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Morphology and Ontogenesis of *Bromeliophrya brasiliensis* gen. n., sp. n., a New Ciliate (Protozoa: Ciliophora) from Brazilian Tank Bromeliads (Bromeliaceae)

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Summary. This is the first of a series of papers describing the morphology and ontogenesis of new ciliates occurring in the cisterns of tank bromeliads, a group of rosette plants of tropical America, entrapping rainwater between the coalescing leaf axils. Likely, most of these new ciliates have a restricted (Gondwanan) geographic distribution, and some might even be specific inhabitants of tank bromeliads. *Bromeliophrya brasiliensis* gen. n., sp. n. was discovered in the tanks of ground bromeliads at the east coast (Mata Atlantica) of Brazil, South America. Its morphology and ontogenesis were investigated using live observation, silver impregnation, and scanning electron microscopy. *Bromeliophrya* belongs to the hymenostome ciliate order Tetrahymenida, where it represents a new family, Bromeliophryidae fam. n., unique by the partially reduced somatic ciliature and migrating somatic kinety fragments during ontogenesis. The oral apparatus, especially a patch ("X-group") of basal bodies originating from the anterior end of adoral membranelle 2, indicates the Glaucomidae as the nearest relatives of the Bromeliophryidae. Further main characteristics of *B. brasiliensis* are: (i) a metopid body shape and ciliary pattern; (ii) two large, unciliated areas: one left of the oral opening, the other with barren basal bodies occupies the right posterior half of the cell; (iii) elongated caudal cilia; (iv) silverlines projecting loop-like in the right side kineties; (v) a large, C-shaped adoral membranelle 3 forming a ring-like oral pattern with membranelle 1 at left margin of buccal cavity; and (vi) an unciliated paroral membrane.

Key words: biodiversity, biogeography, bromeliad tanks, *Bromeliophrya brasiliensis* gen. n., sp. n., Mata Atlantica, South America.

INTRODUCTION

Finlay *et al*. (1996) claim that the main biotopes of the Earth have already been sufficiently investigated for free-living ciliates and estimate a low total diversity of only 3000-4000 species. Both statements are contradicted by evidence discussed in Foissner (1999) and Foissner *et al*. (2002). For instance, ciliates have not been investigated in detail in South America and Australia, nor in the 25 world species diversity hotspots selected by Myers *et al*. (2000), except for the Mediterranean Basin. Likewise, soil ciliates are largely unknown, as shown by a recent study describing 130 new species from 73 soil samples of Namibia (Foissner *et al*. 2002).

Here, I present a new ciliate world discovered in the water cisterns (tanks) of bromeliads, which are well-known for inhabiting a rich, more or less specific fauna and flora, but whose protists, although obviously being common and abundant, were never studied in detail (Picado 1913, Laessle 1961, Maguire 1971, Janetzky and

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Vareschi 1992, Esteves and da Silva Neto 1996, Little and Hebert 1996, Martinelli 2000). Only testate amoebae were investigated in some extent (Van Oye 1923), and recently three new species were described from Brazilian tank bromeliads, one even representing a new family (Torres-Stolzenberg 2000).

Bromeliads are rosette plants, the most famous of which is the pine-apple. The tanks are formed by the coalescing leaf axils, which collect the rain water, and the rosettes of the larger species may entrap up to 30 litres. During holidays in Brazil, I collected some of the entrapped, muddy water of a ground bromelia, just for fun, because I supposed that, if there were specific ciliates at all, they would either be killed by the putrefying water or eaten by the micrometazoans during the long transport to my Salzburg laboratory. Fortunately, this did not happen, likely because the water was surprisingly acidic (pH 5) and abundances were too high to be exhausted by rotifers, small crustaceans, and mosquito larvae. It was a great surprise to discover two new species, each even representing a new genus, in this sample. Thus, I asked a Brazilian colleague, who later visited me in Salzburg, for further samples. Unfortunately, he could collect only one small sample from an epiphytic bromeliad, but this again contained a new species and genus (Foissner and Cordeiro 2000). Finally, another new species, *Urosomoida reticulata*, was discovered in the dry mud from the tanks of a tree bromeliad in Costa Rica. Later, this species was found in soil from Venezuela and Namibia (Foissner *et al*. 2002). Five other new species and two new genera, I discovered recently in a few tank bromeliads from the Dominican Republic.

These data indicate that tank bromeliads contain many undescribed ciliate species. We do not know how many, but the number must be considerable, considering that there are more than 3000 bromelia species, many of which have special life styles and grow in peculiar environments; some or even most release specific substances into the tank water, whose chemical and physical properties show great local and temporal differences providing innumerable niches for the minute protists (Maguire 1971, Martinelli 2000).

MATERIALS AND METHODS

The sample, which contained *Bromeliophrya brasiliensis* sp. n., was collected in 1996 at the Atlantic Sea coast (Mata Atlantica) in the surroundings of the village of Praia do Forte (S14°33' W38°; 1500 mm annual precipitation), that is, about 81 km North of the town of Salvador, Bahia, Brazil. About 500 ml leaf axil water was collected from two specimens of a large ground bromelia and transported without special precautions to Salzburg. Unfortunately, I could not identify the bromelian species; possibly, it was *Aechmea* sp. The tank water contained partially decomposed leaf litter, mud, various small metazoans, and had a brownish colour and strongly acidic reaction (pH 5).

In the laboratory, the water was filtered through a 500 µm net to remove larger metazoa. Part of the sample was fixed for preparations, while the rest was used to set up cultures enriched with 1-3 squashed wheat grains. *Bromeliophrya brasiliensis* and some other ciliates occurred in the native tank water and grew well in the wheat grain cultures, where they fed on bacteria. Thus, ontogenesis could be studied as well; cultures set up mainly with Eau de Volvic (French table water) developed poorly, indicating that the tank water contained substances promoting growth of ciliates.

Cells were studied *in vivo* using a high-power, oil immersion objective and differential interference contrast optics. The infraciliature and various cytological structures were revealed by scanning electron microscopy and the silver impregnation techniques described in Foissner (1991). Counts and measurements on prepared specimens were performed at a magnification of x1000. *In vivo* measurements were conducted at magnifications of x100-1000. Although these provide only rough estimates, it is worth giving such data as specimens may change in preparations. Illustrations of live specimens were based on video-records and micrographs, while those of prepared cells were made with a camera lucida. Terminology is according to Peck (1974), Corliss (1952, 1979) , and Foissner (1996).

RESULTS

Bromeliophryidae fam. n.

Diagnosis: medium-sized Tetrahymenida with partially reduced somatic ciliature and a patch ("X-group") of basal bodies between the anterior ends of adoral membranelles 1 and 3. Stomatogenesis glaucomid, while somatogenesis is unique in that some left side ciliary rows split and the anterior fragments migrate to the left margin of the oral apparatus.

Type genus: *Bromeliophrya* gen. n.

Bromeliophrya gen. n.

Diagnosis: *Metopus*-shaped Bromeliophryidae with some right side ciliary rows rectangularly curved to abut on dorsal side rows preorally; two large, unciliated areas: one left of oral opening, the other with barren basal bodies occupies the right posterior half of the cell. Oral apparatus in second quarter of cell; adoral membranelle 1 short and concave, forms ring-like pattern with the large, C-shaped membranelle 3; membranelle 2 short and convex, extends along proximal part of membranelle

3. Paroral membrane unciliated. Silverlines projecting loop-like from kineties in second quarter of right side.

Type species: *Bromeliophrya brasiliensis* sp. n.

Etymology: composite of the plant generic name *Bromus* (Bromeliaceae) and the Greek noun *ophrya* (eyebrow ~ cilia ~ ciliate), meaning a "ciliate associated with bromeliads". Feminine gender.

Description of *Bromeliophrya brasiliensis* sp. n.

Diagnosis: size about 55 x 35 µm *in vivo*. One broadly ellipsoidal macronucleus and micronucleus in centre of cell. Contractile vacuole in posterior fifth of body. Extrusomes mainly left of ciliary rows, rod-shaped, about 2 x 0.8 µm. On average 32 ciliary rows, of which 16 are distinctly shortened postorally and left laterally; two left lateral kinety fragments at left margin of oral apparatus; about 5 elongated caudal cilia originating from right side kineties. Adoral membranelle 1 composed of one ciliated row; membranelle 2 four-rowed with anterior row shortened at right; membranelle 3 composed of three rows.

