Multiple species of *Phoreiobothrium* from the blacktip shark, *Carcharhinus limbatus*, in the Gulf of Mexico

by

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## Author's Disclaimer

All taxonomic actions in this work are hereby disclaimed for nomenclatural purposes, as recommended in Article 8 of the International Code of Zoological Nomenclature (Ride et al., 1999).

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**Abstract**

During a survey of the adult tapeworm fauna of sharks from the Gulf of Mexico, the blacktip shark, *Carcharhinus limbatus* (Müller and Henle, 1839), was found to host cestodes in the genus *Phoreiobothrium*. *Carcharhinus limbatus* inhabits the world's tropical and warm temperate waters. As yet, 34 species of cestodes representing the orders Tetraphyllidea, Cathetocephalidea, and Trypanorhyncha have been reported to parasitize the species throughout its range. Little is known about tetraphyllidean diversity in *C. limbatus* in the Gulf of Mexico; no records exist for *Phoreiobothrium* from *C. limbatus* in this region. Between 2005 and 2007, 6 specimens of *C. limbatus* were collected off Ocean Springs, Mississippi and 14 specimens were collected off Panama City, Florida, and their spiral intestines examined for cestodes. Whole mounts and histological sections of the cestode specimens were prepared for examination with light microscopy; scoleces were prepared for scanning electron microscopy. Overall, *C. limbatus* was found to host 4 species of Trypanorhyncha and 11 species of Tetraphyllidea. In addition to 1-2 species each in the tetraphyllidean genera *Disculiceps*, *Anthobothrium*, and *Paraorygmatobothrium*, *C. limbatus* hosted 6 species of *Phoreiobothrium*. The diversity of *Phoreiobothrium* species is of special interest: all are new to science and collectively represent an unusually high number of congeners in a single host species. The new species of *Phoreiobothrium* from *C. limbatus* can be distinguished from the known species and each other based on characters such as scolex dimensions, number of subloculi, presence or absence of papillae, and distribution of vitellaria. Despite its

cosmopolitan distribution, it has been suggested that several distinct populations of *C. limbatus* exist in the Gulf of Mexico. The complex species assemblage of *Phoreiobothrium* in *C. limbatus* in the Gulf of Mexico has the potential to inform us about its population structure of the host.

**Introduction**

During a study to survey the adult tapeworm fauna from sharks and rays in the Gulf of Mexico, cestodes of the genus *Phoreiobothrium* Linton, 1889 were found parasitizing the blacktip shark, *Carcharhinus limbatus* (Müller and Henle, 1839). In general, little is known about the cestode diversity of elasmobranch species in the Gulf of Mexico; the most recent list of cestode parasites in the Gulf of Mexico was composed by Asa Chandler in 1954. More specifically, no records exist for *Phoreiobothrium* from *C. limbatus* in this region. Currently, there are 34 species of cestodes reported from *C. limbatus* throughout its range, representing members of the cestode orders Tetraphyllidea, Cathetocephalidea, and Trypanorhyncha (Palm, 2004; Healy, 2002; Palm and Overstreet, 2000; Schmidt and Beveridge, 1990; Carvajal et al., 1976; Linton, 1924). There are no tetraphyllidean species of cestode reported from *C. limbatus* in the Gulf of Mexico; this study aims to expand on the tetraphyllidean fauna of *C. limbatus* in the Gulf of Mexico.

In addition to describing the adult cestodes present in the spiral intestine of *C. limbatus*, I was interested in examining the *Phoreiobothrium*-*C. limbatus* parasite-host infection patterns to see if it would be possible to use these cestodes as biological tags to distinguish between potential stocks of blacktip sharks on either side of Mobile Bay, Alabama. There is some evidence to suggest that Mobile Bay serves as a biogeographic boundary for many clades of organisms, both freshwater and marine (e.g. Wiley and Mayden, 1985; McClure and McEachran, 1992). Mitochondrial DNA evidence suggests that there is a boundary between populations of *C. limbatus* in this

region, and that individuals from either side of the boundary do not interbreed (Keeney et al., 2005). I was interested in exploring the question of whether or not host these data were supported by the parasite fauna present in *C. limbatus*—that is to say, is the composition of cestode species, specifically *Phoreiobothrium*, different on opposite sides of Mobile Bay?

## Background

### *Gulf of Mexico*

The Gulf of Mexico is an ocean basin bordered by the United States, Mexico, and Cuba. Gulf waters are circulated by the Loop Current from the Caribbean Sea through the Straight of Yucatan, northward into the central Gulf, then eastward to Florida, where it flows down the coast and into the Atlantic Ocean through the Straights of Florida (Hoese and Moore, 1998). The habitats of the Gulf are richly varied and include bays, estuaries, marshes, and swamps as well as offshore tropical reefs (Hoese and Moore, 1998). Bottom types in the Gulf include mud, beds of turtle grass, and a mix of coarse sand and shell; a wide variety of salinities, temperatures, and dissolved oxygen levels are also found (Hoese and Moore, 1998).

Traditionally, provinces on land are defined by biotic differences—each province has its own unique assemblage of endemic flora and fauna; marine provinces are usually defined more by each province's unique ecology (Lomolino et al., 2006). The marine biotas of the Gulf Coast of the United States are divided into two coastal biogeographic provinces based on climate and ocean currents—the

temperate Louisianan Province from Texas to Tampa Bay, Florida, and the tropical West Indian Province from Tampa Bay to Jupiter Inlet, Florida (Engle and Summers, 2000). Additional studies have suggested a further subdivision within the Louisianan Province at Mobile Bay, Alabama; based on the endemic ranges of several freshwater species pairs, such as the killifishes *Fundulus confluentus* Goode and Bean, 1879 and *F. pulvereus* (Evermann, 1892) and the freshwater darters *Etheostoma chlorosoma* (Hay, 1881) and *E. davisoni* (Hay, 1885), it has been suggested that a vicariant event occurred at Mobile Bay which separated the previously contiguous ranges of these fishes (Wiley and Mayden, 1985). Additionally, along the Gulf Coast at least 14 clades of organisms are parapatrically distributed with a hybrid zone between Eastern Mississippi and Northwestern Florida. These include the sea robins *Prionotus alatus* Goode and Bean, 1883 and *P. paralatus* Ginsburg, 1950 (McClure and McEachran, 1992), the moray eels *Gymnothorax saxicola* Jordan and Davis, 1891 and *G. nigromarginatus* (Girard, 1858) (Böhlke et al., 1989), the snake eels *Bascanichthyes scuticaris* (Goode and Bean, 1880) and *B. bascanium* (Jordan, 1884) (Leiby and Yerger, 1980), the naked soles *Gymnachirus melas* Nichols, 1916 and *G. nudis* Kaup, 1858 (Dawson, 1964), and the sheepsheads *Archosargus probatocephalus probatocephalus* (Walbaum, 1792) and *A. p. oviceps* (Ginsburg, 1952) (Caldwell, 1965). This barrier also divides species found on both sides of the bay into distinct populations between which there is little gene flow, as indicated by mtDNA markers of the toadfish *Opsanus beta* (Goode and Bean, 1880) (Avise et al., 1987), and, as

will be discussed later, by mtDNA markers of *Carcharhinus limbatus* (Keeney et al., 2005; Keeney and Heist, 2006).

### ***Genus*** **Phoreiobothrium**

The genus *Phoreiobothrium* is a member of the Order Tetraphyllidea, and the Family Onchobothriidae Braun, 1900. It was first described by Edwin Linton in 1889 from specimens of the dusky shark, *Carcharhinus obscurus* (Leseur, 1818) (as *Carcharias obscurus* [Lesueur, 1818]) collected off of Woods Hole, Massachusetts during the course of his summer surveys of Entozoa at the U.S. Fish Commission's summer station between 1884-1885 (Linton, 1889). The type species described by Linton was *Phoreiobothrium lasium* Linton, 1889; host records of this species include 11 additional shark species in both Carcharhinidae and Sphyrnidae include *Carcharhinus limbatus* (see Caira et al., 2005). Recent reevaluation of early host identifications suggested that *P. lasium* is actually a species complex, with each species of worm in the complex infecting a different species of host (Caira et al., 2005). To date, there are 17 species of *Phoreiobothrium*, six of which are considered *species inquirendae* (Caira et al., 2005). Of these six, *P. arabiansi*, *P. ratnagiriensis*, *P. shindei, P. girjamami,* and *P. vinodae* are described from a single host species, "*Carcharias acutus*", which is a synonym of *Rhizoprionodon acutus* (Rüppel, 1837) This species of shark is very similar to several other species which are particularly difficult to distinguish. Additionally, these five species along with *P. puriensis*, lack

available type material. This leaves 11 species in *Phoreiobothrium* that are currently recognized as valid (see Table 1; Caira et al., 2005).

Species of *Phoreiobothrium* are restricted to seven species of requiem sharks (Family Carcharhinidae Jordan and Evermann, 1896) and five species of hammerhead sharks (Family Sphyrnidae Rafinesque, 1810) in the Order Carcharhiniformes. Only four species of *Phoreiobothrium* have been described from species in the genus *Carcharhinus* (see Table 1), which to date contains 31 species (Compano et al., 2005). Seventeen species of *Phoreiobothrium* have been reported from sharks from the western Atlantic Ocean as far north as New York and as far south as the Bahamas, including the Gulf of Mexico, from the Pacific Ocean off the coast of Australia, and from the Indian Ocean near India. The genus is characterized mainly by its scolex features; members possess four bothridia, each divided into a pre-hook loculus and a posterior loculus separated by a pair of hooks which are most often tri-pronged, but are in two cases bi-pronged. The posterior loculus is further subdivided horizontally; the posterior of these subloculi is subdivided vertically into subloculi.

