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Morphological and Molecular Characterization of a New Protist Family, Sandmanniellidae n. fam. (Ciliophora, Colpodea), with Description of *Sandmanniella terricola* n. g., n. sp. from the Chobe Floodplain in Botswana

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ABSTRACT. *Sandmanniella terricola* n. g., n. sp. was discovered in soil from the Chobe floodplain, Botswana, southern Africa. Its morphology and 18S rDNA gene sequence were studied with standard methods. *Sandmanniella terricola* is very likely an adversity strategist because it reaches peak abundances 6–12 h after rewetting the soil and maintains trophic food vacuoles with undigested bacteria in the resting cyst, a highly specific feature suggested as an indicator for an adversity life strategy. Possibly, the energy of the stored food vacuoles is used for reproduction and support of the cyst wall. Morphologically, *Sandmanniella terricola* is inconspicuous, having a size of only 50 × 40 μm and a simple, ellipsoidal shape. The main characteristics of the genus are a colpodid silverline pattern; a perioral cilia condensation; a flat, dish-shaped oral cavity, in the centre of which originates a long, conical oral basket resembling that of certain nassulid ciliates; and a vertically oriented left oral polykinetid composed of brick-shaped adoral organelles. This unique mixture of features and the gene sequence trees, where *Sandmanniella* shows an isolated position, suggest establishing a new family, the Sandmanniellidae n. fam., possibly related to the families Colpodidae or Bryophryidae. The curious oral basket provides some support for the hypothesis of a common ancestor of colpodid and nassulid ciliates.

Key Words. Biodiversity, nassulid ciliates, phylogeny, resting cyst, r-strategy, soil ciliates.

THE reviews of Foissner (1993), Foissner, Agatha, and Berger (2002), and Lynn (2008) show the colpodids to be one of the most well circumscribed ciliate classes. The Colpodea comprise nearly 200 valid species, living mostly in terrestrial habitats, where a highly specialized group of fungivorous taxa evolved (Foissner 1993). The 200 species are distributed over 66 genera (Foissner et al. 2002), rather many of which are monotypic, indicating a considerable pool of species waiting to be discovered. Indeed, the senior author holds over 20 undescribed species in his unpublished material. One of these is *Sandmanniella terricola* n. g., n. sp., a new monotypic genus from southern Africa.

The knowledge on the distribution of individual species is sparse and will be a major challenge for the future. Although a global census has been made on soil ciliates (Chao et al. 2006), many habitats and large regions of the Earth have never been carefully analysed for protists. Thus, Foissner, Chao, and Katz (2008) calculated that over 80% of the ciliate diversity is still undescribed.

The new ciliate described herein is from floodplain soil, an almost untouched, highly diverse global biotope possibly containing thousands of undescribed protist species. This is not a speculation: Foissner et al. (2008) discovered 137 undescribed ciliate species in 13 soil samples from just nine floodplain sites. Such figures are sustained by the floodplain sample that contained *S. terricola* n. g., n. sp.: we found 150 ciliate species, of which more than 40 were undescribed. Thus, we disagree with Finlay et al. (1996) and Finlay (2001) that global free-living ciliate diversity amounts to only about 3,000 species, and the majority of ciliates has been already described. Our doubts are corroborated by the simple fact that only a few specialists have provided excellent descriptions of nearly 300 new ciliate species between 1996 and 2008 (for reviews, see the *Zool. Record*).

MATERIALS AND METHODS

Materials. *Sandmanniella terricola* was discovered in soil from the Chobe River floodplain in the Chobe National Park, Botswana, southern Africa, 17°50′E 25°S. The sampling area was overgrown with grass only and is about 25 km west of the town of Kasane, i.e. on the so-called Kabolebole Peninsula of the Chobe Riverfront. The sample was collected in February 2001, when the river was almost desiccated. Small amounts of soil and litter from the upper 10-cm layer were collected from the riverbank and from elephant pits in the surroundings; these subsamples were mixed to a composite, filling a Petri dish 13 cm across and 2 cm high. The soil of the composite sample was wet, almost black, rather sandy, and very humic; it was air dried for 3 wk and then sealed in a plastic bag.

In August 2006, the sample was rewetted to set up a non-flooded Petri dish culture, as described by Foissner et al. (2002). The culture produced a huge number of species, several of which could not be studied in detail. Thus, the culture was air dried after 4 wk of investigation and rewetted again after some months of rest. This dry–wet cycle was repeated several times, until about 150 ciliate species had accumulated, including more than 40 undescribed taxa. Sterilized wheat straw was added to the sample after the third cycle to improve the nutrient situation and increase the abundance of the ciliates. *Sandmanniella terricola* n. g., n. sp. was recognized during the fifth dry–wet cycle in February 2008. Possibly, it remained unrecognized in the earlier cycles because it appears quite rapidly a few hours after rewetting and disappears 2 or 3 d later when, routinely, the first analysis of a non-flooded Petri dish culture is performed (Foissner et al. 2002).

Encystment was induced by transferring specimens into concave microscope slides either with Eau de Volvic or with centrifuged soil eluate from the non-flooded Petri dish culture. The preparations were stored in a wet chamber and observed every 2 d for 2 wk.

Morphological methods. Field material as obtained with the non-flooded Petri dish method was used for all investigations because several culture attempts failed. Living cells were studied using a high-power oil immersion objective and differential interference contrast microscopy. Preparations were performed as described in Foissner (1991). Counts and measurements on silvered specimens were conducted at a magnification of 1,000X. In vivo measurements were performed at magnifications of 100–1,000X. Illustrations of live specimens were based on free-hand sketches and micrographs, while those of prepared cells were made with a drawing device.

Molecular analyses. To extract genomic DNA for 18S rDNA phylogenies, about 10 specimens were picked with a micropipette and transferred into 180 μl ATL buffer (Qiagen, Hildesheim,

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Germany) and 20 µl Proteinase K (20 mg/ml) (Sigma, Taufkirchen/Munich, Germany). Subsequently, the genomic DNA was extracted using the protocol for cultured animal cells of the DNEasy Tissue Kit (Qiagen). 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 each 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 (ca 1.8 kb in case of the 18S 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. After an initial GenBank BLAST analysis, which identified *Bresslaua vorax* (accession number AF060453) as closest relative (95.8% sequence similarity), we aligned the 18S rDNA sequence to available colpodean sequences using CLUSTALX (Thompson et al. 1997). The alignments were manually refined in MacClade, according to conserved regions.

We first calculated phylogenetic trees on the class-level (Fig. 27). As the position of the new taxon under study within the order Colpodida was uncertain, we performed a second phylogenetic analysis on the order-level (Fig. 28). The reasoning for this is that an analysis with fewer taxa, which are relatively closely related to each other, allows for the use of a higher number of unmasked, unambiguously aligned nucleotide characters, possibly resulting in a better resolved and supported phylogeny. The class-level alignment included 1,506 positions, while the order-level in the alignment included 1,690 positions. We applied the program Modeltest (Posada and Crandall 1998) to choose the model of DNA substitution that best fits our data sets. The models suggested by the Akaike information criterion were TrN+I+G in case of the Colpodea phylogeny (Fig. 27) and GTR+I+G in case of the Colpodida phylogeny (Fig. 28).

Maximum parsimony and neighbour joining evolutionary distance (NJ) analyses were carried out in PAUP* v4.0b8 (Swofford 2002). Bayesian inference (BI) 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. The 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 used to improve the Markov Chain Monte Carlo sampling of the target distribution. We ran 2,000,000 generations and sampled every 1,000th generation, resulting in 2,001 samples from the posterior probability distribution. We assessed the relative stability of tree topologies using 1,000 bootstrap replicates and posterior probabilities of 1,501 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 1,000 replicates using RAxML with the setting as described in Stamatakis, Hoover, and Rougemont (2008). Maximum-likelihood and BI analyses were conducted online on the CIPRES Portal V 1.15 (http://www.phylo.org) The GenBank accession numbers of sequences used in our phylogenetic analyses are given in Table 1. All individual data sets are available from the authors.

