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Morphological and Molecular Characterization of Some Peritrichs (Ciliophora: Peritrichida) from Tank Bromeliads, Including Two New Genera: *Orborhabdostyla* and *Vorticellides*

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Summary. Using standard methods, we studied the morphology and 18S rDNA sequence of some peritrich ciliates from tank bromeliads of Costa Rica, Jamaica, and Ecuador. The new genus *Orborhabdostyla* differs from *Rhabdostyla* by the discoidal macronucleus. Two species from the literature and a new species from Ecuadoran tank bromeliads are combined with the new genus: *O. previpes* (Claparède and Lachmann, 1857) nov. comb., *O. kahli* (Nenninger, 1948) nov. comb., and *O. bromelicola* nov. spec. *Orborhabdostyla bromelicola* is a slender species with stalk-like narrowed posterior half and operculariid/epistylidid oral apparatus. An epistylidid relationship is also suggested by the gene sequence. *Vorticella gracilis*, described by Dujardin (1841) from French freshwater, belongs to the *V. convallaria* complex but differs by the yellowish colour and the number of silverlines. The classification as a distinct species is supported by the 18S rDNA, which differs nearly 10% from that of *V. convallaria* s. str. Based on the new data, especially the very stable yellowish colour, we neotypify *V. gracilis* with the Austrian population studied by Foissner (1979). *Vorticella gracilis* forms a strongly supported phyloclade together with *V. campanula*, *V. fusca* and *V. convallaria*, while *Vorticellides astyliformis* and *Vorticella microstoma* branch in a separate, fully-supported clade that includes *Astylozoon* and *Opisthonecta*. The new genus *Vorticellides* comprises five small (usually < 60 µm), barrel-shaped species with two epistomial membranes: *V. aquadulcis* (Stokes, 1887) nov. comb., *V. astyliformis* (Foissner, 1981) nov. comb., *V. platysoma* (Stokes, 1887) nov. comb., *V. infusionum* (Dujardin, 1841) nov. comb., and *V.* (*Spinivorticellides*) *echini* (King, 1931) nov. comb. Two of these species are redescribed in the present study: *V. astyliformis* and *V. aquadulcis*, which is neotypified with a Costa Rican population. *Pseudovorticella bromelicola* nov. spec. differs from the congeners by the location of the two contractile vacuoles and the number of silverlines.

Key words: 18S rDNA, Costa Rica, Ecuador, neotypification, *Pseudovorticella*, *Rhabdostyla*, *Vorticella*.

INTRODUCTION

The peritrichs are classified into two assemblages (Jankowski 2007, Lynn 2008): the free-living Sessilida and the parasitic Mobilida with their characteristic adhesive disk. The Sessilida, which includes the species described here, comprises about 105 (Lynn 2008) to 140 (Jankowski 2007) genera, showing the great diversity of the group. Although a comprehensive recent review is not available, these genera comprise at least 800 described species. Many of the taxa are epibionts on a great variety of aquatic and semiterrestrial metazoans

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(Matthes 1982). For instance, some 80 peritrich species and about 10 suctorians and chonotrichs have been reported from freshwater gammarids globally (Schödel 1987). Very likely, the undescribed epibiontic peritrich diversity is much greater than the described one because detailed investigations are rare outside Europe. Likewise, the marine ecosystems are poorly explored, providing a continuous flow of new species (Song and Wang 1999, Sun *et al.* 2007). Although being comparatively well investigated, new genera and species are still discovered in freshwaters (Ji and Kusuoka 2009, Norf and Foissner 2009). Further, some interesting habitats escaped the attention, for instance, soil and the little water bodies of tank bromeliads (Foissner *et al.* 2002, 2003). These habitats contain a considerable number of new species, some of which are described in the present study.

Several peritrich genera contain more than 50 nominal species, for instance, *Vorticella* and *Epistylis*. A first split of the former into *Vorticella* (silverlines in transverse rings) and *Pseudovorticella* (silverline pattern reticulate) was performed by Foissner and Schiffmann (1974), and has been widely accepted (Warren 1987, Song *et al.* 2009). In the present paper, we propose a second split, using the number of epistomial membranes as a main distinguishing feature. Both splits are supported by 18S rDNA sequences, in which *Vorticella* appears non-monophyletic (Martin-Cereceda *et al.* 2007, Li *et al.* 2008; present paper, Fig. 25).

Peritrichs attracted comparatively many protistologists but reliable species descriptions became available only in the seventies of the past century. We emphasize not only the need of silver preparations and morphometry but also a detailed photographic documentation of the live cells because most species have a characteristic shape difficult to grasp by line drawings. However, the great success of Kahl's monographs is partially based on his outstanding ability to recognize the representative body shape and to show it by "simple" line drawings (Foissner and Wenzel 2004).

MATERIALS AND METHODS

The geographic origin of the material is provided in the individual species descriptions. Most samples were from tank bromeliads and were collected and sent to Salzburg by colleagues (see Acknowledgements).

In the Salzburg laboratory, the samples were screened for the species present. To study the peritrichs, which were attached to mud particles in low numbers, we used the following method: The tank water and the mud were sieved through a 500 µm net to remove crustaceans, insect larvae, and large rotifers. Then, the sample was transferred into a Petri dish and enriched with some partially crushed wheat grains to stimulate growth of the natural bacterial community. Concomitantly, coverslips were put on the sample (water) surface, where the peritrichs attached and developed considerable abundances within a few days. With this simple method, sufficient material was obtained for live observations and preparations. Further, such material was used to start a pure culture of *Vorticella gracilis*. This species was cultivated over half a year in Eau de Volvic (French table water) enriched with some crushed wheat grains. Specimens attached to the bottom of the Petri dish, bacterial flocks, and the coverslips on the culture surface.

Morphological and presentation methods followed Foissner (1991) and Foissner *et al.* (2002). Briefly, living cells were studied using a high-power oil immersion objective and differential interference contrast. Preparations were performed as described in Foissner (1991). Counts and measurements on silvered specimens were conducted at a magnification of × 1000. *In vivo* measurements were performed at magnifications of × 100–1000. Illustrations of live specimens were based on free-hand sketches and micrographs, while those of prepared cells were made with a drawing device. Terminology is according to Corliss (1979) and Lynn (2008).

To extract genomic DNA for 18S rDNA phylogenies, about 10 specimens of each species were picked with a micropipette and transferred into 180 µl ATL buffer (Qiagen) and 20 µl Proteinase K (20 mg/ml). Subsequently, the genomic DNA was extracted using the protocol for cultured animal cells of the DNEasy Tissue Kit (Qiagen, Hildesheim, Germany). The 18S rDNA was amplified using the universal eukaryotic primers EukA and EukB (Medlin *et al.* 1988). The amplification reaction contained 10–20 ng of DNA template, 2.5 U HotStar Taq DNA polymerase (Qiagen) in the manufacturer-provided reaction buffer, 1.5 mM $MgCl_2$, 200 µM of dNTP, and 0.5 µM of each oligonucleotide primer. The final volume was adjusted to 50 µl with sterile distilled water. The PCR protocol for 18S rDNA gene amplification consisted of an initial hot start incubation of 15 min. at 95°C followed by 30 identical amplification cycles (i.e., denaturing at 95°C for 45 s, annealing at 55°C for 1 min., and extension at 72°C for 2.5 min.), and a final extension at 72°C for 7 min. Negative control reactions included *Escherichia coli* DNA as a template. The resulting PCR products were cleaned with the PCR MinElute Kit (Qiagen) and cloned into a vector using the TA-Cloning kit (Invitrogen, Carlsbad, CA). Plasmids were isolated with Qiaprep Spin Miniprep Kit (Qiagen) from overnight cultures and PCR-reamplified using M13F and M13R primers to screen for inserts of the expected size (about 1.8 kb in case of the SSu-rDNA fragment). Three clones were sequenced bidirectionally (M13 sequence primers) with the Big Dye terminator kit (Applied Biosystems, Foster City, CA) on an ABI 3730 automated sequencer.

For a first assessment of the approximate phylogenetic placement of *O. bromelicola*, *V. astyliformis* and *V. gracilis*, their 18S rDNA sequences were aligned to all 18S rDNA sequences of peritrich ciliates available in GenBank. As an outgroup, we chose a representative each from the orders comprising the class Oligohymenophorea (for a review, see Lynn 2008). Alignments were constructed, using ClustalX (Thompson *et al.* 1997), and were manually refined in MacClade (Maddison and Maddison 2003), according to conserved regions. Distance, Maximum Likelihood and Bayesian

analyses showed a monophyly of the sessilid and mobilid peritrichs, in accordance with the analyses of Gong *et al.* (2006). Trees with support values are available from the authors.

We then performed a second phylogenetic analysis with a taxon sampling restricted to all 18S rDNA sequences of sessilid peritrichs available from GenBank and the oligohymenophorean *Urocentrum turbo* as outgroup taxon. The rationale behind this approach is that an analysis with less taxa, which are comparatively closely related to each other, allows the use of a higher number of unmasked, unambiguously aligned nucleotide characters, possibly resulting in a better resolved and supported phylogeny. The respective alignment included 1571 positions. We applied the program Modeltest (Posada and Crandall 1998) to choose the model of DNA substitution that best fitted our data sets. The model suggested by the AIC (Akaike Information Criterion) was GTR + I + G with the proportion of invariable sites (I) being 0.3739 and the gamma distribution shape parameter (G) being 0.5571. Neighbour joining evolutionary distance (NJ) analyses under maximum likelihood criteria were carried out in PAUP* v4.0b8 (Swofford 2002). Bayesian inference trees were obtained by using Mr. Bayes (Ronquist and Huelsenbeck 2003). For the Bayesian trees we ran two simultaneous, completely independent analyses starting from different random trees. This analysis also employed GTR + I + G as the DNA substitution model with the gamma distribution shape parameter, the proportion of invariable sites, base frequencies, and a rate matrix for the substitution model as assessed by Mr. Bayes. Metropolis coupling with three "heated" chains and one "cold" chain was employed to improve the Markov Chain Monte Carlo sampling of the target distribution. We ran 10,000,000 generations and sampled every 10,000th generation, resulting in 1001 samples from the posterior probability distribution. The relative stability of tree topologies was assessed, using 1000 bootstrap replicates and posterior probabilities of 751 Bayesian trees (25% burnin). Heuristic searches for bootstrap analyses employed stepwise addition, starting trees with simple addition of sequences and TBR branch-swapping. Maximum-likelihood bootstrapping analyses were carried out with 1000 replicates using RAxML with the setting as described in Stamatakis *et al.* (2008). ML and BI analyses were conducted online on the CIPRES Portal V 1.15 (http://www.phylo.org) The GenBank accession numbers of sequences obtained in this study are as follows: *O. bromelicola*: GQ872428; *V. astyliformis*: GQ872427; *V. gracilis*: GQ872429. All individual data sets are available from the Stoeck laboratory.

RESULTS

Orborhabdostyla nov. gen.

Diagnosis: Solitary Epistylididae (?) with discoidal to ellipsoidal macronucleus and transverse-striate silverline pattern.

Type species: *Orborhabdostyla bromelicola* nov. spec.

Etymology: Composite of the epistylidid genus *Rhabdostyla* and the Latin noun *orbis* (circle), meaning a *Rhabdostyla*-like ciliate with globular nucleus. Feminine gender according to Aescht (2001).

Species assignable: In addition to the type, two species may be referred to the new genus, viz., *Orborhabdostyla brevipes* (Claparède and Lachmann, 1857) nov. comb. (basionym: *Epistylis brevipes* Claparède and Lachmann, 1857) and *Orborhabdostyla kahli* (Nenninger, 1948) nov. comb. (basionym: *Rhabdostyla kahli* Nenninger, 1948) nov. comb. Nenninger (1948) established this species for the *Rhabdostyla* sp. described by Kahl (1935) because she found several populations matching Kahl's description. For details, see description and comparison of *O. bromelicola.*

Comparison with related genera: The new genus matches *Rhabdostyla* Kent, 1881, except for the shape of the macronucleus: horseshoe-like in *Rhabdostyla*, discoidal or globular in *Orborhabdostyla*. The same split has been performed by Lust (1950) in the genus *Opercularia*, referring species with discoidal or globular macronucleus to a new genus, *Orbopercularia*. This split has been widely accepted, for instance, by Corliss (1979) and Lynn (2008). Thus, we split *Rhabdostyla*, too. See Foissner (1979) and Foissner *et al.* (1999) for modern descriptions of *Rhabdostyla* species.

Traditionally, rhabdostylids are classified in the Epistylididae. However, the myoneme system and the more or less stalked peristomial disc suggest an operculariid relationship (Foissner 1981). In contrast to the epistylids (Foissner and Schubert 1977), the peristomial disc is not connected to the oral bulge myonemes which, in *O. bromelicola*, produce three thin, short branches connecting the oral bulge to some ventral body myonemes (Figs 10, 19). Vorticellids have, like epistylids, well developed oral bulge myonemes, which are neither connected to the peristomial disc nor the body myonemes (Foissner 1977).

Orborhabdostyla bromelicola branches in a well-supported (NJ/BI/ML – 99/100/79) clade together with *Epistylis chrysemydis*, *E. urceolata,* and *E. wenrichi* (Fig. 25). Thus, the 18S rDNA gene suggests an epistylidid rather than an opercullariid affiliation. Even though the support for this relation is very strong, phylogenetic analyses cannot exclude an opercularid relation, as both, opercullariids and orborhabdostylids are heavily undersampled and only represented by a single sequence each. Also, it is unfortunate that to date no representative of *Rhabdostyla* has been sequenced in order to evaluate the phylogenetic relation of *Orborhabdostyla* and *Rhabdostyla*.

Table 1. Morphometric data on *Orborhabdostyla bromelicola* nov. spec.

Characteristics[1]	Method	$\bar{x}$	M	SD	CV	Min	Max	n
Body, length (field specimens)	IV	64.5	65.0	6.8	10.1	50.0	75.0	15
Body, width (field specimens)	IV	14.8	15.0	1.5	10.0	13.0	18.0	15
Body length: width, ratio (field specimens)	IV	4.4	4.3	0.6	13.5	3.3	5.2	15
Peristomial disc, width (field specimens)	IV	10.0	10.0	0.7	7.3	8.0	11.0	16
Body, length (cultivated)	IV	50.5	45.0	9.3	18.0	40.0	70.0	19
Body, width (cultivated)	IV	15.1	15.0	1.7	11.0	13.0	20.0	19
Body length: width, ratio (cultivated)	IV	3.4	3.5	0.5	15.1	2.4	4.7	19
Peristomial disc, width (cultivated)	IV	10.4	10.0	1.2	11.4	8.0	13.0	15
Stalk, length (without adhesive disc)	P	5.4	4.0	2.8	51.0	3.0	12.0	21
Stalk, width	P	2.0	2.0	–	–	1.6	2.5	21
Macronucleus, length (excluding hollow parts)	P	9.8	10.0	2.1	21.8	7.0	17.0	21
Macronucleus, width (excluding hollow parts)	P	6.6	7.0	1.0	15.5	4.0	9.0	21
Macronucleus, thickness (approximate)	P	3.7	4.0	–	–	3.0	5.0	21
Silverlines from oral end to AAW, number	KF	62.8	63.0	2.4	3.9	57.0	68.0	21
Silverlines from AAW to scopula, number	KF	16.4	16.0	1.5	9.2	13.0	20.0	21
Pellicular pores in 100 µm, number	KF	19.7	20.0	3.7	18.9	14.0	26.0	21

[1] Data from silvered specimens based on randomly selected specimens from coverslip cultures. Measurements in µm. AAW – anlage of aboral ciliary wreath; CV – coefficient of variation in %; IV – *in vivo*; KF – Klein-Foissner "dry" silver nitrate method; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; P – protargol (Foissner's method); SD – standard deviation; $\bar{x}$ – arithmetic mean.

