

# Morphology and ontogenesis of *Lambornella trichoglossa* nov. spec., a new tetrahymenid ciliate (Protozoa, Ciliophora) from Brazilian tank bromeliads (Bromeliaceae)

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This is the second of a series of papers describing the morphology and ontogenesis of new ciliates occurring in the cisterns of tank bromeliads, a group of rosette plants of tropical America, entrapping rainwater between the coalescing leaf axils. The new species described here was discovered in the tanks of ground bromeliads from the east coast (Mata Atlantica) of Brazil, South America, and in tank bromeliads of the Dominican Republic, Caribbean Sea. Its morphology and ontogenesis were investigated on cultivated specimens, using live observation, silver impregnation, and scanning electron microscopy. Morphologically, the new ciliate resembles the tetrahymenine genus *Lambornella*, while the habit is different in that it does not parasitize and produce cuticular cysts on mosquito larvae. Thus, it could be considered as representative of a new genus. Likewise, the Brazilian and Dominican populations are probably not conspecific. Basically, *Lambornella trichoglossa* nov. spec. has a tetrahymenid somatic and oral ciliary pattern with characteristics likely diagnostic for the, unfortunately, still insufficiently described type species of *Lambornella*, viz., an increased number (8) of postoral kineties, a spatulate preoral suture, a curved adoral membranelle 1, a sigmoidal membranelle 2, loop-like intrameridional silverlines, and a synchronous replacement of the parental oral apparatus during cell division, a conspicuous ontogenetic feature. *Lambornella trichoglossa* differs from the two described congeners mainly by the large size ( $200 \times 50 \mu\text{m}$  vs.  $\leq 100 \times 50 \mu\text{m}$ ), the fusiform to claviform shape (vs. ovoidal), the location of the contractile vacuole (near mid-body vs. posterior body third), a  $25 \mu\text{m}$  long caudal cilium (vs. lacking), and the arrangement of adoral membranelle 2 (directed to right vs. left). Considering that *L. trichoglossa* has not been found in Europe, though it is a large, conspicuous ciliate, it might be restricted to South America or Gondwana.

**Key words:** Biodiversity; Bromeliad tanks; Mata Atlantica; South America; *Lambornella trichoglossa* nov. spec.; Tetrahymenida.

## Introduction

Taxonomic research on tetrahymenid ciliates stagnated after a bloom between the fifties and seventies (Corliss 1952, 1979; Elliott 1973). Presently, the order Tetrahymenida comprises 14 genera (Lynn 1994; Lynn and Small 2002). The last new genus was described in 1985 by Small and Lynn, seemingly supporting the hypothesis of Finlay et al. (1996) that most free-living ciliates are known. However, in my

opinion the stagnation is caused by a dramatic decrease of educated taxonomists and of alpha-taxonomic research in general. This is emphasized by the discovery of a distinct tetrahymenid genus in the water cisterns of bromeliads, viz., *Bromeliophrya* Foissner, 2003, and the new species described in the present paper, *Lambornella trichoglossa*, which probably also represents a new genus. See Foissner (2003) and the last section of the present paper for a brief description of the tank bromeliad habitat.

## Material and methods

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

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

I discovered *L. trichoglossa* in December 1996 and investigated it "as usual" because I did not expect that generic classification would pose many problems, that is, I pre-identified it as a new, gigantic *Tetrahymena* species. However, when I studied the slides in detail and put together the data for publication in spring 2002, I recognized that it possesses some unusual ontogenetic features and could belong to *Lambornella*, a genus similar to *Tetrahymena* morphologically, but unique in infecting mosquito larvae and forming special "cuticular cysts" on their surface. Unfortunately, I could not retrieve the missing data on ontogenesis, cysts, and infectivity because the cultures declined and were discarded in 1997.

Fortunately, I spent my holidays in the Dominican Republic in August 2002. Here, I re-discovered the Brazilian species in bromeliads from two sites, and could thus not only collect additional morphological data but also the missing details mentioned. The specimens were cultivated as described above. Methodological details from the investigations on cysts and infectivity are provided in the respective Results sections.

Cells were studied *in vivo* using a high-power, oil immersion objective and differential interference contrast optics. The infraciliature and various cytological structures were revealed by scanning electron microscopy and the silver impregnation techniques described in Foissner (1991). Counts and measurements on prepared specimens were performed at a magnification of  $\times 1,000$ . *In vivo* measurements were conducted at a magnification of  $\times 100$ – $1,000$ . Although these provide only rough estimates, it is worth giving such data as specimens may

change in preparations. Illustrations of live specimens were based on video-records and micrographs, while those of prepared cells were made with a camera lucida. Terminology is according to Peck (1974), Corliss (1952, 1979), and Foissner (1996).

## Results

### Morphology

As conspecificity of the Brazilian and Dominican Republic populations is questionable, data are kept separate and the diagnosis contains only the Brazilian type population. Further, all observations are, if not mentioned otherwise, from cultivated specimens. Morphometric data shown in Tables 1 and 2 are repeated in the description only as needed for clarity.

### Description of Brazilian type population of *Lambornella trichoglossa* nov. spec. (Figs 1–12, 14–29, 33–38; Table 1)

**Diagnosis:** Size about  $200 \times 50$  µm *in vivo*. Indistinctly fusiform, usually slightly sigmoidal and widest postorally due to a ventral hump. Nuclear apparatus postoral in second quarter of cell. Contractile vacuole near mid-body, usually 2 excretory pores at end of kiny 6. Extrusomes rod-shaped, about  $2.5 \times 0.25$  µm. On average 47 ciliary rows, of which 8 are postoral, the others about preorally along a spatulate suture; distance between kiny 1 and n distinctly increased postorally; 1 caudal cilium. Adoral membranelle 1 curved, membranelle 2 sigmoidal and directed right, membranelle 3 minute. Silverlines loop-like projecting between basal bodies.

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

**Type material:** 1 holotype slide each of cultivated, protargol-impregnated and Chatton-Lwoff silver nitrate-impregnated morphostatic and dividing specimens and 15 paratype slides (protargol, Chatton-Lwoff and Klein-Foissner silver nitrate) have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI). Unfortunately, most Klein-Foissner preparations bleached due to a preparation mistake, including the specimens shown in Figures 21, 22, 25, 26, 29. Further, voucher slides with cultivated morphostatic and dividing specimens from the Dominican Republic have been deposited, viz., 5 protargol slides (Foissner's method), 5 Chatton-Lwoff silver

nitrate slides, and 12 silver carbonate slides (method of Augustin et al. 1984). The specimens shown in Figures 6, 7, 12, 13, 15–17, 39–52 and many other well-impregnated cells are individually marked by a black ink circle on the cover glass.

**Etymology:** Composite of the Greek nouns *thrix* (hair ~ cilia) and *glossus* (tongue), referring to the conspicuous, tongue-shaped adoral membranelle 1.

**Description:** Cultivated specimens  $120\text{--}250 \times 30\text{--}70 \mu\text{m}$  *in vivo*, usually near  $200 \times 50 \mu\text{m}$ , as calculated from some *in vivo* measurements and the morphometric data shown in Table 1; protargol-

impregnated cells smaller than Chatton-Lwoff-silvered specimens by about 18%, likely due to some shrinkage during preparation; specimens from declining cultures and the material transported to Salzburg considerably smaller, viz.,  $100\text{--}150 \times 25\text{--}35 \mu\text{m}$  *in vivo*. Lateral shape view highly characteristic, viz., slightly sigmoidal and fusiform to rather distinctly claviform due to a conspicuous postoral hump; thus widest in second quarter, gradually narrowing anteriorly and especially posteriorly, forming a more or less distinct tail; both ends narrowly rounded; unflattened (Figs 1–3, 7, 16, 18, 24). Nuclear apparatus invariably in second