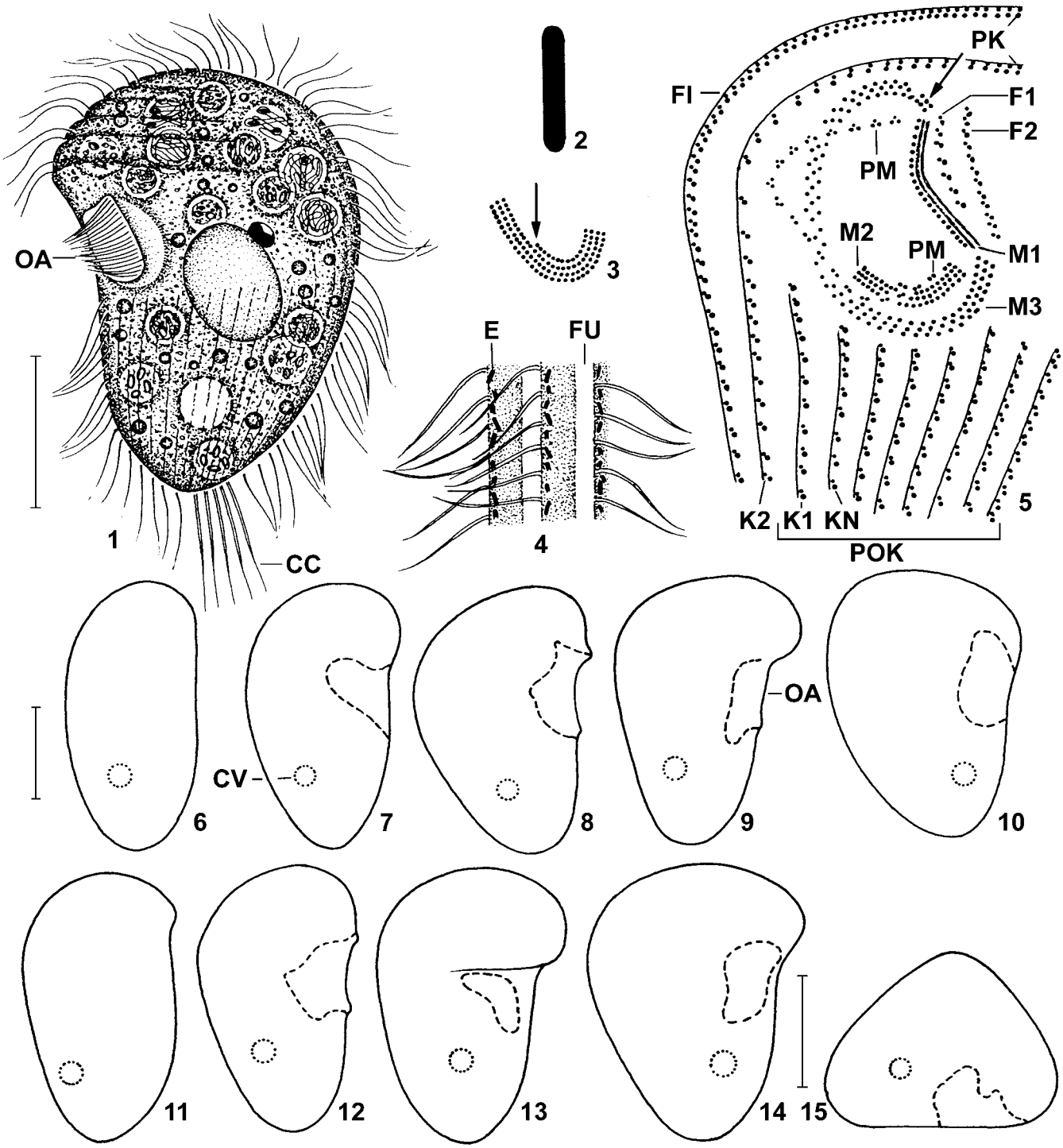
Type location: tanks of ground bromeliads in the surroundings of the village of Praia do Forte (S14°33' W38°), Atlantic Sea coast of Bahia, Brazil.

Type material: 1 holotype slide each of cultivated, protargol-impregnated, Chatton-Lwoff silver nitrate-impregnated, and Klein-Foissner silver nitrate-impregnated specimens and 10 paratype slides have been deposited in the Oberösterreichische Landesmuseum in Linz (LI). Specimens shown in Figures 16-34 and some other well-

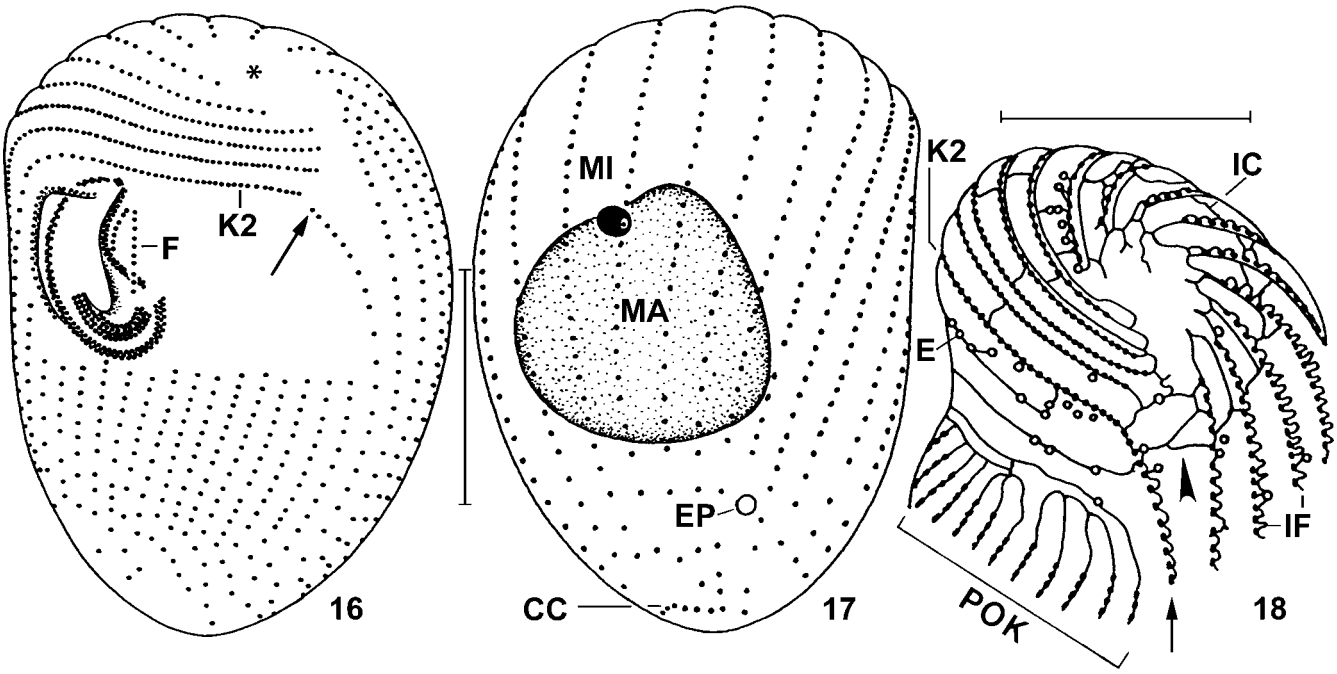
Table 1. Morphometric data on *Bromeliophrya brasiliensis* sp. n.

Characteristics[a]	Method	x	M	SD	SE	CV	Min	Max	n
Body, length	CHL	50.6	51.0	4.5	1.0	8.9	42	58	21
Body, width in lateral view	CHL	34.9	34.0	3.1	0.7	8.8	30	40	21
Body, width in ventral view	CHL	30.3	30.0	2.5	0.5	8.2	26	35	21
Anterior end to upper mouth margin, distance	CHL	12.5	13.0	2.3	0.5	18.4	7	16	21
Anterior end to lower mouth margin, distance	CHL	24.6	25.0	2.5	0.6	10.3	18	28	21
Anterior end to adoral membranelle 3, distance	CHL	9.4	9.0	1.5	0.3	15.7	6	12	21
Anterior end to proximal summit of adoral membranelle 3, distance	CHL	27.0	27.0	1.7	0.4	6.0	25	30	21
Anterior end to macronucleus, distance	CHL	19.4	19.0	3.7	0.8	19.0	9	27	21
Anterior end to distal end of 1st preoral kinety (=K2), distance	CHL	13.9	14.0	1.6	0.4	11.5	12	18	21
Anterior end to last postoral kinety, distance[h]	CHL	29.2	29.0	2.1	0.5	7.3	25	34	21
Posterior end to excretory pore, distance	CHL	9.6	10.0	1.9	0.4	19.4	6	13	21
Macronucleus, length	CHL	14.4	15.0	2.9	0.6	20.3	10	20	21
Macronucleus, width	CHL	11.3	11.0	1.9	0.4	16.6	8	15	21
Micronucleus, length	CHL	2.7	2.5	0.7	0.1	23.7	2	4	21
Micronucleus, width	CHL	2.6	2.5	0.6	0.1	22.7	2	3.5	21
Oral opening, width[b]	CHL	9.5	10.0	0.8	0.2	7.9	8	11	21
Oral opening, width[c]	CHL	13.0	13.0	1.6	0.3	11.9	10	16	21
Buccal cavity, depth[d]	CHL	14.3	14.0	1.5	0.3	10.4	12	18	21
Somatic kineties, total number[e]	CHL	31.5	32.0	0.8	0.2	2.6	29	33	21
Postoral kineties, number[e,h]	CHL	15.8	16.0	0.7	0.2	4.3	14	17	21
Left lateral kinety fragments, number[f]	CHL	2.0	2.0	0.0	0.0	0.0	2	2	21
Kinetids in somatic kinety 2, number	P	77.0	80.0	11.2	2.5	14.6	49	94	21
Kinetids in postoral kinety 7, number[h]	CHL	12.4	12.0	2.3	0.5	18.2	7	16	21
Kinetids in left lateral fragment 1, number	P	7.4	7.0	1.1	0.2	15.1	5	10	21
Kinetids in left lateral fragment 2, number	P	12.1	12.0	1.4	0.3	11.4	9	14	21
Caudal cilia, number[g]	P	5.3	5.0	-	-	-	5	7	21

[a] Data based on cultivated, mounted, randomly selected, morphostatic specimens. [b] Distance from paroral membrane to first kinety left of oral opening (=kinety fragment 1). [c] Distance from outer margin of adoral membranelle 1 to outer margin of membranelle 3. [d] From laterally orientated specimens (Fig. 22). [e] Without the two kinety fragments left of oral opening. [f] Two kinety fragments left of oral opening, see ontogenesis. [g] Approximate values because often indistinctly separated from somatic kinetids. [h] Postoral kineties include left lateral kineties, which are also shortened and thus inseparable. All measurements in µm. CHL - Chatton-Lwoff silver nitrate impregnation, CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, P - protargol impregnation (Wilbert's method), SD - standard deviation, SE - standard error of arithmetic mean, x - arithmetic mean.



