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Dermocystidium sp. (Mesomycetozoea: Dermocystidiaceae) primary ocular infection in a koi carp (Cyprinus carpio var. koi)

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**Dermocystidium sp. (Mesomycetozoea: Dermocystidiaceae)  
primary ocular infection in a koi carp (Cyprinus carpio var.  
koi)**

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3 ***Dermocystidium* sp. (Mesomycetozoa: Dermocystidiaceae) primary ocular infection in a koi**  
4 **carp (*Cyprinus carpio* var. *koi*)**

5

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14 **Running head:** *Dermocystidium* sp. ocular infection in a koi

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25 **Keywords:** *Dermocystidium* sp., Mesomycetozoa, koi carp, eye

26 *Dermocystidium* spp. (Mesomycetozoea: Dermocystidiaceae) comprises several species able to  
27 infect a wide range of marine and freshwater fish (Gozlan et al., 2014; Rowley et al., 2013). The  
28 taxonomic position of *Dermocystidium* is still under debate, even though Dyková and Lom (1992)  
29 observed hyphae associated to *Dermocystidium koi* infection, suggesting its fungal nature.  
30 Currently, the genus is classified in the superclass Mesomycetozoea which includes protists  
31 “fungus-like” in the supergroup Opisthokonta (Gozlan et al., 2014).

32 The lesions caused by *Dermocystidium* spp. in fish are chronic and characterized by visible cysts or  
33 nodules on skin and fins (Feist et al., 2004; Wildgoose, 1995; Zhang & Wang, 2005), but severe  
34 gills and systemic infections causing mortality have been described in several species (Höglund et  
35 al., 1997; Olson & Holt, 1995; Athanassopoulou, 1998; Landsberg & Paperna, 1992; Kirkbright et  
36 al., 2016; Fujimoto et al., 2018).

37 The present study described a primary ocular infection by *Dermocystidium* sp. in a koi carp  
38 (*Cyprinus carpio* var. *koi*). The koi carp was found dead by the owner of a private pond and sent  
39 refrigerated to the laboratory. The fish presented an evident subconjunctival whitish exophytic  
40 tissue, surrounded by reddish edges, that protruded over both ocular bulbs (Fig. 1a). The fish was  
41 necropsied and tissues fixed in 10% buffered formalin. The ocular globes were then dissected under  
42 stereomicroscope and smears of the exophytic tissue placed on slides, clarified with lactophenol or  
43 stained with China ink, and examined under light microscope. For histopathology, tissues were  
44 routinely processed, sections were cut at 4  $\mu\text{m}$  and stained with Haematoxylin and Eosin (H&E),  
45 Periodic Acid Schiff (PAS) and Gomori.

46 At the stereomicroscope, the peribulbar tissue and anterior chamber presented numerous filiform 3-  
47 mm-length white hyphal-like cysts (Fig. 1b). At the light microscope, the cysts were filled with  
48 round variably sized (5.53-12.90  $\mu\text{m}$  in diameter; mean=9.71; sd=1.99; n=80) mature spores  
49 referable to *Dermocystidium* sp. admixed with developmental stages (>5  $\mu\text{m}$ ). The spores showed a  
50 large refractile body or central vacuole and a peripheral cytoplasm with a nucleus and three or more  
51 vacuoles (Fig. 1c).

52 At histology, the peribulbar soft tissues until muscle and the choroid space of the posterior chamber,  
53 adjacent to the retina, were infiltrated by numerous elongated cysts (Fig. 2f-h). The cysts had a  
54 thick eosinophilic wall and contained numerous spores (Fig. 2h,i). Around the cysts, a  
55 granulomatous inflammatory reaction was visible (Fig. 2h). The iris and the ciliary body were also  
56 replete by numerous free mature spores. An intense mixed inflammatory infiltrate with  
57 multinucleated giant cells, vascular congestion and abundant granulation tissue were observed (Fig.  
58 2g). Haemorrhagic and necrotic areas were observed. Some basophilic granular aggregates referable  
59 to bacteria were also found. Neither developmental stages of *Dermocystidium* sp. nor inflammatory  
60 reaction were seen in internal organs. PAS and Gomori stains helped to visualize the developmental  
61 stages (Fig. 1d,e) and exclude the presence of spores in other organs.

