The Journal of Eukaryotic Microbiology

Published by the International Society of Protistologists

Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

Description of *Glaucomides bromelicola* n. gen., n. sp. (Ciliophora, Tetrahymenida), a Macrostome Forming Inhabitant of Bromeliads (Bromeliaceae), Including Redescriptions of *Glaucoma scintillans* and *G. reniformis*

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Keywords
Biodiversity; Bromeliophryidae; Glaucomidae; neotropics; *Polytomella* sp.

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Received: 11 July 2012; revised 26 September 2012; accepted September 26, 2012.

doi:10.1111/jeu.12016

ABSTRACT

Glaucomides bromelicola n. gen., n. sp. is a tetrahymenid ciliate common in tank bromeliads of Central and South America. The new genus is characterized by having a kinety fragment along the left mouth margin, an unciliated dorsolateral area, a tetrahymenid silverline pattern, and the ability to produce macrostomes when bacterial food is depleted. I provide a detailed description of the microstome and the macrostome morph, using several morphological methods. This showed that *G. bromelicola* does not belong to the Glaucomidae, but to the Bromeliophryidae. However, various morphological traits are highly similar to those of *Glaucoma reniformis* and *G. scintillans*, which are thus redescribed and compared with *G. bromelicola*. Most differences are inconspicuous, showing that new tetrahymenids must be described very carefully. The morphological and molecular data suggest a common ancestor for *Glaucoma* and *Glaucomides*, both performing their own radiation, the former in ordinary limnetic habitats, the latter in tank bromeliads.

WITH few exceptions, bromeliads occur only in Central and South America. Most have their leaves arranged in such a way that small cisterns (tanks) are produced that can keep up to 3 litres of rain water in the larger species. The tanks are a specific habitat for various specific organisms, ranging from protists to amphibians (Benzing 1980, 1990; Picado 1913). Foissner et al. (2003) briefly reviewed the knowledge of bromeliad protists, showing that they constitute a severely under-sampled habitat. Thus, they found, without specific effort, several undescribed ciliate genera and species, some of which were spectacular, e.g. *Bromeliothrix metopoides*, a colpodid that can make macrostomes and division chains (Foissner 2010). Foissner et al. (2003) speculated that bromeliad tanks possibly contain hundreds of undescribed ciliate species. Indeed, intensified morphological and molecular research increased the number of supposedly specific bromeliad ciliates to about 40 taxa and showed the number of potential endemic species increasing as more bromeliads were sampled (Dunthorn et al. 2012).

There is convincing evidence that some ciliates, especially tetrahymenids, speciated within the bromeliad tanks, producing clades with two or more species (Foissner et al. 2003; present study). One such species, *Glaucomides bromelicola*, is described in this article. I have studied this ciliate since I first discovered it 10 years ago. The morphological investigations suggest a close relationship with the Glaucomidae Corliss 1971 while the earlier and the recent molecular data show a closer relationship with the Bromeliophryidae Foissner 2003b; a sister clade of the Glaucomidae, with many features developing either from a common ancestor or convergently (Dunthorn et al. 2012; Foissner et al. 2003, present study).

Morphological research on tetrahymenids is rather advanced. More than half of the approximately 28 genera recognized by Jankowski (2007) and Lynn (2008) have been investigated or reinvestigated with modern methods. This showed that several genera and species differ by fairly inconspicuous features, for instance, *Dexiostoma campylum* and species of the *Tetrahymena pyriformis*-complex (for a review, see Foissner et al. 1994), two highly similar species belonging to different families (Jankowski 2007; Lynn 2008). Obviously, several features developed from a common ancestor or convergently.

This study provides a very detailed description of *Glaucomides bromelicola*, a new genus and species very likely endemic to bromeliad tanks. *Glaucomides bromelicola* produces microstomes and macrostomes both being so different that they require separate description

and documentation with various methods. Furthermore, *G. bromelicola* is highly similar to *Glaucoma reniformis* and *G. scintillans,* both lacking detailed morphometrics and some important morphological data. Thus, four taxa had to be described.

The long work on *G. bromelicola* was responsible for mentioning the name in some previous papers, producing a nomen nudum nomenclaturally (Dunthorn et al. 2012; Foissner et al. 2003; Fried and Foissner 2007). This was caused by the fact that I discovered many more ciliates than I could describe in reasonable time and quality. The ontogenesis of *G. bromelicola* will be described in a forthcoming paper.

MATERIALS AND METHODS

Material and identification of species

Three species were investigated. Nonclonal cultures were set up for *Glaucomides bromelicola* and *Glaucoma reniformis* in Eau de Volvic (French mineral water) enriched with some squashed wheat grains to stimulate growth of indigenous bacteria and flagellates. Environmental specimens were used for *Glaucoma scintillans*.

Glaucomides bromelicola was discovered in various species of 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, 70°40′W 19°50′N (type locality).

Glaucoma reniformis Schewiakoff 1892 was found in a stream on the outskirts of the town of Salzburg, Austria, 13°02′E 47°47′N. The identification was based on Schewiakoff (1892, 1893) and Foissner et al. (1994). I agree with McCoy (1975) that *G. chattoni* Corliss 1959 (and Corliss 1971) is a junior synonym of *G. reniformis*. For this species, I also studied a lectotype (USNM no. 24209) and a paralectotype (USNM no. 24210) slide deposited by J. O. Corliss in the Smithsonian Institution under the name *G. chattoni*.

Glaucoma scintillans Ehrenberg 1830 (type of genus) was found in a forest pond near Lake Grabensee on the outskirts of the town of Seekirchen, Salzburg, Austria, 13°5′E 47°50′N. The identification was based on Corliss (1971), McCoy (1975), and the review of Foissner et al. (1994).

Methods

Cells were studied in vivo at 1,000× using bright field and differential interference contrast optics. The infraciliature and various cytological structures were revealed by scanning electron microscopy (SEM) of ciliated and deciliated specimens and by four silver impregnation methods (protargol, silver carbonate, silver nitrate after Chatton-Lwoff and Klein-Foissner), all described in Foissner (1991, 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 and those of prepared cells were made with a drawing device. Terminology is according to Corliss (1952, 1979), Foissner (2003b), and Lynn (2008).

RESULTS

Glaucomides bromelicola: description of the microstome morph

Body size (Table S1; Fig. 1, 2, 5, 6, 63). The size of microstome *G. bromelicola* is highly variable, as shown by the variation coefficients: 27.1% for body length and 19.4% for body width. The average values are rather similar in protargol- and silver nitrate-impregnated specimens as well as in cells prepared for SEM. Assuming 5% preparation shrinkage in the Chatton-Lwoff silver nitrate preparations, *G. bromelicola* has a mean size of 57 × 37 μm (extremes: 33–82 × 21–60 μm) and a length:width ratio of 1.5–1.6:1. However, the in vivo length:width ratio is $\bar{x}$1.9:1 (M = 1.9, SD = 0.2, CV = 7.8, Min = 1.6, Max = 2.2, n = 23) in freely motile specimens from a blooming culture, showing that cell width is rather distinctly inflated in the silver and SEM preparations. Thus, I estimate a common in vivo size of 57 × 30 μm. Furthermore, *G. bromelicola* is usually moderately (~ 1.6:1) flattened laterally (Fig. 2, 63). Environmental specimens may even be so flattened as to appear leaf-like (2–2.5:1, Fig. 6). The environmental specimens are usually also much smaller (Fig. 5–7): 25–38 × 15–25 μm ($\bar{x}$ = 27 × 17 μm, n = 7, protargol-impregnated).

Body shape (Table S1; Fig. 1, 5, 7, 11, 18, 19, 30, 31, 34, 37, 46, 50, 52, 79, 98, 99). The body shape is as variable as the body size. The environmental specimens are frequently obovate with a narrowly rounded to acute posterior body end (Fig. 5, 7). Cultivated specimens are frequently broadly (< 1.5:1) to ordinarily (~ 2:1) ellipsoidal, usually 1.9:1 in vivo (Fig. 1, 3, 18, 34, 37, 46, 50, 98). Small cells are often rather stout (Fig. 11). Eight of 100 specimens have a short but distinct tail (Fig. 10), and 15 of the 100 cells are obovate with acute body end (Fig. 5, 7); in an environmental, blooming sample from Jamaica, even half of the specimens were tailed.

Nuclear apparatus (Table S1; Fig. 1, 4, 5, 7, 10, 20, 22, 29, 32, 34, 37). The nuclear apparatus is slightly posterior and left to mid-body and body's midline respectively (Table S1; Fig. 1, 5, 20, 22). The macronucleus is broadly ellipsoidal and has an average size of 18 × 14 μm in protargol preparations, where it frequently shows small concavities and convexities. Its periphery contains many argyrophilic granules ≤ 1 μm in diameter. The micronucleus, which is about 2 μm across in protargol preparations, is usually attached to the macronucleus, but not in fixed position; rarely, it is some μm away from the macronucleus (Table S1; Fig. 1, 5, 7, 10).

Contractile vacuole and cytopyge (Table S1; Fig. 1, 4, 5, 13, 16, 43–45, 52, 99). The excretory pore, which is of usual structure and 0.8–1.5 μm in diameter, is on average at 82% of body length and thus near to the posterior body

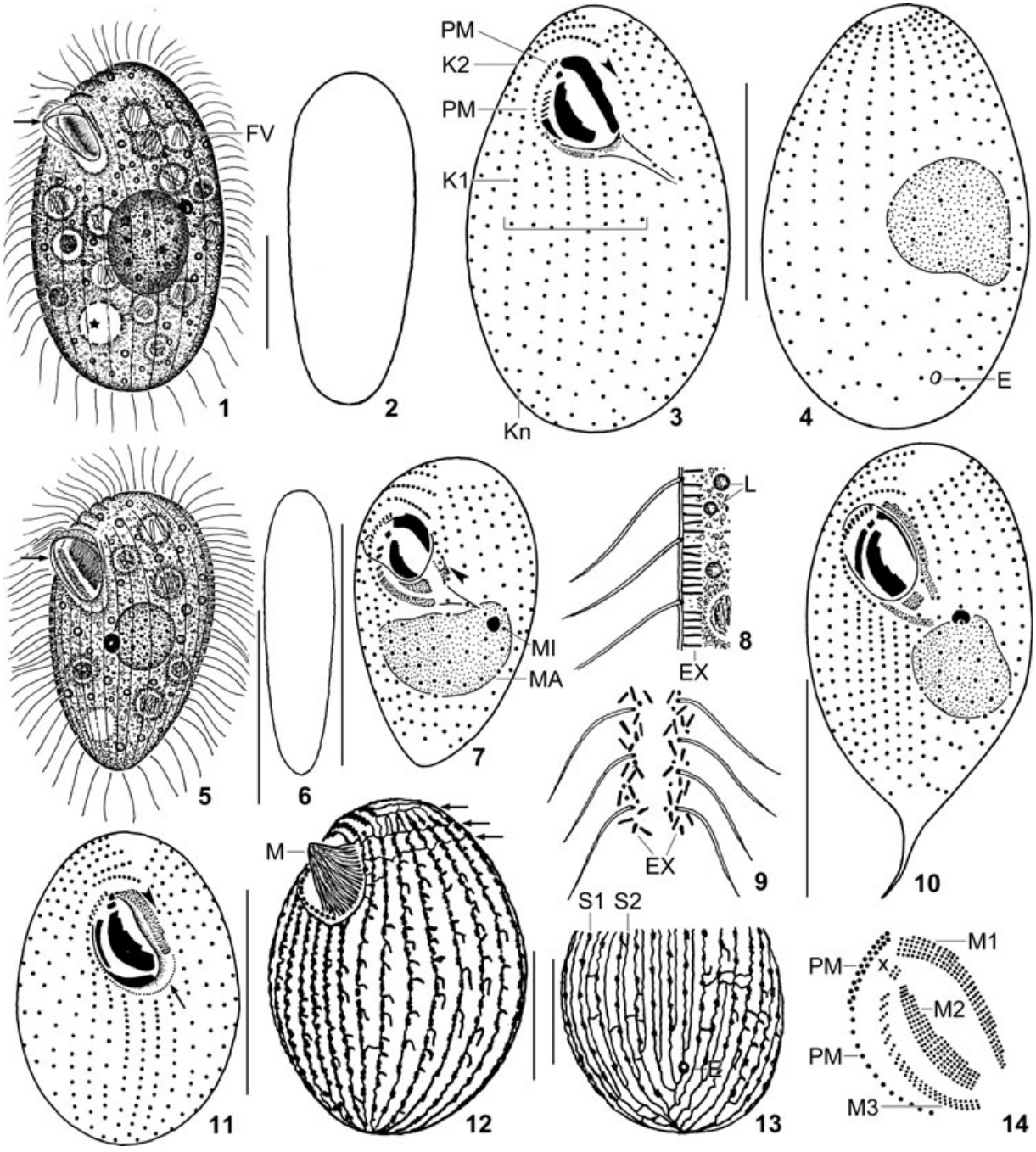

Fig. 1–14. *Glaucomides bromelicola* from life (1, 2, 5, 6, 8, 9), after protargol impregnation (3, 4, 7, 10, 11, 14), and after silver nitrate impregnation (12, 13). **1, 2.** Ventrolateral and dorsal view of a representative specimen from a pure culture. Arrow marks buccal lip. The contractile vacuole (asterisk) shines through from right side of cell. **3, 4.** Ciliary pattern of ventral and dorsal side of the microstome hapantotype, length 41 μm. Parenthesis denotes postoral kineties. Arrowhead marks kinety fragment left of oral opening. **5–7.** Live view and ciliary pattern of environmental specimens. Arrow marks buccal lip. Arrowhead denotes kinety fragment left of oral opening. **8, 9.** Optical section and surface view, showing the about 1-μm-long extrusomes, likely mucocysts. **10, 11.** Ciliary pattern of ventral side of a tailed and of a small specimen. Arrow marks margin of buccal cavity. Arrowhead denotes kinety fragment left of oral opening; the tailed cell (10) lacks this fragment. **12.** Tetrahymenid silverline pattern of a representative specimen. Arrows mark intermeridional connectives. **13.** Rare silverline pattern with distinct secondary meridians. **14.** Oral ciliary pattern (semi-schematic). The (x) marks a small part of adoral membranelle 2. E, excretory pore; EX, extrusomes; FV, food vacuole; K1, 2, somatic kineties; Kn, last kinety; L, lipid droplets; M(1, 2, 3), adoral membranelles; MA, macronucleus; MI, micronucleus; PM, paroral membrane; S1, 2, primary and secondary silverline meridians; x, "X-body". Scale bars 20 μm.

