**Ciliatosporidium platyophryae** nov. gen., nov. spec. (Microspora incerta sedis), a Parasite of *Platyophrya terricola* (Ciliophora, Colpodea)

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**SUMMARY**

*Ciliatosporidium platyophryae* nov. gen., nov. spec. is a microsporidian parasite affecting the soil ciliate *Cirrophrya terricola* Foissner, 1987. It was found in a soil sample from Utah (USA), pure cultures were not available. The developmental stages seen are uninucleate and divide by binary fission (disporoblastic). Each sporont is enveloped by a membrane-like exospore precursor and a parasitophorous vacuole deriving from host endoplasmic reticulum and persisting during spore maturation. The spore is rod-shaped with hemispherical ends and measures about 4 x 2 μm. It has a short, uncoiled polar filament and conspicuous, possibly transient sacs in the wide anterior lamellae of the bipartite polaroplast. The exospore has the appearance of a unit membrane. Maturing spores induce the formation of appendages ("feet") at the posterior end of the host which was thus formerly assigned to the genus *Cirrophrya*. However, our study shows that the feet are a pathological phenomenon and the host thus belongs to the genus *Platyophrya*: *Platyophrya terricola* (Foissner, 1987) nov. comb. Despite its unusual host, the fine structure of *C. platyophryae* is very similar to that of microsporidia from other invertebrate and vertebrate hosts. Very likely, *C. platyophryae* is related to the genera *Oligosporidium*, *Encephalitozoon*, *Unikaryon* and *Endoreticulatus*, presently assigned to the families *Encephalitozoonidae* and/or *Unikaryonidae*. However, for a definite classification of *C. platyophryae* more detailed data on its life cycle and membranous envelopes are necessary. Thus, we prefer an incerta sedis position.

**Introduction**

Microsporidian infections are widespread among multicellular animals [2, 3] but rare in protists [24]. Most are hyperparasites of gregarines, only three infections have been reported from ciliates [20, 24]: *Nosema balantidii* Lutz & Splendore, 1908 in the parasitic trichostome *Balantidium* sp., *Gurleya nova* Hovasse, 1950 in the parasitic astomate *Spirobuetschliella chattoni* and *Glugea campanella* Krüger, 1956 in the free-living peritrich *Campanella umbellaria*. All are poorly known, i.e. have not been investigated electron microscopically, with the exception of an unnamed, very briefly described microsporidium found in the free-living heterotrichs *Stentor roeseli* and *S. polymorphus* [12].

However, some infections could have been unrecognized or misinterpreted. In the present paper, we report on a microsporidium causing foot-like appendages used by taxonomists as a differential character for two genera of colpodid ciliates, viz. *Platyophrya* and *Cirrophrya* [9, 11].

**Material and Methods**

*Ciliatosporidium platyophryae* occurred in a population of *Cirrophrya terricola* Foissner, 1987 found on 24. 06. 1989 in...
the litter layer (0–3 cm) of a popular lowland near the Virgin River, Rockville, Zion National Park, Utah, USA (W 115°, N 37°30′). Material from raw cultures (non-flooded petri dish method [9]) was used for all investigations. Unfortunately, pure cultures were not tried, because we did not realize the nature of the “feet” at that time and could not find another infected population later.

Host identification was according to the original description [6] and the most recent revision of the group [9], using methods described in [8]. Transmission electron microscopy was performed as described earlier [7].

Terminology for the host is according to [9], that for the parasite according to [3, 4, 17, 18, 22, 23]. Specifically, a parasitophorous vacuole is a vacuole of host origin, while a sporophorous vesicle is of parasite origin.

Results

Ciliatosporidium nov. gen.

Diagnosis. Microsporidia with uninucleate sporonts and uninucleate spores having uncoiled polar filament and conspicuous (transient?) sacs within anterior polaroplast lamellae. Sporonts and sporoblasts enclosed by two membrane-like layers, very likely a parasitophorous vacuole and an exospore primordium. Sporogony by binary fission, disporoblastic; all stages isolated in their own parasitophorous vacuoles.

