RE-DISCOVERY OF A "MISSING LINK" CILIATED PROTOZOON

G. F. Esteban*, J. L. Olmo & B. J. Finlay

Centre for Ecology and Hydrology, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 OLP, United Kingdom

ABSTRACT

The characteristic features of the enigmatic ciliate *Psilotricha acuminata* Stein, 1859 are presented. *Psilotricha acuminata* is a peculiar ciliate insofar as it resembles the hypotrich *Euplotes* but it also bears characteristics of a completely different family, the Oxytrichidae. We provide observations on its morphology, and some remarks on the ecology and geographic distribution of this ciliate. Finally, we examine some current practises in describing new ciliate species.

Keywords: biodiversity, ciliates, ubiquity, taxonomy

RESUMEN

La presente investigación describe las características morfológicas de Psilotricha acuminata *Stein, 1859, ciliado considerado "enigmático" por presentar características propias del hipotrico Euplotes y de una familia completamente distinta, la familia Oxytrichidae. En este estudio incluímos, asimismo, algunas observaciones sobre la ecología y distribución geográfica de este ciliado y, para terminar, analizamos ciertas prácticas taxonómicas contemporáneas empleadas para describir especies nuevas de ciliados.*

Palabras clave: biodiversidad, ciliados, distribución geográfica, taxonomía

INTRODUCTION

There is a dilemma that frequently surfaces when naming new ciliated protozoan species: when are we justified in calling a new variant a new morphospecies, and how can we be sure that a description of the creature is not lurking in the historical literature. We have first-hand experience of this dilemma. **A** ciliate with a superficial resemblance to the hypotrich *Euplotes* recently appeared in samples of dried soil from grassland in Scotland. The main characteristic of the ciliate from Scotland is that its shape, low number of cirri, and movements are that of the commonly found ***Euplotes***. In contrast with the latter, it bears one marginal row of cirri on the right and one on the left of the ventral surface of the cell, and consequently the organism can easily match hypotrichs of a totally different family, i.e. Oxytrichidae. Furthermore, the divisional process of this ciliate seems to follow a pattern close to that of *Gonostomum* within the family Oxytrichidae (Esteban *et al.*, 2001a). We therefore seemed to be dealing with the long-searched-for "missing link" between the families Euplotidae and Oxytrichidae.

An initial survey of the published literature, particularly that describing hypotrichs using silver impregnation techniques, yielded no identical or even similar species. And yet, we were troubled with a nagging doubt. We had no difficulty in repeatedly obtaining the ciliate in crude culture after a few days of re-wetting the soil samples, whereupon it soon reached numbers of several hundred per ml. The ciliate was apparently quite common, or remarkably easy to culture, or both. How could such a ciliate not have been described previously? This enigma was especially peculiar

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because the hypotrichs are a group whose taxonomy has received much attention over the years. Kahl (1935), for example, includes 314 species; and at least 425 new hypotrich species have been described since 1936 (re *Zoological Record).* If we had a new hypotrich, it had to differ uniquely from nearly 800 previously described species. It was only after a thorough search in the old literature that we found the matching organism – a ciliate described by Stein in 1859 as *Psilotrichn acuminata.* In the present investigation we describe some characteristic features of living and silver-impregnated specimens of this ciliate species; we explain its ecology and geographic distribution, and we examine some current practise of describing new ciliate species.

MATERIAL AND METHODS

Psilotricha acuminata was found in samples from an experimental site at the Sourhope Research Station of the Macaulay Land Use Research Institute, near Kelso in Southern Scotland. The site is representative of mid-altitude (300 m) temperate upland grassland. *Agrostis capillaris* is the dominant plant species. Average annual rainfall is 952 mm. Grazing stock have been excluded from the site since April 1998. Prior to that date the area was grazed, and periodically fertilised with manure. Further information about the site can be found in Finlay *et al.* (2000).