**Table 1.** Morphometric data on the Brazilian type population of *Lambornella trichoglossa*.

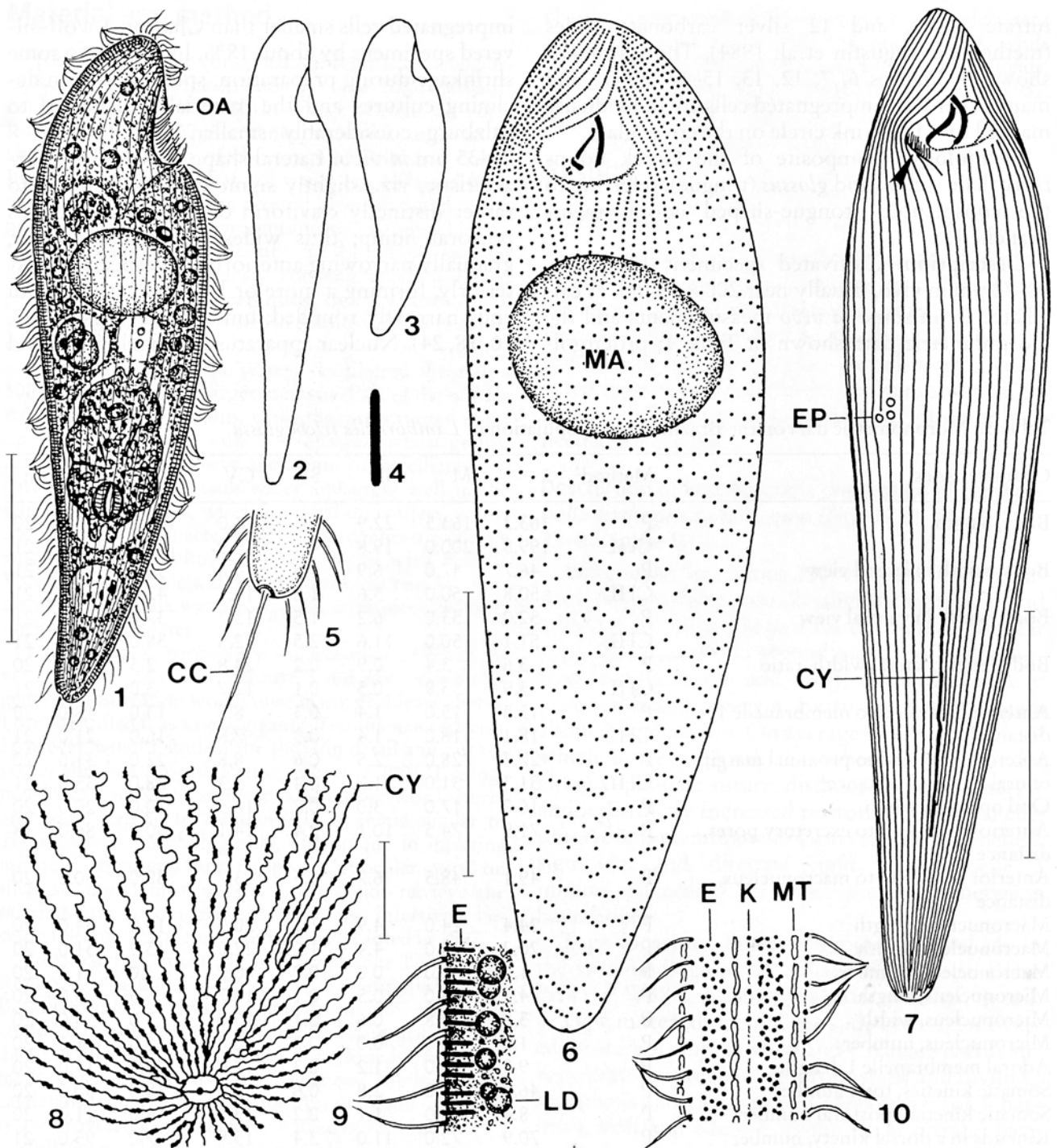
Characteristics <sup>1</sup>	Method <sup>2</sup>	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	P	163.3	163.5	22.9	5.1	14.0	105.0	207.0	20
	CHL	199.8	200.0	19.8	4.3	9.9	175.0	245.0	21
Body, width in lateral view	P	46.7	47.0	6.9	1.5	14.8	30.0	60.0	21
	CHL	50.8	50.0	5.6	1.2	11.1	42.0	67.0	21
Body, width in ventral view	P	52.0	53.0	6.2	1.5	13.7	37.0	62.0	21
	CHL	51.1	50.0	11.6	2.5	22.8	38.0	95.0	21
Body length: lateral width, ratio	P	3.6	3.4	0.9	0.2	24.8	2.3	5.8	20
	CHL	3.9	3.9	0.5	0.1	12.7	3.0	5.1	21
Anterior body end to membranelle 1, distance <sup>3</sup>	P	15.5	15.0	1.4	0.3	8.8	13.0	18.0	20
	CHL	18.1	18.0	1.4	0.3	7.8	16.0	21.0	21
Anterior body end to proximal margin of oral opening, distance <sup>3</sup>	P	27.7	28.0	2.5	0.6	8.8	22.0	31.0	20
	CHL	31.3	31.0	1.7	0.4	5.3	28.0	35.0	21
Oral opening, width	P	16.0	17.0	3.0	0.7	18.5	10.0	20.0	20
Anterior body end to excretory pores, distance	P	71.9	74.5	10.1	2.4	14.1	50.0	86.0	18
Anterior body end to macronucleus, distance	P	49.1	48.5	6.7	1.5	13.7	39.0	60.0	20
Macronucleus, length	P	24.4	24.0	4.9	1.1	20.0	14.0	34.0	20
Macronucleus, width	P	20.2	18.0	4.4	1.0	22.0	13.0	31.0	20
Macronucleus, number	P	1.0	1.0	0.0	0.0	0.0	1.0	1.0	20
Micronucleus, length	P	4.1	4.0	0.5	0.1	11.7	3.5	5.0	20
Micronucleus, width	P	3.8	3.8	0.6	0.1	15.0	3.0	5.0	20
Micronucleus, number	P	1.0	1.0	0.0	0.0	0.0	1.0	1.0	20
Adoral membranelle 1, length	P	9.5	10.0	1.2	0.3	12.6	8.0	11.0	20
Somatic kineties, total number	P	46.7	47.0	2.8	0.6	6.0	38.0	50.0	20
Somatic kineties, postoral number	P	8.4	8.0	1.1	0.2	13.1	6.0	11.0	20
Kinetids in a dorsal kinety, number	P	70.9	72.0	11.0	2.4	15.5	47.0	95.0	21
Excretory pores at end of kinety	P	5.8	6.0	0.6	0.1	9.8	5.0	7.0	17
Excretory pores, number	P	2.3	2.0	0.6	0.1	24.8	1.0	3.0	19

<sup>1</sup>Data based on mounted, randomly selected specimens from two wheat grain cultures; values thus not directly comparable. Measurements in  $\mu\text{m}$ . CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean,  $\bar{x}$  – arithmetic mean.

<sup>2</sup>CHL – silver nitrate impregnation after Chatton-Lwoff, as described in Foissner (1991), P – protargol impregnation, protocol A in Foissner (1991).

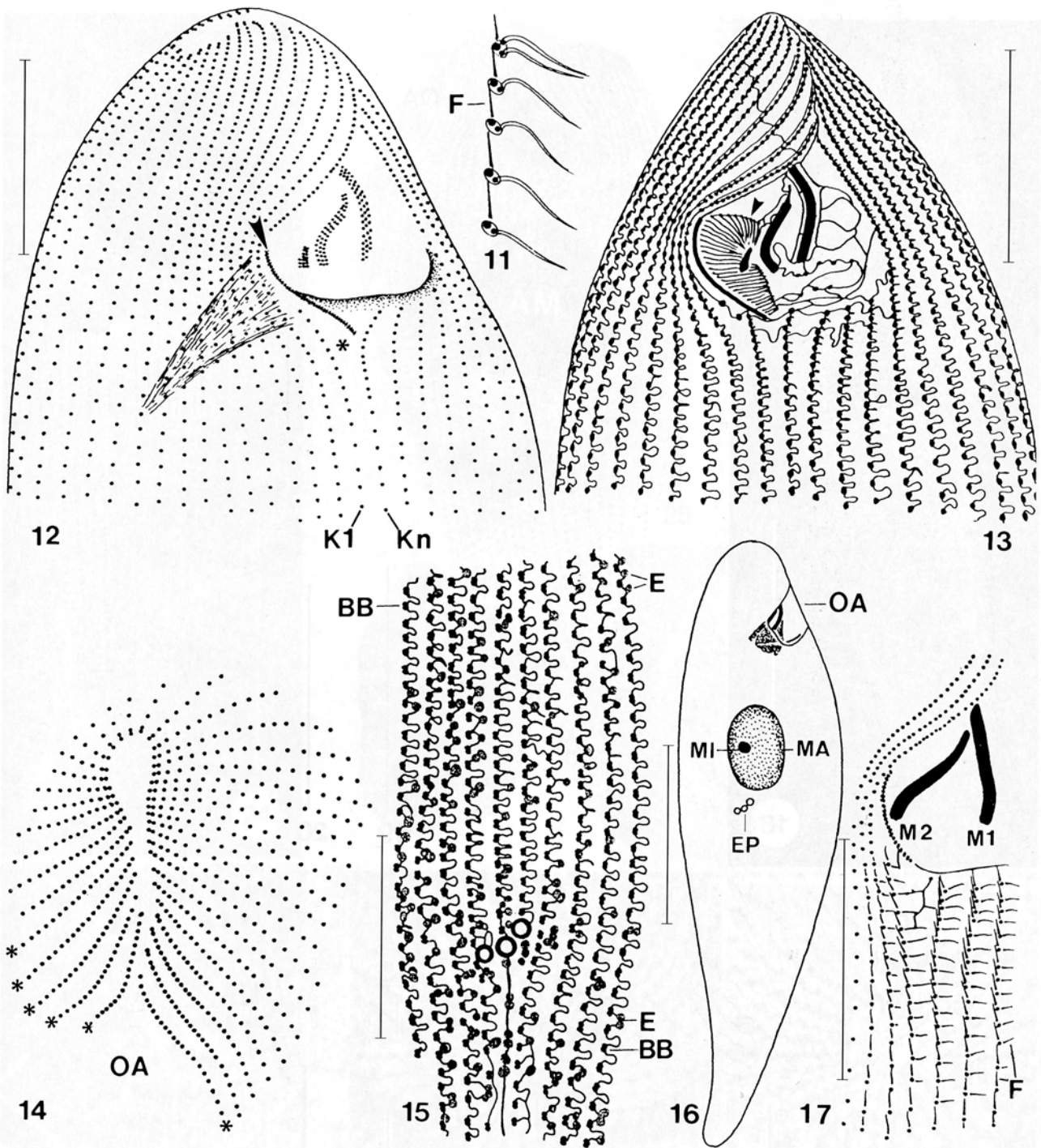
<sup>3</sup>The difference between the two features roughly equals the length of the oral opening.



