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

Domingo Heber, Thorsten Stoek & Wilhelm Foissner

**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 *Urospina 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; *Urospina* 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 Bünchli, 1889 and the genera contained.

**Keywords**

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

**Correspondence**

W. Foissner, Universität Salzburg, Fachbereich Organische 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

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 *Urospina* 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. Boror (1972) followed Dingfelder (1962) and raised the subfamily to family rank: *Psilotrichidae*. Stiller (1974) classified *Psilotricha* into the Holostichidae Fauré-Fremiet 1961; synonymized *Urospina* 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 gelei*.

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 Stichotrichidae 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 fronto-ventral 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 dish cultures 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 (Biologizentrum 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, Hilden, Germany), 18S rDNA amplification with eukaryote specific primers EuK A and EuK B (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 chooretrichs 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-1 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...
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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. hawaiensis nov. spec.

Psilotrichides hawaiensis has a size of 55–75 x 40–50 µm in vivo, usually it is about 65 x 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. hawaiensis 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 x 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 x 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 hawaiensis feeds on colorless flagellates of the genera Polytoma and Hylagonium, 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 the right and the left marginal row (Table 1; Fig. 1A, K, 2B, D–G and 3B).

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*

| Characteristicsa | 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, numberb | 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, numberd | 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 | 24.0 | 6.0 | 13.0 | 21 |
| Adoral zone, percentage of body length | 43.3 | 42.4 | 3.2 | 0.7 | 37.9 | 50.0 | 21 |
| Adoral membranelles, number | 21.0 | 21.0 | 1.0 | 0.2 | 19.0 | 23.0 | 21 |
| Adoral membranelles, length of widest base (µm) | 7.6 | 8.0 | 0.8 | 0.2 | 10.7 | 6.0 | 9.0 | 21 |
| Buccal cavity, width (µm) | 10.1 | 10.0 | 1.2 | 0.3 | 12.2 | 8.0 | 13.0 | 21 |
| Paroral, distance to anterior body end (µm) | 11.5 | 11.0 | 0.7 | 0.2 | 6.5 | 11.0 | 14.0 | 21 |
| Paroral, length (µm) | 11.8 | 12.0 | 1.3 | 0.3 | 10.9 | 10.0 | 15.0 | 21 |
| Endoral, distance to anterior body end (µm) | 9.9 | 10.0 | 1.1 | 0.2 | 11.2 | 8.0 | 13.0 | 21 |
| Endoral, length (µm) | 32.4 | 32.0 | 2.6 | 0.6 | 8.1 | 27.0 | 37.0 | 21 |
| Left marginal cirral row, distance to anterior body end (µm) | 16.0 | 16.0 | 2.5 | 0.5 | 15.7 | 11.0 | 20.0 | 21 |
| Left marginal row, second cirrus to posterior BE, distance (µm) | 11.8 | 12.0 | 1.3 | 0.3 | 10.9 | 10.0 | 15.0 | 21 |
| Left marginal 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 | 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 | 25.5 | 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; x = arithmetic mean.

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

bThree to four nodules in four of 51 specimens.

cZero in one specimen; not included in morphometry.

dNot recognizable in two of 23 specimens.

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*Psilotrichides hawaiiensis* nov. gen., nov. spec.
Psilotrichides hawaiiensis nov. gen., nov. spec.

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).

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*Journal of Eukaryotic Microbiology* 2014, 61, 260–277
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).
Ontogenesis of \textit{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 \textit{U. succisa}.

**Figure 3** A–G. \textit{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 \mu m (C–F), 15 \mu m (B), and 25 \mu m (A, G).
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 rows 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

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**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.
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 dikineties 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 orient increasingly parallel to the proximal part of the adoral...
The 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 cilium (Fig. 6F, H).

**Late dividers (Fig. 5E and 7A–C)**

When the division furrow becomes recognizable, the prot-er forms a new posterior body end at the left margin (Fig. 7A, B). The buccal cavity of the proter commences redee-pening and the undulating membranes finish reorganiza-tion 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 cirr-al 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 phyloge-netic 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](https://example.com/fig7.png)  
**Figure 7** A–D. *Psilotrichides hawaiensis* (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.
Psilotrichides hawaiiensis nov. gen., nov. spec.

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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.

Psilotrichides hawaiiensis as a new genus and species

Foissner (1989) characterized the oxytrichid genera Oxytricha, Stylonychia, Cyrtophymena, 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

(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, Cyrtophymena, 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
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 apparatus 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; ML = micronucleus; PM = paroral membrane; RI = buccal ridge; RM = right marginal cirral row. Scale bar 30 μm.

