

Short Communication

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Molecular identification of tapeworm infection in a bottlenose dolphin, *Tursiops* sp., in South Carolina, USA

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Abstract

A bottlenose dolphin, *Tursiops* sp., stranded on the coast of South Carolina, USA was found to be heavily infected in its intestine by tapeworms, which we identified molecularly. Sequencing of portions of the mitochondrial cytochrome *c* oxidase I (COI) and nuclear large subunit ribosomal RNA (28S rRNA) genes showed the cestodes to be *Diphyllbothrium stemmacephalum*, commonly known as a broad tapeworm. Infections of marine mammals by *Diphyllbothrium* have been previously reported in the Northwestern Atlantic Ocean, but only to genus level. Infection by tapeworms may be rare in dolphins in South Carolina, but because this species is zoonotic, its presence indicates the potential for an emerging public health concern.

Introduction

As long-lived apex predators closely associated with the coastal zone, bottlenose dolphins (*Tursiops* spp.) can be environmental sentinels by providing early indication of health stressors, including parasites (Bossart 2011; Moore 2008). Obtaining samples from marine mammal carcasses via necropsies allows for opportunistic insight into the health of these animals (Rowles *et al.* 2001). Bottlenose dolphins have been reported to be infected with broad tapeworms *Diphyllbothrium* spp. around the world (e.g., Hatsushika and Shirouzu 1990; Quiñones *et al.* 2013; Shirouzu *et al.* 1999; Zam *et al.* 1971) including *D. stemmacephalum* in the Gulf of Mexico (Sánchez *et al.* 2018; Ward and Collins 1959). However, none has been identified to species level in the Northwestern Atlantic Ocean (Ridgway 1965; Zam *et al.*, 1971). Herein we report the finding and molecular identification of *D. stemmacephalum* from the intestine of a bottlenose dolphin *Tursiops* sp. that was stranded on the coast of the Northwestern Atlantic Ocean in South Carolina (SC), USA.

Material and methods

The bottlenose dolphin (Field Number SC1910) was found dead, stranded on Sullivan's Island, SC (32°76'18"N, 79°86'25"W) on 3 March 2019 and identified as *Tursiops truncatus* at the time (Figure 1). However, the species *T. erebennus* was reinstated as a valid species residing in SC after examination of the individual occurred (Costa *et al.* 2022; see discussion below) and no further identification was possible. Therefore, as it is unclear whether this animal belongs to *T. truncatus* or *T. erebennus*, herein we identify it as *Tursiops* sp.

The individual was a ~1.5-year-old, 169 cm long male. Organs, including the gastrointestinal tract, were stored at –20°C for later examination (Figure 1). A tapeworm infection in the intestine was noted during a later assessment of the dolphin's gastrointestinal tract on 18 October 2022. The frozen stomach and intestines were thawed, weighed, and examined. During dissection, the entire length of the intestine was divided into eight equal-length samples, which sectioned the parasite(s). Part of the strobilae was fixed in 10% neutral buffered formalin and part in 100% ethanol for molecular studies. However, the integrity of intestinal helminths, and cestodes in particular, is quickly compromised when collected from stranded and/or frozen hosts (Kuchta and Scholz 2017), as in this case. Hence, identification of this worm was based solely on sequencing data.

Genomic DNA from the parasite specimen was extracted using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the protocol of the manufacturer. Portions of the mitochondrial cytochrome *c* oxidase I (COI) and nuclear large subunit ribosomal RNA (28S rRNA) genes were amplified and sequenced. The COI PCR was done using primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3'; Bowles *et al.* 1995) and COI-R trema (5'-CAA-CAAAATCATGATGCAAAAGG-3'; Miura *et al.* 2005). A 25-μl total reaction contained 1X Promega GoTaq® Flexi PCR Buffer (Madison, WI, USA), 0.4X Invitrogen Rediload™ loading

