MONSTERS OF THE SEA SERPENT: PARASITES OF AN OARFISH, *REGALECUS RUSSELLII*

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ABSTRACT: Examination of a small portion of the viscera of an oarfish (*Regalecus russellii*) recovered from Santa Catalina Island, southern California, revealed numerous tetraphyllidean tapeworm plerocercoids, *Clistobothrium* cf. *montaukensis*; 2 juvenile nematodes, *Contracaecum* sp.; and a fragment of an adult acanthocephalan, family Arhythmacanthidae. This suggests that the fish was relatively heavily parasitized. The presence of larval and juvenile worms suggests that oarfish are preyed upon by deep-swimming predators such as the shortfin mako shark, *Isurus oxyrinchus*, known to be a definitive host for the adult tapeworm, and also by diving mammals such as sperm whales, *Physeter catodon* L., hosts of *Contracaecum* spp. nematodes.

Sightings of the legendary "sea serpent" are often attributed to the infrequent surfacing of the longest bony fish (reaching ~10 m), the oarfish, *Regalecus* spp. Knowledge of the biology of the oarfish is limited but thoroughly summarized by Roberts (2012). Midwater observations are limited to a few remotely operated vehicle sightings (with a blurry image of a probable cymothoid isopod attached to a fin) (Benfield et al., 2013) and submersible sightings. Most specimens examined have, for unknown reasons, come to the surface and were moribund or dead, and often damaged. Roberts (2012) notes, concerning the natural enemies of oarfish, whereas a few parasites have been recorded, information on their predators is almost nonexistent. Only 3 oarfish have been previously necropsied for parasites (Hutton, 1961; Villareal and Dailey, 1993), and there are a few other observations made in passing concerning their parasites (Forbes, 1891; Monticelli, 1900; Benfield et al., 2013).

In October 2013, a large (>5 m), recently dead oarfish, identified as *R. russellii* (Cuvier, 1817), based on the disjunct geographic distribution of Pacific oarfish species (Roberts, 2012), was recovered in good condition by staff of the Catalina Island Marine Institute, Santa Catalina Island, California. We were able to obtain, for parasitological examination, a small portion of the frozen viscera, including the gills, gall bladder, part of the stomach, and the gastric cecum; a few centimeters of either pyloric cecum or intestine adhering to the gall bladder; the spleen; and samples of the liver and musculature. Here, we report these findings and discuss their implications for predation on oarfish.

MATERIALS AND METHODS

The oarfish viscera were thawed and examined under stereomicroscopes. As is often the case with stranded oarfish, the people who graciously salvaged and shipped the viscera were not familiar with the internal anatomy of oarfish, accounting for the small piece of gut tissue that was retained and sent for our examination. Liver, spleen, and musculature samples were examined using squash plates to flatten tissues and reveal encysted parasites. After visible worms were removed from the gall bladder and gut, those organs were squashed and examined for encysted parasites. Bile fluid and scrapings from the inside of the gall bladder were examined for myxozoans. All parasites were photographed, and cestodes were measured to the nearest millimeter, before preservation. Due to the manner in which the tissue samples were packaged and transported, there is a possibility that some of the tapeworms were displaced from their original sites. Most parasites recovered were fixed in 95% ethanol for molecular investigations. Some of the tapeworms were fixed in 10% neutral buffered formalin for preparation of whole mounts. Cestodes were examined with a combination of molecular methods (the partial nuclear gene 28S rDNA [D1–D3] was sequenced) and morphological methods (light microscopy or scanning electron microscopy [SEM] following protocols in Pickering and Caira [2013]). A subset of cestode specimens were deposited in the Lawrence R. Penner Parasitology Collection (LRP), Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut (LRP 8484 and 8485).

RESULTS

Twenty larval cestodes were recovered from the gut or gall bladder of *R. russellii*: 18 were examined in detail. Based on morphological characteristics of the scoleces along with molecular data from 2 specimens (i.e., sequencing of ~1,200 bp of 28S rDNA), these plerocercoids were identified as belonging to the genus *Clistobothrium* Dailey and Vogelbein, 1990. One of the specimens sequenced was an identical match to GenBank AF286957, an adult specimen of *Clistobothrium montaukensis* collected from *Isurus oxyrinchus*, the shortfin mako shark, *Isurus oxyrinchus*, from the coast of Montauk, New York (Olson et al., 2001). The scolex of this specimen was mounted on a slide, as a voucher, and deposited in the LRP collection (LRP 8484): the sequence is deposited in GenBank under accession KM272991.