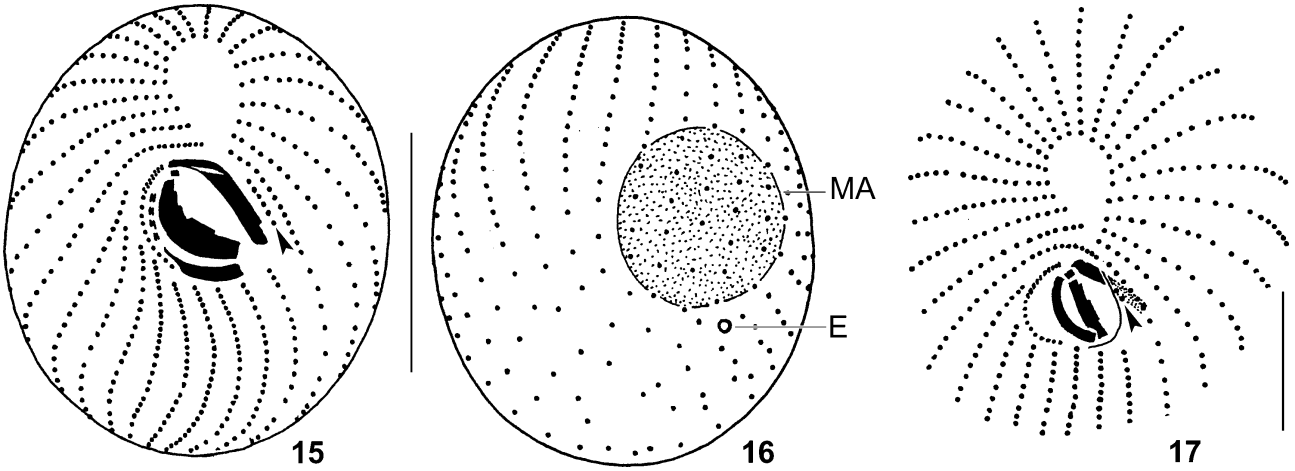

Fig. 15–17. *Glaucomides bromelicola* after protargol impregnation. **15. 16.** Oblique anterior and posterior polar view. Arrowhead marks kinety fragment left of oral opening. Note scattered basal bodies and the excretory pore of the contractile vacuole in posterior pole area. **17.** Anterior polar view showing the obovate, nonciliated pole area. Arrowhead marks kinety fragment left of oral opening. E, excretory pore; MA, macronucleus. Scale bars 10 μm (17) and 20 μm (15, 16).

end. It is located at the end of kinety 5, rarely of kinety 6 right of the stomatogenic kinety 1. Very rarely, there are two excretory pores one after the other (Fig. 52). The cytopyge is at the end of stomatogenic kinety 1. It is recognizable only in silver nitrate preparations (Fig. 43, 44).

Cortex and extrusomes (Fig. 8, 9, 30, 31, 42, 82, 83, 85). *Glaucomides bromelicola* has an ordinary and very flexible cortex. The extrusomes, likely mucocysts, are about 1 μm long, rather refractive rods; they are attached to the intrameridional cross-silverlines (Fig. 43) and to the primary silverline meridians, that is, located around and between the basal bodies of the cilia (Fig. 85). Here, they appear as minute circles when attached to the cortex and as minute granules when released (Fig. 41, 42, 56). In the SEM, minute holes can be observed in the cortex when extrusomes have just been released (Fig. 82, 83).

Cytoplasm (Fig. 1, 5, 20, 21, 63). In cultures, the cytoplasm is hyaline and contains many food vacuoles and lipid droplets. The food vacuoles are 3–6 μm in diameter and contain oblong bacteria. The lipid droplets are moderately refractive and 0.5–2 μm across.

Movement. Glaucomides bromelicola has an ordinary movement. It glides and swims rapidly and continuously, rotating about the main body axis.

Resting cyst. All attempts to get resting cysts failed, both from isolated specimens and in the culture dishes. When not fed, the cells become smaller and smaller, eventually dying after some weeks. Results were the same with a Jamaican population.

Somatic ciliary pattern (Table S1; Fig. 1, 3–5, 7, 10, 11, 15–17, 24, 28–31, 32, 34, 38, 46, 48–52, 79, 82, 83, 98, 99, 105). *Glaucomides* has a glaucomid ciliary pattern with, however, two conspicuous specializations. First, the basal bodies in the middle third of the right dorsolateral area are barren, producing a conspicuous, unciliated area (Fig. 52, 99). The naked area may be small or large, but is always much more distinct than in *Glaucoma scintillans* and *G. reniformis,* which are sparsely ciliated, but never naked in this region (Fig. 90, 102). Second, there is a kinety fragment composed of one to seven cilia at the left margin of the oral opening (Table S1; Fig. 3, 7, 11, 15, 17, 28, 32, 34, 37, 48, 49, 79, 82, 105); rarely, there are two fragments (Fig. 51) or none (Fig. 10). The kinetofragment originates by a split of the leftmost postoral kinety and becomes attached to the left mouth margin during cell shaping, possibly including some migration of the fragment (W. Foissner, unpubl. data on ontogenesis).

All cilia are single, although the basal body is accompanied by a second granule in silver carbonate preparations (Fig. 35, 38). However, this granule is usually impregnated more lightly, likely being a parasomal sac. There are neither dikinetids at the anterior end of the kineties nor elongated caudal cilia, although some cilia are stiffer than the others in the posterior pole area. The cilia are 6–8 μm long in vivo and arranged in 25–32 ($\bar{x}$ = 29, CV = 6.6) meridional rows following the body curvature and extending at the right margin of minute ridges being more distinct in the oral than postoral area; the ciliary pits are inconspicuous (Fig. 79, 82, 83). The number of cilia in a row is highly variable, e.g. in kinety 3 (Table S1): 21–51, $\bar{x}$ = 32, CV = 29.1. There are 6–8 more narrowly spaced and more densely ciliated postoral kineties, frequently producing nice metachronal ciliary waves (Fig. 10, 11, 15, 32, 37, 46, 50, 98).

The ciliary rows, except the postoral ones, commence preorally leaving a small, bare obovate frontal field (Fig. 3, 15, 17, 24, 41, 42, 83). The intrakinetidal ciliary distances increase from anterior to posterior, producing a sparsely ciliated posterior pole area (Fig. 16, 28, 29, 34, 52, 99). Kinety 1, which commences right of the posterior half of the buccal cavity, contains the oral primordium (Fig. 80) and the cytopyge (Fig. 43, 44). Kinety 2 is longest and

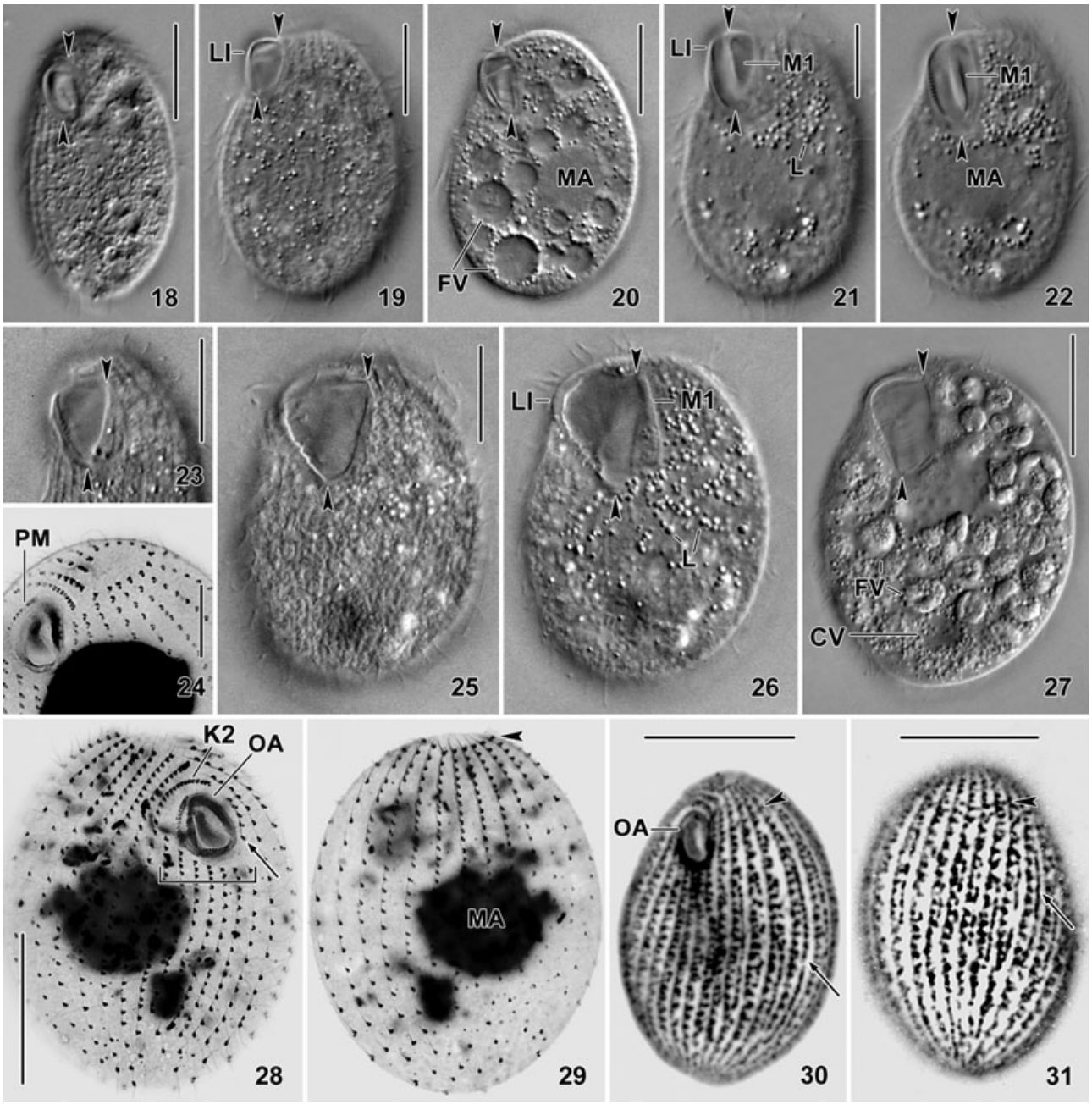

Fig. 18–31. *Glaucomides bromelicola*, microstome (18–22, 24, 28–31) and macrostome (23, 25–27) specimens from life (18–23, 25–27), after silver carbonate preparation (24, 28, 29), and silver nitrate impregnation (30, 31). **18–22.** Ventral (18) and ventrolateral (19–22) views of freely motile (18, 21, 22) and slightly pressed (19, 20) specimens from a pure culture. The longitudinal axis of the oral opening is marked by arrowheads. The distinct buccal lip, a main feature of glaucomids, is recognizable only in lateral views (19–22). The cilia of adoral membranelle 1 form a scintillating plate (21, 22). **23.** Transition to the macrostome morph. Arrowheads mark oral opening. **24.** Anterior polar view, showing the obovate, nonciliated anterior body end. **25–27.** A slightly pressed (by coverslip) cell in three focal planes. When focused on cell surface (25), the triangular oral opening becomes visible (arrowheads). At a slightly deeper focus (26), the adoral membranelle 1 and the buccal lip become recognizable. When focused to body centre (27), many food vacuoles containing the heterotrophic flagellate *Polytomella* sp. become visible. The contractile vacuole shines through from the right side. **28, 29.** Ventral and dorsal view of ciliary pattern. The parenthesis marks the postoral kineties. The arrow denotes the kinety fragment left of the oral opening. The arrowhead in (29) marks the slightly enlarged kinetids at the beginning of kineties. **30, 31.** Ventral and dorsal view, showing the silverline pattern. Arrows mark secondary silverline meridians. Arrowhead denotes intermeridional connectives. Note the many intrameridional silverlines, forming minute, transverse structures. CV, contractile vacuole; FV, food vacuoles; K2, somatic kinety 2; L, lipid droplets; LI, buccal lip; MA, macronucleus; M1, adoral membranelle 1; OA, oral apparatus; PM, paroral membrane. Scale bars 10 μm (24), 15 μm (18–23), and 25 μm (25–31).