The experimental site consists of 30 main plots, covering an area of approximately one

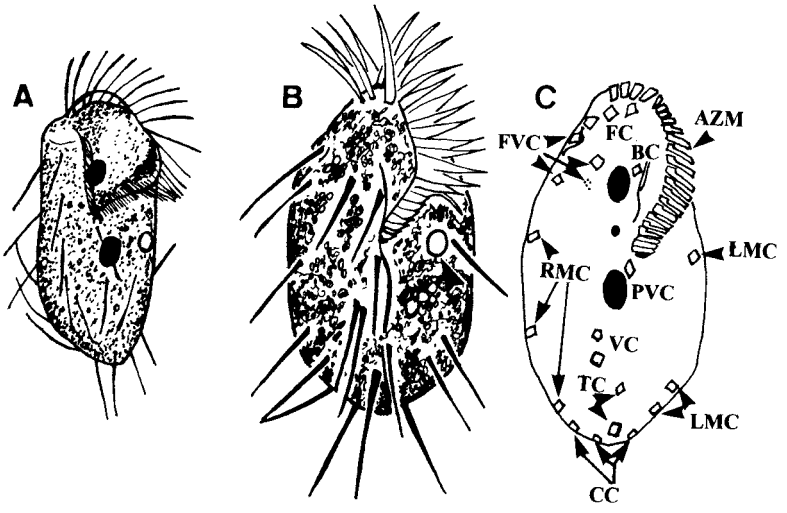


Figure 1. *Psilotricha acuminata* Stein, 1859. A) After Stein (1859b). B) Isolate from Scotland. Arrowhead to the contractile vacuole. C) Silver-impregnated infraciliature (Protargol) showing the arrangement of the cirri in the ventral surface of the ciliate. AZM, Adoral Zone of Membranelles; BC, buccal cirrus (1 cirrus); CC, caudal cirri (3 cirri); FC, frontal cirri (3 cirri); FVC, fronto-ventral cirri (4 cirri, one of them is non-ciliated); LMC, left marginal row of cirri (3 cirri); PVC, post-oral ventral cirrus (1 cirrus); RMC, right marginal row of cirri (3 cirri); TC, transverse cirri (2 cirri); VC, ventral cirri (2 cirri). The two black-filled ellipsoids are the macronuclei, and the small black spot between the macronuclei is the micronucleus. Psilotricha acuminata *Stein, 1859. A) Según Stein (1859b). B) Individuo aislado procedente de Escocia. La flecha señala a la vacuola contractil C) Infraciliación tras la impreganación con plata (Protargol) mostrando la disposición de los cirros en la superficie ventral del ciliado.* **AZM,** *zona adoral de las membranelas: BC cirro bucal (1 cirro); CC, cirros caudales (3 cirros): FC, cirros frontales (3 cirros): FVC, cirros fronto-ventrales (4 cirros, uno de ellos carece de cilios); LMC, hilera marginal izquierda de cirros (3 cirros): PVC, Cirro post-oral ventral (1 cirro): RMC, hilera marginal derecha de cirros (3 cirros): TC, cirros transversos (2 cirros); VC, cirros ventrules (2 cirros). Los elipsoides negros corresponden a los macronúcleos y el punto de color negro entre ambos macronúcleos es el micronúcleo.*

hectare. Soil samples from *25* of these plots were taken between March 1999 and July *2000*. The location of each sample within each plot was selected at random. Samples were taken with a 6cm diameter (steel) corer. Each sample consisted of the upper organic soil (top 5cm). The samples were dried at room temperature for one week. Thereafter, each sample was sieved and mixed together. Five grams of this soil was rehydrated with filtered rainwater to stimulate growth of protozoa.

The morphology of *Psilotricha acuminata* was investigated using living and OsO_4-fixed (2% final concentration) specimens. The infraciliature of the ciliates was revealed using the Protargol silver impregnation technique (Wilbert, 1975).

RESULTS

The cell shape is oblong and dorso-ventrally flattened, resembling ciliates of the genus *Euplotes* (Figs. 1, 2, 3). The shape can be acuminate (Fig. 2), or rounded at the posterior pole (Fig. 3). The cirri, reduced in number, are long and sparse (Figs. 2, 3, 4). With the exception of two cirri (one frontoventral and one transverse, see below and Figs. 3, 4, 5) that are slightly longer than the rest, all the cirri in living organisms are morphologically alike; even the caudal cirri can be confused with marginal cirri (Figs. 2, 3, 4).

There is one marginal row of cirri on the right, and one on the left of the ventral side of the cell (Figs. 1, 3), each with only three or four cirri (see below). The actual number of cirri in the marginal rows can be determined only after observing specimens during morphogenesis (a process that also includes cell division); otherwise, the right marginal row seems to be formed by five or six cirri (see Figs. 3, 5), when, in fact, the anterior two-three cirri are frontoventral cirri. Consequently, the right marginal row of cirri starts towards the cell equator (Figs. 1-C, 5), and is formed by three-four cirri.