Figs 1-15. *Bromeliophrya brasiliensis* sp. n. from life (1, 2, 4, 6-15) and after silver carbonate impregnation (3, 5). **1** - left side view of a representative specimen; **2** - extrusome, 2 x 0.8 µm; **3** - adoral membranelle 2 consists of four basal body rows, of which the anterior row is distinctly shortened (arrow); **4** - the cortex of "wild" specimens is distinctly furrowed; **5** - slightly schematised ventral view of oral and circumoral ciliature. Arrow marks a patch ("X-group") of basal bodies between the anterior ends of adoral membranelles 1 and 3. The somatic kinetids are composed of two granules, of which the posterior one bears a cilium, while the anterior granule is likely a parasomal sac. Note that paroral kinetids are composed of three granules in the anterior half of the organelle; **6-14** - body shape-changes of two specimens rotating from dorsal (6, 11) to right side (10, 14), redrawn from video records; **15** - transverse view. CC - caudal cilia; CV - contractile vacuole; E - extrusomes; F1 - fibre; FU - furrow; F1, 2 - kinety fragments; K1, 2, N - kineties 1, 2, N; M1, 2, 3 - adoral membranelles; OA - oral apparatus; PK - preoral kineties; PM - paroral membrane; POK - postoral and left lateral kineties. Length of cells: 55 µm (1), 60 µm (6-10), 50 µm (11-14). Scale bars 20 µm.

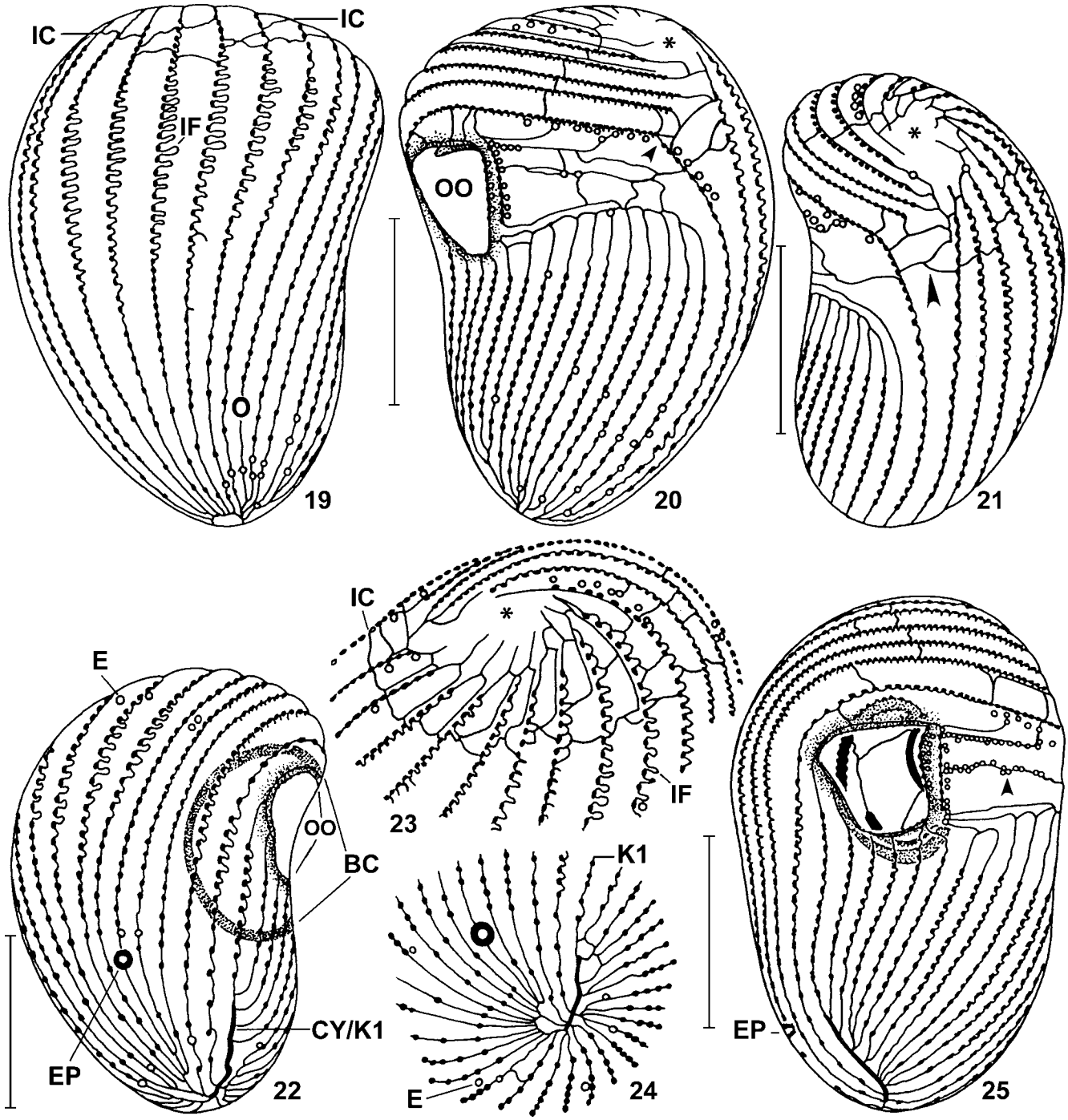


Figs 16-18. *Bromeliophrya brasiliensis* sp. n. after protargol (Wilbert's method; 16, 17) and silver nitrate (Chatton-Lwoff method; 18) impregnation. **16, 17** - ciliary pattern of left and right side of holotype specimen. Note the large, unciliated area left of the oral opening and that basal bodies are unciliated, except for the caudal cilia, in the right posterior half (cp. fig. 38). Arrow denotes the last dorsal kinety, which is so strongly curved that it abuts to the anterior end of right side kinety 2. Asterisk denotes the unciliated anterior pole field, which contacts the bare, obtriangular area between the last and penultimate dorsal kinety. For details on oral structures, see fig. 5; **18** - oblique left side view of anterior body portion showing lack of silverlines in the unciliated pole area. Arrow marks curved last dorsal kinety, which abuts to right side kinety 2. Arrowhead denotes silverlines connecting last and penultimate dorsal kinety. Resting extrusomes (E) are surrounded by a silverline. CC - caudal cilia; E - resting extrusome; EP - excretory pore of contractile vacuole; F - kinety fragments left of oral opening; IC - intermeridional connectives; IF - intrameridional cross-fibres; K2 - somatic kinety 2; MA - macronucleus; MI - micronucleus; POK - postoral and left lateral kineties. Length of left specimen - 54 µm. Scale bars 20 µm.

impregnated cells are individually marked by a black ink circle on the cover glass.

Etymology: named after the native country.

Description: size 45-65 x 30-40 µm *in vivo*, usually about 55 x 35 µm (Table 1). Shape rather constant, cells, however, distinctly asymmetrical and thus showing a variety of shapes, depending on side and angle viewed (Figs 1, 6-15, 35, 36, 38, 39); lateral view highly characteristic because similar to that of members of the genus *Metopus*, that is, with broad, projecting preoral dome and rounded obconical postoral portion. Anterior body end broadly rounded, posterior narrowly rounded to bluntly pointed; ventral body half more distinctly flattened than dorsal, producing more or less rounded triangular outline in ventral, dorsal, and transverse view. Nuclear apparatus in or near body centre. Macronucleus globular to ellipsoidal (2:1), on average broadly ellipsoidal (1.3:1); nucleoli scattered, inconspicuous. Micronucleus attached to macronucleus, globular. Contractile vacuole in posterior fifth of cell, that is, distinctly subterminal, invariably with a single excretory pore at end of a shortened ciliary row in or near midline of right side. Cytopyge extends between posteriorly shortened (stomatogenic) kinety 1 and posterior pole centre, appears as a thick silverline in silver nitrate preparations (Figs 1, 19, 22, 24-26, 38). Extrusomes numerous, slightly left of ciliary rows, left of kinety fragments, at both sides of adoral membranelle 3, and attached to silverlines traversing unciliated areas, about 2 x 0.5 µm in size and rod-shaped *in vivo*, do not impregnate; resting extrusomes surrounded by a heavily impregnated silverline ring, while minute argyrophilic granules mark sites of discharged extrusomes (Figs 4, 20, 23, 25, 51, 56). Cortex of "wild" specimens distinctly furrowed right of ciliary rows (Fig. 4), while almost smooth in cultivated specimens (Figs 35, 36, 38, 39, 57). Cytoplasm colourless, packed with food vacuoles, mainly in anterior body half, and some scattered, up to 5 µm-sized lipid droplets. In



