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CASE STUDY

A case study of *Dermotheca gasterostei* (=*Dermocystidium gasterostei*, Elkan) isolated from three-spined stickleback (*Gasterosteus aculeatus*) captured in lake Vättern, Sweden.

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During the early summer of 2021 several sticklebacks with massive *Dermocystidium* infestation were caught in lake Vättern, Sweden. In this brief report we present a histological investigation of the host-parasite relationship, and by 18S rRNA gene sequencing identify the parasite as *Dermocystidium gasterostei* Elkan, closely related to *Dermocystidium percae*.

Mesomycetozoea is a class of fungus-like protist pathogens close to the branching between the animal and fungal kingdom within the holozoan supergroup (Kirkbright et al. 2015; Mendoza, Taylor, and Ajello 2002). During the last decades the composition of the class Mesomycetozoea have gone through significant changes with an increasing number of species being added. Originally being named Ichthyosporea to reflect their occurrence in fish, these pathogens have now also been identified in invertebrates, birds, and mammals (Glockling, Marshall, and Gleason 2013). However, the parasites are predominantly found in aquatic organisms, both marine and freshwater, usually occurring under cold water conditions. These parasites, which mainly infect the skin, fins, and gills, but occasionally also internal organs, are normally placed in the genus *Dermocystidium* (Langenmayer et al. 2014). The genus currently contains about 20 various pathogenic organisms, but the genus is likely to contain more members in the future as the majority of pathogens have only been described to genus level. The pathogens infect a magnitude of aquatic animals (Kirkbright et al. 2015), where *Dermocystidium* spp. produces clearly visible cysts in host tissue, and each cyst is filled with small spherical spores. In each spore, a relatively large refractile body (vacuoplast) that pushes the cytoplasm and nucleus to the periphery of the cellular body is present (Shamsi et al. 2020). To date, all described organisms within Mesomycetozoea have been found to cause anything from mild sub-clinical infections to aggressive fatal infections. This study is a case report on the first case of *Dermocystidium* recorded in the lake Vättern in Sweden.

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Figure 1. Macroscopic images of infested Sticklebacks.

A) Ethanol fixed adult three-spined stickleback covered with worm-like white cysts. B) zoomed in view revealing the sub-epidermal position of the cysts. Note several melanophores on the cysts.

In May 2021, several three-spined sticklebacks (*Gasterosteus aculeatus* L.) infested with "white skin worms" (Figure 1A) were caught in lake Vättern in Sweden. The discovery was made by a local aquarium that capture wild fish and display them to the public for educational purposes. Capture of wild stickleback is carried out several times every year and until this point, they had never seen such infestation. After consultation with the National Veterinary Institute, some of the fish were fixed in 70% denatured ethanol after they had died (of natural causes, the lifetime of Sticklebacks in the aquarium is short) and sent for analysis. Three individuals were collected, all around 55 mm in length, with multiple 3-5 mm long worm-like cysts embedded under the skin epithelium. This was evident as melanophores were sometimes visible as spots overlying the cysts (Figure 1B).

The cysts could easily be isolated using pointy tweezers. The dissected cysts had a very loose consistency and during initial investigation under the microscope they all fell apart, revealing small individual unicellular organisms. Further characterization of the cysts was performed by histological examination. A whole fish body was embedded in paraffin, cross-sectioned behind the head and stained with hematoxylin and eosin (HE) (Figure 2A-C). Histology verified the macroscopic observation that the cysts were superficial but underlying the epidermis. Each cyst was filled with spores containing a large eosinophilic vacuoplast and a de-centralised nucleus, a common characteristic of the *Dermocystidium* genus (Pekkarinen et al. 2003; Shamsi et al. 2020). The tissue surrounding the cysts was largely unaffected except for the displacement of overlying epidermis. We did not note any aseptate hyphae formation, that has been reported in the past (Höglund, Alfjorden, and Nikkilä 1997; Pekkarinen et al. 2003). None of the cysts showed any evidence of inflammatory response. This contrasts with *Dermocystidium* infection in Atlantic salmon (*Salmo salar*), where granulomatous inflammation is observed (Höglund, Alfjorden, and Nikkilä 1997). This may suggest that stickleback *Dermocystidium* infection is, as previously described, more of a physiological burden to the animal than a disease. No cysts were observed in internal organs.