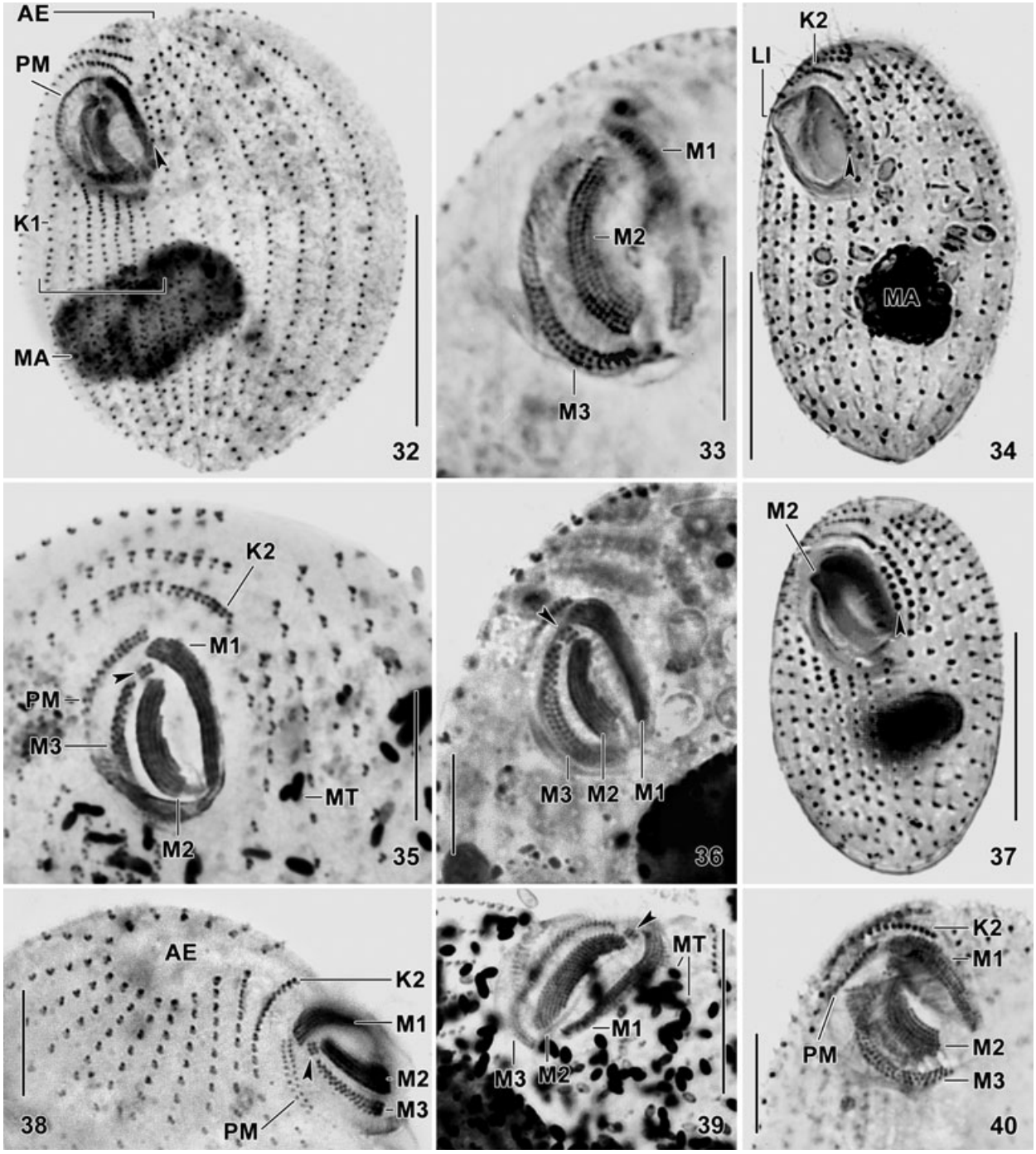

Fig. 32–40. *Glaucomides bromelicola*, oral and somatic ciliary pattern of microstome specimens after protargol impregnation (32–34, 37, 40; heavily squashed, except of Fig. 34, 37) and silver carbonate impregnation (35, 36, 38, 39). In Fig. 35 and 38, the somatic kinetids are composed of paired granules, i.e. of a basal body and a parasomal sac each. **32.** Ventrolateral view, showing the nonciliated, obovate anterior body end and the polymerization of kinetids in the anterior portion of most postoral kineties (parenthesis). The arrowhead marks the kinety fragment at left margin of oral opening. **33, 35, 36, 38–40.** Oral ciliary pattern as shown semi-schematically in Fig. 14. There are three adoral membranelles, each with a specific ciliary pattern, and a paroral membrane with ciliated dikinetids anteriorly and barren monokinetids posteriorly. The "X-body" (arrowheads) is part of adoral membranelle 2 and usually composed of six basal bodies. At right and anteriorly, the oral opening is surrounded by somatic kinety 2, which is densely ciliated anteriorly and slightly recurved. **34, 37.** Ventrolateral and ventral view of ciliary pattern of well-preserved specimens (Stieve fixative improved with some drops of osmium acid). Arrowheads denote kinety fragment at left margin of oral opening, a main difference to *Glaucoma* spp. AE, anterior body end; K1, 2, somatic kineties; LI, buccal lip; MA, macronucleus; MT, mitochondria; M1, 2, 3, adoral membranelles; PM, paroral membrane. Scale bars 10 μm (33, 35, 36, 38–40) and 25 μm (32, 34, 37).

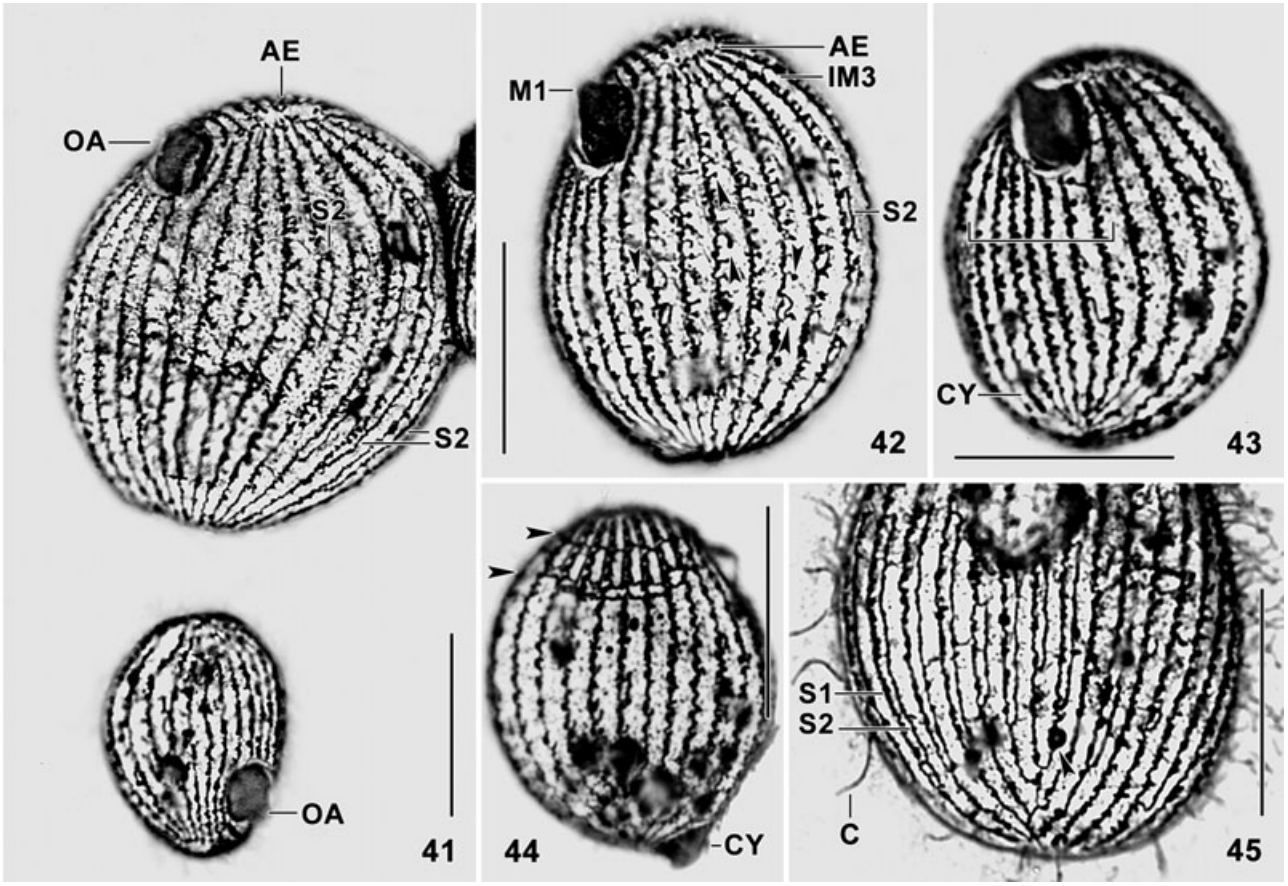

Fig. 41–45. *Glaucomides bromelicola*, silverline pattern after Klein-Foissner silver nitrate impregnation. Basically, *G. bromelicola* has a tetrahymenid silverline pattern, i.e. the basal bodies of a ciliary row are connected by a silverline (first-order meridian), which produces many minute, transversely oriented intrameridional silverlines. **41.** Ventrolateral view of an ordinary and of a very small specimen. The ordinary specimen has many intrameridional silverlines and some second-order meridians, both lacking in the very small specimen. **42.** Ventrolateral view of a representative specimen with many intrameridional silverlines (arrowheads) and some short second-order meridians (S2). The postoral ciliary rows (first-order meridians) are narrower spaced than the lateral ones. **43.** Ventral view of a specimen with many minute intrameridional silverlines, providing the first-order meridians (ciliary rows) with a serrate appearance. Second-order meridians are absent. The postoral ciliary rows (parenthesis) are narrower spaced than the lateral ones. **44.** Dorsal view of a small specimen, showing two of the three (Fig. 42) intermeridional connectives (arrowheads). Both, intrameridional silverlines and second-order meridians are rare or lacking. **45.** An ordinarily sized specimen with distinct second-order meridians (S2), resembling the glaucomid silverline pattern. The arrowhead marks the excretory pore of the contractile vacuole. AE, anterior body end; C, ordinary somatic cilium; CY, cytopyge; IM3, intermeridional connective 3; M1, cilia of adoral membranelle 1; OA, oral apparatus; S1, 2, silverline meridians. Scale bars 25 μm.

densely ciliated anteriorly, where it curves around the mouth margin (Fig. 3, 10, 11, 15, 28, 34, 40, 48, 49, 79, 82, 105); in about half of the cells, the anterior portion recurves slightly, i.e. extends along the left mouth margin with one to three basal bodies; furthermore, the densely spaced anterior cilia of kineties 2 and 3 are distinctly shortened, i.e. only 2–4 μm long in scanning micrographs (Fig. 48, 49). Otherwise, kineties 3 to n are morphologically highly similar, except for the naked area described above. However, this area is unrecognizable in silver preparations because the basal bodies impregnate as those in the ciliated parts of the kineties.