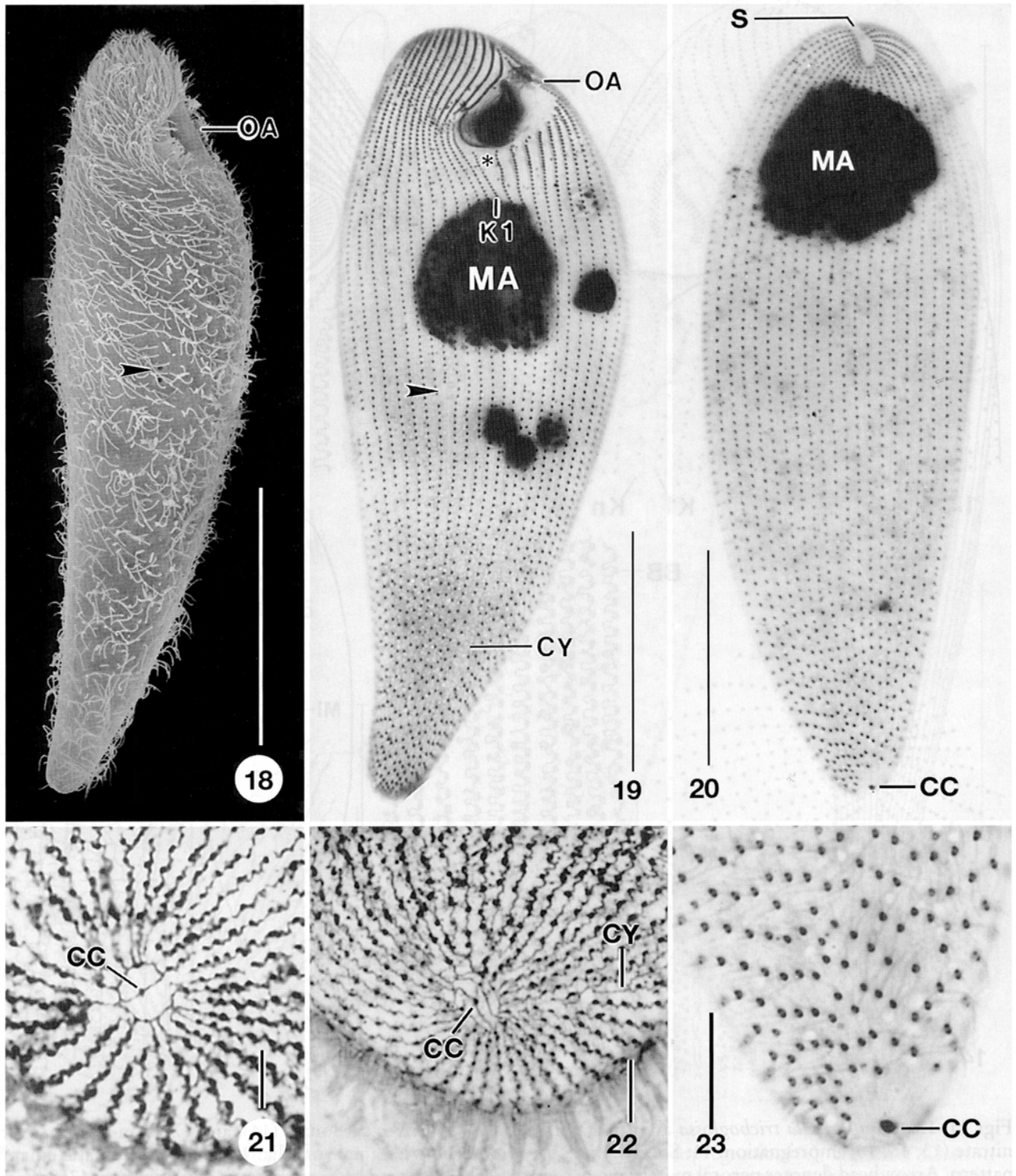


Figs 1–10. *Lambornella trichoglossa* from life (1–5, 9, 10), after protargol impregnation (6), and after Chatton-Lwoff (7) and Klein-Foissner (8) silver nitrate impregnation. 1: Right side view of a representative specimen having ingested a large rotifer. 2, 3: Slender and stout shape variants. 4: Extrusome, length 2.5  $\mu\text{m}$ . 5: Posterior body end with caudal cilium 25–30  $\mu\text{m}$  long. 6: Ciliary pattern of ventral side of holotype specimen. 7: Ventral overview of a well-preserved specimen. Arrowhead marks striated right wall of buccal cavity. 8: Silverline pattern in posterior pole region showing caudal cilium within and surrounded by a silverline. 9, 10: Optical section and surface view of cortex. CC – caudal cilium, CY – cytophyge, E – extrusomes, EP – excretory pores, K – kinety, LD – lipid droplets, MA – macronucleus, MT – mitochondria, OA – oral apparatus. Scale bars 50  $\mu\text{m}$  (1, 6, 7) and 10  $\mu\text{m}$  (8).

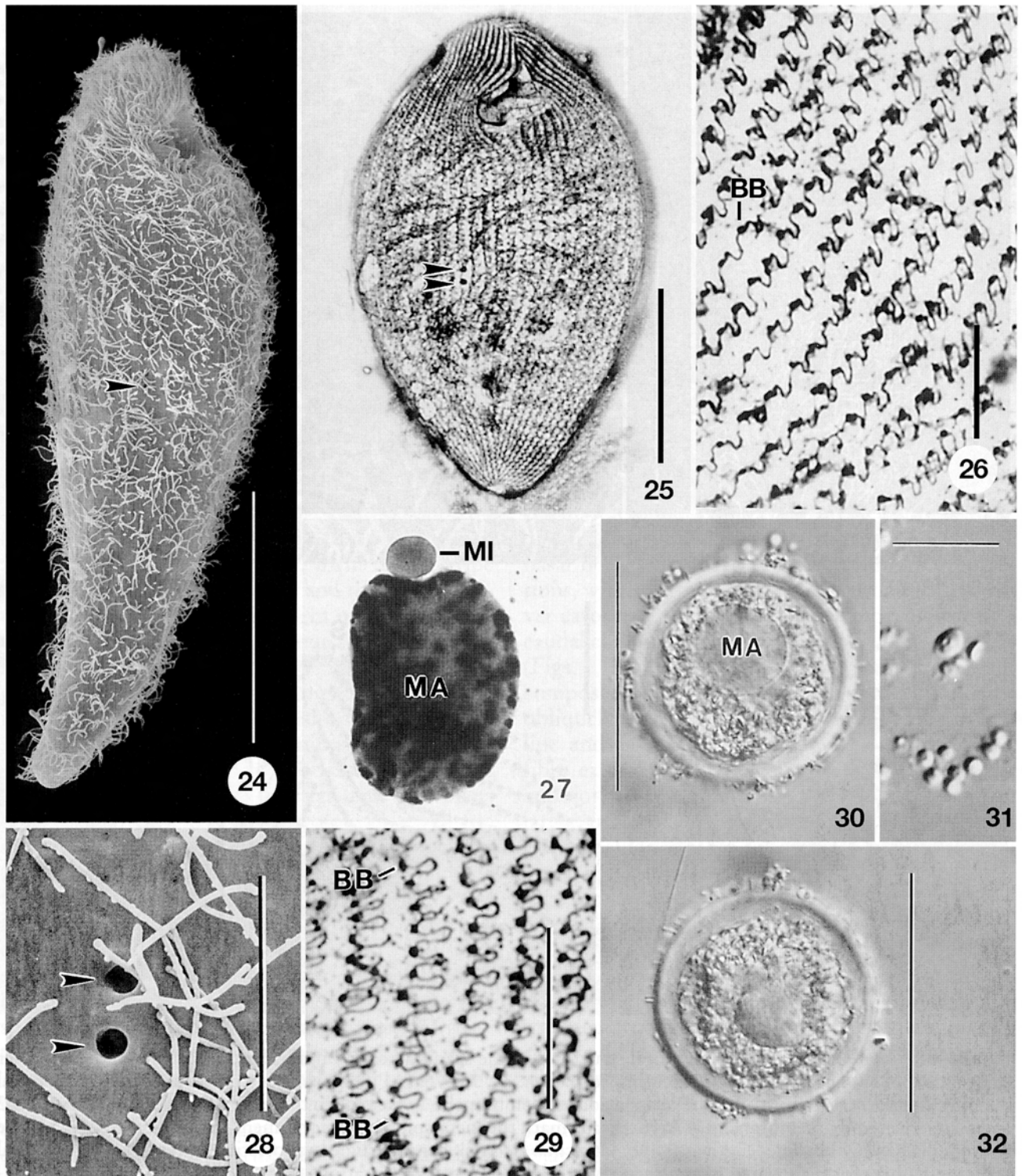




Figs 11–17. *Lambornella trichoglossa* after protargol (11, 12, 17), silver carbonate (14), and Chatton-Lwoff silver nitrate (13, 15, 16) impregnation. 11: Somatic kineties commence with a pair of cilia. 12: Oral and somatic ciliary pattern. Arrowhead denotes paroral membrane. Asterisk marks widening between kinety 1 and kinety n. 13: Silver-line pattern of a specimen from the Dominican Republic. Arrowhead marks buccal fibres left of paroral. 14: Spatulate anterior pole suture. Most kineties commence with a dikinetid, except those marked by an asterisk. 15: Silver-line pattern in excretory pore region. 16: Right side view of a representative specimen. 17: The postoral kinetids are associated with distinct fibres. BB – basal bodies, E – extrusomes, EP – excretory pores, F – fibres, K1, Kn – kineties 1 and n, MA – macronucleus, MI – micronucleus, M1, 2 – adoral membranelles, OA – oral apparatus. Scale bars 20  $\mu$ m (12, 13, 15, 17) and 50  $\mu$ m (16).

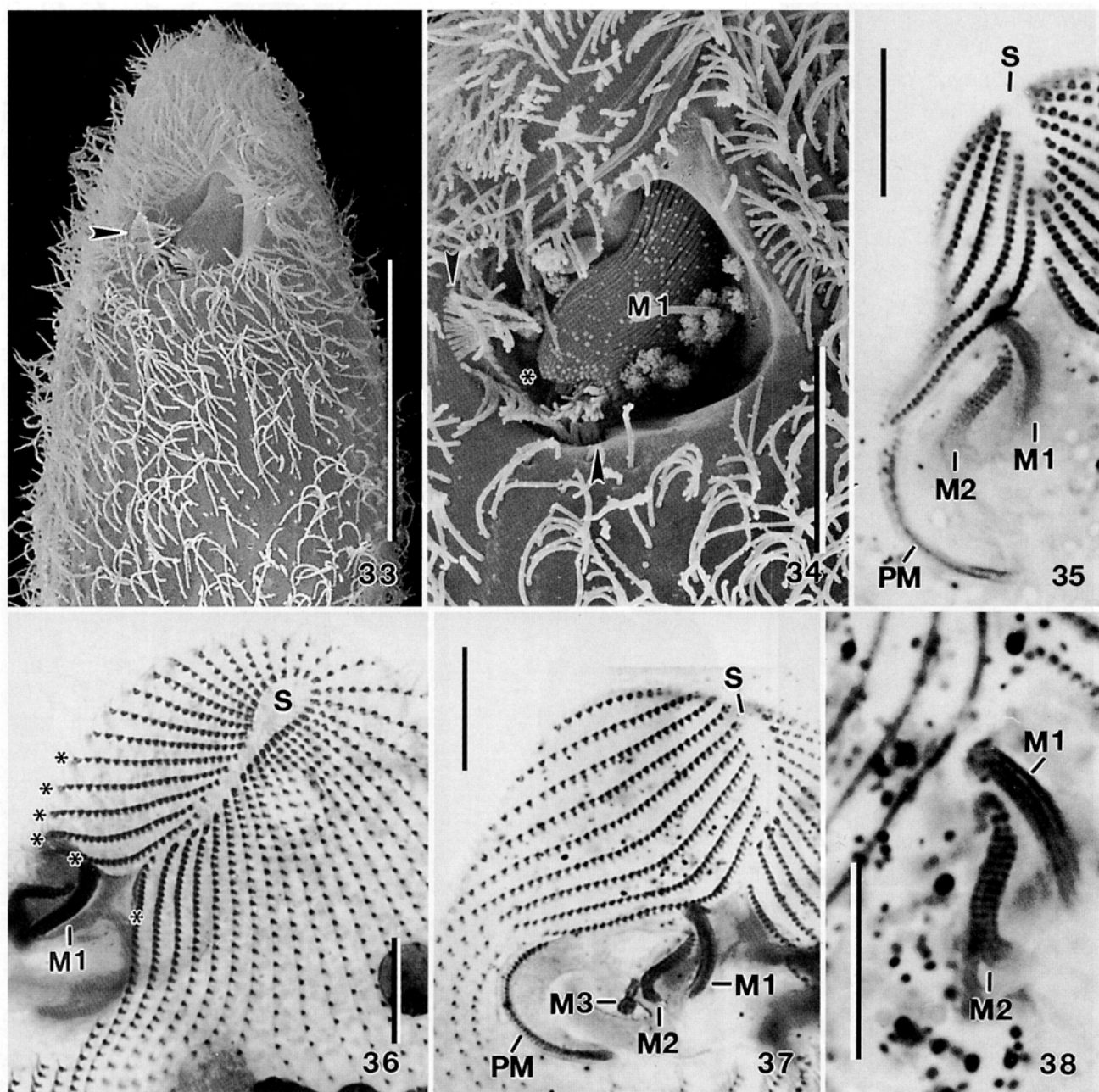


**Figs 18–23.** *Lambornella trichoglossa* in the SEM (18) and after silver carbonate (19, 20, 23) and Klein-Foissner silver nitrate (21, 22) impregnation. **18:** Right side view. Arrowhead marks excretory pores. **19, 20:** Ciliary pattern of ventral and dorsal side. Asterisk denotes widening between kinety 1 and n. Arrowhead marks excretory pores. **21–23:** Posterior polar views showing caudal cilium (CC) surrounded by a silverline ring. CC – caudal cilium, CY – cytophyge, K1 – kinety 1, MA – macronucleus, OA – oral apparatus, S – preoral suture. Scale bars 50  $\mu\text{m}$  (18–20) and 10  $\mu\text{m}$  (21–23).