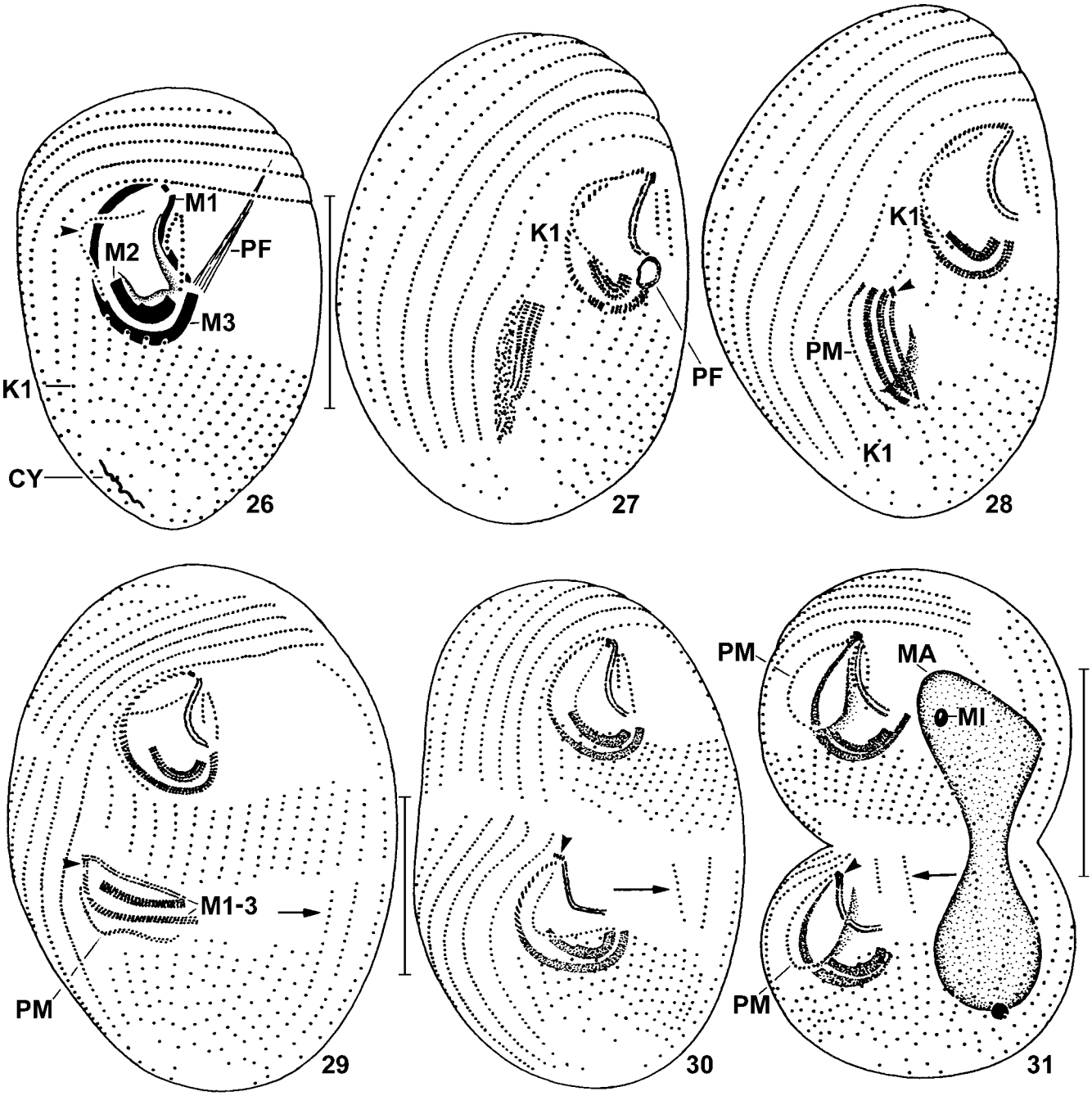
Figs 19-25. *Bromeliophrya brasiliensis* sp. n., silverline and ciliary pattern after Chatton-Lwoff silver nitrate impregnation. **19, 20** - right and left side view of same specimen. Note the conspicuous, loop-like outgrowths (IF) of the intrameridional silverlines in the second quarter of the right side and the wide-meshed silverline pattern in the large, unciliated area left of the oral apparatus. Right side kinety 2 extends to near dorsal side, where it abuts to the curved anterior end of the last dorsal kinety (arrowhead); **21** - dorsolateral view showing silverlines connecting last and penultimate dorsal kinety. Asterisk marks unciliated anterior pole area. Arrowhead denotes silverline connecting last lateral and first dorsal ciliary row; **22** - ventrolateral view showing deep buccal cavity; **23** - anterior portion showing the unciliated pole area (asterisk) partially occupied by the intermeridional connectives, which extend subapically; **24** - posterior polar view showing the thick cytopyge silverline in kinety 1; **25** - ventral view of a representative specimen showing many extrusomes (arrowhead), which are surrounded by minute silverline rings, attached to the silverlines in the unciliated area left of the oral apparatus. BC - buccal cavity; CY - cytopyge; E - extrusomes; EP - excretory pore of the contractile vacuole; IC - intermeridional connectives; IF - intrameridional cross-fibres; K1 - somatic kinety 1; OO - oral opening. Length of cells: 56 µm (19, 20), 48 µm (21), 52 µm (22), 57 µm (25). Scale bars 20 µm.

culture feeds exclusively on about 4 µm long bacterial rods digested in 4-6 µm-sized vacuoles, except the bacterial spores, which often impregnate with protargol and silver carbonate, making photographic documentation of the ciliary pattern difficult (Figs 1, 37, 62, 63). When undisturbed, cells dance conspicuously on bacterial flocks and accumulate at the dark side of the Petri dish; when transferred to the microscope slide, they swim rather rapidly, frequently changing direction by short jumps.

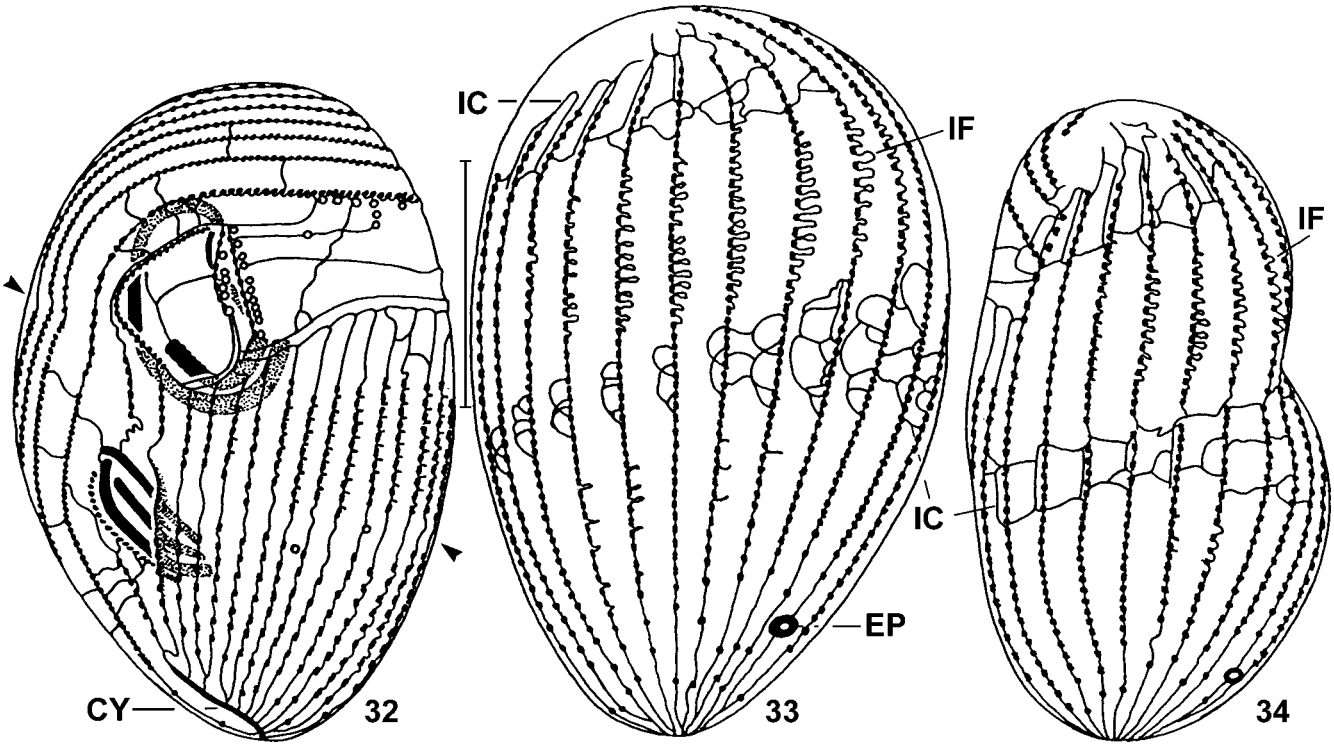
Ordinary somatic cilia about 10 µm long *in vivo*, single throughout; kinetids, however, composed of two obliquely arranged granules in silver carbonate and silver nitrate preparations, but only the larger posterior granule is ciliated, while the smaller anterior granule is likely a parasomal sac (Figs 1, 4, 5, 16, 17, 35, 38-40, 53, 57, 58). Caudal cilia in a roughly transverse array formed by last kinetids of some right side kineties, longer (15 µm) and stiffer than ordinary somatic cilia and thus well recognizable *in vivo* (Figs 1, 17, 38, 43). Ciliary pattern complicated by several unciliated areas and specializations (Figs 1, 5, 16-26, 35-39, 41-48; Table 1). On average 32 ciliary rows, those on right side slightly more widely spaced than those on left; distances between individual kinetids increase from anterior to posterior, especially in right side kineties, cells thus more densely ciliated anteriorly than posteriorly; accompanied by a fibre at right. Somatic kinety 1 is stomatogenic and distinctly shortened anteriorly, where it commences at right margin of oral opening, and posteriorly, where it abuts to the cytopyge. Right of kinety 1, six to eight, usually seven, very long ciliary rows, rectangularly curving around upper mouth margin and extending transversely as preoral kineties to near dorsal side of cell; kinety 2 loosely ciliated in oral area, abuts to curved end of last dorsal kinety; other preoral kineties do not abut, producing a rather large, elliptical, unciliated anterior pole area; transverse portion of all preoral rows very densely ciliated, while basal bodies lack cilia, except for caudal cilia, in kineties 3/4 to 8/9, that is, in posterior right half of cell, which is thus unciliated. Rest of right side and dorsal kineties extend longitudinally from anterior to posterior end of cell; last dorsal kinety anteriorly curved right to abut on kinety 2 as described above, producing an obtriangular, bare area, which contacts the unciliated anterior pole field, between last and penultimate dorsal kinety. Left side kineties strongly shortened and indistinguishable from postoral kineties (thus, all may be designated as postoral), producing large, bare area left of oral opening; bare area anteriorly bordered by kinety 2, at left by last dorsal kinety, and at right by two kinety fragments originating from leftmost postoral kineties (see ontogenesis). The silverline pattern shows that most of the postoral kineties abut to the cytopyge to form some kind of suture with kinety 1 (Figs 1, 5, 16-26, 35-39, 41-48; Table 1).

