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New records of the ectoparasitic flagellate *Colpodella gonderi* on non-*Colpoda* ciliates

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Summary. *Colpodella gonderi* is the only ectoparasitic flagellate of ciliated protozoa described thus far. This investigation reveals new records of *C. gonderi* retrieved from soil samples in southern Scotland, UK. Of fourteen ciliates species identified in one single occasion, three of them, *Colpoda steinii, Pseudoplatyophrya nana* and *Grossglockneria acuta*, were infested with the parasite. These results provide further evidence that *C. gonderi* is not host-specific of the ciliate genus *Colpoda*. [**Int Microbiol** 2011; 14(4):207-211]

Keywords: *Colpoda steinii* · *Colpodella* · *Spiromonas* · colpodids · flagellated protozoa · parasitism

Introduction

The only ectoparasitic flagellate of ciliated protozoa described thus far is *Colpodella gonderi* [8,14]. First reported by Stein in 1878 in fresh waters in Germany, *C. gonderi* has subsequently been found in hay infusions also in Germany [9], in fen pond samples in France [2], and more recently in saline soils in eastern Austria [8], and in a decaying-wood sample in a forest in Slovakia [16].

Nearly every record of *C. gonderi* in the literature is of flagellates attached to the pellicle of ciliates of the genus *Colpoda*. Consequently, the organisms were traditionally considered to be host-specific of that ciliate genus [8]. However, in 2004 [16], *C. gonderi* was observed parasitizing some individuals of the scuticociliate *Pseudocohnilembus pusillus*, making it the first record of the ectoparasitic flagellate on non-*Colpoda* ciliates.

The research presented here reveals new records of infection in two further ciliate genera, *Grossglockneria* and *Pseudoplatyophrya*, providing additional evidence that *Colpodella gonderi* is not host-specific of the genus *Colpoda*. In the work described herein, *C. gonderi* was found in grassland soils in Scotland.

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Material and methods

Study site and soil sampling. Soil samples were obtained from a 1-ha upland grassland experimental site at the Sourhope Research Station of the Macaulay Land Use Research Institute, near Kelso in Southem Scotland (grid reference NT 854196) [7]. The site is mid-altitude (304–312 m a.s.l.) temperate upland grasslands on base-poor mineral soils, with the common bent (colonial bentgrass) *Agrostis capillaris* as the dominant plant species. The average annual rainfall is ca. 950 mm. The soils are shallow brown forest soils with localised gleying on andesite and undifferentiated intermedi-

ate igneous rock type, with the pH ranging from 4.54 to 4.81 across the experimental plots. Livestock have been excluded from the site since 1998.

Samples were taken to a depth of ca. 5 cm using a 6-cm diameter steel soil corer. The soil consisted mainly of the upper soil organic layer. Samples were kept in the dark in a cool box at 4 °C for transport to the laboratory. Details of the sampling treatment, the protocol to estimate protozoan growth potential, and the techniques used to investigate protozoan cell morphology are in [4,5,7]. Briefly, the protocol we followed to study protozoa from soils consisted of removing above-ground foliage from the soil core sample, and breaking up and mixing the remaining soil. The soil was spread as an even layer in 15-cm diameter glass Petri dish bases in a containment room at room temperature (18–22 °C) for six days. After that time, the soil was passed through a 3.35-mm sieve and homogenised. This material is referred to as 'air-dried soil'. To determine its oven-dried weight, 5 g of air-dried soil was heated to 80 °C and weighed at 24 h and 48 h [7].

Enumeration and characterisation of ciliates. Five grams of air-dried soil prepared as explained above was placed into a plastic Petri dish. The sample was incubated by adding a measured volume of filtered (0.2-µm pore-size filters) rain water sufficient to produce a slurry. This slurry was obtained by adding 10–15 ml of filtered water to 5 g of air-dried soil (see [7] for full details of procedure). The water was added to just beyond field capacity so that the crumb structure of the soil was retained and excess water drained to the edge of the Petri dish. The sample was incubated in the dark at 15° C for four days. Fifty µl drops were then removed from the runoff and examined in a Sedgewick-Rafter counting chamber. Median abundance was calculated and the results were subsequently extrapolated to obtain numbers of individuals per gram of oven-dried soil weight [7]. For ciliate species identification, silver impregnation techniques (Protargol and the pyridinated silver carbonate methods [3,6]) were used to reveal the ciliates' infraciliature in order to characterise and identify the different species.

