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FLAGELLATED SPORES OF *Dermocystidium cyprini*

Kaja LOTMAN[a] and Jüri KASESALU[b]

[a] Institute of Zoology and Hydrobiology, University of Tartu, Vanemuise 48, EE-2400 Tartu, Estonia
[b] Institute of Animal Husbandry, Estonian Agricultural University, Kreutzwaldi 1, EE-2400 Tartu, Estonia

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Abstract. A previously unknown flagellated lifestage of the gill pathogen *Dermocystidium cyprini* (Červinka et Lom, 1974) from common carp is described. The flagellated spores were 2–3 µm in diameter and had a single at least 8 µm long flagellum with nine peripheral and two central microtubules. The flagellated spores were found both in mature cultured cysts incubated in water and in the biggest fresh cysts removed from the gills of fingerling common carp in May. Implications of the finding for the systematics of the genus *Dermocystidium* are discussed.

Key words: *Dermocystidium cyprini*, fish parasites, flagellated spores, life stages, spore ultrastructure, systematics.

INTRODUCTION

Dermocystidium cyprini (Červinka et Lom, 1974) is a gill pathogen of common carp (*Cyprinus carpio*), which can cause sporadic outbreaks with high mortality but mostly causes moderate losses of wintering fingerlings in fish farms (Lopukhina, 1968; Červinka et al., 1974; Kasesalu & Lotman, 1994, 1995). The diagnostic features and some of the life stages of this species were described by Červinka et al. (1974). The parasite is recognized by white cysts of moderately ovoid shape that reach about 2 mm in diameter. The development of cysts is supposed to be associated with transformation of uninucleate cells into multinucleate plasmodia. After fragmentation of plasmodia, spores develop inside the cysts. The fresh spores are 4–5 µm in diameter and have a refractile central inclusion.

The genus *Dermocystidium* is of uncertain taxonomical relationship, assigned by some investigators to protists (Garkavi et al., 1980; Nash et al., 1989), by others to the lower fungi (Pauley, 1967; Allen et al., 1968).

Perkins (1976) described a flagellated stage of *D. marinum* that has a rudimentary apical complex. Levine (1978) therefore included this species into the newly established phylum Apicomplexa under the generic name *Perkinsus*. Other species of *Dermocystidium* did not receive a clear taxonomic status in Levine's system.

Olson et al. (1991) reported formation of uniflagellated zoospores within discharged spores of *D. salmonis*. These flagellated stages, lacking any apical complex, represented the agents of disease transmission. It is worth noting here that the disease transmission mechanisms for other species of *Dermocystidium* are still unknown.

This paper is the first report of flagellated spores of *D. cyprini*.

MATERIAL AND METHODS

The cysts of *Dermocystidium cyprini* were removed from the gills of common carp fingerlings in February, March, April, and May 1996. The cysts were observed under a light microscope, washed repeatedly in fresh water, and incubated in petri dishes in fresh water at 4 °C in the absence of antibiotics (Olson et al., 1991). The observed cysts were in three different developmental stages: plasmodia ($n = 17$, removed in February), dividing plasmodia ($n = 21$, removed in March), and early sporogenesis ($n = 20$, removed in April). In cultures where maturation did not occur the experiment was terminated after 60 days of culture.

Mature cultured cysts as well as the biggest fresh cysts removed from gills of fingerling common carp were examined using both light and electron microscopy. For the latter purpose, the cultures were centrifuged and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4 °C for 24 h. The samples were post-fixed for 1 h with 2% OsO_4 in the same buffer. The fixed material was dehydrated and embedded in EPON. The sections were stained with uranyl acetate and lead citrate prior to examination with TESLA BS 500. For light microscopy, smears of cysts were airdried, fixed in methanol, and stained with May Grünwald-Giemsa.

RESULTS

The cysts in the stage of early sporogenesis matured normally within two weeks. Of 21 cysts in the dividing plasmodia stage 3 matured after three weeks, others did not mature.

Flagella-like structures were found in well developed cysts between typical spores (Fig. 1). Electron microscopy revealed a large lipid-like osmiophilic body, small peripheral nuclei, a small amount of endoplasmatic reticulum, and one or two mitochondria with tubular cristae in mature spores. The spores contained several small lipid-like inclusions, which fused into a large one in the process of maturation. Some of the spores were kidney-shaped with a lipid-like inclusion in one side and a flagellum in the other (Fig. 2). The lipid-like inclusions were smaller in spores with a flagellum. Flagellated spores were 2–3 μm in diameter, being therefore smaller than those without a flagellum. They had a single flagellum with nine peripheral and two central microtubules, indistinct nuclei, and not very clearly resolved mitochondria. The flagellum was at least 8 μm long.