Table **1.** Taxon sampling and GenBank accession numbers used in this study.

Taxon	GB#
Aristerostoma marinum	EU264562
Aristerostoma sp. ATCC 50986	EU264563
Bardeliella pulchra	EU039884
Bresslaua vorax	AF060453
Bresslauides discoideus	EU039885
Bryometopus pseudochilodon	EU039887
Bryometopus sphagni	AF060455
Bursaria sp. MSD-2007-1	EU039889
Bursaria sp. MSD-2007-2A	EU039890
Bursaria sp. MSD-2007-2B	EU039891
Bursaria truncatella	U82204
Chain-forming colpodid	AY398684
Colpoda aspera	EU039892
Colpoda cucullus	EU039893
Colpoda inflata	M97908
Colpoda steinii strain SP1	DQ388599
Colpoda henneguyi	EU039894
Colpoda lucida	EU039895
Colpoda magna	EU039896
Colpoda minima	EU039897
Colpoda sp. PRA 118	AY905498
Cyrtolophosis mucicola Austria	EU039899
Cyrtolophosis mucicola Brazil	EU039898
Hausmanniella discoidea	EU039900
Ilsiella palustris	EU039901
Mykophagophrys terricola	EU039902
Notoxoma parabryophryides	EU039903
Ottowphrya dragescoi	EU039904
Platyophrya sp. MSD-2007	EU039905
Platyophrya bromelicola	EU039906
Platyophrya vorax	AF060454
Pseudoplatyophrya nana	AF060452
Pseudocyrtolophosis alpestris	EU264564
Rostrophrya sp.	EU039907
Sagittaria sp.	EU039908
Sorogena stoianovitchae	AF300285
Sandmanniella terricola	**FJ610254**
Outgroup	
Anophryoides haemophila	U51554
Furgasonia blochmanni	X65150
Obertrumia georgiana	X65149
Anoplophrya marylandensis	AY547546

The newly sequenced taxon is in bold.

Terminology. Terminology is according to Corliss (1979) and Foissner (1993); for an update, see Lynn (2008). We propose the new term ''perioral cilia condensation'', which designates small but distinct accumulations of somatic kinetids in the surroundings of the oral apparatus. The tightly spaced, ciliated kinetids, which can be within or at the ends of one or several somatic ciliary rows (Fig. 2, 6, 8, 9), produce membrane-like structures likely supporting food acquisition.

RESULTS

Description of *Sandmanniella terricola* n. g., n. sp. (Table 2 and Fig. 1–7, 10–26). The body length and width are highly variable in vivo and in preparations, ranging from 35–85 × 25–70 µm,

Table 2. Morphometric data on *Sandmanniella terricola*.

Characteristics[a]	Method	Mean	M	SD	CV	Min	Max	n
Body, length[b]	CH	60.5	59.0	8.4	13.8	44.0	76.0	15
Body, width[b]	CH	52.2	50.0	7.7	14.7	40.0	65.0	15
Body, length[c]	P	40.8	42.0	3.6	8.9	30.0	46.0	21
Body, width[c]	P	30.6	30.0	3.2	10.4	25.0	40.0	21
Body, length[b]	OS	49.4	50.0	3.4	6.9	40.0	55.0	14
Body, width[b]	OS	38.5	40.0	3.7	9.7	30.0	45.0	14
Anterior body end to left oral polykinetid, distance	CH	11.9	12.0	3.1	25.8	6.0	18.0	15
Anterior body end to proximal end of PC, distance	CH	21.6	21.0	2.3	10.8	18.0	26.0	15
Oral area, length (left adoral organelle to end of PC)	CH	11.0	11.0	1.4	12.4	9.0	14.0	15
Oral area, width (right margin of ROP to left margin of LOP)	CH	9.4	9.0	1.0	10.5	8.0	11.0	15
Oral basket, width at distal end	P	1.3	1.3	0.2	17.5	1.0	1.8	21
Anterior body end to macronucleus, distance	P	17.2	17.0	4.5	26.1	7.0	30.0	21
Macronucleus, length[b]	P	7.4	7.0	0.7	10.0	6.0	9.0	21
Macronucleus, width[b]	P	7.0	7.0	0.7	10.4	6.0	9.0	21
Macronucleus, length[c]	P	3.5	3.5	—	—	3.0	4.0	21
Macronucleus, width[c]	P	2.8	3.0	—	—	2.0	3.0	21
Ciliary rows, number	CH, SC	13.9	14.0	0.7	5.3	13.0	15.0	21
Kinetids, number in third row right of oral apparatus	CH, SC	20.0	20.0	2.9	14.3	16.0	26.0	21
Kinetids, number composing the PC	SC	12.7	12.0	1.1	8.8	10.0	15.0	21

[a]Data based on wet (SC, OS) or mounted (CH, P), and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm.

[b]From culture rewetted 24 h before.

[c]From same culture, but rewetted only 12 h.

CH, silver nitrate after Chatton Lwoff; CV, coefficient of variation in %; LOP, left oral polykinetid; M, median; Max, maximum; Min, minimum; n, number of specimens investigated; OS, fixed with osmium vapours; P, protargol; PC, perioral cilia condensation; ROP, right oral polykinetid; SC, silver carbonate; SD, standard deviation.

depending on the age of the culture and the nutrition state. The smallest specimens occur a few hours after rewetting of the sample when the newly excysted cells are not yet packed with food vacuoles. When fixed 12 h after rewetting of the sample, the specimens are about one-third smaller than those fixed after 24 h (Table 2), although they are packed with food vacuoles at both times, suggesting considerable growth during the life cycle. In vivo, ''young'' specimens are about 45 × 33 μm in size, while ''old'' cells measure about 65 × 55 μm. The shape of *S. terricola* is broadly ellipsoidal with the ventral side more or less distinctly flattened and the anterior or posterior region slightly widened; it is not flattened laterally and not indented in the oral area (Fig. 1, 2, 4, 7, 12–14, 17, 18).

The location of the nuclear apparatus is highly variable (CV 26.1%; Table 2), possibly because it is sometimes displaced by the large food vacuoles; on average it is in or near to the body centre. The macronucleus is globular to slightly ellipsoidal, in vivo about 10 μm across, and contains many small, pale structures, possibly nucleoli (Table 2; Fig. 1–3, 7, 14, 17, 19, 26). The hemispherical micronucleus is attached to the macronucleus and is comparatively large, about 4–5 μm in vivo; it impregnates only lightly with silver carbonate and protargol (Table 2; Fig. 1, 2, 26).

The contractile vacuole is in the rear body end and has a single excretory pore in the pole centre (Fig. 1, 14, 21). The cortex, which is very flexible and of ordinary thickness, contains colourless granules, possibly mucocysts, arranged in the same pattern as the silverlines (Fig. 10, 11, 12, 15); the individual granules are about 1 × 0.5 μm in size and become extruded but do not stain when methyl-green pyronin is applied. The colourless cytoplasm is usually packed with food vacuoles 2–10 μm (most 4–7 μm) across and some up to 3-μm-sized lipid droplets. The food vacuoles are compact, containing rather regularly arranged bacterial rods (Fig. 1, 14, 16, 17).

Sandmanniella terricola n. g., n. sp. swims rather rapidly rotating about the main body axis. Its somatic cilia are about 10 μm long in vivo and are arranged in an average of 14 equidistant rows extending sigmoidally from right anterior to left posterior end of body, leaving blank (i) a short, slightly curved region preorally; (ii) the oral area, which produces four postoral kineties; and (iii) the surroundings of the excretory pore of the contractile vacuole (Table 2; Fig. 3, 6, 21, 22). The ciliary rows are composed of dikinetids much more narrowly spaced anteriorly than posteriorly, especially on the right side; both basal bodies are ciliated in the oral third of the body, while only the posterior basal body is ciliated postorally (Table 2, Fig. 1–6, 18–23). The perioral cilia condensation, which is composed of an average of 13 very narrowly spaced dikinetids just posterior to the oral region, occupies the anterior portion of the first postoral kinety (Table 2; Fig. 1–4; 6, 18, 19, 22, 25).

