Morphology and phylogeny of the ciliate *Psilotricha silvicola* n. sp. (Alveolata, Ciliophora) from woodland soils in the United Kingdom

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Short title: A new soil ciliate: *Psilotricha silvicola* n. sp.

ABSTRACT

The genus *Psilotricha* was established by Stein in 1859, with *P. acuminata* as the type species within the family Oxytrichidae. This species lacked a full description until it was re-discovered in 2001, showing that its morphological and morphogenetic characters confirmed the inclusion in the family Oxytrichidae. Since then, the genus *Psilotricha* has had a convoluted taxonomy despite the morphological evidence available. In this paper, we describe a new *Psilotricha* species, *Psilotricha silvicola* n. sp., from woodland soils in Southern England (United Kingdom). The morphology was investigated in live and protargol-impregnated specimens. Our findings show that *P. silvicola* n. sp. shares morphological characteristics with *P. acuminata,* including the distinctive cell shape and the long and sparse cirri. Phylogenetic analysis of the 18S rRNA gene places this new species within the family Oxytrichidae, nested apart from the family Psilotrichidae (which includes the genera *Urospinula*, *Psilotrichides* and *Hemiholosticha*), in a clade containing species of the family Oxytrichidae. Furthermore, the morphology of another *Psilotricha* species, *P. viridis*, found in a freshwater pond in the same woodland area, is also here described, bringing additional insight into the taxonomy of the genus. Our findings provide further evidence for inclusion of the genus *Psilotricha* within the oxytrichids.

Introduction

Psilotricha acuminata was described as the type species of the hypotrich genus *Psilotricha* by Stein in 1859 (Stein 1859a, b) but its infraciliature remained unknown until 2001, when Esteban et al (2001a, b) re-discovered the species in grassland soils from Scotland (UK). Unfortunately, no molecular data are available for this species. Stein (1859b) had proposed the inclusion of the genus *Psilotricha* within the family Oxytrichidae, which was then corroborated by Esteban et al. (2001a) following morphological and morphogenetic findings in *P. acuminata*: having two rows of marginal cirri (one left and one right), reduced number of transverse cirri, presence of caudal cirri, and having long primary primordia in the early stages of the morphogenesis. Lynn (2008) and Heber et al. (2014) placed the genus in the family Psilotrichidae Bütschli, 1889, along with *Urospinul*a, *Hemiholosticha*, and *Psilotrichides* based on the diagnostic characters of absence of caudal cirri, even though *Psilotricha* as described by Stein (1859a) and Esteban et al. (2001a) does have three caudal cirri. The phylogenetic analysis performed by Heber et al. (2014) placed *Urospinula succisa*, the only sequenced species within the family Psilotrichidae at that time, in a clade containing species from the families Oxytrichidae, Kahliellidae and Halteriidae, although neither the position of *Urospinula* nor the position of its clade members were statistically supported. Recently, Luo et al. (2019) increased the taxon sampling within the family Psilotrichidae, including the description of a *Psilotrichides hawaiiensis* population (Heber et al., 2014), and a new species, *Hemiholosticha kahli*, both from Guam. The 18S rRNA gene phylogeny grouped them with *Urospinula succisa*, in a well-supported monophyletic group, reinforcing their assignment to the family and shedding some light on the phylogeny of the Psilotrichidae.

Here, we describe a new species within the genus *Psilotricha* Stein, 1859, similar to, but distinct from *P. acuminata*, and we provide for the first time the 18S rRNA gene sequence of a species in the genus *Psilotricha.* Our findings provide further evidence that the genus *Psilotricha* does not belong to the family Psilotrichidae, but to the Oxytrichidae, as established by Stein (1859a, b) and Esteban et al. (2001a).

