The Journal of
Eukaryotic Microbiology

Published by the International Society of Protistologists

Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

Morphology and Ontogenesis of *Psilotrichides hawaiiensis* nov. gen., nov. spec. and Molecular Phylogeny of the Psilotrichidae (Ciliophora, Hypotrichia)

Domingo Heber[a], Thorsten Stoeck[b] & Wilhelm Foissner[a]

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

Keywords
Biodiversity; development of oral primordium in a deep pouch; Hawaiian archipelago; Japanese rice field; migrating kinetofragment; oral pattern; revision.

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

Received: 24 July 2013; revised 9 December 2013; accepted December 29, 2013.

doi:10.1111/jeu.12104

ABSTRACT

The Psilotrichidae are a family of middle-sized hypotrichs with unique morphological and ontogenetic features (e.g. the oral primordium develops in a deep pouch) that, however, did not provide a definite phylogenetic signal. Thus, we studied the 18S rRNA gene of *Urospinula succisa* (Müller 1786) Esteban et al., 2001 as well as the morphology and ontogenesis of *Psilotrichides hawaiiensis*, a new genus and species from an ephemeral swamp on Oahu Island, Hawaii. The molecular data classify the psilotrichids into the oxytrichids but without clear branching position. A brief revision, using the structure of the oral apparatus, the location of the contractile vacuole, and three ontogenetic features, showed four distinct genera: *Psilotricha* Stein, 1859; *Urospinula* Corliss, 1960; *Hemiholosticha* Gelei, 1954; and *Psilotrichides* nov. gen., which differs from the confamilials mainly by the obliquely oriented buccal cavity and the shape of the undulating membranes as well as by a distinct ridge along the right buccal margin. The pyriform species, *P. hawaiiensis*, is about 65 × 45 μm in size and is easily recognized by the table tennis racket-shaped appearance due to the elongated last cirrus of the left marginal row. Refined diagnoses are provided for the family Psilotrichidae Bütschli, 1889 and the genera contained.

THERE is a long-lasting confusion about the Psilotrichidae, a family of curious hypotrichs, because most species descriptions are old and thus not based on protargol preparations (Esteban et al. 2001; Foissner 1983). The genus *Psilotricha* was established by Stein (1859a) with *P. acuminata* as the type species. Bütschli (1889) classified *Psilotricha* and *Balladyna* Kowalewskiego 1882 into his new oxytrichid subfamily Psilotrichina, which was adopted by Roux (1901). Kahl (1932) added *Balladyna viridis* Penard 1922; for which Tagliani (1922) established the genus *Pigostyla*, and questioned the presence of transverse cirri because he observed a *P. viridis* population lacking them.

Based on Nigrosin preparations, Gelei (1944) described the new genus *Urospina*, changed by Corliss (1960) to *Urospinula* because of preoccupation, with three new species: *U. bicaudata*, *U. calcibia*, and *U. sinistrocaudata*. Further, Gelei (1954) described a new genus and species, *Hemiholosticha viridis*, which was synonymized with *Psilotricha viridis* by Dingfelder (1962), based on observations of a German population. Borror (1972) followed Dingfelder (1962) and raised the subfamily to family rank: Psilotrichidae. Stiller (1974) classified *Psilotricha* into the Holostichidae Fauré-Fremiet 1961; synonymized *Urospinula* with *Psilotricha*; and realized that *P. acuminata* sensu Dingfelder (1962) is *U. bicaudata* Gelei 1944. Further, she accepted *B. viridis* Penard 1922 and *H. viridis* Gelei 1954; which resulted in secondary homonymy. Thus, Stiller (1974) replaced *H. viridis* Gelei 1954 by a nomen novum: *Psilotricha geleii*.

Based on protargol impregnation, Grolière (1975) described a new species, *Psilotricha dragescoi*, which Esteban et al. (2001) considered as incertae sedis; we agree. A few years later, Foissner (1983) described the morphology and ontogenesis of *Psilotricha succisa* established by Müller (1786) as *Trichoda succisa*. He synonymized *U. bicaudata* (Gelei 1944) and *P. acuminata* sensu Dingfelder (1962) with *P. succisa* but accepted *U. calcibia* and *U. sinistrocaudata* because of the different dorsal infraciliature. Foissner (1983) and Lynn (2008) further supported the family status of *Psilotricha* because of its unique ontogenesis. Foissner (1983) did not classify the Psilotrichidae at the ordinal level while Lynn (2008) put it into the Stichotrichida Fauré-Fremiet 1961; transferring

Urospinula Corliss 1960 into the Amphisiellidae, and *Psilotricha* Stein 1859a and *Hemiholosticha* Gelei 1954 into the Psilotrichidae.

Esteban et al. (2001) revised the Psilotrichidae and redescribed the type species, *P. acuminata*, and accepted two genera, *Psilotricha* and *Urospinula*, and several species. They classified *Psilotricha* into the Oxytrichidae and *Urospinula* to the Orthoamphisiellidae, following Eigner (1997). However, Berger (2011) rejected the transfer of *Urospinula* to the Orthoamphisiellidae because the frontoventral cirral rows do not originate via primary primordia and the anlagen A1 and A2 of the opisthe originate from the oral primordium (Foissner 1983).

Obviously, the morphological and ontogenetic data did not unambiguously reveal the phylogeny of the Psilotrichidae. Thus, we applied molecular methods to *Urospinula succisa* whose morphology and ontogenesis were described by Foissner (1983). This showed an unexpected position of the Psilotrichidae within the oxytrichid clade. Further, we describe a new psilotrichid genus and species, showing that the psilotrichid diversity is not yet exhausted.

MATERIALS AND METHODS

Materials

Psilotrichides hawaiiensis was discovered in a sample of dry surface soil and litter (0–3 cm) from an ephemeral swamp grown with fern (*Marsilea* sp.) on Koko Head, Oahu Island, Hawaiian archipelago, W157°41′44″ N21°15′52″. Unfortunately, we did not store specimens for sequencing because the species was discovered 20 yr ago when molecular characterization just began.

The sample was analyzed with the "nonflooded Petri dish method" as described by Foissner (1987, 1992). Briefly, this simple method involves placing 50–500 g air-dried terrestrial material (soil, leaf litter, roots, etc.) in a Petri dish (13–18 cm wide and 2–3 cm high) and saturating, but not flooding it, with distilled water. Such cultures are analyzed for ciliates by inspecting about 2 ml of the runoff on days 2, 7, 14, 21, and 28. To obtain sufficient dividers, raw cultures were set up in Petri dishes containing Eau de Volvic (French Table water), a few ml of the eluate from the nonflooded Petri dish culture, and some crashed wheat kernels to stimulate growth of food, i.e. bacteria and protists.

Urospinula succisa was collected from a nonflooded Petri dish culture with soil from a rice field in the surroundings of the Lake Biwa Museum, Japan. It was cultivated as described for *P. hawaiiensis* above. Three voucher slides, reg. no. 2013/47-49, have been deposited in the Biology Centre of the Museum of Upper Austria (Biologiezentrum des Oberösterreichischen Landesmuseums), Linz (LI). Relevant specimens have been marked by black ink circles on the coverslip.

Hemiholosticha sp. was found in the Simmelried, i.e. in a moorland pond in Bavaria, N49°2′ E10°45′. For details, see Kreutz and Foissner (2006). Environmental specimens were used for the investigations because the species was rather abundant; it will be described in a forthcoming paper.

Morphological methods

Living cells were studied using a high-power oil immersion objective and differential interference contrast microscopy. Protargol impregnation and scanning electron microscopy (SEM) were performed as described by Foissner and Xu (2007). For protargol impregnation, *P. hawaiiensis* was fixed with Stieve's solution, which produced rather mediocre preparations while alcohol and Da Fano fixation produced very good results in *Hemiholosticha*.

Counts and measurements of silvered specimens were performed at a magnification of 1,250X. In vivo measurements were conducted at magnifications of 40–1,000X. Drawings of live specimens were based on free-hand sketches and micrographs, those of impregnated cells were made with a drawing device. In the ontogenetic stages, parental structures are shown in outline while newly formed structures are shaded black. Each of the stages depicted has been seen in at least two specimens.

Terminology is according to Foissner (1983) and Lynn (2008) while details of the oral apparatus are according to Foissner and Al-Rasheid (2006).

Molecular methods

DNA isolation using the DNEasy Tissue Kit (Qiagen, Hildesheim, Germany), 18S rDNA amplification with eukaryote specific primers EukA and EukB (Medlin et al. 1988), cloning of DNA fragments with the TA cloning kit (Invitrogen, Carlsbad, CA, USA), and bidirectional M13-Sanger sequencing followed the protocol described by Foissner and Stoeck (2011).

Phylogenetic analyses

Prior to phylogenetic analyses, sequences were quality checked, and PHRED/PHRAP analyses were carried out using CodonCode Aligner v.3.0 (CodonCode Corporation, Dedham, MA). Vector and primer nucleotides were trimmed off. The sequence of *U. succisa* was first subjected to a BLAST analysis (Altschul et al. 1997) against GenBank's nr database. Then, the 18S rDNA sequence was aligned to available hypotrich families and choreotrichs as outgroup using MUSCLE (Edgar 2004) as implemented in SEAVIEW (Gouy et al. 2010), and subjected to Gblocks (Castresana 2000) for refinement. Manual inspection for further editing of the alignment was conducted in MacClade v4.05 (Maddison and Maddison 2005). The GTR-I-Γ evolutionary model was best fitting selected by the AIC in jModeltest v0.1.1 (Guindon and Gascuel 2003; Posada 2008). The resulting alignment used for phylogenetic analyses included 62 sequences and 1750 characters. Maximum likelihood (ML) analyses were carried out in RaxML-HPC v7.2.5 (Stamatakis et al. 2008). Support came from a majority rule consensus tree of 1,000 multiparametric bootstrap replicates. An evolutionary distance

tree using Neighbor Joining (NJ) algorithm was calculated in SeaView (Galtier et al. 1996). Support came from 1,000 bootstrap replicates. Trees were visualized with FigTree v1.3.1 (Rambaut 2006). The GenBank accession number for *U. succisa* is KF411460.

RESULTS

Description of *P. hawaiiensis* nov. spec.

Psilotrichides hawaiiensis has a size of 55–75 × 40–50 μm in vivo, usually it is about 65 × 45 μm, as calculated from in vivo measurements and the morphometric data in Table 1, adding 15% preparation shrinkage. The bluntly pyriform or table tennis racket appearance of the body is caused by the prominent terminal cirrus belonging to the left marginal row. Rarely, the cells are broadly ellipsoidal with acute rear end or have a sigmoidal left body margin. Usually, *P. hawaiiensis* is dorsoventrally flattened up to 2:1. In lateral view, most cells are roughly hemiellipsoidal, i.e. have a convex ventral side and a flat or sigmoidally curved dorsal side (Table 1; Fig. 1A–F, I–K and 2A–C, E–G).

The nuclear apparatus is slightly anterior of the central quarters of the cell and left of body midline, usually being composed of two macronuclear nodules and one micronucleus in between. The macronuclear nodules are rotund to ellipsoidal, on average 14 × 10 μm in protargol preparations; the average distance between the nodules is 3 μm, occasionally they are connected by a fine strand; the nucleoli are usually rotund, 0.5–2 μm across, rarely up to 4 μm. The micronucleus is globular to broadly ellipsoidal, on average 3.6 × 2.9 μm in protargol preparations (Table 1; Fig. 1A, J and 2C).

The contractile vacuole is in midbody at the left cell margin, and the cytopyge is near the acute posterior end (Fig. 1A, B). The cortex is inflexible, colorless, and lacks specific granules. The cytoplasm is colorless and studded with food vacuoles up to 12 μm in diam., some ordinary crystals about 4 μm in size, and lipid droplets 1–5 μm across (Fig. 1A, I). *Psilotrichides hawaiiensis* feeds on colorless flagellates of the genera *Polytoma* and *Hyalogonium*, both having a red eye-spot subapically; most specimens are packed with this kind of food, both in the nonflooded Petri dish culture and in the raw cultures (Fig. 1A, I and 2A). The ciliate glides slowly to rapidly on the microscope slide and can perform short, fast jumps.

There are 18–26 cirri in four ventral, one postoral, and in the right and the left marginal row (Table 1; Fig. 1A, K and 2B, E). The length of the rows is highly variable (CV usually > 20%, Table 1) while the total number of cirri is rather stable (CV 9.5%). Most cirri are thin and long, i.e. are composed of 3 × 3 to 3 × 4 (rarely 5) cilia 20–25 μm long in vivo, those of the left marginal row are short or even absent (but the basal bodies are present, Fig. 2D, E), except of an approximately 30 μm long terminal cirrus that inserts on the acute body end and is composed of cilia of various lengths (Fig. 3B); frontal, buccal and transverse cirri are not distinguishable. The cirri are widely spaced and their number in the individual rows is highly variable (CV usually > 20%), making the pattern difficult to discern. The anterior cirrus of ventral row R1 is near the distal end of the adoral zone and thus possibly homologous to frontal cirrus III/3 of other hypotrichs; the distance between the two anteriormost cirri of ventral row R3 is often increased; and the cirri of ventral row R4 usually form a group each at the ends of the row (Table 1; Fig. 1A, K, 2B, D–G and 3B).