Type species. Ciliatosporidium platyophryae nov. spec.

Derivatio nominis. Composite of the Greek words “Ciliophora” and “Sporidium”, meaning a spore forming organism parasitizing ciliated protozoa. Neutrum.

Ciliatosporidium platyophryae nov. spec.

Diagnosis. Sporonts roundish and with short, microtubule-bearing extensions, near and within stacks of endoplasmic reticulum cisterns. Spores rod-like with hemispherical ends, about 4 × 2 μm, in foot-like appendages at posterior end of host. Polar filament iso-filar, straight to slightly curved, about 5/8 of spore length. Polaroplast bipartite: anterior portion with (very likely four) membrane-bound sacs enclosed by wide, membranous lamellae; posterior portion with narrow, indistinct membranes.

Type host. Platyophrya terricola (Foissner, 1987), Protozoa, Ciliophora.

Type location. Soil in Zion National Park, Utah, USA (W 115°, N 37°30′).

Derivatio nominis. The name refers to the occurrence in ciliates of the genus Platyophrya.

Type material. Epon-embedded material, as used for electron microscopy deposited in the Oberösterreichische Landesmuseum in Linz (L1), accession number: 10/95.

Figs. 1–5. Light microscopic morphology of Platyophrya (formerly Cirrophrya) terricola infected with a microsporidian parasite, Ciliatosporidium platyophryae (from [9]). — Figs. 1, 2, 3. Right and left lateral views of live cells (length about 50 μm) with small appendages (feet) at posterior end containing spores of C. platyophryae; spores are also found in the cytoplasm. — Fig. 3. Possibly, the spore-containing appendages are used for anchoring the cell to a substrate, because the posterior cortex becomes stretched when the organism leaves a soil particle (but see Discussion). — Fig. 4. Oral and somatic infraciliature after silver impregnation; arrows mark paired cilia between somatic ciliary rows, forming a membranoid structure left of the adoral organelles. AO = adoral organelles, CV = contractile vacuole, Ma = macronucleus, OA = oral apparatus, P = microsporidian parasites, i.e. spores of Ciliatosporidium platyophryae, PA = paroral (undulating) membrane, SC = somatic ciliary rows.
Figs. 6–8. Presporal stages of *Ciliatosporidium platyophryae* in a cross-sectioned *Platyophrya terricola*. – Fig. 6. Early developmental stages of the parasite are irregularly roundish and found near stacks of host ER adjacent to the macronucleus. – Fig. 7. The parasite is enclosed by a parasitophorous vacuole derived from the host ER (arrowheads). Extensions contain microtubules which protrude toward the centre of the parasite. Arrow indicates inconspicuous parasite ER. – Fig. 8. The parasitophorous vacuole bears ribosomes, the exospore primordium is close to the parasite cell membrane. Vesicles occur in dilated regions between exospore primordium and parasite cell membrane and in host ER remote (arrow) from the parasite. E = extension, Ex = exospore primordium, M = microsporidium, Ma = macronucleus, Mt = microtubules, Mu = ciliate mucocyst, PM = parasite cell membrane, PV = parasitophorous vacuole, Ve = vesicle. Bars = 2 µm (Fig. 6) and 400 nm (Figs. 7, 8).
Description of *Ciliatosporidium platyophryae* nov. spec.

Host, prevalence and pathology. The host, *Platyophrya terricola* (Foissner, 1987), is a rare, medium-sized colpodid ciliate. Its morphology has been described in detail by Foissner [6, 9]. Thus, some figures and their explanations should suffice to orientate the reader (Figs. 1–5).