Orborhabdostyla bromelicola nov. spec. (Figs 1–50; Table 1)

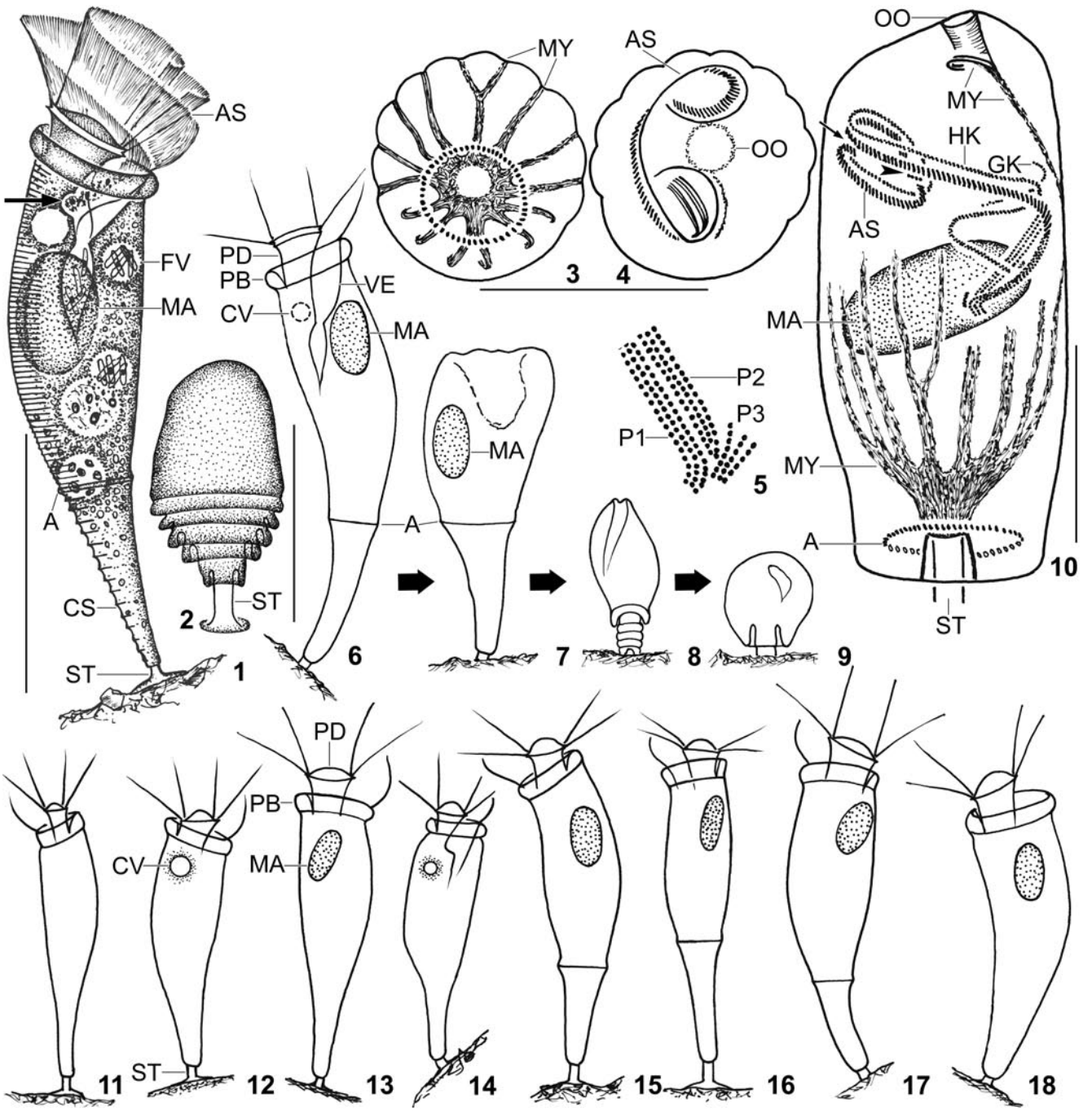
Diagnosis: Size about 65 × 15 µm in field specimens, while 50 × 15 µm in laboratory cultures. Narrowly conical with stalk usually < 10 µm long and 2 µm wide. Contractile vacuole and cytoproct on dorsal wall of vestibulum slightly posterior of oral bulge. On average 63 silverlines from oral end to anlage of aboral ciliary wreath and 16 silverlines from anlage to scopula. Peniculus 2 shortened posteriorly, peniculus 3 composed of three minute kineties. Freshwater, possibly restricted to tank bromeliads and not colonizing any host.

Type locality: Tanks of *Guzmania musaica* from Ecuador, province Esmeraldas, underneath Alto Tambo, 270 m above sea level, N01°02′13.7″, W78°37′13.7″. The molecular investigation was performed on a population from Jamaica.

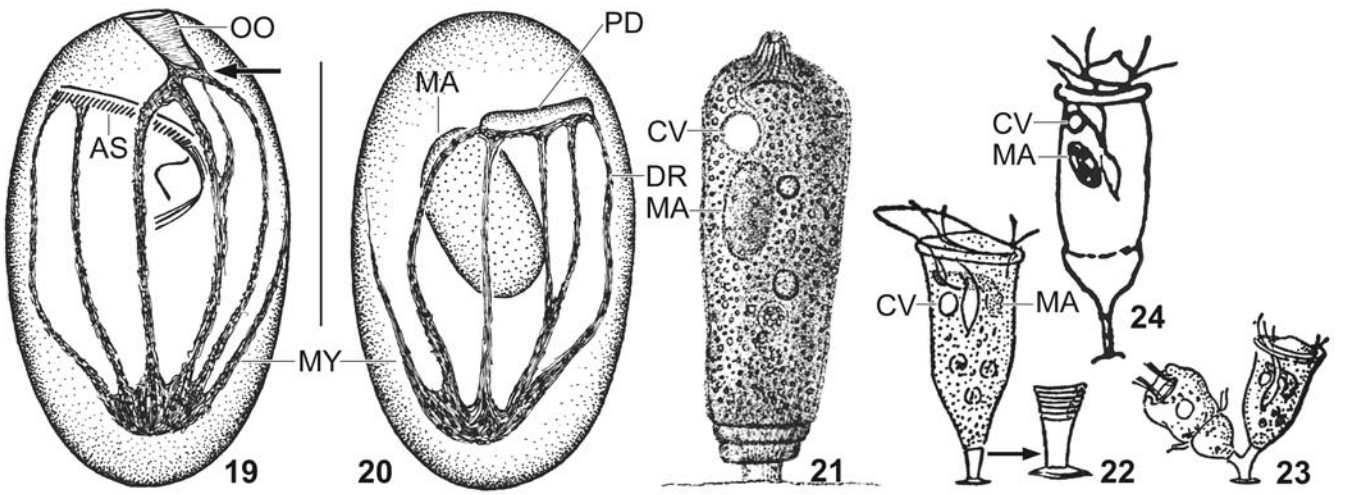
Type material: Three slides each with silver nitrate and protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). The holotypes and other relevant specimens were marked by black ink circles on the coverslip.

Description: The diagnosis and description are based on field and cultivated specimens, as described in the method section. Size and shape were studied in both, while protargol and silver nitrate data were obtained only from cultivated cells.

Size 50–75 × 13–18 µm *in vivo*, on average 65 × 15 µm in field specimens, while 40–70 × 13–20 µm, on average 50 × 15 µm in cultivated cells with high coefficient of variation (18%); average length: width ratio thus conspicuously different, viz., 4.3:1 vs. 3.5:1, with extremes of 2.4:1 to 5.2:1 (Table 1). Shape thus conical to narrowly conical, more or less asymmetrical, anterior end transverse to slightly obliquely truncate, posterior third frequently stalk-like narrowed (Figs 1, 6, 11–18, 26, 27, 35, 36). Highly contractile, especially in posterior third, i.e., from anlage of aboral ciliary wreath to scopula. Fully contracted field specimens about 25 µm long with anterior end rounded or slightly projecting (Figs 2, 9, 19, 41); when contracting, conspicuous transverse folds appear in posterior half and the anterior body section folds more or less over the contracted part, giving cells an ellipsoidal to globular shape with a deep indentation containing part of the strongly contracted posterior third (Figs 6–9, 30–32). Distinct folds usually



Figs 1–18. *Orborhabdostyla bromelicola* from life (1, 6–9, 11–18) and after protargol impregnation (2–5, 10). **1** – lateral view of a representative specimen, length 65 μm. Note the wide cortical striation in the stalk-like narrowed posterior third and the rather distinctly stalked peristomial disc. Arrow marks cytoproct; **2** – contracted specimen; **3**, **4** – aboral and oral view showing the body myoneme system not extending to the anterior end of the body; **5** – proximal portion of adoral ciliary spiral, showing peniculus 3 to be composed of three minute, fan-like spread rows of basal bodies; **6**–**9** – an extended specimen slightly, moderately, and fully contracted. Note the distinctly folded posterior third (8); **10** – infraciliature of holotype specimen, length 30 μm. Arrow marks begin of adoral ciliary spiral, arrowhead denotes epistomial membrane. Myoneme system only partially shown; **11**–**18 –** shape variability of body and peristomial disc; redrawn from micrographs. A – anlage of aboral ciliary wreath, AS – adoral ciliary spiral on peristomial disc, CS – cortical striation, CV – contractile vacuole, FV – food vacuole, GK – germinal kinety, HK – haplokinety, MA – macronucleus, MY – myonemes, OO – oral opening, PB – peristomial bulge, PD – peristomial disc, P1–3 – peniculi (adoral membranelles), ST – stalk, VE – vestibulum. Scale bars: 15 μm (Figs 2, 3, 4, 10) and 30 μm (1).



Figs 19–24. *Orborhabdostyla bromelicola* (19, 20) and related species (21–24) from life (21–24) and after protargol impregnation (19, 20). **19**, **20** – myoneme system. Arrow marks region where ventral and oral bulge myonemes merge; **21** – *Orborhabdostyla brevipes*, length 80–90 µm (from Claparède and Lachmann 1857); **22**, **23** – *Rhabdostyla brevipes* (from Penard 1922) is not identical with *O. bromelicola* because it is much smaller (46–52 µm with stalk, 40–46 µm without) and has a longish macronucleus; **24** – *Orborhabdostyla kahli* (from Kahl 1935), length 60 µm, is attached to *Lumbriculus sp.* AS – adoral ciliary spiral, CV – contractile vacuole, DR – disc retractors, MA – macronucleus, MY – myonemes, OO – oral opening, PD – peristomial disc. Scale bar: 15 µm.

absent from protargol-impregnated specimens (Figs 10, 19), i.e., rarely cells appear as shown in Figure 2. Macronucleus usually in anterior dorsal body half near cell's periphery; rarely ventral or lateral; usually ellipsoidal, rarely globular; flattened by about 50% and curved like a hollow hand, one margin frequently folded inwards; on average 10 × 7 µm in protargol preparations, while about 13–15 µm long *in vivo*; nucleoli minute and numerous. Micronucleus usually close to macronucleus, rarely far away, about 3 × 2 µm in size (Figs 1, 6, 10, 20, 27, 29, 30, 33–35, 38, 39–42; Table1). Contractile vacuole and cytoproct on dorsal wall of vestibulum slightly to distinctly posterior of oral bulge (Figs 1, 6, 12, 14, 26, 28, 36). Cytoplasm finely granulated, cell thus transparent; granules slightly concentrated in region of anlage of aboral ciliary wreath. Some food vacuoles mainly in posterior body half, 4–6 µm across, contain only few bacteria (Figs 1, 26, 27).

Cortex smooth between oral opening and anlage of aboral ciliary wreath, while rugged between anlage and scopula due to distinct transverse ridges (Fig. 1). Pellicular pores mainly within ridges (silverlines), conspicuous because up to 1 µm across, but loosely arranged, i.e., only about 20 pores/100 µm² (Figs 43, 44; Table 1). Specimens strongly contract, especially in posterior third, when air-dried for silver nitrate impregnation; silverlines thus difficult to count in that region, as indicated by the comparably high coefficient of variation, average possibly slightly higher than shown in Table 1. Silverline pattern basically narrowly striate (average distance between silverlines < 1 µm), except in posterior third, where it is widely striate (> 1 µm, see above). Pattern without peculiarities, some rings bifurcating or ending blindly (Figs 43–45). On average 63 silverlines between oral opening and anlage of aboral ciliary wreath and 16 from there to scopula (Table 1).

Stalk not branched in over 200 specimens analysed; minute, i.e., 1–3 µm long in field specimens, while 3–12 × 1.6–2.5 µm in protargol-prepared cultivated cells, 20 µm long in one field specimen; attached to organic and inorganic debris with adhesive disc up to 5 µm across (Figs 1, 2, 11–18, 29, 30, 35; Table 1). Myoneme system opercularid (see above). Body system comprising 10–15 thick, partially branched strands extending from scopula to anterior body end, where about half become disc retractors attaching to dorsal half of peristomial disc. Oral bulge myonemes inconspicuous, form very thin layer near inner margin of bulge, producing three short branches connected to the ventral body myonemes (Figs 3, 4, 10, 19, 20, 37). No myonemes recognizable