The dorsal bristles are 3–4 μm long in vivo and in protargol preparations. They are arranged in three meridional rows slightly (rows 2 and 3) to distinctly (row 1) shortened anteriorly; some parental bristles are preserved in six of 23 specimens. Caudal cirri are absent (Table 1; Fig. 1J and 2C, F).

The adoral zone of membranelles occupies an average of 43% of body length; the largest bases are 6–7 μm wide in vivo. When the cell is viewed ventrally, the zone looks like a question mark cut proximally while it is semicircular when the cell is viewed apically (Table 1; Fig. 1A, G, K, 2A, B, E and 3A). On average, the adoral zone is composed of 21 ordinary membranelles with the intermembranellar distances strongly increasing from proximal to distal. Likewise, the length of their cilia gradually decreases from about 20 μm distally to 5 μm proximally; further, the cilia of the short fourth row are only 1.5 μm long. The 10–12 frontal membranelles, which have ciliary row 4 distant from the centripetal end, are often separated from the ventral membranelles by a membranelle composed of only three kineties (Fig. 1G, H). In vivo, the frontal membranelles are partially covered by an approximately 3 μm high scutum that is strongly asymmetrical, i.e. the right half is much shorter than the left one which merges into the margin of the membranellar stripe (Fig. 1A and 2A). Under the scutum is an accumulation of lipid droplets up to 5 μm across. Lateral membranellar cilia, intermembranellar ridges, and membranellar bolsters are absent (Table 1; Fig. 1A, G, H, K, 2A, B, E and 3A).

The buccal cavity is about half as long as the ventral portion of the adoral zone, i.e. about 11 μm long, 8 μm wide, and 4 μm deep in the protargol preparations. The right margin is occupied by a conspicuous, 2–3 μm thick ridge anteriorly merging into the frontal cortex and posteriorly into the buccal vertex. A buccal seal is not recognizable (Table 1; Fig. 1A, E, 2E and 3A, C, D).

The paroral membrane inserts in a shallow slit at the right margin of the buccal ridge and forms an acute to very acute angle (22–44°) with the longitudinal axis of the cell. The cilia produce an undulating, up to 7 μm high membrane gradually decreasing to 3 μm at both ends. In many specimens more or less large parts of the paroral are doubled or even triplicated. The endoral membrane extends side by side with the paroral, and its cilia form always an approximately 4 μm long plate, indicating that they are motionless (Fig. 3C, D). The pharyngeal fibers extend obliquely backwards and are 8–10 μm long in protargol preparations (Table 1; Fig. 1A, G, H, K, 2E and 3C, D).

Table 1. Morphometric data on *Psilotrichides hawaiiensis*

Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	*n*
Body, length (μm)	58.7	59.0	5.1	1.1	8.7	50.0	66.0	23
Body, width (μm)	38.8	39.0	3.2	0.7	8.2	34.0	44.0	23
Body length:width, ratio	1.5	1.5	0.1	0.0	7.5	1.2	1.7	23
Macronuclear nodules, number[b]	2.0	2.0	0.0	0.0	0.0	2.0	2.0	23
Macronuclear nodules, distance in between (μm)[c]	2.9	3.0	1.1	0.2	39.1	1.0	6.0	21
Anterior macronuclear nodule, distance to anterior BE (μm)	8.3	8.0	1.6	0.4	19.5	6.0	13.0	21
Anterior macronuclear nodule, length (μm)	14.3	15.0	2.1	0.5	14.5	10.0	18.0	21
Anterior macronuclear nodule, width (μm)	9.8	10.0	0.9	0.2	9.1	8.0	11.0	21
Micronuclei, number[d]	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length (μm)	3.6	3.5	0.5	0.1	13.0	2.5	4.5	21
Micronucleus, width (μm)	2.9	3.0	0.4	0.1	14.6	2.0	3.5	21
Anterior BE to proximal end of adoral zone, distance (μm)	25.6	25.0	1.4	0.3	5.5	24.0	29.0	21
Adoral zone, percentage of body length	43.3	42.4	3.2	0.7	7.5	37.9	50.0	21
Adoral membranelles, number	21.0	21.0	1.0	0.2	4.9	19.0	23.0	21
Adoral membranelles, length of widest base (μm)	4.9	5.0	–	–	–	4.5	5.0	21
Buccal cavity, width (μm)	7.6	8.0	0.8	0.2	10.7	6.0	9.0	21
Paroral, distance to anterior body end (μm)	10.1	10.0	1.2	0.3	12.2	8.0	13.0	21
Paroral, length (μm)	11.5	11.0	0.7	0.2	6.5	11.0	14.0	21
Endoral, distance to anterior body end (μm)	11.8	12.0	1.3	0.3	10.9	10.0	15.0	21
Endoral, length (μm)	9.9	10.0	1.1	0.2	11.2	8.0	13.0	21
Left marginal cirral row, distance to anterior body end (μm)	32.4	32.0	2.6	0.6	8.1	27.0	37.0	21
Left marginal row, second cirrus to posterior BE, distance (μm)	16.0	16.0	2.5	0.5	15.7	11.0	20.0	21
Left marginal row, number of cirri[e]	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Postoral cirral row, distance to anterior body end (μm)	33.3	33.0	4.3	0.9	12.8	26.0	40.0	21
Postoral cirral row, distance to posterior body end (μm)	18.8	19.0	3.9	0.9	20.7	12.0	28.0	18
Postoral cirral row, number of cirri	2.1	2.0	0.6	0.1	29.8	1.0	3.0	21
Cirral row R1, distance from last cirrus to anterior BE (μm)	13.3	13.0	1.6	0.4	12.2	10.0	16.0	21
Cirral row R1, number of cirri[f]	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
Cirral row R2, distance to anterior body end (μm)	21.0	21.0	3.7	0.8	17.5	15.0	26.0	21
Cirral row R2, distance to posterior body end (μm)	29.2	31.0	4.5	1.0	15.5	18.0	34.0	20
Cirral row R2, number of cirri	2.1	2.0	0.4	0.1	18.8	1.0	3.0	21
Cirral row R3, distance to anterior body end (μm)	20.7	18.0	5.9	1.3	28.6	15.0	34.0	21
Cirral row R3, distance to posterior body end (μm)	19.5	20.0	4.2	0.9	21.3	12.0	29.0	21
Cirral row R3, number of cirri	3.1	3.0	0.8	0.2	26.9	2.0	5.0	21
Cirral row R4, distance to anterior body end (μm)	12.0	12.0	2.4	0.5	19.9	7.0	17.0	21
Cirral row R4, distance to posterior body end (μm)	9.2	9.0	3.0	0.7	32.3	5.0	16.0	21
Cirral row R4, number of cirri	4.3	4.0	1.0	0.2	22.3	2.0	6.0	21
Right marginal cirral row, distance to anterior body end (μm)	12.1	12.0	2.8	0.6	22.9	8.0	18.0	21
Right marginal cirral row, distance to posterior body end (μm)	8.9	9.0	3.5	0.8	39.6	2.0	15.0	21
Right marginal row, number of cirri	6.1	6.0	1.5	0.3	24.8	4.0	10.0	21
Cirri, total number	22.6	23.0	2.2	0.5	9.5	18.0	26.0	21
Dorsal kinety 1, distance to anterior body end (μm)	29.4	29.0	4.0	0.9	13.5	23.0	37.0	21
Dorsal kinety 1, number of bristles	7.7	8.0	0.8	0.2	11.0	6.0	9.0	21
Dorsal kinety 2, distance to anterior body end (μm)	18.2	18.0	3.8	0.8	21.0	13.0	26.0	21
Dorsal kinety 2, number of bristles	9.4	9.0	1.2	0.3	12.8	7.0	12.0	21
Dorsal kinety 3, distance to anterior body end (μm)	11.7	12.0	2.5	0.5	21.1	8.0	18.0	21
Dorsal kinety 3, number of bristles	16.1	16.0	1.3	0.3	8.3	14.0	19.0	21
Dorsal bristles in kineties 1–3, total number	33.2	33.0	2.4	0.5	7.4	29.0	38.0	21

BE = body end; CV = coefficient of variation in %; M = median; Max = maximum; Min = minimum; *n* = number of individuals investigated; SD = standard deviation; SE = standard error of arithmetic mean; $\bar{x}$ = arithmetic mean.

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a raw culture.

[b]Three to four nodules in four of 51 specimens.

[c]Zero in one specimen; not included in morphometry.

[d]Not recognizable in two of 23 specimens.

[e]Four cirri in one of 22 specimens.

[f]Three cirri in one of 22 specimens.

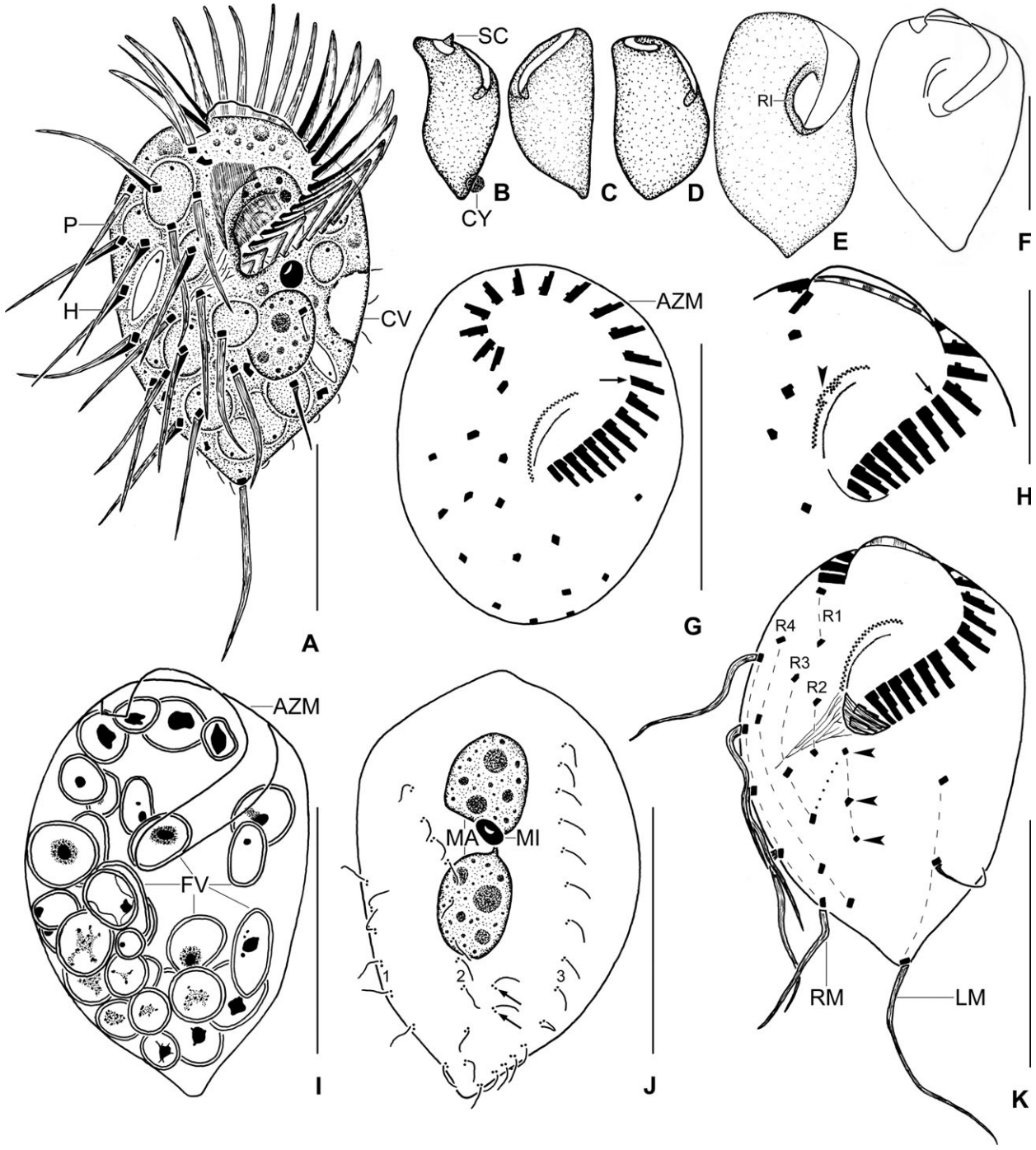

Figure 1 **A–K.** *Psilotrichides hawaiiensis* from life (A, B, E), after protargol impregnation (F–K), and in the SEM (C, D). **A.** Ventral view of a representative specimen, length 65 μm. **B–D.** Lateral views, showing outline variations. **E, F.** A specimen with sigmoidal left body margin, and another with deltoid outline. **G.** Oblique apical view. The frontal and ventral membranelles are separated by a membranelle with only three kineties (arrow). **H.** Ventral view of oral region of a specimen with partially duplicated paroral membrane (arrowhead). The arrow denotes a membranelle composed of only three kineties separating frontal and ventral membranelles. **I–K.** Holotype specimen, length 51 μm, in optical section, and dorsal and ventral view. The arrows in (J) mark parental kinetids while the arrowheads in (K) denote the postoral cirral row, which is connected with a dotted line to R3 from which it originates. Most specimens were studded with food vacuoles when fixed for preparation (I). 1–3 = dorsal kineties; AZM = adoral zone of membranelles; CV = contractile vacuole; CY = cytopyge; FV = food vacuoles; H = an ingested *Hyalogonium*; MA = macronuclear nodules; MI = micronucleus; LM = left marginal cirral row; P = an ingested *Polytoma*; R1–4 = ventral cirral rows; RI = buccal ridge; RM = right marginal cirral row; SC = scutum. Scale bars 20 μm (H) and 30 μm (A, F, G, I–K).