Figs 24–32. *Lambornella trichoglossa* in the SEM (24, 28), after Klein-Foissner (25, 26, 29) and silver carbonate (27) impregnation, and in vivo (30–32). 24, 28: Ventrolateral views with excretory pores marked by arrowheads. 25, 26, 29: Silverline pattern, ventral overview and details showing the conspicuous silverline loops between the basal bodies of the kineties. Arrowheads mark excretory pores. 27: Nuclear apparatus. 30–32: Resting cysts have a 5–6  $\mu\text{m}$  thick, hyaline wall and contain many globular structures 1.5–3  $\mu\text{m}$  across and, frequently, with a central globule (31). BB – basal bodies, MA – macronucleus, MI – micronucleus. Scale bars 50  $\mu\text{m}$  (24, 25, 30, 32) and 10  $\mu\text{m}$  (26, 29, 31).





Figs 33–38. *Lambornella trichoglossa*, oral structures in the scanning electron microscope (33, 34) and after silver carbonate (36, 37) and protargol (35, 38) impregnation. 33, 34: Ventral views showing the rounded triangular oral opening and the conspicuous, tongue-shaped adoral membranelle 1 inserting in the left buccal wall; membranelles 2 and 3 are covered by membranelle 1. Arrowheads mark the inconspicuous paroral membrane composed of a single row of cilia having the same length as the somatic cilia. Asterisk denotes slightly projecting fibres in the right buccal wall; these fibres form a striated structure in vivo and silver nitrate preparations (Figs 7, 13). 35, 37, 38: The oral ciliature is tetrahymenid, that is, consists of three adoral membranelles at left and a paroral membrane at right. All membranelles are composed of three ciliary rows (Fig. 12). Membranelle 1 is the longest and slightly curved, membranelle 2 is sigmoidal and has a short tail anteriorly, membranelle 3 is reduced to a few more or less distinctly scattered basal bodies. The paroral membrane is curved and composed of a single row of cilia. 36: Anterior polar view showing the spatulate preoral suture and the ciliary rows commencing with a dikinetid, except of the postoral kineties and those marked with an asterisk. M1–3 – adoral membranelles, PM – paroral membrane, S – preoral suture. Scale bars 40  $\mu\text{m}$  (33) and 10  $\mu\text{m}$  (34–38).

quarter of cell, that is, between oral apparatus and mid-body. Macronucleus globular to slightly ellipsoidal, contains a reticulate nucleolus *in vivo*, while many globular nucleoli, connected by fine strands, are recognizable in silver stains. Micronucleus usually in small indentation, rarely up to 10  $\mu\text{m}$  apart from macronucleus, about 5  $\mu\text{m}$  across *in vivo* (Figs 1, 6, 16, 19, 20, 27; Table 1). Contractile vacuole slightly to distinctly above mid-body, on average 46% distant from anterior body end, forms from several small, coalescing vacuoles during diastole; with one to three, on average two minute excretory pores at end of one, rarely two shortened ciliary rows, viz., kineties five to seven, usually kinety six, whose silverline meridian continues posteriorly; pores usually one after the other, rarely in oblique or transverse line, or scattered (Figs 1, 7, 15, 16, 18, 19, 24, 25, 28; Table 1). Cortex smooth, except of right oral and preoral area, where kineties extend in shallow furrows (Figs 24, 28, 33, 34). Extrusomes, likely mucocysts, attached to cortex, numerous, form dense stripe between each two ciliary rows and the oblong mitochondria serially arranged right of kineties; rod-shaped with rounded ends, about  $2.5 \times 0.25 \mu\text{m}$  *in vivo* (Figs 9, 10); occasionally impregnate with protargol. Cytophyge subterminal in posterior third of cell at end of shortened kinety 1, appears as argyrophilic line in silver nitrate preparations and as slightly widened, colourless area in silver carbonate and protargol-impregnated specimens (Figs 7, 8, 19, 22). Cytoplasm colourless, well-fed specimens, however, dark at low magnification ( $\leq \times 100$ ) due to food vacuoles and many highly refractive lipid droplets up to 10  $\mu\text{m}$  across. Feeds on large rotifers, both in the native sample and raw cultures, digested in vacuoles up to  $100 \times 50 \mu\text{m}$  in size (Fig. 1); in wheat grain cultures, also ingests bacteria, flagellates (*Peranema* sp., *Polytomella* sp.), and starch grains. Swims rather rapidly by rotation about main body axis; never rests.

Cilia about 8  $\mu\text{m}$  long *in vivo*, form rather pronounced metachronal waves in densely ciliated anterior body half, arranged in an average of 47 equidistant, mostly meridional rows distinctly curving along right margin of oral opening, similar to *Colpidium* (Ganner and Foissner 1989); much more narrowly spaced anteriorly than posteriorly, where some rows are shortened and the row pattern becomes indistinct due to the wide spacing of the basal bodies (Figs 1, 6, 7, 12, 18–20, 24; Table 1). Ciliary rows commence along straight, conspicu-

ously spatulate and slightly projecting preoral suture with a ciliated dikinetid, except for an average of eight postoral kineties, rows two to six right of oral opening, and first row left of oral opening; dikinetids distinct only in lucky preparations. Postoral and kineties right of oral apparatus frequently with small irregularities, such as minute breaks and unevenly spaced basal bodies, one or several rows occasionally slightly shortened anteriorly and especially posteriorly, where they end at the excretory pores, subterminally, and/or gradually shorten along the right and left cytophyge margin, producing a more or less distinct suture. Postoral kineties 1 and n curved anteriorly, producing V-shaped area occasionally containing some aligned or scattered basal bodies, likely remnants from last stomatogenesis (Figs 6, 7, 12, 14, 19, 20, 35–37). Caudal cilium inserted slightly dorsally, surrounded by small, bare area, originates from distinct ciliary pit, about 25  $\mu\text{m}$  long and obliquely spread dorsally *in vivo*, distal third very thin and thus easily overlooked; basal body not enlarged in silver nitrate preparations, while distinctly enlarged in protargol and silver carbonate-impregnated specimens, indicating a caudal cilium complex, as defined by Corliss (1952) (Figs 1, 5, 8, 20–23). Ordinary somatic kinetids composed of two (three in anterior dikinetids) obliquely oriented granules surrounded by a faint line and associated with a short (kinetodesmal?) fibre extending anteriorly at right side of kinety; anterior granule (parasomal sac?) usually slightly larger and deeper-impregnated than ciliated posterior one (Figs 11, 23, 37); anterior 10–15 kinetids of postoral kineties associated with a special fibre system, occasionally recognizable in protargol-impregnated specimens (Fig. 17).

Oral apparatus subapical in first sixth of cell on average, small (about  $15 \times 15 \mu\text{m}$ ) as compared to size of cell (about  $200 \times 50 \mu\text{m}$ ). Oral opening rounded triangular, posterior and left margin sharply defined and slightly thickened, right gradually merges into somatic cortex. Buccal cavity only slightly larger than oral opening and rather flat, exposing adoral membranelle 1 almost completely. Pharyngeal fibres only about 20  $\mu\text{m}$  long, oral funnel thus inconspicuous, extends obliquely dorsad and posteriad (Figs 1–3, 6, 7, 12, 13, 16, 18, 19, 33, 34; Table 1). Invariably three adoral membranelles each composed of three rows of basal bodies, right row accompanied by a granular, lighter impregnated line (parasomal sacs?). Membranelle 1 anchored in left buccal wall, oriented in main body axis and

slightly to distinctly convex, conspicuous because composed of three unshortened rows of about 10  $\mu\text{m}$  long cilia forming a thick, tongue-shaped bundle invariably directed to right in scanning electron micrographs and thus covering membranelles 2 and 3. Membranelle 2 in mid of buccal cavity, distinctly sigmoidal and obliquely directed to right, forming a triangular pattern with membranelle 1; individual basal body rows slightly shortened anteriorly with anterior membranelar end composed of only one or two rows. Membranelle 3 close to proximal end of membranelle 2, minute, that is, consists of few, sometimes slightly scattered basal bodies. Paroral membrane at right posterior half of oral opening with proximal third extending into buccal cavity, slightly curved (distinctly in squashed preparations, likely due to distortion of shape-giving fibrillar systems), in protargol preparations composed of very narrowly spaced dikinetids likely having ciliated only the right (outer) basal body, while the left (inner) is associated with a fibre extending into the buccal wall; buccal wall fibres form plate-like, striated structure projecting from body proper about 3  $\mu\text{m}$  (Figs 1, 6, 7, 12, 13, 17, 33–38; Table 1).

Silverline pattern simple due to the lack of secondary meridians. Primary meridians loop-like projecting to left between kinetids, especially in middle quarters of cell, where loops are frequently associated with granular silver accumulations, likely marking extrusome attachment sites. Silverline loops become smaller anteriorly and posteriorly, where meridians abut to a circular silverline sur-

rounding the caudal cilium complex associated with one, rarely two or three silverlines crossing bare area (Figs 8, 13, 15, 21, 22, 25, 26, 29).

### Dominican Republic population (Figs 13, 30–32; Table 2)

**Morphology:** The Dominican specimens match the Brazilian type population morphologically, while morphometrics differ considerably due to the smaller size in general and the size decrease occurring during prolonged cultivation and starvation (Table 2). These and other observations will be summarized in the following paragraphs.

(1) The native tank ciliates have a length of 90–150  $\mu\text{m}$  *in vivo*, just as those from Brazil. When cultivated for two weeks, length increased to about 130–200  $\mu\text{m}$  with an average of 143  $\mu\text{m}$  in prepared cells (Table 2). The Brazilian specimens reached 200  $\mu\text{m}$  under similar conditions (Table 1). After two months of cultivation, length decreased to about 90–150  $\mu\text{m}$  with 100–120  $\mu\text{m}$  being common *in vivo* and 105  $\mu\text{m}$  in silver nitrate preparations (Table 2). In old, declining cultures and in specimens starved for encystment, length decreased to under 100  $\mu\text{m}$  with smallest specimens only about 40  $\mu\text{m}$  long.