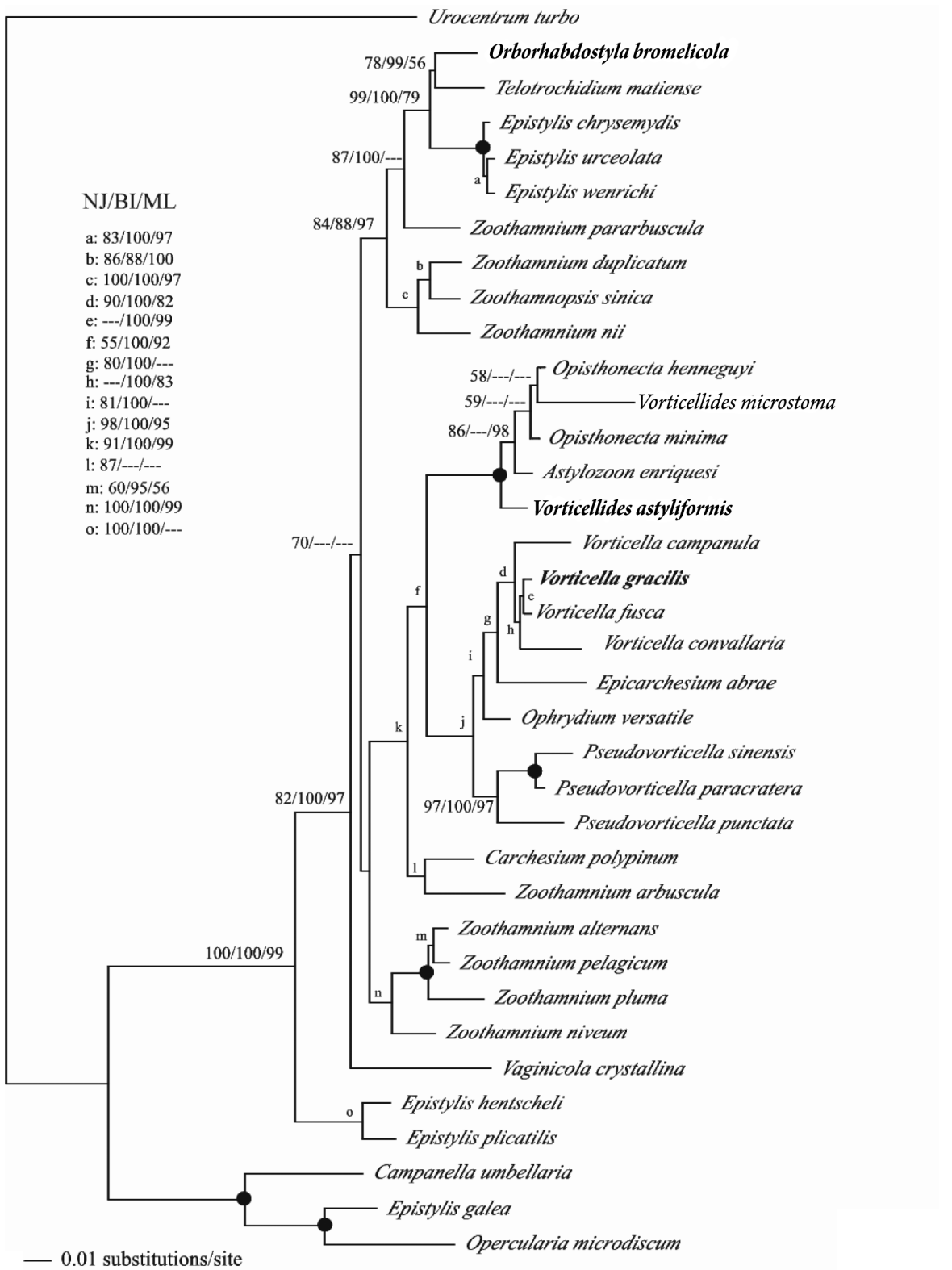
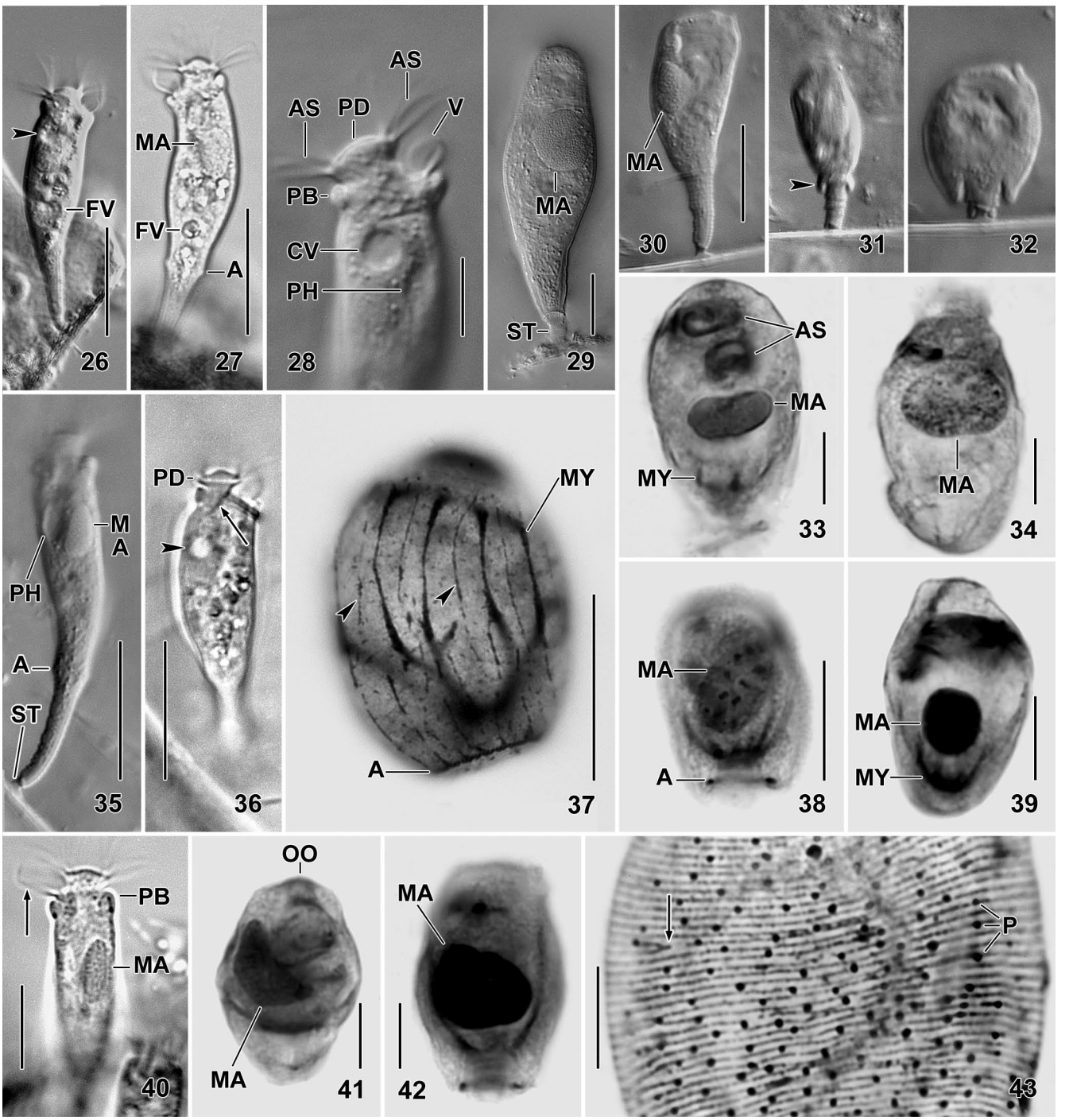
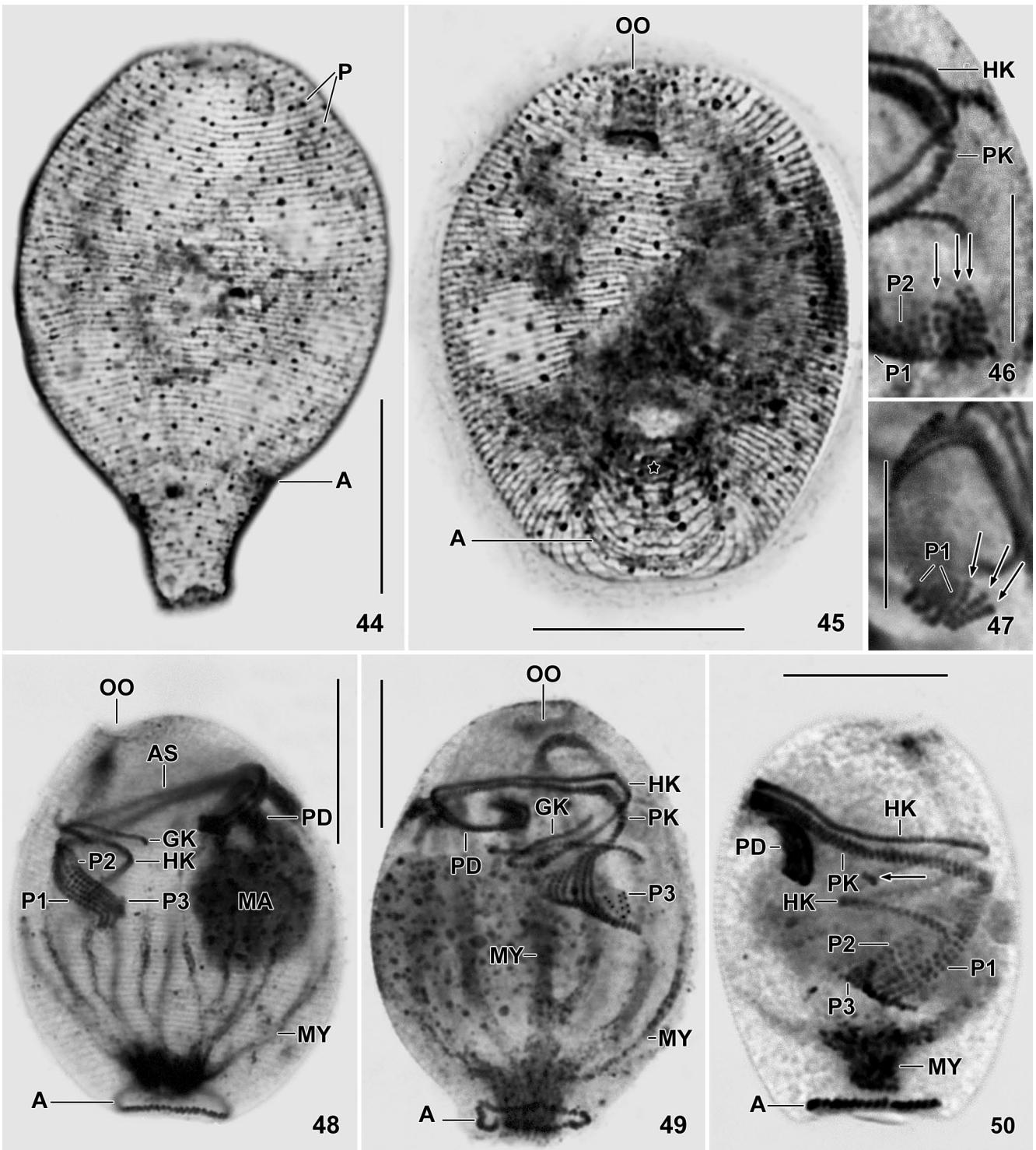


Fig. 25. 18S rDNA tree of sessilid peritrichs, with the species investigated in the present study shown in bold. The tree is a combination of three methods, the inset showing the bootstrap values for the neighbour-joining evolutionary distance (NJ), Bayesian inference (BI), and maximum-likelihood (ML) criteria. As concerns *Vorticellides microstoma*, see "Key to five *Vorticellides* species."



Figs 26–43. *Orborhabdostyla bromelicola* from life (26–32, 35, 36, 40) and after protargol (33, 34, 37–39, 41, 42) and silver nitrate (43) impregnation. **26**–**29** – representative specimens showing the slender body, the globular macronucleus, details of the oral apparatus, and the contractile vacuole on the dorsal wall of the vestibulum (26, arrowhead; 28); **30**–**32** – a contracting specimen showing the conspicuous bulge (31, arrowhead) developing in posterior third. All at same scale, bar: 20 µm; **35** – a very slender specimen with stalk-like posterior third; **36** – the peristomial disc is stalked (arrow) and the contractile vacuole (arrowhead) is on the dorsal wall of the vestibulum; **37** – the myoneme system consists of thick strands. Arrowheads mark a second system of very thin strands; **33**, **34**, **38**, **39**, **41**, **42** – the globular to ellipsoidal macronucleus is discoidal, i.e., flattened about 2:1; **40** – dorsolateral view showing the long cilia of the adoral spiral (arrow); **43** – silverline pattern in mid-body. The arrow marks a branching line. A – anlage of aboral ciliary wreath, AS – adoral ciliary spiral, CV – contractile vacuole, FV – food vacuoles, MA – macronucleus, MY – myonemes, OO – oral opening, P – pellicular pores, PB – peristomial bulge, PD – peristomial disc, PH – pharynx, ST – stalk, V – entrance to the vestibulum. Scale bars: 10 µm (Figs 28, 29, 33, 38, 39, 41–43), 20 µm (30–32, 37, 40), and 30 µm (26, 27, 35, 36).



Figs 44–50. *Orborhabdostyla bromelicola*, silverline and ciliary pattern after silver nitrate (44, 45) and protargol (46–50) impregnation. **44**, **45** – the area between the anlage of the aboral ciliary wreath and the scopula is strongly contracted, showing only part of the silverlines actually present. A fortunate preparation shows the wide striation in the stalk-like narrowed posterior third (45, asterisk; cp. Fig. 1); **46**, **47** – proximal portion of oral ciliary pattern, showing the minute peniculus 3 composed of three fan-like spread rows of basal bodies (arrows); **48**–**50** – heavily squashed specimens, showing the ciliary pattern and part of the myoneme system. Note the small peristomial disc contracted like in *Opercularia*. Arrow in (50) marks epistomial membrane. A – anlage of aboral ciliary wreath, AS – adoral ciliary spiral, GK – germinal kinety, HK – haplokinety, MA – macronucleus, MY – myonemes, OO – oral opening, P – pellicular pores, PD – peristomial disc, PK – polykinety, P1–3 – peniculi. Scale bars: 5 µm (Figs 46, 47) and 20 µm (44, 45, 48–50).

in peristomial disc. Another system, composed of fine, rather widely spaced fibres extends whole body length in some protargol-impregnated specimens (Fig. 37).

Oral apparatus epistylidid, however, with rather distinctly stalked peristomial disc resembling operculariids (Figs 1, 6, 11–18, 26–28, 36, 40; Table 1). Peristomial bulge of ordinary distinctness, upper margin very rarely crenelated. Peristomial disc slightly projecting from body proper, average diameter about 10 µm *in vivo*, inconspicuously to distinctly convex, never umbilicate; obliquely retracted into cell in contracted specimens. Cytopharynx spacious, extends obliquely to near midbody. Ciliature as typical for peritrichs. Cilia about 15 µm long, adoral ciliary spiral performs a 360° turn each around peristomial disc and in cytopharynx; peniculus 2 shortened posteriorly ending between peniculi 1 and 3; peniculus 3 composed of three minute, fan-like spread kineties. Epistomial membrane about halfway between begin of adoral ciliary spiral and vestibular opening, composed of three to five basal bodies (Figs 10, 50).

Anlage of aboral ciliary wreath *in vivo* recognizable as slight convexity at beginning of posterior body third (Figs 1, 6, 7, 27, 35); often difficult to recognize in Klein-Foissner silver nitrate preparations, while composed of deeply impregnated, narrowly spaced, oblique dikinetids (?) in protargol slides (Figs 3, 10, 37, 38, 48–50); in Klein-Foissner preparations consists of two narrowly spaced silverlines associated with body silverlines, one anteriorly and another posteriorly, producing a more or less prominent four line pattern (Figs 44, 45). Swarmers cylindroid, about 40 µm long, swim very fast, not studied in detail.

Occurrence and ecology: As yet found at type locality, as described above, and in a tank bromeliad from Jamaica. Few specimens occurred in the field samples; however, *O. bromelicola* reproduced rapidly in a culture with tank water containing some squashed wheat grains to stimulate bacterial growth. In field attached to inorganic and organic debris, including insect remnants; did not colonize various dipteran larvae present in the sample.

Comparison with related species: Of the *Rhabdostyla* species reviewed in Kahl (1935) and Stiller (1971), only two are similar to *O. bromelicola*: *O. kahli* (Nenninger, 1948) and *O. brevipes* (Claparède and Lachmann, 1857). *Orborhabdostyla kahli* (Fig. 24) differs from *O. bromelicola* by the location of the contractile vacuole (ventral vs. dorsal), body shape (cylindroidal vs. conical), and the habitat (epizooic on *Lumbriculus* vs. attached to dead material). *Orborhabdostyla brevipes* (Fig. 21) is possibly very similar to *O. bromelicola*, but so incompletely described that any identification is arbitrary. Claparède and Lachmann (1857) figured only a contracted specimen because the "extended state is like that of *Epistylis plicatilis*." *Orborhabdostyla brevipes* is 80–90 µm long and has been discovered attached to larvae of dipterans in a pond in Berlin; the stalk is minute but broad according to the description and figure of Claparède and Lachmann (1857). Assuming a length of 60 µm of the contracted specimen illustrated by Claparède and Lachmann (1857), the stalk is about 5 µm wide. Taken together, there are three features which distinguish *O. brevipes* and *O. bromelicola*: body size (80–90 µm vs. 65 × 15 µm), stalk width (about 5 µm vs. 2 µm), and habitat (epizooic vs. attached to dead matter; our species did not colonize the dipteran larvae present in the field sample!). Synonymy of the populations cannot be excluded until a European population has been characterized with modern methods. However, considering the special habitat (tank bromeliads) and the much narrower stalk, *O. bromelicola* is likely distinct from *O. brevipes*. *Rhabdostyla brevipes*, as described by Penard (1922), is a different species classified by Kahl (1935) as *R. brevipes* Penard, 1922 (Figs 22, 23).