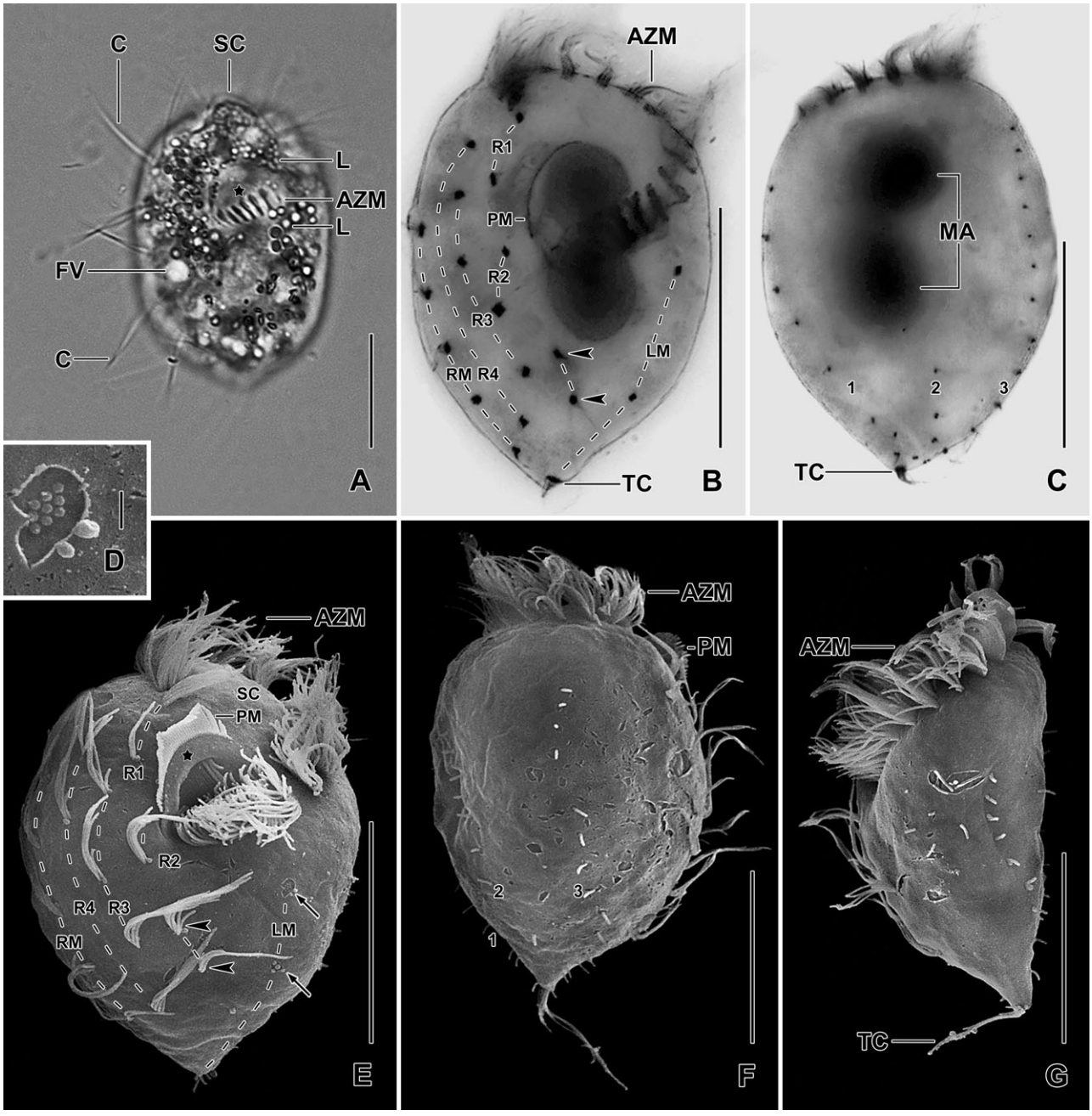

Figure 2 **A–G.** *Psilotrichides hawaiiensis* from life (A), after protargol impregnation (B, C), and in the scanning electron microscope (D–G). **A.** Ventral view of a broadly ellipsoidal specimen with acute posterior end, length 65 μm. The asterisk marks the buccal cavity. Note the asymmetric scutum (SC) and the thin, long cirri some of which cross optically. The specimen is studded with lipid droplets and food vacuoles. **B, C.** Ventral and dorsal view of broadly pyriform specimens, showing the infraciliature and the macronuclear nodules. The arrowheads denote the postoral cirral row. The anterior portion of dorsal bristle row 2 is hidden by the macronuclear nodules. **D.** The unciliated, anteriormost left marginal cirrus of the specimen shown in (E). **E.** Ventral view, showing the cirral pattern and the buccal ridge marked by an asterisk. The arrowheads denote the cirri of the postoral row. The unciliated cirri of the left marginal row are marked by arrows; the terminal cirrus disappeared by the preparation procedures. **F.** Dorsal view, showing the bristle rows and the long terminal cirrus on the acute posterior body end, an important feature of this species. **G.** Lateral view of a specimen with a convex ventral and a flat dorsal side. Note the conspicuous terminal cirrus (TC) 1–3 = dorsal kineties; AZM = adoral zone of membranelles; C = cirri; FV = food vacuole; L = lipid droplets; LM = left marginal cirral row; MA = macronuclear nodules; PM = paroral membrane; R1–4 = ventral cirral rows; RM = right marginal cirral row; SC = scutum; TC = terminal cirrus. Scale bars 1 μm (D) and 25 μm (A–C, E–G).

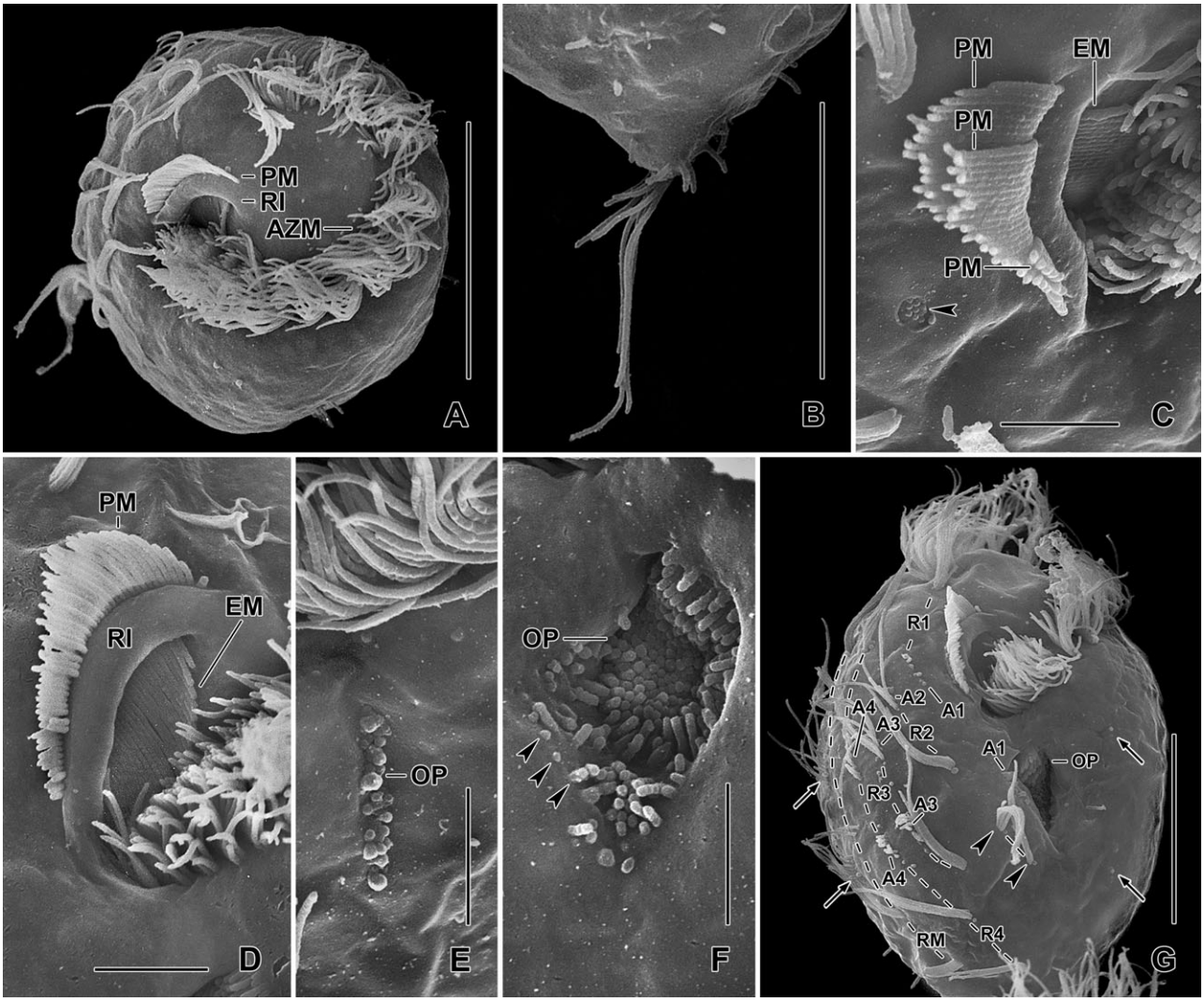

Figure 3 **A–G.** *Psilotrichides hawaiiensis* in the scanning electron microscope. **A.** Apical view, showing the semicircular adoral zone and the buccal ridge. **B.** Dorsal view of posterior body end, showing the elongated terminal cirrus composed of cilia of various lengths. **C, D.** Oral apparatus, showing the endoral and paroral membrane, which is partially triplicated (C) as well as the unique buccal ridge (RI). The arrowhead marks an unciliated ventral cirrus. **E.** The oral primordium originates on the cell surface. **F.** Invaginating oral primordium of an early divider; the basal bodies at the right margin (arrowheads) remain on the surface to form opisthe's anlage A1. **G.** A more advanced early divider, showing the invaginated oral primordium and cirral anlagen. The arrowheads mark the parental postoral cirri while arrows denote the marginal cirral anlagen, those of the right row being much more advanced than those of the left row. A1–4 = cirral row anlagen; AZM = adoral zone of membranelles; EM = endoral membrane; OP = oral primordium; PM = paroral membrane; R1–4 = ventral cirral rows; RI = buccal ridge; RM = right marginal cirral row. Scale bars 5 μm (C–F), 15 μm (B), and 25 μm (A, G).

Ontogenesis of *P. hawaiiensis*

Very early dividers (Fig. 3E and 4A–C)

The oral primordium, i.e. an anarchic field of basal bodies, is formed de novo on the cell surface between the postoral and the left marginal row. The macronuclear nodules show a reorganization band. The dorsal infraciliature is unchanged.

