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Dermocystidium infection in common carp broodstock (Cyprinus carpio L.) from Croatia

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Abstract

This paper presents the first finding of *Dermocystidium* sp. in Croatia. The species was found on a fish farm, in broodstock of common carp (*Cyprinus carpio* L.). The localisation of the hyphae and morphological characteristics of spores confirms that the species is identical with *Dermocystidium* sp. described by Novotny & Smolova (2006). Hyphae were 109 ± 44 μm (mean ± SD) wide, with 4.8 ± 1.3 μm thick homogeneous walls. Mature and immature spores measured 8.3 ± 1.5 μm and 5.1 ± 0.2 μm, respectively. The refractile body was 4.7 ± 0.9 μm in diameter. Despite small variations in size, the authors tentatively identified these species as *Dermocystidium koi* Hoshina & Sahara, 1950.

Introduction

Dermocystidium infection in various freshwater fish has been studied worldwide. To our knowledge, so far only a few *Dermocystidium* spp. have been reported in fish belonging to the Cyprinidae family, namely *Dermocystidium koi* Hoshina & Sahara, 1950, *Dermocystidium kamilovi* Allamuratov, 1965, *Dermocystidium kobiacevi* Allamuratov, 1965, *Dermocystidium cyprini* Cervinka & Lom, 1974 and *Dermocystidium erschowi* Garkavi, Denisov & Afanasjev, 1980. Furthermore, Novotny & Smolova (2006) described *Dermocystidium* sp. in the skin of common carp originally imported to the Czech Republic from Hungary, and Molnár et al. (2008) described *Dermocystidium* sp. in the eye of crucian carp in Hungary. In general, *Dermocystidium* infections are manifested as cysts with different localization and morphology of the spores, depending on the parasites species (Novotny & Smolova, 2006). However, unlike other species of the genus, *Dermocystidium koi* forms long spore-producing hyphae (Dyková & Lom, 2007). Garkavi et al. (1980) also mentioned a fungoid form of cysts in the skin of carp infected with *Dermocystidium erschowi.* As *Dermocystidium* infections have not been previously reported in Croatia, the aim of this study was to determine the presence and prevalence of *Dermocystidium* sp. and to describe histopathological changes found in the fins of common carp. Furthermore, this paper presents the morphological characteristics of the parasite from fresh preparations and histological sections.

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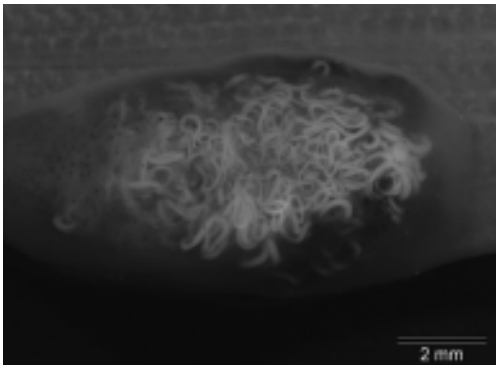

Figure 1. Fin lesion with numerous white hyphae inside the lesion.

Materials and methods

This study involved common carp (*Cyprinus carpio* L.) obtained from one fish farm in Croatia in April. A total of 450 specimens comprising one-year old carp, two-year old carp and carp broodstock were examined. Fresh preparations of fin lesions were made. The fins of infected carp were removed and fixed in 10% buffered formalin and Bouin's solution. The fixed material was embedded in paraffin and serially sectioned at 2 μm. Sections were stained with hematoxylin and eosin (H&E) and the Periodic-acid Schiff (PAS) reaction (Sheehan & Hrapchak, 1980). Some sections were also stained with Toluidine blue (TB) (Pearse, 1968), Mallory's aniline blue and Azan (Sheehan & Hrapchak, 1980). The sections were studied by light microscopy (Olympus BX41) at magnifications of up to 2000x. Hyphae and spores were measured using a digital camera (Olympus DP12) and Cell B software (Soft Imaging System). Measurements were taken from fresh preparations and histological sections. Mean values of all measurements are presented in μm, and ranges are provided in parentheses.

Results

Red coloured fin lesions, 3 – 18 mm in size, were found in 45 (30%) of 150 carp broodstock specimens, but were not found in any of the examined one- or two-year old carp. Lesions were predominantly found in the dorsal and caudal fins, and occasionally in other fins, and were randomly distributed along the entire length of the fins. In cases of intensive infection, lesions were also found in the skin.