Very little is known about the life cycle of cestodes that parasitize sharks. It is hypothesized that the adult tapeworm releases its eggs into the water, which are eaten by copepods; the hexacanth larvae then hatch and infect the host copepod. These copepods are then consumed by another intermediate host (usually a teleost) which in turn is infected by the cestode larvae. This second intermediate host is then consumed by a shark, in the spiral intestine of which the adult cestode takes up residence (Caira, 1990). Whether additional hosts between the second intermediate

host and the definitive host are necessary for a successful life cycle is not known, nor is the duration of individual adult cestode infections.

**Table 1.** Described species of Phoreiobothrium (sensu Caira et al., 2005).

| Species | Host(s) | Type Localities |
|---|---|---|
| Recognized as valid: | | |
| *Phoreiobothrium robertsoni* Caira, Richmond, & Swanson, 2005 | *Carcharhinus brachyurus* | Southern and Western Australia |
| *Phoreiobothrium lasium* Linton, 1889 | *Carcharhinus obscurus* | Woods Hole, MA, U.S.A. |
| *Phoreiobothrium triloculatum* Linton, 1901 | *Carcharhinus obscurus* | Woods Hole, MA, U.S.A. |
| *Phoreiobothrium blissorum* Caira, Richmond, & Swanson, 2005 | *Carcharhinus plumbeus* | Woods Hole, MA, U.S.A.; Long Island, NY, U.S.A |
| *Phoreiobothrium perilocrocodilus* Caira, Richmond, & Swanson, 2005 | *Negaprion acutidens* | Darwin Harbor, Australia |
| *Phoreiobothrium anticaporum* Caira, Richmond, & Swanson, 2005 | *Negaprion brevirostris* | Florida Keys, U.S.A. |
| *Phoreiobothrium lewinense* Caira, Richmond, & Swanson, 2005 | *Sphyrna lewini* | North Carolina, U.S.A. |
| *Phoreiobothrium manirei* Caira, Healy, & Swanson, 1996 | *Sphyrna mokarran* | Tampa Bay, FL, U.S.A. |
| *Phoreiobothrium tiburonis* Cheung, Nigrelli, & Ruggieri, 1982 | *Sphyrna tiburo* | Florida Keys, U.S.A. |
| *Phoreiobothrium exceptum* Linton, 1924 | *Sphyrna zygaena* | Woods Hole, MA, U.S.A. |
| *Phoreiobothrium pectinatum* Linton, 1924 | *Sphyrna zygaena* | Woods Hole, MA, U.S.A. |
| *Species inquirendae:* | | |
| *Phoreiobothrium arabiansi* Shinde, Jadhav, & Mohekar, 1984* | *"Carcharias acutus"* | Ratnagiri, India |
| *Phoreiobothrium ratnagiriensis* Shinde & Jadhav, 1987 | *"Carcharias acutus"* | Ratnagiri, India |
| *Phoreiobothrium shindei* Shinde, Jadhav, & Jadhav, 1990 | *"Carcharias acutus"* | Bombay, India |
| *Phoreiobothrium girjamami* Shinde, Motinge, & Pardeshi, 1993 | *"Carcharias acutus"* | Ratnagiri, India |
| *Phoreiobothrium vinodae* Jadhav, 1993 | *"Carcharias acutus"* | Bombay, India |
| *Phoreiobothrium puriensis* Srivistav & Capoor, 1982 | *Eusphyra blochii* | Puri, India |

***Blacktip Shark Biology***

The blacktip shark, *Carcharhinus limbatus,* is a circumglobally distributed species found in tropical and subtropical continental waters (Compagno, 1984). *Carcharhinus limbatus* is a large shark (up to 2.55m total length) most readily identified by the black tips on its second dorsal fin, lower caudal lobe, and pectoral fins (Garrick, 1982). It is one of the most economically important sharks in the southeastern United States, and is fished both commercially and for sport (Castro, 1996). Throughout its life, *C. limbatus* is primarily a piscivore; clupeid and sciaenid fishes comprise the majority of its diet (Hoffmayer and Parsons, 2003; Bethea et al., 2004). Additional sources of food include sharks, rays, crustaceans, and cephalopods (Compano, 1984), but it is through consumption of teleost that the shark is most likely infected with cestodes.

Although *C. limbatus* is a cosmopolitan species in which individuals are capable of dispersing as far as 1,159 nautical miles over an unspecified duration of time, but not exceeding 7.3 years (Kohler et al., 1998), there is morphological evidence that has been used to suggest that there are distinct sub-species and populations of the shark (Garrick, 1982). Meek and Hildebrand (1923) described *Carcharhinus natator*, which they described as being distributed from the tropical Pacific coast of the Americas to a zone of intergradation at the Mississippi River in the Gulf of Mexico. These Texas blacktips, when compared to blacktips from Florida and the Antilles, are to all appearances quite different—for example, their snouts are noticeably shorter, but in 1950, Springer determined that this species was actually a

subspecies of *C. limbatus*. Morphological evidence suggesting stock separation also includes observations of variation in the fin tip markings of *C. limbatus* populations on either side of the Americas; the outer pectoral fin tip in Atlantic blacktips has a clear straight or convex inner border, whereas Pacific blacktips have a more oblique mark that does not extend as far along the anterior margin as it does along the distal margin (Garrick, 1982). However, variations in these fin marks with the age and preservation of the specimens preclude the use of these data to define blacktip stocks (Garrick, 1982).

Life histories, specifically age and length at maturity, have also been examined as possible modes for identifying stocks in the South Atlantic Bight and the eastern Gulf of Mexico, but have proved inconclusive (Carlson et al., 2006). Artificial tagging data further elucidated the pattern of stock structure and suggested that *C. limbatus* showed philopatric tendencies which may lead to genetic stock structure. In one study conducted along the Florida Gulf coast, juvenile Blacktips tagged at nursery sites in Florida returned to the same site every summer for their first three years, and female adults continued to return to the same nursery every year to pup (Hueter et al., 2004).

These phylopatric tendencies may serve to explain patterns suggested by phylogeographic studies using mitochondrial DNA markers. These sorts of mitochondrial studies can be used to estimate maternal gene flow—mtDNA is always passed from mother to offspring without inclusion of paternal DNA. In a world-wide study by Keeney and Heist (2006), blacktip sharks inhabiting the western Atlantic

Ocean, Gulf of Mexico, and Caribbean Sea were shown to have a distinct haplotype from blacktip sharks in the eastern Atlantic Ocean, Indian Ocean, and Pacific Ocean. Within the western Atlantic, Gulf of Mexico, and Caribbean Sea, mitochondrial and microsatellite DNA have suggested further stock divisions; five distinct populations exist in the eastern and western Gulf of Mexico, the Western Atlantic, off of the Yucatan Peninsula, and off of Belize (Keeney et al., 2005).

### *Using Parasites as Biological Tags*

For over a century, questions of organismal ranges and migration patterns have been studied using mark-recapture techniques to determine ranges and migration patterns of highly vagile animals. These traditional artificial tag studies, whether they focused on terrestrial or aquatic animals, can suffer from complications ranging from expense to biological practicality (Mosquera and Castro, 2003). Artificial tagging requires separate collections to mark and recapture study animals—not often a cheap or logistically easy research protocol. For deep-sea fishes that are often damaged or killed in the process of capture, artificial tagging is impossible (Williams et al., 1992); crustaceans and other arthropods shed artificial tags with their shells as they molt (Mosquera and Castro, 2003). Compared to artificial tags, biological tags eliminate doubt as to abnormal behavior of tagged animals, as well as being cheaper to execute as there need not be separate expeditions to tag the study animals and to collect them later (MacKenzie and Abaunza, 1998). As a result, artificial tag protocols are gradually being supplanted by protocols utilizing biological tags such as

morphological markers, stable isotopes, and genetic data with mixed success (Fallon et al., 2006).

Increasingly, researchers are examining non-pathogenic parasites as a useful source of information as biological tags. The number of papers written on the subject ballooned from nine in the 1950s to over 140 in the 1980s (Williams et al., 1992). There is one very basic principle behind the concept of using parasites as biological tags—parasites will only infect hosts that pass through the endemic range of the parasite (MacKenzie, 2004). An example of a short-term application of this principle is found in a study that Bullard and collegues conducted in Central America in 2004. A number of bull sharks (*Carcharhinus leucas* [Müller and Henle, 1839]) that were recovered from the Colorado River in Costa Rica and the San Juan River in Nicaragua were parasitized by a species of monogenic trematode that is endemic to saltwater habitat. This worm cannot survive in a low-saline environment for longer than a few days, a fact which the researchers suggested could be used to estimate the length of a bull shark's stay in a freshwater environment (Bullard et al., 2004).