Early dividers (Fig. 3F, G, 4D–F, 5A, B and 6A)

The oral primordium increases in size and begins to invaginate. When the first protomembranelles, which are composed of only two kineties, are formed, the pouch becomes deep and prominent (Fig. 3F, G, 4D–F and 5A). Some basal bodies remain on the right margin of the pouch and will later form opisthe's anlage A1 (Fig. 3F, G, 4F and 5A). The parental undulating membranes begin to reorganize (Fig. 4F, 5B and 6A). Four streaks of basal bodies appear within ventral cirral rows R3 and R4 to form the anlagen A3 and A4 in proter and opisthe (Fig. 3G, 4D–F and 5B). In the protargol preparations (Fig. 4D–F) but not in the scanning electron micrographs (Fig. 3G and 5B), the two opisthe streaks are posteriorly connected by scattered basal bodies, as in *U. succisa*

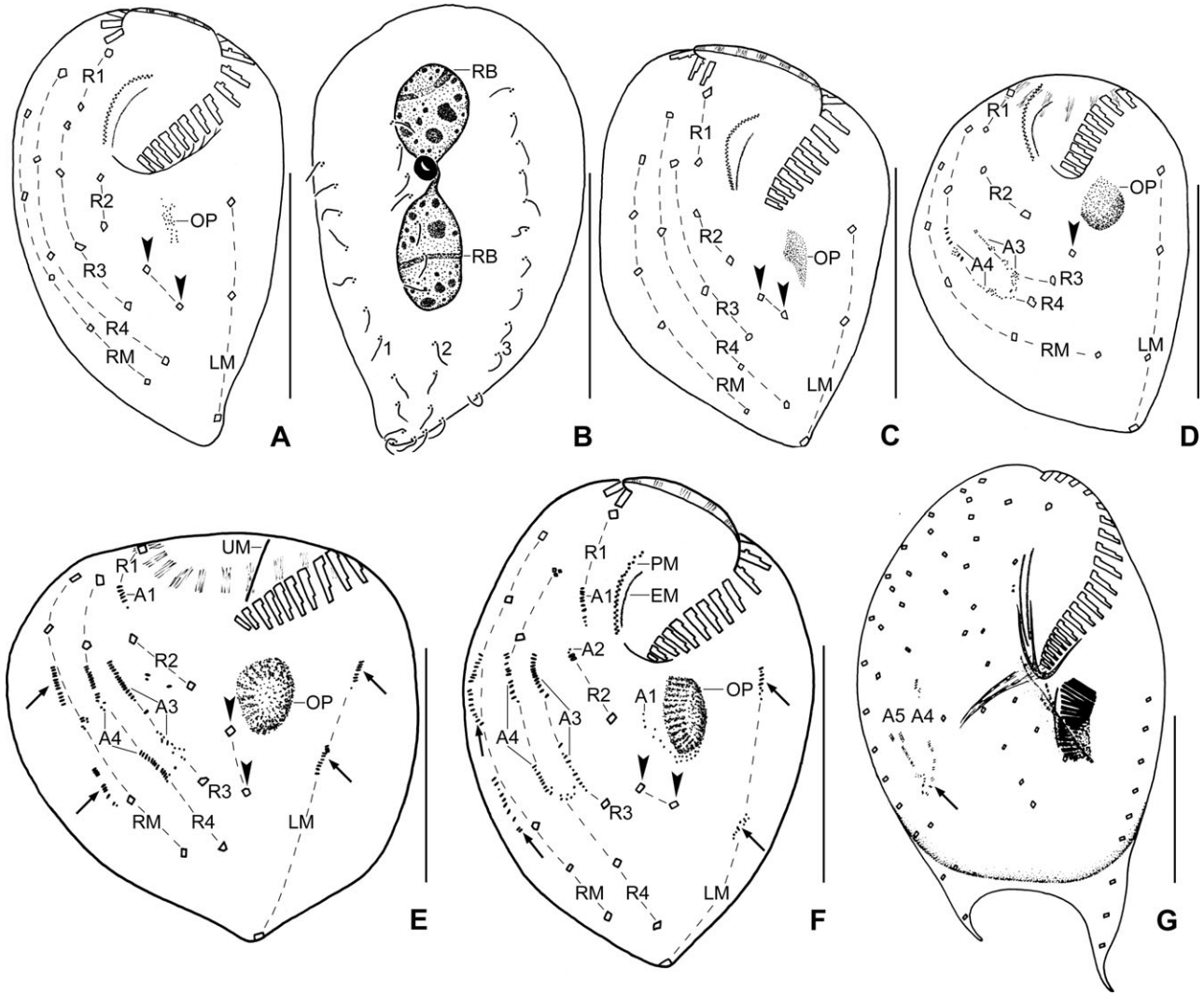

Figure 4 **A–G.** *Psilotrichides hawaiiensis* (A–F) and *Urospinula succisa* (G, from Foissner 1983), very early (A–C), and early (D–G) dividers after protargol impregnation. Dashed lines show the cirral rows and arrowheads denote the postoral cirri. **A–C.** Ventral and dorsal view of very early dividers, showing the forming oral primordium and a reorganization band in the macronuclear nodules. **D.** Ventral view of an early divider, showing the invaginating oral primordium as well as cirral anlagen A3 and A4, which are connected by scattered basal bodies posteriorly. **E, F.** Ventral view of early dividers, showing the oral primordium in a distinct pouch where the anarchic basal bodies arrange to protomembranelles and cirral anlage A1 separates from the oral primordium. The proter begins to reorganize the paroral membrane (F) and anlagen develop in the marginal cirral rows (arrows). **G.** Ventral view of an early divider of *Urospinula*. The anlagen A4 and A5 are homologous to the anlagen A3 and A4 in *Psilotrichides* because they are connected posteriorly (arrow) by scattered basal bodies in both genera (cp. D,F). 1–3 = dorsal kineties; A1–5 = cirral row anlagen; AZM = adoral zone of membranelles; EM = endoral membrane; LM = left marginal cirral row; OP = oral primordium; PM = paroral membrane; R1–4 = ventral cirral rows; RB = reorganization band; RM = right marginal cirral row; UM = undulating membrane. Scale bars 30 μm.

which produces opisthe anlage A4 in this way (Fig. 32 in Foissner 1983 and Fig. 4G in the present publication). The proter anlage A1 is formed by the dedifferentiated posterior cirrus of row R1, and anlage A2 by the anterior cirrus of row R2. The opisthe's anlage A1 is produced by the oral primordium while anlage A2 is formed by the dedifferentiated posterior cirrus of row R2 (Fig. 3G, 4E, F, 5B and 6A). Some cirri of the right and left marginal row disintegrate and form the marginal anlagen in proter and opisthe (Fig. 3G, 4E, F, 5A and 6A).

Early mid-dividers (Fig. 6B–D)

The distal half of the oral primordium, which is still growing and differentiating, evaginates. The last adoral membranelles are formed inside the pouch, which becomes partially covered by the cortex. An anlage for the undulating membranes of the opisthe now separates from the right posterior portion of the oral primordium (Fig. 6D) and is thus not visible in the SEM (Fig. 5B). The parental undulating membranes continue reorganization. All cirral anlagen have been formed and produce cirri. Supernumerary

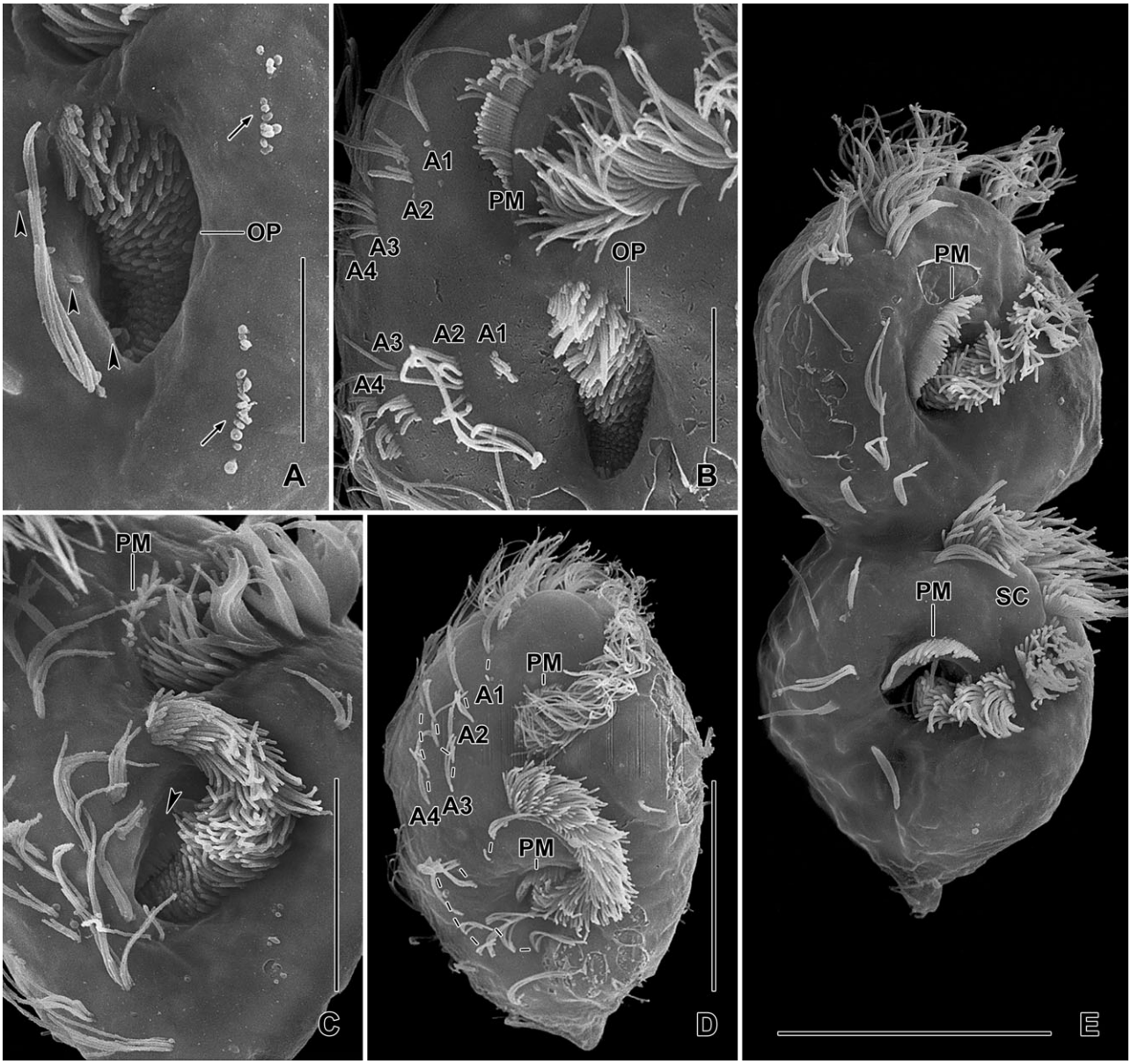

Figure 5 **A–E.** *Psilotrichides hawaiiensis*, dividers in the SEM. **A, B.** Early dividers with invaginated oral primordium and opisthe's anlage A1 (A, arrowheads) which soon migrates rightwards (B). Anlagen develop in the marginal rows (A, arrows), and the paroral membrane of the proter begins to reorganize (B). **C, D.** Mid-dividers, showing reorganization of proter's paroral membrane (C) and flattening of its buccal cavity (D). In the opisthe, the new adoral zone begins to evaginate, and the buccal cavity as well as the undulating membranes develop (C, arrowhead) and orient transversely to main body axis (D). **E.** A late divider, which lost most cirri during the preparation. A new paroral membrane developed in the proter. The opisthe's adoral zone of membranelles evaginated and the undulating membranes oriented transversely to the main body axis. The buccal ridge has not yet formed. A1–4 = cirral row anlagen; OP = oral primordium; PM = paroral membrane; SC = scutum. Scale bars 10 μm (A, B), 20 μm (C), and 30 μm (D, E).

minute anlagen and basal bodies occur frequently between the anlagen A2 and A3 (Fig. 6B, D). The dorsal kineties form within-row primordia in proter and opisthe; both basal bodies of the dikinetids are ciliated, making the anlagen prominent (Fig. 6C). The micronucleus slightly inflates and shows a fibro-granular structure, which remains up to nuclear division (Fig. 6C).