Vorticella gracilis Dujardin, 1841 (Figs 51–79; Table 2)

Material: Great numbers of this species developed in a culture of tank water from a Costa Rican bromeliad. Thus, we could study it in detail and make various preparations to supplement the redescription by Foissner (1979), which is based on life observations and silver nitrate preparations of a population from the Aus-

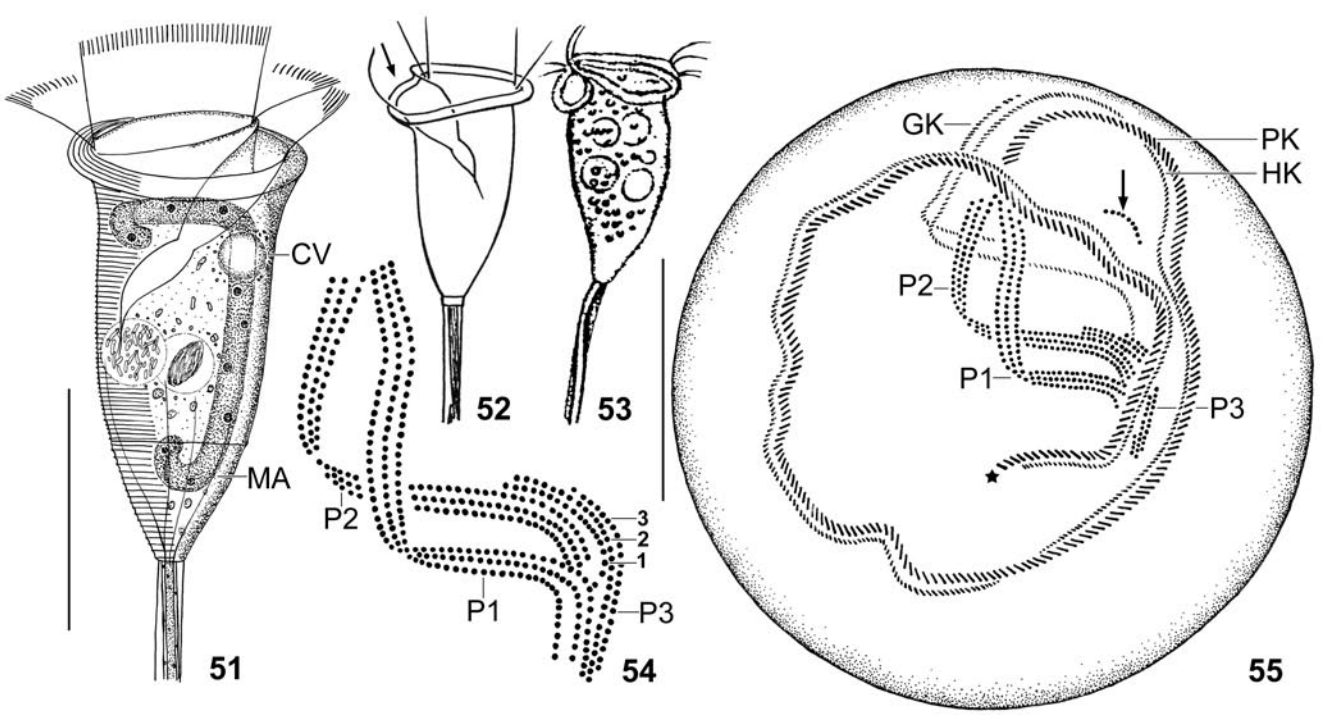
▸▸

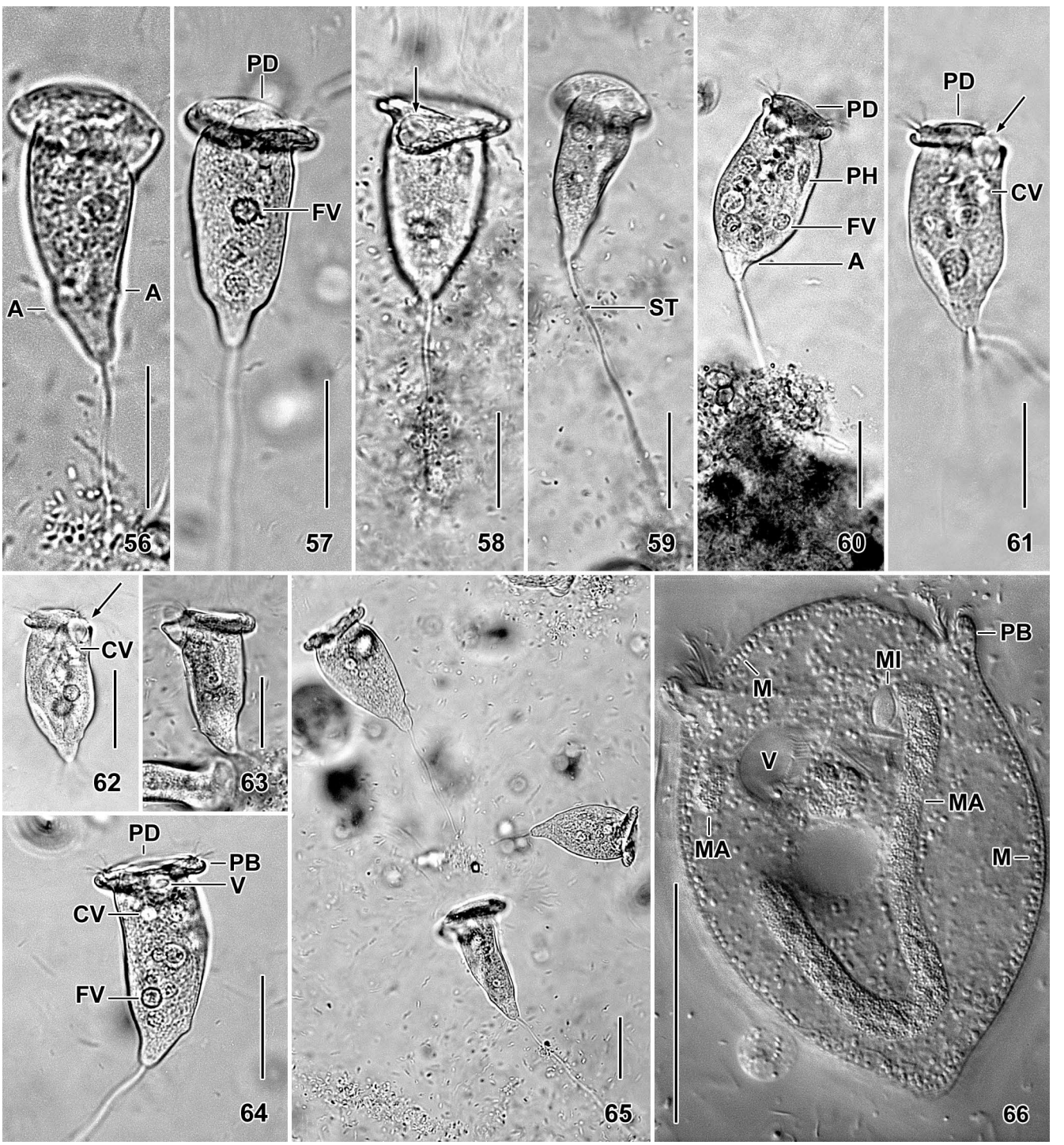
Figs 51–55. *Vorticella gracilis*, Austrian (51, from Foissner 1979), French (53, from Dujardin 1841), and Costa Rican (52, 54, 55; originals) specimens from life (51–53) and after protargol impregnation (54, 55). **51**–**53** – ventrolateral view of representative specimens from Austria, Costa Rican bromeliads, and France; length 75 µm, 80 µm, 55 µm. Note the high similarity in body shape and size. Arrow marks entrance to the vestibulum; **54**, **55** – oral ciliary pattern. The arrow marks the epistomial membrane, the asterisk indicates the distal end of the adoral ciliary spiral. Note the complex structure of peniculus 3 (54). CV – contractile vacuole, GK – germinal kinety, HK – haplokinety (paroral membrane), MA – macronucleus, P 1–3 – peniculi (adoral membranelles) originating from the polykinety (PK). Scale bars: 20 µm (Fig. 55) and 40 µm (51).

Table 2. Morphometric data on *Vorticella gracilis* Dujardin, 1841 and similar species.

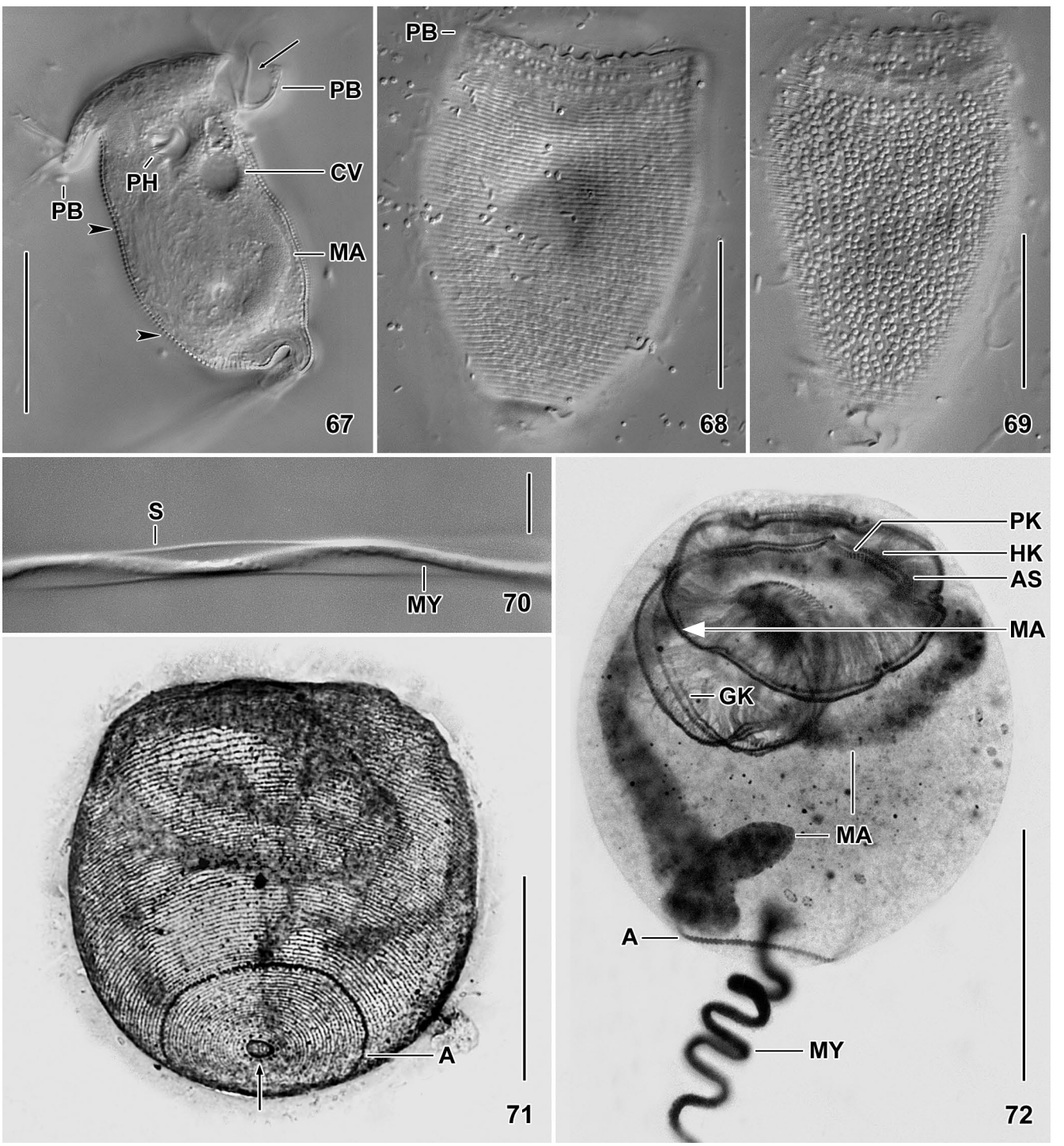
Characteristics [a]	Species[b]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body length: width ratio, *in vivo* [c]	*Vorticella gracilis* [2]	2.6	2.3	0.6	0.1	24.8	1.6	3.7	21
Silverlines from oral end to anlage	*Vorticella gracilis* [1]	71.6	72.5	9.3	2.3	13.0	55	85	16
of aboral ciliary wreath, number	*Vorticella gracilis* [2]	69.2	68.0	3.3	0.7	4.7	65	77	21
	Vorticella convallaria [1]	79.7	79.5	4.2	0.9	5.3	73	90	20
	Vorticella convallaria [3]	77.3	77.0	11.0	2.5	14.0	58	110	20
	Vorticella compacta [1]	87.5	87.5	7.6	2.7	8.7	75	97	8
	Vorticella similis [4]	73.4	73.0	1.8	0.4	2.5	70	76	20
	Vorticella similis [5]	77.6	78.0	2.5	0.7	3.3	74	82	14
	Vorticella similis [6]	80.3	–	–	–	–	78	84	7
Silverlines from anlage of aboral ciliary	*Vorticella gracilis* [1]	19.1	19.5	3.0	0.8	15.0	14	25	14
wreath to scopula, number	*Vorticella gracilis* [2]	21.5	21.0	1.0	0.2	4.8	20	24	21
	Vorticella convallaria [1]	22.2	22.5	1.6	0.4	7.2	20	25	20
	Vorticella convallaria [3]	22.3	22.5	2.4	0.5	11.0	16	27	20
	Vorticella compacta [1]	20.0	20.0	1.0	0.4	5.0	19	22	7
	Vorticella similis [4]	30.9	31.0	1.9	0.4	6.1	28	34	20
	Vorticella similis [5]	30.7	30.0	1.5	0.4	4.9	28	34	14
	Vorticella similis [6]	29.8	–	–	–	–	28	32	7

[a] Silverline data based on specimens impregnated with the Klein-Foissner "dry" method. CV – coefficient of variation in %; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.
[b] Data from Foissner 1979 [1], Costa Rica [2, original], Augustin and Foissner 1992 [3], Foissner and Schiffmann 1975 [4], Foissner 1981 [5], and Song and Wilbert 1989 [6].
[c] Based on micrographs; width – widest site underneath oral bulge.

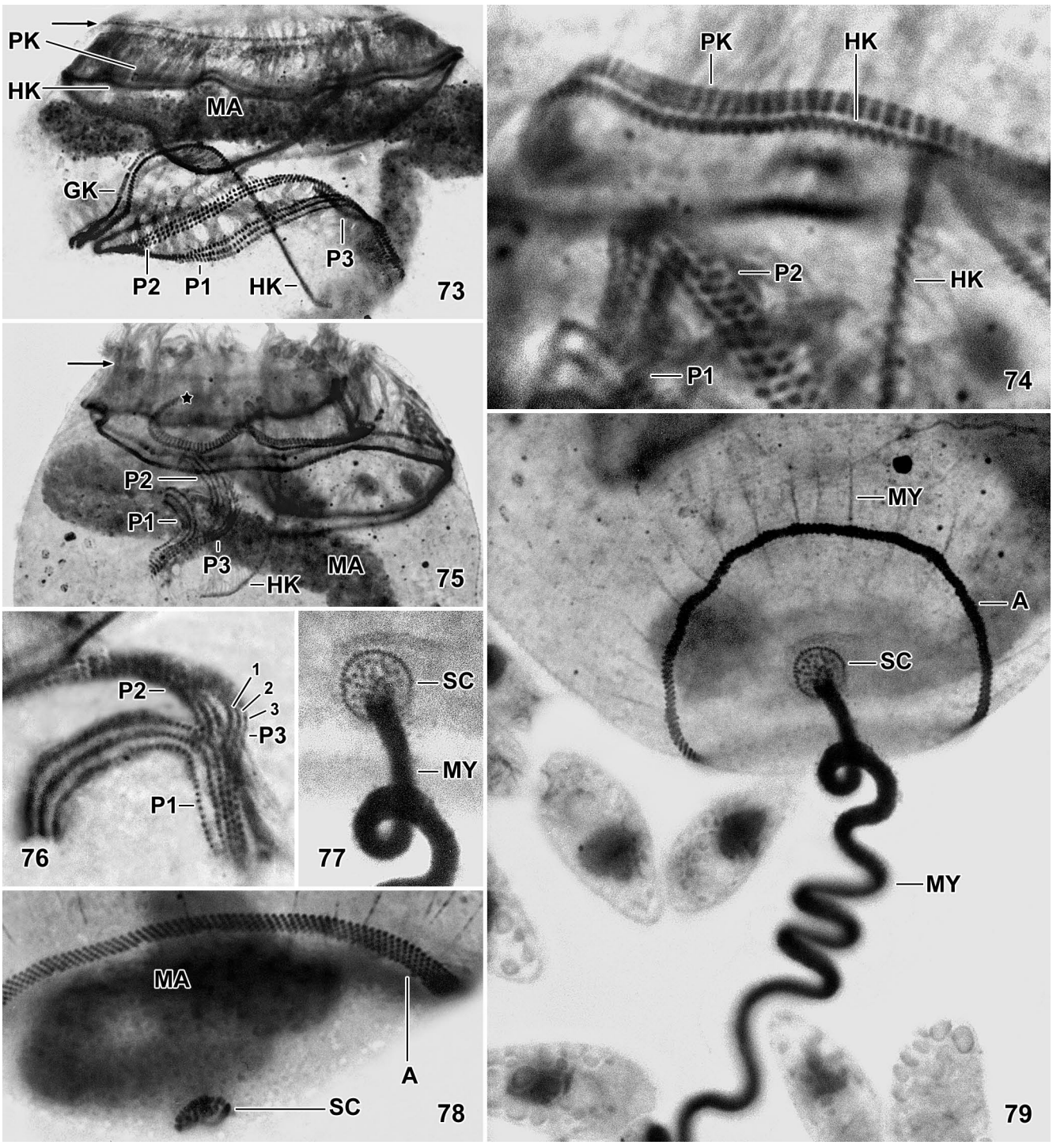




Figs 56–66. Cultivated *Vorticella gracilis* from a Costa Rican tank bromeliad. The specimens are slightly to distinctly yellowish. **56–64** – extended, swirling specimens showing, *inter alia*, the high variability of body shape and size (60–85 × 25–45 µm). The arrows mark the vestibular entrance. The peristomial bulge projects slightly (60–62) to distinctly (56–59, 63, 64) from body proper, while the flat peristomial disc only slightly overtops the peristomial bulge. Figures 61 and 62 show the same specimen photographed in an interval of a few seconds to demonstrate the contractile vacuole on the ventral wall of the vestibulum; **65** – the whirlpool-like water currents produced by the oral ciliature become recognizable due to floating bacteria; **66** – a flattened, dying specimen showing the J-shaped macronucleus and the ellipsoidal micronucleus. The mitochondria produce a dense, granular layer underneath the cortex (see also Fig. 69). A – anlage of aboral ciliary wreath, CV – contractile vacuole, FV – food vacuoles, M – mitochondria, MA – macronucleus, MI – micronucleus, PB – peristomial bulge, PD – peristomial disc, PH – cytopharynx, ST – stalk, V – vestibulum. Scale bars: 30 µm (Figs 58, 60), 35 µm (56, 57, 61, 62), and 40 µm (59, 63–66).