We also found flagellated spores in some cysts that were removed from the fingerlings of common carp in May. These cysts were swollen and fragile.

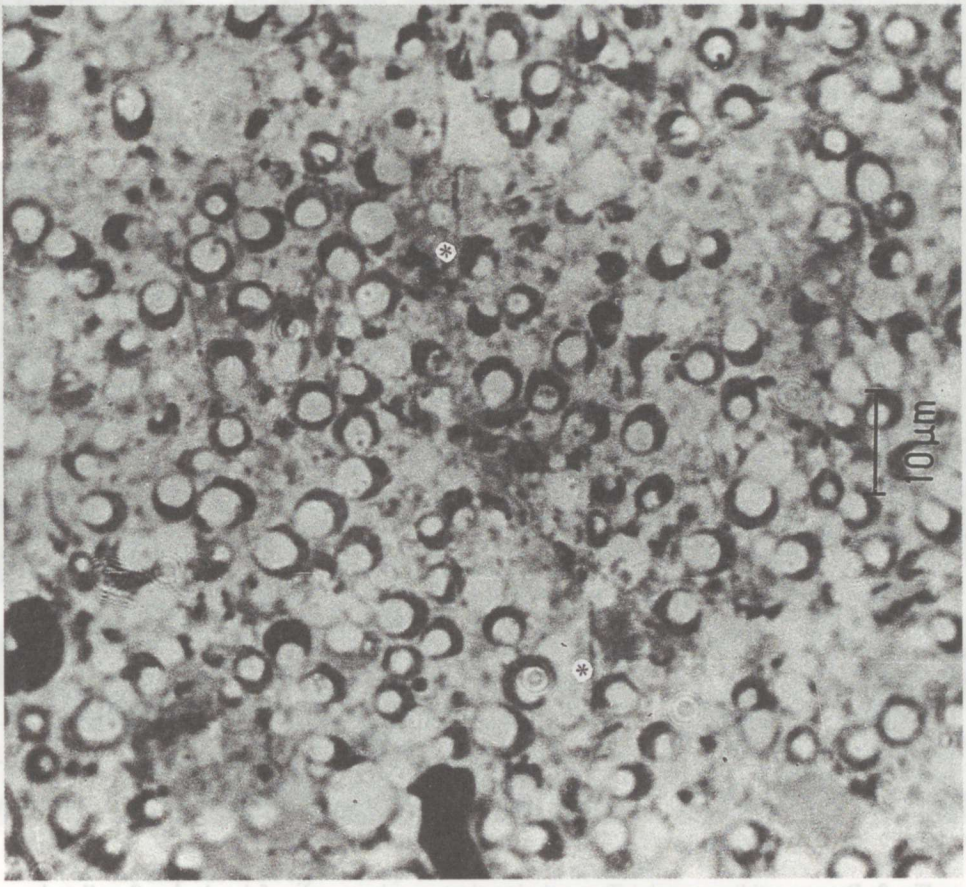

Fig. 1. *Dermocystidium cyprini.* Smear of the mature spores that where incubated at 4 °C (May Grünwald-Giemsa). * flagellated spores among unflagellated spores.