To meet with the increasing standards of rigorous testing and legitimate research demands, MacKenzie (2004) and Mosquera and Castro (2003) summarized the following set of necessary characters of any parasite to be used as a biological tag: the parasite should also have a life span that lasts for the duration of the investigation and the infection rates should be the same from year to year, or else researchers may develop a false estimate of relevant statistics such as prevalence and intensities of infection; for the sake of effective use of time, as many of these studies involve large

sample sizes of both hosts and parasites, the parasites must be easily detected and identified; and the parasite should not have a markedly pathological effect on the host or otherwise affect the host's behavior, lest they exhibit the same drawbacks artificial tagging regimes have experienced. Most importantly, there should be significant difference in the levels of infection of the host being studied in different parts of the study range (MacKenzie, 2004; Mosquera and Castro, 2003). As the ranges of many potential parasite tags are unknown, this is often the first stage of such an investigation.

A wealth of studies using parasite tags to answer questions of fish population distribution has been conducted in the last thirty years. One such study was presented in a 2003 paper by Yamaguti and colleagues on the starspotted dogfish *Mustelus manazo* Bleeker, 1854 in the western Pacific. Regional variation in several aspects of the life history and morphology of the starspotted dogfish between populations in Japan and Taiwan had been previously noted by researchers, and the authors assessed the feasibility of using parasites as discriminators among these populations (Yamaguti et al., 2003). They examined 1,038 specimens from seven localities in Japan and Taiwan, recovering several species of copepods, cestodes, myxosporeans, and one species of nematode. Of these parasites, cestodes were selected as having the most potential for use as a biological tag due to the large number of species and their high prevalence of infection in the host, as well as their established pattern of high host specificity. Using their data, Yamaguti and collegues tested whether, using only parasite tags, they could determine whether the host had been collected from Tokyo

Bay or Aomori. Sharks from Tokyo Bay were correctly identified 100% of the time, and sharks from Aomori were correctly identified 84.6% of the time (Yamaguti et al., 2003). This suggested a separate shark population in Tokyo Bay could be identified with a high degree of confidence using parasite data (Yamaguti et al., 2003).

A similar study was conducted by Moore and colleagues in 2003—parasite data was tested for use in defining individual populations, or stock structures, of the narrow-barred Spanish mackerel *Scomberomorus commerson* Lacepède, 1800 off the northern coast of Australia. For this study, the full range of parasites infecting the fish—copepods, monogenes, cestodes, and nematodes—were considered, and statistically significant differences in infection rate were observed (Moore et al., 2003). These data were then compared to previous studies which assessed a variety of genetic data from *S. commerson* collected from off the coast of Indonesia as well as from four commercial fisheries off the northern coast of Australia. Low abundances of juvenile parasites in the outgroup of mackerel from Indonesia compared to those of northern Australia determined that these were two distinct fisheries—a hypothesis undisputed by the high genetic divergence between mackerel from these two regions (Moore et al., 2003). However, when parasite assemblages were compared among the four fisheries hypothesized to represent distinct stocks in northern Australia, the parasite data indicated distinct stocks which were corroborated with otolith stable isotope data, whereas genetic data indicated a single homogeneous population. The authors argued that a relatively small amount of genetic exchange (as little as 5%) among the mackerel stocks could explain this phenomenon.

Additionally, they suggest that the conflict between parasite and genetic data (which can be found in studies from the North Sea in Europe as well) indicate that the shorter generation times of parasite species mean that they differentiate faster and may be more useful for fine-scale stock structure studies than host genetic data (Moore et al., 2003).

Still, there are confounding factors researchers must keep in mind when embarking on a study using parasites to answer biogeographic questions. Parasite infections are dynamic, and may change from season to season or over longer periods than those over which the tag parasite has been studied (Mosquera and Perez-Villar, 2000). The gaps in our knowledge of the parasite fauna of interest may also serve as a barrier—unknown environmental or life cycle barriers may affect parasite ranges in ways that we cannot predict (MacKenzie, 2004). If the parasite has a complex life cycle (cestodes have at least two intermediate hosts in most cases), the range of the parasite may reflect the range of an intermediate host of a life cycle stage other than the host being studied, and may fluctuate over time in correlation with that intermediate host (Mosquera and Perez-Villar, 2000).

However, these challenges to conducting a meaningful study in parasitological biogeography are not insurmountable. Researchers such as Moore and colleagues (2003) have begun collecting data on the widest possible variety of parasites and integrating it into a bigger picture. These scientists are hard at work developing new and better methods of assessing whole parasite communities and performing multivariate analyses on such information to construct more intelligible data sets

(MacKenzie, 2004). Parasite data is being woven into the multidisciplinary fabric of much broader biogeographic studies utilizing a variety of data ranging from gene sequences to stable isotope tags to elucidate host ranges (Moore et al., 2003).

## Materials and Methods

### *Collection of Specimens*

Between 2005 and 2007, cestodes were collected from the spiral intestines of the following specimens of *Carcharhinus limbatus*: 6 collected in collaboration with the University of Southern Mississippi Gulf Coast Research Laboratory in Mississippi using long line (2 males off Horn Island in June of 2005; 1 male off Round Island in June of 2005; 2 females off Ship Island in July of 2006; 1 female off Horn Island in June of 2007) and 14 collected in Florida in collaboration with the National Marine Fisheries Service Panama City Laboratory using gill nets (1 male off St. Joe's Bay in October of 2006; 1 male off Crooked Island Bay in October of 2006; 1 female off Indian Pass in October of 2006; 1 female off St. Andrew Bay in October of 2006; 3 males and 7 females collected off Indian Pass in May of 2007). The exact localities of these collections are depicted in Figure 1. Shark taxonomy follows Compagno (1984).

The body cavity of each shark was opened with a longitudinal incision and the spiral intestine removed. Each spiral intestine was opened with a longitudinal incision and fixed in 10% formalin; intestines were transferred to 70% ethanol at the University of Kansas. Spiral intestines were subsequently examined under a

dissecting microscope for cestodes. Worms were stored in 70% ethanol following removal from the host's mucosa.

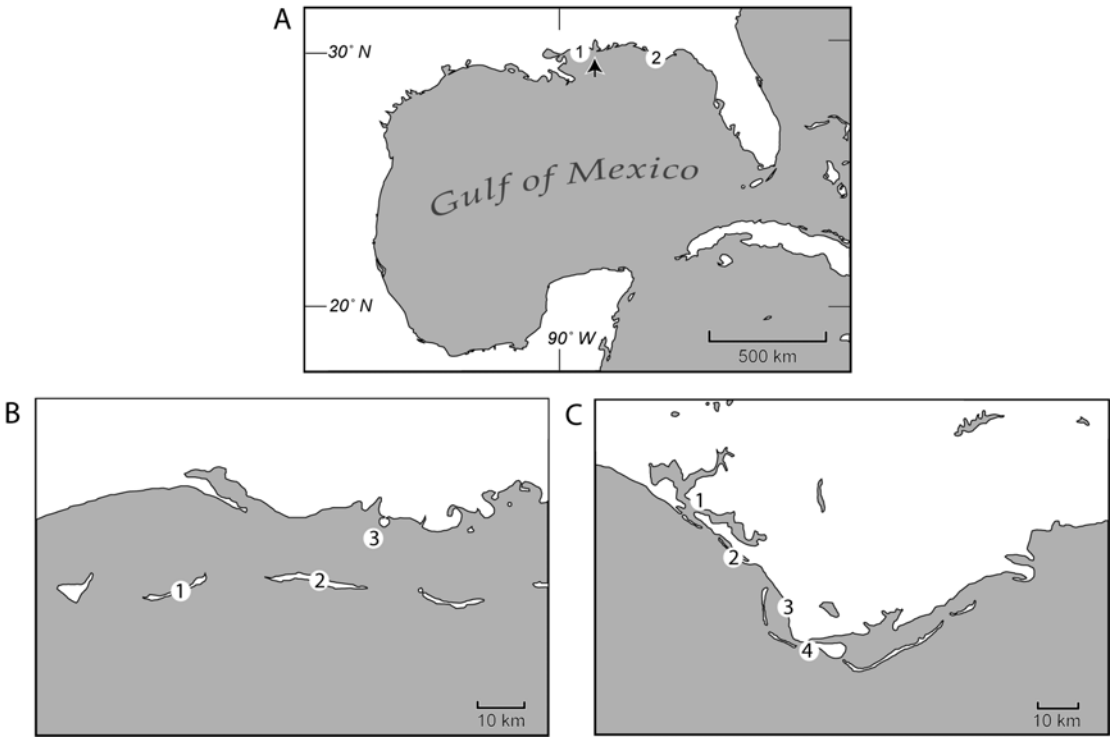


**Figure 1.** Collection localities. **(A)** Map of Gulf of Mexico denoting Mississippi collection sites (1) and Florida collection sites (2). Arrow indicates proposed biogeographic boundary at Mobile Bay, Alabama. **(B)** Detail map of Mississippi collection sites: Ship Island (1), Horn Island (2), and Round Island (3). **(C)** Detail map of Florida collection sites: St. Andrew Bay (1), Crooked Island (2), St. Joe Bay (3), Indian Pass (4).