Oral opening in second quarter of cell, rounded triangular to broadly reniform due to a small convexity that bears membranelle 1 at left; margin slightly thickened; anteriorly and posteriorly smaller than buccal cavity, adoral membranelles thus only partially recognizable *in vivo* and scanning electron micrographs (Figs 1, 20, 22, 25, 35, 36, 39, 41, 57, 58, 60; Table 1). Buccal cavity about 14 µm deep and roughly hemispherical in silver nitrate preparations. Oral ciliature inconspicuous *in vivo* due to lack of paroral membrane and deep location of adoral membranelles, only cilia of membranelle 1 project distinctly from buccal cavity. Adoral membranelle 1 at left wall of buccal cavity, well recognizable *in vivo* and SEM micrographs because located on small convexity at margin of oral entrance, composed of a single row of cilia, at left accompanied by one or two argyrophilic lines in silver carbonate and protargol preparations, possibly unciliated kineties because at least two basal body rows are recognizable in early and middle dividers (Figs 28, 29). Adoral membranelle 2 extends on posterior bottom of buccal cavity and is thus concave and not recognizable in ordinary SEM preparations; ciliated *in vivo* and SEM of broken cells, composed of four ciliary rows, the upper row is distinctly shortened at right. Adoral membranelle 3 also deep in buccal cavity and thus only partially recognizable in SEM micrographs, extends along upper, right and posterior buccal wall and is thus large and C-shaped, composed of three ciliary rows with cilia more narrowly spaced in left than right and upper portion of organelle. "X-group", that is, a small array of about six basal bodies located between anterior ends of membranelles 1 and 3; likely, it originates from membranelle 2 (see ontogenesis). Paroral membrane extends along right and posterior half of oral entrance, unciliated and thus not recognizable *in vivo* and SEM micrographs; composed of single, weakly impregnated granules in protargol and silver nitrate preparations, while silver carbonate reveals groups of three granules in anterior half and paired granules in posterior half, or paired granules throughout. Pharyngeal fibres inconspicuous, recognizable only in protargol preparations, originate at left end of membranelles 1 and 3 and extend obliquely to anterior left quadrant of cell.

Silverline pattern complex, as shown by Figs 18-25 and 49-56, connects all main cortical organelles, such as



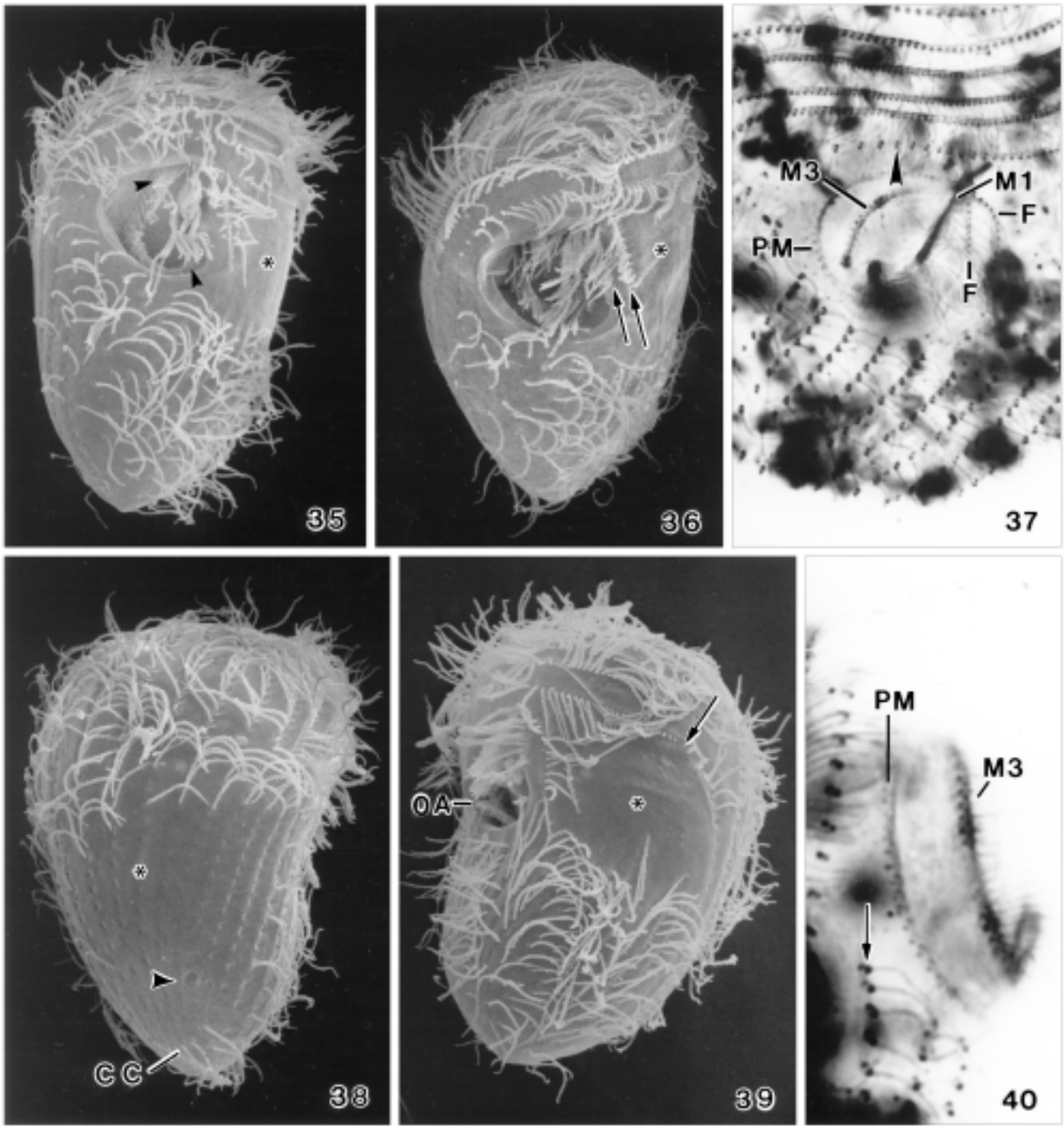
Figs 26-31. *Bromeliophrya brasiliensis* sp. n., ventral views of ciliary pattern of a morphostatic (26) and dividing (27-31) specimens after protargol impregnation with Wilbert's (26, 29, 30) and Foissner's (27, 28, 31) protocol. **26** - a representative, morphostatic specimen with pharyngeal fibres supplemented from another specimen. Note that the unciliated basal bodies of the paroral membrane (arrowhead) are smaller than those of the ciliated somatic kinetids; **27-31** - ontogenesis, especially stomatogenesis, occurs in the typical tetrahymenid manner. The parental oral apparatus is not involved and does not reorganize. Arrowheads mark a patch of basal bodies ("X-group"), likely separating from the anterior end of membranelle 2, which becomes four-rowed and distinctly shortens in middle and late dividers (29, 30). Arrows mark the two leftmost postoral kineties, which proliferate basal bodies anteriorly, producing kinetofragments which migrate to the left margin of the buccal cavity. Note that the shortened postoral and left lateral ciliary rows cause the division axis to become oblique in early dividers (28); however, the disparity is balanced by an increased growth of the left lateral area in middle dividers, generating the broad, blank area left of the buccal cavity and transverse cytokinesis in late dividers (29-31). CY - cytopyge; K1 - kinety 1; MA - macronucleus; MI - micronucleus; M1-3 - adoral membranelles; PF - pharyngeal fibres; PM - paroral membrane. Length of cells: 42 µm, 47 µm, 49 µm, 60 µm, 58 µm, 50 µm. Scale bars 20 µm.