Lesions contained numerous white aseptate hyphae visible through the epidermis (Figure 1). The hyphae, 109 (40 – 183) wide, with 4.8 (2.9 – 7.4) thick hyphal wall, were filled with mature or immature spores (Figure 2). Mature

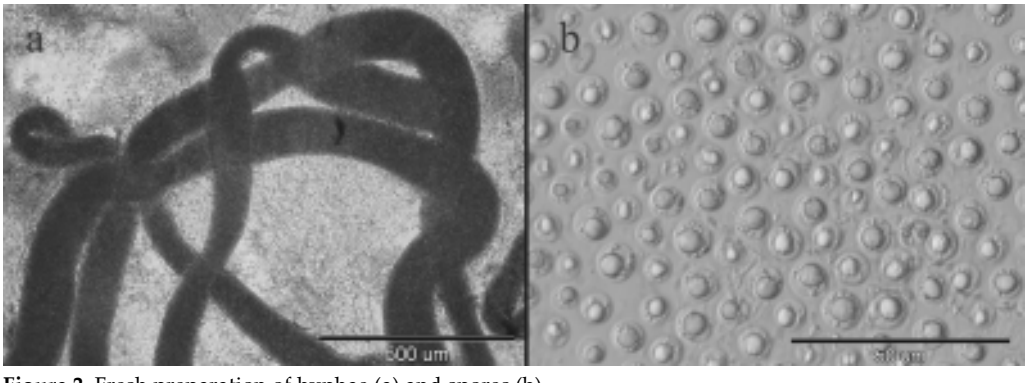

Figure 2. Fresh preparation of hyphae (a) and spores (b).

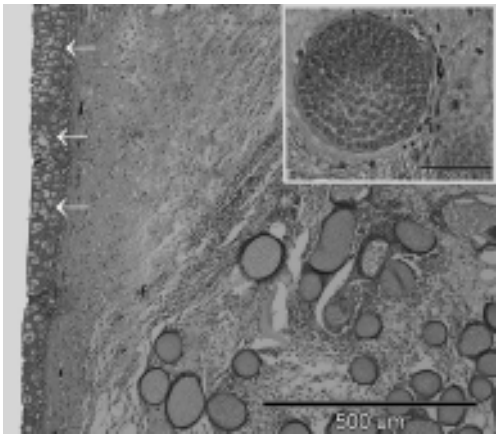

Figure 3. Histological section of the fin showing numerous hyphae of *Dermocystidium koi* in the dermis of common carp. Increased number of club cells in the periphery of the lesion (arrow) (H&E). Inset: Hypha in transverse section with PAS-positively stained spores.

spores measured 8.3 (5.6 – 13.1), and immature spores measured 5.1 (4.7 – 5.6). Round mature spores had an eccentrically situated nucleus and a large refractile body 4.7 (2.4 – 7.5) in diameter. The cytoplasm contained many round granules.

In histological sections, the hyphae appeared as oval or elongate cyst-like structures filled with spores (Figure 3). Early stage hyphae were also observed (Figure 4). Oval "cysts" (hyphae in transverse section) were 80 (52 – 125) in diameter, while elongate "cysts" were 351 (200 – 948) long. The hyphal wall was 2.7 (1.5 – 4.5) thick, and stained blue after Mallory's aniline blue and metachromatic with TB. Mature spores measured 6.4 (4.7 – 7.8), and immature spores measured 3.2 (2.5 – 3.8). The refractile body was 4.1 (2.9 – 5.3) in diameter, and stained pink after H&E (Figure 5), orange after Mallory's aniline blue, and red with a clearly visible area around the refractile body after Azan. The cytoplasm stained metachromatic and the vacuole stained orthochromatic with TB. PAS staining showed PAS-positive content within spores (refractile body weakly PAS-positive).

Histopathology

Histopathological examination of the fins revealed changes representative of *Dermocystidium* sp. infection.