62 In literature, only three descriptions of ocular infection by *Dermocystidium* sp. are reported in fish  
63 (Elkan, 1962; Molnar et al., 2008; Fujimoto et al., 2018). The present case is the first report of a  
64 primary ocular infection in a koi carp. The spore size and morphology are consistent with literature  
65 reports of *Dermocystidium koi* (Gjurcevic et al., 2008; Hoshina & Sahara, 1950). The life cycle of  
66 *Dermocystidium* spp. is partly unknown. The parasite produces motile zoospores which encyst in  
67 the host tissues, form sporonts by division of the plasmodia until subsequent maturation of spores  
68 (Lotman et al., 2000; Mahboub & Shaheen, 2020). In experimental trials, it has been demonstrated  
69 that fish can acquire the infection through intra-gastric and immersion routes. The pathogen cycle  
70 begins from the ingestion of spores through feeding on infected fish or contaminated feed or via  
71 penetration of spores into the gills (Mahboub & Shaheen, 2020).

72 Considering the economic value of koi carps and the possible direct transmission of the infection in  
73 optimal temperatures condition, *Dermocystidium* spp. infection should be considered as an  
74 important disease also in ornamental species.

75

#### 76 **Conflicts of interests**

77 The authors declare to have no conflict of interests.

78

79 **Data availability**

80 The data that support the findings of this study are available from the corresponding author upon  
81 request.

82

83 **References**

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136

### 137 **Figure legends**

138 **Figure 1.** (a) Koi carp showing an ocular exophytic whitish tissue. (b) Numerous filiform hyphal-  
139 like cysts visible at the stereomicroscope (bar=100  $\mu\text{m}$ ). (c) The cysts are filled with round mature  
140 spores morphologically referable to *Dermocystidium* sp. (bar=10  $\mu\text{m}$ ). The spores show a large  
141 refractile body (RB) and a peripheral cytoplasm with a nucleus and vacuoles (inset, arrow). (d)  
142 Gomori stain shows cytological details of the spores (bar=10  $\mu\text{m}$ ). (e) PAS stain evidences the  
143 developmental stages of the parasite. Four zoospores are visible inside a spore (arrowhead) (bar=10  
144  $\mu\text{m}$ ).

145

146 **Figure 2.** (f) The choroid space near the retina is infiltrated by numerous cysts (asterisk) (H&E,  
147 bar=500  $\mu\text{m}$ ). (g) The iris and the ciliary body are replete by numerous free mature spores  
148 (arrowhead). A severe inflammatory reaction and granulation tissue are evident. Note the  
149 multinucleated giant cells (inset) (H&E, bar=50  $\mu\text{m}$ ). (h) Around the cysts, a granulomatous  
150 inflammatory reaction characterized by a wall of epithelioid cells is visible (inset) (H&E, bar=100  
151  $\mu\text{m}$ ). (i) The cysts show a thick eosinophilic wall and contain spores at different developmental  
152 stages (H&E, bar=50  $\mu\text{m}$ ).