Video clips of living *Colpodella gonderi* attached to different species of colpodids have been deposited with Spanish National Research Council (CSIC), at the National Museum of Natural Sciences, Madrid, Spain: http://www.cienciatk.csic.es/index.php?module=search&search=colpodella

Results and Discussion

A flagellate attached to the surface of ciliates unexpectedly occurred in one soil sample out of 150 soil samples analysed on a single occasion. This ectoparasitic flagellate had the following morphological characteristics: two flagella of equal

Table 1. Ciliate species identified in soil from upland grassland in Scotland (UK). The species are sorted from highest to least median abundance in the sample. The percentage of cells infested with the ectoparasitic flagellate is also recorded

Species	Median abundance of ciliates per gram dry weight soil	% of population infested
Colpoda steinii	812	26
Grossglockneria acuta	560	7
Gonostomum affine	280	0
Pseudoplatyophrya nana	252	11
Colpoda inflata	154	0
Holosticha sp.	140	0
Colpoda cucullus	98	0
Nivaliella plana	70	0
Cyrtolophosis elongata	42	0
Leptopharynx costatus	42	0
Platyophrya vorax	42	0
Sathrophilus muscorum	42	0
Cyclidium glaucoma	28	0
Aspidisca turrita	14	0

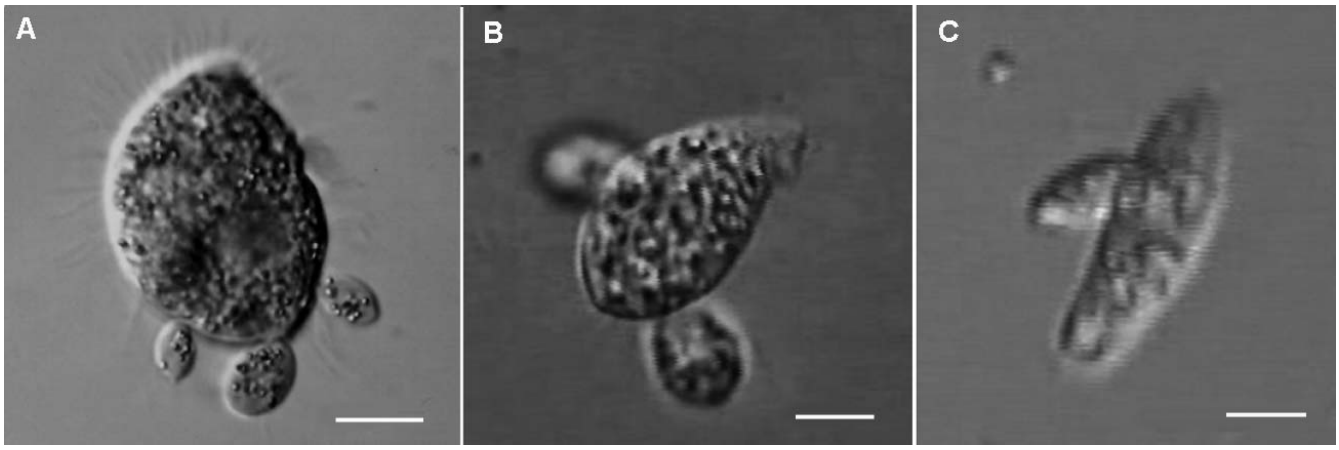

Fig. 1. Ciliates infested with the flagellate *Colpodella gonderi*. (**A**) *Colpoda steinii* with three flagellates attached to its surface; (**B**) *Pseudoplatyophrya nana* with two flagellates; (**C**) *Grossglockneria acuta* with one flagellate. Scale bars: ca. 10 μm.

length of 1.5–2 times the cell length; the size of the well-nourished individual was 10–12 μm long by 8–10 μm wide, and nearly spherical; the cytoplasm contained few granules, sometimes a large spherical or kidney-shaped inclusion was observed at the posterior end; the nucleus was placed approximately in the middle of the cell; no contractile vacuole was observed. These characteristics match those of the ectoparasitic flagellate *Colpodella gonderi* [8].

Fourteen ciliates species were identified in this soil sample. Species names, median abundances and the percentage of infection by the parasitic flagellate are shown in Table 1.