The scolex morphological features of the plerocercoids found in these oarfish specimens match the those of plerocercoids described in Hutton (1961), and these most closely resemble the adult cestodes of *C. montaukensis* Ruhnke, 1993 as described in the monograph of Ruhnke (2011) on phyllobothriid cestodes. The scolex of *C. montaukensis* is characterized by having 4 foliose bothridia, each with 1 muscular, round anterior accessory sucker (Ruhnke, 2011). Two of the plerocercoid specimens were examined with SEM.

One of those specimens (Fig. 1) was also sequenced and differs by 3 bp from *C. montaukensis*, thus being identified as *Clistobothrium* sp.: the sequence is deposited in GenBank under accession KM272992, and a portion of the larval body is mounted on a slide, LRP 8485. Based on morphology, the other specimen (Fig. 2) also appears to belong to the genus *Clistobothrium*.

One large cestode fragment was found in the gall bladder, but it was unable to be identified due to its lack of a scolex and its larval state. Attempts to amplify 28S rDNA were unsuccessful; thus, no molecular data are available at this time.
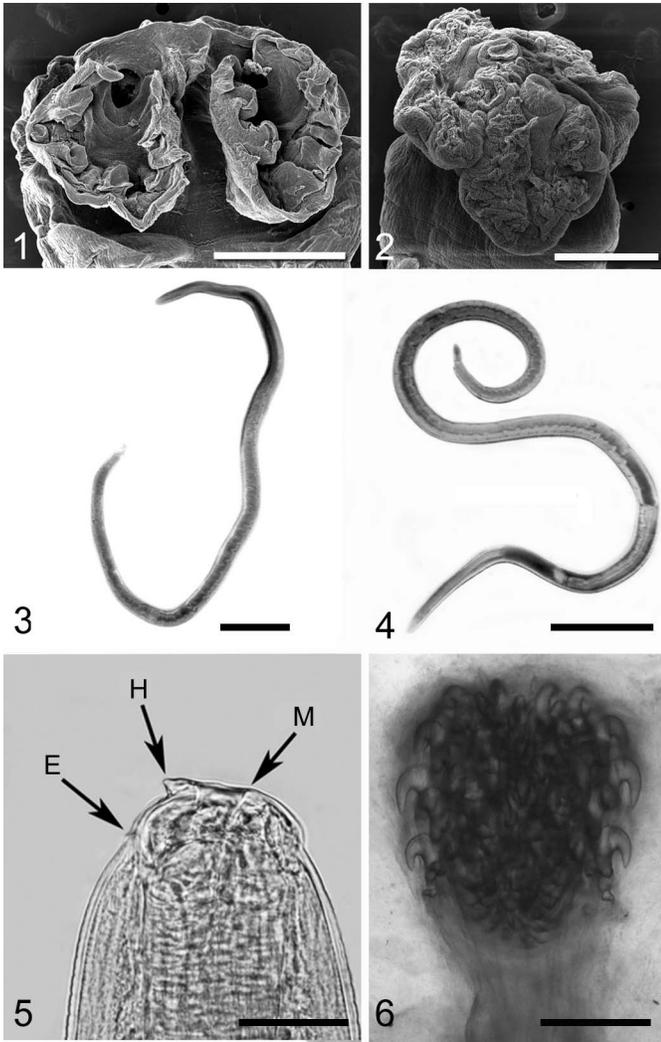
FIGURES 1–6. (**1**) Scanning electron micrograph of a larval cestode from the gut of an oarfish. Scolex of *Clistobothrium* cf. *montaukensis* (identification verified with 28S rDNA sequence data matching 99.8% with GenBank AF286957, *C. montaukensis*). Bar = 500 µm. (**2**) Scanning electron micrograph of a larval cestode from the gut of an oarfish. Scolex identified as *Clistobothrium* sp. based on morphology. Bar = 500 µm. (**3**) Partially ensheathed second stage juvenile *Contracaecum* sp. from the oarfish. Bar = 1.3 mm. (**4**) Third stage juvenile *Contracaecum* sp. from the oarfish. Bar = 1.4 mm. (**5**) Head of third stage juvenile *Contracaecum* sp. from the oarfish. E, excretory pore; H, labial hook; M, mouth opening. Bar = 55 µm. (**6**) Photograph of the proboscis of the acanthocephalan fragment showing hook characteristics and extent of invagination. Bar = 200 µm.

Two unencysted nematodes (Figs. 3, 4) in the lumen of the gall bladder of the oarfish were identified as members of the anisakid genus *Contracaecum* Raillet and Henry, 1912. One specimen was a partially ensheathed second stage juvenile 10.8 mm in length. The second specimen was a third stage juvenile 12.1 mm in length.

