



Title	Diphyllobothrium stemmacephalum (Cestoda: Diphyllbothriidae) found from a harbor porpoise in northern Japan, with comments on a geographical gap with human infection cases in southern Japan
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Citation	Parasitology international, 87, 102487 <a href="https://doi.org/10.1016/j.parint.2021.102487">https://doi.org/10.1016/j.parint.2021.102487</a>
Issue Date	2022-04
Doc URL	<a href="http://hdl.handle.net/2115/89248">http://hdl.handle.net/2115/89248</a>
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Type	article (author version)
File Information	Manuscript_HK et al_revised.pdf



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1 ***Diphyllobothrium stammacephalum* (Cestoda: Diphylobothriidae) found from a harbor**  
2 **porpoise in northern Japan, with comments on a geographical gap with human infection**  
3 **cases in southern Japan**

4  
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18  
19 **ABSTRACT**

20 Even though the cetacean tapeworm *Diphyllobothrium stammacephalum* occurs in both cold  
21 and warm waters, human infections and final host occurrences have been confined to temperate  
22 areas in and near Japan. We recently obtained a strobila of this cestode that was excreted from  
23 a harbor porpoise accidentally caught offshore of Hokkaido of northern Japan. Genetic analysis  
24 of 28S rDNA and *cox1* genes confirmed that the cestode was *D. stammacephalum*. Our finding  
25 sets the northernmost record of *D. stammacephalum* in the western Pacific, suggesting that the  
26 risk of human infections by this parasite in northern Japan deserves further attention.

27  
28 **Keywords:** harbor porpoise; Cestoda; *Diphyllobothrium stammacephalum*; genetic  
29 identification; distribution

31 *Diphyllobothrium stemmacephalum* (Cestoda: Diphylobothriidae) is a zoonotic tapeworm.  
32 Human infections occur by eating raw marine fish, but the causative fishes remain to be  
33 determined [1]. The patients infected with this parasite have been sporadically reported from  
34 the lower latitudes of Japan and Korea [1]. Human infections are generally asymptomatic, but  
35 sometimes mild symptoms such as abdominal discomfort, light diarrhea and nausea have been  
36 reported [1]. A taxonomic review has resulted in the specimens previously identified as  
37 *Diphyllobothrium yonagoense*, which was first recovered from a patient of Tottori Prefecture  
38 in western Japan [2], being synonymized with *D. stemmacephalum* (morphologically in [3,4]  
39 and molecularly in [1]).

40 This cestode has been found from marine mammals such as those of the Phocoenidae and  
41 Delphinidae in the northern Atlantic, Baltic Sea, Black Sea and the Gulf of Mexico regardless  
42 of whether or not they are cold or warm areas [5,6]. Contrary to this, however, the distribution  
43 of *D. stemmacephalum* seems to be limited to the temperate areas in Japanese and Korean  
44 waters (Fig. 1a) [1]. Three odontocete species are known to have been infected with the  
45 tapeworm, which are the Risso's dolphin, *Grampus griseus*, from off Shimane Prefecture in the  
46 southern part of the Sea of Japan [7], the short-beaked common dolphin, *Delphinus delphis*,  
47 from off Mie Prefecture in western Japan facing the Pacific Ocean [8], and the Pacific white-  
48 sided dolphin, *Lagenorhynchus obliquidens*, in the Toba Aquarium of Mie Prefecture [9].

49 We recently observed a cestode strobila identified as *D. stemmacephalum* from the harbor  
50 porpoise, *Phocoena phocoena*, in Hokkaido of northern Japan. This report provides its genetic  
51 profiles and updates information about the northern limit of this parasite's distribution around  
52 Japan. The possible causes of the geographical gap with the known human infection areas are  
53 also considered.

54 The male porpoise host was accidentally caught by a coastal large set net fishery targeting  
55 tuna, squid, and salmon off Hakodate (42°01'03"N, 140°52'50"E) of southern Hokkaido on 10  
56 May 2017 (SNH17013-2, <https://www.gbif.org/occurrence/3319849495>) [10]. The body length  
57 of the porpoise was 120.5 cm, indicating that it was immature. The porpoise was cared for in a  
58 tank (ca. 6.0 m in diameter with a water depth of about 110-120 cm) temporarily set up at the  
59 Usujiri Fisheries Station of Hokkaido University, and was released two days later, after  
60 confirming the progress of its recovery.