Results

Morphology of *Psilotricha silvicola* n. sp. (U.K. population)

Order Sporadotrichida Fauré-Fremiet, 1961

Family Oxytrichidae Ehrenberg, 1830

Genus *Psilotricha* Ehrenberg, 1831

New species *silvicola*

Diagnosis: Medium-sized (usually 55-80 µm long) soil hypotrich ciliate with oblong or slightly rounded outline. Dorso-ventrally flattened with two sharp ribs on the dorsal side, one being longer and twisted, and more pronounced than the other. Cirri are very long and sparse. Lateral view of some cells shows a caudal tail produced by the twisted long dorsal rib. Adoral zone of membranelles occupying 46 % of cell length, on average composed of 23 membranelles. The ventral side includes one right marginal row of cirri, and one left marginal row of cirri. The infraciliature on the ventral surface includes three frontal cirri, four fronto-ventral cirri, one post-oral ventral cirrus, one pre-transverse ventral cirrus, three transverse cirri, and three caudal cirri, the latter originating from dorsal kineties 1, 3 and 4, respectively. The dorsal infraciliature is composed of five dorsal kineties, including one dorso-marginal kinety. Two macronuclear nodules, with one micronucleus placed between them; some cells may have additional one micronucleus anterior to the first macronuclear nodule.

Etymology: *silvicola*, meaning living in or inhabiting a forest, forests or woods, referring to the woodland area from where the new species was isolated at the East Stoke Fen Nature Reserve.

Type material: holotype and paratypes: a permanent preparation slide with protargol-impregnated specimens has been deposited in the Natural History Museum, London (United Kingdom). The holotype is marked with a black circle. Accession number: NHMUK 2020.8.12.1. The species is registered with ZooBank (urn:lsid:zoobank.org:pub:483C0224-F61D-4EA3-8818-02128BEA36CC).

Type locality: East Stoke Fen Nature Reserve, East Stoke, Wareham, Dorset (United Kingdom). Grid reference SY 86531 86614.

A new soil ciliate: *Psilotricha silvicola* n. sp.

Gene sequence: The 18S rRNA gene sequence of *Psilotricha silvicola* has been deposited in the GenBank under the accession number: MN567690.1. It is 1,766 base pairs long.

Description: the size of the living cells is 55-80 µm long x 30-50 µm wide, usually about 65 x 40 µm (Table 1). Cells are colourless but can also be slightly greyish due to the presence of particles in the cytoplasm (Fig. 1A, B). The cirri are very long and sparse - a common morphological feature of the genus *Psilotricha*. *P. silvicola* n. sp. has three types of movement: swimming, crawling, and filter-feeding (Fig. 1D), as also observed in *P. acuminata* (Esteban et al. 2001a).

Living cells are oblong or slightly rounded in outline, dorso-ventrally flattened (Fig. 1A, B), resembling ciliates of the genus *Euplotes*. Cells of older populations are more elongated than younger ones. The anterior and posterior ends are rounded but the latter can also be pointed (Figs 1A and 2A). The single contractile vacuole is located in the middle of the cell, towards the left margin (Fig. 1E). The dorsal surface has two longitudinal ribs positioned to the left and right of the main longitudinal axis (Fig. 2A), with the one on the right being longer than the one on the left. The lateral view of some cells shows a caudal tail due to the twisted dorsal rib (Fig. 1C, D). All cells investigated had two macronuclear nodules, about 9 x 3 µm, one in the anterior half of the cell, the other in the posterior half. There is one micronucleus, 3 µm across on average, positioned between the two macronuclei, and close to the equator of the cell (Fig. 1A, Table 1). However, 36% of the cells investigated presented two micronuclei, one between the two macronuclei, and the other one anterior to the first macronuclear nodule.

The adoral zone of membranelles (AZM) is well developed (occupies an average of 46 % of body length) and consists of 19-26 polykinetids (Figs 1 and 2, Table 1), each formed by three rows of kinetosomes, one of them is shorter than the rest. The cilia are long in anterior part of the AZM, and they decrease in length in the posterior part of the AZM. In protargol-stained specimens, the paroral membrane is formed by a zig-zag line of basal bodies that optically intersects the endoral membrane (Fig. 4A) although in several specimens it follows a parallel pattern.