Oral apparatus (Table S1; Fig. 1, 3, 5, 7, 10, 11, 14, 17, 18–22, 28, 32–35, 38, 40, 46, 48–51, 79, 82, 98). The microstomes of *Glaucomides bromelicola* have a subapical oral apparatus with adoral membranelle 1 beginning on average 7 μm from the anterior end of the cell (Table S1). The oral opening is usually almost quadrangular or slightly obovate and has an average size of 10 × 6 μm in Chatton-Lwoff silver nitrate preparations (Fig. 46, 48, 50, 51). It is slightly to rather distinctly (5–30°) oblique to the main body axis. The margin of the oral opening is not thickened, but the anterior and right margin are modified to a typical glaucomid lip slightly projecting from body proper (Fig. 1, 5, 19, 21, 34, 46, 48, 50, 51, 79, 82). The paroral membrane extends along the lip base. In the anterior third, it is composed of zigzagging dikinetids, of which only one basal body has a cilium 4–5 μm long in vivo. The middle and posterior third of the paroral are composed of comparatively widely spaced, unciliated monokinetids

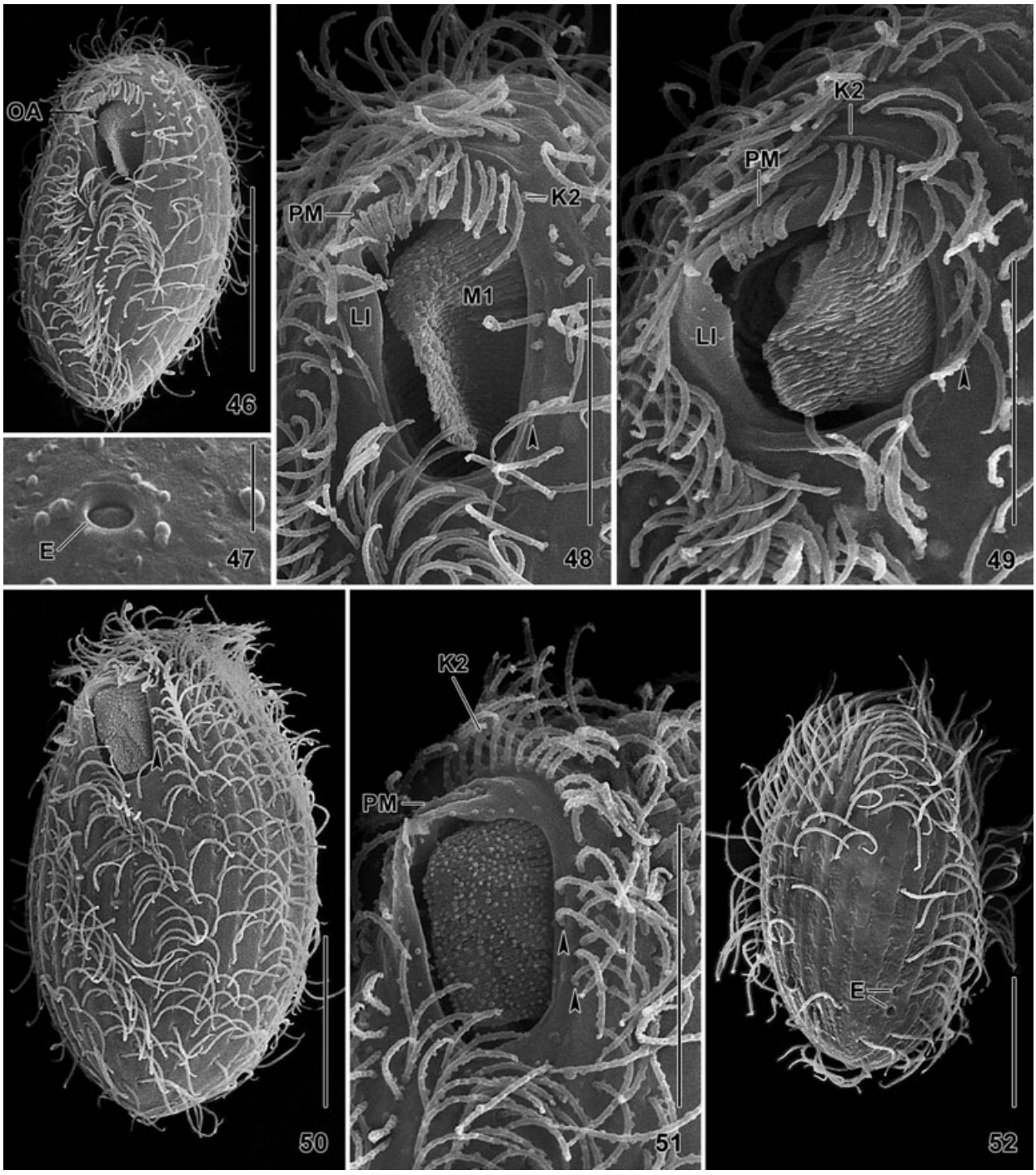

Fig. 46–52. *Glaucomides bromelicola*, microstome specimens in the scanning electron microscope. **46, 48, 50, 51.** Ventral overviews and details of oral apparatus. Metachronal ciliary waves occur in the more densely ciliated postoral kineties. The oral opening is slightly obovate: the right and anterior margin are modified to a distinct buccal lip while one (48, 50) or two (51) kinety fragments occur at its left margin (arrowheads). The large adoral membranelle 1 covers the oral opening and performs the scintillating movement so typical for glaucomid ciliates. Only the anterior quarter of the paroral membrane has slightly shortened cilia, which are monokinetidal in the SEM and dikinetidal in silver preparations (Fig. 3, 7, 14); the monokinetidal posterior quarters are unciliated and thus not recognizable in the SEM. **47.** Excretory pore of contractile vacuole. **49.** A specimen with a triangular oral opening. The arrowhead marks the kinety fragment at left margin of the oral opening. **52.** Dorsal view, showing the barren centre, which, however, contains basal bodies (Fig. 4, 16). E, excretory pores; K2, somatic kinety 2; LI, buccal lip; M1, adoral membranelle 1; OA, oral apparatus; PM, paroral membrane. Scale bars 2 μm (47), 10 μm (48, 49, 51, 52), and 20 μm (46, 50).

(Fig. 3, 7, 10, 11, 14, 15, 17, 28, 32, 35, 38, 40, 46, 48, 49, 51, 98).

The buccal cavity is 6–9 μm, on average 6.7 μm (n = 16) deep and slightly larger than the oral opening, especially posteriorly. The cavity contains three adoral membranelles (M) of different shape and structure (Table S1; Fig. 1, 5, 7, 10, 11, 14, 18–22, 28, 32, 33, 35–40, 46, 48, 50, 51, 79, 82, 98). Membranelle 1, which usually covers the oral opening and M2 and M3, performs the scintillating movement so typical for glaucomid ciliates (Fig. 21, 22, 46, 48–51). It is moderately convex, 9 μm long on average, and composed of five to six ciliary rows strongly decreasing in length from right to left. The cilia of the individual rows are also of different length (Fig. 50, 51): those of the left rows are 4–7 μm long while those of the right rows are 8–12 μm long in vivo. Adoral membranelle 2 is slightly concave, about 7 μm long on average, and composed of five to eight ciliary rows markedly decreasing in length from right to left; the cilia are about 6 μm long in vivo. The X-body belongs to M2, according to the ontogenetic data (unpubl.) and consists of six, rarely of four basal bodies (Fig. 3, 14, 35, 36, 38, 39). Membranelle 3 is distinctly concave, 9 μm long on average, and composed of three ciliary rows with 2–4 μm long cilia much more widely spaced anteriorly than posteriorly (Fig. 14, 35, 36, 38). The pharyngeal fibres are short and extend dorsally and slightly posteriorly.

Silverline pattern (Fig. 12, 13, 30, 31, 41–45). The microstomes of *Glaucomides bromelicola* have a tetrahymenid silverline pattern with many intrameridional cross-fibres originating from the first-order silverline meridians, which contain the basal bodies of the cilia. There are three intermeridional connectives in the oral portion (Fig. 12, 42, 44). Depending on the life cycle, three variations of the silverline pattern can be distinguished. Ordinarily sized specimens have many intrameridional cross-fibres and few, short second-order silverline meridians in Klein-Foissner preparations (Fig. 12, 41, upper specimen; 42). Small specimens have very short intrameridional cross-fibres or lack them at all; likewise, second-order silverline meridians are usually absent (Fig. 41, lower specimen; 43, a transition stage; 44). The third variation is very rare in Klein-Foissner preparations (Fig. 45): there are few intrameridional cross-fibres while second-order silverline meridians are prominent in various regions of the cell. Both the intrameridional cross-fibres and the second-order silverline meridians are more common in cells prepared with the Chatton-Lwoff method (Fig. 30, 31).

Most silverlines contain few to many granules representing attached or just released extrusomes (Fig. 42). Silverlines occur also in the buccal cavity, but were not studied in detail.

Distribution. *Glaucomides bromelicola* is one of the most frequent bromeliad ciliates. It occurred in 11 of 13 bromeliad species in Jamaica (Dunthorn et al. 2012), suggesting a very wide ecological range. Very likely, *G. bromelicola* occurs in the entire bromeliad area, i.e. Mexico, Central America, and South America. I have records from every region I collected: Mexico, Dominican Republic, Jamaica, Venezuela, Ecuador, Peru, and Chile. In Jamaica, I tried to find *G. bromelicola* in rivers and ponds without success, suggesting that it is restricted to Neotropical phytotelmata, i.e. small pools of water within or upon plants.

Micro- and macrostome transformation

When cultivated with bacteria alone, only microstome cells develop (Fig. 18–22). When cultivated with bacteria and an up to 40-μm-long flagellate (likely undescribed) of the genus *Polytomella*, macrostomes are generated as bacteria become depleted (Fig. 23–27). I tried various other flagellates and ciliates (*Chilomonas* sp., *Peranema* sp., *Colpoda steinii*, *Cyrtolophosis mucicola*) as food without success, suggesting that *G. bromelicola* feeds specifically on *Polytomella*, which is very common in bromeliad tanks.

The macrostome transformation requires three to four divisions, and the two morphs can be easily distinguished in the bright field microscope: the microstomes are small (~ 55 × 30 μm) and hyaline (Fig. 18–22, 63); the macrostomes are large (~ 80 × 50 μm) and dark due to the high refractivity of the flagellate food vacuoles (Fig. 27, 63). Morphologically, the transition is indicated by a slightly increased body size (~ 70 × 35 μm); an obtriangular or triangular, slightly enlarged oral opening (Fig. 23, 49, 58–60); and the simultaneous occurrence of food vacuoles with bacteria and a few flagellates.

Glaucomides bromelicola: description of the macrostome morph

Body size (Table S1; Fig. 25, 26, 53, 63). The size of macrostome *G. bromelicola* is moderately variable, showing variation coefficients of up to 15%. The protargol-impregnated and the SEM specimens (values not shown) are smaller than the Chatton-Lwoff prepared cells by 14% and 29% respectively. The length:width ratio is 1.4 and 1.6, indicating some broadening in the protargol preparations. Considering these values and assuming 5% shrinkage in the silver nitrate preparations, I estimate an average in vivo size of 80 × 50 μm, that is, an increase of 39% in body length and of 36% in body width relative to the microstomes.

Body shape (Table S1; Fig. 25, 26, 53, 54, 58–65, 68, 74, 75, 78, 80, 100). The body shape is moderately variable. Most specimens are broadly ellipsoidal (average length:width ratio 1.6:1) and are slightly to distinctly obovate (Fig. 25, 53, 54, 58, 59, 61–65, 68, 78, 80, 100); rarely, cells are ellipsoidal (~ 2:1) or have a distinctly acute posterior end (Fig. 60, 75, 76).

Nuclear apparatus, cortex and extrusomes, cytopyge, movement. These features are as in the microstomes.

Contractile vacuole (Table S1; Fig. 27, 53, 55, 56, 65, 68). The location of the contractile vacuole is as in the microstomes. In contrast, the number of excretory pores increased from an average of one to two. The pores are 1–3 μm apart.

