anlagen are absent, even in distinctly furrowed specimens (Figs 10e, 16a–c, 17a).

Basically, multiple fission is as binary fission, as described in the section before. Thus, the description emphasizes two specialities: anlagen formation for the oral apparatuses occurs much later than in binary fission and a special holdfast develops, connecting the pairs of a chain.

After the reorganization, the ciliary rows are strongly spiralized and studded with new basal bodies. Then, cell division commences: the specimens become ellipsoidal (~35 × 25 µm), the division furrow becomes recognizable, and the nuclear apparatus elongates (Figs 10e, 16a–c, 17a). Some specimens still have remnants of the parental somatic and oral infraciliature (Fig. 16a). When the dividers become dumbbell-shaped, the nuclear apparatus has divided, while oral anlagen are still absent (Fig. 16c). These develop next, i.e. when the ciliary rows divide a second time, cell fission is almost complete, body length increased to about 40 µm, and the holdfast develops (Figs 16e, 17a). The development of the oral anlagen (Fig. 17b, f, g) is as in binary dividers, to which the reader is referred to. The holdfast, which binds together the divided cells, is close above the developing left oral polykinetid and is possibly organized like a snap fastener, i.e. the opisthe develops a plug about 2 µm in diameter and 1 to 1.5 µm high, while the proter has an appropriate deepening in or near to the posterior pole centre (Figs 16d, e–g, 17c–i). Rather frequently, very late dividers have the oral apparatuses not in line but more or less turned, indicating that the pairs can turn independently, i.e. that the holdfast is not fixed. However, a preparation artifact cannot be excluded. *In vivo*, the holdfast is too small and fragile to be observed properly.

The holdfast produces division chains with four globular offspring. The chains are slightly curved and have an average size of 78.3 × 23.8 µm (length: M 75, SD 5.6, CV 7.1, Min 70, Max 85; width: M 22, SD 4.3, CV 18.0, Min 20, Max 30). They swim slightly slower than morphostatic cells and offer a highly unusual sight (Figs 9h, i, 17a). Macrostome chains contain several food vacuoles with only partially digested flagellates, possibly prompting post-divisional growth of the cell and, especially, of the cilia.

Post-divisional shaping of body and ciliature (Figs 1a, 18a–f): The fate of the post-dividers depends on the food available, if the other environmental parameters are favourable. A post-divisional macrostome will become a mature macrostome, if sufficient flagellates are available. When the flagellates are depleted but sufficient bacteria are available, a trophont will develop via a transition division. When bacteria are also depleted or the environmental factors are unfavourable, the cell will encyst (Fig. 1a).

The early post-dividers are globular and show many growing cilia. The anterior cilium of the pairs is often still a short stump (Fig. 18a–c). The ciliary rows are less spiralized than in mature reorganizers, but are still accompanied by a minute ridge (Fig. 18c). The oral polykinetids are vertically arranged and slightly invaginated, so that the buccal vertex becomes recognizable (Fig. 18a, b). The cilia of the right polykinetid are fully developed, while those of the left are still rather short and growing (Fig. 18b, c). Middle post-dividers are still globular (Fig. 18d), but the ciliary rows are arranging in the species-specific pattern, i.e. the large, barren area between the anterior ends of rows 2 and 3 becomes recognizable. The ridge at the right margin of the kineties disappeared. The oral polykinetids spread V-like because the left polykinetid moves posteriad like the hand of a clock, but its anterior end is still near the dome kineties (Fig. 18d). Late post-dividers are broadly ellipsoidal and have the species-specific ciliary pattern (Fig. 18e). The left polykinetid, whose cilia are fully developed, moves further posteriad becoming distinctly concave. The large oral field becomes recognizable and deepens from left to right (Fig. 18e). Very late post-dividers are "young macrostomes" and have a typical, bluntly reniform body shape (Figs 5a, 18f). The left polykinetid stretches becoming a cuneate stripe of ciliary rows decreasing in length from distal to proximal. The irregularly distributed basal bodies of the right polykinetid begin to arrange in short, only slightly disordered rows. The large oral field deepens further and the strongly curved cytostome becomes recognizable (Fig. 18f, cp. with Figs 2e, 9b).

DISCUSSION

Suprafamilial classification of *Bromeliothrix*

The morphological and genetic (Fig. 8) data show that *Bromeliothrix* belongs to the class Colpodea and the order Colpodida, as defined by Foissner (1993) and Lynn (2008). Decisive morphological features for the class are the somatic and oral ciliary pattern and the somatic fibrillar system, showing conspicuous LKm-fibres associated with the dikinetids (Fig. 3a, b). The classification in the order Colpodida Puytorac *et al*., 1974 is based on the colpodid silverline pattern, the

Colpoda-like oral structures, and the division in a highly modified *Colpoda* pattern.