The somatic ciliature of *P. silvicola* n. sp. on its ventral surface includes: three frontal cirri close to the edge of the anterior part of the cell, four fronto-ventral cirri, one post-oral ventral cirrus, one pre-transverse ventral cirrus, and three transverse cirri close to the posterior end of the cell, one to the right and two to the left of the cell axis (Figs 3A and 4A, Table 1). The pre-transverse ventral cirrus (PTVC) is smaller than the transverse cirri (TC) and placed very close to the right-most transverse cirrus (Figs 3-A).

The right marginal row of cirri (RMC) and the left marginal row of cirri (LMC) are non-confluent posteriorly. As common to the genus *Psilotricha*, and typical of other oxytrichids, the RMC starts close to the cell equator and two of the fronto-ventral cirri (Figs 3A and 4A).

The RMC consists invariably of three cirri (Figs 3A and 4A). Meanwhile the LMC comprises four, or sometimes three, cirri (Table 1; Figs 3A and 4A).

The dorsal infraciliature is composed of five dorsal kineties (DK) including one dorsomarginal kinety (DMK) (Figs 3B and 4B). Only two out of the 30 cells investigated presented four dorsal kineties, i.e. they lacked DK4. However, we cannot exclude that we overlooked DK4 in these two cells, since it is very short and it can go unnoticed due to the poorer impregnation of the dorsal infraciliature of those two specimens. DK1 starts at about the level of the proximal end of AZM; DK2 occupies only the middle third of the body; DK3 is bipolar but slightly shortened anteriorly; DK4 is very short and located in the posterior quarter of the cell (Fig. 4), and DK5/DMK extends from slightly above the level of the anterior end of DK3 to the level of the first right marginal cirrus (Figs 3B and 4B, Table 1). There are three caudal cirri, each at the posterior end of dorsal kineties 1, 3 and 4 (Figs 3 and 4), respectively.

Occurrence and ecology

Soil sample collection was carried out for 19 months; *P. silvicola* n. sp. was recorded in all sampling occasions after rewetting air-dried soils for ten days (see Methdos). The ciliate grows well in rewetted soils at room temperature if some boiled wheat grains are added to encourage bacterial growth. *P. silvicola* n. sp. thrives in these enriched cultures for approximately ten days.

Phylogenetic relationships of *Psilotricha silvicola* n. sp.

The investigated 18S rRNA gene sequence of *Psilotricha silvicola* n. sp. was 1,766 base pairs long and had a 45.5% GC content.

The maximum likelihood (ML)-derived genealogy was in general congruent with the Bayesian inference (BI) topology, thus we present here the ML tree with node supports from both methods (Fig. 7). *Psilotricha silvicola* n. sp. clusters away from the well-supported subclade (98.2% ML) containing species from the family Psilotrichidae (*Psilotrichides hawaiiensis*, *Urospinula succisa* and *Hemiholosticha* *kahli*), which is sister to another well-supported subclade (95% ML, 1.00 BI) containing two species from the family Urostylidae (*Uroleptus lepisma* and *Uroleptus pisces*). By contrast, *P. silvicola* n. sp. is nested within a clade composed of species of the family Oxytrichidae (*Hemiurosomoida longa, Paraurostyla weissei*, *Rubrioxytricha ferruginea*, *Cyrtohymena citrina*, *Onychodromopsis flexilis*, *Sterkiella histriomuscorum*, *Gastrostyla steinii,* and *Stylonychia mytilus*), with moderate to high support (84.4% ML, 1.00 BI). The evolutionarily-intriguing obligate gut symbiont of lumbricid earthworms *Plagiotoma lumbrici* (family Plagiotomidae, Obert and Vd'ačný 2019) also nests within this group.