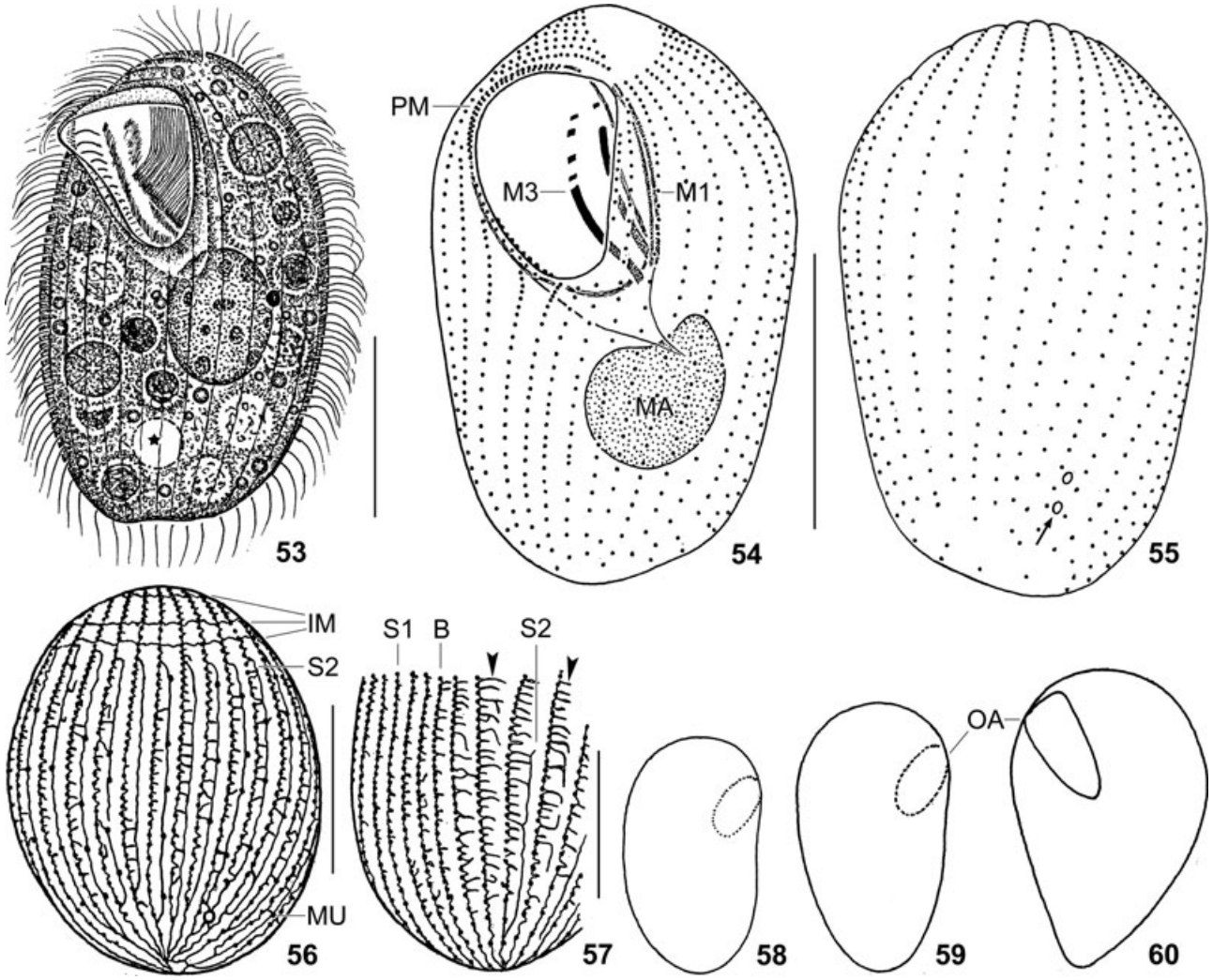

Fig. 53–60. *Glaucomides bromelicola*, macrostome cells in vivo (53, 58–60), after protargol impregnation (54, 55; fixative improved with osmium tetroxide to maintain body shape), and after Chatton-Lwoff silver nitrate impregnation (56, 57). **53.** Ventrolateral view of a representative specimen, length 80 μm. The cell is studded with food vacuoles containing *Polytomella* sp. The asterisk marks the contractile vacuole in right side of cell. **54, 55.** Ventral and dorsal view, showing the ciliary pattern of the macrostome hapantotype; length 63 μm. The arrow (55) marks the excretory pores of the contractile vacuole. **56.** Silverline pattern of dorsal side. The secondary (S2) silverline meridians are pronounced. **57.** Postoral silverline pattern with many intrameridional cross-silverlines (arrowheads). **58.** Right side view of a specimen developing to a macrostome. **59, 60.** Right and left side view of macrostomes narrowed posteriorly. B, basal body; IM, intermeridional connectives; MA, macronucleus; MU, mucocyst; M1, 3, adoral membranelles; OA, oral apparatus; PM, paroral membrane; S1, 2, silverline meridians. Scale bars 20 μm (57) and 30 μm (53–56).

Cytoplasm (Fig. 26, 27). The macrostomes are usually studded with up to 15-μm-sized food vacuoles containing *Polytomella* sp. and many lipid droplets up to 5 μm in diameter.

Somatic ciliary pattern (Table S1; Fig. 53–55, 61, 62, 64, 65, 68–71, 73–78, 80, 100). The general structure and pattern of the ciliature is as in the microstomes, but with an important difference: the barren dorsolateral area of the microstomes becomes sparsely ciliated in the macrostomes (Fig. 76). There are the following morphometric differences (Table S1): on average, there are not 29 but 31 ciliary rows; kinety 2 never recurves (vs. slightly recurving in one-third of microstome cells); 7 vs. 8 postoral kineties; one vs. two kinetofragments at the left margin of the oral opening; three vs. seven basal bodies in the rightmost fragment; and the number of cilia/row increases from 32 to 68 on average. The preoral, unciliated area increases in size and is occasionally slightly convex (Fig. 80).

Oral apparatus (Table S1; Fig. 25–27, 53, 54, 60–62, 64, 67, 68, 69, 71, 73, 75, 77, 78, 100). The macrostome cells of *G. bromelicola* are impressive both in vivo and in preparations (Fig. 61–63, 78, 100). The most distinct changes are related to the oral apparatus, especially the oral opening which now commences almost apically and increases in size from 10 × 6 μm to 26 × 17 μm on average in Chatton-Lwoff silver nitrate preparations (Table S1; Fig. 18, 25, 53, 54, 61, 62, 78, 80, 100). The outline of the oral opening changes from obliquely quadrangular to obliquely obtrian-

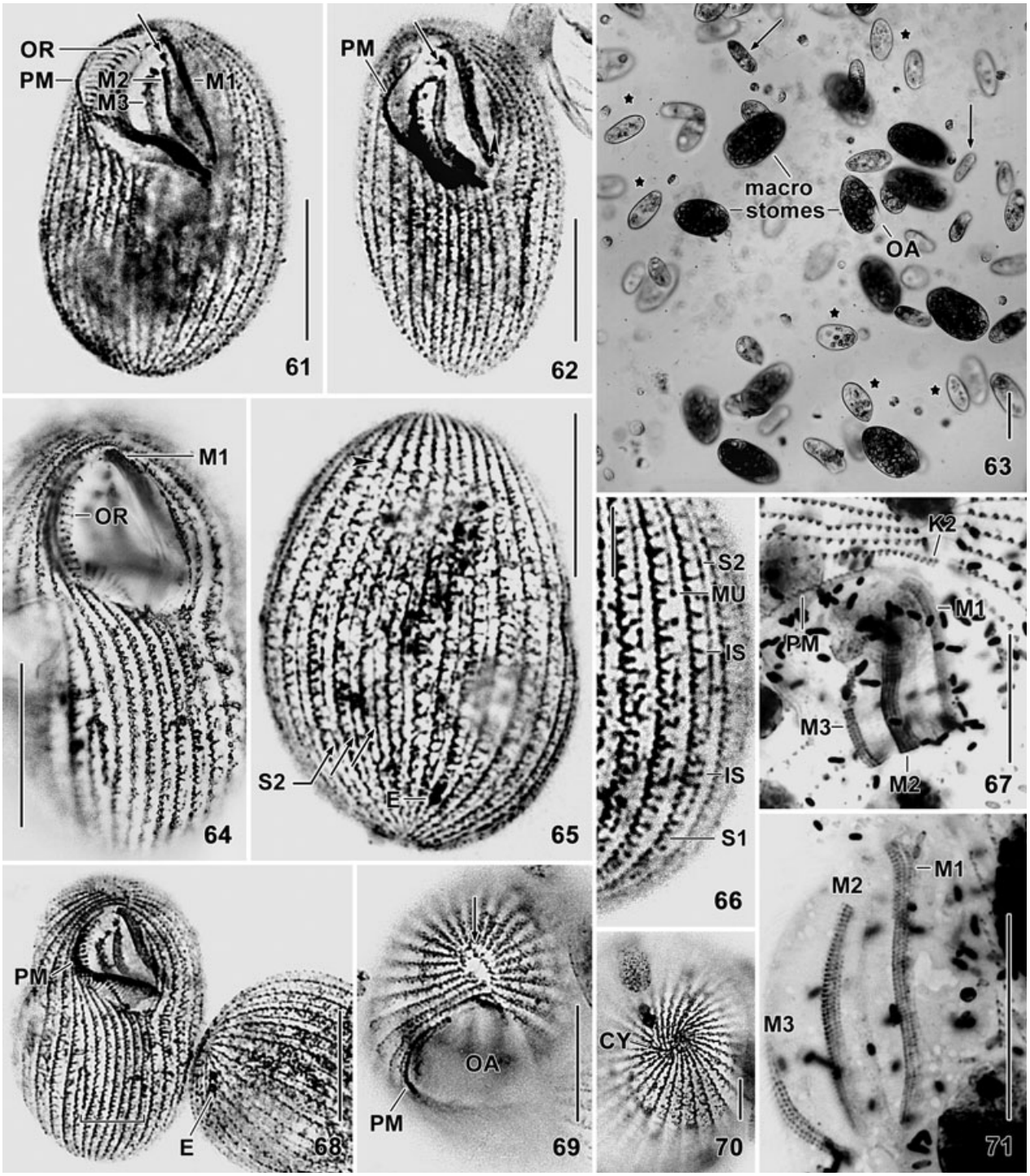

Fig. 61–71. *Glaucomides bromelicola*, macrostome cells in vivo (63), after Chatton-Lwoff silver nitrate impregnation (61, 62, 64–66, 68–70), and after silver carbonate impregnation (67, 71). **61, 62, 64, 68.** Ventral overviews. Arrows in (61, 62) denote the X-body of adoral membranelle 2. The arrowhead in (62) marks two kinetofragments. Parenthesis in (68) embraces postoral kineties. **63.** Bright field micrograph, showing macrostome and microstome (asterisks) cells. The microstomes are distinctly flattened (arrows). The macrostomes appear very dark because they are studded with food vacuoles. **65, 66.** Dorsal overview and detail, showing the silverline pattern. The arrowhead in (65) denotes the third intermeridional connective. **67.** Oral apparatus during microstome–macrostome transformation. **69.** Anterior polar view with barren anterior end marked by arrow. **70.** Posterior polar view. **71.** Adoral membranelles. CY, cytopyge; E, excretory pores of contractile vacuole; IS, intrameridional cross-silverlines; K2, kinety 2; M1, 2, 3, adoral membranelles; MU, mucocyst; OA, oral apparatus; OR, oral ribs; PM, paroral membrane; S1, 2, first and second silverline meridians. Scale bars 10 μm (66, 70), 20 μm (67, 69, 71), 30 μm (61, 62, 64, 65, 68), and 50 μm (63).

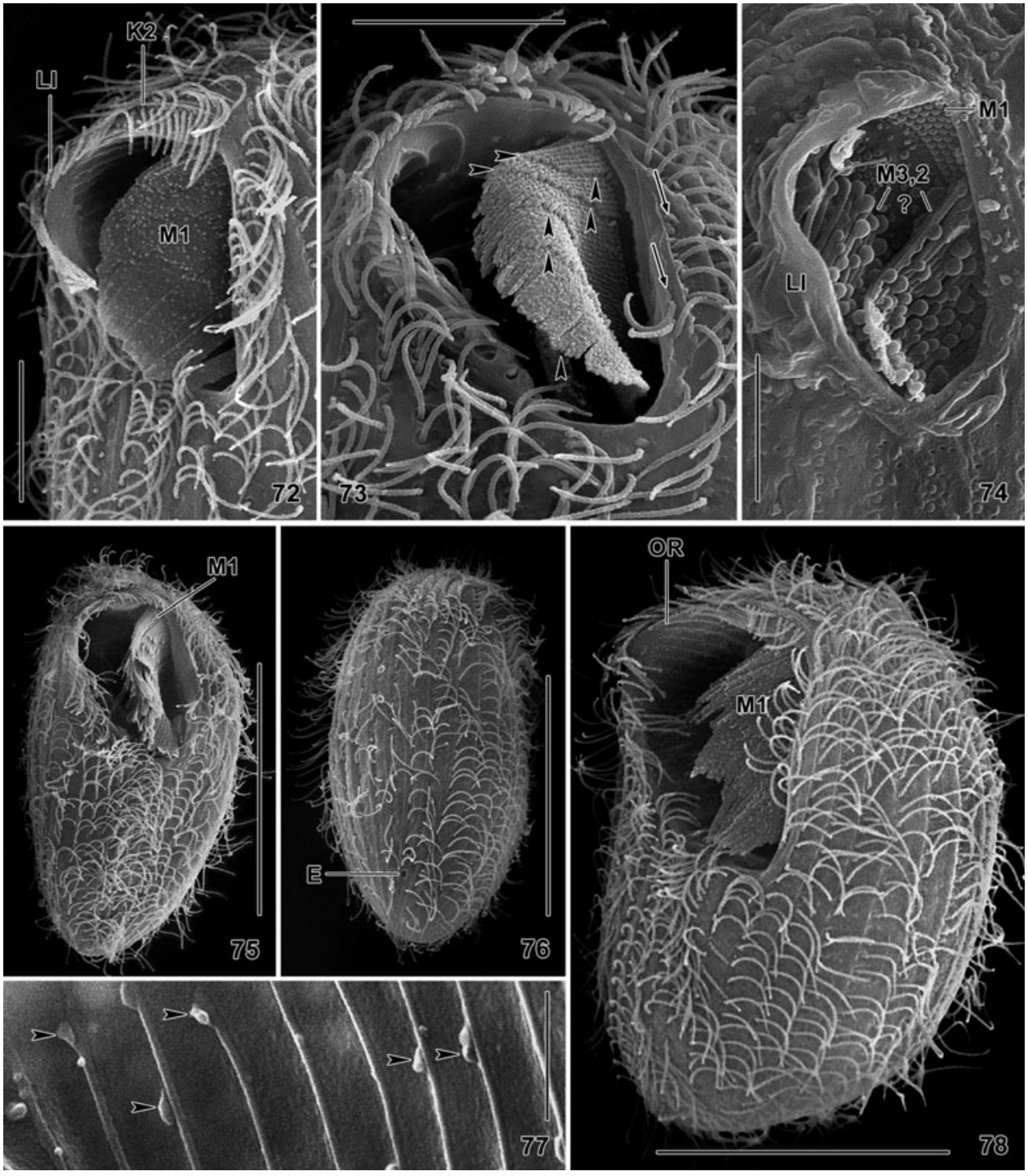

Fig. 72–78. *Glaucomides bromelicola*, macrostomes in the scanning electron microscope. **72.** A microstome specimen developing to a macrostome with larger, obtriangular oral opening. **73.** A fully developed macrostome with large, obtriangular oral opening and a conspicuous adoral membranelle 1 with cilia up to 20 μm long; the arrowheads mark the ciliary rows, which become recognizable due to the different length of the cilia. The arrows mark two kinetofragments at left margin of oral opening. **74.** A deciliated specimen, showing the basal bodies of membranelle 1. **75, 78.** Ventral and ventrolateral view of fully developed macrostomes, showing the huge buccal cavity and the conspicuous adoral membranelle 1, which is shown frontally (75; cp. Fig. 21, 22) and laterally (78; cp. Fig. 19, 20, 26), giving an impression of its scintillating movement. Note the dense postoral ciliature. **76.** Dorsolateral view, showing the subterminal excretory pore of the contractile vacuole. **77.** Oral ribs. Arrowheads mark granular structures attached to the ribs. E, excretory pore; K2, somatic kinety 2; LI, buccal lip; M1, 2, 3, adoral membranelles; OR, oral ribs. Scale bars 2 μm (77), 5 μm (74), 10 μm (72, 73), and 40 μm (75, 76, 78).