Of fourteen ciliates species retrieved, only three species, *Colpoda steinii, Pseudoplatyophrya nana* and *Grossglockneria acuta,* had *Colpodella gonderi* attached to their surfaces. Other *Colpoda* species observed in the soil sample, i.e. *C. inflata* and *C. cucullus*, which have been previously reported as hosts [2,8,9,15], did not bear any flagellates. The highest infection occurred on *Colpoda steinii* (Fig.1, Table 1) with 26 % of the population infected by the flagellate; 11 % of the ciliates *Pseudoplatyophrya nana* (Fig. 1) and 7 % of *Grossglockneria acuta* were infested by *Colpodella gonderi* (Fig. 1).

These three ciliate species also registered the highest population abundances (except for *Gonostomum affine*, which was not infected) out of the fourteen ciliate species recorded in the sample (Table 1). Higher ciliate abundances might, therefore, have facilitated flagellate infestation. Infestation with *C. gonderi* in large ciliate populations has also been reported in the finding of the ectoparasitic flagellate on the non-*Colpoda* ciliate *Pseudocohnilembus pusillus* [16].

The number of flagellate parasites attached to ciliate individuals was usually one, two or three, but in some cases up to ten. *Colpoda steinii* was the species with the highest numbers of parasites while *Pseudoplatyophrya nana* and *Grossglockneria acuta* usually had only one or two parasites. *Colpodella gonderi* attached most commonly to the posterior end of the host (Fig. 1) although they were also observed attached to the ciliate cell equator (Figs. 1 and 2). When ciliates are severely infested or in the last stage of infestation, the ciliate cytoplasm becomes vacuolated, and they eventually round up and burst.

Flagellates of the genus *Colpodella* are small (< 20 μm) alveolate protozoa. The genus includes seven species [14]: *Colpodella edax, C. gonderi, C. perforans, C. pugnax*, and *C. vorax*, *C. angusta* and *C. turpis*. While the latter two species are also considered as within the *Colpodella* genus, this needs further taxonomic support. All species are predatory free-living or parasitic; the latter, after attaching to their prey's surface, suck the cytoplasm by means of a rostrum. Their preys are other single-celled eukaryotes such as microalgae and ciliates [8,14].

Colpodella is the genus with free-living species most closely related to apicomplexan parasites [14], which comprise animal parasites: coccidia, gregarines, *Plasmodium* and *Babesia*, amongst others [13]. Molecular phylogenetic studies [10,11] suggested colpodellids as potential ancestors of apicomplexans. It is now recognised that colpodellids constitute a sister group of parasitic apicomplexa and that they probably have a common origin [1].

Colpodella gonderi was originally described as *Spiromonas gonderi* [8]; the genus *Spiromonas* was established by Perty in 1852 (see, e.g. [8]). However, the type species was not actually a flagellate and the genus name was