The phylogenetic analyses showed that the family Oxytrichidae might be polyphyletic, which is consistent with previous publications (Chen *et al*., 2017; Jung et al. 2015; Kumar et al. 2014; Schmidt et al. 2007; Obert and Vd'ačný 2019).

Psilotricha viridis sensu Kahl 1932

We had the opportunity to investigate the morphology and infraciliature of a population of *P. viridis* that we found in a freshwater pond, also at the East Stoke Fen Nature Reserve (Fig. 6). Unfortunately, we could not obtain molecular data for *P. viridis*. However, the morphological study showed that this *P. viridis* population does not match the diagnostic features of the genus *Psilotricha* but rather those of *Hemiholosticha viridis* Gelei, 1954 (Esteban et al. 2001a), *Psilotricha viridis* sensu Kahl, 1932 (Esteban et al. 2001a), a German population of *Hemiholosticha* sp*.* showed in Heber et al. (2014), and *H. kahli* Luo et al. (2019). Our UK population also has intracellular algae with one or more eye spots, and Chlorella-like endosymbionts of very small size (approx. 5µm) and difficult to notice due to the presence of the larger intracellular algae.

Discussion

Comparison of *Psilotricha silvicola* n. sp. with *P. acuminata* and other ciliate species

The morphological differences between *Psilotricha silvicola* n. sp. and related ciliates, including *P. acuminata* Stein, 1859 (Esteban et al. 2001a), *Urospinula succisa* Müller, 1786 (as described by Foissner 1983), *Psilotrichides hawaiiensis* Heber et al. (2014), and *Hemiholosticha kahli* (Luo et al., 2019) are shown in Table 2.

Psilotricha silvicola n. sp. from the East Stoke Fen soils (Dorset, UK) and *P. acuminata* Stein, 1859 from Sourhope soils (Scotland, UK) (Esteban et al. 2001a) share common characteristics, such as habitat type, arrangement of the cilia and cirri on the dorsal and ventral surfaces, long and sparse cirri, types of motion, cell shape, having one right and left marginal ventral rows of cirri, presence and number of caudal cirri, deep and wide oral area with very long cilia in the anterior half of the membranelles, one post-oral ventral cirrus, variability of number of macronuclei and micronuclei and their position in the cell (Esteban et al. 2001a) (Fig. 5). However, *P. silvicola* n. sp. differs from *P. acuminata* (Esteban et al. 2001a) in: (i) absence of buccal cirrus *vs.* buccal cirrus present; (ii) three cirri on the right marginal row and three/four cirri on the left marginal row (usually four cirri) *vs.* three/four cirri in the right marginal row and two/three in the left marginal row (usually three in both rows); (iii) absence of ventral cirri *vs.* two ventral cirri present; (iv) one pre-transverse ventral cirrus *vs.* none; (v) three transverse cirri of similar size *vs.* two transverse cirri one longer than the other; (vi) colourless or slight grey cytoplasm *vs.* dark cytoplasm (Table 2; Figs 4 and 5). Although, unfortunately, no molecular data of *P. acuminata* are available, the morphological similarities between these two species merit their assignment to the genus *Psilotricha* Stein, 1859.

The case of “*Psilotricha viridis*”

P. silvicola n. sp. differs from *P. viridis* sensu Kahl, 1932 in having caudal cirri, lacking endosymbiotic algae and the ventral cirral rows (Kahl 1935; Esteban et al. 2001a). Also, in contrast to *P.* viridis sensu Kahl, 1932, *P. silvicola* was found in soil samples, not in a freshwater environment. *Hemiholosticha viridis* Gelei, 1954, *Psilotricha viridis* sensu Kahl, 1932 and *P. geleii* (Gelei 1954) Stiller, 1974 are conspecific within the genus *Hemiholosticha* Gelei, 1954, as is the *Hemiholosticha* population in Heber et al. (2014). Therefore, we consider the population of *P. viridis* (see Fig. 6) found in the freshwater pond at East Stoke Fen (UK) to be *Hemiholosticha viridis* Gelei, 1954. On the basis of the infraciliature pattern, and the presence in the cytoplasm of intracellular algae with eye spot, it seems that the German population of *Hemiholosticha* sp. showed in Heber et al. (2014), and *H. kahli* (Luo et al. 2019) are also most probably *H. viridis* Gelei, 1954.