### *Specimen Preparation*

Specimens prepared as whole mounts were hydrated, stained in Delafield's hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted on glass slides in Canada balsam. Specimens prepared for histology were lightly stained in Fast Green to improve visibility in wax blocks, dehydrated in a

graded ethanol series, and embedded in paraffin. Sections were cut at 4 μm intervals using an Olympus CUT2020 B retracting rotary microtome. Sections were mounted on glass slides flooded with 2.5% sodium silicate and dried on a slide warmer overnight. Specimens were then stained in Delafield's hematoxylin and eosin, cleared in xylene, and mounted on glass slides in Canada balsam. Specimens prepared for scanning electron microscopy (SEM) were transferred to distilled water, postfixed in 1% osmium tetroxide overnight, dehydrated in a graded ethanol series, transferred to hexamethyldisilizane (Electron Microscopy Sciences, Fort Washington, Pennsylvania) for approximately 15 min and, following removal of the bulk of the hexamethyldisilizane, allowed to air dry. Specimens were subsequently mounted on aluminum stubs with carbon tape, sputter-coated with approximately 35 nm of gold and examined using a LEO/Zeiss DSM982 Gemini field emission scanning electron microscope.

***Material Examined***

For comparative taxonomic purposes, museum material was in the form of type material and voucher specimens borrowed from the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. The following specimens were borrowed and examined: 1 slide (USNPC No. 35874) containing a total of 4 specimens collected from *Carcharhinus obscurus* near Woods Hole, Massachusetts and identified by MacCallum as *P. lasium*, including the neotype (Caira et al., 2005); 3 slides (USNPC No. 35876) containing a total of 6 specimens collected from

*Carcharhinus obscurus* near Woods Hole, Massachusetts and identified by MacCallum as *P. lasium*; the holotype and paratype of *P. tiburonis* (USNPC No. 76691); 4 slides (USNPC Nos. 96731, 96732) containing a total of 4 specimens collected from *Sphyrna tiburo* from Pine Island Sound, Florida (USNPC No. 96731) or near Tampa Bay, Florida (USNPC No. 96732) and identified as *P. tiburonis* (Caira, et al., 2005); 3 slides (USNPC No. 96737) containing a total of 3 paratypes of *P. blissorum*; 2 paratypes (USNPC No. 96744) of *P. robertsoni*. Other museum abbreviations used: Lawrence R. Penner Parasitology Collection (LRP), University of Connecticut, Storrs, Connecticut, U.S.A.; and Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, Nebraska, U.S.A.

***Descriptions***

Measurements of cestodes were taken using a Leica DFC320 digital camera mounted on a Zeiss Axioskop 2 using the image analysis software OpenLab Demo 4.0.4. All measurements are in micrometers unless otherwise noted. For each measurement the range is presented in the text, followed by the mean, standard deviation, number of worms measured, and the number of measurements taken if more than one measurement was taken per worm. Hook measurements taken follow Caira (1985) and are illustrated in Figure 2; six measurements were taken of both the lateral and medial hooks; lateral hook measurements are represented by capital letters and corresponding medial measurements are represented by the corresponding capital letter prime. These measurements consist of: A (A'),distance from abaxial prong tip

to point uniting axial and abaxial prongs; B (B'), distance from axial prong tip to most elevated point uniting axial and abaxial prongs; C (C'), distance from axial extremity of base to most elevated point uniting axial and abaxial prongs; D (D'), distance from axial extremity of base to tip of axial prong; E (E'), distance from tip to base of basal prong; F (F'), length of talon from rounded posterior extremity to base. Morphological terminology for the scolex and strobila follows Caira and colleagues (1999) and Caira and Jensen (2001). Measurements of the scolex features are illustrated in Figure 2. Illustrations of each proposed species were drawn with the aid of a camera lucida drawing tube.

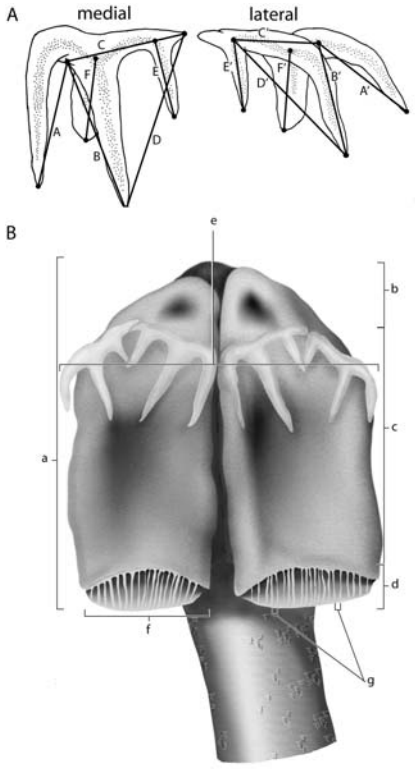


**Figure 2**. Measurements taken. **(A)** Hook measurements. **(B)** Scolex measurements: scolex length (a), apical loculus length (b), bothridium width (c), subloculus length (d), scolex width (e), bothridium width (f), subloculus width (h).

## Results

### *Descriptions of New Species*

Worms in the genus *Phoreiobothrium* were recovered from 19 of the 20 specimens of *Carcharhinus limbatus* collected in numbers ranging from 2 to 344, but averaging 70. In all, approximately 1,615 worms of the genus *Phoreiobothrium* were recovered; 899 whole mounts, 9 scolex mounts for SEM, and 1 last mature proglottid cross section mount were prepared from this collection. Based on measurements of these specimens, six new species could be identified and are described below.

### ***Phoreiobothrium* n. sp. 1**

(Figs. 3-4)

*Description:* Based on 20 whole mounts and 1 scolex mounted for SEM. Worms euapolytic, 6.5-12.1 mm long (8.4±1.4, 20), 17-30 (24±3.7, 20) total proglottids; greatest width 377-528 (463±40.7, 20), at level of last mature proglottid. Scolex 255-320 (305±15.4, 20) long by 250-304 (278±16.4, 20) wide, consisting of scolex proper with 4 bothridia. Bothridia 215-295 (250±18.9, 20) long by 132-181 (160±12.7, 20) wide, each with one pair of tri-pronged hooks, anterior prehook region in form of loculus, and posthook region divided into anterior and posterior loculi. Anterior prehook region 31-49 (41±5.4, 20) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 32-53 (44±6.5, 20) long, divided into 10-16 (14±1.3, 18, 24) subloculi; subloculi 7-13 (10±1.6, 20, 23) wide. Hooks tri-pronged with blunt talon

embedded in musculature of scolex; talon of medial hook shorter and thicker than that of lateral hook; prongs and talon hollow. Bases of medial and lateral hooks slightly spaced apart; accessory piece lacking. Hook surfaces covered with thin layer of tissue. Axial prongs longest; basal prongs greater than 1/2 length of axial prongs; abaxial prongs greater than 3/4 length of axial prongs, conspicuously more extended toward horizontal axis on lateral hook than on medial hook. Lateral hook lengths: A 34-62 (47±9.1, 15), B 41-72 (55±8.9, 15), C 38-62 (51±6.4, 14), D 55-76 (65±5.4, 14), E 18-41 (33±6.6, 18), F 24-40 (30±4.1, 20). Medial hook lengths: A' 37-58 (49±6.1, 20), B' 45-73 (61±7.3, 20), C' 38-60 (53±6.2, 20), D' 52-87 (72±8.8, 20), E' 22-46 (35±6.4, 20), F' 23-41 (32±3.9, 19).

Distal and proximal surfaces of bothridia covered with short filitriches (Fig. 4C, D).; no spinitriches seen in these regions. Cephalic peduncle covered with long filitriches and bladelike spinitriches oriented with points directed posteriorly (Fig. 4E).

Proglottids acraspedote. Immature proglottids 14-27 (24±3.7, 20) in number, initially wider than long, becoming longer than wide with maturity, last immature proglottid 469-982 (683±127, 20) long by 287-423 (347±36.3, 20) wide. Mature proglottids longer than wide, 2-4 (3±0.6, 20) in number, 1,013-1,792 (1,372±200.1, 20) long by 377-528 (463±40.4, 20) wide. Gravid proglottids not seen. Testes 58-95 (77±9.1, 20) in number, 8-12 (10±1.3, 20) in postvaginal field, oval, 28-62 (42±7.6, 20, 60) long by 48-98 (69±12.2, 20, 60) wide, arranged in 4-5 columns, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus

sac and uterus. Genital pore lateral, 44-74% proglottid length from posterior end; irregularly alternating. Cirrus sac oval, 149-229 (180±18, 19) long by 46-100 (81±15, 19) wide, extending 34-65% of proglottid width into proglottid, containing coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, located at posterior end of proglottid, 305-470 (391±51.3, 20) long by 229-363 (297±41.3, 20) wide; posterior margin of ovarian bridge 45-89% from posterior of ovary. Vagina wide, extending anteriorly from ootype region along medial line of proglottid, then curving laterally across anterior margin of cirrus sac, opening anterior to cirrus sac into genital pore. Uterus extending into anterior 2/5 of proglottid. Vitellaria follicular, 11-24 (16±2.7, 20, 60) long by 22-83 (40±12.9, 20, 60) wide; in two lateral bands with a dorsal and ventral row in each band, extending into field of testes, extending from posterior margin of proglottid, stopping short of anterior margin of testes field, extending laterally into the field of testes uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* Indian Pass, Gulf of Mexico, Florida, U.S.A.

*Additional localities:* None.

*Prevalence:* 3 of 20 blacktip sharks.

*Site of infection:* Spiral intestine.