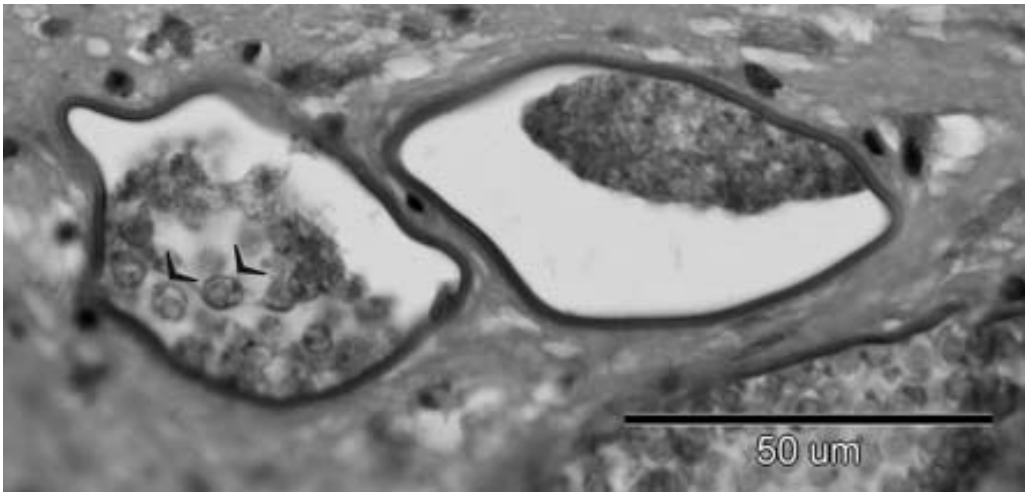

Figure 4. Early stage hyphae with thick walls. Note immature spores (arrowhead) within the hypha (H&E).

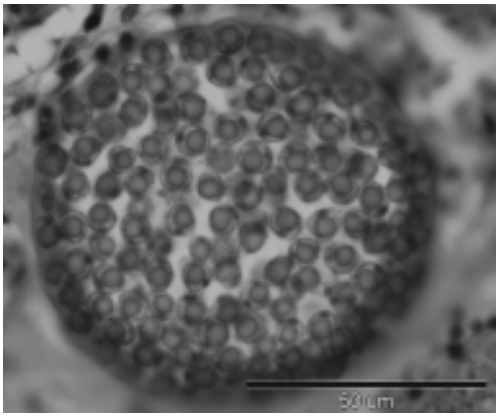

Figure 5. Mature spores of *Dermocystidium koi* showing eccentrically situated nucleus and a large refractile body (H&E).

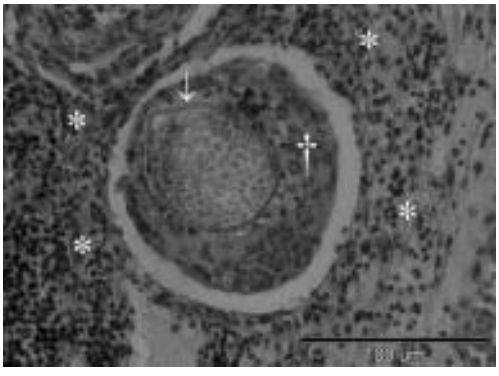

Figure 6. Host response in the dermis (H&E). The hypha is surrounded by granulomatous tissue (†). Lymphocyte infiltration in the periphery (*); Hyphal wall (arrow).

Histopathological changes in the dermis included a huge infiltration of mononuclear cells, in particular lymphocytes and macrophages. Moderate plasma cell infiltration was found occasionally. Oedema and disseminated focal haemorrhages were also evident in the areas of infection. The host response frequently included the formation of multiple granulomas with severe mononuclear cell infiltration around the granulomas (Figure 6). Although the majority