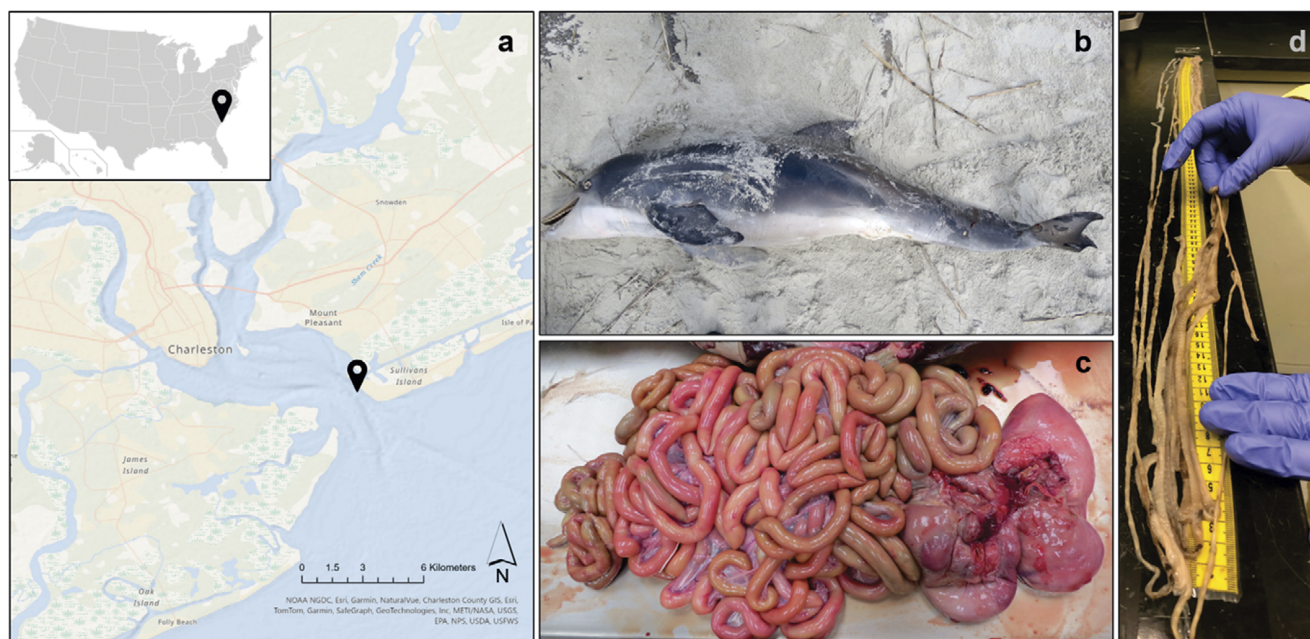


Figure 1. (a), stranding location of bottlenose dolphin SC1910 on Sullivan's Island, South Carolina, USA; (b), stranded dolphin *Tursiops* sp. on site; (c), gastrointestinal tract excised during necropsy; (d), strobila of cestode (later identified molecularly as *Diphyllbothrium stemmacephalum*) collected from one segment of the dolphin's small intestine.

buffer (Thermo Fisher Scientific, Waltham, MA, USA), 2 mM MgCl_2 , 0.5 mM dNTPs, each primer at 0.3 μM , 1 U Promega GoTaq[®] DNA polymerase, and 3 μl template DNA. Cycling was as follows: 5 min at 95°C was followed by 35 cycles at 95°C for 30 s, 48°C for 30 s, and 72°C for 45 s, followed by 72°C for 5 min. The 28S PCR was done using primers digl2 (5'-AAGCATATCACTAAGCGG-3'; Tkach *et al.* 1999) and 1500R (5'-GCTATCCTGAGGGAAGTTCG-3'; Tkach *et al.* 2003), and reagents were the same as above except 1.5 mM MgCl_2 , 0.2 mM dNTPs, 0.5 μM of each primer, and 1 μl of template were used. Cycling was as follows: 3 min at 94°C was followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. Products, including PCR positive and negative controls, were electrophoresed on 1% agarose gels stained with GelRed (Biotium, Fremont, CA, USA) and visualised under UV light. The product was then cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and sent to Eurofins MWG Operon LLC (Louisville, KY, USA) for direct, bi-directional sequencing using the same primers as above in addition to internal primer 300F (5'-CAAGTACCGTGAGGGAAGTTG-3'; Littlewood *et al.* 2000) for the 28S rRNA gene sequence. Complementary sequences were assembled, compared to their chromatograms, and edited accordingly using Sequencer version 5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA). The resulting sequences were compared to those in the National Center for Biotechnology Information's GenBank database using megaBLAST (Morgulis *et al.* 2008).