Figs 67–72. Cultivated *Vorticella gracilis* from a Costa Rican tank bromeliad from life (67–70) and after Klein-Foissner silver nitrate (71) and protargol (72) impregnation. **67** – longitudinal optical section showing the contractile vacuole on the ventral wall of the vestibulum and part of the J-shaped macronucleus. The cortical striation (arrowheads) is visible on the cell margin. The arrow indicates the vestibular entrance; **68** – surface view showing the cortical striation; **69** – same specimen as in Fig. 68 but at a slightly deeper focal level to show the granular mitochondria underneath the cortex; **70** – extended stalk; **71** – silverline pattern. Arrow marks scopula; **72** – a squashed specimen showing the adoral ciliary spiral performing 1.3 turns around the peristomial disc before plunging into the vestibulum. Arrow marks epistomial membrane. A – anlage of aboral ciliary wreath, AS – begin (distal end) of adoral ciliary spiral, CV – contractile vacuole, HK – haplokinety, MA – macronucleus, MY – stalk myoneme, PB – peristomial bulge, PH – cytopharynx, PK – polykinety, S – stalk sheath. Scale bars: 30 μm (Figs 68–70) and 35 μm (67, 72).



Figs 73–79. Cultivated *Vorticella gracilis* from a Costa Rican tank bromeliad after protargol impregnation. Scale bars deliberately omitted because all micrographs are based on specimens strongly flattened by the coverslip. **73**, **75** – oral ciliary pattern. The adoral ciliary spiral, which consists of a haplokinety (paroral membrane) and a polykinety (adoral membranelles), performs about 1.3 turns around the peristomial disc (75, asterisk) before plunging into the vestibulum, where it splits into 3 peniculi (P1–3). Arrows denote margin of oral opening; **74**, **76** – high magnifications of oral ciliature. In the vestibulum, the polykinety splits into 3 peniculi, each consisting of three rows (1–3) of basal bodies; **77**–**79** – specimens transforming to the swarmer stage, where the anlage (A) of the aboral ciliary wreath grows to short, oblique, densely spaced ciliary rows (78). The scopula forms the stalk, of which only the thick stalk myoneme and the scopular organelles, which form the stalk sheat, are impregnated. A – anlage of aboral ciliary wreath, GK – germinal kinety, HK – haplokinety, MA – macronucleus, MY – body and stalk myonemes, P 1–3 – peniculi, PK – polykinety, SC – scopula.

trian Central Alps (Fig. 51). The life and silver nitrate data from the Costa Rican specimens match Foissner's redescription perfectly (Table 2). Thus, only main characteristics and some additional observations will be mentioned.

Observations: *Vorticella gracilis* is bell-shaped but the size (60–85 × 25–45 µm) and the length: width ratio are highly variable, as shown by Figs 56–65 and the morphometric data (Table 2): $\bar{x}$ = 2.6:1, M = 2.3, SD = 0.6, CV = 24.8, Min = 1.6, Max = 3.7, n = 21. One of the most important features is the yellowish colour, which was stable for the cultivation period, i.e., for over half a year in ordinary Petri dish cultures set up with Eau de Volvic (French Table water) and some crushed wheat grains. These cultures also contained *V. astyliformis,* which remained colourless. In this connection, it must be emphasized that the re-evaluation of the original notes on the Austrian specimens mention a "yellowish cytoplasm," not only "yellowish cytoplasmic granules," as stated in the redescription by Foissner (1979). The macronucleus is J-shaped and its long middle portion extends in the ventral body half, as in the Austrian specimens (Figs 51, 66, 72). The protargol preparations show that *V. gracilis* has an oral ciliature of the *convallaria*-type, i.e., the adoral ciliary spiral performs about 1.3 turns around the peristomial disc, and peniculus 3 is composed of 3 ciliary rows, of which row 1 is distinctly shortened proximally (Figs 54, 55, 72–76); further, there is an epistomial membrane (Figs 55, 72). For ordinary details, see Figures 52, 67–71, 77–79.

Identification: This species was described by Dujardin (1841) only in the Figure section: "Longeur 55 µm. Dans l'eau de marais conservée pendant long temps. Elle n'est pas décrite dans le texte" (Fig. 53). Foissner (1979) provided a solid redescription and based the identification on the rather slender shape, the sole feature recognizable in Dujardin's "description." Indeed, *V. gracilis* is usually more slender than vorticellas of the *convallaria*-type (~ 2.5:1 vs. 1.8:1, see Table 2 and Foissner *et al.* 1992), but the most distinctive feature is its yellowish colour, which is very stable within and between populations. In contrast, the yellowish "variety" of *V. convallaria*, i.e., *V. citrina*, is likely only an "ecovariety" possibly caused by algal food (Foissner *et al*. 1992). Interestingly, the number of silverlines is also unique, i.e., it does not match well those of various populations of *V. convallaria* and *V. similis* (Fig. 71; Table 2): the former has slightly more silverlines from the anterior body end to the anlage of the aboral ciliary wreath (81 vs. 71), the latter has distinctly more silverlines from the anlage of the aboral ciliary wreath to the scopula (31 vs. 19). Thus, we do not have doubts that the populations represent a distinct species. This is supported by the molecular data (Fig. 25), where *V. gracilis* branches in a well-supported clade (NJ/BI/ML – 90/100/82) containing *V. campanula*, *V. fusca* and *V. convallaria*. Genetically, *V. gracilis* is very distinct from *V. convallaria* with a sequence dissimilarity of 9.8%. Even tough, we currently have no verified guidelines how to translate 18S rDNA sequence similarities into taxonomic hierarchies, such a high dissimilarity clearly points to distinct species. Thus far, to our knowledge no ciliate species have been reported with such a high intergeneric 18S rDNA gene divergence (Li *et al.* 2008)! The closest known 18S rDNA-relative of *V. gracilis* is *V. fusca* with 99.14% sequence similarity. *Vorticella fusca* has been described rather superficially by Precht (1935). It differs from *V. gracilis* by the habitat (freshwater vs. marine, colonizing the alga *Enteromorpha*), the dark-brown colour (vs. yellowish), the distinctly granulated (vs. smooth) stalk myoneme, the location of the contractile vacuole (ventral vs. dorsal), and the structure of peniculus 3 (Song *et al* 2009).

Neotypification: Considering the very incomplete original description and several similar species, all discussed by Foissner (1979), *V. gracilis* needs neotypification. Although the data from the Costa Rican population are more detailed than those from the Austrian Central Alps, we suggest using the Austrian population as a neotype because it is from the same biogeographic region as Dujardin's specimens (Palaearctic, Europe). Based on these and the present data, we fix the species diagnostically.

Diagnosis: Size about 80 × 30 µm *in vivo*; narrowly to ordinarily campanulate. Macronucleus J-shaped. Single contractile vacuole at ventral wall of vestibulum. Cytoplasm yellowish. About 70 silverlines from anterior end to anlage of aboral ciliary wreath and about 20 from there to scopula. Kinety 1 of peniculus 3 distinctly shortened proximally.

Type locality: Meltwater pool near the Fuschertörl (site 23 in Foissner 1980), Grossglockner-Hochalpenstrasse, Salzburg, Austria, N 47° E 12°, about 2400 m above sea-level.

Type material: Six slides with silver nitrate-impregnated specimens from a puddle in the Austrian Central Alps (Grossglockner area) have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Further, we deposited voucher slides from the Costa Rican population, viz., four slides each with

silver nitrate and protargol-impregnated cells. The neotypes and other relevant specimens are marked by black ink circles on the coverslip.

***Vorticellides* nov. gen.**

Diagnosis: Small, barrel-shaped Vorticellidae with two epistomial membranes and transverse-striate silverline pattern. Peniculus 3 usually composed of two ciliary rows.

Type species: *Vorticella aquadulcis* Stokes, 1887.

Etymology: Composite of the generic name *Vorticella* and the substantivated adjective *ides*, referring to the overall similarity with *Vorticella*. Masculine gender.

Species assignable: In addition to the type, four other species, formerly classified in *Vorticella*, may be referred to the new genus *Vorticellides*: *V. astyliformis* (Foissner, 1981) nov. comb. (briefly redescribed below); *V. platysoma* (Stokes, 1887) nov. comb., as redescribed by Foissner *et al.* 1999 (second epistomial membrane not marked but recognizable in Figure 15, p. 489); *V. infusionum* (Dujardin, 1841) nov. comb. (see below and Figs 113, 114); and *V.* (*Spinivorticellides*) *echini* (King, 1931) nov. comb., as redescribed by Foissner *et al.* (2002). The terminations of the species names remain unchanged because *aquadulcis* and *platysoma* are appositions; *echini* is a genitive; *infusionum* is a genitive plural; and *astyliformis* has the same termination in the feminine and masculine gender (see ICZN 1999).

As there are many small, barrel-shaped *Vorticella* species (Kahl 1935, Warren 1986, Song *et al.* 2009), one may expect that several of them will be transferred to *Vorticellides* on detailed reinvestigation. Possibly, the second epistomial membrane, which is minute and near the anterior (distal) end of the adoral spiral, has been sometimes overlooked or considered as an irregularity of the adoral ciliary spiral.

Justification of a second split of the genus *Vorticella*: About 200 nominal *Vorticella* species have been described (Kahl 1935, Corliss 1979, Warren 1986). Many of them have been considered to be junior synonyms (Warren 1986). However, more recent research resurrected some of the synonyms and added a considerable number of new species (Foissner 1979; Foissner *et al.* 1992, 1999; Song *et al.* 2009). Obviously, *Vorticella* and *Vorticella*-like peritrichs are much more divers than supposed previously.

In 1974, Foissner and Schiffmann split the genus in *Vorticella* (with transverse-striate silverline pattern) and *Pseudovorticella* (with reticulate silverline pattern). This split has been widely accepted and greatly refined the taxonomy of the vorticellids (Warren 1986, 1987; Song *et al.* 2009); now, the split is supported also by molecular data (Martin-Cereceda *et al.* 2007, Li *et al.* 2008; Fig. 25 of the present study).

Here, we propose a second split of *Vorticella*, using a rather difficult feature, viz., the number of epistomial membranes (one or two). The epistomial membranes are difficult to recognize because they consist of only few basal bodies, forming minute rows at the anterior (distal) end of the adoral ciliary spiral (epistomial membrane 2) and/or near the level of the vestibular entrance (epistomial membrane 1). Further, the epistomial membranes are ciliated only in the swarmer stage, except of *Opisthonecta*, where membrane 1 forms a conspicuous tuft in the trophic cells (Foissner 1975). Nothing is known about the function of the epistomial membranes, but the activation in the swarmer stage suggests some role in habitat selection. Likewise, nothing is known on their genesis and whether membrane 1 and 2 are homologous at all.

Most peritrichs have only one epistomial membrane near the anterior end or at the level of the vestibular entrance of the adoral ciliary spiral. However, Foissner *et al.* (1992) reported two epistomial membranes in a species of the *Vorticella infusionum*-complex (Fig. 114). Since then, we found two epistomial membranes in some other vorticellas (Foissner *et al.* 1999, 2002), and here we describe them in detail for *Vorticella aquadulcis* Stokes, 1887. The five species now collected in *Vorticellides* have not only two epistomial membranes in common but also a small body size (usually < 60 µm), a barrel-like body shape, and a minute peniculus 3 consisting of only two kineties. However, the most important and unique feature is the two epistomial membranes, one near the anterior (distal) end of the adoral ciliary spiral, the other rather far away from the anterior end, i.e., at level of the vestibular entrance.

The second split is also supported by the molecular data, where the genus *Vorticella* appears biphyletic (Martin-Cereceda *et al.* 2007, Li *et al.* 2008, Fig. 25 in the present paper). One clade contains, *inter alia*, "typical" campanulate species, viz., *Vorticella gracilis* and *V. convallaria*, while the other clade consists of small, barrel-shaped species, such as *V. astyliformis* and *V. microstoma*, as well as of several stalkless peritrichs, such as *Opisthonecta* spp. and *Astylozoon enriquesi*. The latter lacks any epistomial membrane (Foissner, unpubl.), while *Hastatella* has two membranes (Foissner, unpubl.).

Key to five *Vorticellides* species: The identification needs silver impregnation, exept of *V.* (*Spinivorticellides*) *echini*, which has distinct spines on the body

surface (for a detailed redescription, see Foissner *et al.* 2002). *Vorticellides infusionum* is usually distinctly larger than *V. astyliformis*, *V. platysoma* and *V. aquadulcis*. *Vorticellides platysoma* and *V. aquadulcis* are highly similar (Table 5), and thus possible synonymous. Further studies are required on the macronucleus (ellipsoidal to reniform vs. elongate reniform to semicircular) and peniculus 3.

We included in the new genus *Vorticellides* also the *Vorticella microstoma* available in GenBank because it was contained in the same clade as *Vorticellides astyliformis*. As far as we know, the morphological identity of the GenBank species has been not documented. We would be not surprised, if the GenBank species belongs to the *Vorticellides infusionum*-complex because species of this complex are very frequent and have been often mixed with *Vorticella microstoma*. See Foissner *et al*. (1992) how to separate species of the *V. microstoma* – and *V. infusionum*-complex.

1 Trophic cells without spines 2
Trophic cells with spines ..... *V.* (*Spinivorticellides*) *echini*
2 Body up to 40 µm long; total number of silverlines < 30 3
Body usually 45–60 µm long; total number of silverlines > 40 ..... *V. infusionum*-complex
3 Mainly in limnetic habitats; less than 50 pellicular pores/100 m^2 4
Mainly in terrestrial habitats; about 100 pellicular pores/100 m^2 ..... *V. astyliformis*
4 Row 1 of peniculus 3 shorter than row 2 ..... *V. platysoma*
Row 1 of peniculus 3 longer than row 2 ..... *V. aquadulcis*

Table 3. Morphometric data on a Costa Rican population of *Vorticellides astyliformis*.

Characteristics [a]	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	IV	31.3	31.0	_	_	_	28.0	35.0	4
Body, width	IV	14.0	12.0	_	_	_	12.0	20.0	4
Body length:width, ratio	IV	2.3	2.4	0.4	0.2	17.0	1.8	2.6	4
Silverlines from anterior end to AAW, number	KF	17.5	18.0	1.1	0.3	6.4	16.0	19.0	15
Silverlines from AAW to scopula, number	KF	3.6	4.0	–	–	–	3.0	4.0	10
Pellicular pores/100 μm^2	KF	117.0	120.0	10.0	3.1	8.9	97.0	130.0	11

[a] Data based on randomly selected specimens from a wheat grain culture. Measurements in µm. AAW – anlage of aboral ciliary wreath; CV – coefficient of variation in %; IV – *in vivo*; KF – Klein-Foissner "dry" silver nitrate impregnation; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.