Unfortunately, nothing is known on the ecology of *P. terricola* [9]. As yet, we have found infected populations in grassland, meadow and arable soils from Austria, USA and Australia. In the raw cultures set up with these soils also many other ciliate species (and small metazoans like nematodes, rotifers and diptera larvae) occurred which were never infected, indicating a high degree of host specificity of *C. platyophryae*. The infected populations were recognized by their foot-like appendages at the posterior end containing the spores of the parasite (Figs. 1, 5). However, the prevalence of the parasite may be greater because the presporal stages are too inconspicuous to be recognized with the light microscope. The motility and the general light microscopic morphology of infected cells were not changed but appendages without spores were never observed, suggesting that their formation was induced by the maturing parasites. The fine structure of the host likewise showed some peculiarities, for instance, prominent stacks of cisterns of endoplasmic reticulum (ER) near developing parasites (Figs. 6, 9, 16). Furthermore, the appendages lacked mucocysts, alveoli and cortical microtubules, which were typical components of the ciliate pellicle in other cell regions (Figs. 21, 22).

The feet were occasionally retracted without obvious reason and the spores dispersed in the posterior body half. Spores outside the host were never observed. Whether the parasite influences the reproduction rate of the host and whether spores occur in its resting cysts is not known, because pure cultures were not available.

Presporal stages. Most cells sectioned contained presporal stages and spores at the same time; cells without mature spores were less frequent.

Earliest presporal stages (sporonts) occur near and within stacks of rough ER cisterns mostly adjacent to the host macronucleus (Figs. 6, 9, 16). Their shape is irregularly roundish with a diameter of about 3 µm,
Figs. 12-15. Spore formation in *Ciliatosporidium platyophryae*. – Fig. 12. Sporoblasts have an ellipsoidal shape and are not surrounded by host ER stacks. – Fig. 13. Cross section showing developing polar filament and ER cisterns studded with ribosomes (arrowhead). Arrow points to vesicular region, possibly the Golgi apparatus. – Fig. 14. Developing anchoring disc. The parasitophorous vacuole is smooth. – Fig. 15. Maturing spore with already distinct endospore developing between parasite cell membrane and exospore (cp. Fig. 14). Note round posterior end of polar filament (arrow). A = anchoring disc, aP = anterior polaroplast region, E = extension, En = endospore, ER = host endoplasmic reticulum, Ex = exospore (precursor), N = nucleus, PF = polar filament, PM = cell membrane, pP = posterior polaroplast region, PV = parasitophorous vacuole, S = sac. Bars = 500 nm.
Figs. 16-18. Spores of *Ciliatosporidium platyophryae* in posterior region of *Platyophrya terricola*. – Fig. 16. Cross-section of host with cross-sectioned spores (asterisks) near contractile vacuole. A presporal stage (arrow) is seen close to a host ER stack. – Fig. 17. Light micrograph (Nomarski interference contrast) of mature spores squeezed out of host. Spores are composed of a dense anterior portion and a lucid posterior vacuole (cp. Fig. 18). – Fig. 18. Longitudinal section through host “feet” containing spores with elongate nucleus. CV = contractile vacuole, ER = host ER stack, FV = food vacuole, L = lipid vesicle, Mu = mucocyst, N = nucleus, V = parasite vacuole. Bars = 2 μm (Figs. 16, 18) and 4 μm (Fig. 17).
and extensions up to 300 nm long may protrude from the surface (Figs. 6, 7, 9). The parasite is enclosed by three layers resembling unit membranes each with a thickness of about 5 nm (Fig. 8). The inner layer apparently is the parasite cell membrane. The outer layer is studded with host ribosomes and is, at some sites, continuous with the host ER, thus being a parasitophorous vacuole. The middle, smooth layer probably represents the exospore precursor (see Discussion). The space between middle layer and parasitophorous vacuole approximates the thickness of thin host ER cisterns (about 30 nm; Fig. 11); dilated regions contain vesicles or short cylinders (Fig. 8, [16]) which seem to pinch off or fuse with the middle layer. The centre of these structures frequently appears electron dense, their surface is covered by a loose coat. They also occur in the host ER remote from the parasite (Fig. 8).

Single and paired microtubules are randomly oriented beneath and parallel to the cell membrane (Fig. 11). Whether they form a continuous layer or network could not be ascertained. Some microtubules are anchored in the cell extensions (Fig. 7) and protrude into the parasite. ER cisterns are inconspicuous among the numerous ribosomes (Fig. 7).

