The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Clistobothrium sp. (Cestoda: Tetraphyllidea) in oarfish (*Regalecus russelii*) stranded on the coast of Akita Prefecture, Japan

Haruyuki HIRATA[1)], Yuki MIBE[1)], Koji ONO[2)], Satsuki SHIZUNO[2)], Kazuyuki SUZUKI[1)] and Mitsuhiko ASAKAWA[1)]*

[1)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[2)]Oga Aquarium GAO, Akita 010-0673, Japan

J. Vet. Med. Sci.
83(10): 1590–1592, 2021
doi: 10.1292/jvms.21-0317

Received: 3 June 2021
Accepted: 17 August 2021
Advanced Epub:
2 September 2021

ABSTRACT. Oarfish (*Regalecus russelii* Cuvier) are mesopelagic fish with little known about their life history. Oarfish live in deep water, making it difficult for researchers to collect specimens; thus, records of their parasitic helminths are limited. Two plerocercoids were found for the first time in an oarfish stranded on the coast of Akita Prefecture, Japan. These plerocercoids were identified as *Clistobothrium* sp. RR-1 using morphological and molecular analyses. It was revealed that oarfish represent one of the intermediate hosts of the genus *Clistobothrium*, and large sharks are the definitive hosts for these parasites.

KEY WORDS: deep-sea fish, first record, oarfish, phylogenetic analysis, plerocercoid

Oarfish (*Regalecus russelii* Cuvier; Regalecidae) is a circumglobal, mesopelagic, and/or epimesopelagic species associated with upwelling events at depths ranging from 30 to 200 m and possibly as deep as approximately 1,600 m [2, 4, 8]. Because oarfish live in deep water, it is difficult for researchers to collect specimens; thus, records of their parasitic helminths, including *Gymnorhadinorhynchus* sp. and immature *Bolbosoma vasculosum,* [10], the adults of which are typically considered parasites of marine mammals [1], are limited. Additionally, an unidentified partial specimen belonging to the families Arythmacanthidae and Gymnorhadinorhynchidae of necropsied oarfish from the coast of Japan has been reported [6, 10, 13].

In the present study, larval cestodes were found for the first time in oarfish, in Japan. In December 2019, an oarfish was stranded on the coast of Akita Prefecture (39°54′9 N and 139°54′7 E), and was autopsied at Oga Aquarium. The oarfish was female, 3.95 m long and 26 cm high, and registered as #19-006 in the aquarium (Fig. 1A). Two larval cestodes were obtained from the intestine of the oarfish (Fig. 1B) and were fixed and preserved in 70% ethanol. The specimens were then stained with acetocarmine. The obtained helminths were assessed by morphological, molecular, and phylogenetic analyses. The anterior parts of the helminths were stained with acetocarmine solution, and permanent specimens were prepared. The larval cestode specimens comprised short (130 mm long) and long (271 mm long) individuals (Fig. 1C, left and right, respectively). These specimens were approximately 5 mm wide at the scolex and 0.3 mm wide at the posterior extremity of the body (Fig. 1D). The scolex was approximately 1.4 mm long and 2.1 mm wide and had four suckers (Fig. 1D, left). The general shape of the specimens, including the scolex, was consistent with that of plerocercoids belonging to the genus *Clistobothrium* (syn. *Phyllobothrium*) in the order Tetraphyllidea [3, 6]. There have been reports of the presence of the genus *Clistobothrium* in individuals of the genus *Regalecus* in Florida and California in the USA [3, 6]. Nevertheless, an overview of the cestode fauna present in sea fish does not include records of the genus *Clistobothrium* in oarfish from Japanese territory as well as from Western Pacific areas [12]. Identification was not performed by morphological analysis because of its larval stage; molecular analysis was performed using a part of the specimens (Fig. 1).

The preparation of the molecular specimens was according to the protocols below. Total genomic DNA (gDNA) was extracted from ethanol-preserved specimens using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Then, gDNA from the aqueous phase was precipitated with ethanol, and the pellet was dried and dissolved in 25 µl of TE buffer (0.1 M Tris-HCl, pH 8.0, 10 mM EDTA). The dissolved gDNA samples were stored at −20°C and subjected to polymerase chain reaction (PCR). The 16S rRNA and internal transcribed spacer 1 (ITS1) region sequences were amplified from gDNA extracts of the samples. Although there are limited molecular biological data on the detected plerocercoids from oarfish, molecular data of the 16S rRNA gene and ITS1 region have been found useful for species recognition in other cestode taxa [7]. A 790-base pair (bp) fragment of the mitochondrial 16S rRNA gene was amplified from a part of the plerocercoid

