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<th>Digenean Metacercariae Parasitic in a Staurozoan Cnidarian</th>
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<td>Author(s)</td>
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<tr>
<td>Citation</td>
<td>Zoological Science, 39(2)</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2022-04</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/88722">http://hdl.handle.net/2115/88722</a></td>
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<td>File Information</td>
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I report digenean metacercariae from Staurozoa, which were not previously known as digenean hosts. The host species, Haliclys tus tenuis Kishinouye, 1910, was collected from algae in Oshoro Bay, Hokkaido, Japan, and contained metacercariae in the mesoglea. The metacercariae were encysted; cysts were oval, 93 μm long by 64 μm wide in one live individual. For the digenean, I generated partial sequences for the 18S rRNA (1585 bp) and 28S rRNA (1672 bp) genes, and the region spanning the 3’ end of the cytochrome c oxidase subunit gene and the 5’ end of the 16S rRNA gene, including the threonine tRNA gene (868 bp in total). Phylogenetic reconstructions based on combined 18S+28S datasets showed the digenean to belong in Opecoelidae, members of which utilize marine or freshwater teleost fishes as definitive hosts, and placed it in Plagiopori naeae (sensu lato) clade C within Opecoelidae.

Key words: COI, fluke, intermediate host, Medusozoa, Platyhelminthes, Scyphozoa, stalked jellyfish, Stauromedusa, Trematoda

INTRODUCTION

It can be challenging to ascertain the intermediate host for a particular parasitic species, and this information is lacking for many parasites. This is especially the case for parasites utilizing unexpected taxa, where novel hosts are generally discovered only fortuitously (e.g., Escobar-Briones et al., 1999; Kakui, 2014; Kakui et al., 2021).

Digenea (Platyhelminthes; Trematoda), a group of endoparasitic metazoans with more than 12,000 described species (Littlewood et al., 2015), utilize second intermediate hosts from diverse animal groups (cf. Lefebvre and Poulin, 2005), and many unexpected host groups may remain undiscovered. In digenean intermediate host surveys, Cnidaria (e.g., corals, jellyfishes, and sea anemones) had not received much attention (Browne et al., 2020). Recent studies revealed that more digeneans utilize cnidarians as their intermediate hosts than expected (e.g., Martin et al., 2018b; Browne et al., 2020), but still many cnidarian groups remain untouched.

Staurozoa (Cnidaria: Medusozoa) is a group of sessile “jellyfishes”, members of which remain attached by a stalk to the substratum throughout their life (Mills and Hirano, 2007). Fifty species have been reported worldwide (Miranda et al., 2018). Staurozoans prey on small crustaceans and in turn are consumed by pycnogonids (sea spiders), nudibranch mollusks (sea slugs), and fishes (Miranda et al., 2018). To my knowledge, there have been no reports of organisms parasitic on staurozoans.

Here I report the first case of a digenean parasitic in a staurozoan, in this case Haliclys tus tenuis Kishinouye, 1910. Because the parasites I found were encysted metacercariae, I attempted to identify them using a molecular phyloge-
The result of BLAST (Altschul et al., 1990) for our 18S sequence indicated that the parasite was a member of the digenean family Opecoelidae, and so a combined 18S + 28S dataset that included sequences from 106 opecoelids and five outgroup taxa (see Supplementary Table S1) was analyzed by maximum likelihood (ML) to place the parasite into a subfamily or clade within Opecoelidae. To construct the 18S + 28S nucleotide dataset, the 18S and 28S data were first aligned independently by using the “Q-INS-i” strategy (Katoh and Toh, 2008) in MAFFT ver. 7 (Katoh and Standley, 2013) and then trimmed with MEGA7 to match the shortest length for each gene (1578 aligned positions for 18S; 1002 for 28S). The optimal substitution model for both genes was GTR + I + G, determined under the corrected Akaike information criterion (AICc) with PartitionFinder 2.1.1 (Lanfear et al., 2017) using a greedy algorithm (Lanfear et al., 2012). An ML analysis of the 18S + 28S dataset was conducted by using RAxML v8.2.10 (Stamatakis, 2014), with nodal support values obtained by analysis of 1000 bootstrap pseudoreplicates. The ML tree was drawn by using FigTree v1.4.4 (Rambaut, 2021). The subfamily/clade nomenclature follows Martin (2020a, b) and Sokolov et al. (2020).