(2) Associated with the small size is a distinct decrease in the number of ciliary rows from 45 to 37 and postoral kineties from 6 to 5. Further, the excretory pores of the contractile vacuole became located underneath mid-body, indicating that the size decrease is caused by shortening of the narrowed posterior body half (Table 2). Likewise,

**Table 2.** Morphometric data on *Lambornella trichoglossa* from the Dominican Republic after two (upper line) and eight (lower line) weeks of cultivation.

Characteristics <sup>1</sup>	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	143.0	145	12.9	2.8	9.1	120	165	21
	105.3	105	14.0	3.1	13.3	77	140	21
Body, width	45.7	45	3.7	0.8	8.0	40	53	21
	45.3	45	4.2	0.9	9.2	38	54	21
Anterior body end to first excretion pore, distance	68.9	70	8.2	1.8	11.9	50	83	21
	62.9	65	7.0	1.5	11.2	48	75	21
Somatic kineties, total number	45.1	45	2.4	0.6	5.4	40	50	16
	36.8	37	1.6	0.4	4.4	34	39	21
Postoral kineties, number	5.6	6	0.7	0.2	12.2	5	7	20
	4.8	5	–	–	–	4	5	21

<sup>1</sup>Data based on mounted, Chatton-Lwoff silver nitrate-impregnated, randomly selected specimens from wheat grain cultures. Measurements in  $\mu\text{m}$ . CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean,  $\bar{x}$  – arithmetic mean.



kinety 1 and n are not or only slightly curved anteriorly, both in large and small specimens, making the bare postoral area, so conspicuous in most Brazilian specimens, indistinct (Fig. 13).

(3) Body shape is sigmoidal, as in the Brazilian type, in the large ( $\geq 130\ \mu\text{m}$ ) and, especially, very large ( $\geq 150\ \mu\text{m}$ ) specimens, while more or less fusiform and widest orally and postorally, as typical for this species, in the smaller cells.

(4) Even rather small cells ( $\sim 100\ \mu\text{m}$ ) ingest rotifers. Frequently, small cells swim very fast and in a dancing manner, that is, the posterior half rotates in a narrower circle than the anterior.

(5) Excellent Chatton-Lwoff silver nitrate preparations were obtained from specimens cultivated for two months (Fig. 13). These preparations confirmed the observations from the Brazilian type population, especially as concerns the silverline pattern, and showed some additional features not or poorly recognizable in the weaker-prepared type material. There are two more or less wrinkled silverlines along the posterior margin of the oral opening. They contact the irregularly meshed silverline pattern extending in the dorsal and left lateral buccal wall. The posterior half of the right buccal wall is supported by fibres likely originating from the paroral kinetids, as in *Tetrahymena*; however, the anterior third of fibres is deep in the buccal cavity and obviously not associated with paroral kinetids, although a minute granule occurs at the anterior end of each fibre. Along the right side preoral kineties, which are connected by an intermeridional silverline, extends a very fine silverline recognizable in only few specimens (Fig. 13).

**Resting cysts:** No resting cysts were observed in the cultures. Thus, I isolated about 50 specimens and starved them in 2 ml of either centrifuged (to remove food, viz., flagellates and most bacteria) culture medium or pure Eau de Volvic. Of five such trials made within two months, only one was partially successful, that is, three resting cysts were found after two weeks. Obviously, this ciliate produces cysts very rarely and/or only under special, not yet known conditions, as also evident from the observations reported in the Occurrence section. Most specimens died within one week when starved, while others became smaller and smaller, as described above, and survived for three to four weeks.

The three cysts produced had a diameter of 48–50  $\mu\text{m}$ , including the about 5  $\mu\text{m}$  thick, glossy, colourless wall, which appeared smooth and structureless even at high magnification; as usual, the

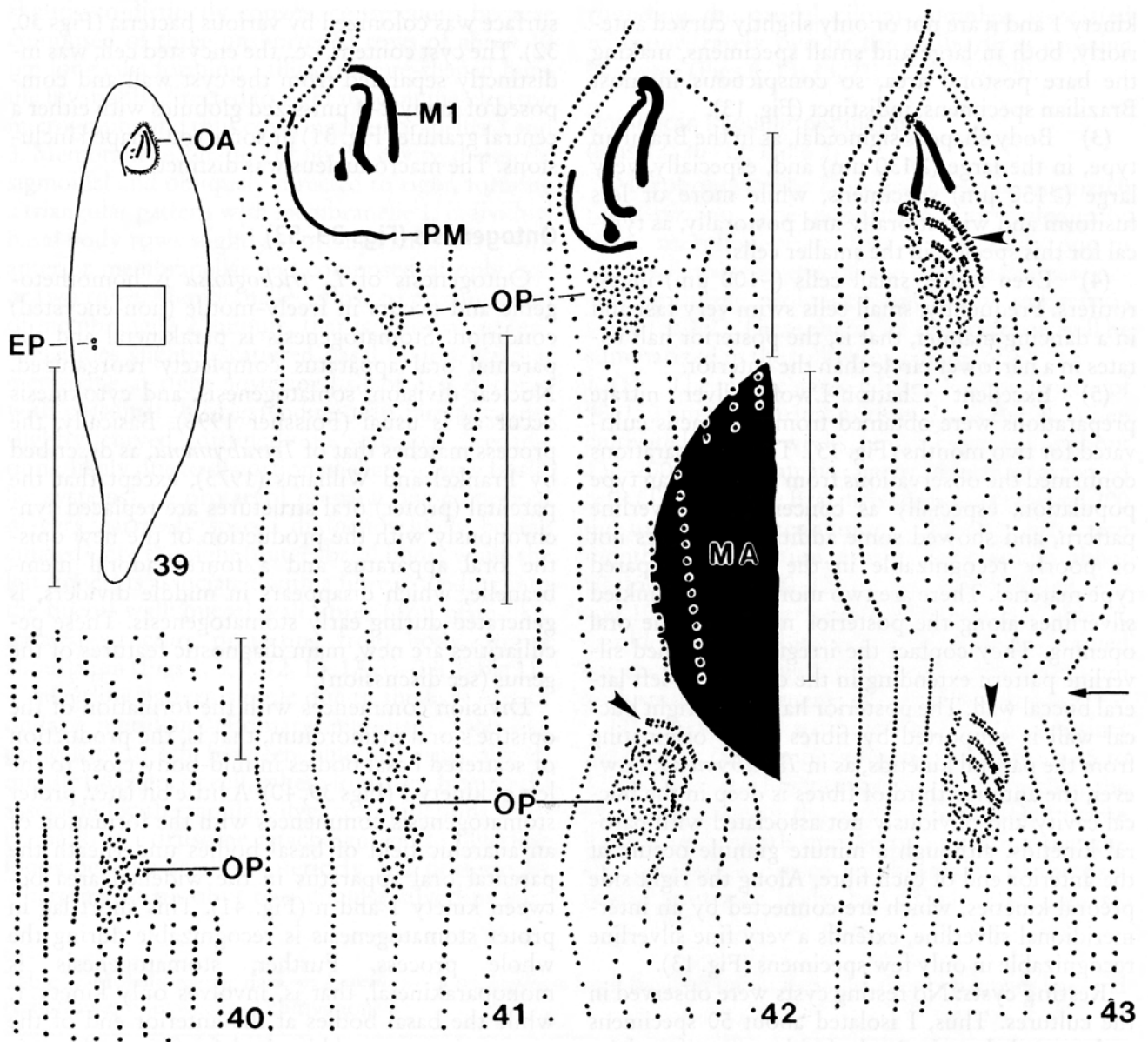
surface was colonized by various bacteria (Figs 30, 32). The cyst content, i.e., the encysted cell, was indistinctly separated from the cyst wall and composed of many 2–4  $\mu\text{m}$ -sized globules with either a central granule (Fig. 31) or some rod-shaped inclusions. The macronucleus was distinct.

## Ontogenesis (Figs 39–53)

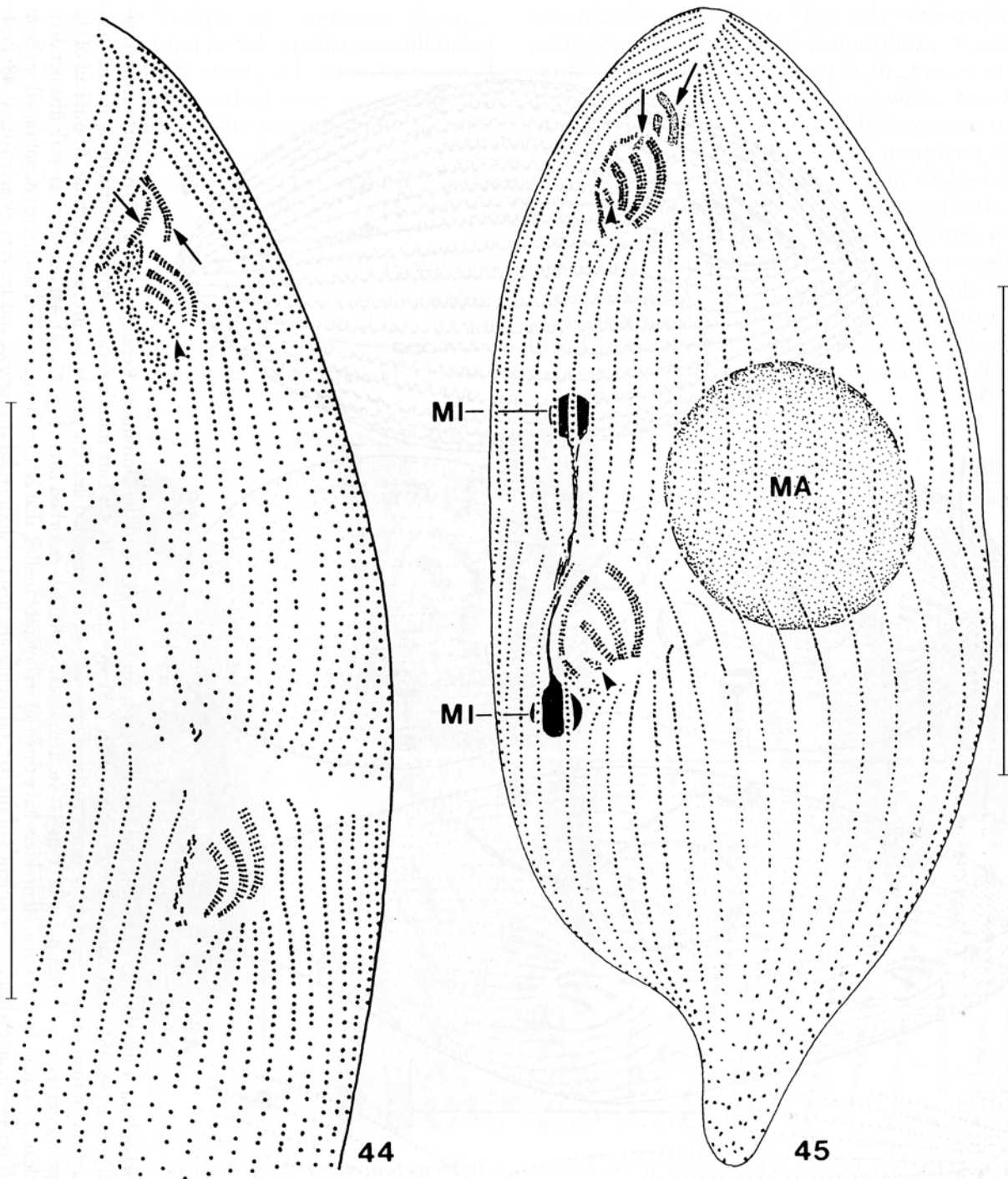
Ontogenesis of *L. trichoglossa* is homothetogenic and occurs in freely-motile (non-encysted) condition. Stomatogenesis is parakinetal and the parental oral apparatus completely reorganized. Nuclear division, somatogenesis, and cytokinesis occur as is usual (Foissner 1996). Basically, the process matches that of *Tetrahymena*, as described by Frankel and Williams (1973), except that the parental (proter) oral structures are replaced synchronously with the production of the new opisthe oral apparatus and a fourth adoral membranelle, which disappears in middle dividers, is generated during early stomatogenesis. These peculiarities are new, main diagnostic features of the genus (see discussion).