61 During its recovery period, the strobila was observed hanging down from the anus of the  
62 porpoise (Fig. 1b). That part of the worm (about 49 cm in length) then dropped off  
63 spontaneously. It was scooped up with a net, preserved in a glass bottle filled with 99.5%  
64 ethanol, and then it was sent to Azabu University in Kanagawa Prefecture, where the following  
65 examinations were conducted.

66 A DNA sample was extracted by lysing a piece of the parasite specimen in 100 µL of Tail  
67 lysis buffer (Nacalai Tesque, Inc., Kyoto, Japan) with 2 µL of Proteinase K (Qiagen K. K.,  
68 Tokyo, Japan, 20mg/ml) at 56°C overnight. The extracted DNA was purified using  
69 Ethachinmate following the manufacturer's instructions (NIPPON GENE, Tokyo, Japan). The

70 targeted genes for polymerase chain reaction (PCR) amplification were nuclear 28S ribosomal  
71 DNA (28S rDNA) and mitochondrial cytochrome *c* oxidase gene subunit I genes (*cox1*). The  
72 PCR was conducted by applying the primer sets LSU-5 and 1500R [11], and Cox1Forward and  
73 Cox1Reverse [5]. The PCRs were run for 30 cycles (94°C for 30 s, 52°C for 30 s and 72°C for  
74 90 s) for 28S rDNA and 35 cycles (95°C for 30 s, 50°C for 30 s, and 72°C for 90 s) for *cox1* in  
75 a total volume of 25 µL. PCR products were purified with the Wizard SV Gel and PCR Clean-  
76 Up System (Promega, Tokyo, Japan) and directly sequenced at Eurofins Genomics (Tokyo,  
77 Japan). The 28S rDNA and *cox1* products were sequenced in both directions using the PCR  
78 primers and internal primers: 300F and ECD2 for the former [11]: and *cox1*F9845-9864 (5'-  
79 TAGCTGCTGCTATTACAATG-3') and *cox1*R10077-10096 (5'-  
80 ACTACCAAGACAAACAATG-3') for the latter. The obtained sequences were aligned and  
81 edited using MEGA-X software [12], and were compared with reference sequences available  
82 in the GenBank/EMBL/DDBJ databases using BLAST (Basic Local Alignment Search Tool).  
83 Phylogenetic trees were constructed based on the partial 28S rDNA and the complete *cox1*  
84 sequences of specimens along with sequences of other related species retrieved from the  
85 GenBank [5,13]. Phylogenetic analysis was performed with MEGA-X software by the  
86 maximum likelihood method. Clades were assessed by bootstrap resampling with 1000  
87 replicates.

88 A total of 1411 and 1566 bp were determined from the sequencing of the targeted regions of  
89 28S rDNA and *cox1*, respectively. These sequence data were registered in the GenBank as  
90 LC644720 and LC644653 for 28S rDNA and *cox1*, respectively. BLAST searches for both  
91 targeted regions showed 100% sequence matches between our specimen and known *D.*  
92 *stemmacephalum* (e.g. KY552825.1 and AP017648.1). Phylogenetic analysis also supported  
93 that the species is *D. stemmacephalum* (Fig. 2a and 2b).

94 The previous records of human infections suggest that *D. stemmacephalum* is mainly  
95 distributed off the southern coast of Japan between 25°N and 35°N (Fig. 1a). The locality  
96 reported in the present study represents the new northernmost record of the species in the  
97 western Pacific.

98 The Risso's dolphin and the short-beaked common dolphin are southern species commonly  
99 occurring in warm-sea environments and are known to be final hosts [14–16]. The Pacific  
100 white-sided dolphin is the other known host, and it is a migratory species that moves between  
101 the cold waters (i.e., the northern region around Hokkaido) and warm waters (i.e., off the  
102 southern coast of Japan), with individuals overwintering off Fukuoka and Yamaguchi  
103 Prefectures that are located in the southern the Sea of Japan and also off the Kii Peninsula along  
104 the Pacific coast of Japan, respectively [17–19]. Although the feeding habits of these cetaceans  
105 have not yet been clarified, some of their prey fishes are expected to serve as a second  
106 intermediate host for *D. stemmacephalum*, which may have been transmitted to humans by  
107 eating them raw.