A feature shared by *Psilotricha*, *Hemiholosticha*, *Urospinula*, and *Hemiholosticha* and, thus, a likely convergent feature, is that the cilia of the adoral zone of membranelles are long in the anterior part, decreasing conspicuously in length in the posterior (proximal) part.

Taxonomy and phylogeny of the genus *Psilotricha*

The taxonomy of the genus has been convoluted in spite of the morphological evidence available in the literature. Stein (1859b) included the genus *Psilotricha* within the family Oxytrichidae; this was confirmed by Esteban et al. (2001a,b), who found and re-described the type species *P. acuminata* Stein, 1859. The inclusion of *Psilotricha* in the family Oxytrichidae has thus far been justified by morphology, i.e. having left and right marginal ventral cirri, reduced number of transverse cirri (although with a few exceptions), and presence of caudal cirri. This infraciliature arrangement is also found in the new *Psilotricha* species here described, *P. silvicola* n. sp. Furthermore, Esteban et al. (2001a) observed the formation of long primary primordia during the early stages of the ontogenesis of *P. acuminata* - a character typical of the family Oxytrichidae. Long primary primordia are considered a key morphogenetic character for classification of hypotrich species into Oxytrichidae (Eigner 1997; Esteban et al. 2001a).

Bütschli (1889) subsequently established the family Psilotrichidae for the genus *Psilotricha*. Lynn (2008) also included *Psilotricha* Stein, 1859 and *Hemiholosticha* Gelei 1954 in that family, and Heber et al. (2014) added two more genera, *Urospinula* Corliss, 1960 and *Psilotrichides*, despite their lack of shared morphological features, and without phylogenetic analysis since no molecular data were available for *Psilotrichides*. Nonetheless, Heber et al. (2014) remarked that *Psilotricha* actually looks like a typical *Oxytricha*, and that the type species, *P. acuminata* (Stein 1859b), re-described by Esteban et al. (2001a, b), certainly presents the features of the family Oxytrichidae. With the discovery of *P. silvicola* n. sp., and its characterization at morphological and molecular (18S rRNA gene) levels we shed light on the phylogenetic relationships of the genus with the three genera included in the family Psilotrichidae, i.e., *Urospinula*, *Psilotrichides* and *Hemiholosticha*. Our finding of *Psilotricha silvicola* n. sp. nesting apart from *Urospinula succisa* in a clade mostly containing species from the family Oxytrichidae, supports the inclusion of *Psilotricha* within this family, as previously proposed by Stein (1859b) and Esteban et al. (2001a, b). Therefore, the family Oxytrichidae now includes *Psilotricha acuminata* Stein, 1859 (Esteban et al. 2001a) (at least at morphological level), and *P. silvicola* n. sp., with the *incertae sedis P. dragescoi* Groliėre, 1975.

The family Psilotrichidae would now consist of the genera *Hemiholosticha*, *Psilotrichides* and *Urospinula*.

Methods

Sampling site and culture methods

Soil samples were collected at the East Stoke Fen Nature Reserve (Wareham, Dorset, United Kingdom; grid reference SY 86531 86614) for 19 months (January 2016 to December 2017). The site is a woodland area with scattered ponds. The soil contained ~30% of organic matter, and the texture is representative of sandy loam with soil pH (H_2O) ranging from 3.8 to 4.5, and soil moisture oscillating from 36 to 55%.