Vorticellides astyliformis (Foissner, 1981) nov. comb. (Figs 80–83; Tables 3, 5)

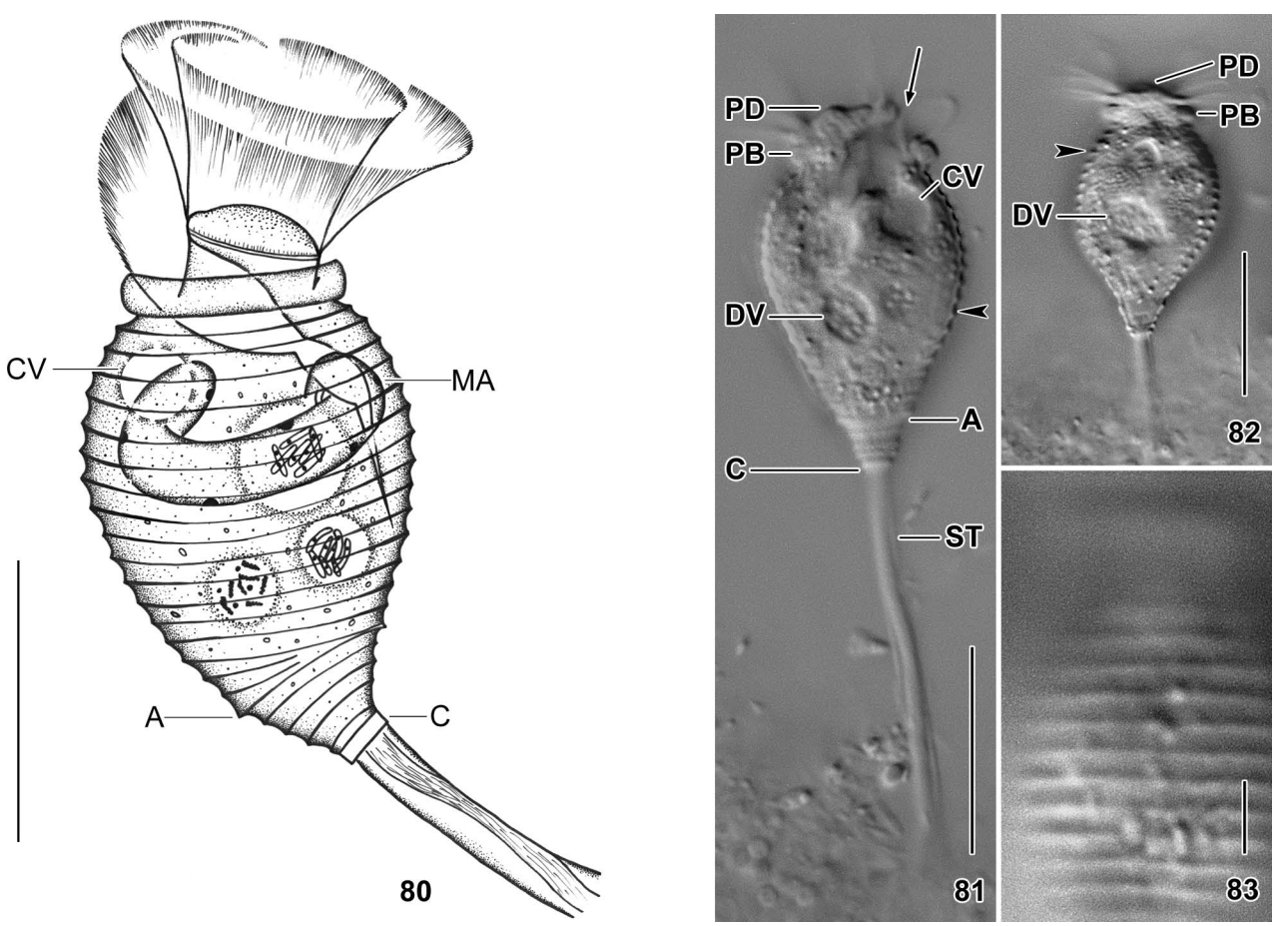
Material and slide deposition: This species was discovered by Foissner (1981) in soil from the Austrian Central Alps. Later, it was found in soils globally (Foissner 1998). We re-investigated a slide from the type series to obtain more detailed data on the epistomial membrane and peniculus 3. The second population was found at Namibian site 23 (Foissner *et al.* 2002), and the third population occurred in the tank of a bromeliad from Costa Rica. The slides investigated have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens have been marked by black ink circles on the coverslip.

Observations: The reinvestigation of the type and the Namibian population showed that both have two epistomial membranes in exactly the same position as *V. aquadulcis*, redescribed below. Thus, we do not provide new figures. The same applies to peniculus 2, which consists of two rows (Foissner 1981) but row 2 is only about half as long as row 1.

The population from a tank bromeliad of Costa Rica is highly similar to the specimens described from Austrian soils by Foissner (1981). Even a sophisticated feature like the number of pellicular pores is quite similar: about 100 pores/100 μm^2 (Tables 3, 5). There are, however, a few slight differences and additional observations. Size 28–35 × 12–20 µm *in vivo*, on average 30 ×

15 µm with a length:width ratio of 1.7–2.5:1, while the Austrian specimens are 30–50 µm long. Stalk 2–3 µm thick. Specimens attached to debris and bacterial flocs. Cortical striation as distinct as in type population, i.e., crest distance > 1 µm (Figs 80–83).

Comparison with similar species: There are many similar species, most depicted and briefly discussed in Foissner (1981) and Foissner *et al*. (1992, 2002). Especially, *V. costata* appears so similar to *V. astyliformis* that synonymy appears likely. However, there is a massive difference in a little used feature, viz., the number of pellicular pores: about 100/100 µm^2 in three populations of *V. astyliformis* (Foissner 1981, Fig. 35; Foissner *et al.* 2002, Fig. 378d; present study), while about 16–30 in *V. costata* and related species (Foissner 1979, Fig. 39; Foissner *et al*. 1992, Figs 22, 23 of the *V. aquadulcis* complex). Further, the oral bulge is considerably narrower in *V. astyliformis* than *V. costata*, which provides the former with a very characteristic appearance (Figs 80–82): ratio of width of body and peristomial bulge 1.4–2.0:1 vs. 1.2:1, as calculated from the present observations, the micrographs and drawings in the literature cited above and in Sommer (1951), and Foissner *et al*. (2002, Fig. 378a).



Figs 80–83. *Vorticellides astyliformis* from a Costa Rican tank bromeliad. **80** – ventro-lateral live view of a representative specimen, length 30 µm. Note, *inter alia*, the bulging body shape, the transverse, semicircular macronucleus, the stalk collar, and the ventral contractile vacuole. The anlage of the aboral ciliary wreath (A) appears as a slight subterminal convexity; **81**, **82** – representative specimens showing the bulging body shape, the conspicuous cortical striation (arrowheads), the vestibular entrance (arrow), the contractile vacuole on the ventral wall of the vestibulum (81), and the distinct dome formed by the narrow peristomial bulge and peristomial disc (82). Both specimens are feeding, having opened the vestibulum and showing a descending food vacuole, length 28–35 µm; **83** – surface view showing the wide (~1.5 µm) cortical striation with crests containing the silverlines. A – anlage of aboral ciliary wreath (telotroch), C – stalk collar, CV – contractile vacuole, DV – descending food vacuoles, MA – macronucleus, PB – peristomial bulge, PD – peristomial disc, ST – stalk. Scale bars: 20 µm (Figs 81, 82), 15 µm (80) and 3 µm (83).

Table 4. Morphometric data on a Costa Rican population of *Vorticellides aquadulcis* [a].

Characteristics [b]	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	IV	33.5	33.5	4.6	1.1	13.7	23.0	40.0	16
Body, width	IV	18.9	19.5	3.0	0.7	15.7	13.0	24.0	16
Peristomial bulge, width	IV	15.5	15.0	1.6	0.4	10.0	12.0	18.0	16
Peristomial disc, width	IV	12.1	13.0	1.2	0.3	9.9	10.0	13.0	16
Stalk, length	IV	47.6	45.0	21.7	5.4	45.5	20.0	105.0	16
Silverlines from anterior end to AAW, number	KF	18.3	18.0	1.2	0.2	6.4	17.0	21.0	25
Silverlines from AAW to scopula, number	KF	5.8	6.0	0.9	0.2	16.2	4.0	7.0	25
Pellicular pores/100 µm^2	KF	31.8	30.0	5.9	1.2	19.0	22.0	45.0	25

[a] Data for an Austrian population, see Table 5.
[b] Data based on randomly selected specimens from a wheat grain culture. Measurements in µm. AAW – anlage of aboral ciliary wreath; CV – coefficient of variation in %; IV – *in vivo*; KF – Klein-Foissner "dry" silver nitrate impregnation; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.

Table 5. Silverline features of six *Vorticellides* populations.

Characteristics [a]	Species [b]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Silverlines from anterior end to anlage of aboral ciliary wreath, number	ACR	18.3	18	1.2	0.2	6.4	17	21	25
	AAU	19.1	19	0.6	0.2	3.4	18	20	20
	PLM	17.6	17	0.9	0.2	5.1	16	19	26
	PPG	13.6	–	–	–	–	13	16	9
	ASR	17.5	18	1.1	0.3	6.4	16	19	15
	ASA	21.0	21	0.0	0.0	0.0	21	21	16
Silverlines from anlage of aboral ciliary wreath to scopula, number	ACR	5.8	6	0.9	0.2	16.2	4	7	25
	AAU	5.9	6	0.5	0.1	8.4	5	7	20
	PLM	5.8	6	0.8	0.2	13.8	5	7	13
	PPG	6.6	–	–	–	–	6	7	9
	ASR	3.6	4	–	–	–	3	4	10
	ASA	3.7	4	0.6	0.2	16.3	3	5	16
Pellicular pores, number per 100 µm^2	ACR	31.1	30	5.9	1.2	19.0	22	45	25
	AAU	25.3	25	3.5	0.8	13.7	20	32	20
	ASR	117.0	120	10.0	3.1	8.9	97	130	11
	ASA	104.7	106	21.9	5.5	20.9	70	150	16

[a] Data based on randomly selected specimens impregnated with the Klein-Foissner silver nitrate method. CV – coefficient of variation in %; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.
[b] AAU – *V. aquadulcis* from a pond in Linz, Austria (original); ACR – *V. aquadulcis* from tank bromeliads of Costa Rica, as described here (original); ASR – *V. astyliformis* from tank bromeliads of Costa Rica, as described here (original); ASA – *V. astyliformis* from alpine soil (from Foissner 1981); PLM – *V. platysoma* from lake Mondsee (from Foissner *et al*. 1999); PPG – *V. platysoma* from a pond in Berlin, Germany (from Song and Wilbert 1989).

Vorticellides aquadulcis (Stokes, 1887) nov. comb. (Figs 84–112; Tables 4, 5)

Material: Two populations were studied from a tank bromeliad of Costa Rica and a pond in the botanical garden of the town of Linz, Austria, respectively. Protargol preparations were made only from the Costa Rican population, which grew well in the natural sample enriched with some wheat grains. The specimens attached to organic debris and to coverslips on the water surface (Figs 94–98).

Original description by Stokes (1887; Fig. 84): "Body ovate or pyriform, very slightly changeable in shape, less than twice as long as broad, slightly constricted beneath the peristome border, the cuticular surface strongly and conspicuously striate transversely; peristome more than one-half the body-centre in breadth, but not equaling it, the border thickened, not everted; ciliary disc obliquely elevated; pedicle from two to three times as long as the body. Length of body, 35 µm. *Hab*.–Fresh water; attached to rootlets of *Lemna*. Solitary, or few together. Contracted body obovate."

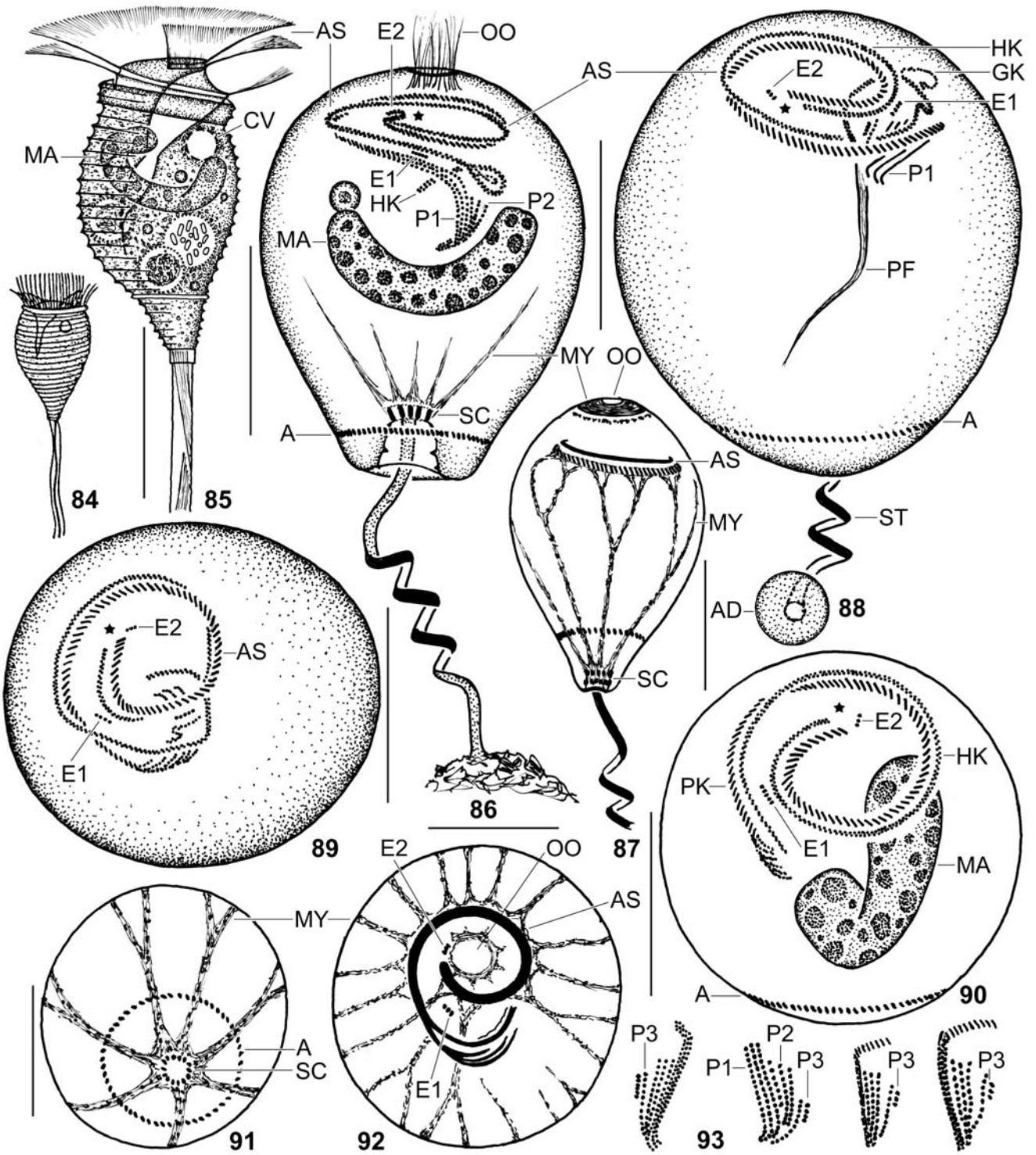
Redescription (if not stated otherwise, data are from the Costa Rican neotype population): Size 23–40 × 13–24 µm *in vivo*, usually about 35 × 20 µm (Table 4), matching Stokes' notion. Shape of Austrian and Costa Rican specimens more variable than in Stokes' population, i.e., usually more or less pyriform or barrel-like, rarely obovate, conical, slenderly conical or globular; never campanulate because oral bulge distinctly narrower than mid-body (Figs 94–98, 110–112; Table 4); contracted specimens globular. Macronucleus in or slightly above mid-body, elongate reniform to semicircular, with conspicuous nucleoli (Figs 85, 86, 90, 99); micronucleus not impregnated with the protargol method used. Contractile vacuole on ventral wall of vestibulum underneath oral bulge (Figs 85, 96, 97). Cytoplasm colourless, usually packed with 4–7 µm-sized food vacuoles and some lipid droplets about 1 µm across. Feeds on bacteria, forming rather transparent masses in the food vacuoles (Figs 85, 94–98).

Cortex as distinctly striate transversely as in Stokes' North American specimens; striation recognizable even at low magnification (~ × 100) because crests comparatively high (~ 1 µm) and about 1.5 µm apart, as measured *in vivo* and calculated from average body length and number of silverlines (Figs 85, 94–98, 110–112; Table 4). Silverline pattern widely striate (average distance between silverlines > 1 µm), without peculiarities, on average 18 silverlines (19 in Austrian specimens) from anterior body end to anlage of aboral ciliary wreath and 6 silverlines from there to scopula, both in Costa Rican and Austrian specimens (Tables 4, 5); two silverlines in oral bulge. On average 31 and 25 pellicular pores/100 µm² in Costa Rican and Austrian specimens, respectively; most pores underneath silverlines in Costa Rican cells, while above in Austrian ones (Figs 85, 102, 105, 106, 108, 109; Tables 4, 5).