The parasite contains one nucleus except in late fission stages. It is roundish, occupies the centre of the cell and measures 1-1.5 μm (Figs. 6, 12). Nuclear pores are 50 nm wide. Nucleoli or chromosomes were not seen.

Serial sections showed that the parasite divides by binary fission (Fig. 9). Mitosis is closed and the nuclear double membrane is depressed and more electron dense at the spindle poles. An electron dense flat vesicle and globular polar vesicles are present near the centriolar plaque (Fig. 10).

The sporoblasts are not surrounded by host ER stacks and the parasitophorous vacuole is smooth, i.e. free of ribosomes (Figs. 12-15). The extensions as well as the submembranous microtubules disappear, and the developing sporad adopts an ellipsoid shape (Figs. 12, 15). The microsporidian ER and the Golgi apparatus become more distinct (Fig. 13). The development and differentiation of the extrusion apparatus are similar to those known from other microsporidia [22], i.e. they commence with the formation of the anchoring disc (Fig. 14) and the polar filament. Later, the polaroplast and the spore wall differentiate (Fig. 15). The parasitophorous vacuole persists, and the space between parasite cell membrane and exospore precursor enlarges due to endospore formation (Fig. 15).

**Spore.** Spores are mainly found in the posterior body half of the host, especially in the appendages ("feet"; Figs. 1, 5, 16, 18). The spore has the shape of a short rod with hemispherical ends. It consists of two distinct regions recognisable even in the light microscope: a dense anterior portion and a lucid posterior portion occupying approximately 1/3 of the spore (Figs. 1, 5, 17, 19). Spores protrude with the anterior or posterior end (Figs. 1, 5, 18). The fresh spore measures about 4 x 2 μm.

The spore is delimited from the host cytoplasm by the parasitophorous vacuole. The exospore resembles a unit membrane, about 5 nm thick (Figs. 22, 23). The electron lucent endospore has a maximum width of 90 nm. The nucleus, about 1.5 x 1 μm, has a slightly elongate, irregular shape and is centrally located (Fig. 18). Conspicuous ER cisterns occur among the densely packed ribosomes and are continuous with the nuclear membrane (Figs. 19, 24). The posterior vacuole contains flocculent material and membranous structures (Fig. 19).

The anchoring disc of the extrusion apparatus is cup-shaped (Fig. 23). The polar filament is straight or slightly bent and up to 2.5 μm long, which corresponds to 3/8 of the spore length. It follows an oblique course from the anchoring disc through the polaroplast. The filament has a uniform diameter of about 140 nm (isofilar) except for the broadened anterior end (polar aperture). The rounded posterior end is closed and terminates near the nucleus (Fig. 15). Layers of different electron densities can be identified in appropriate sections of the polar filament (Figs. 20, 23, 24). The polaroplast is bipartite (Fig. 19) and belongs to type V [17]. Transverse sections show the anterior portion composed of probably four membrane-bound sacs ("wheel segments" [17]), surrounded by a system of concentrically arranged membranous lamellae (Fig. 20). The sacs are very distinct and have a finely granular content of medium electron density (Figs. 15, 19, 20, 23). The lamellae have an electron density of 500 nm (Fig. 19), 300 nm (Figs. 20, 23), and 200 nm (Figs. 21, 22, 24).