Mid-dividers (Fig. 5C, D and 6E–H)

The oral primordium is still evaginating and curves to the right so that the membranelles form a convex zone; the proximal third of the primordium is still in the pouch (Fig. 5C and 6E). The buccal cavity is developing and the undulating membranes, which are already ciliated and orient increasingly parallel to the proximal part of the adoral

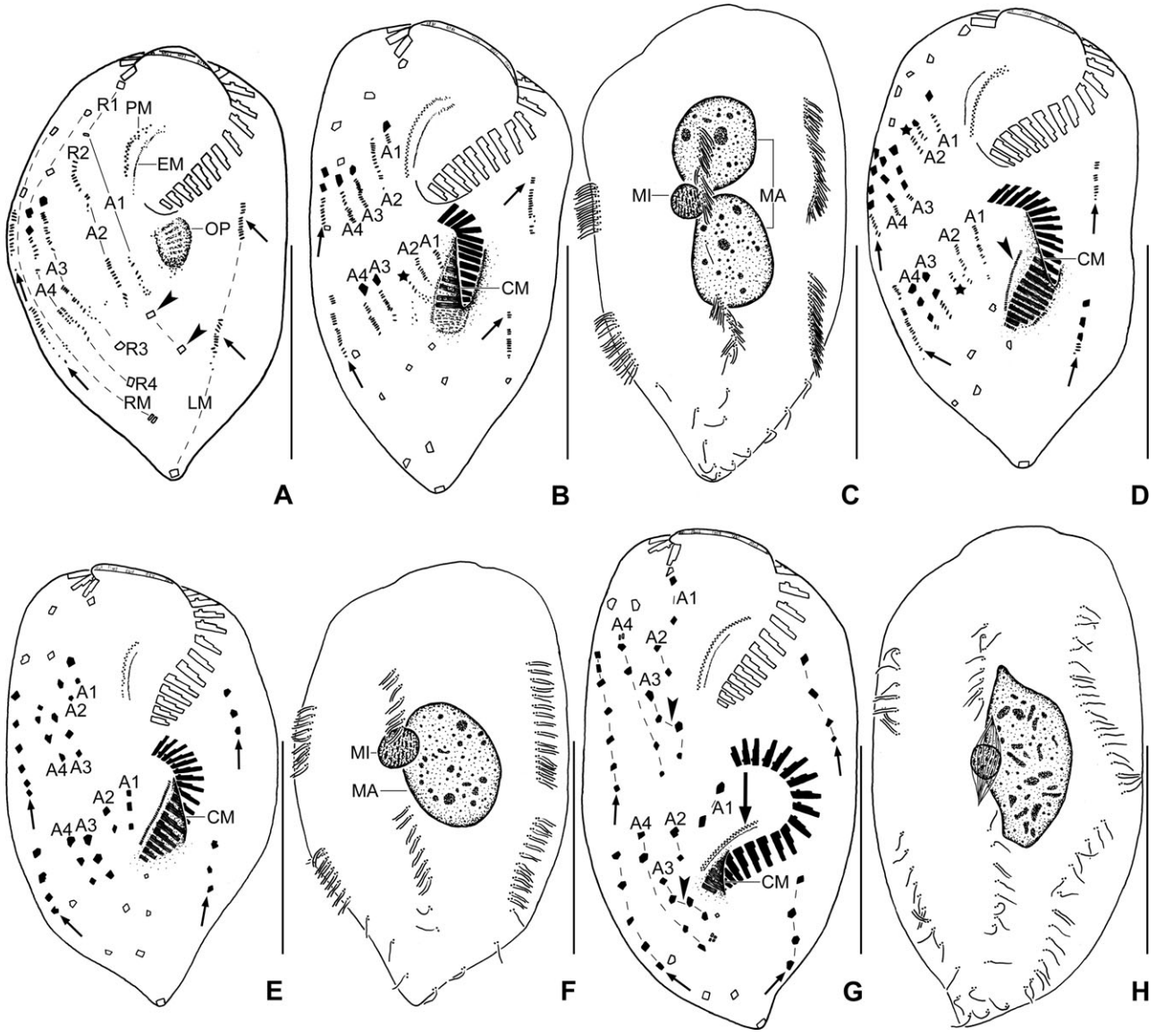

Figure 6 **A–H.** *Psilotrichides hawaiiensis*, dividers after protargol impregnation. Small arrows denote marginal cirral anlagen. **A.** Ventral view of an early divider, showing protomembranelles in the oral pouch and anlagen in the parental cirral rows connected by dashed lines. Arrowheads mark postoral cirral row. **B–D.** Early mid-dividers with fusing macronuclear nodules. Cirri form in the anlagen, and the oral primordium begins to evaginate, showing the opisthe's undulating membranes (D, arrowhead). Small, supernumerary anlagen (asterisks) are common in this stage. Note the ciliated dikinetids in the anlagen for the dorsal kineties. **E–H.** Mid-dividers with fused macronuclear nodules. New cirri developed in the anlagen (E) and migrate to their specific positions, especially the posterior portion of row 3 that migrates leftwards to become the postoral cirral row (G, arrowheads). The new adoral zone becomes distinctly curved and the paroral and endoral membrane separate (G, large arrow). The posterior bristle of the new dorsal dikinetids is resorbed (H). A1–4 = cirral row anlagen; CM = cortical margin of pouch; EM = endoral membrane; LM = left marginal row; MA = macronuclear nodules; MI = micronucleus; OP = oral primordium; PM = paroral membrane; R1–4 = ventral rows; RM = right marginal row. Scale bars 30 μm.

zone of membranelles, migrate onto its dorsal wall; the buccal ridge is not yet recognizable (Fig. 5C, D). In late mid-dividers, a remarkable process occurs (Fig. 5D and 6G): the undulating membranes which are separating, and the proximal third of the adoral zone become inclined and orient almost transversely to the main body axis. The parental buccal cavity and undulating membranes are reorganizing, i.e. the cavity disappears and the cilia shorten, according to the SEM investigations (Fig. 5C, D). The parental membranellar zone does not show any sign of reorganization.

In the anlagen, the new cirri are migrating to their specific sites, in both proter and opisthe (Fig. 5C, D and 6E, G). Anlage A1 migrates to the distal end of the

adoral zone. Anlage A2 migrates to the buccal vertex. Anlage A3 splits: the anterior portion remains at the level of the gap in anlage A4 while the posterior portion migrates leftwards to become the postoral row, as does anlage A4 in *U. succisa* (Fig. 49 in Foissner 1983 and Fig. 7D in the present publication). Anlage A4 elongates and splits in two portions separated by a wide gap. Cirri not involved in anlagen formation become resorbed.

The macronuclear nodules have fused in the cell center and the micronucleus commences division (Fig. 6F, H). The new dorsal bristle rows elongate, replace the parental bristles, and the dikinetids lose the posterior bristle (Fig. 6F, H).

Late dividers (Fig. 5E and 7A–C)

When the division furrow becomes recognizable, the proter forms a new posterior body end at the left margin (Fig. 7A, B). The buccal cavity of the proter commences redeepening and the undulating membranes finish reorganization and obtain their final position (Fig. 5E). The adoral zone and undulating membranes of the opisthe are inclined to the longitudinal body axis by about 90° or, in other words, the posterior half of the adoral zone and cirral row R1 are oriented transversely to the main body axis (Fig. 5E and 7B). Parental cirri are continuously resorbed. The new dorsal bristle rows reach their final length and most dikinetids lose the posterior cilium (Fig. 7C). The macronucleus elongates and divides. The micronucleus has already divided but both are still connected by a fibrous structure (Fig. 7C).

Molecular phylogeny of *U. succisa* (Müller 1786)

The Japanese population is morphologically highly similar to that from Austria studied by Foissner (1983). Thus, a redescription is not necessary. The 18S rRNA sequence is 1,671 base pairs (bp) long and its closest relative sequence deposited in public databases is the 18S rRNA sequence of *Bistichella variabilis*, an unclassified genus (accession number HQ699895.1, He and Xu 2011). The two taxa share a sequence similarity of 97.79%. In spite of this comparatively high sequence similarity, *Urospinula* and *Bistichella* are at rather different sites in the phylogenetic tree (Fig. 8). However, all clades involved have poor statistical support, indicating that clade composition and phylogenetic relationships are not yet settled and thus may change significantly when more sequences become available. Morphologically, *Urospinula* and *Bistichella* have little in common: cortex rigid vs. highly flexible; cirri hardly differentiated vs. highly differentiated into, e.g. frontal, buccal, and transverse cirri; oral primordium in deep pouch vs. on cell surface.

The phylogenetic analyses show that *Urospinula* branches with the oxytrichid *Onychodromopsis flexilis* (accession number AM412764.1), *Kahliella* sp. TT2005 (accession number EU079472.1), *Oxytricha lanceolata*

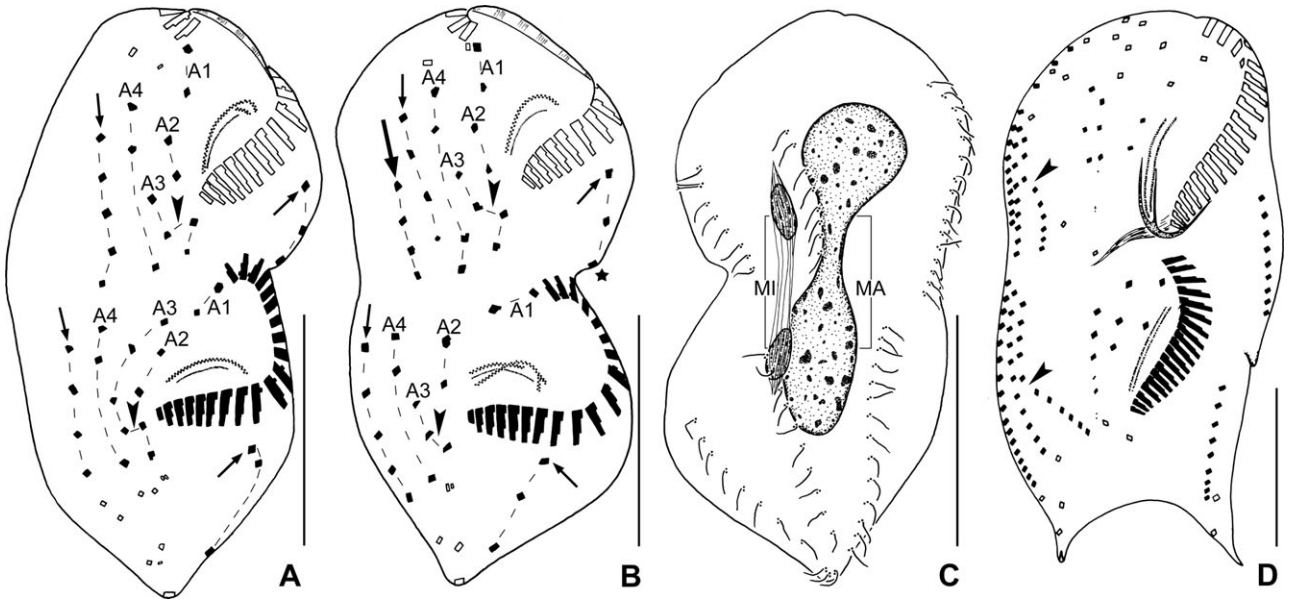

Figure 7 **A–D.** *Psilotrichides hawaiiensis* (A–C) and *Urospinula succisa* (D, from Foissner 1983), late dividers (A–C), and a late mid-divider (D) after protargol impregnation. Dashed lines connect cirri developed from the same anlage, and small arrows denote the new marginal cirral rows. The arrowheads mark the terminal segregation of anlage A3 (A4 in *U. succisa*), which later forms the postoral cirral row. **A.** Ventral view of a late divider. The adoral zone of membranelles of the opisthe approached the left body margin and is inclined orthogonally to the main body axis. **B, C.** Ventral and dorsal view of another late divider. The large arrow denotes a supernumerary cirral row, and the asterisk marks the new posterior body end of the proter. The macronuclear nodule divides, and a fibrous spindle separates the micronuclei. **D.** Ventral view of a very late mid-divider of *Urospinula*, showing the segregation of the posterior portion of anlage A4 (arrowheads), an important similarity to *Psilotrichides* (cp. Fig. 6G, 7A, B). A1–4 = cirral row anlagen; MA = macronuclear nodules; MI = micronuclei. Scale bars 30 μm.