The silverline pattern is colpodid, consisting of comparatively large, rectangular meshes connecting all somatic and oral ciliary structures (Foissner 1993); the bottom of the flat oral cavity contains a diaphragm-like silverline pattern at the centre of which is the opening of the oral basket. The excretory pore of the contractile vacuole is surrounded by very small silverline meshes (Fig. 4, 5, 18, 20, 21).

The oral apparatus is located subapically, occupying about 18% of body length; the high variability (CV 25.8%; Table 2) in the distance from the anterior body end to the left oral ciliary field is partially caused by the proper orientation of the cell (flat vs. more or less oblique). The oral region is a very flat, dish-shaped concavity whose posterior half is covered over by the somatic cortex to form a very hyaline lip, which is difficult to recognize (Fig. 1, 7, 12, 17). The crescentic right ciliary field, which is upright when the cell is viewed ventrally, is composed of four curved kineties having cilia about 3 μm long (Table 2; Fig. 1–3, 6, 13, 17, 18, 22, 24, 25). The innermost kinety with respect to the centre of the oral region is the longest, composed of narrowly spaced dikinetids, while the outermost kinety is slightly shorter and composed of monokinetids; the middle two kineties are distinctly shortened and composed of slightly disordered monokinetids. The ribbon-like left ciliary field, which is obliquely (∼ 45°) attached to the wall of the oral cavity, is a series of closely spaced polykinetids composed of aligned dikinetids. It is bipartited in an anterior and a posterior portion, both having cilia about 5 μm long forming scintillating bundles (Fig. 1, 12–14). The anterior portion is composed of two slightly oblique rows each comprising four to six cilia. The posterior portion is much longer than the anterior and composed of about 12 short, possibly paired kineties forming a conspicuous, slightly curved ribbon (Table 2; Fig. 1–4, 6, 18, 19, 22, 25).

Reproduction. There was not a single divider among about 1,000 specimens seen in the protargol slides. Thus, *S. terricola* very likely divides in reproductive cysts, as common in many colpodids (Foissner 1993).

Molecular sequencing. The 18S rDNA sequence of *S. terricola* n. g., n. sp. is 1,784 bp long and available under GenBank

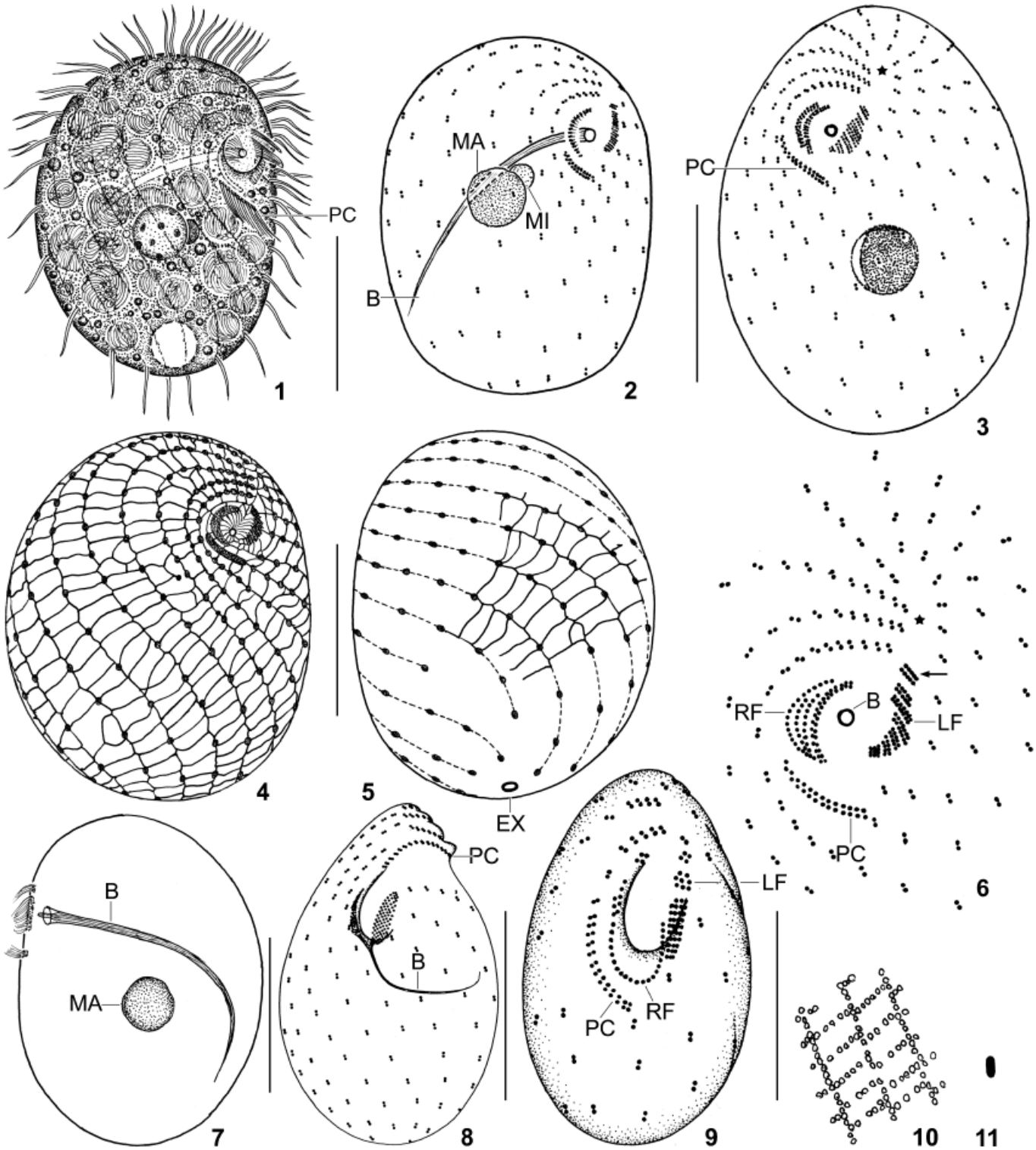

Fig. **1–11.** *Sandmanniella terricola* n. g., n. sp. (1–7, 10, 11), *Apocolpoda africana* (8; from Foissner 1993), and *Pseudokreyella australis* (9; from Foissner 1993) from life (1, 10, 11) and after protargol (2, 3, 7, 8), silver nitrate (4, 5), and silver carbonate (6, 9) impregnation. **1.** Right side view of a representative specimen, length 60 µm. **2, 3, 6.** Somatic and oral (6) ciliary pattern of right side (2) and ventral view of holotype specimen (3; length 47 µm). Asterisks mark preoral suture; arrow (6) denotes the first adoral polykinetid slightly set off from the others. **4, 5.** Ciliary and silverline pattern of right and left side. **7.** Optical section showing the long oral basket. **8, 9.** *Apocolpoda* has an oralized somatic membranoid (M) along the upper mouth margin, while the membranoid of *Pseudokreyella* extends along the right posterior mouth margin, just as in *S. terricola* (3). **10.** Surface view showing mucocyst pattern. **11.** Mucocyst, about 1 × 0.5 µm. B, oral basket; EX, excretory pore; LF, left oral ciliary field (polykinetid); MA, macronucleus; MI, micronucleus; PC, perioral cilia condensation; RF, right oral ciliary field (polykinetid). Scale bars 30 µm (Fig. 1), 20 µm (Fig. 2–5, 7, 8), and 15 µm (Fig. 9).