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**Figs. 19-24.** Spores of *Ciliatosporidium platyophryae* and cortex of *Platyophrya terricola*. – Fig. 19. Longitudinal section showing uncoiled polar filament, bipartite polaroplast and conspicuous ER cisterns (arrowhead). Enlarged detail of extrusion apparatus see Fig. 23. – Fig. 20. Cross section through anterior polaroplast region. Four membrane-bound sacs, containing granular material, are clearly recognizable within the wide polaroplast lamellae. – Figs. 21, 22. The normal cortex of *Platyophrya terricola* (Fig. 21) consists of a cell membrane, membrane-bound alveolar sacs, microtubules and mucocysts, whereas the "foot" cortex (Fig. 22) enveloping the maturing spores is reduced to the cell membrane. – Fig. 23. Longitudinal section through anterior end of extrusion apparatus. Note the conspicuous sacs within anterior portion of polaroplast. – Fig. 24. Cross-section close beneath polaroplast sacs, i.e. at boundary of anterior and posterior polaroplast region (cp. Fig. 23). Note that posterior portion of polaroplast surrounds proximal part of anterior portion. A = anchoring disc, Al = alveolus, aP = anterior polaroplast region, CM = ciliate cell membrane, En = endospore, Ex = exospore, Mt = microtubule, Mu = mucocyst, PF = polar filament, PM = parasite cell membrane, Pp = posterior polaroplast region, PV = parasitophorous vacuole, S = sac, V = vacuole. Bars = 500 nm (Fig. 19), 300 nm (Figs. 20, 23), and 200 nm (Figs. 21, 22, 24).
lucent lumen, about 15 nm wide, and the membranes of neighbouring lamellae are tightly compressed, forming a distinct pattern of light and dark lines. Cross sections at sac-level show two to three lamellae between polar filament and sacs and four to seven lamellae outside the sacs (Fig. 20). In longitudinal sections, sacs and surrounding lamellae appear ear-shaped with the pointed end directed posteriorly (Figs. 19, 23). The posterior portion of the polaroplast is less distinct and of nearly uniform electron density, probably consisting of tightly spaced lamellae and/or tubules (Figs. 23, 24).

**Systematic Position of Ciliatosporidium platyophryae**

*Ciliatosporidium platyophryae* clearly differs from microsporidia found in other ciliates. *Nosema balantidii* Lutz & Splendore, 1908 has pyriform or oval spores which are sometimes enclosed in cysts [19]; *Gurleyanovia Hovasse, 1950 has bi- and tetranucleate sporulation stages and oval spores [14]; and *Glu gea campanellae* Krüger, 1956 has paired, elongate-ovoidal spores [15].

According to the classification by Sprague et al. [21], *Ciliatosporidium* belongs to the class Haplophasea (because it is very likely uninucleate) and the order Glugeida (because it has a well-developed polaroplast). Within that order, the family Encephalitozoonidae Voronin [26] is defined as having “isolated nuclei during the developmental cycle and a disporoblastic sporogony.” Sprague et al. [21] refined the diagnosis by including a fine structural character, namely that “all developmental stages are enveloped by a host membrane”. However, they assign to the Encephalitozoonidae not only the disporoblastic *Encephalitozoon* but also the polysporoblastic *Endoreticulatus*. Possibly, their “disporoblastic sporogony” in the family diagnosis is a simple mistake.

According to our and the reviewers’ interpretation of the micrographs, all developmental stages seen in *C. platyophryae* are surrounded by at least one membrane of host ER. Thus, *C. platyophryae* would fit into the Encephalitozoonidae. The family comprises two genera according to Sprague et al. [21]: *Encephalitozoon* is enclosed by a single host membrane, is disporoblastic and proliferates by binary fission like our organism, but is apparently restricted to vertebrate hosts [18] and forms conspicuous chains of cells within the parasitophorous vacuole [4]; and *Endoreticulatus*, which is polysporoblastic and proliferates by binary or multiple division [1]. Voronin [26] also includes the genus *Loma* which, however, belongs to the family Glugeidae because an interfacial envelope is produced by the sporont [21].

One of the reviewers supported our suggestion of a relationship between Encephalitozoon and Ciliatosporidium and indicated affinities with Unikaryon and Oligosporidium. According to his opinion these genera are closely related and should be united in the Unikaryonidae Sprague [20], which is the oldest family name available. However, the genera assigned to the Unikaryonidae, respectively Encephalitozoonidae, vary considerably [3, 20, 21, 26]. Furthermore, we possibly do not know all stages of the life cycle of Ciliatosporidium. We cannot exclude, for instance, the existence of a diplokaryotic stage. Thus, an incerta sedis position seems appropriate at the present state of knowledge. Ciliatosporidium is possibly most closely related to Oligosporidium, differing mainly by the uncoiled polar filament, the conspicuous polar sacs and the production of only one cell within each parasitophorous vacuole [5]. These differences apply also to the other genera mentioned. Unikaryon
seems to be more distant, because it lacks a parasitophorous vacuole, i.e. is not separated from the host's cytoplasm [3].