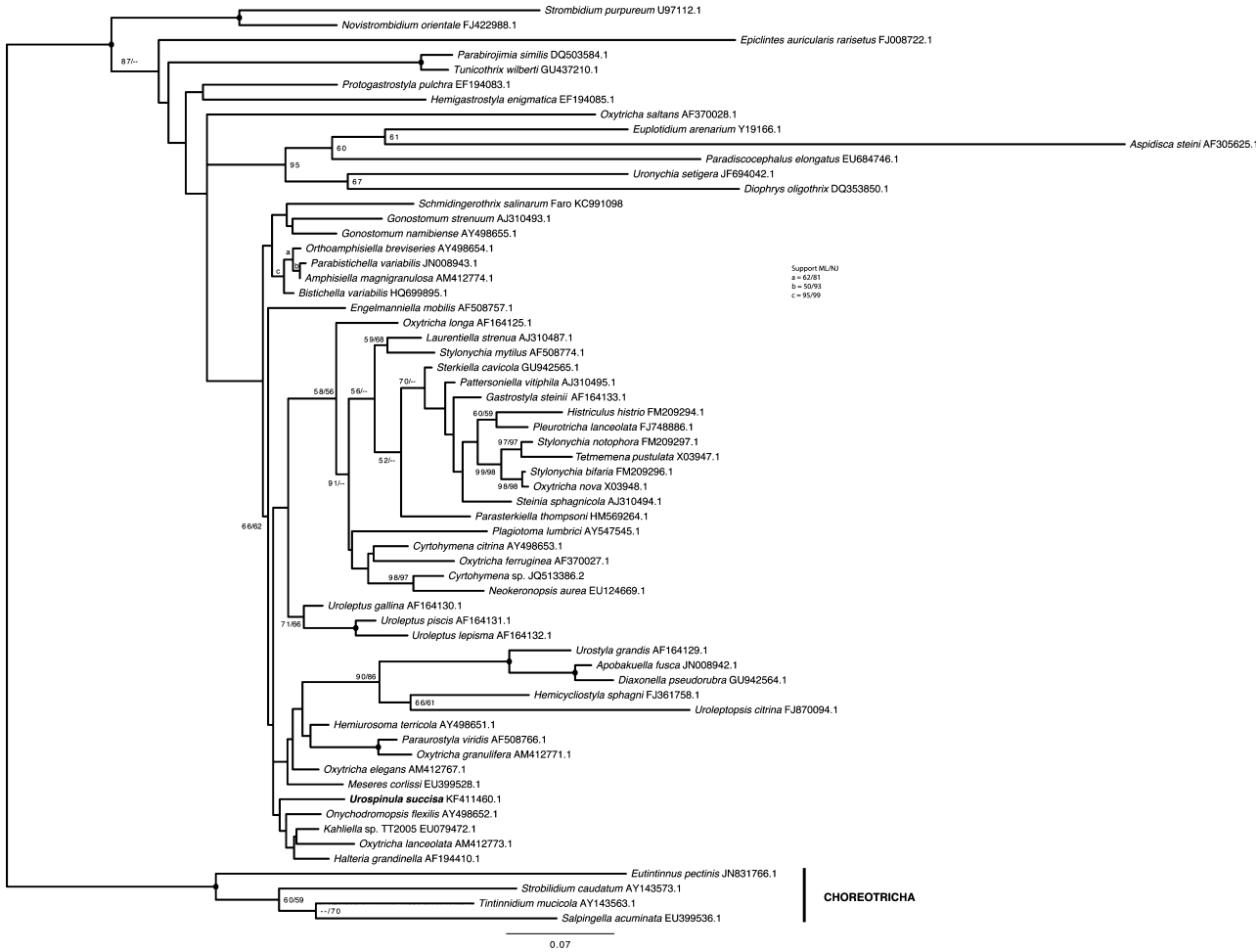

Figure 8 Phylogenetic maximum likelihood (ML) tree, showing the phylogenetic position of *Urospinula succisa* (in bold) based on its 18S rRNA gene sequence. Bootstrap support values above 50 from 1,000 ML trees/1,000 NJ trees are given at the individual nodes. Dots at nodes indicate full support. For details, see Methods section.

(accession number AM412773.1), and *Halteria grandinella* (accession number AF194410.1). However, the position of *U. succisa* as well as the positions of its clade members are not supported statistically, neither by ML nor by NJ analyses.

DISCUSSION

Psilotrichides hawaiiensis as a new genus and species

Foissner (1989) characterized the oxytrichid genera *Oxytricha*, *Stylonychia*, *Cyrtohymena*, and *Steinia* by the shape of the buccal cavity and undulating membranes. This has been widely acknowledged (for a review, see Berger 1999). A similar (convergent?) diversity occurs in the Psilotrichidae where *Psilotrichides* is unique in having a strongly oblique buccal cavity and undulating membranes (Fig. 9A–D). A further character is the buccal ridge, which is not only unique to the family but very likely to the entire subclass. *Psilotrichides* differs from *Urospinula*, the sole genus whose ontogenesis has been investigated (Foissner 1983), mainly by the oral apparatus (Fig. 9A–D) and the number of cirral anlagen originating from the oral primordium (see below).

Psilotrichides hawaiiensis is unique in having a pyriform body with a narrow posterior end, where an elongated left marginal cirrus causes a table tennis racket-shaped appearance of the cell. The body shape resembles *P. acuminata* (Fig. 10A–H) and, especially, *B. viridis* Penard 1922 (Fig. 11N). However, Penard's species has the contractile vacuole posterior of the buccal vertex (vs. at left body margin) and possibly possesses transverse cirri and symbiotic green algae both absent from *P. hawaiiensis*.

Ontogenetic comparison

There are three distinct differences and similarities each in *P. hawaiiensis* and *U. succisa*, as described by Foissner (1983): the postoral cirral row is ontogenetically inactive in

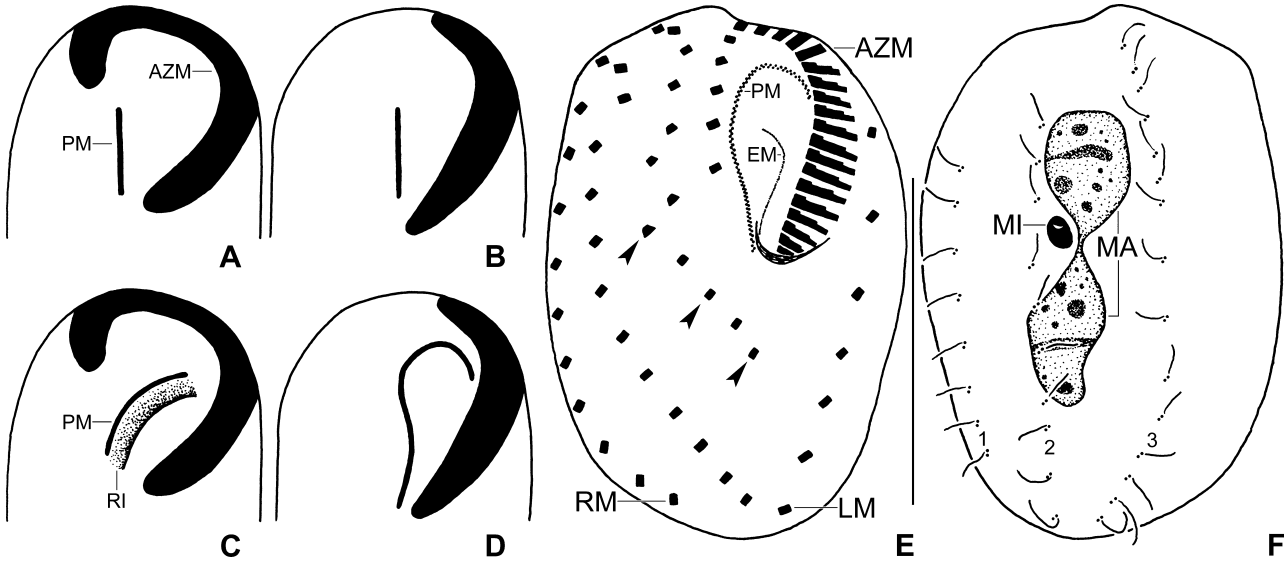

Figure 9 **A–F.** Schematic drawings of oral patterns in the psilotrichids (A–D), and *Hemiholosticha* sp. (E, F) from the Simmelried in Germany (Kreutz and Foissner 2006) after protargol impregnation. **A–D.** Oral apparatus of *Psilotricha* (A), *Urospinula* (B), *Psilotrichides* (C), and *Hemiholosticha* (D). **E, F.** Ventral and dorsal view of *Hemiholosticha* sp. The arrowheads mark the postoral cirral row ("postorale Schrägreihe" in Foissner 1983) which is highly similar to that of *Urospinula succisa* (7D, 10J). Note the "cyrtohymenid" paroral membrane. For details, see Discussion. 1–3 = dorsal kineties; AZM = adoral zone of membranelles; EM = endoral membrane; LM = left marginal cirral row; MA = macronuclear nodules; MI = micronucleus; PM = paroral membrane; RI = buccal ridge; RM = right marginal cirral row. Scale bar 30 μm.

Psilotrichides while it produces cirral row R3 in *Urospinula*; the parental undulating membranes are reorganized in *Psilotrichides* while they appear unchanged in *Urospinula*; and the oral primordium produces one cirral row in *Psilotrichides* while two in *Urospinula*. The similarities are: the oral primordium develops in a deep pouch, as in euplotids and oligotrichs (Foissner 1996; Lynn 2008) while it develops on the surface or in a shallow concavity in the stichotrichs, e.g. in *Steinia sphagnicola* (Voß and Foissner 1996); the undulating membranes do not produce cirri, as in *Schmidingerothrix extraordinaria* (Foissner 2012); and both have a migrating kinetofragment that produces the postoral cirral row.

Phylogeny of the Psilotrichidae

Neither morphology nor ontogenesis could unambiguously classify the Psilotrichidae (Foissner 1983). Likewise, the family state and contents were questioned by several authors. For instance, Corliss (1979) classified *Psilotricha* and *Hemiholosticha* into the Psilotrichidae while *Urospinula* into the Spirofilidae. Following Eigner (1997), Esteban et al. (2001) classified *Psilotricha* in the Oxytrichidae and *Urospinula* in the Orthoamphisiellidae. Only Foissner (1983), Tuffrau (1987), and Jankowski (2007) recognized the close relationship of *Psilotricha*, *Urospinula*, and *Hemiholosticha*, and thus collected them into a single family, either Kahliellidae (Tuffrau 1987) or Psilotrichidae (Jankowski 2007).

The 18S rRNA sequence classifies *Urospinula* into the large *Oxytricha*-clade with a tentative relation to *Kahliella*, which would support the above mentioned assignment to the family *Kahliellidae*. However, statistically, this position is unsupported and the branching of the Psilotrichidae in phylogenetic analyses remains elusive. However, there are two strong ontogenetic arguments that *Psilotricha*, *Psilotrichides*, *Urospinula,* and *Hemiholosticha* belong to the same family: the oral primordium develops in a deep pouch and a migrating part of a ventral cirral row becomes the postoral cirral row. Unfortunately, the deep pouch development of the oral primordium might be a plesiomorphic and thus a phylogenetically weak character because it is found also in the euplotids (Foissner 1996). An oxytrichid relationship, already proposed by Stein (1859a,b), is indicated by the enigmatic genus *Pachycirrus* whose organization is not very different from that of a typical *Oxytricha* (Fig. 11I–K). Indeed, an oxytrichid relationship of the psilotrichids is indicated by the migrating cirri of row 4, which are reminiscent of the cirri in anlage VI of the 18-cirri hypotrichs (the frontoterminal cirri are distinctly separated from the corresponding pretransverse and transverse cirri), and rows R1–4 which might be homologuous to the rows formed from anlagen III–VI. Increased taxon sampling as well as the analyses of genes with different rates of evolution may shed light on the phylogenetic position of the Psilotrichidae as well as of other oxytrichid families.