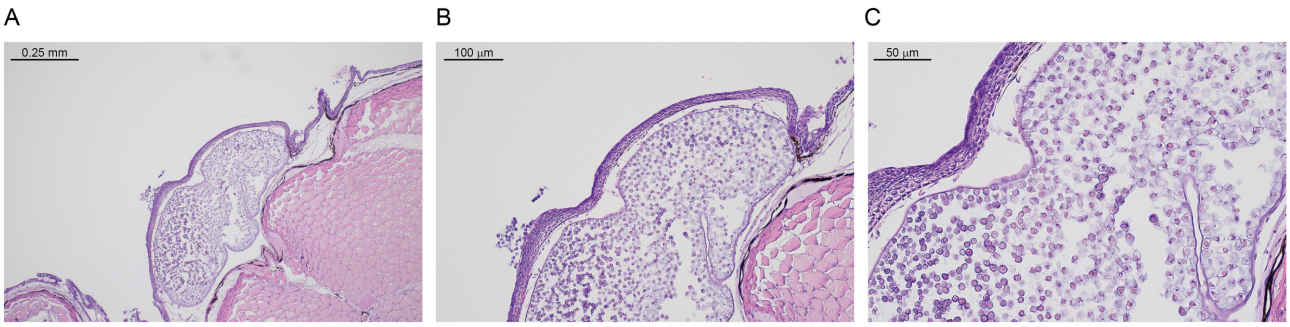

Figure 2. Histological preparation with cross-section of the abdominal cavity.

A) 10X magnification with several visible sub-epidermal cysts, B) 20X, C) 40X magnification revealing typical Dermocystidium spores with central vacuoplasts and marginalized nuclei.

Based on macroscopic and microscopic investigation, parasites of the genus *Dermocystidium* were suspected. A morphologically similar parasite was described in three-spined stickleback and nine-spine stickleback (*Pungitius pungitius* syn. *Gasterosteus pungitius* L.) by Elkan in 1962 and named *Dermocystidium gasterostei* (Elkan 1962). *Dermocystidium gasterostei* was described as producing cylindrical cysts covering the body surface, including the eyes and fins, and even dissecting the tissue between the skull and the eye. Size of the cysts were not described. Cysts contained numerous spores with a large vacuoplast. Figures showing infected stickleback and sections through cysts were similar to our findings (Elkan 1962; Reichenbach-Klinke and Elkan 1965). Because the genus contains several different species and there are no previous reports of *Dermocystidium* infection in sticklebacks in Sweden, we sequenced a segment of the 18S rRNA gene to determine species.

Single parasite cysts were picked by using fine tweezers under the microscope. First the skin layer of the fish was moved to the side exposing the unicellular parasites and using a scalpel blade the sausage like cysts could be isolated. Single cysts were transferred to Eppendorf tubes containing lysis buffer for DNA extraction. Genomic DNA was purified using the GeneJETGenomic DNA Purification Kit (Fermentas, St. Leon-Rot, Germany), according to the manufacturer's instructions, with the modification that dissected parasites transferred to lysis buffer first were mashed by bead-beating, using a FastPrep -24™ homogenizer (MP Biomedicals, Irvine, CA, USA), with the settings 6.5m/s and MP24x2, for 2x60 seconds. Purified DNA was subjected to PCR using the anti-metazoan primers 574*f (CGGTAAYTCCAGCTCYV) and UNonMet_DB (CTTTAARTTTCASYCTTGCG), as described by Bass and del Campo (Bass and Del Campo 2020). PCR products were cleaned-up using Fast-AP treatment (Fermentas) and sent for Sanger sequencing to Macrogen (Amsterdam, the Netherlands). Sequences were edited, assembled, and analysed by using CLC Main Workbench version 21.0.3 (Qiagen, Hilden, Germany).