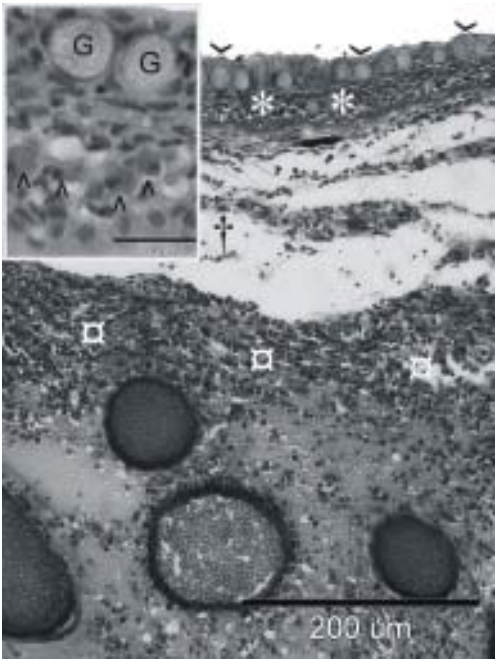

Figure 7. Huge mononuclear cell infiltration in the dermis (¤) with oedema (†) and epidermal vacuolization (*); Goblet cells (arrowhead) (TB). Inset: Eosinophilic granule cells infiltration (arrowhead) and vacuolization of basal cells (PAS); Goblet cells (G).

of the hyphae usually had an intact hyphal wall, some were ruptured, releasing spores within the damaged tissue. In addition to these histopathological changes in the dermis, some changes were also observed in the epidermis, such as focal vacuolization of basal cells and hyperplasia of epithelial cells. A moderate infiltration of eosinophilic granule cells and lymphocytes with an increased number of goblet cells were also recorded in the epidermis of this area (Figure 7). However, these changes were only observed sporadically. More often, the hyphae, which were located near the epidermis, caused atrophy of the overlying epidermis (Figure 8). In several cases, disseminated epidermal

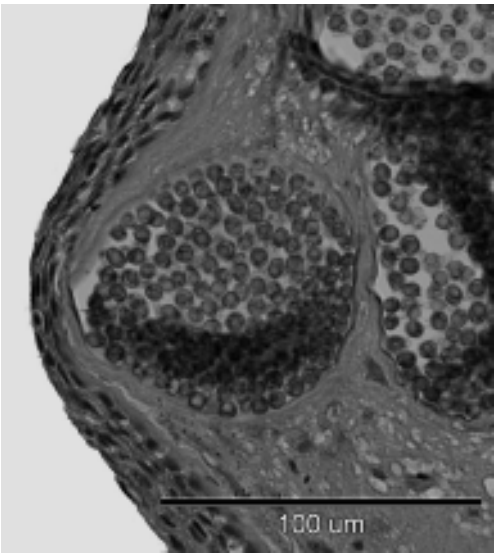

Figure 8. Atrophy of epidermis without club and goblet cells (PAS).

erosion was observed. In some areas, the epidermis was five cells thick on average, with complete disappearance of club and goblet cells. An increased number of club cells were present in the peripheral area of the lesion. Compared with uninfected samples, club cells were almost completely absent from the infected parts of the fin.

Discussion

Our research on the presence of *Dermocystidium* sp. in common carp cultured in Croatia revealed that 30% of the examined common carp broodstock were infected with *D. koi.* The majority of these carp broodstock were originally imported from Hungary few years ago. A similar finding was also reported by Novotny & Smolova (2006), according to which the prevalence of the species in common carp imported to Czech Republic from Hungary was approximately 40%. The finding of *Dermocystidium* sp. in the fins of carp is hereby reported in the Republic of Croatia for the first time. No *Dermocystidium* infection was found in any of the examined one- or two-year old carp, thus, we believe that the species was imported to Croatia. Therefore, it is essential to subject carp importation to quarantine and regular health monitoring.

Several authors have studied *Dermocystidium* infection in the Cyprinidae (Table 1). To our knowledge, only *D. kamilovi* (Allamuratov, 1965) and *D. cyprini* (Cervinka et al., 1974) have been found in the gills of carp. The species observed in this study differs from the two mentioned above in certain morphological characteristics. It also differs from *D. erschowi*, which has been found in the skin of carp (Garkavi et al., 1980). Hoshina & Sahara (1950) and Dyková & Lom (1992) gave a detailed description of *D. koi* found in the skin of koi carp. The morphometrical values provided by these authors are similar, with the exception of cysts/hyphae description. According to Hoshina & Sahara (1950), the cysts were 40 – 300 μm in diameter with a 1.8 – 2.0 μm thick membrane, whilst according to Dyková & Lom (1992) the hyphae were up to 70 μm. In this study, morphometrical values are in line with the findings of Hoshina & Sahara (1950) and Dyková & Lom (1992) with the exception of small differences in the size of the hyphae. The species studied here, also resembled *Dermocystidium* sp. reported in the fins of common carp (Novotny & Smolova, 2006). These authors reported the presence of cysts filled with spores, and the report was based on histological sections. Thus, we believe that described cysts are actually hyphae in transverse section. According to Lom &