The diagnostic characters of *Contracaecum* that the juveniles possessed are as follows: lips lacking teeth and ridges, no cervical collar, presence of interlabia, presence of ventricular (pharyngeal) and intestinal ceca, and excretory pore situated between latero-ventral lips (Fig. 5). The closely related genus *Anisakis* Dujardin, 1845 has no intestinal cecum, and each lip bears a row of small teeth. Also, the labial hook is often on the dorsal side in *Anisakis* rather than the ventral side as is typical in *Contracaecum* (Fig. 5) (Delyamure, 1955; Yamaguti, 1961).

A possible unique character not noted in previous juvenile species of *Contracaecum* is the presence of a ventral mouth opening (Fig. 5). Since the juveniles were not encysted, they may have used their mouth hooks to move through the tissues of the host.

The proboscis of an acanthocephalan was embedded in the intestinal mucosa (Fig. 6). Based on its location, we presume it was from an adult worm. The proboscis was not fully evaginated, so only some hook characters were evident. Hooks were arranged spirally with 12 longitudinal rows of hooks and an estimated 12–15 hooks per row. The most basal 2 hooks per longitudinal row were spine-like (unrooted) and much smaller than those above (basal hooks 1 and 2 = 27 × 13.5 µm; third or fourth hook: 99.5 × 45.6 µm). The proboscis length was estimated at 908 µm. DNA could not be amplified (positive controls were successful), likely due to the small amount of tissue available.

DISCUSSION

Hutton (1961) reported tetraphyllidean plerocercoid larvae from 2 oarfish specimens from the Gulf of Mexico coast of Florida, identified as belonging to the genus *Phyllobothrium*. Due to taxonomic changes since that time, specifically the erection of the genus *Clistobothrium* Dailey and Vogelbein, 1990, upon reexamining Hutton's drawings and descriptions and recognizing that they are consistent with the larvae we found in this study, we consider that the specimens in Hutton (1961) and the present specimens most closely resemble the genus *Clistobothrium*. Our molecular sequence data support this identification. However, it remains uncertain whether there is a single species, *C. montaukensis*, or whether there were 2 *Clistobothrium* species present. The genus *Clistobothrium* lives as an adult in the spiral intestines of large lamniform sharks (i.e., shortfin mako shark) (Ruhnke, 2011).

There are 2 likely possibilities concerning how oarfish become infected with these larval plerocercoid cestodes. (1) Most likely, they consume first intermediate hosts. These are probably small crustaceans (see Caira and Reyda, 2005; Jensen and Bullard, 2010) for general information on elasmobranch cestode life cycles and larval stages. Oarfish have been reported to eat euphausiid crustaceans and small fishes (Roberts, 2012). (2) It is also possible that oarfish served as a paratenic host, consuming a second intermediate host that contained a plerocercoid. The longfin Patagonian squid, *Loligo gahi*, has been reported as another host for larval *Clistobothrium* cf. *montaukensis* (Brickle et al., 2001). Oarfish have been known to eat small squid. Adult worms from the genus *Clistobothrium* have been described from lamniform sharks. Although not all lamniform sharks have been examined for cestodes, there are 2 species reported: *C. montaukensis* Ruhnke, 1993 from the shortfin mako shark and *C. carcharodoni* Dailey and Vogelbein, 1990 from the great white shark, *Carcharodon carcharias*. If the oarfish does play such a role in the life cycle of *Clistobothrium*, and is not a "dead end host," then it is likely that a lamniform shark, perhaps the shortfin mako shark, is the definitive host. The oarfish is reported from depths that overlap with shortfin mako shark (Last and Stevens, 1994; Roberts, 2012).

A recent survey of deeper water sharks from the Azores revealed larval *Clistobothrium* cf. *montaukensis* specimens from the spiral intestine of the birdbeak dogfish, *Deania calcea*, and the longnose velvet dogfish, *Centroselachus crepidater* (see figs. 22 and 24 in Caira and Pickering, 2013). This information, coupled with this oarfish report, indicates that the genus *Clistobothrium* appears to be widespread in deepwater taxa from different host groups (i.e., larvae from squid and teleosts, adults from elasmobranchs).

Although Forbes (1891) and an unpublished observation cited in Roberts (2012) report thousands of nematodes in the liver, intestines, and esophagus of an oarfish, here we provide the first partial description of nematode parasites of the oarfish. Potential definitive hosts of the juvenile *Contracaecum* sp. would be mammalian, probably large predators considering the size of the oarfish. One possibility is the sperm whale, *Physeter catodon* L., since unidentified anisakid juveniles have been recovered from sperm whales in the Pacific Ocean (Delyamure, 1955).