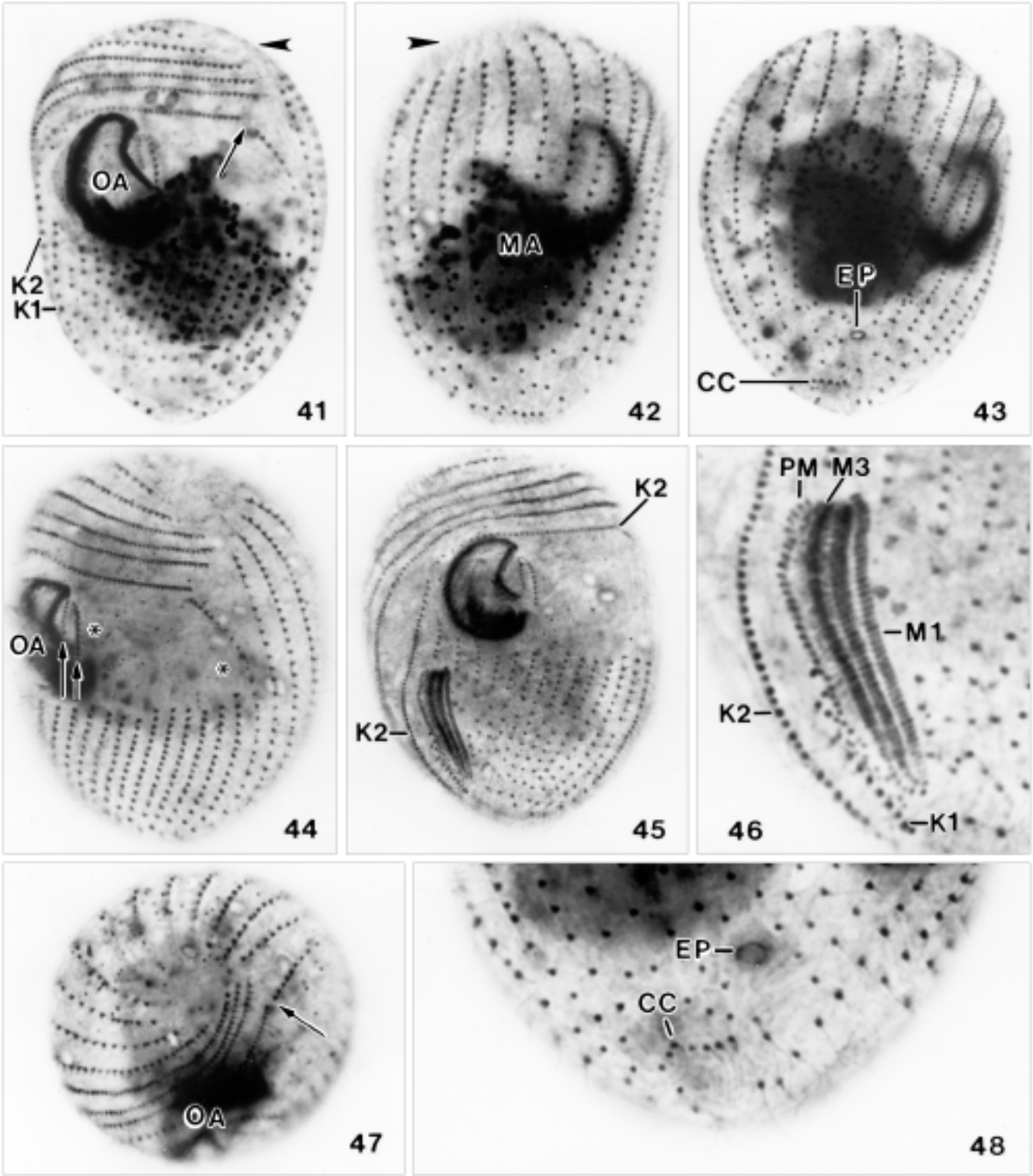
Figs 32-34. *Bromeliophrya brasiliensis* sp. n., ciliary and silverline pattern of middle (32, 33) and late (34) dividers after Chatton-Lwoff silver nitrate impregnation. **32** - ventral view of an early-middle divider showing the invaginating opisthe oral apparatus and the oblique division axis (arrowheads) produced by the strongly shortened left lateral ciliary rows (for details, see text and explanation to figures 27-31). Note that the dividing kineties remain connected by the intrameridional silverlines; **33, 34** - right side view of an early divider and left side view of a late divider showing the origin of the intermeridional connectives from foamy silverline arrays developing at the right margin of the left and right side and dorsal ciliary rows. The arrays develop underneath the prospective division furrow and are, likely, outgrowths of the intrameridional silverline connecting the basal bodies within a row. The proter (parental) connectives are not reorganized. Note that the loop-like intrameridional cross-fibres in the anterior portion of the right side ciliary rows develop very late and are thus not yet recognizable in the late divider shown in figure 34. CY - cytopyge; EP - excretory pore of contractile vacuole; IC - intermeridional connectives; IF - intrameridional cross-fibres; PM - paroral membrane. Length of cells: 56 µm, 61 µm, 54 µm. Scale bar 20 µm.

basal bodies, oral apparatus and excretory pore, and extends, left of oral apparatus, into the unciliated area, which is thus less conspicuous in silver nitrate than protargol preparations. Basal bodies of individual ciliary rows connected by an intrameridional silverline having conspicuous, loop-like outgrowths (intrameridional cross-fibres) in second quarter of right-side; silverlines of individual ciliary rows end in the unciliated anterior pole area or merge into the intermeridional connectives, posteriorly they merge into each other or abut to the cytopyge silverline. Intrameridional silverlines of shortened postoral and left lateral kineties extend into the unciliated area left of the oral apparatus to form a wide-meshed pattern with silverlines extending between oral apparatus, kinety 2, and last dorsal kinety. A wide-meshed silverline pattern occurs also in the buccal cavity and connects the adoral membranelles with each other and with some intrameridional somatic silverlines; short, longitudinal silverlines connect the preoral kineties. A single, narrow area with intermeridional connectives, probably originating from intrameridional silverlines, occurs subapically and extends via anteriorly directed, straight silverlines also into the unciliated anterior pole area. Resting extrusomes surrounded by a minute silverline, found mainly left of kineties in the intrameridional cross-fibres and attached to the silverlines crossing the unciliated area left of oral apparatus.

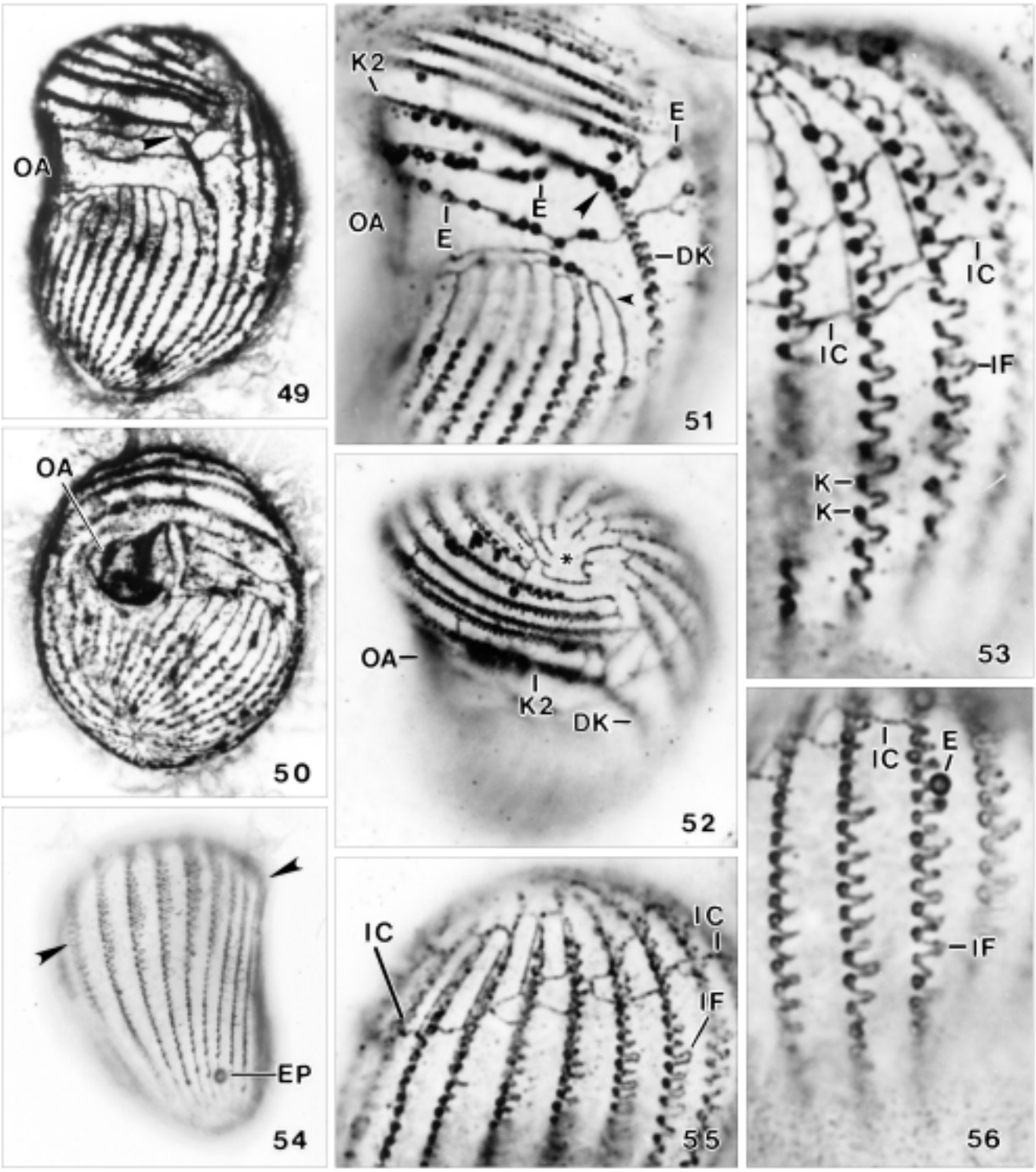
Ontogenesis (for terminology, see Foissner 1996) of *B. brasiliensis* is homothetogenic and occurs in freely-motile (non-encysted) condition. Stomatogenesis is monoparakinetal, as in most members of the group, and the parental oral apparatus is transmitted to the next generation without distinct signs of reorganization, which must be very rare in interphase specimens because I did