Species (reference)	Characteristics (dimensions in μm)	Spore size (μm)	Host and site of infection
Present study			
(fresh preparations)	Aseptate hyphae, 40 - 183	5.6 - 13.1	*Cyprinus carpio*; fins
(histological sections)	Cyst-like structures: oval 52 - 125, elongate 200 - 948	4.7 - 7.8	
D. koi (Hoshina & Sahara, 1950)	Cysts elongate, 40 - 300 in diameter	6.3 - 14.4 (skin) 5.4 - 12.9 (muscle)	*Cyprinus carpio*(koi carp); skin, muscle
D. koi (Dyková & Lom, 1992)	Hyphae like a thick-wall cylinders or thin-wall tubes, 10 - 70	6.5 - 15	*Cyprinus carpio*(koi carp); skin, fins
D. koi (Wildgoose, 1995)	Intradermal cystic lesions comprising a thin-wall hyaline capsule. White, aseptate hyphae of varying thickness	8.9 - 14.6	*Cyprinus carpio*(koi carp); skin, fins
D. kamilovi (Allamuratov, 1965)	Cysts elongate, 651 - 1880 x 237 - 1374	7.5 - 10.5	*Cyprinus carpio*; gill
D. kobiacevi (Allamuratov, 1965)	Cysts oval, 171 - 204 x 122 - 155	10.5 - 14.2	*Cyprinus carpio*; skin
D. cyprini (Cervinka et al., 1974)	Cysts ovoid, 600 - 2000	4 - 5	*Cyprinus carpio*; gill
D. erschowi (Garkavi et al., 1980)	Cysts elongate, 100 - 160 x 2000	14 - 16	*Cyprinus carpio*; skin
Dermocystidium sp. (Ehab Elsayed et al., 2002)	Cysts round, 500 - 3000	9 -36 x 6 - 33	*Cyprinus carpio*; head
Dermocystidium sp. (Novotny & Smolova, 2006)	Spherical cysts 50 - 110, elliptic cysts 150 - 550	5 - 6	*Cyprinus carpio*; fins
Dermocystidium sp. (Molnár et al., 2008)	Convoluting hyphae, 280 - 500	9 - 15	*Carassius carassius* (crucian carp); eye

Table 1. *Dermocystidium* species in fish from Cyprinidae family.

Dyková (1992) and Bruno et al., (2006), *Dermocystidium koi* is characterized by great variability in the size of spores and formation of ramified hyphae with a thick homogeneous wall. The results of this study show that fixation of material affects the size of hyphae and spores. These observations suggest that fresh material should be used for the determination of *D. koi*, and this might explain some of the morphological differences reported by other authors (Hoshina & Sahara, 1950; Dyková & Lom, 1992; Novotny & Smolova, 2006). Despite variations in size, we believe that the species we studied is *Dermocystidium koi* Hoshina & Sahara, 1950. However, further research (molecular) is needed to establish the validity of *Dermocystidium koi* as an independent species.

Furthermore, Allamuratov (1965) described *Dermocystidium kobiacevi* in the skin of common carp. The morphometrical values provided by Allamuratov (1965) are in line with the finding of Hoshina & Sahara (1950), and we therefore consider that *D. kobiacevi* could be a synonym of *D. koi*.

There is little information available on the histopathology associated with *Dermocystidium koi* infection. Hence, this paper describes histopathological findings observed in the fins of common carp. The basic histopathological changes described in the dermis show a huge infiltration of mononuclear cells accompanied with oedema, disseminated focal haemorrhages, and formation of multiple granulomas. This histopathological finding is in line with the findings described for koi carp by Dyková & Lom (1992). We also observed some changes in the epidermis that were not described by these authors. The changes include epidermal atrophy with complete disappearance of club and goblet cells as well as focal vacuolization of basal cells and epithelial hyperplasia with a moderate infiltration of eosinophilic granule cells and lymphocytes. The present study shows that *Dermocystidium* infection causes only local changes in fins, and we therefore believe that this species could not be a serious pathogen of common carp broodstock.

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