The experimental plot within the fen was 1 x 2 m. Samples were taken with a 4 cm-diameter stainless steel corer from the topsoil to a depth of 5 cm, which includes the litter layer. The overall method to collect and process the soil samples followed Finlay et al. (2000). On each sampling occasion, eight core samples were taken randomly from within the experimental plot to ensure samples were representative of the plot. The soil collected was removed from the corer and placed in a labelled plastic bag to form a bulk sample for that plot, and transferred immediately to the laboratory. The content of the bag was mixed well in a 30 cm diameter glass bowl. A sub-sample of soil was then taken and spread out as a layer in a 15 cm-diameter glass Petri dish, and dried at room temperature (18-22°C) for six days. Five grams of the air-dried soil was rewetted with filtered rain water (Finlay et al. (2000) to stimulate population growth of ciliates, including *Psilotricha silvicola* n. sp.

Morphological methods

Morphometric measurements were performed on the images of living cells taken at 40-400x magnification, and on protargol-impregnated specimens at 1,250x by using QCapture Pro software (QImaging) through an Olympus BH-2 light microscope mounted with a Micropublisher 3.3 RTV camera (QImaging). To reveal the infraciliature, the protargol method as described by Foissner (2014) was followed. Drawings of impregnated cells were based on free-hand sketches and micrographs.

Terminology is according to Berger (1999), Esteban et al. (2001a) and Lynn (2008).

Molecular methods

P. silvicola n. sp. was isolated from enriched soil cultures and washed in PCR water using a hand-drawn micropipette. Cells were put in PCR tubes, frozen at -30°C until they were sequenced.

The 18S rRNA gene from ciliate cells was amplified using the KOD Hot Start DNA Polymerase kit (Merck-Millipore, Burlington, USA) following the manufacturer’s standard protocol and the primers for the eukaryote 18S rRNA gene described by (Embley et al. 1992) (forward 5’-AYCTGGTTGATYYTGCCAG-3´; reverse 5’-TGATCCATCTGCAGGTTCACCT-3´). The PCR product was purified from a 1% agarose gel using a QIAquick Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany), ligated into pJET 1.2 plasmids, and cloned using a CloneJET PCR Cloning Kit (Life Technologies, Carlsbad, USA) into DH5α competent *E. coli* cells. Plasmids were purified from overnight cultures using a QIAprep Spin Miniprep Kit (QIAGEN GmbH) and Sanger sequencing of three clones for the PCR product in both directions was performed by GATC Biotech using plasmid-specific sequencing primers provided in the cloning kit. Sequencing reads were assembled using the program Sequencher 5.4.6 (Gene Codes Corporation).

Phylogenetic analyses

The 18S rRNA gene sequence of *P. silvicola* n. sp. was aligned with 60 gene sequences downloaded from the GenBank database, using MUSCLE (Edgar 2004) implemented in MEGA 7 software (Kumar et al. 2016), under the default parameters. Accession numbers are provided in Fig. 7. Ambiguous regions and gaps were identified and then removed by Gblocks 0.91b with all three options checked for a less stringent selection (Castresana 2000; Talavera and Castresana 2007), leaving 1,742 unambiguous positions. The Bayesian inference (BI) analysis was performed with MrBayes v.3.2.5 (Ronquist and Huelsenbeck 2003) using the TIM2 + *I* + *G* as the best model selected by the jModelTest v.2.1.10 software (Darriba et al. 2012) using Akaike Information Criterion with correction (AICc). Two Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations with a sampling frequency of every 100 generations and a burn-in of 25,000 trees (25%). The maximum likelihood (ML) analysis was computed by MEGA 7 (Kumar et al. 2016) using 1000 bootstrap replicates. Phylogenetic trees were visualized using the free software package FigTree v1.4.3 by A. Rambaut (http://tree.bio.ed.ac.uk/software/figtree/).

