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ORIGINAL ARTICLE

Morphology of *Bromeliophrya quadristicha* n. spec., an Inhabitant of Tank Bromeliads (Bromeliaceae), and Phylogeny of the Bromeliophryidae (Ciliophora, Tetrahymenida)

Wilhelm Foissner[a] & Thorsten Stoeck[b]

a Department of Organismic Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria
b Department of Ecology, University of Kaiserslautern, Erwin-Schrödinger-Strasse 14, D-67663 Kaiserslautern, Germany

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Correspondence
W. Foissner, University of Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria
Telephone number: +43-(0)662-8044-5615;
FAX number: +43-(0)662-8044-5698;
e-mail: wilhelm.foissner@sbg.ac.at

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ABSTRACT

Using morphological, morphometric, and molecular methods, we describe *Bromeliophrya quadristicha* n. spec. from tank bromeliads of Jamaica and the Dominican Republic. The new species differs from the single congener, *B. brasiliensis*, mainly in having four (vs. 2) left lateral kinetofragments, 23 (vs. 32) ciliary rows, and a short (vs. long and C-shaped) adoral membranelle 3. Both the morphological and molecular phylogenies show *Bromeliophrya* and *Glaucomides* as sister group of the Glaucomidae. Thus, they should have the same (family) rank.

TANK bromeliads contain a rich protistan and metazoan fauna (Foissner et al. 2003; Kitching 2004). Several of these species are highly adapted to this habitat, both in protists (e.g. *Bromeliothrix metopoides*; Foissner 2010) and in metazoa (e.g. *Metopaulias depressus*; Diesel and Schubart 2000). As concerns protists, reliable data are available only for ciliates, where Foissner and colleagues discovered about 40 new species in about 200 bromeliad samples, mainly from the Caribbean area (Dunthorn et al. 2012; Foissner et al. 2003). To discover these new ciliates, which are usually among a mass of euryoecious cosmopolitans, and to describe them properly is a great challenge. As yet, we described the following species (ordered after year of publication): *Bromeliophrya brasiliensis* Foissner 2003a (a new genus); *Lambornella trichoglossa* Foissner 2003b; *Coriplites proctori* Oertel et al. 2008; *Platyophrya bromelicola* Foissner and Wolf 2009; *Orborhabdostyla bromelicola* Foissner et al. 2009 (a new genus); *Bromeliothrix metopoides* Foissner 2010 (a new genus); *Leptopharynx bromelicola* Foissner et al. 2011; *Leptopharynx bromeliophilus* Omar and Foissner 2011; *Cotterillia bromelicola* Foissner and Stoeck 2011; and *Glaucomides bromelicola* Foissner 2013 (a new genus). Some other new genera and species are briefly described in Foissner et al. (2003). Very likely, most of these species occur only in bromeliads because we could not find them in ponds or rivers of the respective region.

Here, we describe a small tetrahymenid, *Bromeliophrya quadristicha*, which is rather common in tree bromeliads of the Dominican Republic and Jamaica. The larger congener, *B. brasiliensis*, occurs in the tanks of large terrestrial bromeliads on the east coast of Brazil (Foissner 2003a). Furthermore, the morphological and molecular data are now sufficient to discuss the family Bromeliophryidae Foissner 2003a, which Lynn (2008) synonymized with the Glaucomidae Corliss 1971.

MATERIALS AND METHODS

Material

Bromeliophrya quadristicha was discovered in various tank bromeliads from the botanical garden on the Pico Isabel de Torres, a 800 m high mountain on the outskirts of the

town of Puerto Plata, Dominican Republic, 19°15′N 70°40′ W (type locality). In Jamaica, *B. quadristicha* occurred at several sites. The population studied is from epiphytic bromeliads on the northern slope of the Blue Mountains, surroundings of the village of Silver Hill, where *Leptopharynx bromelicola* Foissner et al. 2011 was discovered (18°12′N 76°40′W).

Nonclonal "raw" cultures containing several ciliates and flagellates were set up in Eau de Volvic (French mineral water) enriched with two to four squashed wheat grains to stimulate growth of indigenous bacteria, the food of *B. quadristicha*. The species did not thrive well and was soon overgrown by *Glaucomides bromelicola* Foissner 2013, another frequent bromeliad ciliate.

Morphological methods

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 several silver impregnation methods (protargol, silver nitrate after Chatton-Lwoff and Klein-Foissner), all described in Foissner (1991).

Counts and measurements on prepared specimens were performed at a magnification of 1,000×. In vivo measurements were conducted at magnifications of 100 to 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. Terminology is according to Corliss (1952, 1979), Foissner (2003b), and Lynn (2008).

Molecular methods

The 18S rDNA of the Jamaican type population of *B. quadristicha* was obtained and analyzed as described in detail previously (Kreutz et al. 2012). In short, genomic DNA was extracted from a mix of 20 *B. quadristicha* using the protocol for cultivated animal cells of the DNEasy Tissue Kit (Qiagen, Hildesheim, Germany). 18S rRNA genes were amplified using the universal eukaryotic primers 82F and 1492R (Lopez-Garcia et al. 2001; Medlin et al. 1988), according to the protocol in Kreutz et al. (2012). PCR products were purified (PCR MinElute Kit, Qiagen, Hildesheim, Germany) and cloned (TA-Cloning Kit, Invitrogen, Carlsbad, CA). Isolated plasmids (Qiaprep Spin Miniprep Kit, Qiagen) were sequenced bidirectionally (M13 sequence primers) with the Big Dye terminator kit (Applied Biosystems, Foster City, CA) on an ABI 3730 automated sequencer.

Sequence alignments were conducted with MUSCLE (Edgar 2004) and refined using Gblocks (Castresana 2000). The resulting curated alignment included 1695 characters and 17 taxa, two of which *Ophryoglena catenula* and *Ichthyophthirius multifiliis* (Ophryoglenina) were included as an out-group for the subsequent phylogenetic tree analyses. The alignment is available from the authors upon request. Evolutionary distance and maximum-likelihood analyses were conducted for phylogenies. Neighbor-joining evolutionary distances (BioNJ) were carried out in the Seaview program package (vers. 4.2, Galtier et al. 1996). Maximum-likelihood bootstrapping analyses were carried out with 1,000 replicates using RAxML with the setting as described by Stamatakis et al. (2008). Pairwise sequence similarities were calculated with the module pairalign, as implemented in the JAguc software package (Nebel et al. 2011).

RESULTS

Morphological description of *Bromeliophrya quadristicha*

Body size (Table 1)

The size of *B. quadristicha* is ordinarily variable, as shown by the moderate variation coefficients ($\leq$ 15%). The average values of body length are similar in the two populations, while body width is considerably different, possibly indicating 30% preparation shrinkage in the protargol slides. When taking into account the in vivo measurements and 5% preparation shrinkage in the osmium-fixed silver nitrate preparations, *B. quadristicha* has a usual in vivo size of about 40 × 30 μm (extremes: 30–50 × 20–40 μm) and a length:width ratio of about 1.4:1. *Bromeliophrya quadristicha* is not or only slightly flattened laterally.