A 556 bp identical sequence was obtained from two separate parasite cysts. The sequence was blastn-searched in NCBI Genbank, and the most similar hits (AF533941, AF533942, AF533943, AF533944, AF533945, AF533946, AF533947, AF533948 and AF533949) plus a representative selection of other lineages in the order Dermocystida (Adl et al. 2019) were downloaded and aligned using the G-ins-I algorithm in MAFFT v7 (Katoh, Rozewicki, and Yamada 2017). These sequences, with *Sphaerothecum destruens* (GenBank Accession FN996945) as an outgroup, were cropped to the same length as the 18S rRNA gene region generated from the parasite from sticklebacks. This alignment was used to produce a Bayesian phylogenetic tree in MrBayes v3.2.6 (Ronquist et al. 2012) hosted on the Cipres server (Miller, Pfeiffer, and Schwartz 2010), with a GTR+gamma model with four rate categories. Two runs of four simultaneous chains were run for 930k generations, until the runs converged at the default stop value of <0.01. The Bayesian posterior probabilities were mapped onto a 50% majority rule consensus tree (Figure 3). The sequence obtained in this study grouped with maximum support with nine other sequences identified as *Dermocystidium percae,* the closest match being AF533948, isolated from European perch (*Perca fluviatilis*), showing an identity of 542/556 (97.5%) including 4 gaps. Within that robustly supported clade the stickleback parasite branched as sister to a subclade containing all *D. percae* sequences. Bayesian posterior probability support (and Maximum Likelihood bootstrap support; data not shown) for the monophyly of the *D. percae* subclade, and therefore its sister relationship to the stickleback parasite was weak, probably due to only partial length 18S sequences being available for the analyses. Additional sequence data (longer 18S/28S sequences) are required to confirm this sister relationship. No sequences from *Dermocystidium* isolated from stickleback were present in GenBank. The identified stickleback sequence has been deposited in GenBank under Accession No. ON000583. The taxonomy of the order Dermocystida has recently been revised (Borteiro et al. 2018), reasonably renaming the *D. percae* clade as *Dermotheca*. As the stickleback parasite very clearly belongs to this clade it should also be regarded as a species of *Dermotheca* under this new taxonomy. However, extending the circumscription of *Dermotheca* to the other lineages as shown on Figure 3 (and proposed in Borteiro et al. 2018) is not supported by our (not all shown), and some others', analyses. Bipartition support between dermocystid lineages is generally weak, but the resulting genus would almost certainly be paraphyletic (as *Dermocystidium* is currently).

We believe that the species observed and isolated in this study is identical with the species *Dermocystidium gasterostei* Elkan, described in 1962 (Elkan 1962). At that time, genetic characterisation could not be performed, and parasite speciation was done based on morphology. The morphological and histological observations in this study match the ones described by Elkan. The closely related *D. percae* has smaller, oval cyst (1-2 mm x 0.18-0.24 mm) and spores of 6-7.75 µm containing a lid (which is lacking in *D. gasterostei*) (Reichenbach-Klinke and Elkan 1965). In addition, *D. percae* is described as