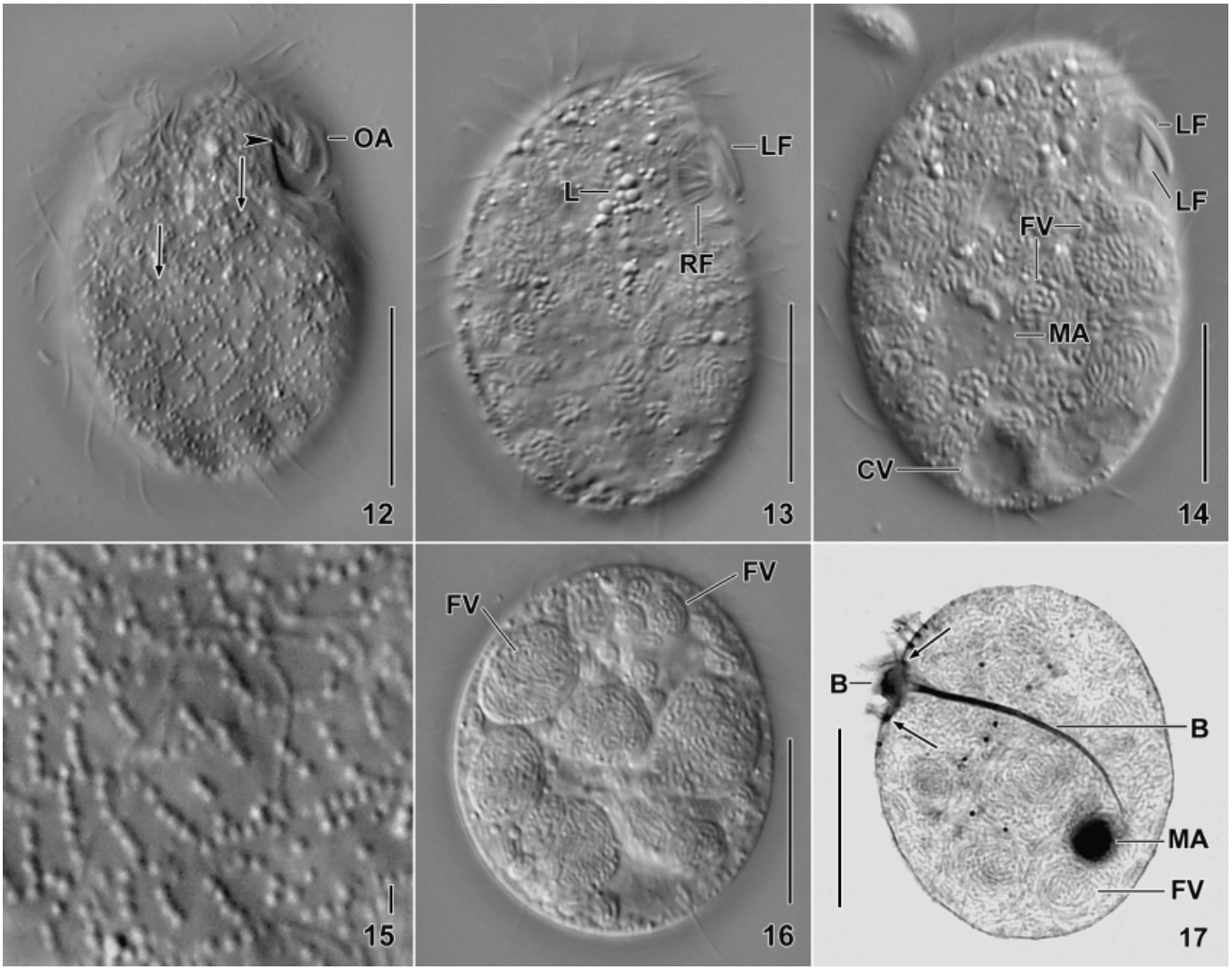

Fig. **12–17.** *Sandmanniella terricola* n. g., n. sp. from life (12–16) and after protargol impregnation (17). **12–14.** Surface view (12) and optical sections (13, 14), showing the hyaline lip covering the posterior half of the oral opening (12; arrowhead), the mucocyst pattern (12; arrows), and the oral ciliary fields (13, 14; LF, RF). **15.** Surface view showing the mucocysts in a slightly flattened specimen. **16.** A slightly flattened specimen filled with small and large food vacuoles (up to 15 μm) containing tightly packed bacterial rods. **17.** Optical section showing the long, conical oral basket. Arrows delimit the flat oral region. B, oral basket; CV, contractile vacuole; FV, food vacuoles; L, lipid droplets; LF, left oral ciliary field; MA, macronucleus; OA, oral apparatus; RF, right oral ciliary field. Scale bars 20 μm (Fig. 12–14, 16, 17), and 1 μm (Fig. 15).

accession number FJ610254. All other sequences used are also from this repository. In both BI and evolutionary distance analyses, the 18S rDNA sequence of *S. terricola* branches with significant support in the order Colpodida (Fig. 27). However, none of the analyses is able to assign *S. terricola* to a described and sequenced family within this order. While the position of *S. terricola* remains unresolved in the NJ evoultionary distance analysis, BI suggests an ancestral position to the family Colpodidae (Fig. 27). This basal position is supported in the order-level phylogeny including only colpodean sequences (Fig. 28). Here, the Sandmaniellidae appears as sister to the family Bryophryidae with significant support from BI and NJ analyses.

Resting cyst (Fig. 29–35). All specimens set up with soil eluate formed resting cysts within 12 h, while cells died in Eau de Volvic. The resting cysts were globular and almost as large as trophic specimens because they still contained the large food vacuoles of the trophic cells: 40 ± 0.9 μm (35–47; n = 12). The cyst wall was inconspicuous, about 2 μm thick, and possibly tripartite: the external layer was slimy and most distinct in old cysts, where organic and inorganic debris and bacteria adhered (Fig. 30, 32, 35); the two internal layers, which were separated by a very narrow, bright zone, appeared structureless and yellowish (Fig. 29–31, 34); the innermost layer became conspicuously thick in old cysts (Fig. 32, 35), indicating that part of the energy contained in the stored food vacuoles, which had mostly disappeared by this time, was likely used to support the cyst wall.

In young, 10–20-hour-old cyst, where the contractile vacuole was still active and the cells rotated slowly within the cyst, the cytoplasm was packed with food vacuoles appearing like those of the trophic specimens (Fig. 29–31, 33, 34). Although the number of such vacuoles decreased, there were still several of them present after 2 wk. Some of these vacuoles were obviously digested during the first week, where 10–15-μm-sized vacuoles with fluffy contents appeared (Fig. 29). The cytoplasm also contained fine granules, minute, sparkling crystals (?), and lipid droplets up to 3 μm across.