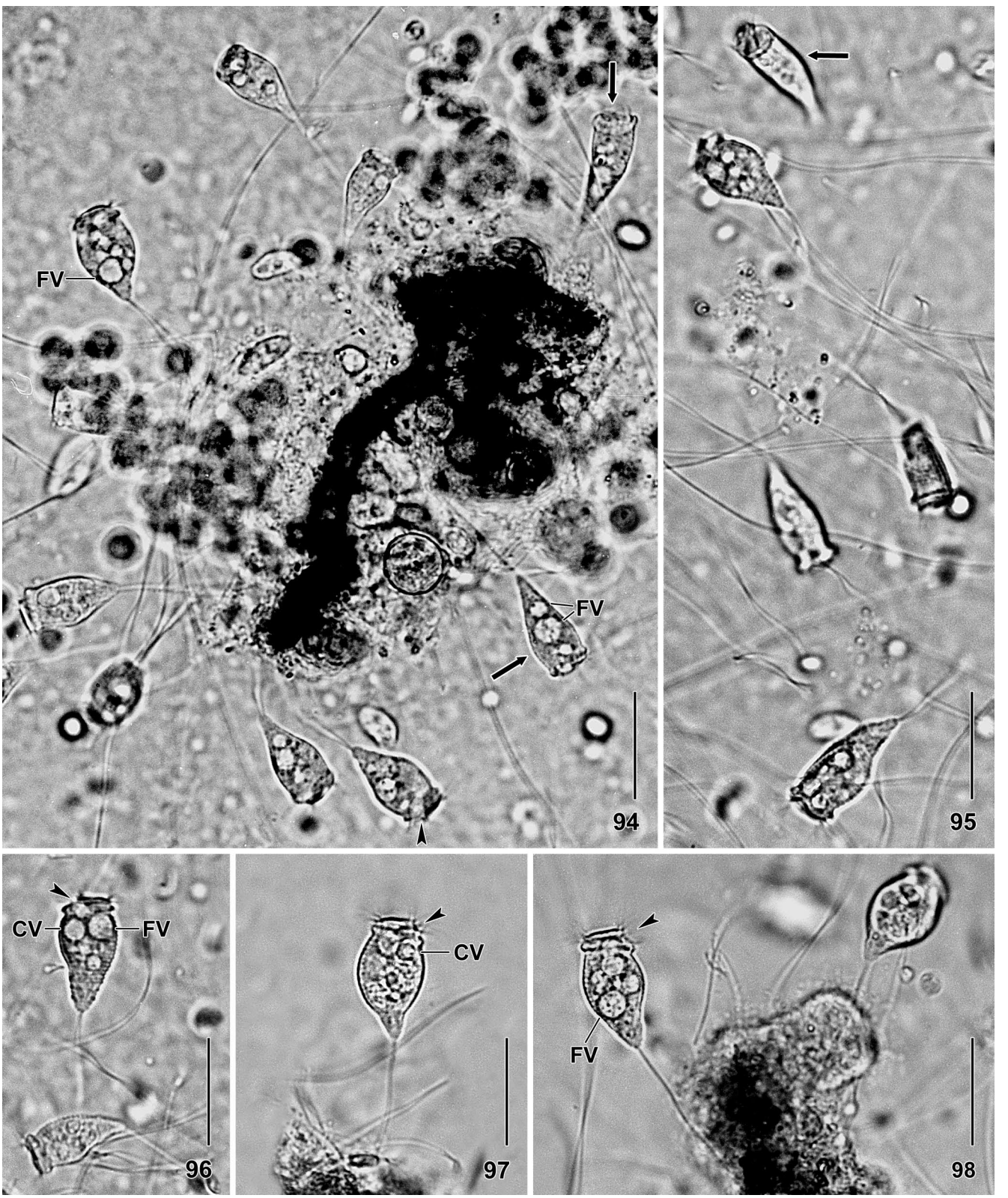
Stalk vorticellid, i.e., with flattened, spirally contracting myoneme; 2–2.5 µm wide and 20–105 µm long, on average 48 µm and thus short, as mentioned by Stokes (1887); attached to debris and coverslips by a leaf-like disc about 3 µm across. Scopular organelles 1–1.5 µm long, in some specimens two rings one upon the other (Figs 85–88, 94–98, 110–112; Table 4). Myoneme system vorticellid and rather loose, i.e., six to eight strands originate in the surroundings of the scopula and extend anteriorly, becoming bi-to quadripartite before attaching to the adoral ciliary spiral (Figs 86, 87, 91, 92, 99). Oral bulge myoneme dense, with short, posteriorly directed extensions, forming a granular ring (Figs 87, 100); no myonemes recognizable in peristomial disc.

Oral apparatus typical, except of the two epistomial membranes, structures very small and thus difficult to analyze (Figs 86, 88–90, 93, 99–101, 103, 104, 107; Table 4). Peristomial bulge and peristomial disc about 5 µm high in feeding specimens, vestibulum of ordinary size, extends obliquely to body centre. Peristomial bulge 2–2.5 µm thick, slightly projecting from body proper and thus distinct, considerably narrower than widest site in mid-body (~ 15 µm vs. 19 µm), shows two transverse striae (silverlines). Peristomial disc slightly narrower than peristomial bulge (12 µm vs. 15 µm; Table 4), surface flat to inconspicuously convex, never umbilicate both in Costa Rican and Austrian specimens; slightly obliquely elevated in feeding cells (Figs 85, 94–98, 110–112). Adoral ciliary spiral performs slightly more than one turn (~ 400°) around peristomial disc, before plunging into the vestibulum, performing a further turn and splitting into three peniculi (Figs 85, 86, 88–90, 92, 99–101). Peniculus 1 and germinal kinety without peculiarities. Peniculus 2 only about 5 µm long, first row slightly shortened proximally. Peniculus 3 composed of only two ciliary rows: row 1 distinctly longer than row 2, slightly curved or sigmoidal, ends near proximal end of peniculus 1; row 2 distinctly shortened proximally, composed of only three to five basal bodies (Figs 93, 99–101, 107). Epistomial membrane 1 at level of vestibular entrance, i.e., rather far away from distal end of adoral ciliary spiral, composed of three to five basal bodies. Epistomial membrane 2 slightly ahead of distal end of adoral ciliary spiral, composed of three basal bodies (Figs 86, 88–90, 92, 103, 104).

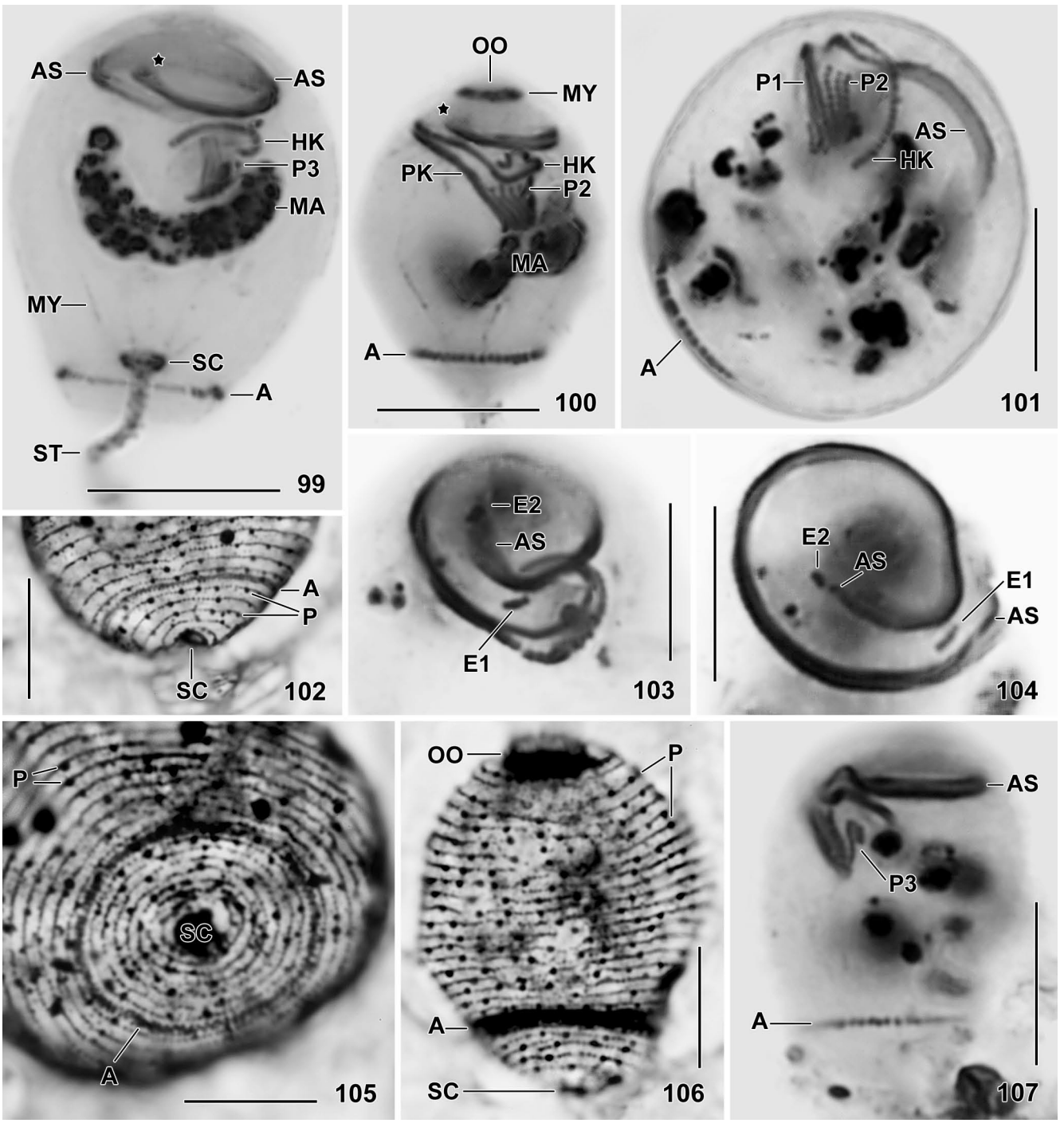
Anlage of aboral ciliary wreath hardly recognizable *in vivo*, in posterior quarter of cell and distinct in silver nitrate preparations, appearing as a granular mass boardered by a silverline each anteriorly and posteriorly (Figs 102, 105); in protargol preparations composed of minute, slightly oblique pairs (?) of basal bodies (Figs 86–88, 90, 91, 99–101, 107); when activated in swarmers, composed of oblique, 2–3 µm long kineties (Figs 106, 108, 109).



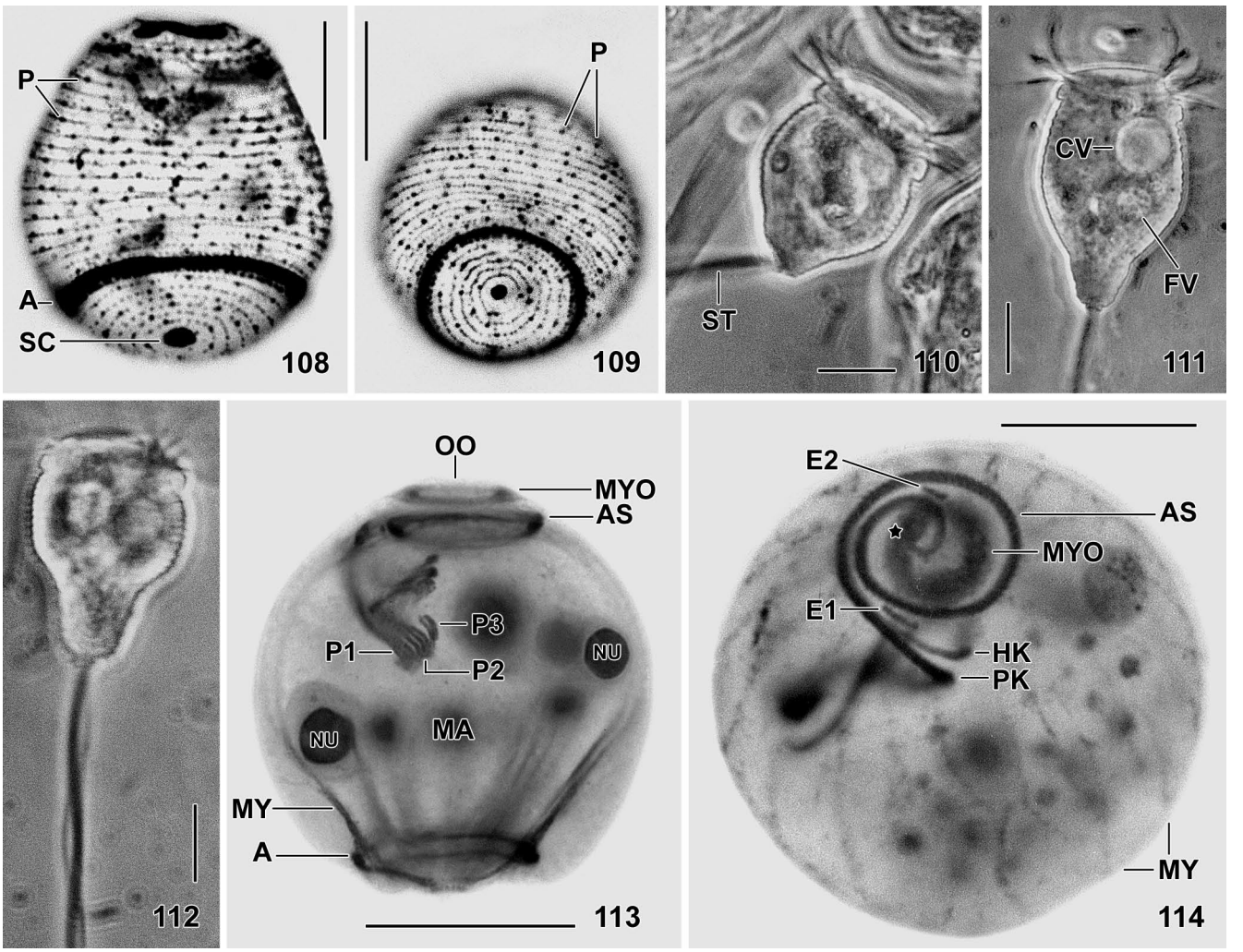
Figs 84–93. *Vorticellides aquadulcis* from life (84, 85) and after protargol impregnation (86–93). **84** – original figure from Stokes (1887), length 35 µm; **85** – a representative neotype specimen from Costa Rica, length 30 µm; **86**, **88**–**90** – various views of the nuclear and ciliary pattern. The asterisks mark the distal end of the adoral ciliary spiral. Note the two epistomial membranes (E1, E2); **87**, **91**, **92** – various views of the myoneme system, which is attached to the adoral ciliary spiral; **93** – proximal end of adoral ciliary spiral, showing the variability of the minute (~ 2.5 µm) peniculus 3, which consists of two ciliary rows, of which row 2 is distinctly shorter than row 1. A – anlage of aboral ciliary wreath, AD – adhesive disc, AS – adoral ciliary spiral, CV – contractile vacuole, E1; E2 – epistomial membranes, GK – germinal kinety, HK – haplokinety, MA – macronucleus, MY – myonemes, OO – oral opening, PF – pharyngeal fibres, PK – polykinety, P1–3 – peniculi, SC – scopula, ST – stalk. Scale bars: 10 µm (Figs 86–92) and 20 µm (85).



Figs 94–98. *Vorticellides aquadulcis*, live Costa Rican neotype specimens from a coverslip culture. Note the high shape variability, though many specimens are more or less pyriform. Arrows in Figures (94, 95) mark conical specimens; the arrowheads in Figures (94, 96–98) denote the vestibular entrance. CV – contractile vacuole on ventral wall of vestibulum, FV – food vacuoles. Scale bars: 30 µm.



Figs 99–107. *Vorticellides aquadulcis*, Costa Rican neotype specimens after protargol (99–101, 103, 104, 107) and silver nitrate (102, 105, 106) impregnation. As the species is very small (20–25 µm) and globular in the protargol slides, sharp micrographs are difficult to obtain. **99–101**, **107** – various views of the ciliary pattern and the macronucleus. Note the short peniculus 2 (100, 101) and the minute peniculus 3 (99, 107), which is composed of only two ciliary rows, of which row 2 is distinctly shortened. The asterisks mark the distal end of the adoral ciliary spiral; **103, 104** – anterior polar views showing the outer portion of the adoral ciliary spiral and the two epistomial membranes (E1, E2), the most unique feature of the new genus *Vorticellides;* **102, 105** – lateral and posterior polar view showing the silverline pattern. Note, *inter alia*, the considerable variability in the number (5 and 7) of silverlines from the anlage of the aboral ciliary wreath to the scopula; **106** – a swarmer with fully developed aboral ciliary wreath (A). Note the moderate number of pellicular pores. A – (anlage of) aboral ciliary wreath, AS – adoral ciliary spiral, E1, E2 – epistomial membranes, HK – haplokinety, MA – macronucleus, MY – myonemes, OO – oral opening, P – pellicular pores, PK – polykinety, P1–3 – peniculi, SC – scopula, ST – stalk. Scale bars: 10 µm.