*Specimens deposited:* Holotype (USNPC No. 0000) and 8 paratype specimens (USNPC Nos. 0000-0000); 7 paratype specimens (LRP Nos. 0000-0000); 4 paratype specimens (HWML Nos. 0000); scolex mounted for SEM and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

*Remarks*

Of the 17 accepted species of *Phoreiobothrium, Phoreiobothrium* n. sp. 1 is readily distinguished from *P. exceceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the posterior border of the anterior loculus of each bothridium, which distinguishes it from *P. pectinatum*, and lacking papillae along the posterior border of each bothridium, which distinguishes it from *P. tiburonis*. *Phoreiobothrium* n. sp. 1 possesses an abaxial prong on each scolex hook, which is absent in *P. manirei* and *P. exceptum*. *Phoreiobothrium* n. sp. 1 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum* and *P. pectinatum*, (10-16 vs. 3, 5, 6, and 6-7 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (10-16 vs. 25-29, 23-31, and 25-30 respectively).

In addition, *Phoreiobothrium* n. sp. 1 has a longer total length than *P. tiburonis* and *P. lewinense* (6.5-12.1 mm vs. 3.8-5.9 mm, and 1.1-4.4 mm respectively) and a shorter total length than *P. blissorum* (6.5-12.1 mm vs. 13-18.9 mm). The ovary of *P.* n. sp. 1 is narrower than that of *P. robertsoni* (229-363 vs.

105-160). It has more testes than *P. perilocrocodilus* and *P. anticaporum* (58-95 vs. 36-49 and 36-54 respectively) and fewer testes than *P. blissorum*, and *P. lewinense* (58-95 vs. 103-127 and 173 respectively). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4-5 vs. 6-7, 7-8, and 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus,* (4-5 vs. 2 and 2 respectively).

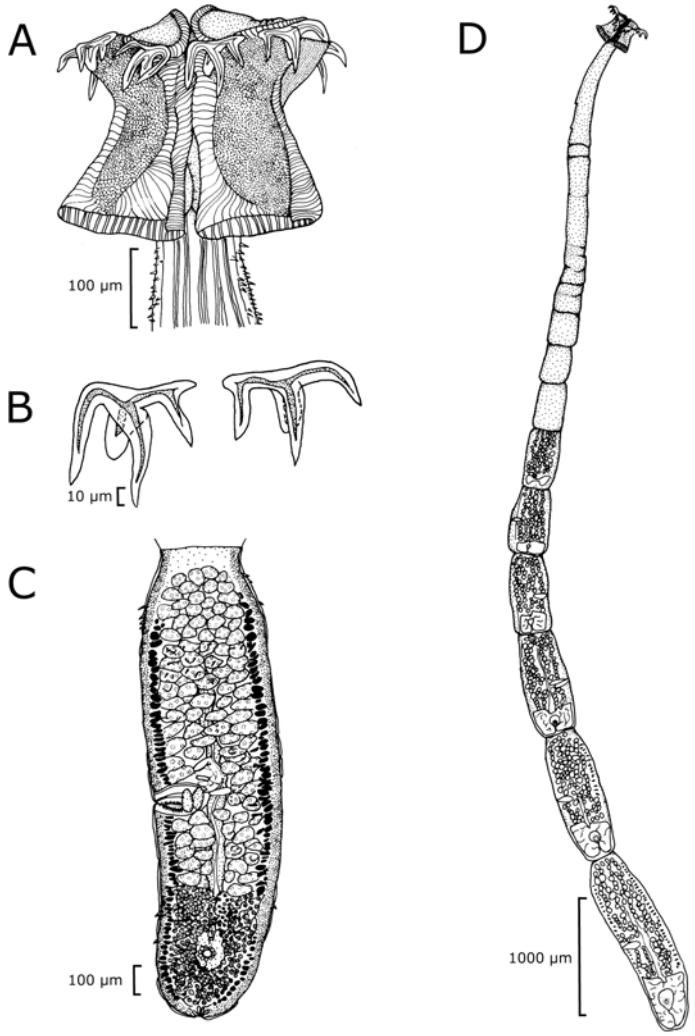


**Figure 3.** Line drawings of *Phoreiobothrium* n. sp. 1. **(A)** Scolex. **(B)** Hooks. **(C)** Mature proglottid. **(D)** Whole worm.

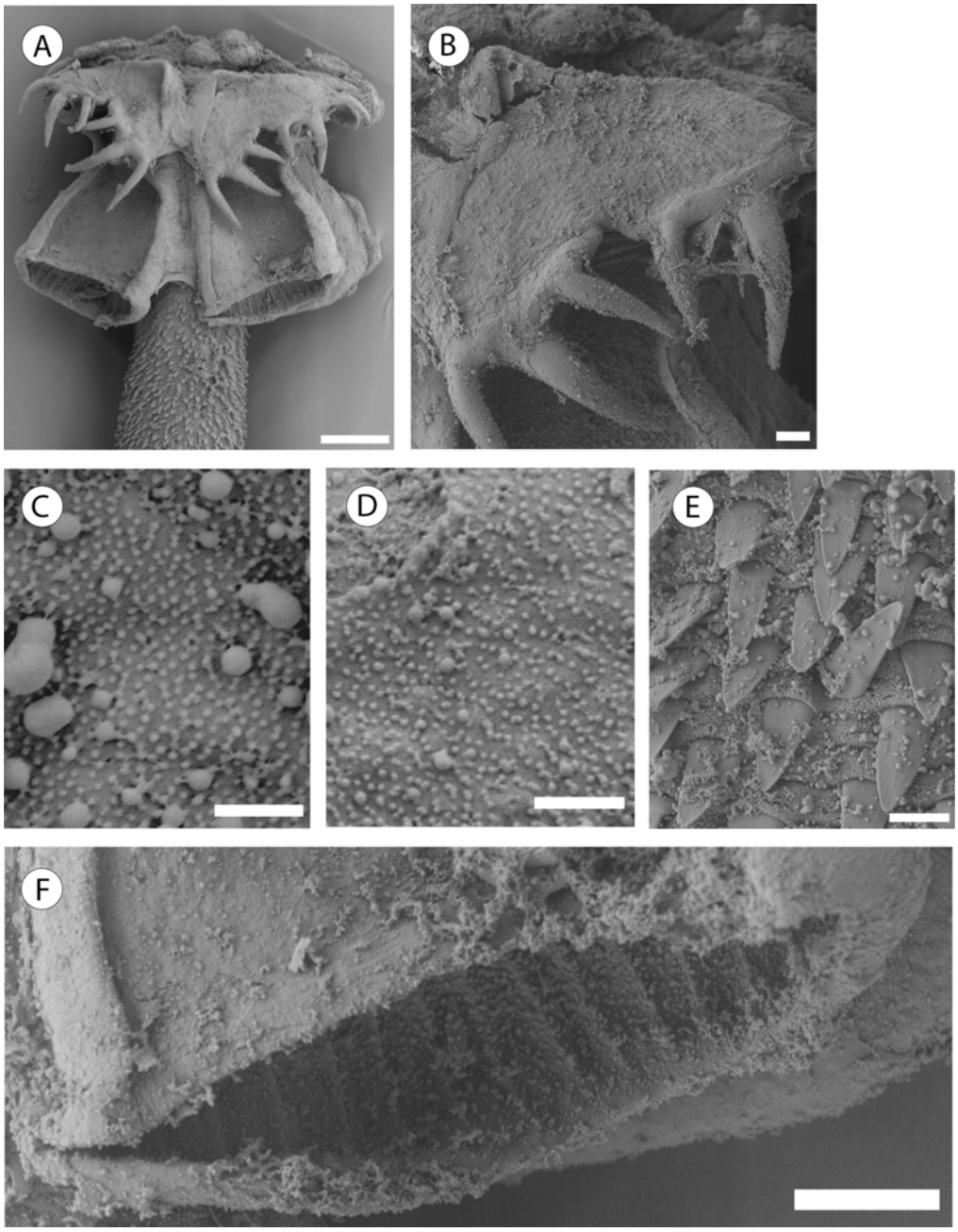


**Figure 4.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 1. **(A)** Scolex; scale bar 50 μm. **(B)** Enlarged view of anterior loculus; scale bar 10 μm. **(C)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(D)** Enlarged view of proximal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(F)** Enlarged view of sublocculi; scale bar 20 μm.

***Phoreiobothrium* n. sp. 2**

(Figs. 5-6)

*Description*: Description based on 6 whole mounts and 1 scolex mounted for SEM. Worms euapolytic, 5.0-9.9 mm long (6.6±2.1, 5), 22-45 (34±9.3, 5) total proglottids; greatest width 364-495 (431±52.2, 5) at level of last mature proglottid. Scolex 331-400 (357±18, 6) long by 319-340 (354±11, 2) wide, consisting of scolex proper with 4 bothridia. Bothridia 291-353 (322±13, 6) long by 151-228 (193±22, 5) wide, each with one pair of tri-pronged hooks, anterior prehook region in form of loculus, and posthook region divided into anterior and posterior loculi. Anterior prehook region 29-49 (40±2, 6) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 27-47 (32±2, 5) long, divided into 14-16 (15±1, 4) subloculi; subloculi 10-16 (12±1, 5) wide. Hooks tri-pronged with blunt talon embedded in musculature of scolex; talon of medial hook longer and thicker than that of lateral hook; prongs and talon hollow. Bases of medial and lateral narrow, typically with a space between; accessory piece lacking. All hook surfaces covered with thin layer of tissue. Axial prongs longest; basal prongs greater than half length of axial prongs; abaxial prong 2/3 length of axial prong, conspicuously more extended toward horizontal axis on lateral hook than on medial hook. Lateral hook lengths: A 55-62 (60±4, 3), B 42-63 (52±9, 4), C 49-56 (54±3, 6), D 41-99 (80±26, 4), E 29-43 (36±5, 5), F 28-36 (34±3, 6). Medial hook lengths: A' 53-71 (63±7, 5), B' 55-75 (63±8, 5), C' 51-71 (61±8, 5), D' 71-126 (97±21, 5), E' 41-48 (44±3, 5), F' 31-45 (40±6, 5).