*Correspondence to: Asakawa, M.: askam@rakuno.ac.jp

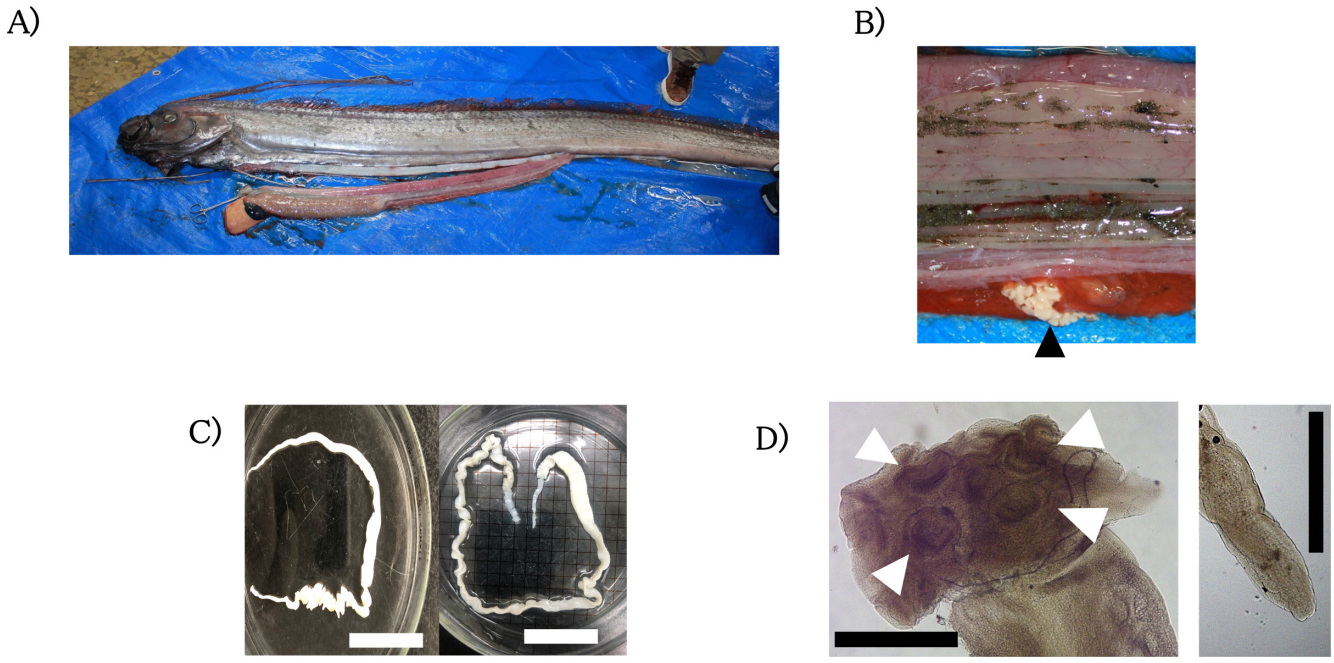

Fig. 1. **(A)** Image of the postmortem examination of the oarfish stranded on the coast of Akita Prefecture in Japan. **(B)** Parasitic state (a gross white lesion shown in the photograph) caused by larval cestodes (arrowhead). **(C)** Whole bodies fixed in ethanol of short (left) and long (middle) plerocercoid individuals obtained from the lesion (scales=3 cm). **(D)** A scolex with suckers (arrowheads; left) and posterior extremity of the fixed body (right) derived from the short individual (scales=1 mm).