Figs 108–114. Austrian populations of *Vorticellides aquadulcis* (108–112, from Foissner *et al.* 1992) and *Vorticellides infusionum* (113, 114, from Foissner *et al.* 1992) after silver nitrate impregnation (108, 109), *in vivo* (110–112), and after protargol impregnation (113, 114). **108**, **109** – the number of silverlines and pellicular pores is highly similar in the Austrian and Costa Rican specimens (cp. Figs 102, 105, 106 and Table 5); **110**–**112** – most Austrian specimens are 30–40 µm long and pyriform, as those from the Costa Rican neotype population (cp. Figs 94–98); **113**, **114** – *V. infusionum* is larger than *V. aquadulcis* (~ 50 µm vs. 30 µm), enhancing recognition of structural details. The lateral and the oblique anterior polar view show, *inter alia*, peniculus 3, which consists of two rows having the same length (113), and the two conspicuous epistomial membranes (E1, E2). The asterisk in Fig. 114 marks the distal end of the adoral ciliary spiral. A – (anlage of) aboral ciliary wreath, AS – adoral ciliary spiral, CV – contractile vacuole, FV – food vacuole, HK – haplokinety, MA – macronucleus, MY – body myonemes, MYO – oral bulge myoneme, NU – nucleoli of macronucleus, OO – oral opening, P – pellicular pores, PK – polykinety, P1–3 – peniculi, SC – scopula, ST – stalk. Scale bars: 10 µm (Figs 108–112) and 20 µm (113, 114).

Occurrence and ecology: The three reliable records available (Stokes 1887 and the two populations described here) indicate that *V. aquadulcis* is probably widespread in various freshwater habitats, ranging from ponds to bromeliad tanks. Many records of this and similar species were reviewed by Foissner *et al.* (1992). They conclude that species of the *V. aquadulcis*-complex prefer rather clean, beta-mesosaprobic habitats, while records from sewage plants are possibly misidentifications.

Comparison with congeners and similar species: See key to species and *V. astyliformis*.

Neotypification: Considering the very incomplete original description and many similar species, most discussed by Warren (1986) and Foissner *et al.* (1992, 1999), *V. aquadulcis* needs neotypification. Ideally, the

neotype should be from the locus classicus or from nearby (ICZN 2009). However, this regulation has been questioned in the case of microscopic organisms, many of which have been very poorly described and have a large areal (Foissner 2002).

Of the two populations studied, only that from Costa Rica has been fully investigated and will thus be fixed as a neotype, although it is about 3300 km away from the type area, i.e., a pond in the surroundings of the town of Trenton, New Jersey, USA, where Stokes lived and worked. To use the Costa Rican population as a neotype is supported by the high similarity with the North American (Stokes 1887) and the European (Figs 84, 108–112; Table 5) populations, indicating a cosmopolitan distribution and wide ecological range (ordinary ponds to tanks of bromeliads).

Diagnosis: Size about 35 × 20 µm *in vivo*. Shape highly variable, usually pyriform, obovate or conical. Macronucleus semicircular in transverse axis of midbody. Single contractile vacuole at ventral wall of vestibulum. Cortex distinctly transverse-striate, with about 19 silverlines from anterior end to anlage of aboral ciliary wreath and an average of 6 silverlines from there to the scopula. Epistomial membrane 1 at level of vestibular entrance, membrane 2 at distal end of adoral ciliary spiral. Peniculus 3 composed of two kineties, with kinety 2 distinctly shortened proximally.

Type locality: Tank bromeliads from Costa Rica, Central America. Unfortunately, a more exact locality cannot be provided because the collector did not specify the site.

Type material: Six slides with protargol-impregnated cells and two slides with silver-nitrate impregnated specimens from the Costa Rican neotype population have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Further, five slides with silver nitrate-impregnated specimens from Linz, Austria, have been deposited at the same locality. The neotypes and other relevant specimens have been marked by black ink circles on the coverslip.

Pseudovorticella bromelicola nov. spec. (Figs 115–129; Table 6)

Diagnosis: Size about 65 × 40 µm *in vivo*; pyriform to campanulate. Macronucleus J-shaped. Two ventral contractile vacuoles. On average 41 silverlines from anterior end to anlage of aboral ciliary wreath and 13 silverlines from there to scopula.

Type locality: Tanks of *Guzmania scherzeriana* (Bromeliaceae) in the garden of the tropic station La Gamba, Costa Rica, N 8°42′, W 83°12′, 70 m above sea-level.

Etymology: Composite of the plant genus *Bromelia* (Bromeliaceae) and the Latin verb *colere* (inhabiting), referring to the habitat in which the species was discovered.

Description: Size 60–75 × 30–50 µm *in vivo*, on average about 65 × 40 µm; shape narrowly pyriform to pyriform and more or less campanulate, length:width ratio 1.3–1.9:1, usually about 1.7:1. Fully contracted specimens globular with projecting peristomial bulge (Figs 115–126; Table 6). Macronucleus J-shaped and extending longitudinally in dorsal half of cell. Micronucleus not observed. Two contractile vacuoles at ventral wall of vestibulum: one in mid-portion, the second near its end. Cytoproct on dorsal wall of vestibulum underneath peristomial bulge (Fig. 115). Cytoplasm colourless, with many food vacuoles 7–9 µm across and containing bacteria. Stalk up to three times body length, i.e., up to 200 µm long and 4–5 µm wide. Stalk myoneme without granules, contracts spirally. Cells attached to debris and bottom of Petri dish (Figs 115, 122–124).

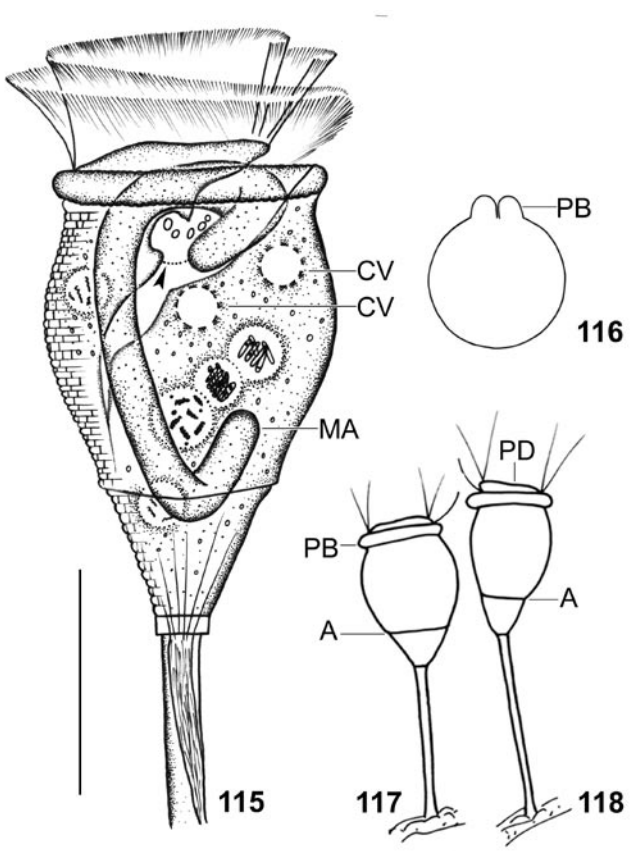
Table 6. Morphometric data on *Pseudovorticella bromelicola* nov. spec.

Characteristics [a]	Method	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	IV	67.3	67.5	4.4	1.4	6.6	60.0	75.0	10
Body, width	IV	41.1	41.5	3.8	1.2	9.3	35.0	45.0	10
Body length:width, ratio	IV	1.7	1.7	0.2	0.0	9.5	1.3	1.9	10
Silverlines from anterior end to AAW, number	KF	40.6	41.0	1.0	0.2	2.4	39.0	43.0	21
Silverlines from AAW to scopula, number	KF	13.1	13.0	1.0	0.2	8.0	11.0	15.0	21

[a] Data based on randomly selected specimens from a wheat grain culture. Measurements in µm. AAW – anlage of aboral ciliary wreath; CV – coefficient of variation in %; IV – *in vivo*; KF – Klein-Foissner "dry" silver nitrate impregnation; M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.

Cortex almost smooth *in vivo* as alveoli flat and without inclusions (Figs 115, 119–125). Silverline pattern reticulate, typical of genus (Figs 127–129). On average 41 transverse silverlines between anterior end and anlage of aboral ciliary wreath and 13 from there to scopula (Table 6). Anlage of aboral ciliary wreath a slight convexity or concavity at beginning of posterior third of body, comprises three or four narrowly spaced silverlines in Klein-Foissner silver nitrate preparations, particularly discernible at cell margin, otherwise appearing as a thick dark line (Figs 115, 117, 119, 120, 127–129).

Oral apparatus of usual structure. Peristomial bulge about 5 µm thick, slightly narrower to slightly wider than broadest body region. Peristomial disc projecting slightly from peristomial bulge, flattened to slightly concave. Vestibulum and cytopharynx spacious, extending obliquely to dorsal side and mid-body (Figs 115, 119–125).



Figs 115–118. *Pseudovorticella bromelicola* nov. spec. from life. **115** – lateral view of a representative specimen, length 65 µm. Note, *inter alia*, the longitudinally arranged, J-shaped macronucleus and the two contractile vacuoles on the ventral wall of the vestibulum. Arrowhead marks the cytoproct on the dorsal wall of the vestibulum; **116** – fully contracted specimen with the projecting peristomial bulge; **117**, **118** –shape variants. A – anlage of aboral ciliary wreath, CV – contractile vacuoles, MA – macronucleus, PB – peristomial bulge, PD – peristomial disc. Scale bar: 30 µm.

Occurrence and ecology: As yet found only at type locality.

Comparison with related species: Unfortunately, we lost the protargol slides, and thus the description is incomplete. However, the differences in the pattern of the contractile vacuoles and the number of silverlines are so distinct that *Pseudovorticella bromelicola* cannot be confused with congeners.

Of the *Pseudovorticella* species reviewed by Warren (1987) and ourselves, three congeners have two contractile vacuoles and a J-shaped macronucleus: *Pseudovorticella foissneri* Sun *et al*., 2007; *P. sphagni* Foissner and Schiffmann, 1974; and *P. monilata* (Tatem, 1870) Foissner and Schiffmann, 1974. *Pseudovorticella foissneri* is a marine species mainly characterized by its extremely flattened, asymmetrical body. *Pseudovorticella sphagni*, as described by Foissner and Schiffmann (1974) and Foissner (1979), differs from *P. bromelicola* by the location of the contractile vacuoles (one each on ventral and dorsal vestibular wall, checked in three populations; vs. both ventral) and the number of silverlines from the anterior end to the anlage of the aboral ciliary wreath (25–30 vs. 39–43) and from the anlage to the scopula (8–10 vs. 11–15). *Pseudovorticella monilata* is possibly most similar to *P. bromelicola* but differs distinctly in the number of silverlines from the anterior end to the anlage of the aboral ciliary wreath (15–23 vs. 39–43), while the number of silverlines from the anlage to the scopula is similar (9–18 vs. 11–15). These values are important because they are based on five populations of *P. monilata* (for a review, see Foissner *et al*. 1992). Further, the cortical alveoli are usually much more prominent in *P. monilata* than in *P. bromelicola* (see micrographs in Foissner *et al*. 1992).

Very recently, Song *et al.* (2009) described and redescribed 25 *Pseudovorticella* species from marine habitats in China. None resembles *P. bromelicola*.

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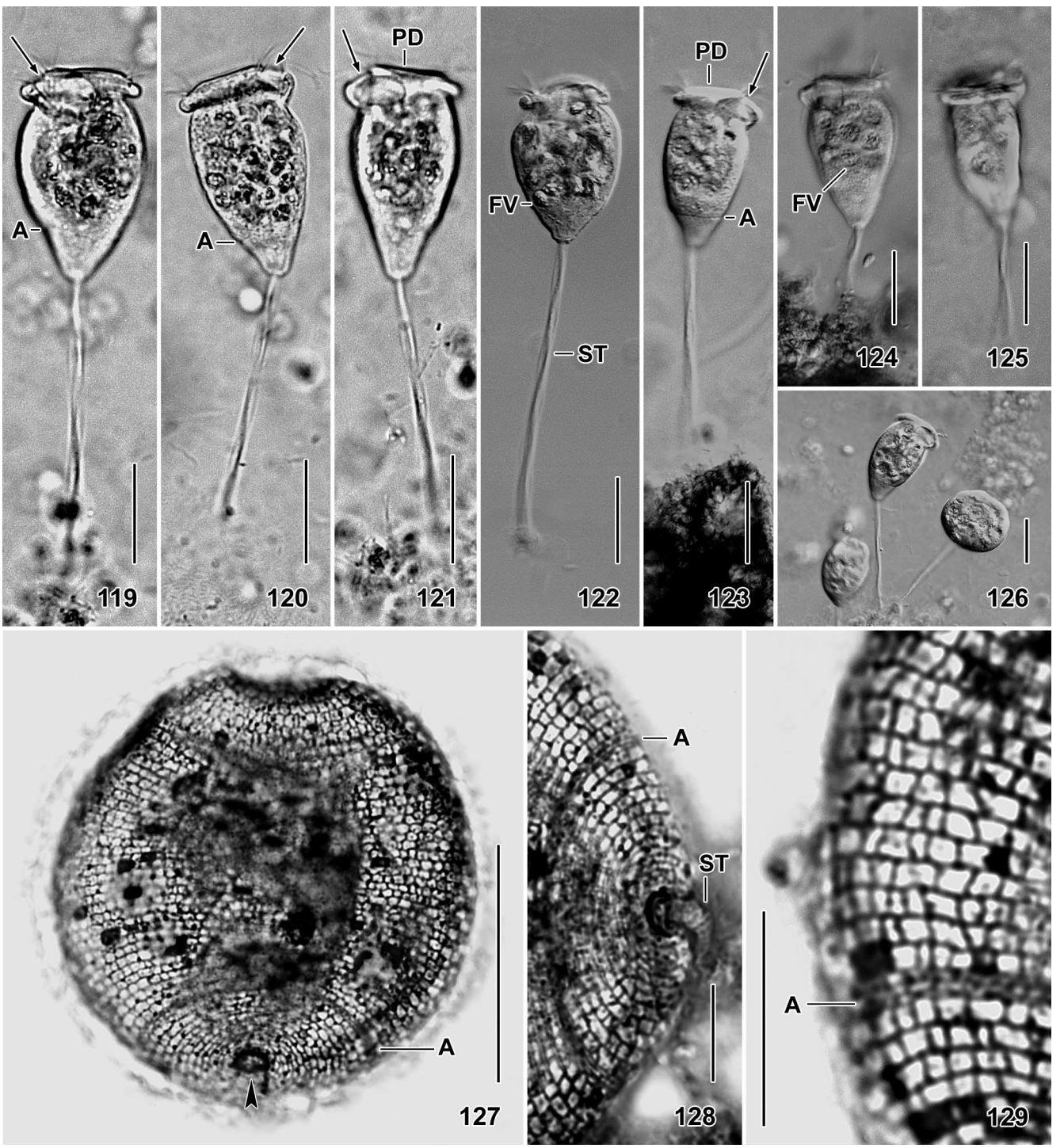

Figs 119–129. *Pseudovorticella bromelicola* nov. spec. from life (119–126) and after silver nitrate impregnation (127–129). **119–125** – extended, swirling specimens showing, *inter alia*, the considerable body shape variability, length 60–75 µm. The pellicle appears smooth because the cortical alveoli, whose margins contain the silverlines, are flat. Arrows mark vestibular entrance; **126** – extended (centre) and partially (left) and fully (right) contracted specimens; **127**, **128** – *Pseudovorticella bromelicola* has a reticulate silverline pattern with, on average, 41 silverlines from the anterior body end to the anlage of the aboral ciliary wreath and 13 silverlines from there to the scopula (arrowhead). These numbers and the two contractile vacuoles (Fig. 115) distinguish *P. bromelicola* from the congeners; **129** – higher magnification showing the anlage of the aboral ciliary wreath consisting of three narrowly spaced silverlines. Some meshes are filled with argyrophilic substance. A – anlage of aboral ciliary wreath (telotroch), FV – food vacuoles, PD – peristomial disc, ST – stalk. Scale bars: 10 µm (Figs 128, 129), 30 µm (119, 127) and 35 µm (120–126).

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