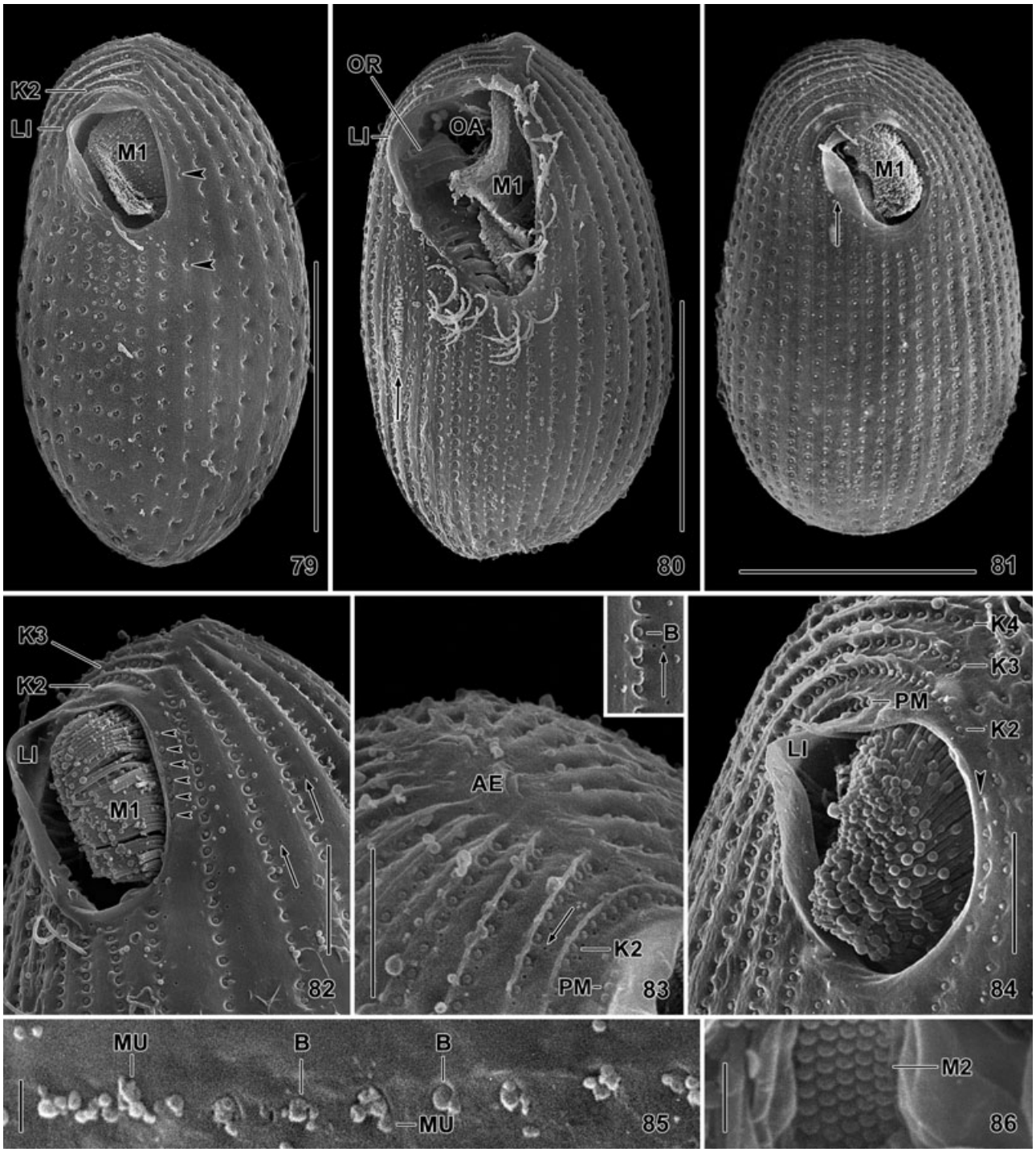

Fig. 79–86. *Glaucomides bromelicola* (79, 80, 82, 83, 85, 86) and *Glaucoma scintillans* (81, 84), deciliated specimens in the scanning electron microscope. **79.** Ventral overview of a microstome specimen. The arrowheads mark an interruption in the first kinety left of the oral opening, producing a kinety fragment. **80.** Ventral overview of a very early macrostome divider with oral primordium marked by an arrow. **81, 84.** Ventral overview and oral detail. Note the buccal lip (arrow), the recurving kinety 2, and the absence of a kinety fragment at left mouth margin (arrowhead). **82, 83.** Ventrolateral and anterior polar view, showing the buccal lip, the kinety fragment at left margin of oral opening (arrowheads), the holes in the cortex left by just extruded mucocysts (arrows and inset), and the obovate, unciliated anterior body end. **85.** Part of a kinety, showing the basal bodies surrounded by just exploding mucocysts. **86.** Part of adoral membranelle 2. AE, anterior body end; B, basal bodies; K2, 3, 4, somatic kineties; LI, buccal lip; M1, 2, adoral membranelles; MU, mucocysts; OA, oral apparatus; OR, oral ribs; PM, paroral membrane. Scale bars 1 μm (85, 86), 5 μm (82–84), and 20 μm (79–81).

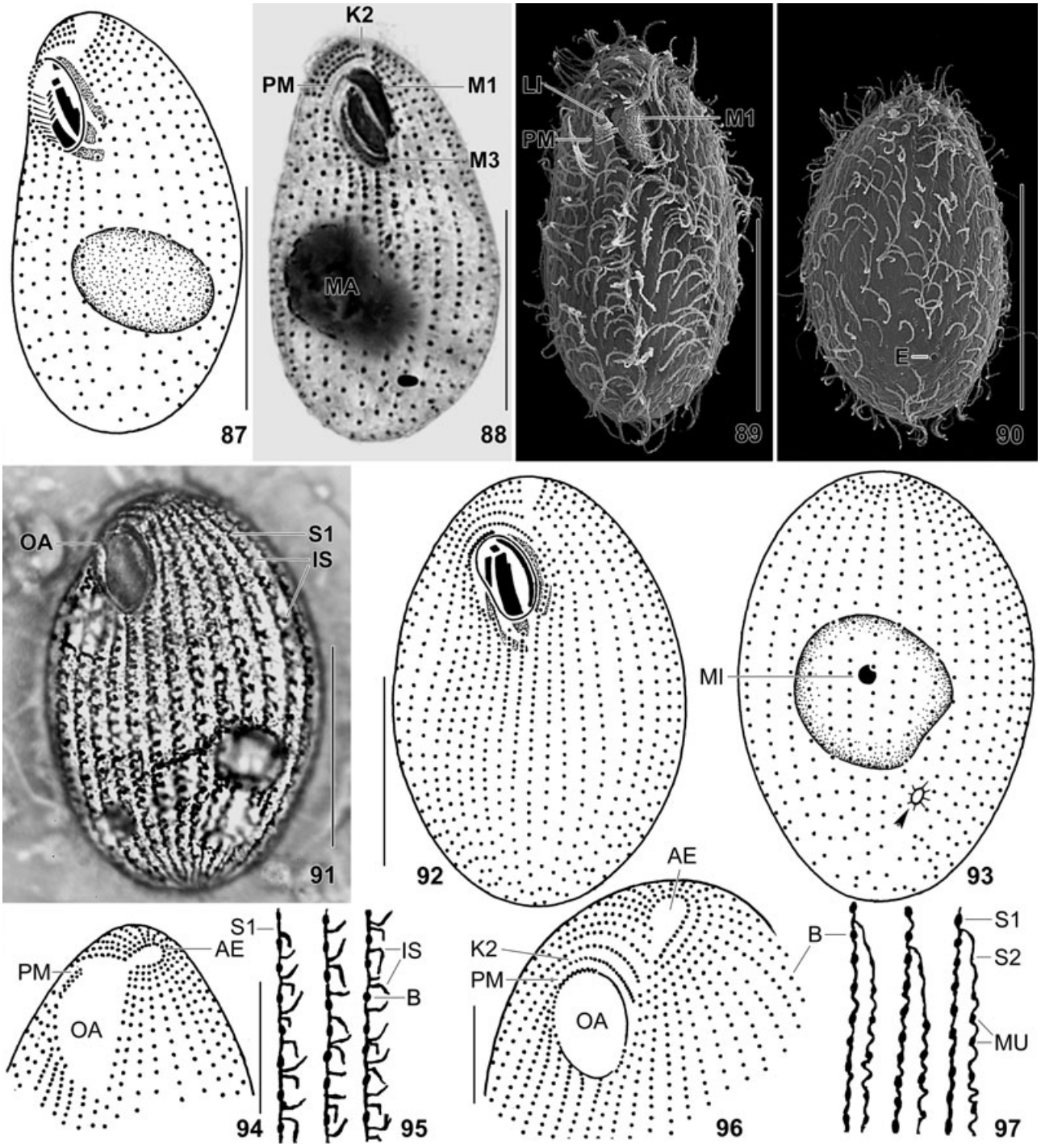

Fig. 87–97. *Glaucoma reniformis* (87–91, 94, 95) and *Glaucoma scintillans* (92, 93, 96, 97) after protargol impregnation (87, 88, 92–94, 96), Klein-Foissner silver nitrate impregnation (91, 95, 97), and in the SEM (89, 90). **87–89, 94.** Ventrolateral (87, 94) and ventral (88, 89) views, showing the ciliary pattern. Somatic kinety 2 does not extend over body's midline. The paroral membrane consists of zigzagging dikinetids anteriorly and of comparatively widely spaced, nonciliated monokinetids posteriorly. Only one basal body of the dikinetids bears a short cilium. **90.** Dorsal view, showing the dispersed ciliature around the excretory pore. **91, 95.** *Glaucoma reniformis* has a tetrahymenid silverline pattern with many intrameridional cross-silverlines. **92, 93, 96.** Ventral, dorsal, and oblique ventral view of the ciliary pattern of *Glaucoma scintillans*. Although the ciliature is slightly dispersed around the excretory pore (arrowhead and Fig. 102), the basal bodies are aligned. Somatic kinety 2 is conspicuously recurved anteriorly. **97.** *Glaucoma scintillans* has sinuous secondary silverline meridians. AE, anterior body end; B, basal bodies; E, excretory pore; IS, intrameridional cross-silverlines; K2, somatic kinety 2; LI, buccal lip; MA, macronucleus; MI, micronucleus; MU, mucocysts; M1, 3, adoral membranelles; OA, oral apparatus; PM, paroral membrane; S1, 2, silverline meridians. Scale bars 10 μm (94, 96) and 20 μm (87–93).

gular. The oral lip is increased in length, but not in width and is thus inconspicuous (Fig. 53, 74, 80). The paroral also increased in length, but the cilia are only 2–3 μm long in scanning micrographs. Conspicuous ribs develop on the right wall of the oral cavity (Fig. 53, 61, 64, 78, 80).

The adoral membranelles strongly increase in length while the number of ciliary rows composing the membranelles is similar to the microstomes (Table S1; Fig. 62, 67, 71, 75, 78, 80); however, most rows of the macrostome membranelles have now the same length (Fig. 67, 71). In the scintillating membranelle 1, the length of the cilia gradually increases from about 5 μm in the leftmost row to 25 μm in the rightmost row (Fig. 73, 75, 78, 100). Membranelles 2 and 3 are in the buccal cavity, which is 15–20 μm deep ($\bar{x}$ = 17.0, n = 11) and usually covered by the cilia of membranelle 1; possibly, their cilia are rather short.

Silverline pattern (Fig. 61, 62, 64–66, 68). The silverline pattern of the macrostomes is tetrahymenid as that of the microstomes. However, the second-order silverline meridians are more common and distinct. Likewise, the intrameridional cross-fibres are more pronounced.

Notes on *Glaucoma scintillans* and *G. reniformis*

The morphometric data and micrographs presented here supplement previous descriptions and the reviews of Corliss (1971) and Foissner et al. (1994).

Glaucoma scintillans Ehrenberg 1830 (Table S1; Fig. 81, 84, 92, 93, 96, 97, 101–104, 106).

This is the type of the genus and has been well described by Bary (1950), Corliss (1971), Dragesco and Dragesco-Kernéis (1986), Foissner et al. (1994), Gelei and Horváth (1931), Klein (1926), Klug (1968, Table 8), and McCoy (1975). To be concise, I mention these authors only when their data do not match earlier and/or the present results.