Family and genus classification of *Bromeliothrix*

Bromeliothrix metopoides has several outstanding morphological and ontogenetic features: (i) the (TH) and (TR) have a *Metopus*-like body shape not found in any other colpodid (Foissner 1993, Foissner *et al*. 2002); (ii) the somatic ciliature, albeit being *Colpoda*-like, is distinct due to the spread kineties 2 and 3; (iii) the pharyngeal fibres extend anteriorly, as in marynid colpodids (Foissner 1993, Foissner *et al.* 2002); (iv) only one basal body is ciliated in the dikinetids of the dikinetal row at the proximal margin of the right oral polykinetid, in contrast to *Exocolpoda augustini* and *Colpoda variabilis*, which have both basal bodies ciliated (Hofmann-Münz 1991, Foissner *et al.* 2002); (v) *Bromeliothrix* can form macrostomes, a rare ability in colpodids (Foissner 1993, Foissner *et al*. 2002, Foissner and Wolf 2009); (vi) the (TH) and (TR) have ellipsoidal resting cysts not known from any other colpodid (Foissner 1993, Foissner *et al*. 2002); (vii) division occurs in freely motile condition, while other genera of the order divide in resting cysts (Foissner 1993), except of *Exocolpoda* (Foissner *et al*. 2002); (viii) reproduction occurs by binary fission or polytomy, producing a unique, motile division chain; and (ix) the dividing pairs are connected by a unique holdfast (Fig. 17c–i). This accumulation of specific characters justifies a distinct morphological family. Here, I assign *Bromeliothrix* tentatively to the family Exocolpodidae because *Exocolpoda* also divides in freely motile condition (Foissner *et al*. 2002). But all other features are different, indicating that *B. metopoides* could represent a distinct family independent from the Exocolpodidae.

The morphological and ontogenetic features characterizing *Bromeliothrix* are hardly represented by the sequence of the small-subunit rDNA, that assigns it to the family Colpodidae, which itself is not monophyletic (Fig. 8). However, the bootstrap values are low, suggesting that this may change when more relatives are included in the analysis. The close relationship with *Colpoda steinii* (only one nucleotide difference!) strongly suggests some decoupling of morphological and genetic evolution, at least with respect to the gene investigated. On the other hand, there are some features which indicate a relationship with *C. steinii* (macronucleus with large central nucleolus, a rare feature in colpodids; small body with 12 ciliary rows each; extrusomes inconspicuous *in vivo*, but with strong affinity to protargol) and the colpodid families (right oral polykinetid with a proximal row of dikinetids and more or less scattered monokinetids), specifically the Hausmanniellidae, which can form macrostomes similar to those of *Bromeliothrix* (Foissner *et al*. 2002). Indeed, *Hausmanniella discoidea* is close to *B. metopoides* in the sequence of the small-subunit rDNA, suggesting that they could have a common ancestor (Fig. 8). And there are some supposed hausmanniellids, viz., the genera *Avestina* and *Dragescozoon*, that have some similarities with *Bromeliothrix* in body shape or ciliary pattern (for reviews, see Foissner 1993 and Foissner *et al*. 2002).

As concerns the genus and species, I did not find any other ciliate in the literature resembling *B. metopoides*. Thus, I classified it as undescribed.

Comparative ontogenesis

Members of the order Colpodida usually divide in cysts covered by a thin membrane (for a review, see Foissner 1993). To date, only one exception is known: *Exocolpoda augustini* that divides, like *B. metopoides*, in freely motile state, but does not generate a division chain (Foissner *et al.* 2002), which is thus unique to *B. metopoides*. Indeed, division chains have not been described from any member of this class, but multiple division (polytomy) within cysts is quite common (Foissner 1993). Thus, *B. metopoides* may be considered as a colpodid that has transferred polytomy into the freely motile state, possibly as an adaptation to the highly competitive habitat (see next section).

Division chains are common in the parasitic/mutualistic astomate ciliates (Corliss 1979, Lynn 2008), while very rare in free-living ones. Actually, I know of only one family, the Deltopylidae, where division chains with four tomites are produced (for a review, see Bourland and Strüder-Kypke 2010). Interestingly, one of the few species included in this family, has two types of resting cysts, like *B. metopoides*.

Scanning electron microscopical investigations on the ontogenesis of colpodids are not available because of the membrane that covers the division cysts, except of the above mentioned *E. augustini*, which has been investigated by Foissner *et al.* (2002) and thus can be compared with *B. metopoides*.

a) In the Colpodida, the right oral polykinetid develops from a stomatogenic kinety and parts of the anlagen for the left polykinetid (Hashimoto 1966, Garcia-Rodriguez *et al*. 1981, Foissner 1993). However, the contribution of the left anlagen has never been shown un-

equivocally by a detailed series of micrographs. Thus, it is not surprising that the subject is controversial, e.g. in *Colpoda steinii*, where Hashimoto (1966) states that two of the four anlagen of the left ciliary field contribute to the right one, while Taylor and Garnjobst (1939) and Perez-Paniagua *et al.* (1979) observed that the right polykinetid is made by only one stomatogenic kinety, as it is possibly the case in *B. metopoides* (Fig. 15d, but see Figs 10j, 11f). In *Colpoda inflata*, the right polykinetid is also generated by only one kinety (Martin-Gonzalez *et al.* 1991). In *Exocolpoda augustini*, the rightmost anlage splits (Foissner *et al.* 2002): the anterior portion produces the right polykinetid, while the posterior portion generates an anlage for the left polykinetid. This has been shown by only one SEM micrograph and should be checked in a more detailed study.