Body shape (Table 1; Fig. 1, 3, 4, 15, 24, 25, 27, 30, 31, 35, 38–40)

When observed laterally, *B. quadristicha* is *Metopus*-shaped, i.e. has a slightly projecting, broadly convex preoral dome and a slightly narrowed, obconical uvula (postoral portion). The dome occupies about one-third of body length, and the ventral projection is usually less distinct in the preparations than in vivo, making cells more or less obovate. The uvula is highly variable, especially the body end which may be broadly rounded (Fig. 1, 27, 30), acute (Fig. 39, 40) or, in 2 of 100 cells, even tailed (Fig. 31). When observed ventrally or dorsally, *B. quadristicha* is obovate (Fig. 15, 24, 25) or ellipsoidal (Fig. 4).

Nuclear apparatus (Table 1; Fig. 1, 3, 13, 26, 27, 29–31)

The nuclear apparatus is posterior of mid-body on average. The macronucleus, which has a size of about 13 × 11 μm in vivo, is globular to broadly ellipsoidal and contains many minute accumulations of argyrophilic material, possibly nucleoli. The micronucleus is attached to the macronucleus and about 2 μm in size; frequently, it does not impregnate with the protargol method used.

Contractile vacuole and cytopyge (Table 1; Fig. 1, 6, 13, 18, 21, 28, 34, 39)

On average, the excretory pore of the contractile vacuole is 23% from the posterior body end, i.e. distinctly subterminal. The pore, which has a diameter of about 2 μm, is associated with either kinety 5 (Jamaican population) or kinety 6 (Dominican population). The cytopyge is at the end of kinety 1 and appears as a thickened line in silver nitrate prepared cells (Fig. 18, 34).

Table 1. Morphometric data on *Bromeliophrya quadristicha* impregnated with protargol (P, Jamaican population) and the Chatton-Lwoff silver nitrate method (CHL, Dominican Republic population).

Characteristics[a]	Method	$\bar{x}$[e]	*M*	SD	SE	CV	Min	Max	*n*
Body, length (μm)	P	32.9	34.0	3.2	0.7	9.8	25.0	37.0	21
	CHL	37.3	37.0	5.0	1.1	13.5	29.0	47.0	21
Body, width in lateral view (μm)	P	21.0	20.0	3.3	0.7	15.7	15.0	27.0	21
	CHL	30.2	31.0	4.0	0.9	13.2	22.0	36.0	21
Body length:width, ratio	P	1.6	1.5	0.3	0.1	19.5	1.2	2.3	21
	CHL	1.3	1.3	0.2	0.1	14.2	0.9	1.7	21
Body, width in ventral view (μm)	P	20.6	21.0	2.7	0.6	13.2	16.0	26.0	21
	CHL	28.2	27.0	5.1	1.3	18.2	22.0	37.0	17
Anterior body end to upper mouth margin, distance (μm)	P	9.7	10.0	1.5	0.3	15.8	7.0	13.0	21
	CHL	12.4	12.0	3.8	0.8	30.4	7.0	20.0	21
Anterior body end to lower mouth margin, distance (μm)	P	17.9	18.0	1.5	0.3	8.5	15.0	21.0	21
	CHL	19.1	19.0	3.1	0.7	16.4	15.0	26.0	21
Anterior body end to adoral membranelle 3, distance (μm)	P	10.5	11.0	1.5	0.3	8.5	8.0	14.0	21
	CHL	14.6	13.0	3.8	0.8	26.2	7.0	20.0	21
Anterior body end to proximal summit of adoral membranelle 3, distance (μm)	P	17.0	17.0	1.2	0.3	7.2	14.0	19.0	21
	CHL	20.8	21.0	2.9	0.6	14.1	16.0	26.0	21
Anterior body end to macronucleus, distance (μm)	P	13.2	14.0	2.8	0.6	20.9	5.0	16.0	21
	CHL	18.1	18.0	2.8	0.6	15.4	13.0	22.0	21
Anterior body end to distal end of first preoral kinety (= K2), distance (μm)	P	7.1	7.0	1.5	0.3	20.9	5.0	10.0	21
	CHL	10.1	10.0	3.4	0.7	33.5	5.0	17.0	21
Anterior body end to last postoral kinety, distance (μm)	P	17.9	17.0	2.3	0.5	13.1	14.0	23.0	21
	CHL	20.4	20.0	3.7	0.8	18.2	15.0	27.0	21
Posterior body end to excretory pore, distance (μm)	P	7.3	7.0	1.5	0.3	20.0	4.0	10.0	21
	CHL	8.7	9.0	2.6	0.6	30.4	3.0	12.0	21
Macronucleus, length (μm)	P	12.6	12.0	2.0	0.4	16.0	9.0	17.0	21
	CHL	12.8	12.0	2.1	0.5	16.8	10.0	18.0	21
Macronucleus, width (μm)	P	10.3	10.0	1.7	0.4	16.6	6.0	13.0	21
	CHL	10.6	10.0	2.0	0.4	20.0	8.0	15.0	21
Micronucleus, length (μm)	P	2.2	2.0	0.5	0.1	20.8	1.8	4.0	21
	CHL	2.0	2.0	0.2	0.1	7.9	2.0	3.0	21
Micronucleus, width (μm)	P	1.8	2.0	0.2	0.1	12.6	1.3	2.0	21
	CHL	1.8	2.0	0.2	0.1	12.9	1.5	2.0	21
Oral opening, width[b] (μm)	P	7.7	7.0	1.0	0.2	13.1	6.0	10.0	21
	CHL	9.1	9.0	1.2	0.3	13.0	7.0	11.0	21
Oral opening, width[c] (μm)	P	6.5	6.0	1.2	0.3	18.0	5.0	9.0	21
	CHL	7.3	7.0	1.1	0.3	14.7	6.0	10.0	21
Buccal cavity, depth[d] (μm)	P	8.9	9.0	1.2	0.3	13.0	6.0	11.0	21
	CHL	8.4	8.0	1.3	0.3	15.7	6.0	10.0	21
Somatic kineties, total number (without kinety fragments)	P	23.6	24.0	0.9	0.2	3.9	21.0	25.0	21
	CHL	23.1	23.0	1.3	0.3	5.7	21.0	25.0	21
Postoral kineties, number (ex. somatic kinety 1)	P	4.6	5.0	0.7	0.1	14.3	3.0	5.0	21
	CHL	4.1	4.0	0.7	0.2	16.0	3.0	5.0	21
Left lateral kinety fragments, number (= left-side kineties)	P	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
	CHL	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
Kinetids in somatic kinety 2, number	P	56.0	54.0	9.5	2.1	16.7	40.0	82.0	21
	CHL	55.3	58.0	7.9	1.7	14.2	45.0	70.0	21
Kinetids in postoral kinety 7, number	P	10.1	9.0	3.0	0.7	29.9	5.0	17.0	21
	CHL	10.8	10.0	3.1	0.7	28.5	6.0	18.0	21
Kinetids in left lateral fragment 1, number	P	9.8	10.0	2.1	0.5	21.3	6.0	13.0	21
	CHL	9.1	9.0	1.1	0.2	12.1	7.0	12.0	21
Kinetids in left lateral fragment 2, number	P	7.9	8.0	1.1	0.2	14.1	6.0	10.0	21
	CHL	7.3	7.0	1.1	0.2	14.5	6.0	10.0	21
Excretory pore in kinety	P	5.1	5.0	0.5	0.1	9.9	4.0	6.0	21
	CHL	6.0	6.0	0.0	0.0	0.0	6.0	6.0	21