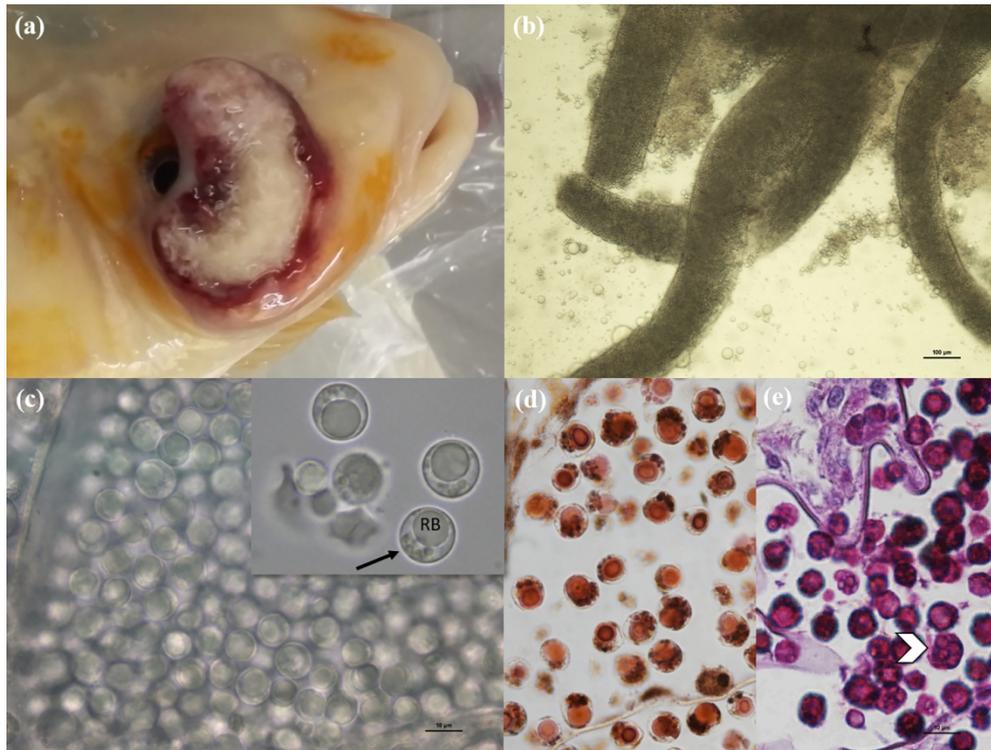


Figure 1. (a) Koi carp showing an ocular exophytic whitish tissue. (b) Numerous filiform hyphal-like cysts visible at the stereomicroscope (bar=100  $\mu$ m). (c) The cysts are filled with round mature spores morphologically referable to *Dermocystidium* sp. (bar=10  $\mu$ m). The spores show a large refractile body (RB) and a peripheral cytoplasm with a nucleus and vacuoles (inset, arrow). (d) Gomori stain shows cytological details of the spores (bar=10  $\mu$ m). (e) PAS stain evidences the developmental stages of the parasite. Four zoospores are visible inside a spore (arrowhead) (bar=10  $\mu$ m).

144x108mm (300 x 300 DPI)

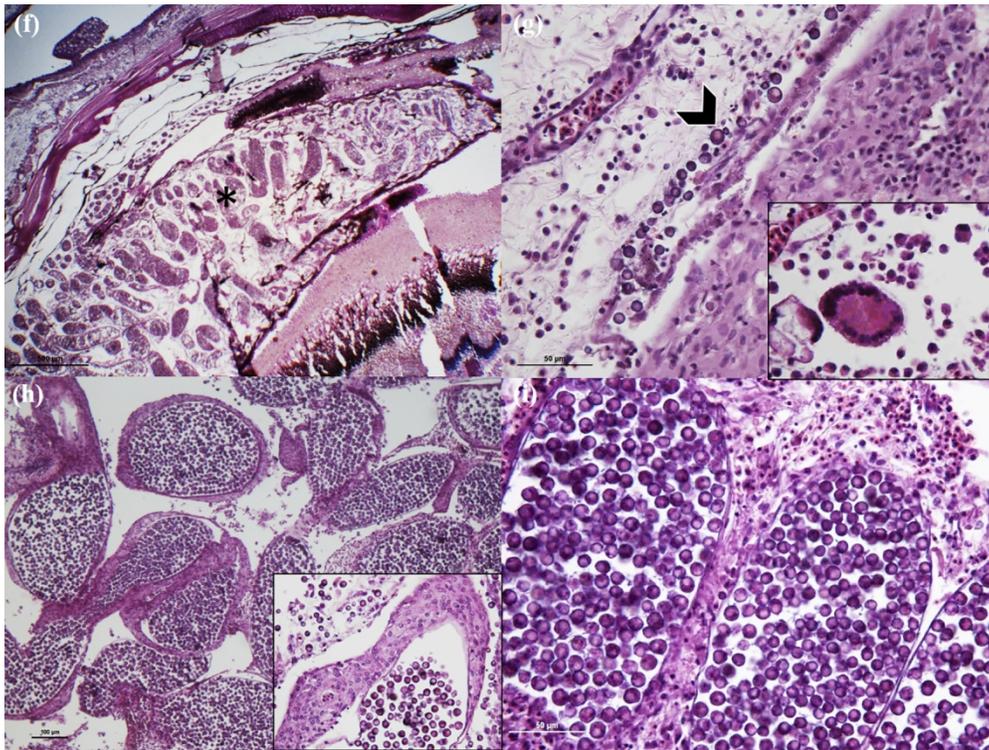


Figure 2. (f) The choroid space near the retina is infiltrated by numerous cysts (asterisk) (H&E, bar=500  $\mu$ m). (g) The iris and the ciliary body are replete by numerous free mature spores (arrowhead). A severe inflammatory reaction and granulation tissue are evident. Note the multinucleated giant cells (inset) (H&E, bar=50  $\mu$ m). (h) Around the cysts, a granulomatous inflammatory reaction characterized by a wall of epithelioid cells is visible (inset) (H&E, bar=100  $\mu$ m). (i) The cysts show a thick eosinophilic wall and contain spores at different developmental stages (H&E, bar=50  $\mu$ m).

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