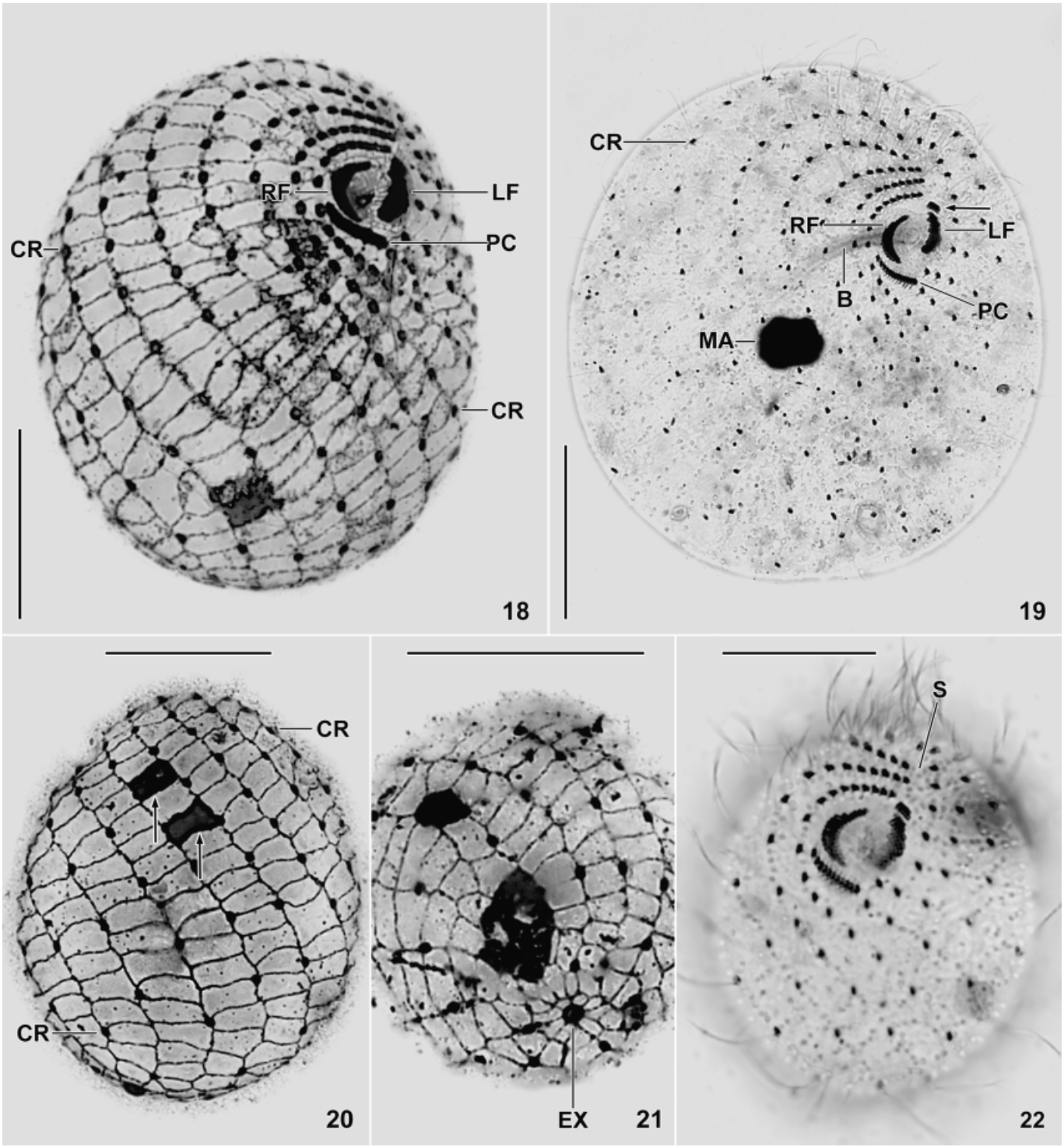

Fig. **18–22.** *Sandmanniella terricola* n. g., n. sp., somatic and oral ciliary and silverline pattern after silver nitrate (18, 20, 21) and silver carbonate (19, 22) impregnation. **18.** Ventrolateral view of the colpodid silverline pattern. The opening of the oral funnel, which is between the right and left oral ciliary fields, is surrounded by a diaphragm-like silverline pattern. The oral ciliary fields and the oralized somatic membranoid form a characteristic pattern typical for the new genus. **19, 22.** Ventral views showing the somatic and oral ciliary pattern. The arrow marks a brick-shaped adoral polykinetid slightly set off from the ribbon-shaped portion of the left ciliary field. **20.** Silverline pattern of left side. Arrows mark two meshes filled with argyrophilic substance. **21.** Posterior polar view showing the excretory pore of the contractile vacuole in the pole centre. CR, ciliary rows; EX, excretory pore; LF, left oral ciliary field; MA, macronucleus; PC, perioral cilia condensation; RF, right oral ciliary field; S, preoral suture. Scale bars 20 μm.

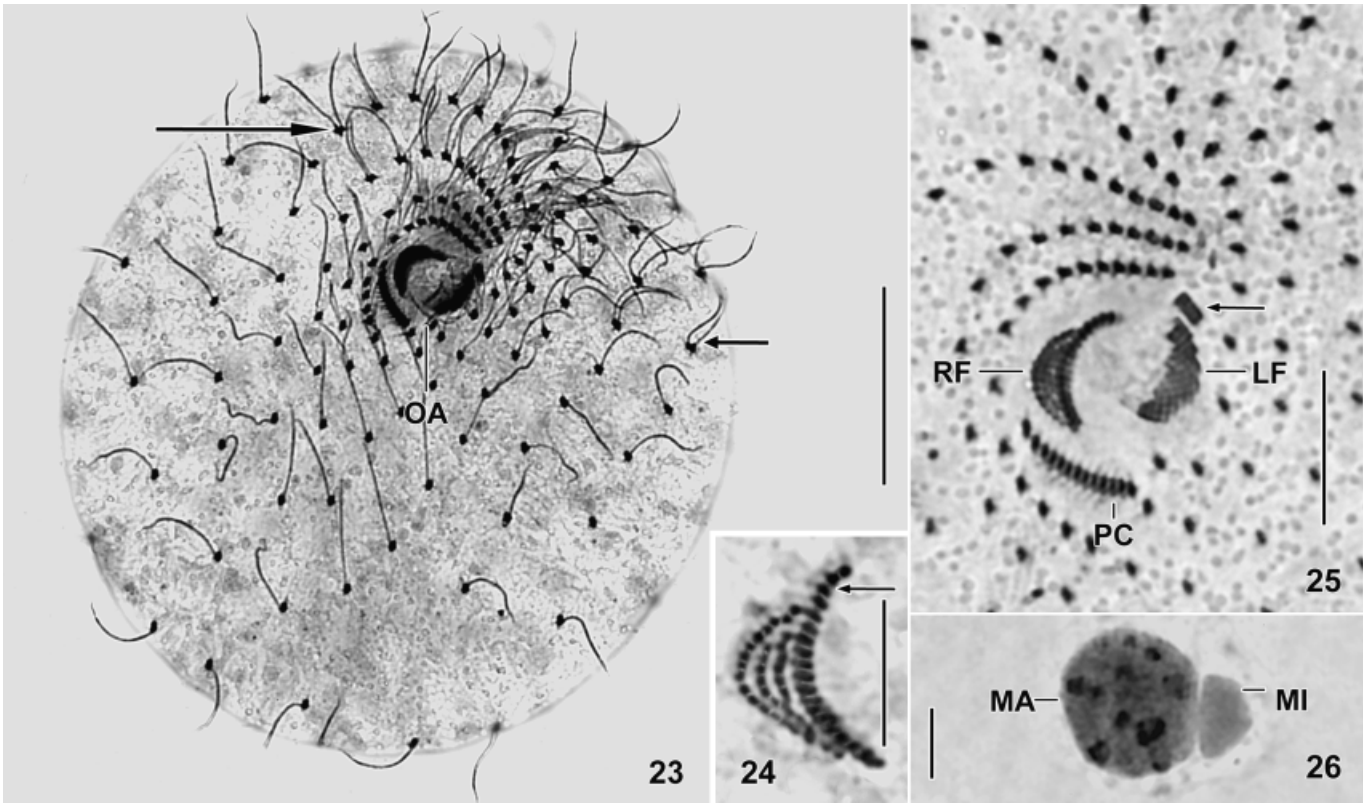

Fig. **23–26.** *Sandmanniella terricola* n. g., n. sp. after silver carbonate impregnation. **23.** A specimen strongly flattened by coverslip pressure, showing paired cilia at the oral portion of the body (arrows). **24.** The right oral ciliary field consists of four kineties, of which the inner kinety is a file of dikinetids (arrow). **25.** Oral ciliary pattern. The arrow marks a brick-shaped adoral polykinetid slightly anterior of the ribbon-shaped posterior portion of the left ciliary field, which is usually composed of six closely associated polykinetids. Note the curious perioral cilia condensation composed of the very tightly spaced dikinetids. **26.** Nuclear apparatus. The hemispherical micronucleus is only lightly impregnated. LF, left oral ciliary field; MA, macronucleus; MI, micronucleus; OA, oral apparatus; PC, perioral cilia condensation; RF, right oral ciliary field. Scale bars 20 μm (Fig. 23), 10 μm (Fig. 24, 25), and 5 μm (Fig. 26).