**RESULTS**

More than 10 metacercariae were observed in the mesoglea of the host staurozoan (Fig. 1A, B). In one living metacercaria, the oral and ventral suckers, excretory vesicles, and inner and outer cyst walls (Fig. 1C) were evident; the cyst was 93 μm long and 64 μm wide. In four ethanol-fixed metacercariae, the oral and ventral suckers and inner and outer cyst walls were evident.

Sequences for the COI cluster (868 bp; INSD accession number LC651419) and parts of the 18S (1585 bp; LC651417) and 28S (1672 bp; LC651418) genes were determined from...
Digenean parasitic in Staurozoa

In a BLAST search, the COI-cluster sequence most similar to the one I determined was from Opecoelidae sp. C (FJ765495; identity score 81.13%, query cover 100%), extracted from the first intermediate gastropod host Diloma aethiops in Otago Harbor, New Zealand (Leung et al., 2009).

In the ML tree based on the 18S + 28S dataset (Fig. 2), the metacercaria lies in “Plagioporinae (sensu lato) clade C” sensu Martin et al. (2018a) with 100% bootstrap support. Scale at bottom of the tree indicates branch length in substitutions per site.

My detection of encysted metacercariae in Haliclystus tenuis is the first example of a digenean utilizing a staurozoan as the second intermediate host, and also the first discovery of any parasite in staurozoans. The 18S + 28S phylogenetic analysis strongly indicates that the digenean parasite is a member of Plagioporinae (sensu lato) clade C within Opecoelidae. Opecoelidae is the most species-rich digenean family and utilizes a highly diverse second intermediate host group, including scleractinian corals, crustaceans, aquatic insects, triclad flatworms, ciliate annelids (oligochaetes and leeches), gastropods, cephalopods, echinoids, and teleost and elasmobranch fishes (Martin et al., 2020b). Here I add staurozoans to this list.

Opecoelids utilize freshwater and marine teleost fishes as their definitive hosts (Martin et al., 2020b). There is one previous report of predation on staurozoans by teleost fishes, although this has been considered an instance of bycatch (Miranda et al., 2018). Information regarding predators on H. tenuis is lacking. The finding of opecoelids in H. tenuis indicates that certain marine teleosts may consume this staurozoan species as a diet item and be the definitive host for the opecoelid species I found.

Martin et al. (2020b) suggested that significant second intermediate host-switch events may have driven the evolution of (some) major lineages within Opecoelidae. For instance, most known opecoelid life cycles involve crustaceans as second intermediate hosts, but some species utilize corals for this role, all of which are contained in the subfamily Polypipapiliotrematinae (Martin et al., 2018b). Haliclystus tenuis is currently the sole example of second intermediate hosts for taxa in Plagioporinae (sensu lato) clade C (cf. Martin et al., 2020b). More data from other members in this clade are needed, but Plagioporinae (sensu lato) clade C may be the lineage defined by the use of staurozoans as their second intermediate host.

I fortuitously discovered the digenean parasite while observing H. tenuis during a feeding experiment (Kakui, in press). This underscores that contributions by non-parasitologists, like this study, can help elucidate the diversity of second intermediate host groups, many of which may remain undetected, for various parasite groups.
ACKNOWLEDGMENTS

I thank Norifumi Sugimoto of the Oshoro Marine Station for the use of facilities during sampling; Yayoi Hirano for information on staurozoans and critical comments on an early draft; and Matthew H. Dick for reviewing the manuscript and editing the English.

COMPETING INTERESTS

I declare no competing interests.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs210099)

Supplementary Table S1. Species included in 18S + 28S dataset to infer the phylogenetic position within Opecoelidae of the metacercaria parasite in H. tenuis.

Supplementary File S1. Aligned dataset used for phylogeny reconstruction (positions 1–1578, 18S rRNA gene; 1579–2580, 28S rRNA gene).

REFERENCES

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SUPPLEMENTARY MATERIALS

(Received October 23, 2021 / Accepted November 4, 2021 / Published online January 3, 2022)