Division commences with the formation of the opisthe's oral primordium, that is, the production of scattered basal bodies in mid-body close to the left of kinety 1 (Figs 39, 40). A little bit later, proter stomatogenesis commences with the formation of an anarchic field of basal bodies underneath the parental oral apparatus in the widened area between kinety 1 and n (Fig. 41). This short lag in proter stomatogenesis is recognizable during the whole process. Further, stomatogenesis is monoparakinetal, that is, involves only kinety 1, while the basal bodies at the anterior end of the prospective postoral kineties left of kinety 1 are either resorbed or incorporated into the growing oral primordium (Figs 40–42).

The oral primordium soon grows to a large field, both in proter and opisthe, composed of scattered monokinetids and indistinct dikinetids. Intense basal body proliferation occurs within the somatic kineties (Figs 41, 42). Next, adoral membranelles assemble in the left anterior area of the anarchic field, leaving back numerous scattered kinetids in the right and posterior area. The membranelles consist of short, dikinetidal pieces which align to curved protomembranelles along the anterior and left margin of the oral primordium (Figs 42, 43). Concomitantly, the resorption of the parental oral apparatus commences, that is, the buccal cavity

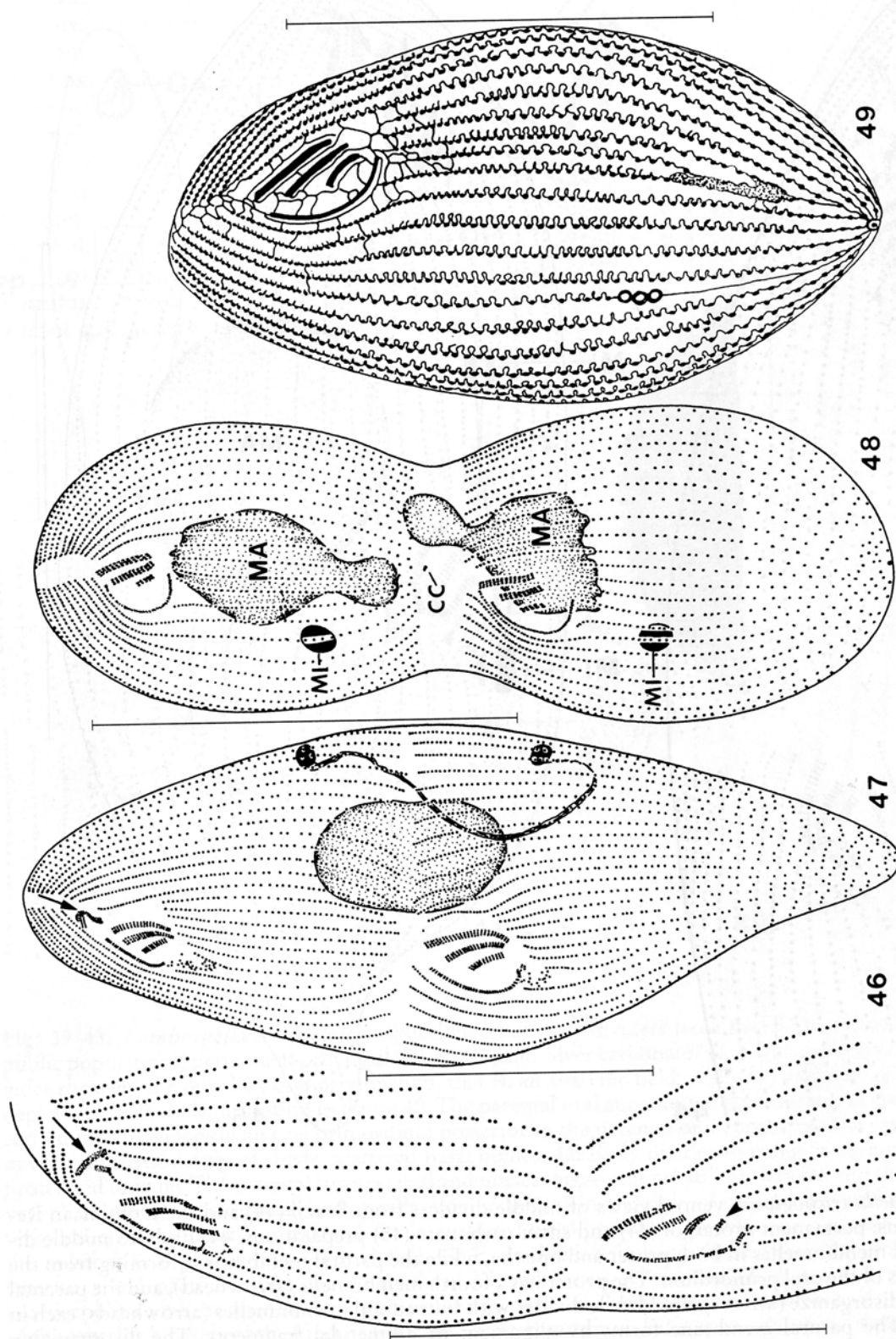


**Figs 39–43.** *Lambornella trichoglossa*, ventral views of early dividers from Brazil (39, 40) and the Dominican Republic populations; permanent protargol (39, 40, 43) and silver carbonate (41, 42) preparations. **39, 40:** Very early divider showing the opisthe's oral primordium, that is, an anarchic field of single basal bodies in mid-body (frame), depicted at higher magnification in figure 40. The parental oral apparatus and postoral area are unchanged. **41:** Very early divider developing an oral primordium posterior to the parental oral apparatus. The opisthe's oral primordium still consists mainly of single, scattered basal bodies. **42:** Early divider showing growth of oral primordium in proter and opisthe. The parental (proter) oral and nuclear apparatus are still unchanged and the somatic kineties not yet split, although intense basal body production occurs in kineties right of the opisthe's oral primordium. The proter's oral primordium still consists of single, scattered basal bodies, while dikinetids organize at the left margin of the opisthe's oral primordium (arrowhead). **43:** Early divider showing slight disorganization of the parental oral structures and protomembranelles (arrowheads) in both oral primordia, which are now sizeable. The somatic kineties split above the opisthe's oral primordium (arrow). The opisthe's postoral kineties develop by resorption (?) of the anterior kinetids of the kineties left of the oral primordium. EP – excretory pores of contractile vacuole, MA – macronucleus, M1 – membranelle 1, OA – parental oral apparatus, OP – oral primordium in proter and opisthe, PM – parental paroral membrane. Scale bars 50  $\mu$ m (39), 20  $\mu$ m (41–43), and 10  $\mu$ m (40).



**Figs 44, 45.** *Lambornella trichoglossa*, ventral views of middle dividers from Brazil (44) and the Dominican Republic (45) populations; permanent protargol (44) and silver carbonate (45) preparations. **44:** Early to middle divider with new adoral membranelles in both proter and opisthe, while the paroral membrane is forming from the remaining basal bodies of the oral primordium. The proter has a fourth membranelle (arrowhead), and the parental adoral membranelles disorganize (arrows). **45:** Middle divider with four adoral membranelles (arrowheads) each in proter and opisthe. The paroral membrane forms by alignment of dikinetidal fragments. The disorganizing parental adoral membranelles (arrows) migrate into the preoral suture. MA – macronucleus, MI – micronucleus. Scale bars 50  $\mu$ m.