108 The harbor porpoise that is newly recognized as the host of *D. stemmacephalum* in this report,

109 is a northern species that does not appear to inhabit southern or western areas (i.e., off Honshu  
110 and off western Hokkaido) with sea surface temperatures exceeding 12-16°C in summer [20,21].  
111 Some individuals are known to migrate south from Hokkaido to Honshu as sea surface  
112 temperatures decrease in winter [20]. The southward limits of harbor porpoises are off Niigata  
113 Prefecture (i.e. 39°N) in the Sea of Japan and off Ibaraki Prefecture (i.e. 36°N) along the Pacific  
114 coast of Japan; but in an exceptional case, one individual was found in a more southwest area  
115 off Taiji of Wakayama Prefecture [20]. Therefore, it seems possible that parasite infections of  
116 harbor porpoises occur during those periods of southward migrations.

117 Alternatively, the recent global warming may have resulted in the parasite being more easily  
118 transported to the north by migrations or habitat expansions of fish intermediate hosts [e.g.  
119 22,23]. Because the harbor porpoise is known to be an opportunistic generalist predator that  
120 uses any available prey at each time and place [24], this potential host might become infected  
121 with *D. stemmacephalum* around Hokkaido as a result of rising water temperatures.

122 The intermediate fish hosts exploited by *D. stemmacephalum* are still uncertain, but have  
123 been assumed from the diets of the patients who frequently eat raw fish. The consumed fish  
124 include the skipjack tuna (*Katsuwonus pelamis*), the Japanese Spanish mackerel  
125 (*Scomberomorus niphonius*), tunas (*Thunnus* spp.), and the Japanese amberjack (*Seriola*  
126 *quinqueradiata*) [25]. In addition to these highly migratory fishes, other hosts may be resident  
127 species such as mackerels (*Rastrelliger* spp.), the silver-stripe round herring (*Spratelloides*  
128 *gracilis*), and the striped beakfish (*Oplegnathus fasciatus*), which are mainly distributed and  
129 consumed in the southern regions [26]. Regardless of the species that caused the infection, the  
130 present finding may suggest that the risk of human infection by this parasite by consuming  
131 fishes caught from the northern cold-water areas needs to be considered, and it may be  
132 necessary to monitor possible fish hosts species.

133

## 134 **Acknowledgements**

135

136 We thank the Matsuishi Laboratory and Cetacean Research Group members, Hokkaido Univ.,  
137 who held the harbor porpoise during its recovery. We also thank two anonymous reviewers for  
138 their careful reading of our manuscript. The tapeworm sample was granted to HK from the  
139 Stranding Network Hokkaido. The rehabilitation of the porpoise was conducted under the  
140 guidelines and approval of the Committee on Animal Experiments of Hokkaido University. This  
141 work was partly supported by JSPS KAKENHI Grant Numbers JP19K15910 to HK and  
142 JP18J30013 to AM.

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- 224

225 **Figure captions**

226

227 Fig. 1. Human infection records around Japan and the *Diphyllobothrium stemmacephalum*  
228 parasite specimen of the present study. (a) Localities of human infections reported in previous  
229 studies (orange closed-circles) [1] and the locality reported in the present study (blue closed-  
230 circle). (b) A video frame-capture (see the video clip in Appendix S1) of the sheltered harbor  
231 porpoise in the holding tank, showing the cestode strobila being excreted.

232

233 Fig. 2. The phylogenetic relationships of *Diphyllobothrium stemmacephalum* reported in this  
234 study and related species. (a) A phylogenetic tree estimated by the maximum likelihood method  
235 based on 28S rDNA sequences using K2P+G. *Spirometra erinaceieuropaei* was used as an out-  
236 group. (b) A phylogenetic tree estimated by the maximum likelihood method based on *cox1*  
237 sequences using GTR+G+I. *Spirometra mansoni* was used as an out-group. Bootstrap values  
238 (> 90) are shown on branches.