The remaining somatic cirri are arranged as follows: three frontal cirri, four fronto-ventral cirri (one of them non-ciliated), one buccal cirrus, one post-oral ventral cirrus, two ventral cirri, two transverse cirri, and three caudal cirri. One of the two transverse cirri is longer and thicker, and has a broom-like end (Fig. *5).*

The structure of the mouth is similar to that of marine interstitial ciliates, i.e. the adoral zone of membranelles (AZM) is formed by an anterior portion with long cilia, and a posterior part with shorter cilia (Fig. 6). The AZM is large and reaches the cell equator. Its full extent is observable in ciliates that are filter feeding. In these, the frontal cirri can be mistaken for the long cilia of the anterior membranelles as both beat in harmony to develop a current flow for filter feeding.

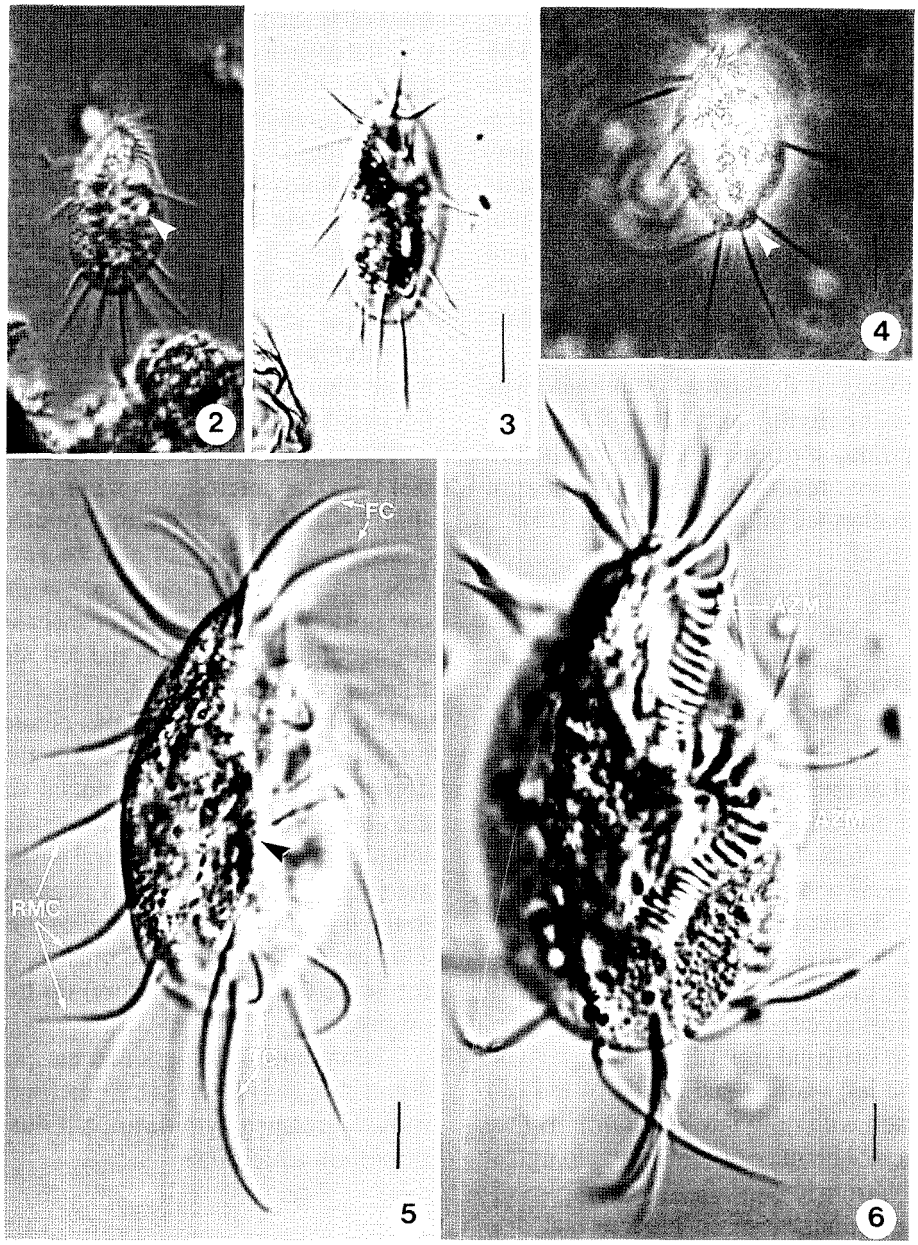
The ciliates typically have two oval macronuclei with one micronucleus placed between them (Fig. 1-C). However, the macronuclei can also be elongate, and there can also be two micronuclei (one anterior to the anterior macronucleus, and one posterior to the posterior macronucleus). The contractile vacuole is on the left side and at the equator of the cell (Fig. *2).*

The dorsal surface of the ciliate bears cilia, placed in three longitudinal kineties and in one dorsomarginal kinety. The dorsal side of the cell also bears two ribs. A further rib runs along the longitudinal axis of the ventral surface of the ciliate (Fig. *5),* and develops a beak-like projection to the left of the posterior end of the cell (Fig. 4). This feature is observable only in specimens left for some time (1 to 2 hours) on the microscope preparation.