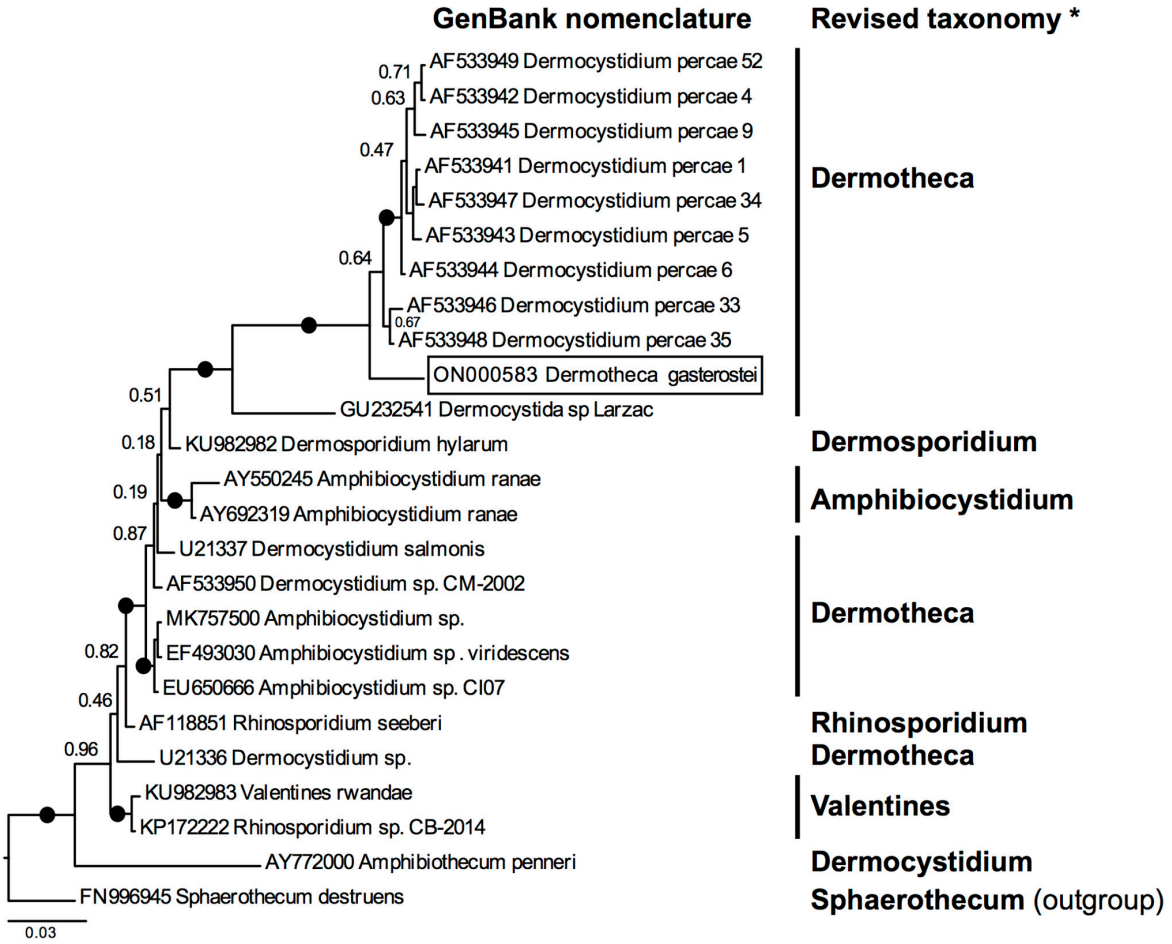

Figure 3. An 18S rRNA gene Bayesian phylogenetic analysis (562 positions analysed) showing the relationship between the isolates of *Dermotheca* (=*Dermocystidium*) *gasterostei* Elkan from this study (boxed on the tree), to known clones of *Dermotheca* (=*Dermocystidium*) *percae*, and other representative lineages of the order Dermocystida, rooted on *Sphaerothecum destruens*.

* The genus names listed on the right of the figure correspond to the taxonomy proposed by Borteiro et al. (2018). The branch labels show the nomenclature of the GenBank Accessions, as of April 2022.

quite host specific, infecting red-fin perch (*Perca fluviatilis*) (Reichenbach-Klinke and Elkan 1965) and ruff (*Gymnocephalus cernua*) (Morley, Campbell, and Lewis 2008; Pekkarinen and Lotman 2003). Thus, we propose that the species described as *Dermocystidium gasterostei* has now been characterised at the 18S rRNA gene level. According to the taxonomic revision of Borteiro et al. (2018), the correct nomenclature is *Dermotheca gasterostei*.

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