The body size is 35–75 × 25–45 μm in vivo, usually about 55 × 30 μm. The body shape is narrowly to broadly ellipsoidal, usually slightly widened posteriorly (Fig. 81, 92, 101, 102). The nuclear apparatus is near the body centre. The macronucleus and the micronucleus are globular. The contractile vacuole is near the rear body third (Table S1; Fig. 93, 102) and has been illustrated by several authors too far posteriorly; depending on the number of ciliary rows, the dorsolateral excretory pore is usually associated with kineties 7–9. The extrusomes, likely mucocysts, are about 1 μm long and thus inconspicuous. The cortex and the cytoplasm are colourless, the latter is usually studded with food vacuoles containing various bacteria (for an ecological review, see Foissner et al. 1994). Swimming is moderately rapid and cells never rest.

Glaucoma scintillans has 30–40, on average about 35 ciliary rows, of which 6–10, usually 8 are postoral; these are more narrowly spaced and more densely ciliated, producing metachronal ciliary waves (Table S1; Fig. 92, 101, 104). The ciliary rows begin at the level of the left margin of the oral opening, and kinety 2, which is very densely ciliated anteriorly, distinctly recurves, i.e. extends around the anterior edge of the oral opening to end on its opposite side almost touching the first kinety left of the oral opening (Fig. 84, 92, 96, 104). The ciliature is slightly dispersed in the region of the excretory pore of the contractile vacuole (Table S1; Fig. 102). There is no kinetofragment at the left margin of the oral opening (Fig. 92, 96, 104).

The oral apparatus of *G. scintillans*, as described by McCoy (1975), is highly similar to that of *Glaucomides bromelicola* (Table S1; Fig. 14, 92, 101). The components are slightly larger and the kineties comprising adoral membranelle 1 are of very similar length (vs. of very different length).

The silverline pattern is glaucomid, i.e. there are primary silverline meridians which contain the basal bodies of the cilia and secondary meridians which contain granules belonging to the extrusomes. The shape of the secondary meridians varies from straight to very sinuous (Fig. 97, 103, 106). The primary meridians lack intrameridional cross-fibres.

The ontogenesis has been described very carefully by Peck (1974). No resting cysts have been reported. My attempts to induce cysts in populations from a variety of habitats failed. When isolated without food, the cells gradually become smaller and finally die.

Glaucoma reniformis Schewiakoff 1892 (Table S1; Fig. 87–91, 94, 95).

This species has been described as *G. chattoni* by Corliss (1959). However, we agree with McCoy (1975) that it is a junior synonym of *G. reniformis*. *Glaucoma reniformis* is rather well known due to the investigations of Corliss (1971), Dragesco and Dragesco-Kernéis (1986), Frankel (1960), Klug (1968), McCoy (1975), Peck (1974), and Schewiakoff (1893). I mention these authors only when their data do not match previous and/or the present results.

Glaucoma reniformis has a size of 35–65 × 20–35 μm in vivo; usually, it is about 45 × 25 μm when a preparation shrinkage of 15% is assumed (Table S1). The body is ovate or narrowly to ordinarily ellipsoidal; the ventral side is slightly to distinctly concave in about one-third of the Salzburg specimens, which thus look reniform in lateral view. The ventral concavity has been emphasized by Schewiakoff (1893) in naming his Australian specimens "*reniformis*". The concavity is usually less distinct in cultures and in silver impregnated specimens (Table S1; Fig. 87–90). The nuclear apparatus is near the body centre. The macronucleus is globular to broadly ellipsoidal (Fig. 87); a micronucleus is not recognizable in the Salzburg specimens, just as in the North American strain investigated by Frankel (1960). The excretory pore of the contractile vacuole is associated with kineties 5–7 (usually kinety 6) and is on average 25% from the posterior body end (Table S1; Fig. 90). The inconspicuous extrusomes, likely mucocysts, are about 1 μm long. The cortex and cytoplasm are colourless, the latter usually packed with food vacuoles containing various bacteria (for an ecological review, see Foissner et al. 1994). Swimming is moderately rapid and cells never rest.

Glaucoma reniformis has 22–30, usually 26–29 ciliary rows, of which 5–7, usually 6 are postoral and more narrowly spaced and more densely ciliated, producing metachronal ciliary waves (Table S1; Fig. 87–89). The ciliary rows are slightly curved apically, except the postoral ones

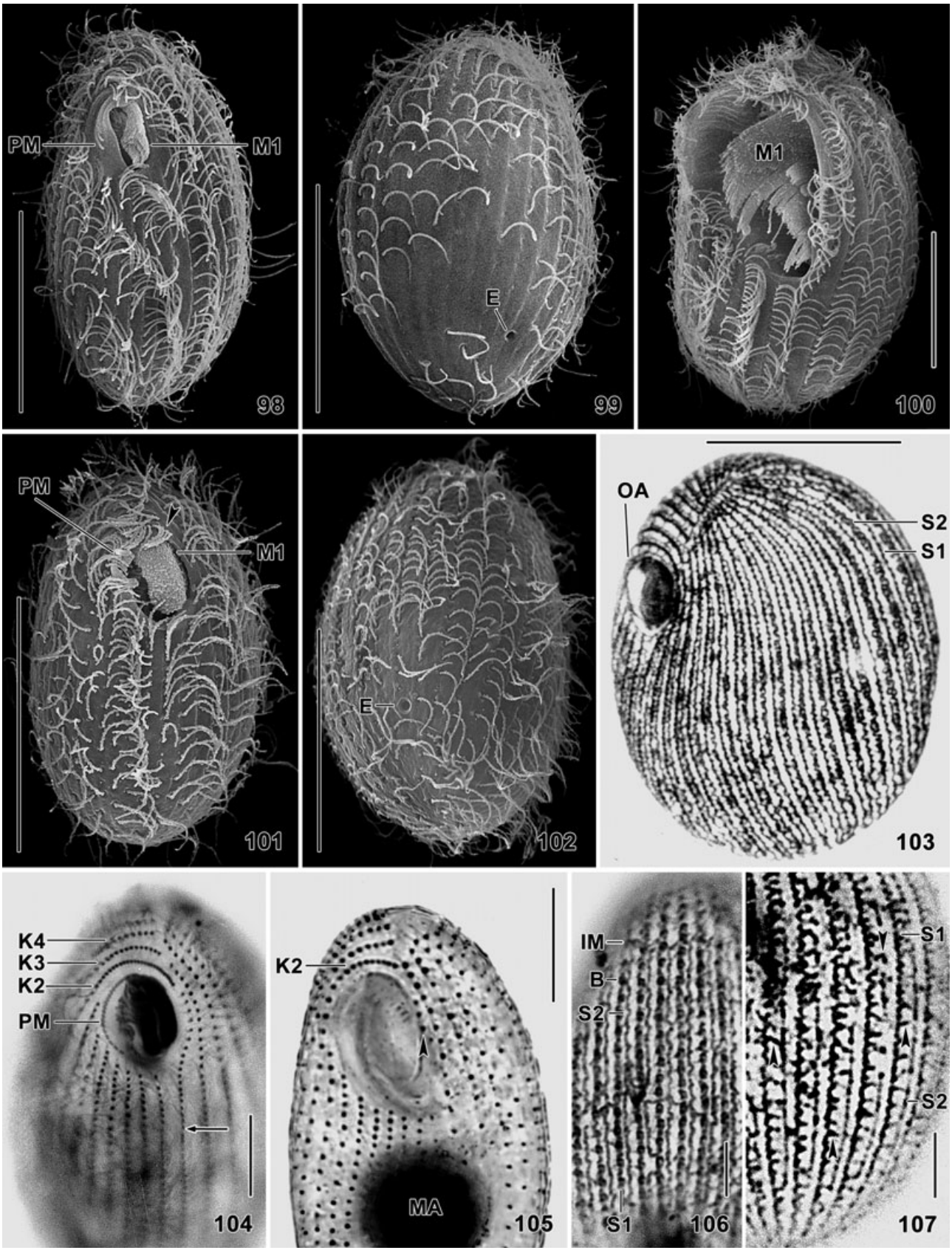

Table 1. Morphometric and morphological differentiation of four glaucomids (arithmetic means are provided; for details, see Table S1 and text).

Characteristics[a]	Methods[b]	*Glaucomides bromelicola* (microstomes)	*Glaucomides bromelicola* (macrostomes)	*Glaucoma scintillans* (Salzburg)	*Glaucoma reniformis* (Salzburg)	*Glaucoma reniformis* (type)
Body, length (μm)	SN	54.4	75.5	48.2	46.6	85.0
Body length: oral opening length, ratio	SEM	4.9	2.8	4.6	3.9	7.4[c]
Body length: anterior end to excretory pore, ratio	P	1.2	1.2	1.4	1.3	1.2
Anterior body end: proximal end of membranelle 1, ratio	P	2.7	2.1	2.3	3.8	5.0
Somatic kineties, total number	P	28.7	30.6	36.7	26.2	26.7
Postoral kineties, number (including kinety 1)	P	7.2	8.2	7.8	5.8	5.7
Kinetofragments at left mouth margin, number	P	1.1	2.0	Usually absent	0.4	Absent
Excretory pore located in kinety	P	5.1	5.3	8.6	6.0	6.3
Anterior end of somatic kinety 2	P	Slightly recurved	Not recurved	Distinctly recurved	Not recurved	Not recurved
Silverline pattern	SN	Tetrahymenid	Tetrahymenid	Glaucomid	Tetrahymenid	Tetrahymenid
Macrostomes	C	Present	–	Absent	Absent	Absent

[a]From cultures.
[b]C, cultures; P, protargol impregnation; SEM, scanning electron micrographs; SN, silver nitrate impregnation.
[c]From silver nitrate preparations (Chatton-Lwoff method).

and kinety 2 is densely ciliated anteriorly and does not recurve. The ciliature is slightly to distinctly dispersed in the region of the excretory pore of the contractile vacuole (Table S1; Fig. 87, 88, 90, 94). Of 25 specimens investigated, 15 do not have a kinetofragment at the left margin of the oral opening, 6 have a fragment, and 4 are intermediate, i.e. one or two kinetids are lacking at the level of the posterior mouth margin (Table S1; Fig. 87, 88, 91, 94). This fragment has been not described by the authors cited above.

The oral apparatus of *G. reniformis*, as described by McCoy (1975), is highly similar, if not to say identical, with that of *Glaucomides bromelicola* (Table S1; Fig. 14, 87–89). It occupies on average 25% of the body length in *G. reniformis* while only 18% in *G. bromelicola* (Table S1).

The silverline pattern is tetrahymenid, i.e. there are primary silverline meridians, which contain the basal bodies of the cilia and produce many intrameridional cross-fibres, which contain granules belonging to the extrusomes (Fig. 91, 95). Secondary silverline meridians are absent.

The ontogenesis has been carefully described by Frankel (1960), and the fine structure has been investigated by Peck (1978). There are no reports on resting cysts.

DISCUSSION

Family classification of *Glaucomides*

This is discussed in Foissner and Stoeck (2013). Briefly, the morphological investigations presented here and the preliminary molecular data in Foissner et al. (2003) and Dunthorn et al. (2012) classify *Glaucomides* into the Bromeliophryidae Foissner 2003b; the sister family of the Glaucomidae Corliss 1971.

Glaucomides bromelicola and *Glaucoma* spp. have much in common; for instance, body shape, most morphometrics, the general somatic and oral ciliature, and even the silverline pattern (*Glaucoma reniformis*). This strongly suggests a common ancestor and, possibly, some recessive

Fig. 98–107. Comparison of *Glaucomides bromelicola* (98–100, 105, 107) and *Glaucoma scintillans* (101–104, 106) in the SEM (98–102), after Klein-Foissner (103) and Chatton-Lwoff (106, 107) silver nitrate impregnation, and in protargol preparations (104, 105). **98, 99.** Ventral and dorsal overview, showing the densely ciliated postoral region and the large, barren area on dorsal side, where many nonciliated basal bodies occur (cp. Fig. 4). **100.** *Glaucomides bromelicola* forms conspicuous macrostomes absent in *Glaucoma scintillans* and *Glaucoma reniformis*. **101, 102.** Ventral and dorsal overview of *G. scintillans*, showing the dense postoral ciliature and the slightly dispersed cilia around the excretory pore, which is much more anteriorly than in *G. bromelicola* (Fig. 99). The arrowhead in (101) marks the densely ciliated, recurved anterior portion of somatic kinety 2. **103, 106, 107.** Comparison of the silverline pattern in *Glaucoma scintillans* (103, 106) and *Glaucomides bromelicola* (107). The former has distinct, sinuous secondary silverline meridians while the latter has many intrameridional cross-silverlines (arrowheads). **104, 105.** *G. scintillans* has a recurving somatic kinety 2 and an uninterrupted kinety (arrow) right of the oral opening while *G. bromelicola* has an ordinarily curved kinety 2 and a kinetofragment at left margin of the oral opening (arrowhead). B, basal body; E, excretory pore of contractile vacuole; IM, intermeridional connective; K2, 3, 4, somatic kineties; MA, macronucleus; M1, adoral membranelle 1; OA, oral apparatus; PM, paroral membrane; S1, 2, primary and secondary silverline meridians. Scale bars 10 μm (104–107) and 25 μm (98–103).

mutations expressing plesiomorphic features of the common ancestor (Mayr 1963; Minelli 2009; Sudhaus and Rehfeld 1992).