The cytoplasm is dark and has particles of different shapes (Figs. 4, 5). Sometimes, the ciliates show a greenish cytoplasm, but this is not due to symbiotic Chlorellae.

Habitat and geographical distribution

Stein (1859a, b) discovered *Psilotricha acuminata* in a manure puddle in the yard of a rectory farm in Germany. We have found it in (manure) grassland in Scotland. Other authors have also reported it from soils: in Italy (Grandori & Grandori, 1934, Coppa, 1921), and in the USA (Bamforth, 1969). The species has been found in fresh water in New Zealand (Maskell, 1886),



Figures 2–6. *Psilotricha acuminata* from Scotland (UK). 2), 3), 4) Living specimens showing the variation in cell shape; 2) Nomarski interference contrast of an acuminate cell; arrowhead to the contractile vacuole. Scale bar = 20µm; **3)** Nomarski interference contrast of a cell with a rounded posterior end. Scale bar = 20µm; 4) Phase contrast of a cell with the typical "beak-like" ventral projection (arrowhead). Scale bar = 20µm; 5) and 6) Osmium-tetroxide fixation. Scale bars = 10µm. 5) FC, two of the three frontal cirri; RMC, right marginal row of cirri; TC, transverse cirri. Arrowhead to the ventral rib. 6) dividing specimen. AZM, adoral zone of membranelles. Psilotricha acuminata *recogida en Escocia (Reino Unido). 2), **3)** y 4) individuos vivos, nótese la variabilidad **de** la forma corporal; 2) Imagen interferencial de contraste de Nomarski de una célula acuminada; la flecha señala la vacuola contractil. Escala 20 µm; 3) Imagen interferencial de Nomarski de una célula con el **rxtrrmo** posterior redondeado. Escala 20 µm; 4) Imagen de contraste de fase de una célula con la típica proyección "en forma **de** pico de ave" (flecha). Escala 20 µm; **5)** y 6) Imágenes tras la fijación con tetraóxido de osmio. Escala 10µm. **5)** FC, dos de los tres cirros frontales; RMC, hilera marginal derecha de cirros; TC. cirros transversos. La flecha señala la costilla ventral. 6) Individuo en división. AZM, membranelas de la zona adoral.*

Switzerland (Roux, 1901), and China (Yang, 1989). It has also been retrieved form the littoral of Lake Baikal in Russia (Gajewskaja, 1934). It has been found in brackish water in Russia by Butschinsky (1897), and in sea water in Germany by Bartsch & Hartwig (1984), respectively. *Psilotricha acuminata* is, therefore, a cosmopolitan ciliate.

DISCUSSION

The history of *Psilotricha acuminata* is curious insofar as it is frequently listed in surveys from the end of the nineteenth century and first part of the twentieth. The species has rarely been recorded in recent times, even though soil is a habitat where this species seems to develop population growth without difficulty. It has not been listed in any recent survey of terrestrial ciliates, and it does not appear in the updated compilation of world soil ciliates (Foissner, 1998). Moreover, Stein's description of *Psilotricha acuminata* in 1859 was the only one available until now, and is based on living organisms. The species has a peculiar arrangement of the ciliature that can only be understood after observing silver impregnated cells (see description of morphological details above). Silver impregnation techniques became crucial for the taxonomy of ciliates in the second half of the twentieth century for they reveal the infraciliature (i.e. the assembly of all kinetosomes and associated subpellicular microtubular structures, both somatic and oral in location [Corliss, 19791) of these organisms, thus facilitating the identification and taxonomy of ciliate species. Such a description was lacking for *P. acuminata,* and consequently, the genus *Psilotricha* became mixed with other hypotrich genera (Esteban *et al.,* 2001a). The reason why this species has not been re-described since 1859 is probably its resemblance with *Euplotes,* a genus that also includes several soil-dwelling species. It is plausible that *P. acuminata* has been regarded as an *Euplotes* species in soil ciliate surveys - but of this we cannot be certain since such surveys are not usually accompanied by illustrations.