Distal bothridial surface covered with short filitriches interspersed with cilia (Fig. 6C). Proximal bothridial surface covered with short filitriches interspersed with small spinitriches. Cephalic peduncle covered with long filitriches and densely packed with bladelike spinitriches of varying lengths and oriented with points directed posteriorly (Fig. 6E). Boundary between anterior and posterior loculi possesses muscular double ledge (Fig. 6F).

Proglottids acraspedote. Immature proglottids 22-45 (32±9, 5) in number, initially wider than long, becoming longer than wide with maturation, last immature proglottid 334-596 (470±100, 6) long by 327-490 (392±47, 6) wide. Mature proglottids 0-3 (2±1, 5) in number, longer than wide, last mature proglottid 570-1,207 (958±230, 4) long by 364-461 (416±47, 4) wide. Gravid proglottids not seen. Testes 82-98 (89±6, 5) in number, 10-13 (11±1, 5) in postvaginal field, oval, 18-35 (27±4, 5, 15) long by 39-64 (52±8, 5, 15) wide, arranged in 4-5 columns, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus sac and uterus. Genital pore lateral, 45-52% of proglottid from posterior end; irregularly alternating. Cirrus sac oval, 125-169 (145±18, 5) long by 30-71 (49±16, 5) wide, extending 28-37% of proglottid width into the proglottid, containing coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, located at posterior end of proglottid, 146-327 (223±63, 5) long by 202-320 (261±44, 5) wide; posterior margin of ovarian bridge 44-64% from posterior margin of ovary. Vagina narrow, extending anteriorly

from ootype region along medial line of proglottid, then curving laterally around anterior margin of cirrus sac, opening anterior to cirrus sac into genital pore. Ovary extending into anterior 1/5 of proglottid. Vitellaria follicular, in two lateral bands with dorsal and central rows of vitellaria extending laterally into field of testes, 5-14 (11±2, 5, 15) long by 15-37 (26±5, 5, 15) wide; extending from posterior to anterior margin of proglottid, stopping short of anterior margin of testes field, extending laterally into the field of testes, uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* Horn Island, Gulf of Mexico, Mississippi, U.S.A.

*Additional localities:* Indian Pass, Gulf of Mexico, Florida, U.S.A.

*Site of infection:* Spiral intestine.

*Prevalence:* 4 of 20 blacktip sharks.

*Specimens deposited:* Holotype (USNPC No. 0000) and 2 paratypes (USNPC Nos. 0000-0000); 1 paratype (LRP No. 0000); 1 paratype (HWML No. 0000); scolex mounted for SEM and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

*Remarks*

Of the 11 accepted species of *Phoreiobothrium,* as well as the species previously described in this paper, *Phoreiobothrium* n. sp. 2 is readily distinguished from *P. exceceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the posterior border of each bothridium, which distinguishes it from *P. pectinatum*. *Phoreiobothrium* n. sp. 1 possesses an abaxial prong on each scolex hook, which is absent in *P. manirei* and *P. exceptum*. *Phoreiobothrium* n. sp. 2 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum* and *P. pectinatum*, (10-18 vs. 3, 5, 6, and 6-7 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (10-18 vs. 25-29, 23-31, and 25-30 respectively).

Additionally, *Phoreiobothrium* n. sp. 2 possesses more testes than *P. anticaporum* and *P. perilocrocodilus* (82-98 vs. 36-54 and 36-49 respectively) and fewer testes than *P. blissorum* and *P. lewinense* (82-98 vs. 103-127 and 173 respectively). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4-5 vs. 6-7, 7-8, and 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus,* (4-5 vs. 2 and 2 respectively). *Phoreiobothrium* n. sp. 2 has a wider ovary than *P. robertsoni*, *P. perilocrocodilus*, and *P. anticaporum* (202-320 vs. 105-160, 53-73 and 80-123 respectively). *Phoreiobothrium* n. sp. 2 has a longer cirrus sac than that of *P. tiburonis* and *P. perilocrocodilus* (125-169 vs. 70-108 and 90-105 respectively). *Phoreiobothrium* n.

sp. 2 has a longer and scolex than *Phoreiobothrium* n. sp. 1 (331-353 vs. 215-295); additionally, *P.* n. sp. 2 has a shorter ovary (146-327 vs. 305-470) and a narrower vagina than *P.* n. sp. 1 that does not cross the cirrus sac. *Phoreiobothrium* n. sp. 2 also possesses spinitriches on its proximal bothridial surface, which *P.* n. sp. 1 lack.

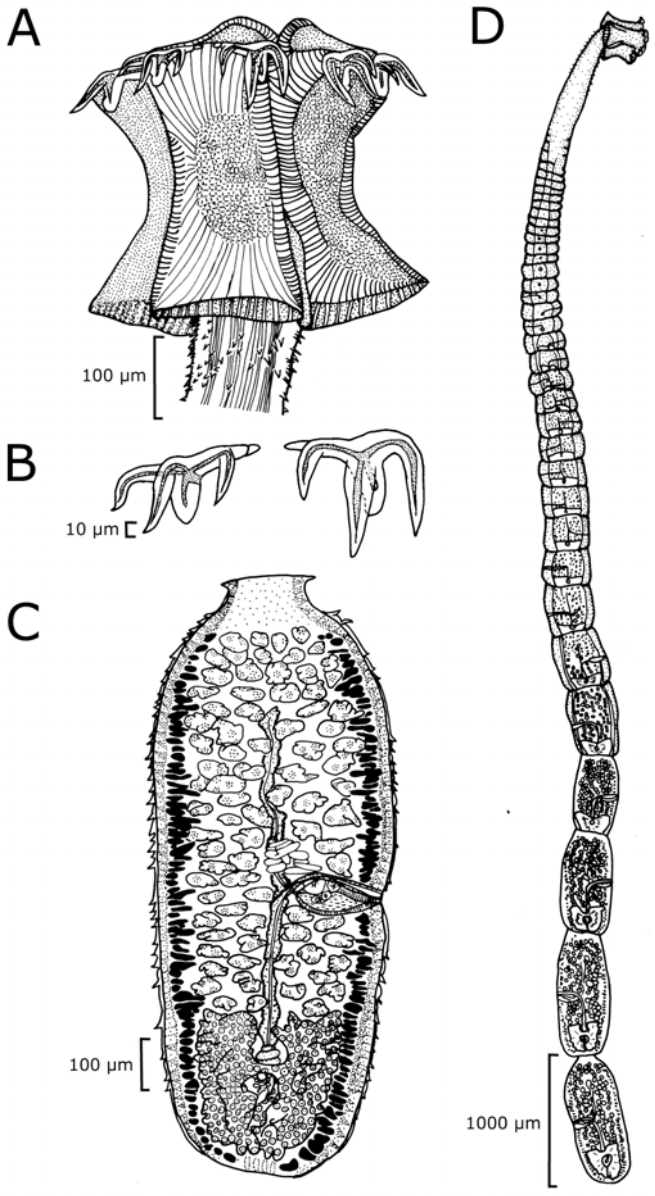


**Figure 5.** Line drawings of *Phoreiobothrium* n. sp. 2. . **(A)** Scolex **(B)** Hooks **(C)** Mature proglottid **(D)** Whole worm.