Comparison of *Glaucomides bromelicola* with related and similar species (Table 1; Fig. 79–107)**.**

Glaucomides bromelicola is easily distinguished from the other known members (two still unpubl., but see Foissner et al. 2003) of the Bromeliophryidae by the body shape: ellipsoidal to slightly ovate or obovate vs. more or less *Metopus*-shaped. Furthermore, the other taxa have a nonciliated area not only dorsolaterally but also left of the oral apparatus (Foissner 2003b; Foissner et al. 2003). However, in vivo, it is difficult to distinguish from another glaucomid discovered in Jamaica (W. Foissner, unpubl.). *Glaucomides bromelicola* is difficult to distinguish from several *Glaucoma* species, especially *G. scintillans* and *G. reniformis*, warranting a detailed comparison.

Glaucoma scintillans

There is hardly any morphometric feature that would separate unequivocally *G. scintillans* from *Glaucomides bromelicola* because both have high intrapopulation variability (Table S1). Usually, *G. scintillans* has more ciliary rows (37 vs. 29) and a different location of the excretory pore of the contractile vacuole: at 69% vs. 82% of body length.

Fortunately, there are several morphological and life cycle features that separate *G. scintillans* unequivocally from *G. bromelicola*. In vivo, they can be distinguished by the ciliature: holotrichous vs. a large, nonciliated area dorsolaterally, but the ciliature of *G. scintillans* is also slightly dispersed in this area (cp. Fig. 52, 99, 102). In cultures, *G. scintillans* and *G. bromelicola* can be distinguished by the ability of the latter to produce macrostomes. Macrostomes have not been described in any *Glaucoma* species, except *G. ferox* discussed below. In silver nitrate and protargol preparations, *G. scintillans* and *G. bromelicola* can be distinguished by (i) the recurved kinety 2 (cp. Fig. 84, 92, 96, 104 with 3, 11, 28, 34, 37, 82, 105), a quite typical feature of *G. scintillans* (Corliss 1971; McCoy 1975), (ii) the absence of a kinety fragment left of the oral opening (cp. Fig. 84, 92, 96, 101, 104 with 3, 11, 15, 17, 28, 34, 37, 51, 73, 79, 82, 105), and (iii) the silverline pattern (glaucomid vs. tetrahymenid; cp. Fig. 97, 103, 106 with 12, 41–45, 107). In the molecular tree, *G. scintillans* and *G. bromelicola* are clearly different (Foissner and Stoeck 2013). Furthermore, both can be differentiated by fluorescence in situ hybridization (Fried and Foissner 2007).

Glaucoma reniformis

This species is even more similar to *Glaucomides bromelicola* than *Glaucoma scintillans*, especially in its morphometric characteristics, most overlapping more or less distinctly (Table 1, S1), except of the length ratio of body and oral opening (3.9 vs. 4.9; 7.4 vs. 4.9 in lectotype) and the length ratio of anterior body end to the proximal end of adoral membranelle 1 (3.8 vs. 2.7; 5.0 vs. 2.7 in lectotype). Studies on further populations are required to estimate the discriminatory value of these ratios.

In vivo, *G. reniformis* and *G. bromelicola* can be distinguished by the body shape (*Tetrahymena pyriformis*-like vs. *Glaucoma scintillans*-like; cp. Fig. 87–91 with 1, 3, 18, 19, 34, 37, 46, 50, 79, 81, 98, 99) and the ciliature (holotrichous vs. a large, nonciliated area dorsolaterally); however, the ciliature of *G. reniformis* is also slightly dispersed in this region (cp. Fig. 90 with 52, 99). In cultures, *G. reniformis* and *G. bromelicola* can be distinguished by the ability of the latter to produce macrostomes (Fig. 53–55, 61–63, 75, 78, 100). In silver nitrate and protargol preparations, the two species can be distinguished by the usual absence of a kinety fragment left of the oral opening in *G. reniformis* (cp. Fig. 87, 88, 94 with 3, 11, 15, 17, 28, 34, 37, 51, 73, 79, 82, 105). In the molecular tree, *G. reniformis* makes a clade with *G. scintillans* and is thus distinctly different from *Glaucomides bromelicola* (Foissner and Stoeck 2013).

Glaucoma ferox

This species, described in detail by Puytorac et al. (1973) and raised to subgeneric level (*Amphiglaucoma*) by Jankowski (2007), resembles *Glaucomides bromelicola* in many features. Unfortunately, the original habitat of *G. ferox* is unknown. *Glaucoma ferox* is a polymorphic species whose life cycle includes four forms all lacking the kinetofragment at left mouth margin so typical for *Glaucomides bromelicola*. The trophotomont (feeding stage of the tomont) of *G. ferox* feeds only on ciliates (macrostome *Glaucomides* only on flagellates) and membranelle 1 consists of 7–12 ciliary rows and membranelle 2 of 12–15 rows (only up to 6 in *Glaucomides*). The "giant cells" resemble the macrostomes of *G. bromelicola,* but are microstomous feeding on bacteria and yeast, while *Glaucomides* feeds on flagellates. The trophozoites of *G. ferox*, which have a length of only 15–25 μm and are cannibalistic, do not occur in *G. bromelicola*. The microstomous vegetative cells are less than 50 μm in size and originate from the trophotomonts and the giant cells. They feed on bacteria and have membranelles 1 and 2 each composed of 4–7 ciliary rows. Thus, they might be difficult to distinguish from the microstomes of *G. bromelicola*, except for the kinetofragment at left mouth margin. Although certain cells of *G. ferox* look quite similar to *Glaucomides bromelicola* (see Fig. 5 in Puytorac et al. 1973), both are very likely only distantly related. This is indicated by the feeding mode: *G. ferox* feeds on various ciliates while *G. bromelicola* forms macrostomes taking up only whole, middle-sized (20–40 μm) flagellates of the genus *Polytomella*.

McCoy (1975) could induce giant cells (~ 80 μm) in several strains of *Glaucoma reniformis* and *Glaucoma scintillans*, using diluted fresh egg yolk as a culture medium, but predatory cells feeding on *Tetrahymena* could be produced only in strain Hz-1 (from the Holz collection) and in strain T (from a stream in Tennessee). The predatory cells looked like the trophotomonts of *G. ferox* and thus were not macrostomous as in *Glaucomides bromelicola*. Unfor-

tunately, McCoy (1975) did not provide any figures for these observations.

Other *Glaucoma* species

There are at least four *Glaucoma* species that resemble microstomous *Glaucomides bromelicola*. *Glaucoma setosa* Schewiakoff 1892 is similar to *G. bromelicola* in many features, but has a caudal cilium (Schewiakoff 1893; Foissner unpubl.). The curious features of *Glaucoma kirki* Bary 1950 were confirmed by McCoy (1975), i.e. it has an anterior knob and a comparatively large macronucleus. *Glaucoma macrostoma* belongs to the *G. scintillans* complex because it has a strongly recurved kinety 2 (Corliss 1971); it is not known whether it has a microstome and a macrostome form. *Glaucoma intermedia* (Gajevskaja 1927) Corliss 1954 from Lake Baikal is poorly known, but separable from *Glaucomides bromelicola* by the extremely large macronucleus (Gajevskaja 1927, 1933).

TAXONOMIC SUMMARY

Order Tetrahymenida Fauré-Fremiet in Corliss 1956.

Remarks. For a modern characterization, see Lynn (2008).

Family Bromeliophryidae Foissner 2003b.

Improved diagnosis. Medium-sized Tetrahymenida with partially reduced somatic ciliature and a patch ("X-group") of basal bodies at anterior end of adoral membranelle 2. Life cycle monophasic or biphasic with rapacious macrostomes. Silverline pattern tetrahymenid. Stomatogenesis glaucomid. Somatogenesis unique, that is, one or several left side kineties split and the anterior fragments migrate to the left margin of the oral opening. Inhabit neotropic phytotelmata (usually tank bromeliads).

Type genus. *Bromeliophrya* Foissner 2003b.

Taxa assignable. Presently, the family comprises only *Bromeliophrya* and *Glaucomides*. However, two further genera are known, but not yet described (Foissner and Stoeck 2013; Foissner et al. 2003b).

Remarks. The family diagnosis of Foissner (2003b) has been improved to include the silverline pattern, the usual habitat, and the biphasic life cycle of *Glaucomides*.

Genus *Glaucomides* n. gen.

Diagnosis. Small to medium-sized Bromeliophryidae with a nonciliated dorsolateral area and a biphasic life cycle with conspicuous macrostomes. Paroral composed of dikinetids in anterior third while of unciliated monokinetids in middle and posterior thirds. Adoral membranelles glaucomid, membranelle 1 scintillating.

Type species. *Glaucomides bromelicola* n. sp.

Etymology. Composite of the generic name *Glaucoma* and the Greek suffix *ides* (similar), referring to the conspicuous similarity with several *Glaucoma* species. Masculine gender.

Remarks. See discussion chapter for distinguishing *Glaucomides* and *Bromeliophrya*. As yet monotypic, but at least one additional species has been observed in tank bromeliads from Jamaica (Foissner and Stoeck 2013).

Species *Glaucomides bromelicola* n. sp.

Diagnosis. Cultivated microstome cells: Size in vivo about 57 × 30 μm, environmental specimens only about 27 × 17 μm, slightly to distinctly flattened laterally. Body usually broadly to ordinarily ellipsoidal or obovate, sometimes even with a short tail. A single, broadly ellipsoidal macronucleus and micronucleus. Contractile vacuole with a single excretory pore about 18% from posterior end of body. Mucocysts numerous, about 1 μm long. On average 29 ciliary rows, seven postoral. A single kinetofragment at left margin of oral opening. Oral apparatus subapical, oral opening obliquely quadrangular or slightly obovate, about 10 × 6 μm in silver nitrate preparations. Oral lip conspicuous. Feeds on bacteria. Widely distributed in tank bromeliads of the neotropics.

Macrostomes: Transforms to macrostomes when bacterial food is depleted, then specifically feeding on an up to 40-μm-long flagellate of the genus *Polytomella*. Size in vivo about 80 × 50 μm, slightly to distinctly flattened laterally. Body usually broadly ellipsoidal or slightly to distinctly obovate. Nuclear apparatus and extrusomes as in microstome cells. Contractile vacuole with two excretory pores on average 15% from posterior body end. On average 31 ciliary rows, eight postoral. Two kinetofragments at left margin of oral opening. Oral apparatus almost apical, oral opening obtriangular, about 26 × 17 μm in silver nitrate preparations. Oral lip inconspicuous. Paroral and adoral membranelles and their cilia greatly elongated. Right wall of buccal cavity with conspicuous oral ribs.

Type locality. In tanks of bromeliads from the Pico Isabel de Torres, an 800-m-high mountain on the outskirts of the town of Puerto Plata, Dominican Republic, 70°40′W 19°50′N.

Etymology. The Latin adjective *bromelicola* refers to the habitat in which the species was discovered.

Type material. I deposited 19 slides with specimens from the Dominican type locality and 13 slides with cells from two Jamaican populations in the Oberösterreichische Landesmuseum in Linz (LI), reg. no. 2012/69–100. In detail, the following material has been deposited from the type locality (all from semi-pure cultures, if not mentioned otherwise): a slide each with protargol-impregnated hapantotypes for the microstome and macrostome morph, four paratype slides, and two slides with protargol-impregnated environmental specimens; two paratype slides with microstomes and macrostomes impregnated with silver carbonate (poor appearance at low magnification, but fine mouth details can be seen with an oil immersion objective); a slide each with silver nitrate-impregnated (Klein-Foissner method) hapantotypes for the microstome and macrostome morph, four paratype slides, and five slides with microstome and macrostome specimens impregnated with the silver nitrate method of Chatton-Lwoff. The protargol slides contain also those specimens, marked with "MG", that were used for studying the ontogenesis. The following voucher slides with specimens from Jamaica have been deposited: nine and four slides, respectively, impregnated with the silver nitrate methods of Klein-Foissner and Chatton-Lwoff.

Furthermore, I deposited voucher slides from *Glaucoma scintillans* and *G. reniformis* at the same locality, reg. no. 2012/101–103 and 2012/104–108 and 114–126. From the former, I deposited two slides with protargol-impregnated specimens and one slide with silver nitrate-impregnated specimens (Chatton-Lwoff method). From the latter, I deposited six slides with protargol-impregnated specimens and three slides each with silver nitrate-impregnated specimens, according to the methods of Klein-Foissner and Chatton-Lwoff. Relevant specimens have been marked with black ink circles on the coverslip.

Remarks. Considering the similarities with some *Glaucoma* species, I have deposited many slides, showing *Glaucomides bromelicola* and *Glaucoma* spp. prepared with four different silver methods. For typification, I use the hapantotype concept (ICZN 1999) because the microstomes and macrostomes have a rather different morphology and life style.

ACKNOWLEDGMENTS

Financial support was provided by FWF Projects P20360-B17 and P22846-317. The technical assistance of B. Harl and R. Schörghofer is greatly acknowledged.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Morphometric data on *Glaucomides bromelicola* microstomes (GB) and macrostomes (GM), *Glaucoma scintillans* (GS), and *Glaucoma reniformis* (GR).