**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 Schrägerei” 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; ML = micronucleus; PM = paroral membrane; RI = buccal ridge; RM = right marginal cirral row. Scale bar 30 μm.

**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 Spirofiliidae. 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 Kahlilidae (Tuffrau 1987) or Psilotrichidae (Jankowski 2007).

The 18S rRNA sequence classifies Urospinula into the large Oxytricha-clade with a tentative relation to Kahlia, which would support the above mentioned assignment to the family Kahlilidae. 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. 111–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 homologous 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*
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 membranes 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 kahliliellid (Fig. 11L); and with B. viridis Penard 1922, which has possibly transverse cirri (Fig. 11M, N).
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 congeneric species. 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) neotyped 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. succissa (Müller 1786) Esteban et al. 2001.

Species assignable. Urospinula succisa (Müller 1786) Esteban et al. 2001 (see above), U. bicaudata (Gelei 1944)
The majority of the diagnosis is based on three
Type species. 

**Genus Hemiholosticha Gelei 1954**

**Improved diagnosis.** Ellipsoidal Psilotrichidae with micro-
**Remarks.** The diagnosis is based on the study of Foissner
(nucleus usually between macronuclear nodules. Contractile
(syn. of U. succisa) and the present data. Possibly, *U. calcibia* (Gelei
vacuole near body center. Adoral zone of membranelles C-shaped, length of cilia abruptly decreasing
vesicular apparatus as follows (Fig. 11A–E): “The peristomial field is not a deepening but appears as a
membranelles with four, and *U. sinistrocaudata* with five. The
membrane distinctly curved (cytohymenid). Two cirral
rows produced by the oral primordium; parental undulating
membranes reorganized; postoral cirral row ontogeneti-
ically 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):
“arotund micrornaucleus 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 unciuated, 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
kinetes. 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, W15°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-impreg-
nated specimens have been deposited in the Biology Cen-
tre of the Museum of Upper Austria (Biologizentrum des
Österreichischen 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 finan-
cial 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 acknowl-
edged. 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 Univer-
sity, Japan) for their hospitality and help with sampling.

**LITERATURE CITED**

Aescht, E. 2001. Catalogue of the generic names of ciliates (Pro-
tozoa, Ciliophora). Denisia, 11:350.

Altschul, S. F., Madden, T. L., 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 pro-
grams. *Nucleic Acids Res.*, 25:3389–3402.

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

Berger, H. 2011. Monograph of the Gonostomatidae and Kahlilie-
dae (Ciliophora, Hypotrichia). *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. Abteilung: Infusoria und Sys-
tem der Radiolaria. *Inr.* Bronn, H. G. (ed.), Klassen und Ordnun-
gen des Tier-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 vor

Esteban, G. F., Olmo, J. L. & Finlay, B. J. 2001. Redescription of

Galtier, N., Gouy, M. & Gautier, C. 1996. SEAVIEW and PHYLO.

Gelei, J. 1944. Különleges planktonikus hypotrichák az időszakos vizekben. Adaiok mág yaroszág csíldső állatvilágához. (XII. közmény.) (Sonderbare planktonische Hypotrichen in den temporären Gewässern. XII. Beitrag zur Ciliatenfauna Ungarns. Múz. Fűz. Kiad. Múz. Egy., Uj Folyam, 2:137–157 (in Hungarian with detailed German summary).

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

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

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

Grolle, C. A. 1975. Descriptions of 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: Ali-mov, A. F. (ed.), Protista Part 2. Nauka, St. Petersburg. p. 415–993.

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

Kowalewskiego, M. 1882. Przyczyny do history naturalnej oxytrichów. Pam. Fyzog., 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–xxiii + 605 p.

Maddison, W.P. & Maddison, D.R. 2006. MacClade v. 4.08. Sina-tistologica, Lawrence. p. B-10.1

Maddison, W.P. & Maddison, D.R. 2005. MacClade v. 4.08. Sina-tistologica, Lawrence. p. B-10.1

Roland, J. & 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 Infusoria 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 environ de Genève. Mém. Inst. Natn. Génev., 19:1–149.

ραπεριμήνα και την απεικόνιση χρησιμοποιούνται για τη δημιουργία και την εικονική μεταφορά των υπολογισμών. Phylogen. Comput. Appl. Biosci., 12:543–548.
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 sugli 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.