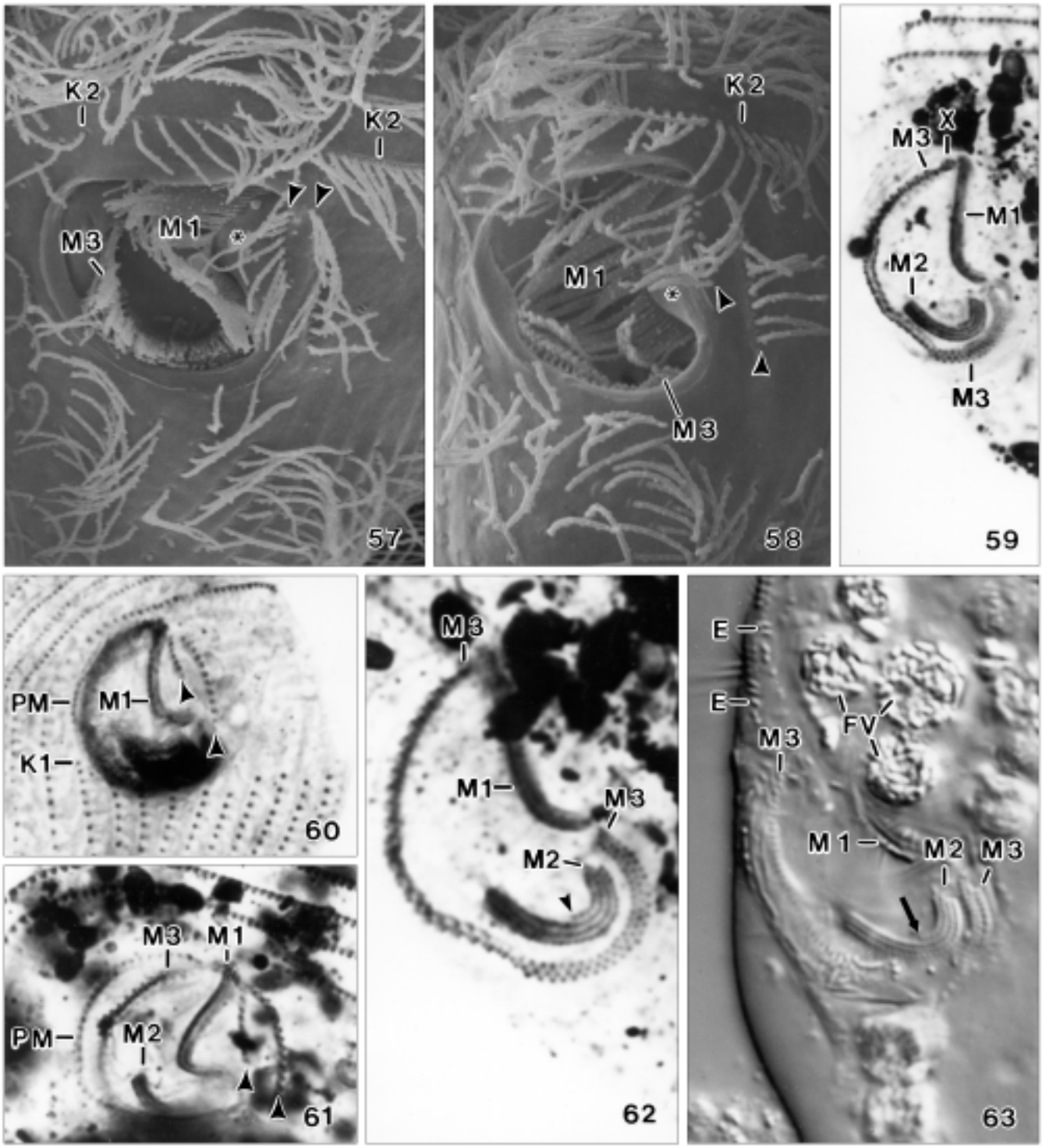
Figs 35-40. *Bromeliophrya brasiliensis* sp. n. in the scanning electron microscope (35, 36, 38, 39) and after silver carbonate impregnation (37, 40). **35, 36** - ventral views showing oral apparatus in second quarter of cell and metopid arrangement of ciliary rows. Asterisks denote barren area left of oral opening. Arrows mark two short ciliary rows, which migrate from dorsolateral to the oral opening, a main family character. Arrowheads denote the large, C-shaped adoral membranelle 3; **38, 39** - right and left side view showing a main feature of the Bromeliophryidae family, viz., the two large, unciliated areas (asterisks): the right posterior half of the cell, where, however, basal bodies are present (Figs 42, 43); and a quadrangular field left of the oral opening, where even basal bodies are lacking. Arrowhead marks excretory pore of contractile vacuole. Arrow denotes site where right side ciliary row 2, which extends preorally and left laterally, abuts to the last dorsal kinety; **37, 40** - oral area of strongly squashed specimens, showing the inconspicuous, unciliated paroral membrane. Note that, in silver carbonate preparations, the kinetids consist of oblique pairs of granules (arrow): the smaller anterior granule is barren and likely a parasomal sac, while the larger posterior granule bears a cilium. Arrowhead in figure 37 marks kinety 2, which is loosely ciliated right of the oral opening. CC - caudal cilia; F - kinety fragments; M1, 3 - adoral membranelles; OA - oral apparatus; PM - paroral membrane.



Figs 41-48. *Bromeliophrya brasiliensis* sp. n., somatic ciliary pattern (41- 44, 47, 48) and ontogenesis (45, 46) after protargol impregnation (Wilbert's method). **41, 42, 47** - ventro-and dorsolateral view of same specimen and anterior polar view of another cell. Note the elliptical, unciliated anterior pole area (arrowheads) and kinety 2, which abuts on the last dorsal kinety (arrows); **43, 48** - dorsal views showing the transverse row of caudal cilia and the unciliated basal bodies of the right side (cp. figure 38); **44** - left side view showing the large, unciliated area left of the oral opening (asterisks), posteriorly bordered by the shortened postoral and left lateral kineties. Arrows mark kinety fragments left of oral opening; **45, 46** - ventral view of an early divider with a conspicuous, dikinetidal paroral membrane. CC - caudal cilia; EP - excretory pore; K1, 2 - somatic kineties; M1, 3 - adoral membranelles; MA - macronucleus; OA - oral apparatus; PM - paroral membrane.



Figs 49-56. *Bromeliophrya brasiliensis* sp. n., silverline and ciliary pattern after Klein-Foissner (49, 50) and Chatton-Lwoff (51-56) silver nitrate impregnation. **49, 51** - left side views showing site where right side kinety 2 abuts on the last dorsal kinety (large arrowheads). Small arrowhead denotes intrameridional silverlines extending into the unciliated area left of oral opening. **50** - ventral view; **52** - the anterior pole (asterisk) is unciliated; **53-56** - right side views showing the loop-like projecting intrameridional cross-fibres (arrowheads in 54) and the subapical intermeridional connectives (IC). DK - last dorsal kinety; E - extrusomes; EP - excretory pore; K - kinetids; K2 - kinety 2; IC - intermeridional connectives; IF - intrameridional cross-fibres; OA - oral apparatus.