[a]Data based on cultivated, mounted, silver-impregnated and randomly selected, morphostatic specimens.
[b]Distance from paroral membrane to first kinety left of oral opening (= kinety fragment 1).
[c]Distance from left margin of adoral membranelle 1 to right margin of membranelle 3.
[d]From laterally orientated specimens, e.g. Fig. 1, 27.
[e]CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of mean; $\bar{x}$, arithmetic mean.

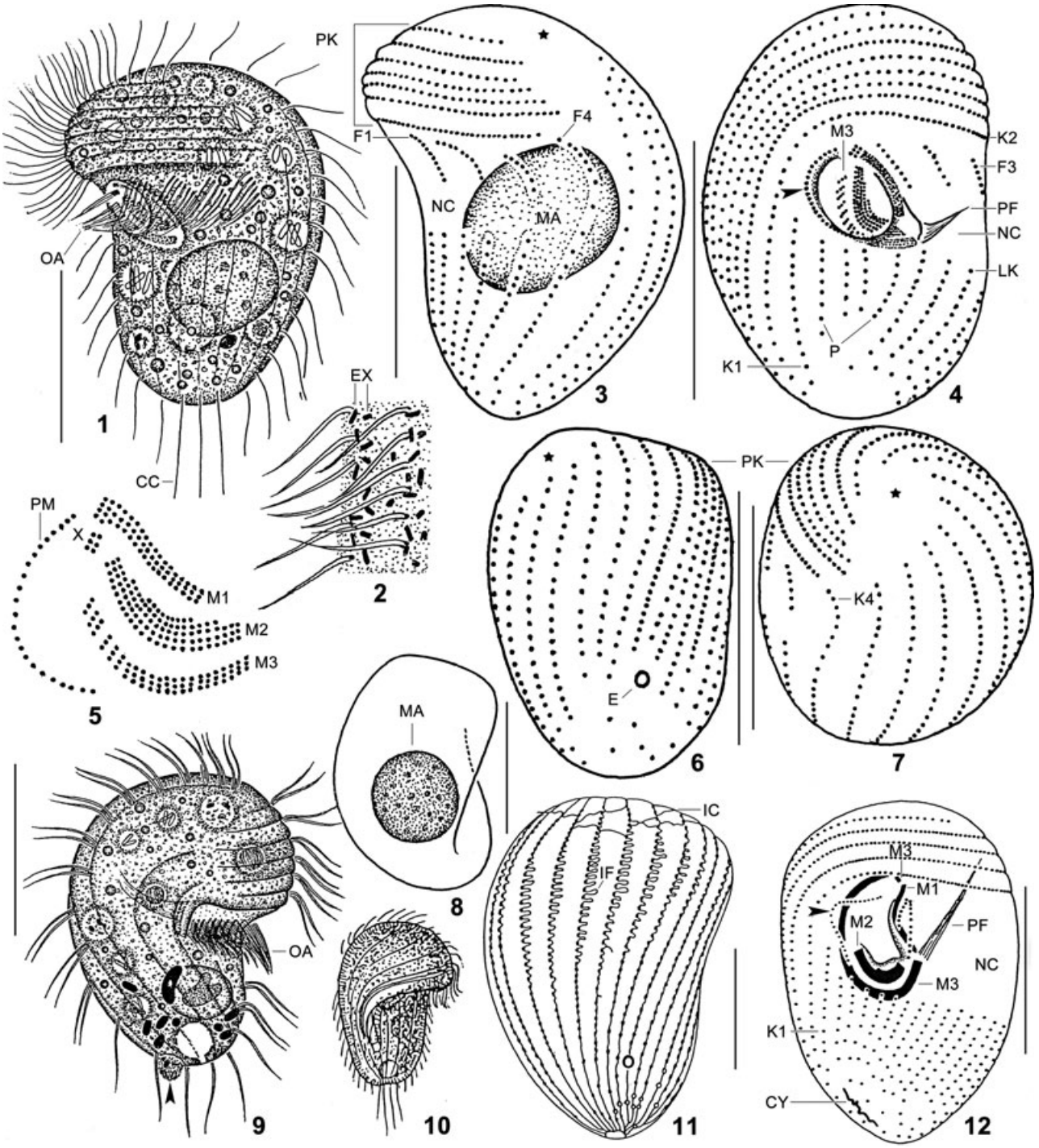

Fig. 1–12. *Bromeliophrya quadristicha* (1–8) and morphologically similar species (9–12) from life (1, 2, 9, 10), after protargol impregnation (3–8, 12), and in a silver nitrate preparation (11). Asterisks mark the large, nonciliated anterior pole region. **1.** Left-side view of a representative specimen, length 40 μm. **2.** Surface view of cortex, showing extrusomes. **3.** Left-side view of ciliary pattern. **4.** Ventral view of holotype specimen, length 32 μm. The arrowhead marks the nonciliated paroral. **5.** Scheme of oral structures. **6.** Right-side view. Most of the basal bodies of the posterior half are not ciliated. **7.** Oblique anterior polar view. **8.** A specimen with overhanging preoral region. **9.** Right-side view of *Bromeliothrix metopoides*, length 56 μm (from Foissner 2010). Arrowhead marks fecal mass. **10.** The shape of certain *Metopus* species, e.g., *M. pullus* (80 μm), is highly similar to that of *Bromeliothrix metopoides* and *Bromeliophrya* spp. (from Kahl 1932). **11, 12.** Right-side and ventral ciliary and silverline (11) pattern of *B. brasiliensis*, length 56 μm and 42 μm (from Foissner 2003a). The arrowhead marks the nonciliated paroral. CC, caudal cilia; CY, cytopyge; E, excretory pore; EX, extrusomes; F1–4, kinetofragments; IC, intermeridional connectives; IF, intrameridional cross-silverlines; K1, 2, somatic kineties; LK, left-side kineties; MA, macronucleus; M1–3, adoral membranelles; NC, nonciliated area; OA, oral apparatus; P, postoral kineties; PF, pharyngeal fibers; PK, preoral kineties; PM, paroral; x, X-body. Scale bars: 20 μm.