Results

Upon necropsy this dolphin was determined to have a body condition of Code 3 (fair to moderate decomposition, organs still intact according to Geraci and Lounsbury (2005)). Gross pathology from necropsy indicated that this dolphin suffered from scoliosis, oral lesions, circular, depressed skin lesions, probable verminous pneumonia caused by lungworms, ruptured spleen, possible

hypertrophic cardiomyopathy, and malnourishment (SC1910 necropsy report, NOAA NCCOS, Charleston, SC, USA). The dolphin's intestine was 23.75 m long and contained 375 g of contents (wet weight), 320 g of which was cestode (wet weight). Strobilae were found in six of the eight sections of intestine with none present in the proximal and distal ends. Strobilae within each section was weighed and the total length was estimated to be approximately 63 m (Figure 1d). Hence, although no scolex was recovered and no number of cestodes could be confirmed, this dolphin was most certainly infected by several tapeworm individuals. No cestode was present in the stomach, which contained 110 g of contents (wet weight) and two cephalopod beaks.

The parasite 28S rRNA gene sequence (1,234 base pairs) was 100% similar with 100% coverage to that of the three 28S rRNA gene sequences of *D. stemmacephalum* Cobbold, 1858 in GenBank (two from the USA found in a bottlenose dolphin (accession number KY552825) from Mississippi (Gulf of Mexico; Waeschenbach *et al.* 2017) and an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) (AF286943) from Massachusetts (Olson *et al.* 2001), and a third from northern Japan in a Harbour porpoise (*Phocoena phocoena*) (LC644720; Katahira *et al.* 2022). The parasite COI sequence (554 bp) was 100% similar with 100% coverage to that of *D. stemmacephalum* (JQ268543) found in *L. acutus* from the USA (Massachusetts) and 99.28–99.64% similar (100% coverage) to five sequences of *D. stemmacephalum* in GenBank (MW034674: *Monachus monachus*, Adriatic Sea (unpublished), LC709257: *T. truncatus*, Japan (Ishisaka *et al.* 2023), KY552885: *T. truncatus*, Gulf of Mexico (Waeschenbach *et al.* 2017), LC644653: *P. phocoena*, Japan (Katahira *et al.* 2022), LC042231: *Homo sapiens*, Japan (Yamasaki *et al.* 2016). Sequences from this study were deposited in GenBank as accession numbers OR588137 (28S) and OR558138 (COI).

Discussion

Diphyllbothrium stemmacephalum belongs to the group of broad fish tapeworms, which have an unarmed scolex and typically a long