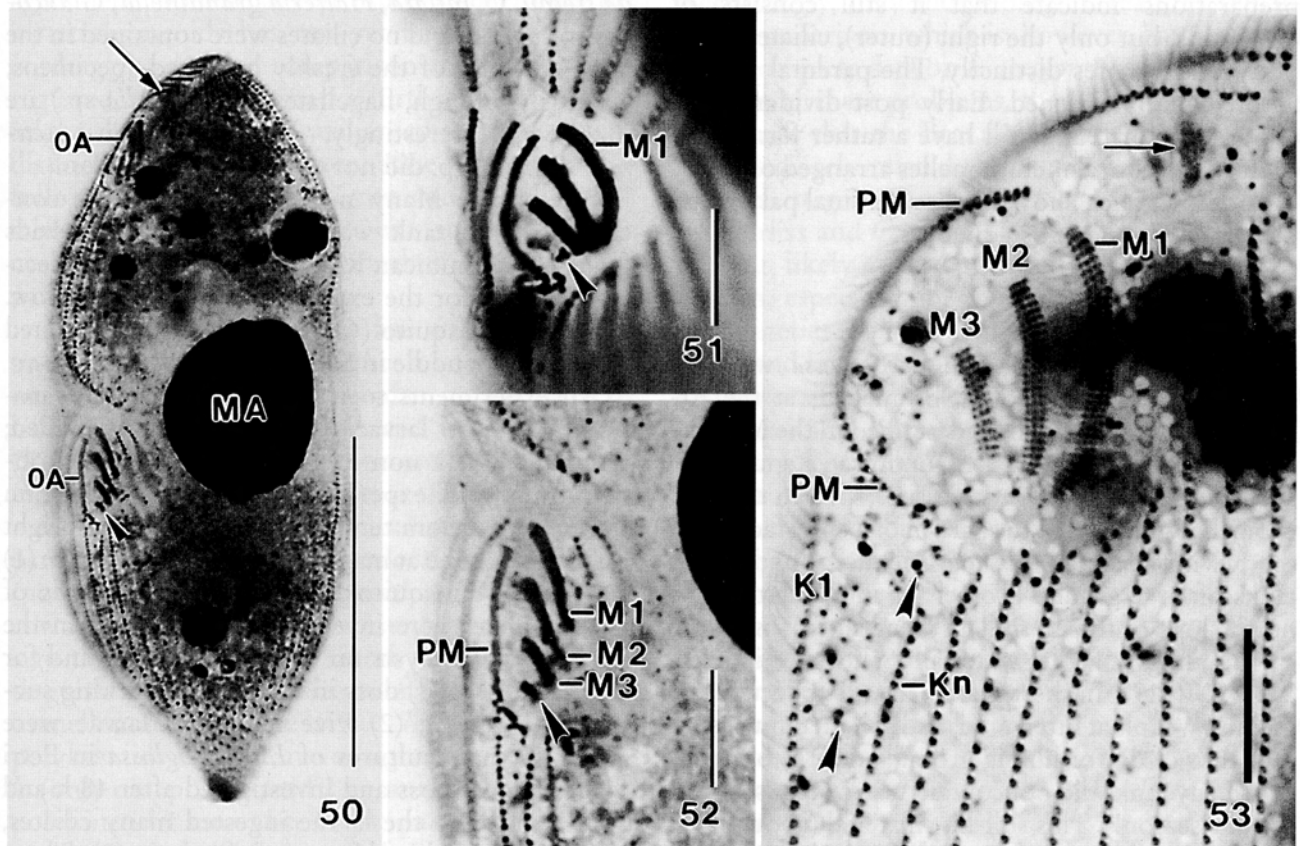


Figs 46–49. *Lambornella trichoglossa*, ventral views of middle and late dividers from Brazil (47) and the Dominican Republic (46, 48, 49) populations; permanent protargol (47), silver carbonate (46, 48), and Chatton-Lwoff silver nitrate (49) preparations. 46, 47: Late middle dividers with oral structures almost completed, but not yet invaginated and in final shape. The parental oral structures (arrows), which migrated into the preoral suture, are resorbing. About half of the specimens have a fourth, supernumerary adoral membranelle (Figs 44–46, arrowhead), which is resorbed later (Figs 47, 48). 48: Late divider with distinct division furrow and divided nuclear apparatus. In this stage, all dividers have three adoral membranelles, that is, they have resorbed the fourth membranelle present in about half of the dividers (Figs 44–46, arrowhead). The kinetids of the paroral membrane lost the dikinetidal appearance and are very closely spaced. 49: Post-divider with invaginating, not yet fully shaped oral structures. CC – newly formed caudal cilium, MA – macronucleus, MI – micronucleus. Scale bars 50  $\mu$ m.

flattens and the undulating membrane disorganizes, while the adoral membranelles are still intact (Fig. 43). I could not clarify whether the parental paroral kinetids are resorbed or incorporated into the oral primordium. The somatic kineties commence to separate in the prospective fission area (Fig. 43), although separation is recognizable earlier in some specimens (Fig. 40). The micronucleus begins to divide.

The next stomatogenic events are the assemblage of the paroral membrane and the ongoing resorption of the parental oral structures (Figs 44–46). Like the adoral membranelles, the paroral membrane assembles from small, dikinetidal fragments

formed from the scattered kinetids in the right and posterior area of the oral primordium. Some supernumerary kinetids remain at the posterior end of the newly formed paroral for a while; eventually, they are resorbed or, possibly, organize to the short ciliary row found in some interphase specimens between the anterior portion of kineties 1 and n (Figs 46, 47, 50–53). Concomitantly, the newly formed adoral membranelles become three-rowed and a small, fourth membranelle is generated either in the proter or opisthe, or in both, in half of 12 appropriate dividers found. Clearly, this fourth membranelle, which is a rather transient structure, is not part of the paroral, but located



Figs 50–53. *Lambornella trichoglossa*, ventral views of middle and late dividers from Brazil (53) and the Dominican Republic (50–52) populations; permanent (50–52) and transient (53) silver carbonate preparations. 50, 52: Overview and opisthe detail of a middle divider, whose opisthe has a supernumerary (fourth) adoral membranelle (arrowheads). Arrow marks resorbing parental adoral membranelles. The paroral membrane consists of clearly recognizable dikinetids. 51: Similar as figures 50 and 52, that is, an opisthe oral apparatus with a supernumerary, fourth adoral membranelle (arrowhead). 53: Proter of a late divider, showing the newly formed oral structures (a paroral membrane and three adoral membranelles) and the almost completely resorbed parental adoral membranelles (arrow). Arrowheads mark scattered postoral basal bodies, that is, vestiges of the oral primordium, which developed between stomatogenic kinety 1 and kinety n. The paroral membrane now consists of a row of single granules. K1 – stomatogenic kinety, Kn – last ordinary somatic kinety, M1–3 – adoral membranelles, MA – macronucleus, OA – oral apparatus, PM – paroral membrane. Scale bars 60  $\mu$ m (50) and 10  $\mu$ m (51–53).

right of membranelle 3 and left of the newly formed paroral (Figs 44–46, 50–53). The parental oral area has flattened and the adoral membranelles, which slowly disorganize, commence to migrate anteriorly into the widened preoral suture (Figs 46, 47, 53). Micronuclear division is almost complete, and the macronucleus begins to divide (Figs 45, 47, 50).

When cell furrowing commences, nuclear division and stomatogenesis are almost complete in both proter and opisthe, and the parental oral structures have been resorbed (Fig. 48). The paroral membrane now consists of very narrowly spaced kinetids which lost the dikinetidal appearance so distinct in the former stages; protargol preparations indicate that it still consists of dikinetids, but only the right (outer), ciliated basal body impregnates distinctly. The parental preoral suture is still widened. Early post-dividers (Fig. 49) are fusiform and still have a rather flat buccal cavity with adoral membranelles arranged obliquely and in parallel, showing that the final patterning occurs only in late post-dividers.

## Ecology

**Occurrence:** *Lambornella trichoglossa* was discovered in tanks of ground bromeliads at the Atlantic sea coast of Brazil, as described in the Material and Methods section. In the Dominican Republic, *L. trichoglossa* was found at two sites, viz., in tanks of bromeliads planted in the garden of a cigar factory in the town of Santiago de los Caballeros and in the tanks of large ground bromeliads in a forest on the north slope of the Cordillera Central, that is, in the surroundings of the waterfall Salto de Jimenoa, W70°30' N19°. In the waterfall area, I collected also dry and wet plant litter, mud, and soil accumulated in the tanks of dry or humid ground and tree bromeliads (many tanks did not contain water because it was the dry season!). This soil-like material (about 800g) was air-dried for seven weeks and used to set up a non-flooded Petri dish culture, as described in Foissner et al. (2002). Over 80 ciliate species could be reactivated from the resting cysts present, but *L. trichoglossa* and some other new tank species (described in forthcoming publications) were not among them, supporting the laboratory observations that cysts are produced very rarely (see above).

**Food and growth:** The data reported above indicate that *L. trichoglossa* is a common inhabitant of bromeliad tanks. It can be easily grown in labo-

ratory cultures (at room temperature, 18–22 °C) containing part of the native bacteria and flagellate community as a food source, especially if some tank water is added to the basal medium (Eau de Volvic). Then, specimens become especially large during the first weeks, both in the Brazilian and Dominican cultures. Later, however, the cultures grow less readily and cells become smaller and smaller (Table 2). Possibly, this decline is related to the lack of tank water and the restricted diet. In the native environment and raw cultures, *L. trichoglossa* also feeds on rotifers, a really remarkable feature considering the rather small oral apparatus and that it does not feed on ciliates. Laboratory trials to adapt it to ciliate food (*Uronema nigricans*, *Colpoda steinii*, *C. inflata*, *Halteria grandinella*, *Glaucoma* sp.) failed, and no ciliates were contained in the food vacuoles of the freshly collected specimens; curiously enough, flagellates (*Polytomella* sp.) are ingested. Interestingly, the heliozoan *Actinosphaerium* sp. did not prey on *L. trichoglossa*.

**Infectivity:** Many mosquito larvae were contained in the tank water of ground bromeliads from the Dominican Republic. They were collected and used for the experiments described below. European mosquito (*Culex*) larvae were collected from a road puddle in Salzburg.

All experiments to infect indigenous or European mosquito larvae with *L. trichoglossa* failed; thus, it is likely non-infective. The following observations and experiments were performed, in triplicate, at room temperature, and with a bright field microscope at magnifications of  $\times 40$ – $\times 125$ : (1) Indigenous mosquito larvae in various stages of development were investigated for ciliates in the haemocoel, for cysts on the body surface, and for black (melanized) dots in the cuticle marking successful invasion; (2) Five mosquito larvae were added to mass cultures of *L. trichoglossa* in Petri dishes 5 cm across and investigated after 48 h and 72 h. Although the larvae ingested many ciliates, sufficient remained for an infection; (3) Three mosquito larvae were punctured with a needle and added to mass cultures of *L. trichoglossa* in Petri dishes 5 cm across. Some larvae died during the experiments observed for 72 h. Ciliates did not enter the larvae, either dead or alive. However, they fed on droplets from the fat body, when the larvae were squashed at the end of the experiments; (4) Cultures from experiments (2) and (3) were investigated for astomatous, infective theronts, as described by Washburn et al. (1988); (5) Adults de-



veloped from experiment (2) were investigated for ciliates in the haemocoel; (6) European *Culex* larvae were added to mass cultures of *L. trichoglossa*; (7) Fragmented larvae of the meal beetle (*Tenebrio molitor*) were used as a food source, but ciliates did not feed on tissue.

## Discussion

### Suprageneric and generic allocation

As a species, *Lambornella trichoglossa* has a clear identity, while generic classification is doubtful because the type species, *L. stegomyiae* Keilin, 1921, is still insufficiently known and general agreement about what constitutes a generic feature in tetrahymenids has not yet been reached. Accordingly, a detailed comparison and discussion is required, especially because further investigations might show that the Brazilian ciliate represents a distinct genus. At the present state of knowledge, I follow Lynn (1994) and Lynn and Small (2002), who introduced a clear, but unfortunately undiscussed generic concept for tetrahymenids (see next paragraph).

*Lambornella trichoglossa* has attributes which unequivocally assign it to the suborder Tetrahymenina, family Tetrahymenidae, as defined by Corliss (1973), Lynn (1994), and Lynn and Small (2002), viz., the first anterior somatic kinetid in most kineties is a dikinetid; a classical tetrahymenid oral apparatus with a ciliated, continuous paroral membrane; and adoral membranelles (polykinetids) each composed of three ciliary rows. Lynn (1994) and Lynn and Small (2002) assign three genera to the Tetrahymenidae and distinguish them by the shape of the adoral membranelles and the life style: *Tetrahymena* (oral polykinetids straight, never sigmoid); *Lambornella* (only oral polykinetid 2 sigmoid; anterior end of polykinetid 1 curved to right; parasites on cuticle of mosquito larvae); and *Deltopylum* (oral polykinetids 1 and 2 sigmoid). Obviously, the Brazilian ciliate does not fit perfectly any of these genera, but is probably nearest to *Lambornella*, which is thus discussed first.