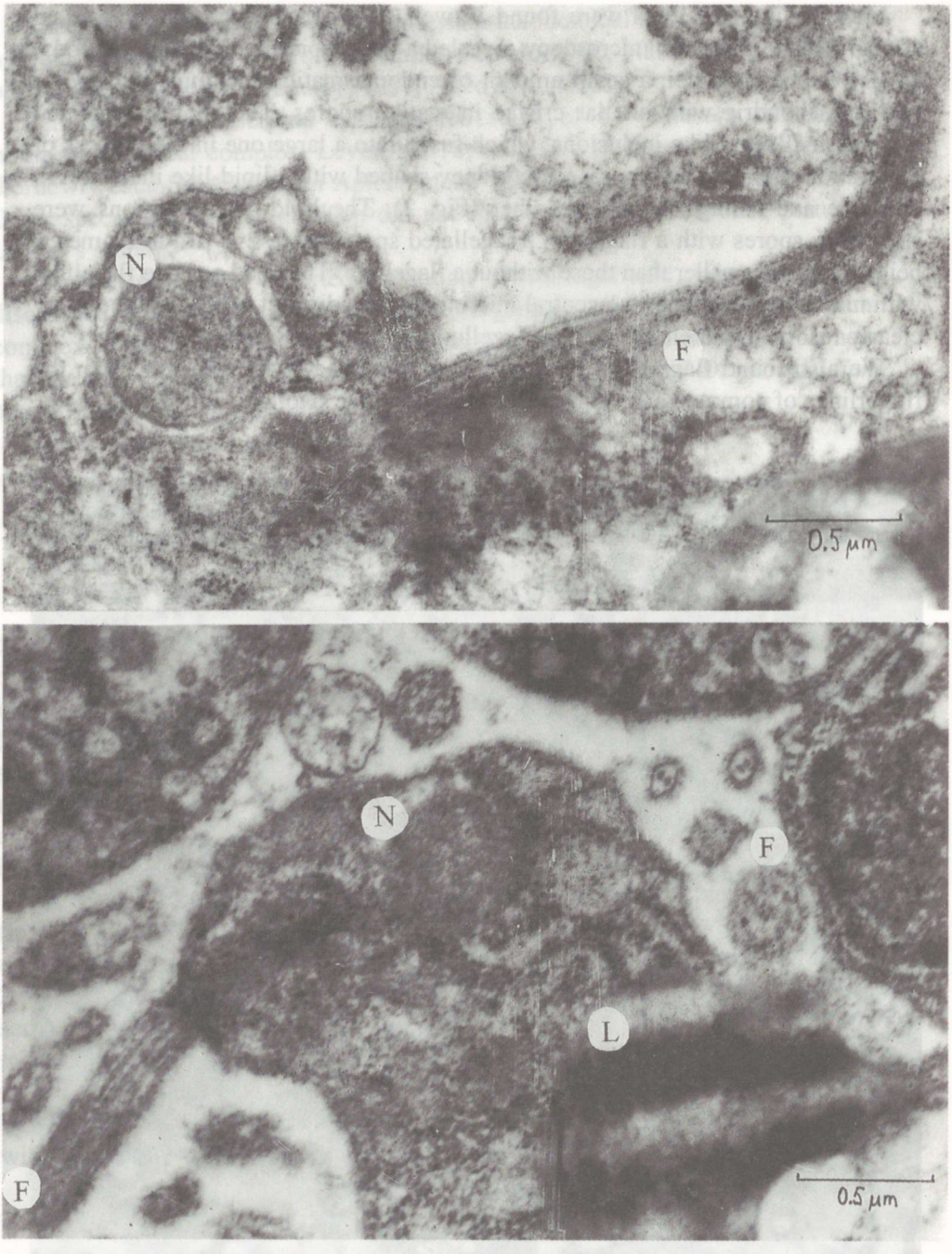

Fig. 2. *Dermocystidium cyprini*. Transmission electron micrographs of developing flagellated spores. L, lipid inclusion; F, flagella; N, nucleus.

DISCUSSION

There are relatively few studies of the spore ultrastructure of *Dermocystidium* in spite of its extreme importance for the taxonomy of the genus. Therefore, several important points are still open for debate.

The features of cysts and spores observed during this study are mostly consistent with those described by Červinka et al. (1974) for *D. cyprini*. However, they did not observe flagellated spores of this species.

We could not find characteristic structures of apical complex as it was described in zoospores of *D. marinum* by Perkins (1976). Neither could we find vacuoplasts, laminated bodies or ramified hyphae, described in some other species of the genus *Dermocystidium* (Lom & Dykova, 1992).

Olson et al. (1991) described the development of motile uniflagellated zoospores in *D. salmonis* and the transmission of the disease by these zoospores. The ultrastructure of the flagellated spores of *D. cyprini* is very similar to that of *D. salmonis* as described by Olson et al. (1991). The presence of flagellated spores in the swollen, fragile cysts in May, at the end of the annual epizootic cycle, and the lack of such spores in earlier samples, suggest the transmission of the disease by the flagellated spores. This further strengthens the similarity between these two species. It is possible that a flagellated stage for disease transmission is common among *Dermocystidium* spp., although this has not been described due to our insufficient knowledge on the lifecycle of the parasite. This hypothesis, however, remains to be confirmed by further investigations. The biology of the genus *Dermocystidium* and similar forms clearly needs further study.

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Dermocystidium cyprini VIBURIGA SPOORID

Kaja LOTMAN ja Jüri KASESALU

On esmakordselt kirjeldatud karpkala parasiidi *Dermocystidium cyprini* viburiga spoore. 2–3 µm läbimõõduga spoorid olid varustatud ühe vähemalt 8 µm pika viburiga, milles oli üheksa servmist ja kaks keskset mikrotorukest. Viburiga spoore leiti nii kultuuris kasvatatud küpseis tsüstides kui ka mai lõpul karpkala lõpustelt eemaldatud tsüstides. Arutelu käsitleb leiu tähendust perekonna *Dermocystidium* süstemaatikale.