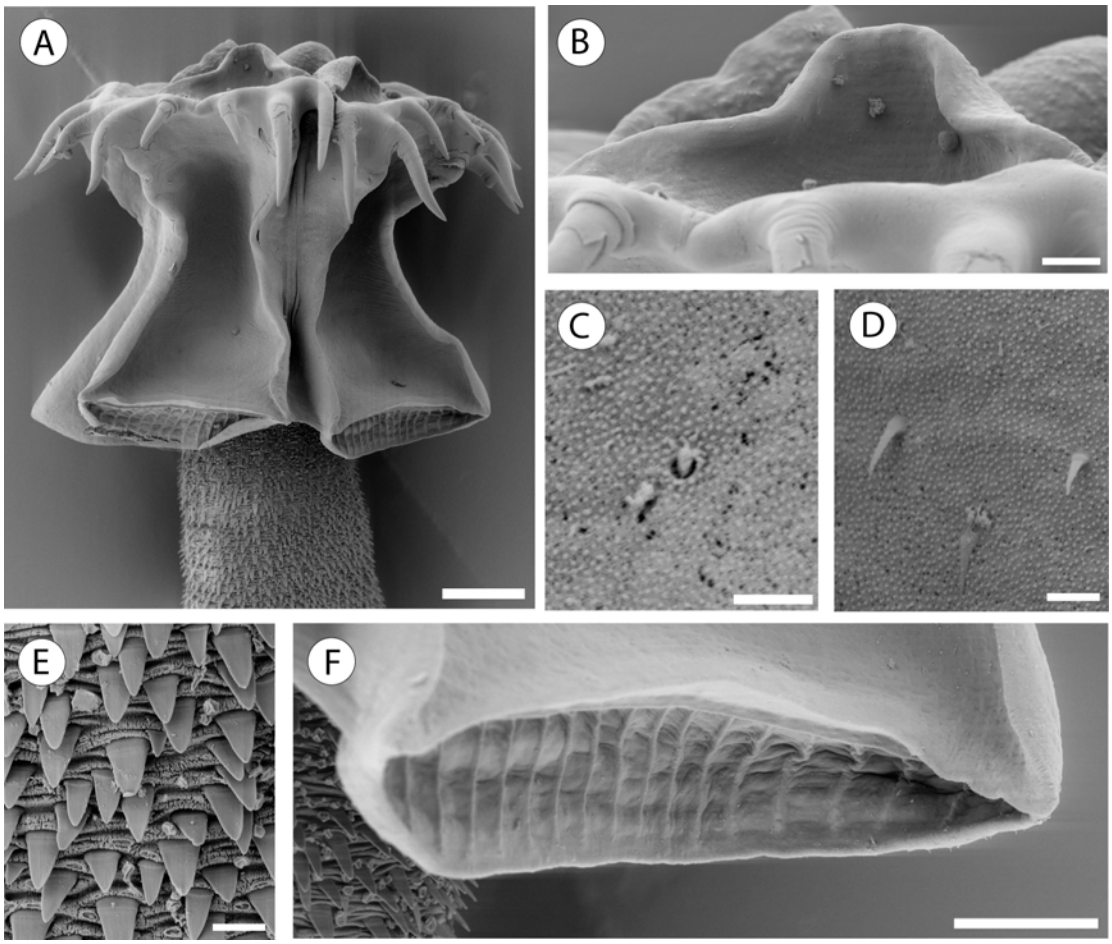


**Figure 6.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 2. **(A)** Scolex; scale bar 50 μm. **(B)** Enlarged view of anterior loculus; scale bar 10 μm. **(C)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(D)** Enlarged view of proximal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(F)** Enlarged view of subloculi; scale bar 20 μm.

***Phoreiobothrium* n. sp. 3**

(Figs. 7-8)

*Description:* Based on 18 whole mounts, 1 scolex mounted for SEM, and 1 last mature proglottid cross section series. Worms euapolytic, 4.8-7.8 mm long

(6.6±9.3, 17), 23-37 (32±4.3, 17) total proglottids; greatest width 274-378 (305±28, 16), generally at level of last mature proglottid. Scolex 281-371 (322±29.1, 17) long by 256-294 (274±12.3, 17) wide, consisting of scolex proper with 4 bothridia. Bothridia 233-307 (263±17.3, 17) long by 133-173 (146±9.8, 17) wide, each with one pair of tri-pronged hooks, anterior prehook region in form of loculus, and posthook region divided into an anterior and posterior loculus. Anterior prehook region 42-75 (55±9.4, 16) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 30-52 (39±6.1, 17) long; divided into 18-20 (19±0.9, 18) subloculi; subloculi 6-9 (7±1, 17) wide. Hooks tri-pronged with blunt talon embedded in musculature of scolex; talon of medial hook longer and thicker than that of lateral hook; all prongs and talon hollow. Bases of medial and lateral hooks in close proximity to one another, often crossing; accessory piece lacking. Hook surfaces covered with thin layer of tissue. Axial prongs longest; basal prongs greater than 1/2 length of axial prongs; abaxial prongs greater than 3/4 of length of axial prongs; lateral abaxial prong slightly more extended toward horizontal axis than medial hook. Lateral hook lengths: A 57-82 (65±6.2, 14), B 64-86 (76±6.8, 15), C 42-69 (59±6.5, 15), D 90-112 (102±6.7, 15), E 34-52 (43±4.9, 17), F 35-46 (39±2.6, 17). Medial hook lengths: A' 61-90 (71±7.1, 17), B' 77-103 (76±6.8, 15), C' 58-82 (70±6.0, 17), D' 91-120 (107±8.8, 17), E' 39-53 (45±3.4, 17), F' 33-47 (42±3.3, 17).

Distal and proximal bothridial surface covered with short filitriches (Fig. 8D). Cephalic peduncle covered with long filitriches and bladelike spinitriches of varying lengths and oriented with points directed posteriorly (Fig. 8E). Boundary between anterior and posterior loculi possesses muscular double ledge (Fig. 8F).

Proglottids acraspedote. Immature proglottids 22-37 (31±4.2, 17) in number, initially wider than long, becoming longer than wide with maturity, last immature proglottid 366-824 (522±132.2, 17) long by 239-338 (289±28.9, 17) wide. Mature proglottids 0-2 (1±0.7, 17) in number, longer than wide, last proglottid 668-1,168 (868±155.7, 12) long by 259-378 (305±32.9, 11) wide. Gravid proglottids not seen. Testes 73-110 (86±10.8, 13) in number, 9-16 (12±2.4, 13) in postvaginal field, oval, 15-30 (23±3.4, 11, 33) long by 30-54 (42±6.1, 11, 33) wide, arranged in 4-5 columns, 1 layer deep in cross section, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus sac and uterus. Genital pore lateral, 46-57% proglottid length from posterior end, irregularly alternating. Cirrus sac oval, 103-142 (120±12.5, 11) long by 28-51 (35±6.2, 11) wide and extending 30-45% of proglottid width into proglottid, containing coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, bilobed in cross section, located at posterior end of proglottid, 158-383 (235±57.6, 12) long by 139-223 (179±30.1, 12) wide; posterior margin of ovarian bridge 48-68% from posterior margin of ovary. Vagina narrow, extending anteriorly from ootype region along medial line of

proglottid, then curving laterally across anterior margin of cirrus sac, opening anterior to the cirrus sac into genital pore. Uterus extends into the anterior 2/5 of proglottid. Vitellaria follicular, in 2 lateral bands extending into field of testes, 7-29 (11±5.1, 11, 33) long by 8-41 (20±6.8, 11, 33) wide, extending from posterior to anterior margin of proglottid, stopping short of anterior margin of testes field, extending laterally into the field of testes, uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* St. Andrew Bay, Gulf of Mexico, Florida, U.S.A.

*Additional localities:* St. Joe Bay, Gulf of Mexico, Florida, U.S.A.

*Prevalence:* 2 of 20 blacktip sharks.

*Site of infection:* Spiral intestine.

*Specimens deposited:* Holotype (USNPC No. 0000) and 8 paratype specimens (USNPC Nos. 0000-0000); 7 paratype specimens (LRP Nos. 0000-0000); 3 paratype specimens (HWML Nos. 0000-0000); scolex mounted for SEM, its voucher, cross section series, and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

Remarks

Of the three new species of *Phoreiobothrium* described so far in this thesis, as well as the three yet to be described, *P.* n. sp. 3 is the most distinctive, with its comparatively flat, pad like bothridia and thick, muscular anterior prehook lobes. In addition to the unique shape of its bothridia, *Phoreiobothrium* n. sp. 3 is readily distinguished from *P. exceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the posterior border of each bothridium, which distinguishes it from *P. pectinatum*. *Phoreiobothrium* n. sp. 3 possesses an abaxial prong on each scolex hook, which is absent in *P. manirei* and *P. exceptum*. *Phoreiobothrium* n. sp. 3 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum, P. pectinatum*, and *P. tiburonis* (18-20 vs. 3, 5, 6, 6-7, and 8-13 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (18-20 vs. 25-29, 23-31, and 25-30 respectively).

Additionally, *P.* n. sp. 3 has a wider ovary than *P. perilocrocodilus* and *P. anticaporum* (139-223 vs. 53-73 and 80-123 respectively). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4-5 vs. 6-7, 7-8, and 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus,* (4-5 vs. 2 and 2 respectively). *Phoreiobothrium* n. sp. 3 can be distinguished from *P.* n. sp. 1 and *P.* n. sp. 2 by their higher number of subloculi (18-20 vs. 10-16, and 14-16 respectively). Additionally, *P.* n. sp. 3 has a narrower scolex than *P.* n. sp. 2 (256-294 vs. 346-361 respectively), and a shorter cirrus than *P.* n. sp. 1 (103-142 vs. 149-228).