using the primer pair 16S-F (5′-TGCCTTTTGCATCATGCT-3′) and 16S-R (5′-AATAGATAAGAACCGCCGACCTGG-3′) [7, 14]. Five hundred and seventy base pairs of the ITS1 region, shown to be useful in resolving interspecific relationships in the phylogenetic analysis of cestodes [7], were amplified from a part of the plerocercoids using the primer pair ITS1-FWD (5′-GTAACAAGGTTTCCGTAGGTG-3′) and ITS1-REV (5′-AGCCGAGTGATCACC -3′). The PCR reaction mixture contained 0.1 µg of template DNA, 5 µl of 10× PCR buffer with 15 mM $MgCl_2$ (TaKaRa Bio Inc., Kusatsu, Japan), 5 µl of dNTP mix (2 mM of each dNTP) (TaKaRa), 2.5 U of TaKaRa LA Taq DNA polymerase (TaKaRa), and 50 pmol of each primer set for the 16S rRNA or ITS1 region-specific primers for PCR. The following cycling program was used: initial denaturation at 94°C for 1 min; 40 cycles of denaturation at 94°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 5 min. The 16S rRNA and ITS1 regions were successfully amplified from the gDNA of plerocercoids using PCR (data not shown). The PCR products were isolated using 1.5% (w/v) agarose gel electrophoresis in TAE buffer and purified using a GENECLEAN kit (BIO 101, Vista, CA, USA). Nucleotide sequences of the 16S rRNA and ITS1 regions were determined directly (Hokkaido System Science Co., Ltd., Sapporo, Japan). The 16S rRNA and ITS1 regions were 793 bp and 551 bp in size, respectively, and their sequences were registered in GenBank under the accession numbers LC626597 and LC617190, respectively. In a BLAST search of the public database, no sequences identical to the 16S rRNA and ITS1 region sequences were found. In a BLAST search of GenBank, the 16S rRNA gene sequence was found to be closely related to that of *Clistobothrium montaukensis* (JQ268541) (94.2% identity). The ITS1 region sequence was found to be related to that of the *Protecephalus* sp. JWJ-2016 (KU212135) (95.9% identity) and *Clistobothrium* sp. JH-2016 (KU724058; 92.3% identity). Although the genetic material of *Protecephalus* sp. and *Clistobothrium* sp. detected in the oarfish was small in amount, we designated the cognate sequence as *Clistobothrium* sp. *Regalecus russelii*-1 (*Clistobothrium* sp. RR-1).

The ITS1 region sequences of the plerocercoid specimens were aligned using the Clustal W program [11]. A phylogenetic tree was constructed from the aligned sequences using the neighbor-joining (NJ) method via phylogenetic analysis in the Mac Vector software package, v.12.5, with gap ignore. Support for tree nodes was calculated from 1,000 bootstrap replicates using the bootstrap tree algorithm [9]. The NJ phylogenetic tree constructed using the ITS1 region sequences separated *Proteocephalus* sp. and *Clistobothrium* sp. into the closely related *Clistobothrium* sp. JH-2016 (KU724058) (Fig. 2). *Clistobothrium* sp. RR-1 was most closely related to *Clistobothrium* sp. isolated from Cape fur seals (*Arctocephalus pusillus pusillus*) [5].

In the present analysis, plerocercoids in a stranded oarfish in Japan were identified as *Clistobothrium* sp. RR-1 using morphological and molecular analyses. The complete life cycle of the genus *Clistobothrium* remains unclear. However, Koltz *et al.* have reported the life cycle with sharks as the definitive hosts; crustaceans as the first intermediate hosts of the plerocercoid larvae; bony fish, squid, or sea turtles as second intermediate hosts of plerocercoid larvae; and pinnipeds as third intermediate hosts of merocercoid larvae [5]. It was revealed that oarfish represent one of the intermediate hosts of the genus *Clistobothrium*, and large sharks are the definitive hosts for these parasites.