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Figure 1. *In vivo* images of *Psilotricha silvicola* n. sp. **(A, B)** Ventral view of an old (A) and young (B) specimens. Note the rounded shape of younger cells *vs.* the elongated shape typical of older cells; **(C, D)** Lateral views of two specimens showing a 'caudal tail' (arrowhead); **(E)** Ventral view showing the contractile vacuole. CC-caudal cirri, CV-contractile vacuole, FC-frontal cirri, FVC- fronto-ventral cirri, LMC-left marginal row of cirri, RMC- right marginal row of cirri, TC-transverse cirri. Scale bars = 10 µm.

Figure 2. *In vivo* images of *Psilotricha silvicola* n. sp. **(A)** Ventral side with slightly pointed posterior end. Arrowheads point to the two dorsal ribs, the right one is longer than the left one. **(B)** Ventral side showing the typical very long caudal and transverse cirri (up to 35 µm) CC-caudal cirri, TC-transverse cirri. Scale bars = 10 µm.

Figure 3. *Psilotricha silvicola* n. sp. after protargol impregnation. **A**. Ventral view of a representative specimen showing the pattern of cirri, including two frontal cirri, three fronto-ventral cirri, one postoral ventral cirrus, one pre-transverse cirrus and three transverse cirri. **B**. Dorsal view of a specimen showing 5 dorsal kineties and three caudal cirri. CC-caudal cirri, DK1-4-dorsal kineties; DMK-dorso-marginal kinety, e-endoral membrane, FC-frontal cirri, FVC-fronto-ventral cirri, LMC-left marginal row of cirri, p-paroral membrane, PTVC-pre-transverse ventral cirrus, PVC-post-oral ventral cirrus, RMC-right marginal row of cirri, TC-transverse cirri. Scale bars = 10 µm.

Figure 4. Line diagrams of *Psilotricha silvicola* n. sp. after protargol silver impregnation. Ventral **(A)** and dorsal **(B)** views, CC-caudal cirri (note that they are located on the dorsal side but are more easily seen from the ventral side), DK1-4-dorsal kineties, DMK-dorso-marginal kinety, e-endoral membrane, FC-frontal cirri, FVC-fronto-ventral cirri, LMC-left marginal row of cirri, p-paroral membrane, PTVC-pre-transverse ventral cirrus, PVC-post-oral ventral cirrus, RMC-right marginal row of cirri, TC-transverse cirri.

Figure 5. *Psilotricha acuminata* after Esteban et al. (2001a) from life **(A)** and after protargol silver impregnation **(B, C)**. **(A)** General morphology of a representative cell. Ventral **(B)** and dorsal **(C)** views. AZM-adoral zone of membranelles. BC-buccal cirrus, CC-caudal cirri, FVC-fronto-ventral cirri, FC-frontal cirri, LMC-left marginal row of cirri, PVC-post-oral ventral cirrus, p-paroral membrane, RMC-right marginal row of cirri TC-transverse cirri, VC-ventral cirri.

Figure 6. *Hemiholosticha viridis,* population from a freshwater pond at East Stoke Fen Nature Reserve (United Kingdom); **(A)** protargol impregnation; **(B)** living specimen; arrows to the intracellular algae with eye spot. Scale bars = 30 µm.

Figure 7. Maximum likelihood (ML) phylogenetic tree inferred from the 18S rRNA gene sequences, showing the position of *Psilotricha silvicola* n. sp. Numerical support values are given at the respective nodes as: Maximum likelihood (ML) bootstrap out of 1000 replicates/Bayesian inference (BI) posterior probability. A hyphen (-) represents differences between ML and BI tree topologies. The GenBank accession numbers of these 18S rRNA gene sequences are provided after the species names. The scale bar corresponds to two substitutions per 100 nucleotide positions.

Table 1. Morphometric data of *Psilotricha silvicola* n. sp. isolated from woodland soils at East Stoke Fen Nature Reserve (Wareham, United Kingdom).