The hook pattern of the acanthocephalan conforms to 1 of the 2 distinguishing features of the Arythmacanthidae Yamaguti, 1935 (Golvan, 1960; Pichelin and Cribb, 1999); the other feature is the possession of 6 cement glands, which cannot be seen in this specimen. The family Arhythmacanthidae is in the class Palae-acanthocephala Meyer, 1931, order Echinorhynchida Southwell and Macfie, 1925. There are 9 genera (Amin, 2013). Classification into these genera is based primarily on the presence or absence of trunk spines (Pichelin and Cribb, 1999). There is a report of an acanthocephalan from the peritoneum of an oarfish (*R. glesne*), *Bolbosoma* (=*Echinorhynchus*) *vasculosum* (Rudolphi, 1819) Porta, 1908 (Monticelli, 1900). Although *Bolbosoma* does have small spine-like hooks at the basal part of the proboscis, that species has 18 longitudinal rows of hooks. The current specimen has only 12 longitudinal rows of hooks. Figure 6 shows the proboscis hook characters and the degree of proboscis invagination.

Roberts (2012) reviewing the sparse literature on oarfish parasites reasonably concludes that oarfish "are almost devoid of parasites." The parasites detected in the small volume of viscera we had available for examination, particularly lacking almost the entire intestine and the ability to inspect the mesenteries and peritoneum, suggest that this was a rather heavily parasitized fish. Since most of the parasites we observed, the tapeworms and nematodes, were larval stages whose adults require trophic transmission to mature in their predator final hosts, it is likely that the larger and older the oarfish, the more it will have accumulated parasites. The 2 fish examined by Hutton (1961) were much smaller (~2 m), and the size of the fish available to Villareal and Dailey (1993) was not reported. The fish we examined, and the fish reported to be heavily parasitized by Forbes (1891), were much larger, >5.5 m, and presumably older. If such large fish have, by dint of size, a very reduced chance of being eaten, then they will continue to accumulate parasites, serving as a population sink for those parasites. However, if they are subject to predators, then the increasing parasite loads may make them more likely to fall prey, with transmission of many larval parasites to their final hosts. Many recent studies of trophically transmitted parasites detect host behavior modification of their prey hosts, to the extent that it is perhaps the rule that such modification usually occurs (Kuris, 1997). This possibility may be involved in the epidemiology of oarfish parasites.

Although the parasites of oarfish are poorly known, information regarding their predators is so scant that there is no information on the predators of oarfish larger than 1 m (Roberts, 2012). Hence, the presence of trophically transmitted parasites may provide the best source of information regarding the likely predators of these large midwater fishes. The size, depth, and the potential host taxa of the adult parasites constrain the possible predator final host range. If the oarfish is indeed a viable part of the life cycle of the cestodes, then they are surely destined for an elasmobranch predator. This indicates a large shark that feeds on midwater prey. The shortfin mako shark and other large sharks seem likely possibilities, especially because the adults of *C. montaukensis* have been recovered from that host (Ruhnke, 2011) and its behavior extends to midwater depths (Last and Stevens, 1994). Furthermore, Stevens (1984) reported finding fragments of an oarfish in the stomachs of 2 shortfin mako sharks *Prionace glauca* and a few blue sharks. However the identification of those fragments is in question (Stevens in Roberts, 2012). The present observations, and those of Hutton (1961), however, support the likelihood of such sharks as predators on oarfish. The juvenile *Contracaecum* sp. nematodes point to a very different type of predator on oarfish since their final hosts are marine mammals. Sperm whales seem the most likely possibility since they have the size and the diving depth to readily feed on oarfish. As with the sharks, Roberts (2012) could find no certain evidence that diving whales feed on oarfish. Assuming that the large oarfish are not simply sinks for those larval parasites, consumption by a suitable predator for the tapeworms essentially hijacks the host destiny of the nematodes and vice versa (Lafferty, 1999).

The most commonly reported food items of oarfish are various euphausiid species (Roberts, 2012). Euphausiids and other small crustaceans are common hosts for various larval species of acanthocephalans (e.g., Gregori et al., 2013). We anticipate that detailed examination of other oarfish will recover more adult acanthocephalans, based on their feeding on pelagic crustaceans, and also larval infections since the specimens reported by Monticelli (1900) were embedded in the peritoneum and are presumably larval stages of another acanthocephalan species.

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