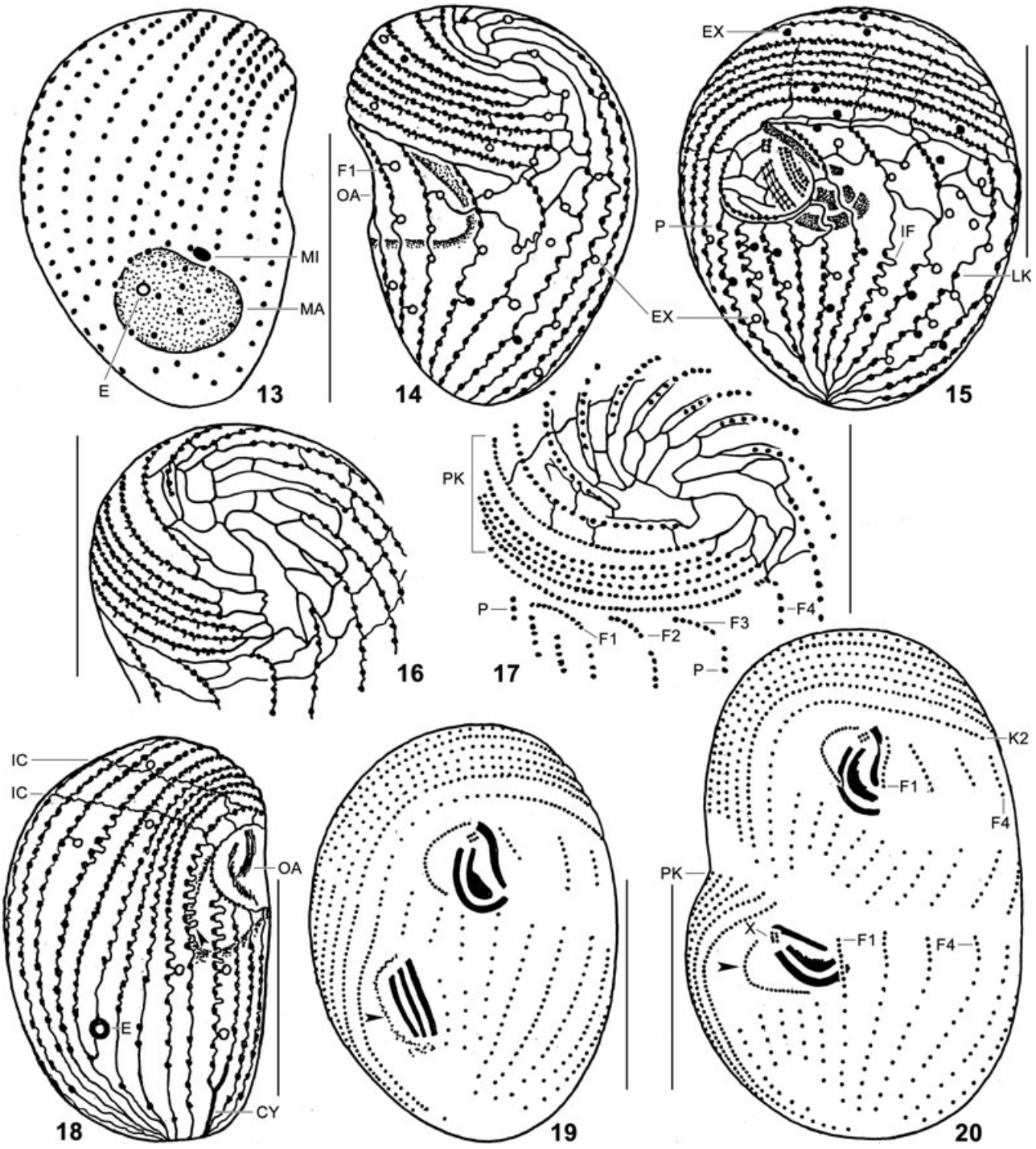

Fig. 13–20. *Bromeliophrya quadristicha*, morphostatic cells after silver nitrate impregnation (13–18) and dividers after protargol impregnation (19, 20). **13, 14.** Right and left-side view of basal body and silverline pattern (14) of same specimen. The basal bodies in posterior third of the right side are not ciliated. **15.** Ventrolateral view of basal body and silverline pattern. When the extrusomes are attached to the cortex, they appear as minute circles, when they were just released, a granule becomes recognizable. **16, 17.** Anterior polar views of silverline pattern. **18.** Ventrolateral view of basal body and silverline pattern. The arrowhead marks loop-shaped intrameridional cross-silverlines. **19.** An early divider, showing the origin of the paroral from many minute granules (arrowhead). **20.** Late divider, showing the origin of the four kinetofragments (F1–4) from a split of the left-side kineties. The arrowhead marks the paroral. CY, cytopyge; E, excretory pore; EX, extrusomes; F1–4, kinetofragments; IC, intermeridional connectives; IF, intrameridional cross-silverlines; K, somatic kinety 2; LK, left-side kineties; MA, macronucleus; MI, micronucleus; OA, oral apparatus; P, postoral kineties; PK, preoral kineties; x, X-body. Scale bars 20 μm.