Materials for a revision of the psilotrichids

Our brief revision is based on Foissner (1983), Esteban et al. (2001), the present and some unpublished data, and two assumptions: *P. acuminata* Stein 1859a; type of the family and genus, has not been restudied and *Pachycirrus*

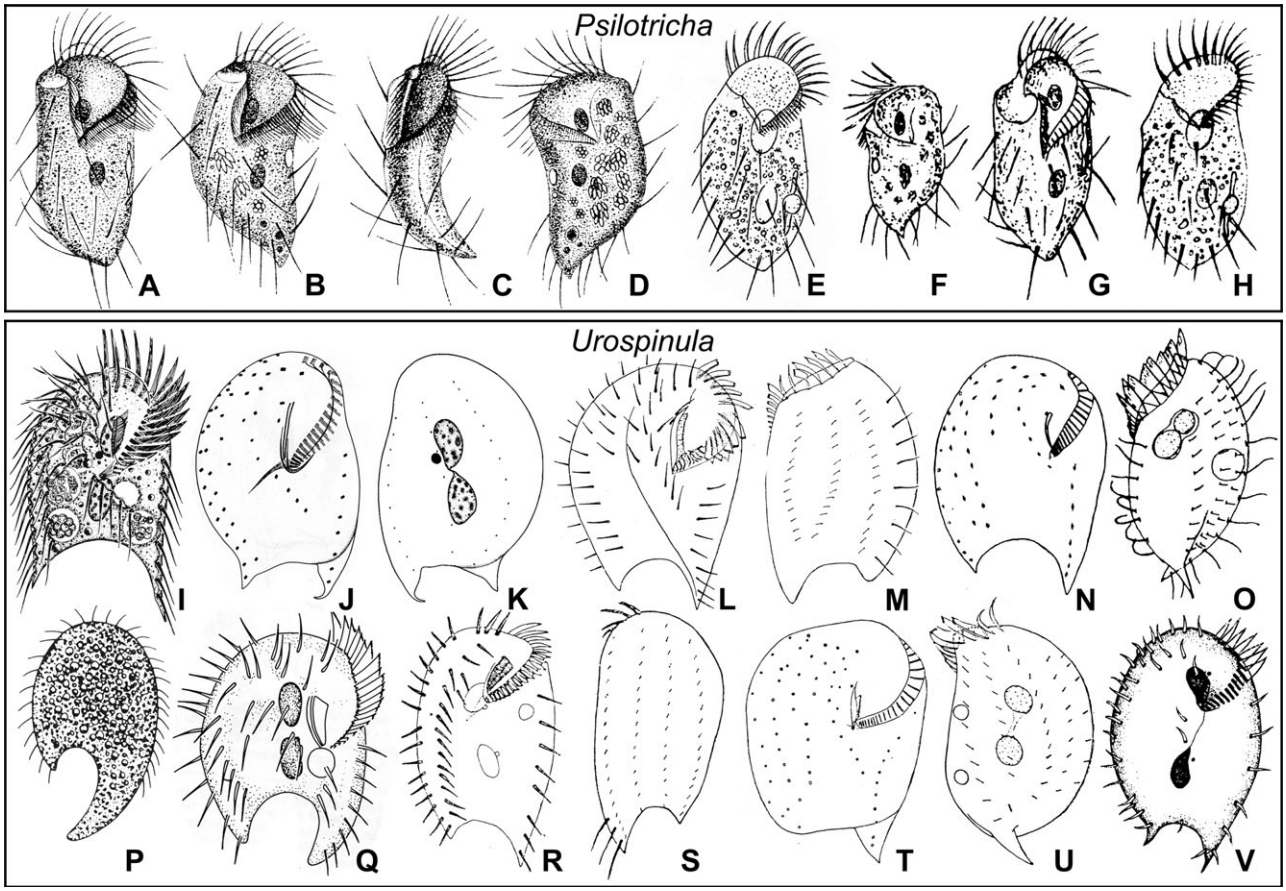

Figure 10 **A–V.** Species classified in the Psilotrichidae, length 40–100 μm. **A–H.** *Psilotricha acuminata* after Stein 1859b (A–D), Roux 1901 (E), Kahl 1932 (F, G), and Grandori and Grandori 1934 (H). **I–Q.** *Urospinula succisa* after Foissner 1983 (I–K), Gelei 1944 (L–O, as *Urospinula bicaudata*), Müller 1786 (P, as *Trichoda succisa*), and Dingfelder 1962 (Q, as *P. acuminata*). **R, S.** *Urospinula calcibia* after Gelei (1944). **T, U.** *Urospinula sinistrocaudata* after Gelei (1944). **V.** *Urospinula simplex* after Dragesco and Dragesco-Kernéis (1986).

costatus Olmo and Esteban 1999 very likely belongs to a distinct family, possibly related to the Psilotrichidae.

How can we be sure that *Urospinula*, *Hemiholosticha*, and *Psilotrichides* are confamilial with *P. acuminata*? First, all have a rigid cortex already described by Stein (1859a,b) as "euplotid". Second, they all have a similar size and an undifferentiated ciliature without, e.g. distinct frontal, marginal, and buccal cirri. Third, *P. hawaiiensis* has a great overall similarity with *P. acuminata*.

Stein (1859a,b) discovered *P. acuminata* in a poorly studied habitat, viz. in a puddle strongly contaminated by liquid manure. All other species and populations described later are from more ordinary limnetic habitats, such as clean and eutrophic ephemeral puddles. The oral apparatus is distinctly different from that of other psilotrichid genera (Fig. 9A–D). It is rather large and has a deep buccal cavity, the right margin of which is occupied by a long, vertical paroral membrane. The adoral zone of membranelles is semicircular, and the length of the cilia abruptly decreases in the proximal third, a distinct feature present also in *Hemiholosticha* spp. (W. Foissner, unpubl. data). In contrast, the somatic cirral pattern is quite similar to that of *Psilotrichides* and *Urospinula* (Fig. 1A and 10I). A further feature shared by *Psilotricha* and *Psilotrichides* is the lateral location of the contractile vacuole while all other described populations have it posterior of the buccal vertex, i.e. near the body center (Fig. 10I, Q and 11C, N).

Pachycirrus costatus, which was discovered in a nonflooded Petri dish culture with grassland soil from Scotland, poses several problems. It has been "fully" described as a new genus and species by Olmo and Esteban (1999) in a congress abstract. Although not recommended, the name is very likely available according to Article 9 of the International Commission on Zoological Nomenclature (1999) because the genus and species were diagnosed in the abstract. Thus, this must be considered as the original description. Later, Esteban et al. (2001) identified this population as *P. acuminata* Stein 1859a. We disagree because *P. costatus* has a quite different oral apparatus (buccal cavity narrow, paroral minute) and three distinct caudal cirri (Fig. 11I–K). We classify *P. costatus* as *incertae sedis* as we do with *P. dragescoi* Grolière 1975, which is possibly a kahliellid (Fig. 11L); and with *B. viridis* Penard 1922, which has possibly transverse cirri (Fig. 11M, N).

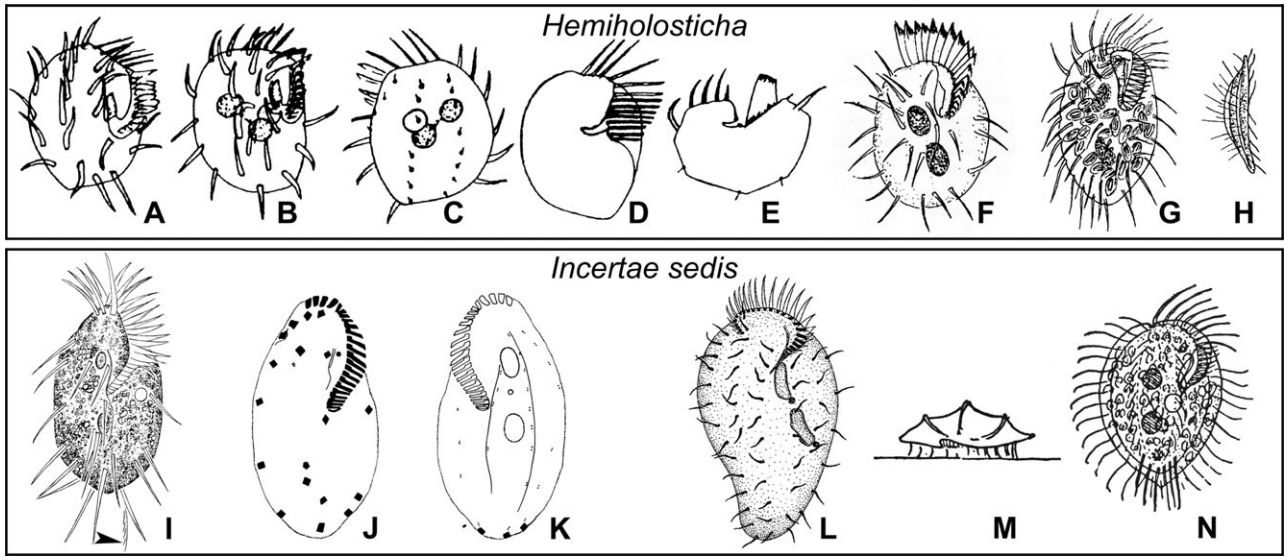

Figure 11 **A–N.** Species classified in the Psilotrichidae, length 40–100 μm. **A–F.** *Hemiholosticha viridis* after Gelei 1954 (A–E) and after Dingfelder 1962 (F, as *Psilotricha viridis*). **G, H.** *Psilotricha viridis* sensu Kahl (1932). **I–K.** *Pachycirrus costatus* Olmo and Esteban 1999 (*Psilotricha acuminata* according to Esteban et al. 2001). **L.** *Psilotricha dragescoi* after Grolière (1975). **M, N.** *Balladyna viridis* after Penard (1922).

TAXONOMIC SUMMARY

Class Spirotrichea Bütschli 1889
Subclass Hypotrichia Stein 1859b
Family Psilotrichidae Bütschli 1889

Improved diagnosis. Medium-sized, ellipsoidal hypotrichs with posterior body end rounded, acute, or with one or two spines. Two macronuclear nodules, usually one micronucleus in between. Contractile vacuole at left body margin or near body center slightly posterior of buccal vertex. Cortex rigid, in some species with distinct ridges. Cirri long and sparse, arranged in several ventral rows, one right and one left marginal row, and a postoral row originating from the posterior, migrating fragment of a ventral row; frontal, buccal, and transverse cirri not distinguishable. Three to five dorsal kineties; caudal cirri absent. Oral apparatus occupies about one-third to one-half of body length, in four distinct patterns (Fig. 9A–D). Oral primordium on body surface, invaginates into a conspicuous pouch; parental undulating membranes maintained or reorganized.

Type genus. *Psilotricha* Stein 1859a

Genera assignable. *Psilotricha* Stein 1859a; *Urospinula* Corliss 1960; *Hemiholosticha* Gelei 1954; and *Psilotrichides* nov. gen.

Remarks. *Pachycirrus* and *B. viridis* Penard 1922; both excluded as explained above. The genus *Balladyna* Kowalewskiego 1882 has a complex nomenclatural and taxonomic history explained by Berger (1999) and Aescht (2001).

Genus *Psilotricha* Stein 1859a

Improved diagnosis. Psilotrichidae with acute posterior end. Contractile vacuole at left margin of body. Adoral zone of membranelles semicircular, length of cilia abruptly decreases in proximal half. Buccal cavity deep, right margin limited by straight undulating membranes along main body axis.

Type species. *Psilotricha acuminata* Stein 1859a (type by monotypy).

Species assignable. *Psilotricha acuminata* Stein 1859a.

Remarks. In the absence of new data (see above), the diagnosis remains incomplete but is sufficient to separate *Psilotricha* clearly from the confamilials. Of particular significance is the abrupt shortening of the cilia in the proximal half of the adoral zone because this rare feature is present also in *Hemiholosticha* (W. Foissner, unpubl. data). One must credit Stein (1859b), who shows this feature clearly in his figures (Fig. 10A, B).

Genus *Urospinula* Corliss 1960

Improved diagnosis. Psilotrichidae with one or two posterior spines. One or two micronuclei between or near macronuclear nodules. Contractile vacuole near midbody. Adoral zone of membranelles C-shaped, length of cilia gradually decreasing from distal to proximal. Right margin of buccal cavity and undulating membranes usually straight in main body axis. Two cirral rows produced by the oral primordium; parental undulating membranes not reorganized; postoral cirral row ontogenetically active.

Type species. *Trichoda succisa* Müller 1786. Foissner (1983) neotypified this species with an Austrian population and combined it with *Psilotricha*. This is outdated, according to the new data. Later, Esteban et al. (2001) combined it with *Urospinula* with which we agree: *U. succisa* (Müller 1786) Esteban et al. 2001.

Species assignable. *Urospinula succisa* (Müller 1786) Esteban et al. 2001 (see above), *U. bicaudata* (Gelei 1944)

Corliss 1960 (syn. of *U. succisa*), *P. acuminata* sensu Dingfelder 1962 (syn. of *U. succisa*), *U. calcibia* (Gelei 1944) Corliss 1960, *U. sinistrocaudata* (Gelei 1944) Corliss 1960, and *Urospinula simplex* Dragesco and Dragesco-Kernéis 1986.

Remarks. The diagnosis is based on the study of Foissner (1983) and the present data. Possibly, *U. calcibia* (Gelei 1944) lacks a postoral cirral row. Species are distinguished by the number of ventral cirri and cirral rows, respectively (about 54 in *U. succisa*, only 15 in *U. simplex*), the number of posterior spines (only one in *U. sinistrocaudata*), and the number of dorsal kineties (*U. succisa* with three, *U. calcibia* with four, and *U. sinistrocaudata* with five). The GenBank code for the Japanese population of *U. succisa* is KF411460.

Genus *Hemiholosticha* Gelei 1954

Improved diagnosis. Ellipsoidal Psilotrichidae with micronucleus usually between macronuclear nodules. Contractile vacuole near body center. Adoral zone of membranelles C-shaped, length of cilia abruptly decreasing in proximal half. Right margin of buccal cavity and paroral membrane distinctly curved (cyrtohymenid). Two cirral rows produced by the oral primordium; parental undulating membranes reorganized; postoral cirral row ontogenetically active.

Type species. *Hemiholosticha viridis* Gelei 1954.

Species assignable. *Hemiholosticha viridis* Gelei 1954 and *P. viridis* sensu Kahl (1932).