b) The ciliary rows of the colpodids extend more or less sigmoidally from anterior to posterior body end. It is widely believed that they despiralize when the cells round up for reorganization and division (for a review, see Foissner 1993). However, this is not the case in *Exocolpoda augustini* (Foissner *et al.* 2002) and *B. metopoides*, where spiralization even increases (Figs 11a, 15a). Further, my unpublished material on several *Colpoda* species and *Maryna umbrellata* do not show despiralization. Thus, I suppose that despiralization is a false impression resulting from the polar view necessary for studying oral anlagen development. Lateral views show that kinety spiralization is similar in morphostatic and dividing cells (Figs 4e, f, i, j, 9a, b, k, 17g).

c) The right oral polykinetid of most or even all members of the order Colpodida consists of two parts. This has been overlooked for a long time (Foissner 1993), but is now firmly established (Hofmann-Münz 1991, Foissner *et al.* 2002). The major part is composed of ciliated basal bodies in a more or less anarchic arrangement. The minor part is a dikinetal (double), ordered row of basal bodies at the proximal (dorsal) margin of the anarchic field. In the genus *Kuehneltiella*, the anarchic field is lacking, indicating that the dikinetal row is plesiomorphic (Foissner 1993). The right oral polykinetid of *B. metopoides* matches this general pattern (Figs 3f, i, j, 6g, 13a, c, f, 14d, h), but the SEM micrographs strongly indicate that only one basal body of the dikinetids is ciliated, i.e. only one ordered row of basal bodies and sprouting cilia is recognizable (Figs 15b, h, 17b, g). This contrasts *Exocolpoda augustini* and *Colpoda variabilis*, in which both basal bodies of the dikinetids bear cilia (Hofmann-Münz 1991, Foissner *et al.* 2002). I do not know the phylogenetic meaning of this difference, but it is a further indicator of the distinctness of the bromeliad ciliate.

d) The following minor differences between *E. augustini*, as redescribed by Foissner *et al.* (2002), and *B. metopoides* should be mentioned: (i) *E. augustini* resorbs the somatic ciliature around the oral apparatus, while *B. metopoides* keeps it; (ii) *E. augustini* resorbs about 50% of the basal bodies in the oral anlagen, while *B. metopoides* very likely keeps most or all basal bodies generated during anlagen development.

e) The following similarities in the ontogenesis of *Exocolpoda augustini* and *B. metopoides* are remarkable: (i) both show a tendency to resorb the distal cilia of the left oral polykinetid slightly later than the proximal ones; (ii) when the daughters separate, they are still globular, and thus body shaping occurs exclusively in post-dividers.

Biogeographic aspects and ecology

In nature, *B. metopoides* is restricted both in habitat and geographic distribution. I did not find it in hundreds of limnetic samples from Austria and Southern Germany and in about 1000 samples from terrestrial habitats globally, e.g. from Europe, Africa, Australia, South America, and Antarctica. It is even absent in many soil samples from the bromeliad region (Central and South America), and in over 50 limnetic habitats from Brazil and Jamaica, where it occasionally occurred in the water and mud accumulating in bamboo stumps (Foissner and Wolf, unpubl.).

Restriction of *B. metopoides* to the highly specialized habitat of bromeliad tanks is difficult to explain in view of its ability to thrive when added to a wide variety of laboratory media including non-flooded Petri dish soil cultures and its ready formation of resting cysts. Food specialization is an unlikely explanation, at least for the theronts and trophonts, which feed on bacteria.

Ciliates with the ability to switch between a bacteriophagous microstome and a predaceous macrostome are much more frequent in tank bromeliads than in other habitats (Foissner *et al.* 2003). *Bromeliothrix metopoides* and *Platyophrya bromelicola* Foissner and Wolf, 2009 are typical examples from the ciliate class Colpodea, where microstome – macrostome transformation is very rare (Foissner 1993, Foissner and Wolf 2009). This supports the assumption of Foissner *et al.* (2003) that the bromeliad habitat is highly competitive because the tanks dry up partially or completely during rainless periods, squeezing together the inhabitants.

Colpodids divide ordinarily, producing two offspring or they divide in cysts, generating four or more offspring (Foissner 1993). Possibly, the division chain of *B. metopoides* is also an adaptation to the highly competitive habitat, needing less time and resources than division cysts. Whatever the reason, this kind of division, as yet known mainly from parasitic/mutualistic astomate ciliates, causes a fast growth of the population, suggesting that *B. metopoides* is more *r*- than *k*-selected. This is sustained by the small body size and the great variety of habitats it can live in nature and in the laboratory.

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