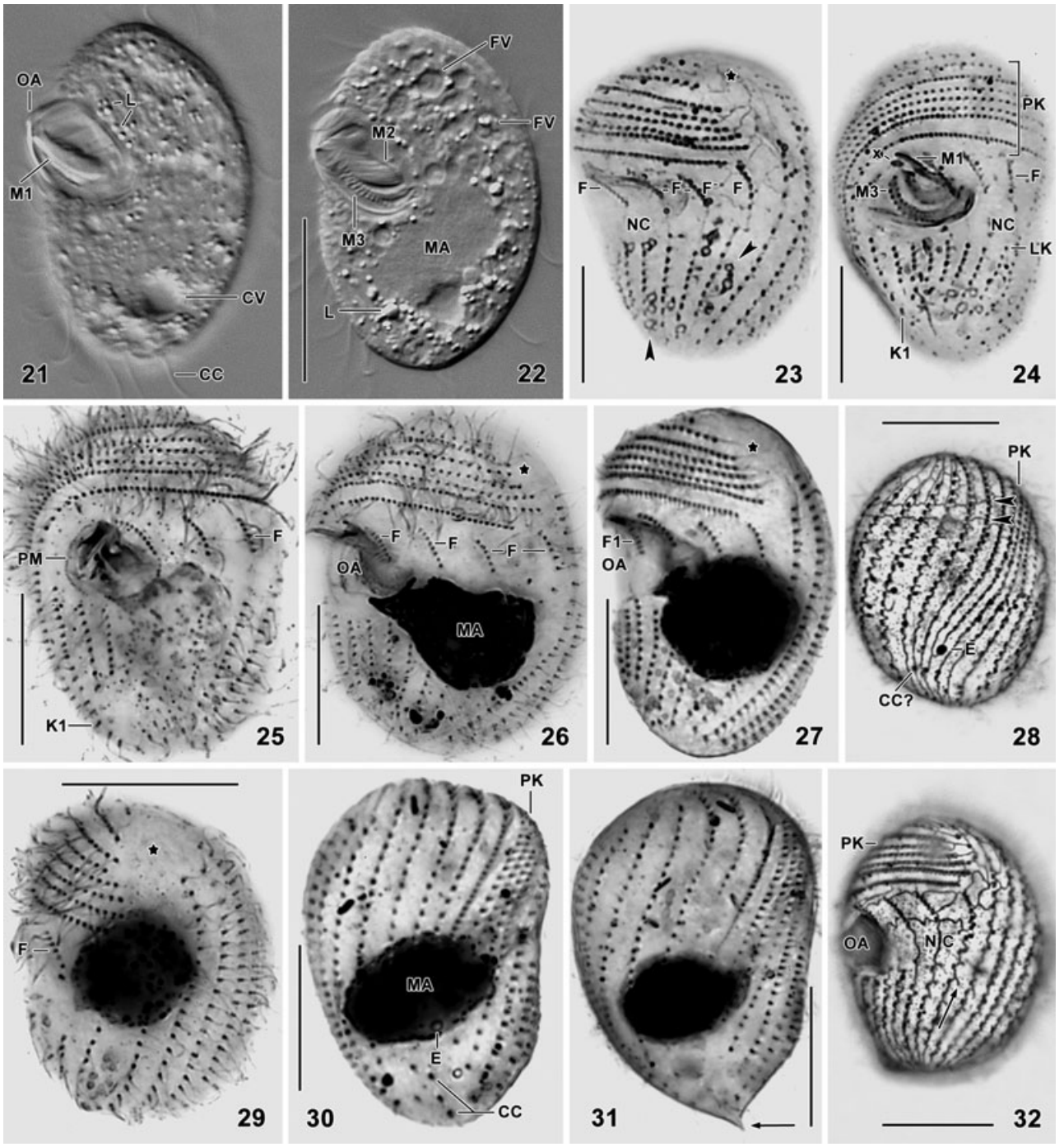

Fig. 21–32. *Bromeliophrya quadristicha* from life (21, 22), after Chatton-Lwoff (23, 24) and Klein-Foissner (28, 32) silver nitrate impregnation, and in protargol preparations fixed with ethanol (25, 26, 29) or Stieve's fluid with some drops of osmium acid (27, 30, 31). Asterisks mark the large, nonciliated anterior pole region. **21, 22.** Same, strongly squashed specimen at two focal planes, showing various organelles. **23.** Left-side view, showing the four species-specific kinetofragments and attached extrusomes (arrowheads). **24, 25.** Ventral views, showing the oral apparatus and the basal body pattern. Note the rather large, nonciliated (NC) area between kinetofragments and left-side kineties. **26, 27.** Left-side views, showing the basal body pattern and the macronucleus. **28, 32.** Dorsolateral and ventrolateral silverline and basal body pattern. Arrowheads mark the intermeridional connectives; the arrow denotes an intrameridional cross-silverline. **29.** Slightly oblique anterior polar view, showing the nonciliated pole area. **30, 31.** Dorsolateral views of a specimen with broadly rounded and tailed posterior body end (arrow), respectively. The basal bodies in the posterior third are rather sparse and not ciliated. CC, caudal cilia; CV, contractile vacuole; E, excretory pore; F(1–4), kinetofragments; FV, food vacuoles; K1, somatic kinety 1; L, lipid droplets; LK, left-side kineties; MA, macronucleus; M1–3, adoral membranelles; NC, nonciliated region; OA, oral apparatus; P, postoral kineties; PK, preoral kineties; PM, paroral membrane; x, X-body. Scale bars 15 μm.