Remarks. The majority of the diagnosis is based on three unpublished species from Germany and Brazil (for an example, see Fig. 9E, F) because the description of Gelei (1954) appears rather incomplete and bewildering. He describes the buccal apparatus as follows (Fig. 11A–E): "The peristomial field is not a deepening but appears as a projecting wedge which runs into the adoral zone. Thus, the lip membrane, which is very short, is associated with a very short row of membranelles, forming a ciliary row". This text is difficult to interpret, especially because Fig. 11E shows a well developed buccal cavity. Generally, the cyrtohymenid oral apparatus in this kind of psilotrichs is difficult to analyze because part of the buccal cavity is covered by the buccal lip. Perhaps, Gelei (1954) recognized only the endoral membrane. We shall discuss this matter in detail when we describe these species.

Genus Psilotrichides nov. gen

Diagnosis. Pyriform Psilotrichidae with micronucleus usually between macronuclear nodules. Contractile vacuole at left margin of body. Adoral zone of membranelles semicircular, length of cilia gradually decreasing from distal to proximal. Buccal cavity and undulating membranes curved and distinctly slanted (~20–45°) to longitudinal axis of cell; with buccal ridge, i.e. conspicuously thickened right margin of buccal cavity. Oral primordium produces one cirral row; parental undulating membranes reorganized; postoral cirral row ontogenetically inactive.

Type species. *Psilotrichides hawaiiensis* nov. spec.

Species assignable. *Psilotrichides hawaiiensis* nov. spec.

Species Psilotrichides hawaiiensis nov. spec.

Diagnosis. Size in vivo about 65 × 45 μm; bluntly pyriform. Two broadly ellipsoidal macronuclear nodules and one rotund micronucleus in between. On average a total of 23 cirri in four ventral, one postoral, and one right and one left marginal row; left marginal cirri usually short and partially unciliated, last cirrus in center of posterior pole and distinctly elongated, providing the species with a table tennis racket shape. On average 33 dorsal bristles in three kineties. Adoral zone occupies about 43% of body length, on average composed of 21 membranelles widely spaced in anterior half.

Type locality. Surface soil and litter (0–3 cm) from an ephemeral swamp on Koko Head, Oahu Island, Hawaiian archipelago, W157°41′44″ N21°15′52″.

Etymology. The epithet refers to the type locality.

Type material. One holotype slide and eight paratype slides with morphostatic and dividing, protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria (Biologiezentrum des Oberösterreichischen Landesmuseums), Linz (LI) reg. no. 2013/38-46. Relevant specimens have been marked by black ink circles on the coverslip.

ACKNOWLEDGMENTS

The Austrian and the German Science Fund provided financial support (FWF project P22846-B17 and DFG project STO4141/3-2). The technical assistance of Mag. Barbara Komm, Johannes Rattey M.Sc., Robert Schörghofer, Hans-Werner Breiner, and Andreas Zankl is gratefully acknowledged. Thanks to Dr. Hubert Blatterer and Dr. Martin Kreutz for collecting the samples, which contained *Psilotrichides hawaiiensis* and *Hemiholosticha* sp., respectively. Further, the senior author thanks Dr. Yasushi Kusuoka (Lake Biwa Museum, Japan) and Dr. Satoshi Shimano (Miyagi University, Japan) for their hospitality and help with sampling.

LITERATURE CITED

Aescht, E. 2001. Catalogue of the generic names of ciliates (Protozoa, Ciliophora). *Denisia*, 1:1–350.

Altschul, S. F., Madden, L. T., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W. & Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, 25:3389–3402.

Berger, H. 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monographiae Biol.*, 78:i–xii +1080.

Berger, H. 2011. Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha). *Monographiae Biol.*, 90: i–xiv + 741.

Borror, A. C. 1972. Revision of the order Hypotrichida (Ciliophora, Protozoa). *J. Protozool.*, 19:1–23.

Bütschli, O. 1889. Protozoa I. III. Abtheilung: Infusoria und System der Radiolaria. *In*: Bronn, H. G. (ed.), Klassen und Ordnungen des Thier-Reichs, wissenschaftlich dargestellt in Wort und Bild. C. F. Winter, Leipzig, p. 1585–2035.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.*, 17:540–552.

Corliss, J. O. 1960. The problem of homonyms among generic names of ciliated protozoa, with proposal of several new names. *J. Protozool.*, 7:269–278.

Corliss, J. O. 1979. The Ciliated Protozoa. Characterization, Classification and Guide to the Literature, 2nd ed. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt. i–xvi + 455 p.

Dingfelder, J. H. 1962. Die Ciliaten vorübergehender Gewässer. *Arch. Protistenk.*, 105:509–658.

Dragesco, J. & Dragesco-Kernéis, A. 1986. Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des ciliés. *Faune Tropicale* (Éditions de l'ORSTOM), 26:1–559.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32:1792–1797.

Eigner, P. 1997. Evolution of morphogenetic processes in the Orthoamphisiellidae n. fam., Oxytrichidae, and Parakahliellidae n. fam., and their depiction using a computer method (Ciliophora, Hypotrichida). *J. Eukaryot. Microbiol.*, 44:553–573.

Esteban, G. F., Olmo, J. L. & Finlay, B. J. 2001. Redescription of *Psilotricha acuminata* Stein, 1859 and revisions of the genera *Psilotricha* and *Urospinula* (Ciliophora, Hypotrichida). *J. Eukaryot. Microbiol.*, 48:280–292.

Fauré-Fremiet, E. 1961. Remarques sur la morphologie comparée et la systématique des ciliata Hypotrichida. *C. R. Hebd. Séanc. Acad. Sci., Paris*, 252:3515–3519.

Foissner, W. 1983. Morphologie und Morphogenese von *Psilotricha succisa* (O. F. Müller, 1786) nov. comb. (Ciliophora, Hypotrichida). *Protistologica*, 19:479–493.

Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Prog. Protistiol.*, 2:69–212.

Foissner, W. 1989. Morphologie und Infraciliatur einiger neuer und wenig bekannter terrestrischer und limnischer Ciliaten (Protozoa, Ciliophora). *Sber. Öst. Akad. Wiss. Mathematisch-naturwissenschaftliche Klasse, Abt. I*, 196 (year 1987):173–247.

Foissner, W. 1992. Estimating the species richness of soil protozoa using the "non-flooded Petri dish method". *In*: Lee, J. J. & Soldo, A. T. (ed.), Protocols in Protozoology, Society of Protozoologists, Lawrence. p. B-10.1–B-10.2.

Foissner, W. 1996. Ontogenesis in ciliated protozoa, with emphasis on stomatogenesis. *In*: Hausmann, K. & Bradbury, P. C. (ed.), Ciliates: Cells as Organisms. Gustav Fischer, Stuttgart, Jena, Lübeck, Ulm. p. 95–177.

Foissner, W. 2012. *Schmidingerothrix extraordinaria* nov. gen., nov. spec., a secondarily oligomerized hypotrich (Ciliophora, Hypotricha, Schmidingerotrichidae nov. fam.) from hypersaline soils of Africa. *Eur. J. Protistol.*, 48:237–251.

Foissner, W. & Al-Rasheid, K. A. S. 2006. A unified organization of the stichotrichine oral apparatus, including a description of the buccal seal (Ciliophora: Spirotrichea). *Acta Protozool.*, 45:1–16.

Foissner, W. & Xu, K. 2007. Monograph of the Spathidiida (Ciliophora, Haptoria). Vol. I: Protospathidiidae, Arcuospathidiidae, Apertospathulidae. *Monographiae Biol.*, 81: i–ix + 485 p.

Foissner, W. & Stoeck, T. 2011. *Cotterillia bromelicola* nov. gen., nov. spec., a gonostomatid, bromeliad-inhabiting ciliate (Ciliophora, Hypotricha) with *de novo* originating dorsal kineties. *Eur. J. Protistol.*, 47:29–50.

Galtier, N., Gouy, M. & Gautier, C. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci.*, 12:543–548.

Gelei, J. 1944. Különleges planktonikus hypotrichák az idöszakos vizekben. Adaiok mágyarország csillós állatvilágához. (XII. közlmény.) (Sonderbare planktonische Hypotrichen in den temporären Gewässern. XII. Beitrag zur Ciliatenfauna Ungarns). *Múz. Fúz. Kiadja erd. Múz. Egy., Uj Folyam*, 2:137–157 (in Hungarian with detailed German summary).

Gelei, J. 1954. Über die Lebensgemeinschaft einiger temporärer Tümpel auf einer Bergwiese im Börzsönygebirge (Oberungarn) III. Ciliaten. *Acta Biol. Hung.*, 5:259–343.

Gouy, M., Guindon, S. & Gascuel, O. 2010. SEAVIEW Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.*, 27:221–224.

Grandori, R. & Grandori, L. 1934. Studî sui protozoi del terreno. *Boll. Lab. Zool. Agr. Bachic. R. Ist. Sup. Agr. Milano*, 5:1–339.

Grolière, C. A. 1975. Descriptions de quelques ciliés hypotriches des tourbières a sphaignes et des étendues d'eau acides. *Protistologica*, 11:481–498.

Guindon, S. & Gascuel, O. 2003. A simple, fast, accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.*, 52:696–704.

He, Y. & Xu, K. 2011. Morphology and small subunit rDNA phylogeny of a new soil ciliate, *Bistichella variabilis* n. sp. (Ciliophora, Stichotrichia). *J. Eukaryot. Microbiol.*, 58:332–338.

International Commission on Zoological Nomenclature 1999. International Code of Zoological Nomenclature. International Trust for Zoological Nomenclature, London. 306 p.

Jankowski, A. W. 2007. Phylum Ciliophora Doflein, 1901. *In*: Alimov, A. F. (ed.), Protista Part 2. Nauka, St. Petersburg. p. 415–993.

Kahl, A. 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Dtl.*, 25:399–650.

Kowalewskiego, M. 1882. Przyczynek do historyi naturalnéj oxytrichów. *Pam. Fizyogr.*, 2:395–413 (in Polish with French explanation of plates).

Kreutz, M. & Foissner, W. 2006. The *Sphagnum* ponds of Simmelried in Germany: a biodiversity hot-spot for microscopic organisms. *Protozool. Monogr.*, 3:1–267.

Lynn, D. H. 2008. The Ciliated Protozoa. Characterization, Classification, and Guide to the Literature, 3rd ed. Springer, Dordrecht, i–xxxiii + 605 p.

Maddison, W.P. & Maddison, D.R. 2005. MacClade v. 4.0.8. Sinauer Assoc., Sunderland.

Medlin, L., Elood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71:491–449.

Müller, O. F. 1786. Animalcula Infusoria Fluviatilia et Marina, quae Detexit, Systematice Descripsit et ad Vivum Delineari Curavit. Hauniae, Mölleri. 367 p.

Olmo, J. L. & Esteban, G. F. 1999. *Pachycirrus costatus* n. g., n. sp., a new hypotrich ciliate (Ciliophora, Hypotrichida) occurring in soil? 3rd European Congress of Protistology and 9th European Conference on Ciliate Biology, Helsingør (Denmark). Book of Abstracts, 56 p.

Penard, E. 1922. Études sur les Infusoires d'Eau Douce. Georg & Cie, Genève, 331 p.

Posada, D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.*, 25:1253–1256.

Rambaut, A. 2006. FigTree. Institute of Evolutionary Biology, University of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree. Accessed on June 2013.

Roux, J. 1901. Faune infusorienne des eaux stagnantes des environs de Genève. *Mém. Inst. Natn. Génev.*, 19:1–149.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A fast bootstrap algorithm for the RAxML web-servers. *Syst. Biol.*, 57:758–771.

Stein, F. 1859a. Characteristik neuer Infusorien-Gattungen. *Lotos*, 9(2–5):57–60.

Stein, F. 1859b. Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. W. Engelmann, Leipzig, i–xii+ 206 p.

Stiller, J. 1974. Ergänzungen der von Fauré-Fremiet vorgenommenen Neuordnung der hypotrichen Ciliaten. *Annls Hist.-Nat. Mus. Natn. Hung.*, 66:129–133.

Tagliani, G. 1922. Studi critico-sistematici sugl' infusori. *Annuar. R. Mus. Zool. Univ. Napoli (Nuova Serie)*, 5:1–13.

Tuffrau, M. 1987. Proposition d'une classification nouvelle de l'ordre Hypotrichida (Protozoa, Ciliophora), fondée sur quelques données récentes. *Annls Sci. Nat. (Zool.)*, 13e Série (years 1986-1987) 8:111–117.

Voß, H.-J. & Foissner, W. 1996. Divisional morphogenesis in *Steinia sphagnicola* (Ciliophora, Hypotrichida): a comparative light and scanning electron microscopic study. *Eur. J. Protistol.*, 31:31–46.