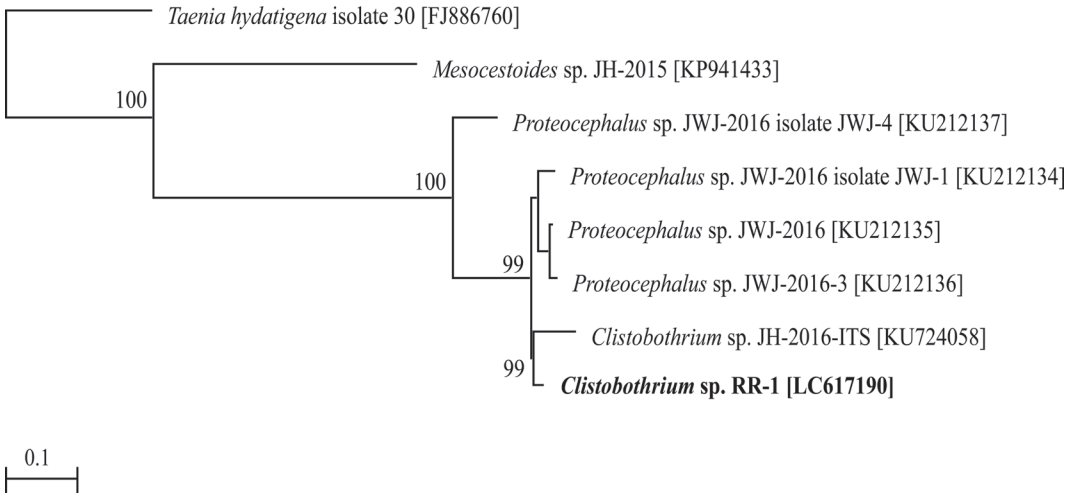

Fig. 2. Neighbor-joining phylogenetic tree showing relationships of internal transcribed spacer 1 (ITS1) region sequences from *Clistobothrium* sp. RR-1, other *Clistobothrium* spp., *Mesocestoides* sp., and *Proteocephalus* sp. isolates. GenBank accession numbers are shown in the tree. The corresponding *Taenia hydatigena* isolate 30 (FJ886760) sequence served as an outgroup. Numbers at the nodes indicate bootstrap support from 1,000 iterations.

CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

REFERENCES

1. Costa, G., Chubb, J. C. and Veltkamp, C. J. 2000. Cystacanths of *Bolbosoma vasculosum* in the black scabbard fish *Aphanopus carbo*, oceanic horse mackerel *Trachurus picturatus* and common dolphin *Delphinus delphis* from Madeira, Portugal. *J. Helminthol.* **74**: 113–120. [Medline] [CrossRef]
2. Forsgren, K. L., Jamal, H., Barrios, A. and Paig-Tran, E. W. M. 2017. Reproductive morphology of oarfish (*Regalecus russellii*). *Anat. Rec. (Hoboken)* **300**: 1695–1704. [Medline] [CrossRef]
3. Hutton, R. F. 1961. A plerocercôid (Cestoda: Tetraphyllidea) from the oar-fish, *Regalecus glesne* (Ascanius), with notes on the biology of the oar-fish. *Bull. Mar. Sci. Gulf Caribb.* **11**: 309–311.
4. Kells, V., Rocha, L. A. and Allen, L. G. 2016. A field fuide to coastal fishes from Alaska to California, Johns Hopkins University Press, Baltimore.
5. Klotz, D., Hirzmann, J., Bauer, C., Schöne, J., Iseringhausen, M., Wohlsein, P., Baumgärtner, W. and Herder, V. 2018. Subcutaneous merocercoids of *Clistobothrium* sp. in two Cape fur seals (*Arctocephalus pusillus pusillus*). *Int. J. Parasitol. Parasites Wildl.* **7**: 99–105. [Medline] [CrossRef]
6. Kuris, A. M., Jaramillo, A. G., McLaughlin, J. P., Weinstein, S. B., Garcia-Vedrenne, A. E., Poinar, G. O. Jr., Pickering, M., Steinauer, M. L., Espinoza, M., Ashford, J. E. and Dunn, G. L. 2015. Monsters of the sea serpent: parasites of an oarfish, *Regalecus russellii*. *J. Parasitol.* **101**: 41–44. [Medline] [CrossRef]
7. Pickering, M. and Caira, J. N. 2013. Differences in microthrix form between stomach and spiral intestine stages of the cestode, Trilocularia: interspecific variation or development? *J. Parasitol.* **99**: 1099–1105. [Medline] [CrossRef]
8. Roberts, T. R. 2012. Systematics, biology, and distribution of the species of oceanic oarfish genus Regalecus. Publications Scientifiques du Museum, Paris.
9. Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. [Medline]
10. Steinauer, M. L., Garcia-Vedrenne, A. E., Weinstein, S. B. and Kuris, A. M. 2019. Acanthocephalan parasites of the oarfish, *Regalecus russelii* (Regalecidae), with a description of a new species of *Gymnorhadinorhynchus* (Acanthocephala: Gymnorhadinorhynchidae). *J. Parasitol.* **105**: 124–132. [Medline] [CrossRef]
11. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680. [Medline] [CrossRef]
12. Yamaguti, S. 1934. Studies on the helminth fauna of Japan. Part 4. *Jpn. J. Zool.* **6**: 1–112.
13. Yamaguti, S. 1961. Studies on the helminth fauna of Japan. 57. Nematodes of fishes. III. *J. Helminthol.* **57** Suppl: 217–228. [Medline] [CrossRef]
14. Zehnder, M. P. and Mariaux, J. 1999. Molecular systematic analysis of the order Proteocephalidea (Eucestoda) based on mitochondrial and nuclear rDNA sequences. *Int. J. Parasitol.* **29**: 1841–1852. [Medline] [CrossRef]