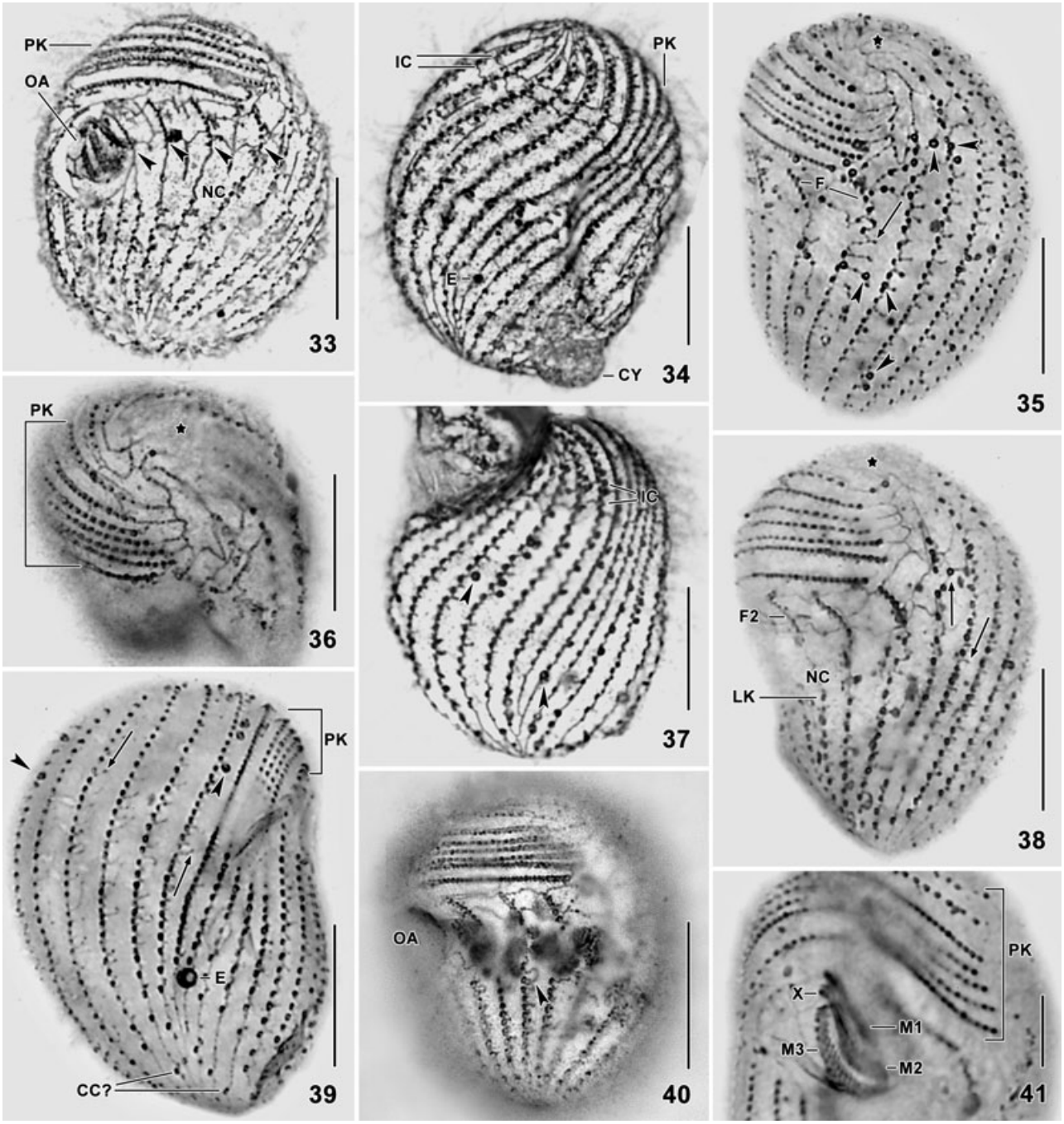

Fig. 33–41. *Bromeliophrya quadristicha*, basal body and silverline pattern after Klein-Foissner (33, 34, 37) and Chatton-Lwoff (35, 36, 38–41) silver nitrate impregnation. Asterisks mark the large, nonciliated anterior pole area. **33.** Ventrolateral view, showing the four species-specific kinetofragments marked by arrowheads. Note the nonciliated stripe (NC) between kinetofragments and postoral kineties. **34.** Dorsolateral view, showing the two intermeridional silverline connectives, the excretory pore of the contractile vacuole, and fecal mass leaving the cytopyge. **35, 38.** Left-side views, showing intrakinetal cross-silverlines (arrows) and attached extrusomes surrounded by a silverline (arrowheads). **36.** Anterior polar view, showing the semicircular course of the preoral kineties. **37.** Dorsal view, showing attached extrusomes (arrowheads) and the sparse, nonciliated basal bodies in the posterior third of the cell. **39.** Right-side view of a specimen with acute posterior body end. Arrows mark intrakinetal cross-silverlines, arrowheads denote attached extrusomes. **40.** Left-side view, showing loop-shaped intrameridional silverlines in the anterior region of the postoral kineties (arrowhead). **41.** Oral structures. CY, cytopyge; E, excretory pore of contractile vacuole; F, kinetofragments; F2, kinetofragment 2; IC, intermeridional silverline connectives; LK, left-side kinety; M1–3, adoral membranelles; NC, nonciliated area; OA, oral apparatus; PK, preoral kineties; x, X-body. Scale bars 5 μm (Fig. 41) and 15 μm (Fig. 33–40).

Extrusomes and cortex (Fig. 1, 2, 14, 15, 18, 23, 35, 37, 39)
The extrusomes belong to the mucocyst type and are about 1.4 × 0.3 μm in size in vivo. They are numerous within and slightly left of the ciliary rows, and are connected with the intrameridional silverline meridians and the intrameridional cross-silverlines, forming minute silverline circles when attached and granules when they have just been extruded. They were not impregnated with the protargol method used. The cortex is without peculiarities. It is slightly furrowed along the ciliary rows and very flexible.

Cytoplasm (Fig. 1, 21, 22)
The cytoplasm is hyaline and usually contains many food vacuoles and moderately refractive lipid droplets 0.5 to 2 μm across. The food vacuoles, which have a diameter of 4 to 6 μm, contain only two to five bacterial rods or their fluffy remnants.

Movement
The movement is conspicuous because *B. quadristicha* rapidly dances up and down.

Somatic ciliary pattern (Table 1; Fig. 1, 3, 4, 6, 7, 13–18, 21, 23–40)
The ordinary cilia are 7 to 8 μm long in vivo and are monokinetidal throughout. The three to four caudal cilia are about 13 μm long in vivo and are in the left posterior pole area; frequently, they are difficult to recognize both in vivo and in preparations, where they are not unequivocally separable from the ordinary cilia (Fig. 1, 21, 28, 30, 39). Four bare areas and some specializations complicate the ciliary pattern.

Bromeliophrya quadristicha has an average of 23 to 24 ciliary rows, in which the distances between the individual kinetids gradually increase from anterior to posterior, especially in the right-side kineties. Somatic kinety 1 is stomatogenic and is distinctly shortened anteriorly, where it commences at the right margin of the oral opening, and posteriorly, where it abuts to the cytopyge (Fig. 4, 18, 24, 25). Right of kinety 1, there are six to eight, usually seven very long kineties that rectangularly curve around the upper mouth margin and extend as preoral kineties over the left side of the cell. The preoral kineties are narrowly spaced, except of kineties 2 and 3, and densely ciliated making the ventral portion of the preoral dome very hairy. Preoral kinety 1 (= somatic kinety 2) is comparatively sparsely ciliated in the oral area and abuts to kinetofragment four; the other preoral kineties end at a similar level, thus contributing to the rather large, obovate, bare anterior pole area (Table 1; Fig. 3, 4, 6, 7, 14, 15, 23–27, 29, 32, 35, 36, 38).

The ventral and left-side kineties are split into an average of four to five, rarely six postoral kineties and four left-side kineties each forming a kinetofragment anteriorly by reduction of some kinetids. Thus, a nonciliated stripe is generated between the kinetofragments and the anterior end of the left-side kineties. The fourth kinetofragment is frequently indistinctly separated from the posterior portion of the kinety (Table 1; Fig. 1, 3, 4, 14, 15, 17, 23–27, 32, 35, 38). Posteriorly, all kineties are slightly to distinctly shortened, producing a bare pole area crossed by the intrameridional silverline meridians (Fig. 4, 6, 15, 18, 24, 27, 28).

Oral apparatus (Table 1; Fig. 1, 4, 5, 15, 21, 22, 24, 33, 41)
The oral opening is in the second quarter of the cell between the preoral dome and the uvula. It is broadly elliptical, about 40% smaller than the buccal cavity, and orientated slightly obliquely to the main body axis. The buccal cavity increases in depth from about 5 μm anteriorly to 10 μm posteriorly. The oral ciliature is rather inconspicuous due to the lack of paroral cilia and the comparatively small, about 10 μm long adoral membranelles whose posterior half is covered in the buccal cavity. Macrostome cells were not observed.

The three adoral membranelles have almost the same length, are more or less concave, and have about 5 μm long cilia (Fig. 1, 4, 5, 15, 24, 41). Membranelle 1 is on the left wall of the buccal cavity and composed of three rows of basal bodies, except of the four-rowed anterior portion. Adoral membranelle 2 is on the dorsal wall of the buccal cavity and is composed of five basal body rows, of which the two leftmost rows are gradually shortened at both ends. Membranelle 3 is on the right wall of the buccal cavity and composed of three rows of basal bodies whose individual distances gradually decrease from anterior to posterior. The "X-group" is at the anterior end of membranelle 2 and composed of six ciliated basal bodies. The pharyngeal fibers are short and extend dorsally and slightly anteriorly. The paroral is a convex row of nonciliated basal bodies along the right half of the oral opening (Fig. 4, 5, 25).