*Lambornella stegomyiae* Keilin, 1921 was re-described by Corliss and Coats (1976), together with the description of a new species, *L. clarki*. However, both descriptions are brief due to the lack of living specimens and good preparations. The generic diagnosis is, indeed, mainly a brief de-

scription of the two species, and the generic comparison lacks clear morphological features distinguishing *Lambornella* from closely related tetrahymenid genera. However, discussion shows that Corliss and Coats (1976), like Keilin (1921), consider the unique cuticular cysts these species produce on their culicine mosquito hosts as the main generic feature. Such cysts are very likely absent in *L. trichoglossa* (see Results section). Further, *Lambornella* can also produce desiccation cysts (Washburn and Anderson 1986) and is not an obligate but a facultative parasite, which may be grown, like the Brazilian ciliate, in ordinary culture media without hosts (Washburn et al. 1988; Norton et al. 1992; Broberg and Bradshaw 1997). Accordingly, only morphological features remain for a comparison, which is, however, hampered by the incomplete data on *L. stegomyiae* and *L. clarki*. In spite of these problems, there are good reasons to assume that the Brazilian ciliate either belongs to or is closely related to *Lambornella*. It matches either *L. stegomyiae* and/or *L. clarki*, as described by Corliss and Coats (1976), in the following important, likely generic features: the somatic ciliary pattern, especially the increased number of post-oral kineties and the spatulate preoral suture; the shape and arrangement of the adoral membranelles and paroral membrane; the ontogenetic pattern, at least basically, because Corliss and Coats (1976) provide a figure showing that the parental oral apparatus is reorganized during ontogenesis.

Thus, the special life cycle ecology and morphology, including astomatous theronts and highly specialized cuticular invasion cysts (Corliss and Coats 1976; Washburn et al. 1988), remain as main differences between *L. stegomyiae*, *L. clarki* and the bromeliad ciliate. Although these differences are conspicuous, I consider them as insufficient for allocating the bromeliad ciliate to a new genus, because many ciliate genera, especially *Tetrahymena*, contain free-living and parasitic species (Batson 1985; Corliss 1973). On the other hand, *Lambornella* has been defined by just those features lacking in the bromeliad ciliate, but likely because Keilin (1921) and Corliss and Coats (1976) did not have solid morphological and ontogenetic data. Thus, I suggest to assign the bromeliad ciliate to *Lambornella*, until this is disproved by a detailed reinvestigation of the morphology and ontogenesis of the type species, *L. stegomyiae* Keilin, 1921.

*Tetrahymena* is a large genus grouped into three "complexes" by Corliss (1973) and three subgen-

era by Jankowski (1967). Considering the generic differences in related taxa, for instance, the scuticociliates, but also the great genetic diversity (Jerome and Lynn 1996, Nanney et al. 1998), *Tetrahymena* is likely under-split. For the following comparison with *L. trichoglossa*, I shall use mainly the excellent reviews by Corliss (1973) and Frankel and Williams (1973).

*Lambornella trichoglossa* differs from *Tetrahymena* spp. mainly by size (200  $\mu\text{m}$  vs.  $\leq 100 \mu\text{m}$ ); shape (fusiform vs. pyriform to ovoidal); location of the contractile vacuole (above mid-body vs. posterior body third); the high number of ciliary rows (47 vs. usually  $\leq 40$ ) and postoral kineties ( $> 5$  vs. usually 2); the shape and arrangement of the adoral membranelles (membranelles 1 and 2 distinctly curved and in triangular pattern vs. straight and side by side or slightly V-shaped); the silverline pattern (intrameridional cross-fibrils conspicuously loop-shaped vs. simple, straight lines and usually a distinct secondary silverline meridian); and ontogenesis (synchronous parental oral replacement vs. irregular interphase replacement). However, there are exceptions, which evolved likely convergently to similar *Lambornella* features: *T. patula* has, like *L. trichoglossa* and *L. clarki* an increased number of postoral kineties, viz. three to five, usually four; and *T. bergeri* shows parental oral replacement, though asynchronously, that is, replacement of the parental (proter) oral apparatus commences when the opisthe oral apparatus is almost complete (Roque et al. 1970).

*Deltopylum* is a monotypic genus assigned to the Tetrahymenidae by Small and Lynn (2002), while Song and Wilbert (1989) put it in a new family, Deltopylidae, due to the *Paramecium*-like silverline pattern. *Deltopylum rhabdoides* has conspicuous similarities with *L. trichoglossa*, viz., large size (150–180  $\times$  40–45  $\mu\text{m}$ ; trophonts up to 300  $\mu\text{m}$ ); contractile vacuole in mid-body; many ( $> 5$ ) postoral kineties; and synchronous replacement of the parental oral apparatus during ontogenesis (Fauré-Fremiet and Mugard 1946; Mugard 1949). There are, however, also distinct differences: the long, nodular macronucleus; the shape of the adoral membranelles (all of same length and sigmoidally curved); the *Paramecium*-like silverline pattern; and the histiophagous life style with a distinct cycle including the production of small tomites and large trophonts.

## Comparison of Brazilian and Dominican population

Both populations of *L. trichoglossa* show pronounced variability (Tables 1, 2), as usual in tetrahymenid ciliates (Corliss 1973). The Dominican population differs from the Brazilian one in three features, viz., the specimens are smaller (usually about 100–150  $\mu\text{m}$  vs. 150–200  $\mu\text{m}$ ), have fewer postoral kineties (4–7 vs. 6–11), and lack the postoral widening between kinety 1 and n. Of these, the different number of postoral kineties, even in large specimens with the same number of ciliary rows as the Brazilian cells, is the most important difference, indicating that the Dominican population is a distinct taxon. Indeed, *L. stegomyiae* and *L. clarki* are separated by features of similar (in)distinctness (Corliss and Coats 1976). However, I prefer a broad species concept in highly variable taxa, and thus consider both populations as belonging to the same morphospecies, but confine the formal diagnosis of the species to the Brazilian type population. Separation should await gene sequence data and morphometric studies on other populations.

## Comparison with related species

Basically, *L. trichoglossa* differs from the two congeners, *L. stegomyiae* Keilin, 1921 and *L. clarki* Corliss and Coats, 1976, by the free-living mode of life. However, there are also distinct morphological and morphometric differences, though these are less obvious due to the high variability and the insufficient data available on the other species. Nonetheless, *L. trichoglossa* can be distinguished from *L. stegomyiae* and *L. clarki* by the following features: size (usually 140–200  $\mu\text{m}$  vs. 50–100  $\mu\text{m}$ ); shape (fusiform with posterior portion often tail-like narrowed vs. ovoid, though *L. clarki* may have a short tail, as mentioned by Corliss and Coats (1976) and recognizable in a micrograph provided by Washburn et al. (1988)); contractile vacuole in mid-body vs. posterior body third; caudal cilium (present vs. likely absent); adoral membranelle 2 oriented to right vs. left causing that membranelles 1 and 2 form a triangular pattern in *L. trichoglossa*, while they extend side by side in the other species. While the last feature might be of generic significance, the other characteristics are likely relevant only at species level. This is emphasized by a comparison of species of the related genus *Glaucoma*,

where *G. frontata* shows exactly the same specializations as *L. trichoglossa* within the genus *Lambornella* (Corliss 1971; Kahl 1931).

## Ontogenesis

Ontogenesis is very similar to that of *Tetrahymena* (Frankel and Williams 1973) and tetrahymenid ciliates in general (Foissner 1996), except of the final shaping of the adoral membranelles, which is thus likely a derived trait. However, the transient occurrence of a minute fourth adoral membranelle is a unique feature of *L. trichoglossa*. It indicates that ancient tetrahymenids probably possessed more membranelles than the extant ones, which invariably have three (Lynn and Small 2002). Indeed, another large tetrahymenid ciliate, which I discovered in Dominican tank bromeliads, has 6–8 adoral membranelles, putting forward not only the idea mentioned above but also the assumption that bromeliad tanks are a relict biotope for ancient, Gondwanan ciliates.

## Towards a refined characterization of *Lambornella* Keilin, 1921

If the species described here is considered as a representative member of *Lambornella*, then it becomes obvious that it differs distinctly from *Tetrahymena*, not only by the cuticular cysts (Keilin 1921; Corliss and Coats 1976) but also morphologically and ontogenetically by the increased number of postoral kineties; the spatulate preoral suture; the loop-like silverlines; the distinctly curved adoral membranelles 1 and 2; and the synchronous replacement of the parental oral apparatus and the anlage of a fourth adoral membranelle during cell division. These features can be considered as diagnostic for *Lambornella*, until the detailed reinvestigation of the type species, *L. stegomyiae*, disprove them, that is, show that the Brazilian species represents a new genus.

## Biogeographical notes

Many potential habitats for ciliates were never carefully investigated, promoting the view of cosmopolitan distribution of free-living ciliates (protists) in general (Finlay et al. 1996; but see Foissner 1999). One such habitat is the rain-water entrapped by the coalescing leaf axils of bromeliads, a group of rosette plants found almost exclusively in

Central and South America. It is well known that these minute cisterns (tanks) contain a rich metazoan life with many specific and/or endemic species (for literature, see Foissner 2003).

During short stays at the Atlantic coast of Brazil and the Caribbean Sea island of Hispaniola (Dominican Republic and Haiti), I collected some leaf axil water from about ten bromeliad species and discovered at least nine new ciliate species, some even representing new genera and families (Foissner 2003; Foissner and Cordeiro 2000; and unpubl.). Considering that there are more than 3000 bromeliad species, many of which have special life styles and/or grow in peculiar environments, one can expect a tremendous number of undescribed protist species in their leaf axil water.

With a length of up to 250 µm and a fusiform shape, *L. trichoglossa* is a very conspicuous ciliate that would be easily recognized in ordinary freshwater samples. It is thus surprising that it was not discovered earlier, though detailed investigations on free-living ciliates of South America and tank bromeliads are very scant (Foissner 2003). Accordingly, any speculation whether or not *L. trichoglossa* is restricted to bromeliad habitats would be premature. On the other hand, it is reasonable to assume that this ciliate is absent from the much better investigated Central Europe. Thus, *L. trichoglossa* is likely a further example of a protozoan species with restricted geographic distribution (Foissner 1999). In this context, it is noteworthy that *L. stegomyiae* has been recorded only from Africa and Asia, while *L. clarki* is possibly restricted to North America (see Corliss and Coats (1976) for an excellent review of records).

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