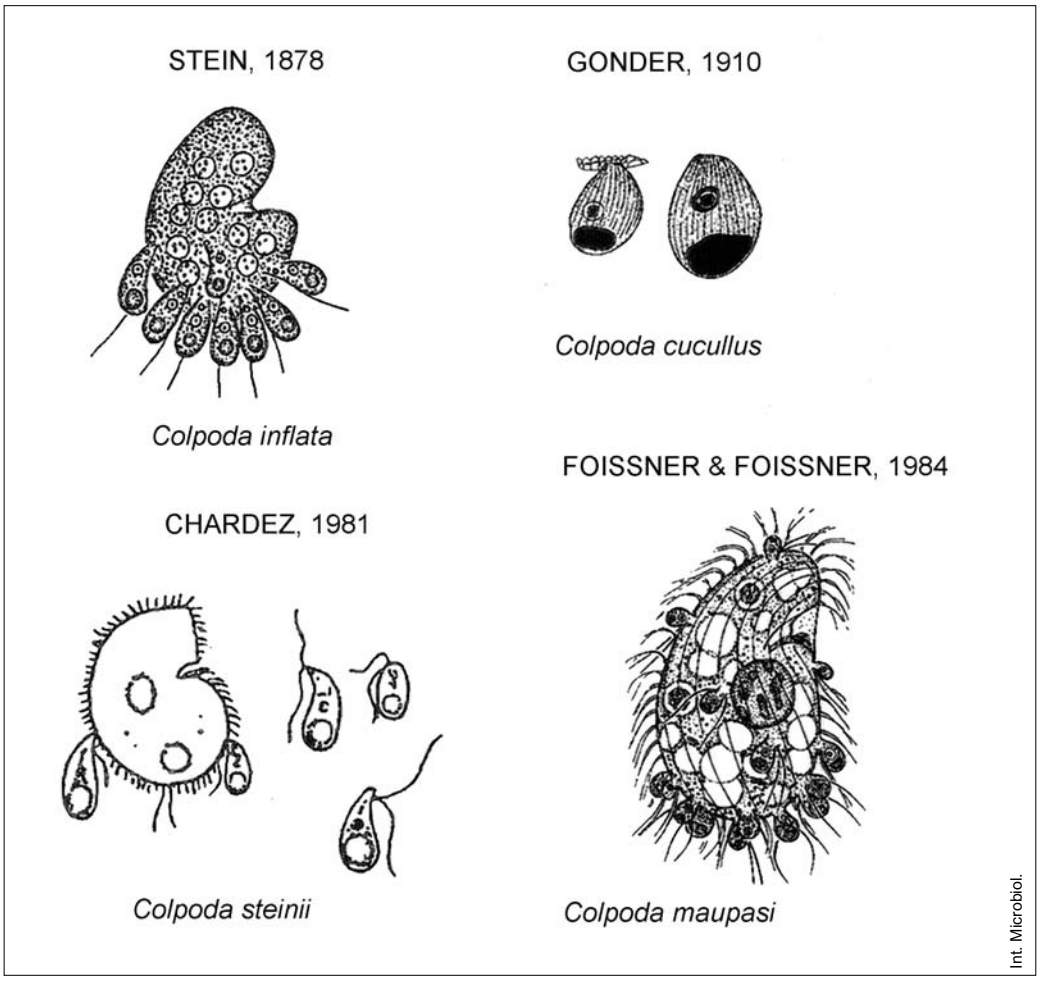

Fig. 2. Previous records of *Colpodella gonderi* on flagellates attached to ciliates of the genus *Colpoda* as published in the scientific literature (after [2,8,9,15]). See text for details on infection of non-colpodid ciliates.

consequently invalidated [12]. Full details and justification of the *Spiromonas* species' status are given in [12,14].

Nearly every record of *C. gonderi* in the scientific literature is of flagellates infecting ciliates of the genus *Colpoda* (Fig. 2): *C. inflata* [15], *C. cucullus* [9], *C. steinii* [2] and *C. maupasi* [8]. Thus, *Colpodella gonderi* was historically considered to be host-specific of *Colpoda* [8]. However, in 2004 [16], *C. gonderi* was observed on the cell surface of the scuticociliate *Pseudocohnilembus pusillus*, making it the first record of the ectoparasitic flagellate on non-*Colpoda* ciliates.

The research presented here provides new, further records of infection in two other ciliate genera, *Grossglockneria* and *Pseudoplatyophrya*, thus serving as additional evidence that *Colpodella gonderi* is not host-specific of the genus *Colpoda*. There is the possibility that *Grossglockneria* and *Pseudoplatyophrya* were 'accidental' hosts, hence the lower infestation rates (Table 1). However, in all instances, the flagellates did attach successfully to the ciliate, causing the host to eventually perish. Had infestation occurred by chance, other ciliate genera and/or colpodids co-existing in the same soil sample would have been expected to be infested, but this was not the case. Furthermore, no flagellates were observed to become detached from their hosts, which might have indicated a non-successful infection. The other colpodids detected in the sample probably had too low an abundance to provide a strong attachment signal and therefore were not infected; but this could not be verified. Research published in the scientific literature has shown that non-colpodid ciliate species can be parasitized [16]. Thus far, the flagellate has been found attached to three genera of colpodid ciliates, and on a scuticociliate, once population abundance of the (potential) host reaches a certain threshold. Its incidence is undeniably higher on colpodids, particularly those of the *Colpoda* genus. It may be that the characteristics of the colpodids' pel-

licle facilitate attachment of the flagellates. Further research —including laboratory experimental work—into the biology of this parasite is essential. Thus far, we can conclude that *Colpodella gonderi* is an ectoparasite flagellate, most commonly found attached to the surface of colpodid ciliates, but not host-specific of the ciliate genus *Colpoda.*

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Competing interests. None declared.

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