Figs 57-63. *Bromeliophrya brasiliensis* sp. n., oral apparatus in the SEM (57, 58), after silver carbonate (59, 61, 62) and protargol (60) impregnation, and *in vivo* (63). Arrowheads in figures (57, 58, 60, 61) mark two kinety fragments at left margin of oral opening. These fragments have a special origin described in the ontogenesis section. Arrow in figure (63) marks shortened kinety in membranelle 2. Asterisks in figures (57, 58) denote convex left margin of oral opening. Basically, the oral apparatus is in the second quarter of the cell (Figs 35, 36) and has a broadly reniform opening (Figs 57, 58). The buccal cavity is rather deep (Fig. 22) and contains three membranelles of different shape and structure (Figs 57-63) described in the results section and diagramatically shown in figure (5). A small patch of basal bodies ("X-group") is between the anterior ends of membranelles 1 and 3 (Fig. 59). The paroral membrane consists of single, unciliated basal bodies (Figs 60, 61) and is thus not recognizable in scanning micrographs (Figs 57, 58). E - extrusomes; FV - food vacuoles; K1, 2, - somatic kineties; M1-3 - adoral membranelles; PM - paroral membrane; X - "X-group" (patch) of basal bodies.

not find a single reorganizer among more than a thousand specimens investigated. Nuclear division and cytokinesis occur as is usual.

Stomatogenesis commences with the formation of an oral primordium in or very near to kinety 1, that is, the posterior half of the rightmost postoral ciliary row, with an intense proliferation of basal bodies, soon forming three axially oriented protomembranelles each consisting of two basal body rows; at right, scattered basal bodies remain and later form the paroral membrane; the anterior portion of kinety 1 is not involved in stomatogenesis and later reorganizes to a new proter kinety 1 (Figs 27, 28). I could not clarify whether basal bodies of kinety 1 are included in the oral primordium. Figures 28 and 45, 46 indicate that this is not the case because a posterior fragment with an ordinary number of basal bodies becomes recognizable after the paroral has formed. Next, protomembranelles 2 and 3 become three-rowed, a patch of basal bodies ("X-group") separates from the anterior end of membranelle 2, and the posterior portion of the membranelles begins to invaginate and rotate clockwise. At right, the paroral is formed from the scattered basal bodies of the oral primordium, leaving back a short (parental?) row of basal bodies forming opisthe's kinety 1 (Figs 28, 32, 45, 46). In middle dividers (Fig. 29), the newly formed oral structures arrange transversely to the main body axis, membranelle 2 becomes four-rowed and begins to shorten, and the paroral membrane is distinctly dikinetidal. I could not clarify whether the fourth row of membranelle 2 originates de novo or by a re-arrangement of existing basal bodies; the latter is more likely, considering that the membranelle becomes shorter. When cytokinesis commences, the newly formed oral structures invaginate and obtain their specific shape and arrangement; further, the dikinetidal structure of the paroral becomes indistinct (Figs 30, 31).

Somatogenesis commences in early dividers with the intrakinetal proliferation of basal bodies and a rather early split of the ciliary rows in the prospective division furrow (Figs 27-29), except row 2, which splits only in late dividers, that is, when cytokinesis commences (Fig. 30). The rows split in the middle, even the strongly shortened postoral and left lateral kineties, producing an oblique division axis in early dividers (Figs 28, 29, 34). However, the disparity is balanced by an increased growth of the left side in middle dividers, generating the blank area left of the buccal cavity and transverse cytokinesis in late dividers (Figs 29-31). At the left margin of the blank area, two opisthe ciliary rows generate additional basal bodies forming two projecting kinetofragments (Fig. 29, arrow), which separate (Fig. 30) and migrate (Fig. 31) to the left margin of the buccal cavity (Fig. 26).

The silverlines connect all parental and newly-formed structures, including the separating ciliary rows (Fig. 32). The parental silverline system is not reorganized. The intermeridional connectives originate from foamy silverline arrays developing at the right margin of the right side and dorsal ciliary rows. The arrays are generated underneath the prospective division furrow and are, likely, outgrowths of the intrameridional silverline connecting the basal bodies within a row (Figs 33, 34). The loop-like intrameridional cross-fibres in the anterior portion of the right side ciliary rows develop in very late dividers.

DISCUSSION

Ordinal and familiar classification

Bromeliophrya has all main features of a tetrahymenide ciliate (Corliss 1979, Lynn 1994), specifically, it has a paroral membrane and three adoral membranelles in a deep buccal cavity and in tetrahymenid position (subapical) and arrangement (Figs 1, 5, 22, 35, 57-62). Further, it has a monoparakinetal stomatogenesis (Fig. 27) and a striated silverline pattern (Figs 19, 20, 49, 53, 54), as all "good" tetrahymenids have (Foissner 1996). Thus, *Bromeliophrya* belongs to the order Tetrahymenida Fauré-Fremiet in Corliss (1956).

Familiar classification is more difficult, although the "X-group" of basal bodies between the anterior ends of adoral membranelles 1 and 3 (Figs 5, 30, 59), the curved anterior portion of some right side somatic kineties (Figs 16, 20, 25, 35, 39, 41, 49), and ontogenesis (transverse orientation of oral anlagen in middle dividers; Fig. 29) suggest the Glaucomidae Corliss, 1971 as nearest relatives (Corliss 1971, Peck 1974, McCoy 1975, Foissner *et al*. 1994, Lynn 1994). In contrast, the somatic ciliature with the two large, unciliated areas and the migrating kinety fragments is unique for the whole order, suggesting separation of *Bromeliophrya* at family level (Figs 16, 29-31, 38, 39). This is emphasized by the unique, metopid body plan (shape) and the silverline pattern, which lacks the secondary meridians so prominent in many tetrahymenids, especially *Glaucoma* and *Colpidium* (Foissner *et al*. 1994). Note that the kineties at the left margin of the buccal cavity of, e.g., *Colpidium*

and *Turaniella*, are also fragments of somatic kineties, however, they do not migrate, as in *Bromeliophrya*, but originate by a simple split of kineties during buccal shaping (Foissner 1970, Iftode *et al*. 1984).

The high (family) rating of the somatic peculiarities of *Bromeliophrya* is based on the Structural Conservatism Hypothesis of Lynn (1976, 1981), which suggests that somatic cortical features are more strongly conserved than oral ones because they are subjected to less selective pressures. Accordingly, the particularities of the oral structures define the new genus *Bromeliophrya*, while those of the body define the new family Bromeliophryidae.

Bromeliophrya brasiliensis as a new genus and species

As mentioned above, the new genus *Bromeliophrya* is defined by its oral structures which are unique in that membranelles 1 and 3 extend along the buccal wall and thus form a ring-like pattern (Figs 5, 25, 26, 57-63), similar to that found in another curious genus, *Bursostoma*, which belongs either to the tetrahymenids or the ophryoglenids (Ganner *et al*. 1988, Lynn *et al*. 1991). Basically, however, the oral apparatus of *Bromeliophrya* resembles that of *Glaucoma*, not only by the X-group of basal bodies at the anterior end of membranelle 2, but also due to the loosened ciliary rows composing the anterior half of membranelle 3; membranelle 2, in contrast, is much more conspicuous in *Glaucoma* than *Bromeliophrya* (Corliss 1971, McCoy 1975, Foissner *et al*. 1994).

Another remarkable feature of *Bromeliophrya* is the unciliated paroral membrane (Figs 5, 26-31, 57-62). In this respect, *Bromeliophrya* resembles *Turaniella* (Iftode *et al*. 1984), which Lynn (1994) classifies in the family Turaniellidae, together with *Colpidium*, which has the paroral ciliated, like *Tetrahymena* in the family Tetrahymenidae. In *Glaucoma*, only the anterior portion of the paroral is ciliated. See Foissner *et al*. (1994) for SEM-micrographs of the paroral of *Tetrahymena*, *Glaucoma*, and *Colpidium*. Obviously, this feature is too progressive to be useful for family classification, but useful for defining genera.

To the best of my knowledge, there is no ciliate described in the literature resembling *B. brasiliensis*. Thus, it is a new species. Both the metopid body shape and the unique somatic ciliary pattern make it easily recognizable *in vivo* and silver preparations (Figs 1, 16, 39, 41-44).

Biogeographic considerations

As mentioned in the Introduction, I found several new ciliates in the tank of Brazilian bromeliads. Most are rather small (~ 100 µm) and thus comparatively inconspicuous. Accordingly, it can not be excluded that they occur also in ordinary freshwaters of South America, but were overlooked up to now. Unfortunately, reliable studies on free-living ciliates of South America are practically non-existant. Most of the literature is outdated, and even the few recent studies did not use modern methods, for instance, Cairns (1966) and Hardoim and Heckman (1996). But there is evidence that the South American ciliate biota contain a large number of undescribed species. Foissner (1997) reported 20 new species in two soil samples from the Amazon flood plain, and Steffens and Wilbert (2002) found several undescribed species in ephemeral flushs of Brazilian inselbergs.

However, we can be rather sure that *Bromeliophrya brasiliensis* and the other new species from tank bromeliads are absent in the much better investigated central Europe, suggesting that they have a restricted geographical distribution, like some other ciliates and testate amoebae (Foissner 1999). There is even some probability that they are restricted to bromelian tanks because one of them has a special life cycle (Foissner and Cordeiro 2000).

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