Occurrence and ecology. *Sandmanniella terricola* n. g., n. sp. appeared a few hours after rewetting the sample, reached moderate abundances after about 30 h, and disappeared 2–3 d later. We repeated the dry–wet cycle 3 times within 1 yr. The outcome was the same.

As yet, we have found *S. terricola* only at its type locality, although we investigated many soil samples from floodplains globally. However, *S. terricola* is a small, inconspicuous ciliate. Thus, we cannot exclude the possibility to have overlooked it in some samples.

DISCUSSION

Suprafamiliar classification. Using the features established by Foissner (1993), the new ciliate belongs to the class Colpodea: it has somatic dikinetids; a colpodid silverline pattern; a right and a left oral polykinetid; and is terricolous. It can be placed in the order Colpodida based on the subapical location of its oral apparatus, the right oral polykinetid composed of a row of dikinetids accompanied by several more or less disordered rows of monokinetids, the macronucleus and micronucleus each with separate nuclear membrane, the cell division presumably occurring in reproductive cysts, and its molecular genetic affinities.

The molecular analyses of the class Colpodea (Fig. 27) basically match those of Breiner, Foissner, and Stoeck (2008) and of Dunthorn, Foissner, and Katz (2008), who included nassulids in the analyses, obtaining a weakly supported relationship of colpodid and nassulid ciliates. Such relationship is supported by *Sandmanniella* n. g., the oral basket of which resembles those of several small nassulids, such as *Furgasonia* and *Wolfkosia*, both described in Foissner et al. (2002). However, electron microscopical investigations are required to substantiate or refuse this similarity.

Family classification. We could not unequivocally assign *Sandmanniella* n. g. to one of the existing colpodid families because it has oral features in a unique combination: a long, conical oral basket originating in the centre of a very flat oral region; a typical colpodid right oral polykinetid; a left oral polykinetid composed of minute, brick-shaped adoral polykinetids oriented in the long axis of the body; and a perioral cilia condensation. These features are discussed in the following paragraphs.

(i) Although several colpodids have a rather distinct oral basket, which arises from the proximal end of the oral polykinetids (Foissner 1993), it very rarely originates between the oral ciliary fields, i.e. in the centre of the oral region. The only known exceptions are the grossglockneriid colpodids, a highly specialized group of fungivores that have evolved a feeding tube in the centre of a very flat oral region (Foissner 1993). Both features resemble the situation in *Sandmanniella*. Thus, we cannot exclude the possibility that *S. terricola* is a secondarily modified grossglockneriid colpodid, although the molecular genetic data do not support such relationship.

(ii) The right oral polykinetid of *S. terricola* matches perfectly that of various small *Colpoda* species (Foissner 1993; Foissner et al. 2002; Hofmann-Münz 1991; note that the dikinetidal row has been overlooked in many old descriptions). This is interpreted

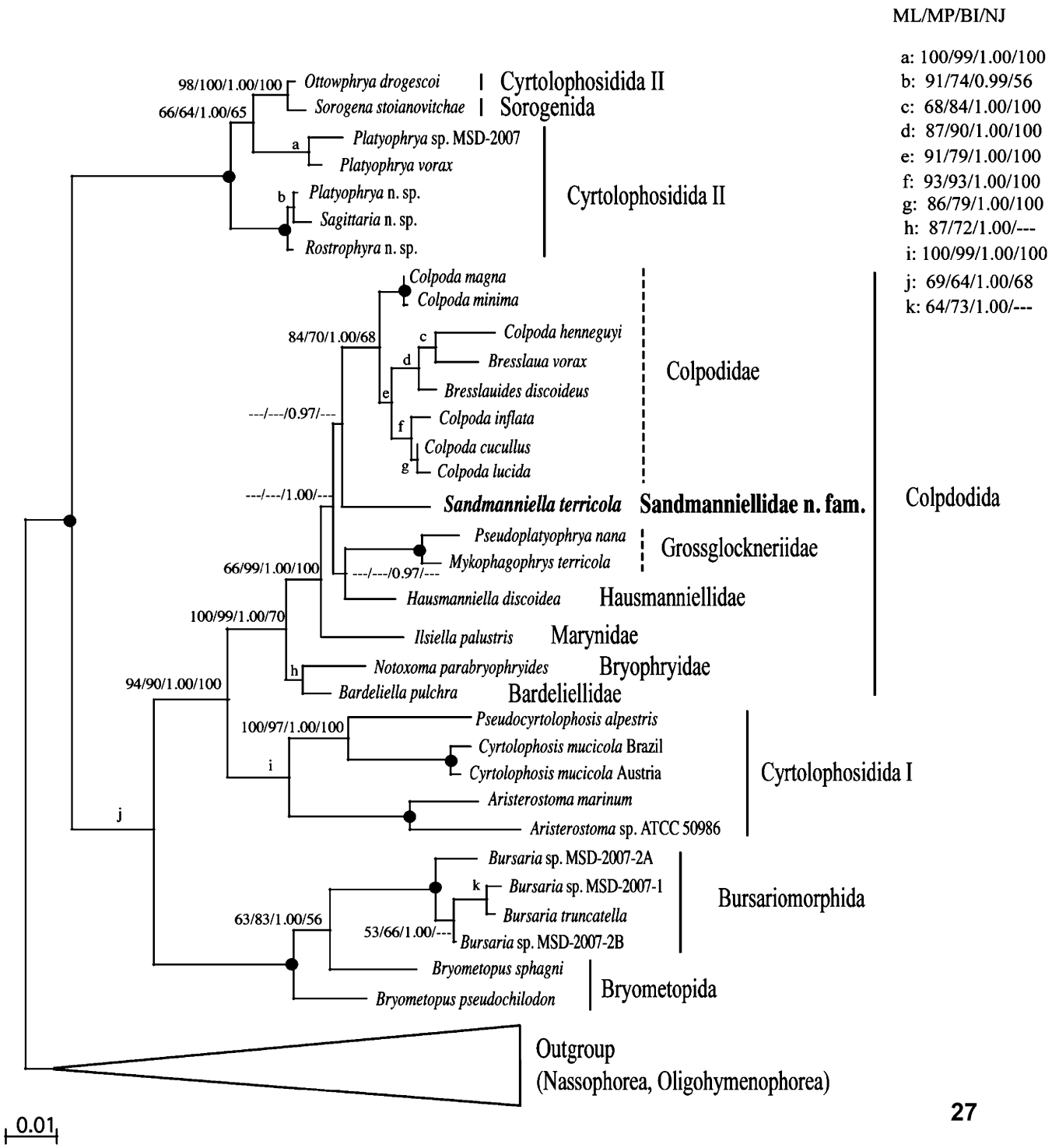

Fig. **27.** A phylogenetic tree on class-level of 18S rDNA sequences derived by Bayesian inference and showing the position of *Sandmanniella terricola* n. g., n. sp. The tree was constructed by using a GTR+I+G DNA substitution model with the variable-site gamma distribution shape parameter (G) as suggested by Mr. Modeltest based on 1,506 unambiguously aligned positions. Support values are given at the respective nodes. Two oligohymenophorean and two nassophorean sequences were chosen as outgroup. Black dots indicate full support in all analyses. The log-likelihood of the best-scoring tree is −8,776. Dashed lines indicate family clades within the order Colpodida and solid lines mark orders. The GenBank accession numbers of sequences used in the phylogenetic analyses are given in Table 1. BI, Bayesian inference; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour joining.