Silverline pattern (Fig. 14–18, 23, 24, 28, 32–40)
Bromeliophrya quadristicha has a complex silverline pattern connecting all main cortical organelles and extending into the nonciliated areas, forming wide-meshed webs. The basal bodies of the individual ciliary rows are connected by the intrameridional silverline having many minute outgrowths to the left (intrameridional cross-silverlines) sometimes forming rather large loops (Fig. 18, 40); anteriorly and posteriorly, the silverline meridians extend into the nonciliated areas, forming wide-meshed, irregular patterns. The intrameridional silverlines continue also into the bare area produced by the split of the left-side kineties and continue into the kinetofragments, forming a cross-silverline each at the ends (Fig. 15, 32, 40). There are two intermeridional connectives in the dome area (Fig. 18, 28, 34, 37). A rather narrowly meshed, irregular silverline pattern occurs in the buccal cavity and connects the adoral membranelles with each other and with the intrameridional silverlines (Fig. 15, 33).

Notes on ontogenesis (Fig. 19, 20)
One well-impregnated specimen in early ontogenetic stage and another in late stage were found in the preparations.

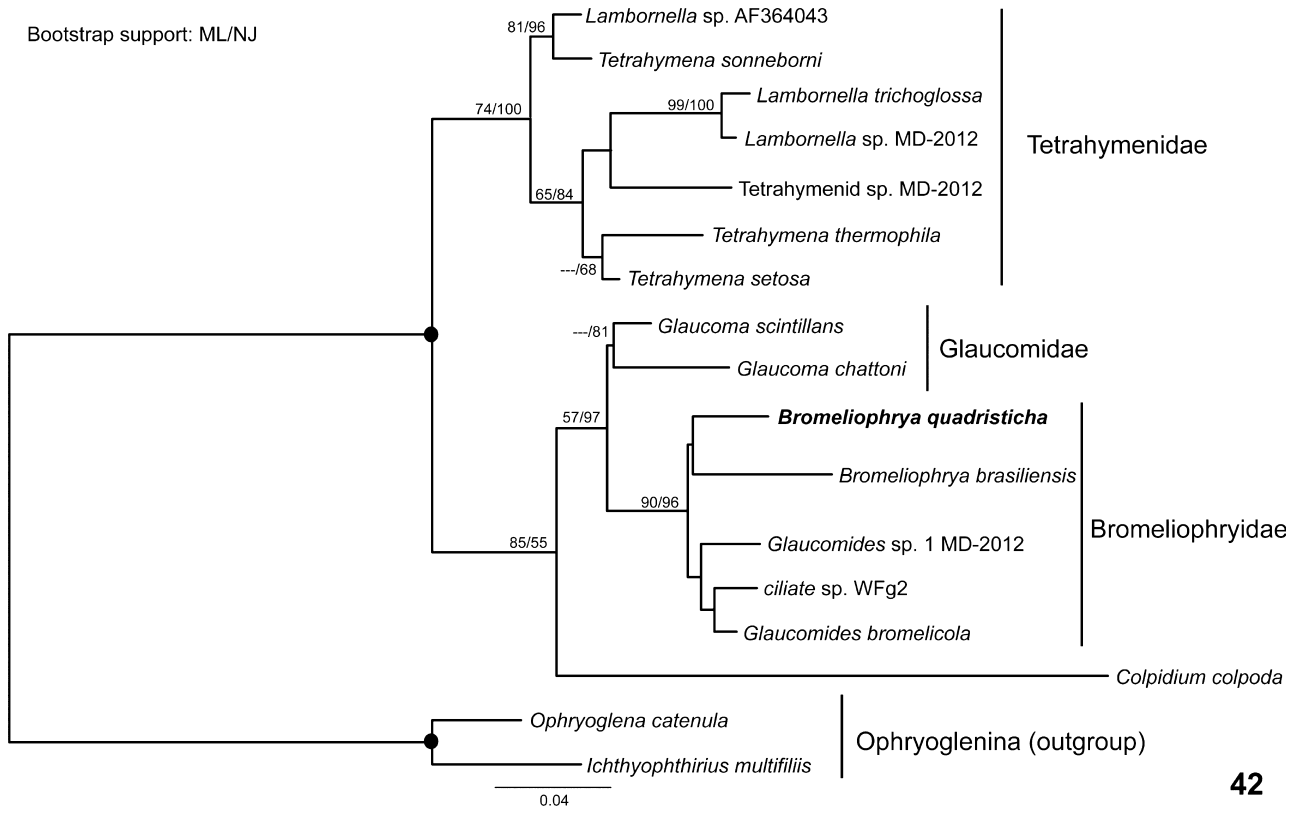

Fig. 42. Maximum-likelihood phylogenetic tree, based on the 18S rRNA genes. Bootstrap values above 50 for the maximum-likelihood (ML, 1000 replicates) and neighbor-joining evolutionary distance (NJ, 1000 replicates) analyses are given at the individual nodes. Filled circle at nodes indicates full support from both tree construction methods. The new species, *Bromeliophrya quadristicha*, branches with significant support from both phylogenetic reconstruction analyses within the family Bromeliophryidae, together with the sole *Bromeliophrya* sequence deposited in a public data base.

The early stage shows three rod-like adoral membranelles in the opisthe, while the paroral is represented by many minute, disordered granules. The late stage shows (i) the origin of the kinetofragments from the left-side kineties, (ii) the assembled adoral membranelles, the "X-body" (likely originating from membranelle 2) and the paroral, and (iii) the origin of the preoral kineties from the posterior half of the parental ones. The proter oral ciliary structures do not reorganize.

Molecular phylogeny of *Bromeliophrya quadristicha* (Fig. 42)

The amplified 18S rDNA sequence of *B. quadristicha* is 1698 bp long and available under GenBank accession number JX561218. The maximum-likelihood tree shows, with high bootstrap support, a common ancestor for tetrahymenids and glaucomids and confirms the notion of Chantangsi et al. (2007) that *Colpidium colpoda* cannot be unambiguously assigned to the Tetrahymenidae.

The glaucomid clade splits into two subclades, one containing *Glaucoma* spp. (Glaucomidae), the other *Bromeliophrya* spp. and *Glaucomides* spp. (Bromeliophryidae), both living in tank bromeliads. The bromeliophryid clade has high bootstrap support from both analyses and is divided into a subclade containing *Bromeliophrya* spp. and another subclade containing *Glaucomides* spp. The *Glaucomides* subclade contains two as yet undescribed species: ciliate sp. WFg2 (GB accession number AJ810076) and *Glaucomides* sp. 1 MD-2012 (GB accession number JQ723968).