Morphometric characters[a]	$\bar{x}$	M	SD	SE	Min	Max	n
Cell length in vivo (µm)	67.4	66.5	5.80	1.10	57.0	77.0	30
Cell width in vivo (µm)	37.8	37.0	4.50	0.80	29.0	47.0	30
Cell length (µm)	45.0	46.0	4.40	0.80	32.0	51.0	30
Cell width (µm)	21.2	21.0	3.10	0.60	17.0	29.0	30
Macronuclear nodules, number	2.0	2.0	0.00	0.00	2.0	2.0	30
Micronuclei, number	1.4	1.0	0.49	0.09	1.0	2.0	30
Macronucleus, length (µm)	9.3	9.1	2.05	0.37	6.15	13.4	30
Micronucleus, length (µm)	3.0	3.0	0.40	0.07	2.10	3.6	30
Adoral membranelles, number	22.9	23.0	1.98	0.36	18.0	26.0	30
Adoral zone, percentage of body length (%)	45.8	45.3	3.61	0.65	38.2	54.2	30
Cirri in right marginal row, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Cirri in left marginal row, number	3.9	4.0	0.3	0.05	3.0	4.0	30
Frontal cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Fronto-ventral cirri, number	4.0	4.0	0.0	0.0	4.0	4.0	30
Post-oral cirri, number	1.0	1.0	0.0	0.0	1.0	1.0	30
Pretransverse ventral cirri, number	1.0	1.0	0.0	0.0	1.0	1.0	30
Transverse cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Caudal cirri, number	3.0	3.0	0.0	0.0	3.0	3.0	30
Dorsal kineties, number	4.9	5.0	0.3	0.06	4.0	5.0	30
Dorsal ribs, number	2.0	2.0	0.0	0.0	2.0	2.0	30

$\bar{x}$: arithmetic mean; M: median; SD: standard deviation; SE: standard error; Min: minimum; Max: maximum; n: number of individuals investigated. [a]Data based, if not mentioned otherwise, on mounted, protargol-impregnated.

Table 2. Comparison between *Psilotricha silvicola* n. sp. from East Stoke Fen (Wareham, United Kingdom), *Psilotricha acuminata* Stein, 1859 (from Scotland, UK; Esteban et al., 2001a), *Urospinula succisa* (Müller, 1789; Foissner, 1983), *Psilotrichides hawaiiensis* (Heber et al., 2014), and *Hemiholosticha kahli* (Luo et al., 2019).

Morphological character	*Psilotricha silvicola* sp. nov.	*Psilotricha acuminata*	*Urospinula succisa*	*Psilotrichides hawaiiensis*	*Hemiholosticha kahli*
Size in vivo (µm)	~ 65x40	~ 70x40	~ 120x70	~ 65x45	~ 63x47
Macronuclear nodules, number	2	2-3	2	2	2
Micronuclei, number	1-2	1-2	1	1	1
Adoral membranelles, number	18-26	18-25	18-21	19-23	19-22
Buccal cirri, number	No	1	Not distinguishable	Not distinguishable	Not distinguishable
Ventral cirral rows[a], number	2	2	7	6	5
Cirri of right marginal row, number	3	3-4	6-10	4-10	9-12
Cirri of left marginal row, number	3-4	2-3	11-16	3	4-6
Post-oral ventral cirri, number	1	1	4-6[b]	1-3[b]	1-2
Frontal cirri, number	3	3	Not distinguishable	Not distinguishable	Not distinguishable
Fronto-ventral cirri, number	4	3+1unciliated cirrus	No	No	No
Ventral cirri, number	No	2	Form ventral rows	Form ventral rows	Form ventral rows
Pre-transverse ventral cirri, number	1	No	No	No	No
Transverse cirri, number	3	2	No	No	No
Dorsal kineties, number	4-5	4-5	3	3	3
Dorsal ribs, number	2	2	No	No	3
Caudal cirri, number	3	3	No	No	No

[a] include right and left marginal row of cirri and cirral ventral rows; [b] cirri form a cirral row.