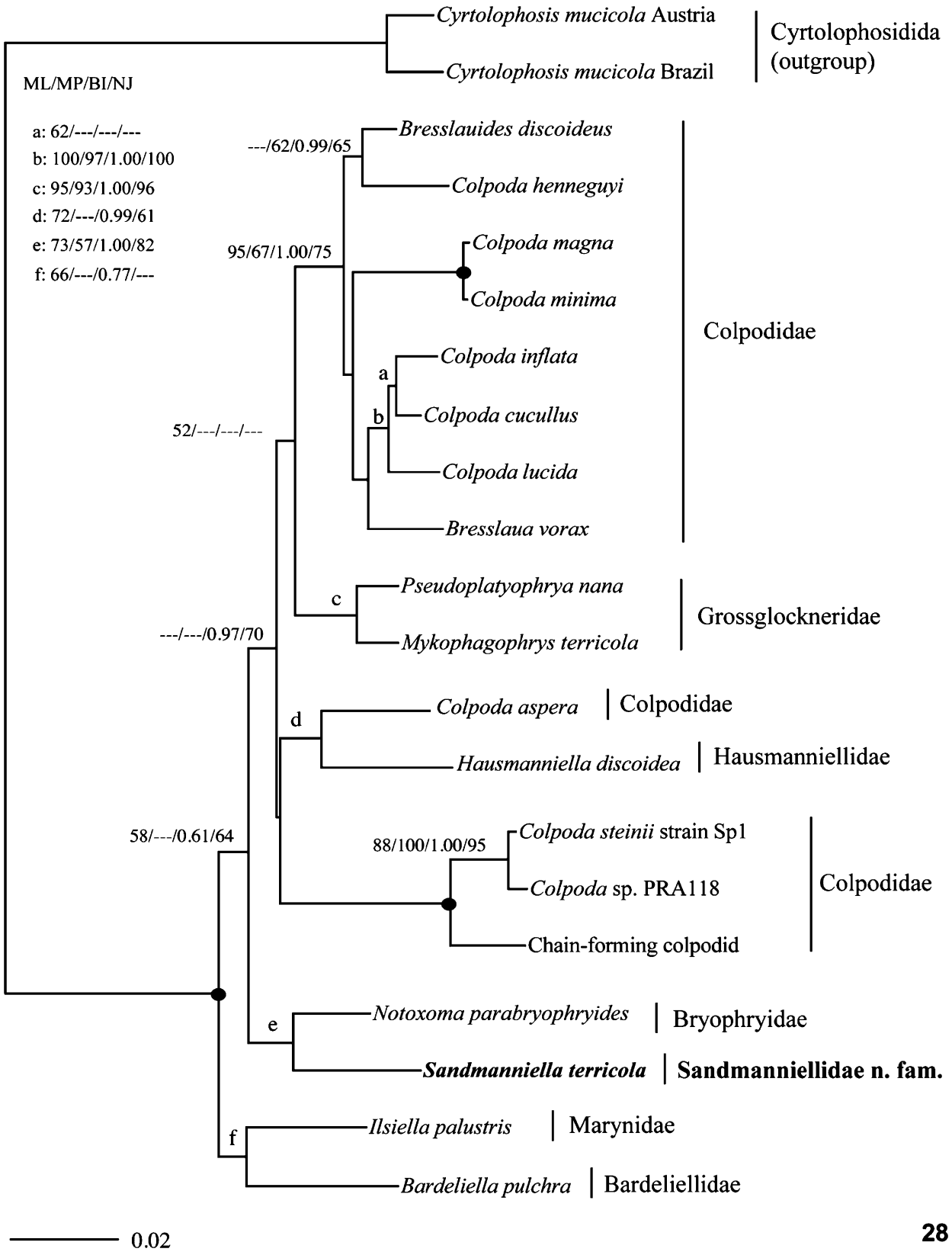

Fig. **28.** Best-scoring maximum likelihood phylogenetic tree of colpodean 18S rDNA sequences showing the position of *Sandmanniella terricola* n. g., n. sp. Support values are given at the respective nodes. Two sequences of *Cyrtolophosis mucicola* were chosen as outgroup. Black dots indicate full support in all analyses. The log-likelihood of the best-scoring tree is −5,573. The GenBank accession numbers of sequences used in the phylogenetic analyses are given in Table 1. BI, Bayesian inference; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour joining.