Hypotrich ciliates are, in general, highly variable in morphology; yet most of the new species currently described belong to this group of ciliates. Within the free-living ciliates as a whole, there are three main reasons for the continuing description of new species. First, neglect of the older literature. Second, inadequate sample size and over-interpretation of minor morphological details; third, the assumption that an isolate from an exotic location must be a new species.

Psilotricha acuminata Stein, 1859 is a remarkable example of the value of the older literature. However, other ciliate species also described long ago, have not been so fortunate. Descriptions based only on silver-impregnated specimens gained such weight in ciliate taxonomy that the morphology of the living cells was not provided. The infraciliature was thus revealed, but the morphology of the living organisms was not. When the identification of the ciliate took place, all the "older descriptions" became superfluous, for they are based on living cells. For example, *Cristigera pleuronemoides* Roux, 1901 is a scuticociliate found in freshwater sediments worldwide. Wilbert (1986), and Dragesco & Dragesco-Kernéis (1991) described its unusual infraciliature in silver-impregnated specimens from Canada and tropical Africa, respectively. However, these authors did not realise that the living organism was that discovered by Roux (1901) in Europe (Esteban & Olmo, 1997). In each case, the ciliate was described as new. This practise also illustrates why some ciliate species still remain unreported since they were discovered.

The majority of recent descriptions of new ciliate species are based on the "morphometry" and morphology of only 10 to twenty organisms, sometimes even less (see some comments in Esteban *et al.,* 1997). This practise is unfortunate, particularly when the species involved differ from very close relatives in cell size only, or in a pair of kinetosomes or in the location of one cirrus. Unnecessary inflation of species numbers could be avoided if the species were described after investigation of a larger number of specimens grown in (clonal or enriched) cultures or,

after examination of large numbers of wild forms. For example, the nuclear apparatus of *Psilotricha ucuminata* was described (Stein, 1859) as formed by two oval macronuclei and one micronucleus placed between the macronuclei (Fig. 1**A**). Our findings show that the nuclear apparatus of this species can vary, and we found the full variability in form and number of nuclei after examining more than one hundred (wild, non-dividing) cells. Some "new" species of hypotrichs have been described that differ in only the number of macronuclei.

Descriptions based on the observation of few cells have other consequences – the over interpretation of morphological details, e.g. of extrusomes, of the silverline system, of the somatic structures, of the nuclear apparatus. In some instances, the investigator decides beforehand which specimens are "suitable" to be a "good" species, and which will be excluded from the description (Foissner, 1997, 1999). Among the latter are those cells with a different nuclear structure, those of unusually small size, and those with "distinctly deviating ciliary pattern" (Foissner, 1997, 1999). However, these forms or "artefacts" are typically found after examining large numbers of individuals for they are part of the natural morphological variation in populations of ciliates (see below). The common ciliate *Prorodon discolor* (Ehrenberg, 1833), for instance, has recently been described as a different species – *Holophrya seyrli* Foissner, 1997 – on the basis of the size of the extrusomes of fourteen specimens (Foissner, 1997). Esteban *et al.* (2000) carried out a prompt emendation.

Some free-living ciliate species are so remarkably distinct that a few tens of cells would suffice to explain the morphology of the organism, e.g. *Atopodinium fibulatum* Kahl, 1932, *Discomorphella pectinata* (Levander, 1894) Corliss, 1960, *Stentor coeruleus* Ehrenberg, 1830, *Tropidoatractus acuminatus* Levander, 1894. But for the majority of remaining cases, the observation of large number of specimens should be carefully planned before describing a ciliate as new.

Finally, a further source for the increase in the number of ciliate nominal species is the taxonomist's predisposition to describe a new ciliate if it is found somewhere remote or exotic (e.g. Blatterer & Foissner, 1988; Foissner, 1988, 1994, 1996; Petz *et al.*, 1995). It has now been shown that those ciliate species once thought to be endemic to the area where they were first found are encountered elsewhere in the world when additional sampling occurs. Several examples of rejected "endemics" can be found in Esteban & Olmo (1997), Finlay *et al.* (1999), and Esteban *et al.* (2001b).

Ciliate taxonomy is currently languishing in a state where it is easier to describe a ciliate as new, than to re-describe it as one of the "old' species. Future thorough research into the taxonomy of these organisms and additional global sampling will challenge some dubious practises in ciliate taxonomy.

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