The second reviewer suggested to consider affinities with the monotypic family Abelsporidae. However, *Abelspora portucalensis* has a marine decapod host, a coiled polar filament, merogonial stages in the host hyaloplasm and produces two spores within one vacuole which originates by fusion of host vesicles [21].

*Ciliatosporidium platyophryae* was formerly suggested as belonging to the Metchnikovellidae because of its short, uncoiled polar filament [10]. However, the very special organization of metchnikovellids, viz. the lack of a polaroplast [25], sets apart *C. platyophryae*. The length of the polar filament varies considerably in genera of different families [17] and is thus of limited systematic value for higher level taxonomy. However, at genus level it appears as an appropriate character because there are few, if any, well-defined genera including species with coiled and uncoiled polar filament.

Transfer of *Cirrophrya terricola* Foissner, 1987 to *Platyophrya* Kahl, 1926

*Cirrophrya* belongs to the cyrtolophosid colpodids and is differentiated from the other genera of the family Platyophryidae, *Platyophrya* and *Platyophryidae*, by its foot-like appendages at the posterior end, allegedly acting as adhesive organelles [9, 11]. Foissner [6], being inexperienced with parasitic protozoa and influenced by Gellert's [11] report on *C. baptica*, did not recognize the parasitic nature of the appendages. He thus assigned the species to the genus *Cirrophrya*. The present results provide convincing evidence that the feet of *C. terricola* are a pathological phenomenon caused by a microsporidian parasite. Since all other characteristics of *C. terricola* match those of the genus *Platyophrya*, the species should be transferred to that genus: *Platyophrya terricola* (Foissner, 1987) nov. comb.

Within the genus *Platyophrya*, *P. terricola* is most similar to *P. vorax* (see [9] for detailed review), which has, however, only about 15-20 paroral dikinetids as opposed to 40-50 in *C. terricola*. Thus, synonymization of *P. terricola* with *P. vorax* would be premature at the present state of knowledge.

Two other *Cirrophrya* species have been described, viz. *C. baptica* Gellert, 1950 [11], type of the genus, and *C. australis* Foissner, 1993 [9]. The appendages of *C. baptica*, although very similar to those of *P. terricola* in size, shape and behaviour, apparently do not contain any structure which could be inter-

Figs. 25–28. Scanning electron micrographs of *Cirrophrya australis* (from [9]), a platyophryid ciliate with club-shaped to fusiform appendages at the posterior end (arrows). The size and shape of the appendages (arrows in Figs. 25, 26) vary greatly, and are in line with the somatic ciliary rows and only slightly thicker than ordinary cilia (arrows in Fig. 26), indicating that the appendages are modified (parasitized?) cilia. OA = oral apparatus. Bars = 10 μm (Fig. 25), 5 μm (Figs. 26, 28), and 3 μm (Fig. 27).
Are the Feet of Cirrophrya Adhesive Organelles?

Gellért [11] describes that C. haptica moves in a leech-like manner by adhering with its posterior appendages to detritus and algae. He also reports that the feet contain a glutinous substance and can be retracted leaving ring-shaped structures in the ciliate cortex. Unfortunately, these observations were never confirmed or disproved because the species has never been found again. However, Foissner [6] reports on similar behaviour of Platophrya terricola (Fig. 3). The present investigations show that the ciliate cortex lacks the alveoli, i.e. is reduced to the cell membrane, where it contacts the mature, protruding spores. This could indeed facilitate the access of secretory vesicles to the cell membrane, i.e. secretion processes. However, no secretions or structures (vesicles) which produce such substances were found. It is possible that the peculiar behaviour of the ciliate is due to the release of mature spores or simply, as suggested by J. Vavra (pers. comm.), by the weight of the spores accumulated at the posterior end of the cell. But we cannot confirm or disprove any hypothesis, because we did not study the behaviour of the population from the United States, assuming an anchoring function of the feet.

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References


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