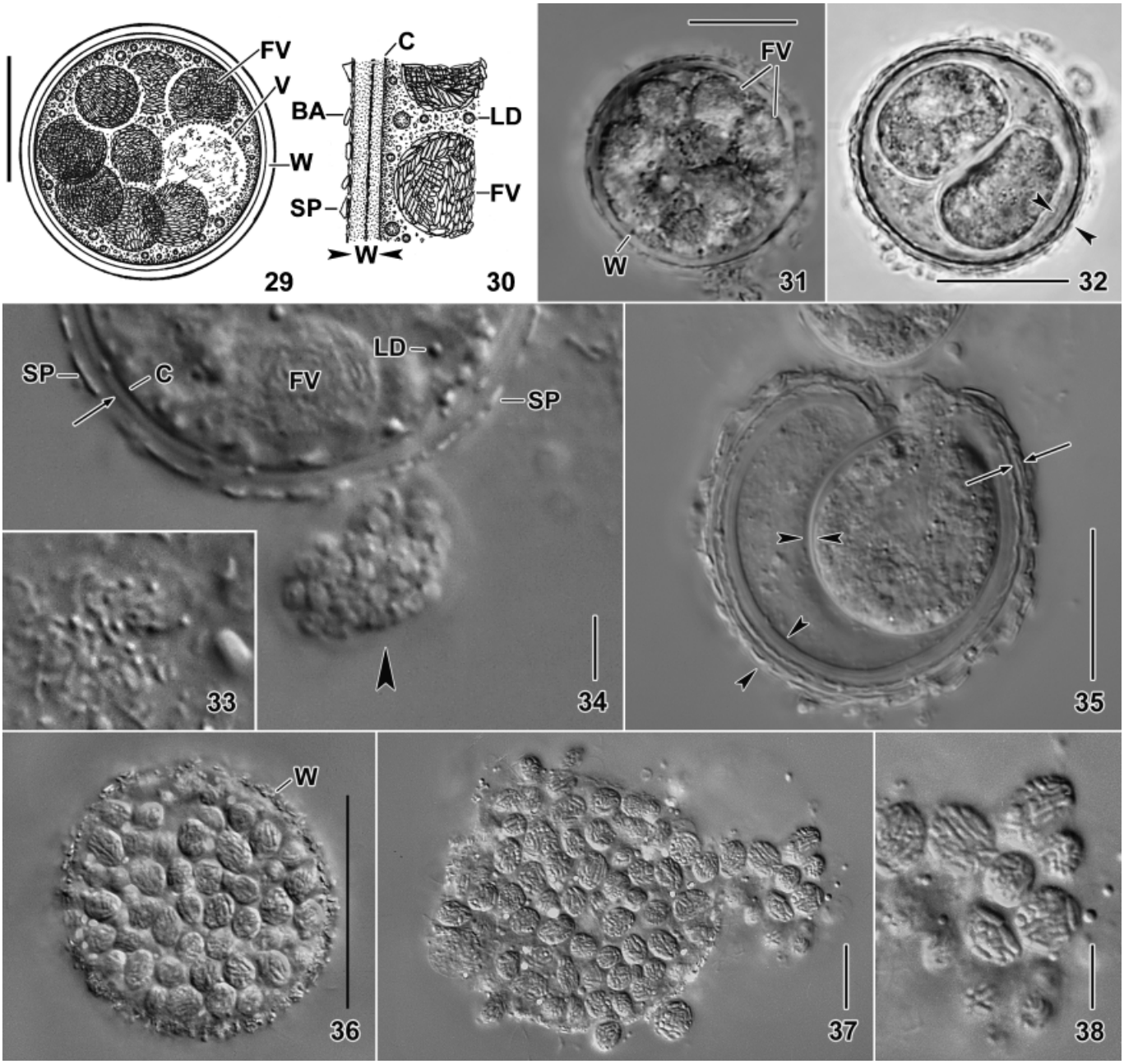

Fig. **29–38.** *Sandmanniella terricola* n. g., n. sp. (29–35) and *Pseudomaryna australiensis* (35–37), resting cysts from life. **29–31, 33, 34.** Overviews (29, 31) and details (30, 33, 34) of 4-d-old cysts. The cyst wall is about 2 µm thick and consists of two hyaline layers separated by a slightly more refractive sheet (29, 30, 34 arrow). The cysts contain many large, compact globules (29, 31) composed of densely packed bacteria, which become evident when the globules are pressed out of the cyst (30, 33, 34 arrowhead). One of the bacterial globules has been digested leaving a large, bright vacuole with fuzzy contents (29). **32, 35.** A 7-d-old cyst, which divided within the cyst. When one of the divided cells was pressed out by the coverslip, a 4-µm-thick outer wall (delimited by arrowheads) and a thinner wall ($\sim$ 1.5 µm) surrounding each divided cell became visible. The thick inner layer of the outer cyst wall is hardly recognizable in the bright field microscope (32, arrowheads). Arrows mark a mucous layer becoming distinct mainly in old cysts. The bacterial globules have been digested (cf. Fig. 30). **36–38.** Micrographs from the population of *P. australiensis* studied by Foissner (2003). The about 1.5-µm-thick cyst wall is, like the trophic cell, covered by inorganic soil particles. The cyst's contents consists of many about 5-µm-sized globules (36). When the globules are pressed out of the cyst (37), it becomes evident that they consist of densely packed bacteria (38). BA, bacteria; C, ciliate cortex; FV, food vacuoles; LD, lipid droplets; SP, soil particles; V, vacuole; W, cyst wall. Scale bars 20 µm (Fig. 29, 31, 32, 35, 36), 10 µm (Fig. 37), and 5 µm (Fig. 34, 38).

as a strong indicator for classifying *Sandmanniella* in or near to the family Colpodidae.

(iii, iv) A left oral ciliary field composed of minute, brick-shaped adoral polykinetids is widespread in cyrtolophosidid and bryometopid colpodids, for instance, in *Cyrtolophosis mucicola* and *Pseudokreyella australis* (Fig. 9). However, it occurs also in *Corticocolpoda*, a large member of the Colpodidae (Foissner 1993). Typically, members of the family Colpodidae have ori-

ented the left oral polykinetid transverse to the main body axis (Foissner 1993). However, *Apocolpoda* (Fig. 8) and *Bardeliella* have the left ciliary field oriented parallel to the main body axis, just like *Sandmanniella*. Obviously, the structure and orientation of the left oral polykinetid are problematic features, very likely having evolved independently several times.

(v) The perioral cilia condensation is a conspicuous feature of *S. terricola*. Again, this character is not unique but occurs in several, possibly only distantly related genera, for instance in *Platyophrya* (where it is called postoral pseudomembrane) and the bryometopid colpodean *Pseudokreyella* (Fig. 9). Nonetheless, when this feature is combined with the structure and orientation of the left oral polykinetid, a rather close relationship of *Sandmanniella* and bryometopid colpodids cannot be excluded (Fig. 6, 9).

The molecular phylogenetic analyses within the class Colpodea (Fig. 28) confirm the isolated position of *Sandmanniella* and relate it to the Bryophryidae, while the class-level phylogenies indicate a basal position to the Colpodidae (Fig. 27). The latter is strongly supported by the morphological investigations, but the former cannot be excluded because the Bryophryidae also contain genera with a flat oral region and brick-shaped adoral polykinetids (for a review, see Foissner 1993). However, they have a different (platyophryid) silverline pattern, a semicircular right oral ciliary field, and an inconspicuous oral basket.

Considering the mixture of morphological features and the isolated position in the molecular trees, we classify *Sandmanniella* in a new family: Sandmanniellidae n. fam. Possibly, *Sandmanniella* is a modified grossglockneriid or bryophryid, as explained above. The former is not unlikely because the grossglockneriids are close relatives of the Colpodidae based on gene sequence data (Fig. 27; Dunthorn et al. 2008).

***Sandmanniella terricola* as a new genus and species.** We could not assign the ciliate population from the Chobe River floodplain to any described ciliate genus or species. Thus, it is classified as an undescribed genus and species.

***Sandmanniella terricola*, an adversity strategist.** Adversity strategists are strongly r-selected organisms (Smith 1978). We believe that *S. terricola* matches this pattern: it appears very soon after rewetting the soil and disappears 1–3 d later when competition with other organisms presumably increases. However, the most convincing argument is the resting cyst, which preserves part of the food vacuoles. We propose that this allows the ciliate not only to divide within the cyst, as do many other colpodids (Foissner 1993) but also to become active in very ''strong'' condition when the environment is advantageous. Further, the ciliate can build part of the cyst wall during disadvantageous periods not wasting time to be active as long as environmental conditions are optimal. Certainly, such strategy is very useful in floodplains where the environmental conditions may vary quite rapidly.

Preservation of food vacuoles in resting cysts has not been described before. However, Foissner (2003) mentioned and illustrated in *Pseudomaryna australiensis*, another floodplain colpodid that the cyst contains ''many opaque globules 1–5 μm across''. A reinvestigation of the original micrographs showed these globules to be composed of bacteria (Fig. 35–37), just like those in *S. terricola* (Fig. 30–33).

Both, *S. terricola* and *P. australiensis* appear well adapted to the flood-pulses typical for floodplain environments. As concerns the colpodids, many of them are r-strategists (Foissner 1993). Obviously, *S. terricola* and *P. australiensis* follow this general pattern and even surpass it by preserving in the resting cyst part of the food vacuoles from the trophic phase of the life cycle. We suggest using this highly specific feature as an indicator for an adversity (extremely r-selected) life strategy. The mechanism of preserving bacteria in the food vacuoles is not known, but could be of great interest for physiologists and ecologists. The ''enslaved'' food vacuoles remind one of the enslaved chloroplasts of mixotrophic ciliates, which sequester plastids from the prey (for a review, see Lynn 2008).

TAXONOMIC SUMMARY

Class Colpodea Small et Lynn, 1981
Subclass Colpodia Foissner, 1985
Order Colpodida Puytorac et al., 1974

Sandmanniellidae n. fam.

Diagnosis. Colpodida with colpodid silverline pattern and flat, dish-shaped oral region associated with a long oral basket originating between the oral ciliary fields.

Type genus. *Sandmanniella* n. g.

Sandmanniella n. g.

Diagnosis. Small Sandmanniellidae with perioral cilia condensation, a right oral polykinetid composed of a dikinetid file on whose right are several files of unordered monokinetids, and a longitudinally oriented left oral field of several oral polykinetids composed of aligned dikinetid units.

Type species. *Sandmanniella terricola* n. sp.

Dedication. The senior author dedicates this genus to Prof. Dr. Wilhelm Sandmann (University Hospital, Düsseldorf), an excellent surgeon, who endowed me with a third life (for my second life, see *Wolfkosia loeffleri* Foissner et al. 2002).

Remarks. The diagnosis is based on the hierarchical classification of Foissner (1993).

Sandmanniella terricola n. sp.

Diagnosis. Size about 50 × 40 μm in vivo; broadly ellipsoidal. On average 14 ciliary rows; perioral cilia condensation attached to anterior end of postoral kinety 1, composed of an average of 13 dikinetids. Right oral polykinetid composed of four kineties, left of about 7 paired rows with anterior pair set off from posterior ones. Resting cyst globular, wall smooth, contains undigested food.

Type locality. Soil from the Chobe River, Kabolebole Peninsula, Botswana, southern Africa, 17°50′E 25°S.

Type material. Two holotype slides, one with protargol and one with silver nitrate-impregnated specimens, as well as five paratype slides have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip. The sequence of the 18S rDNA has been deposited in Genbank, accession number: FJ610254.

Etymology. Named after the soil habitat in which it was discovered.

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