(1–30 m) strobila (Kuchta and Scholz 2017). These tapeworms are found throughout the Northern Hemisphere, have marine mammals as definitive hosts, and are zoonotic (Yamasaki *et al.* 2016). The species is the type species of the genus; it was originally described from a Harbour porpoise (*Phocoena phocoena*) from the Scottish North Sea (Cobbold 1858) and reported again in *P. phocoena* by Andersen (1987) off the Netherlands and Denmark, by Katahira *et al.* (2022) in the western Pacific in Japan, and most recently by Striwe *et al.* (2025) in the North and Baltic Seas off Germany. This species has also been identified in other marine mammals, including bottlenose dolphins from the Gulf of Mexico (Sánchez *et al.* 2018; Ward and Collins, 1959). Additional occurrences of *Diphyllbothrium* infection have been reported in a variety of odontocetes, including a Beluga whale (*Delphinapterus leucas*) in the estuary and Gulf of Saint Lawrence (Brunel *et al.* 1998), a bottlenose dolphin (*T. truncatus*) off Florida's northeastern coast (Ridgway 1965; Zam *et al.* 1971), and pygmy sperm whales (*Kogia breviceps*) and short-finned pilot whales (*Globicephala macrorhynchus*) off the coast of Brazil (Carvalho *et al.* 2010). However, none of these tapeworms were identified to species level. While Sánchez *et al.* (2018) noted that the few specimens of *D. stemmacephalum* collected from bottlenose dolphins from the Gulf of Mexico were all significantly larger than those from other hosts and localities, to our knowledge no specimen >30 m long has ever been reported, supporting our notion that the 63 m of tapeworm collected from this dolphin was comprised of several individuals. This is the first report of *D. stemmacephalum* infection in a bottlenose dolphin from SC.

Bottlenose dolphins stranded in SC could be from one of several stocks that frequent coastal waters including the Charleston Estuarine System Stock, the Southern Migratory Stock, the South Carolina-Georgia Coastal Stock, the Northern Georgia-Southern South Carolina Estuarine System Stock, and the Northern South Carolina Estuarine System Stock (Hayes *et al.* 2020). As such, dolphins in SC may belong to *T. truncatus*, the Atlantic bottlenose dolphin, or to *T. erebennus*, the Tamanend's bottlenose dolphin, which was previously considered to be a coastal ecotype of *T. truncatus* and was recently reinstated as a valid species residing within estuarine environments (Costa *et al.* 2022). Parasitic infections of *Tursiops* spp. are commonly observed during necropsies, but no tapeworm infection was reported in a SC study carrying over sixteen years of data (McFee and Lipscomb 2009). Although infections by *D. stemmacephalum* appear to be overall infrequent (Striwe *et al.* 2025), their occurrence in various odontocetes around the world and in bottlenose dolphins, which are the most common marine mammals in our region, is significant as this parasite is zoonotic (Yamasaki *et al.* 2016).

The life cycle of *D. stemmacephalum* has yet to be unravelled. However, while this group of tapeworms was recently revised and several species of *Diphyllbothrium* with known life cycles were moved to genus *Dibothriocephalus* (see Waeschenbach *et al.* 2017, WoRMS, 2022), it is expected that the life cycles of parasites remaining in genus *Diphyllbothrium* follow the same general complex pattern as other broad tapeworms (e.g., *Dib. dentriticus* or *Dib. ditremum*, see Kuchta *et al.* 2013 and Borgstrom *et al.* 2017, respectively): in brief, these tapeworms use copepods and fishes as first and second intermediate hosts, respectively, and piscivorous fishes as paratenic hosts (e.g., Ikuno *et al.* 2018). To our knowledge, no fish in the Northwestern Atlantic have been reported to be infected with plerocercoids of this particular species, but it is a reasonable assumption that dolphins become infected via ingestion of infected fish. Furthermore, while no human case of infection by

D. stemmacephalum is known to date from the USA, 24 human infections have been reported in Japan and Korea (Lee *et al.* 1988; Yamane *et al.* 1981; in Scholz *et al.* 2009; Yamasaki *et al.* 2016). Hence, the infection of a stranded bottlenose dolphin indicates the potential for the emergence of a public health risk for raw/undercooked fish consumers in our area and warrants continuous monitoring of this sentinel species.

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Competing interests. The authors declare none.

Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki of 1975, as revised in 2008. Samples were collected under NOAA's authority under the MMPA Section 109(h).

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