Fig. 1

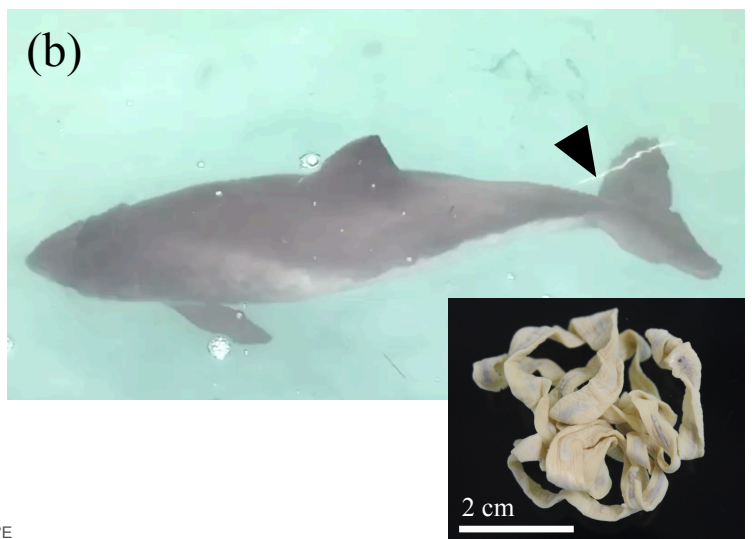
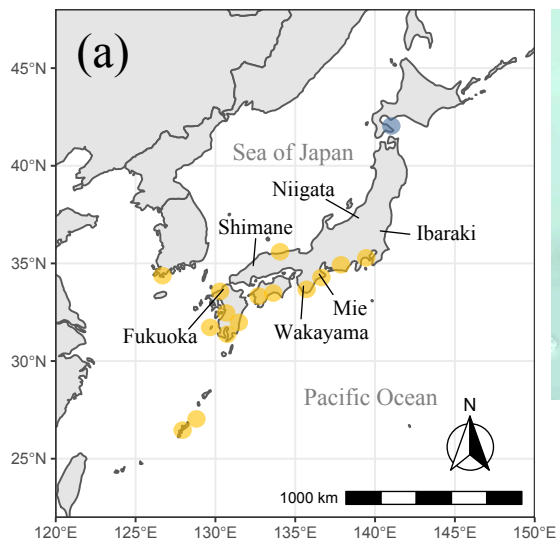
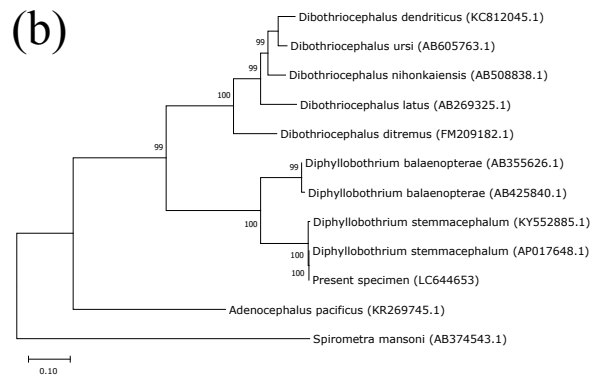
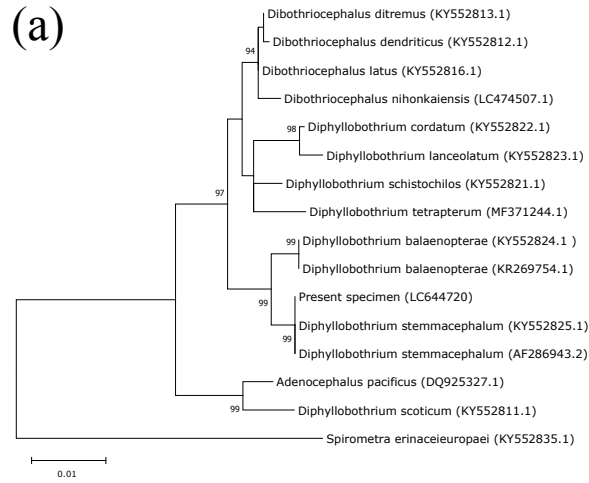


Fig. 2



# Graphical abstract