DISCUSSION

Family classification of *Bromeliophrya* and *Glaucomides*

The morphology of *Glaucomides bromelicola* has been described by Foissner (2013). Its molecular sequence was published by Foissner et al. (2003) and Dunthorn et al. (2012). In the present study, we add new sequences and discuss the phylogenetic relationship with the Glaucomidae Corliss 1971 that appear to be the most closely related taxon.

Both morphology (Foissner 2003a, 2013) and molecular sequences (Fig. 42) classify *Bromeliophrya* and *Glaucomides* in a moderately supported clade with *Glaucoma*, suggesting a common ancestor. Unfortunately, the *Glaucoma* clade consists of only two *Glaucoma* species, although the family contains about 10 genera and many

species not yet sequenced (Lynn 2008). However, the *Glaucoma* clade is clearly distinct from the *Bromeliophrya/Glaucomides* clade with 97% NJ bootstrap support and more than 50% ML support. Furthermore, the bromeliophryid clade, and very likely also the glaucomid clade, shows its own evolution obviously different from that of the glaucomids. Thus, both clades should have family rank, especially from a cladistic point of view (Hennig 1982). Lynn (2008) synonymized the Bromeliophryidae Foissner 2003a with the Glaucomidae Corliss 1971 without providing any reason.

Although the molecular trees strongly support family rank for *Bromeliophrya* and *Glaucomides*, we would like to add a morphological and an ecological reason: (i) the Bromeliophryidae are supported by morphological (± large, bare area on left and/or dorsal side) and ontogenetical (split of somatic kineties to provide migrating kinetofragments at left mouth margin) features (Foissner 2003b, present study); (ii) as yet, bromeliophryid ciliates have been found only in phytotelmata (organisms that inhabit small pools of water within or upon plants), suggesting that they underwent an independent evolution there while glaucomids occur globally in ordinary standing and running waters.

The *Glaucomides* clade contains two not yet described species, which can be distinguished also morphologically and will be described later. Obviously, this clade is rich in species while we know of only two *Bromeliophrya* species. The tetrahymenid clade contains two or three *Lambornella* species, of which only *L. trichoglossa* Foissner 2003b has been described. The tetrahymenid MD-2012 is a new, still unpublished genus from a tree-hole in Jamaica.

Differentiating *Bromeliophrya quadristicha* and *B. brasiliensis*

In vivo, the two species are rather difficult to distinguish because body shape (metopoid) and size (~ 40 × 30 vs. 55 × 35 μm) are rather similar. Thus, the best feature is the number of kinetofragments on the left body side (4 and widely spaced vs. 2 along the left margin of the oral opening). In silver preparations, *B. quadristicha* and *B. brasiliensis* are easily distinguished by body size (37 × 30 μm vs. 51 × 35 μm on average in Chatton-Lwoff silver nitrate preparations); the non-overlapping number of ciliary rows (21–25 vs. 29–33, $\bar{x}$23 vs. 32) and kinetofragments (4 vs. 2); the length of adoral membranelle 3 (about as long as membranelles 1 and 2 vs. about three times longer, cp. Fig. 4 with Fig. 12); and the right-side silverline pattern (without or with few vs. with many loop-like processes of the primary silverline meridians, cp. Fig. 11 with Fig. 18, 34, 37, 39). These are pronounced differences, indicating the presence of further *Bromeliophrya* species.

Comparison with other ciliates

Bromeliophrya has a highly characteristic, metopoid shape (Fig. 1). This, however, makes it prone to misidentifications. In tank bromeliads, the most similar species is the colpodid ciliate *Bromeliothrix metopoides* Foissner 2010, which has a quite similar body shape and size and a small oral apparatus. In vivo, these species can be distinguished mainly by the structure of the macronucleus (with a central dense mass vs. with many minute dense masses) and the shape and size of the micronucleus (broadly ellipsoidal and about 2–2.5 μm in size vs. discoidal and about 4 μm across and 2 μm thick). Of course, the oral structures are also quite different but difficult to analyze in vivo. In silver preparations, *Bromeliophrya* spp. and *Bromeliothrix metopoides* are easily distinguished by the tetrahymenid vs. colpodid organization, for instance, in having somatic monokinetids vs. dikinetids and three vs. one left-side oral structures ("membranelles"). Basically, this applies also to small members of the genus *Colpoda*, especially *C. steinii*, which is frequent in tank bromeliads. In vivo, it can be distinguished from *Bromeliophrya* by the macronucleus (with large, dense central mass vs. many minute dense masses) and the ciliature in the anterior third of the body (cilia paired vs. single).

Another group of ciliates in vivo easily mixed with *Bromeliothrix* (see Foissner 2010) and *Bromeliophrya* spp. is the metopoids, especially, *Metopus* s. str., of which several occur in tank bromeliads, e.g. *M. pullus* (Fig. 10) and *M. setosus*. For these ciliates, the oral apparatus should be studied (comparatively small and with broadly ellipsoidal entrance vs. many thin adoral membranelles, forming a sigmoidal ribbon).

TAXONOMIC SUMMARY

Class Oligohymenophorea Puytorac et al. 1974
Order Tetrahymenida Fauré-Fremiet in Corliss 1956
Family Bromeliophryidae Foissner 2003a
Genus *Bromeliophrya* Foissner 2003a
Species *Bromeliophrya quadristicha* n. sp.

Diagnosis (based on two populations)

Size in vivo about 40 × 30 μm. Macronucleus and micronucleus posterior to mid-body, broadly ellipsoidal. Excretory pore of contractile vacuole on average 23% from posterior body end. Extrusomes mainly left of ciliary rows, oblong, about 1.4 × 0.4 μm in size. On average 23 to 24 ciliary rows, of which 8 are distinctly shortened postorally and left laterally, leaving space for four kinety fragments and a nonciliated, transverse stripe; three or four caudal cilia originating from right-side kineties. Adoral membranelles of similar length, membranelles 1 and 3 three-rowed, membranelle 2 five-rowed.

Type locality

Tank bromeliads on the northern slope of the Blue Mountains, Jamaica, Silver Hill village, 18°12′N 76°40′W.

Type material

We have deposited the following material from the Jamaican type population in the Biologiezentrum of the

Oberösterreichische Landesmuseum in Linz (LI), reg. no. 2012/127–2012/139: one holotype and four paratype slides with protargol-impregnated specimens; three paratype slides with specimens impregnated with the Chatton-Lwoff silver nitrate method; and five paratype slides impregnated with the Klein-Foissner silver nitrate method. Furthermore, we deposited in the same repository voucher material from the Dominican Republic, reg. no. 2012/140–2012/145: four slides with silver nitrate impregnated specimens (Chatton-Lwoff method) and two slides with some protargol-impregnated, environmental specimens. All methods were as described by Foissner (1991). GenBank accession number: JX561218.

Etymology

The epithet is a composite of the Latin numeral *quadri* (four) and the Greek noun *stichus* (row), referring to the four kinetofragments on the left side.

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