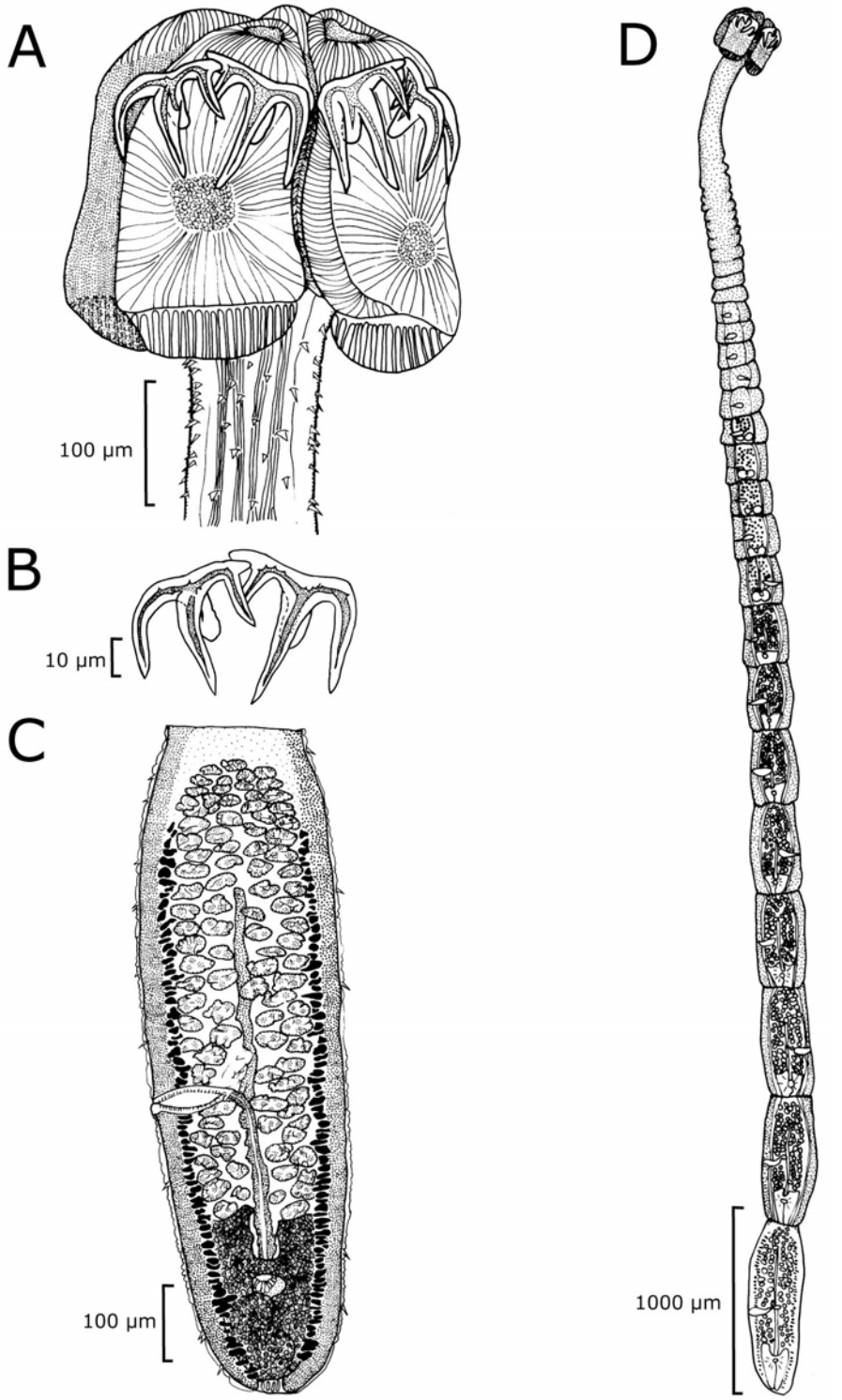


**Figure 7.** Line drawing of *Phoreiobothrium* n. sp. 3. **(A)** Scolex. **(B)** Hooks. **(C)** Mature proglottid. **(D)** Whole worm.

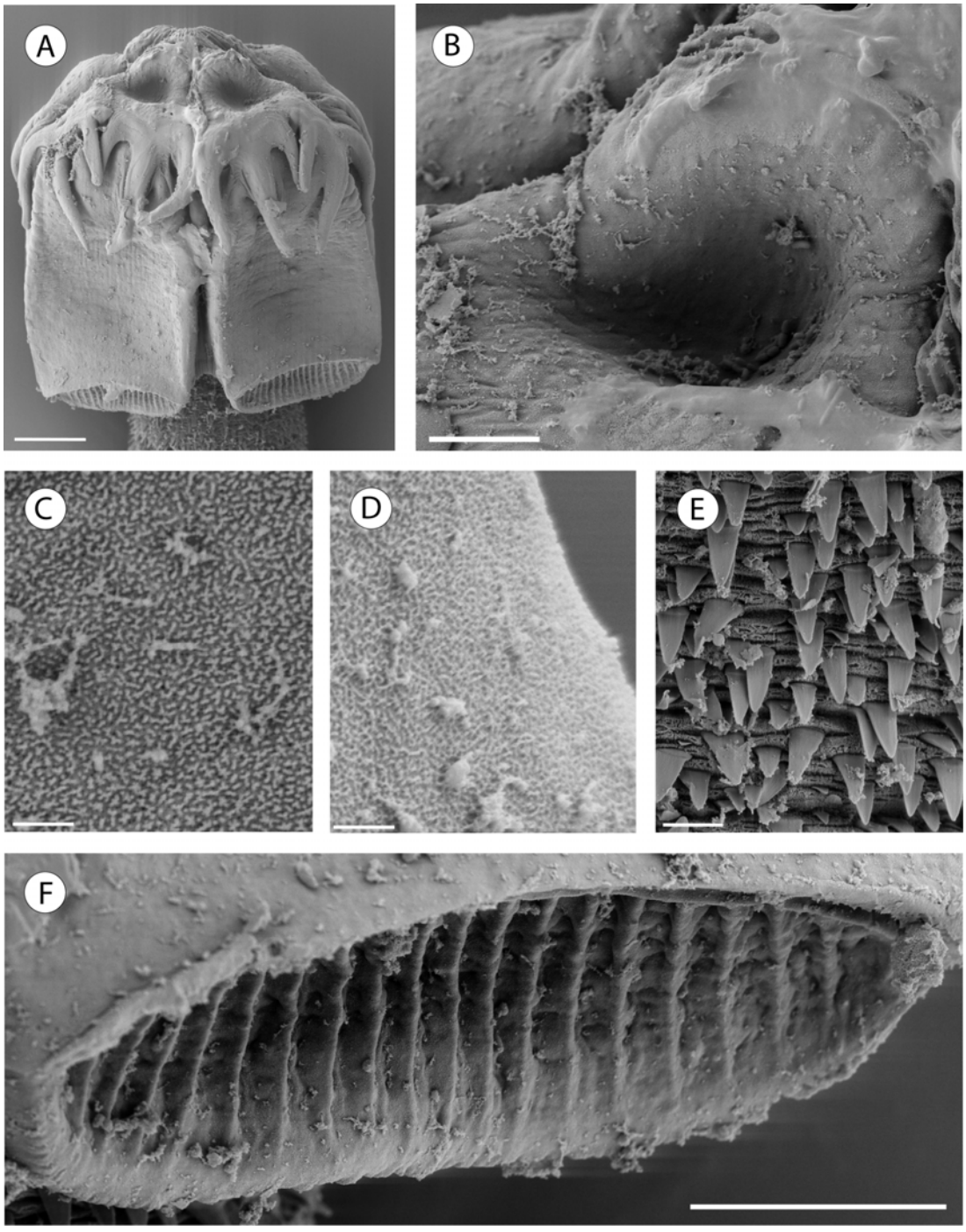


**Figure 8.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 3. **(A)** Scolex; scale bar 50 μm. **(B)** Enlarged view of anterior loculus; scale bar 10 μm. **(C)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(D)** Enlarged view of proximal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(F)** Enlarged view of subloculi; scale bar 20 μm.

***Phoreiobothrium* n. sp. 4**

(Figs. 9-10)

*Description:* Based on 15 whole mounts and 1 scolex mounted for SEM. Worms euapolytic, 6.1-10.5 mm long (8.1±1.3, 15), 26-44 (37±5.5, 15) total proglottids; greatest width 312-529 (402±59.7, 15), generally at level of last mature proglottid. Scolex 318-389 (357±20.3, 15) long by 287-489 (329±51.7, 13), consisting of scolex proper with 4 bothridia. Bothridia 293-349 (318±18, 15) long by 150-196 (178±13.2, 15) wide, each with one pair of tri-pronged hooks, anterior prehook region in form of loculus, and posthook region divided into an anterior and posterior loculus. Anterior of prehook region 26-46 (36±7.3, 12) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 31-48 (36±4.9, 14) long, divided into 14-18 (17±1.3, 14) subloculi; subloculi 8-11 (10±1, 15) wide. Boundary between anterior and posterior loculi possesses muscular double ledge. Hooks tri-pronged with blunt talon embedded in musculature of scolex; talon of medial hook longer and thicker than that of lateral hook; prongs and talon hollow. Bases of medial and lateral hooks in close proximity to one another; accessory piece lacking. Hook surfaces covered with thin layer of tissue. Axial prongs longest; basal prongs less than 1/2 length of axial prongs; abaxial prong 4/5 length of axial prong, conspicuously more extended toward horizontal axis on lateral hook than on medial hook. Lateral hook lengths: A 62-67 (65±3.5, 2), B 50-67(59±12, 2), C 58-65 (62±2.3, 6), D 96-115 (106±8.2, 6), E 36-48 (42±4.5, 9), F 31-47 (38±4.0, 14). Medial hook lengths: A' 52-97 (74±11.5, 15), B'

74-101 (89±8.8, 15), C' 56-77 (68±7.5, 14), D' 77-137 (104±18.3, 14), E' 35-52 (45±5.3, 15), F' 31-54 (45±6.4, 14).

Distal and proximal bothridial surfaces covered with short filitriches (Fig. 10C, D). Cephalic peduncle covered with long filitriches and bladelike spinitriches oriented with points directed posteriorly (Fig. 10E). Boundary between anterior and posterior loculi possesses muscular double ledge (Fig. 10F).

Proglottids acraspedote. Immature proglottids 26-44 (34±5.4, 15) in number, initially wider than long, becoming longer than wide with maturity, last immature proglottid 272-626 (522±98.6, 15) long by 211-427 (342±57, 15) wide. Mature proglottids 1-4 (2±1, 15) in number, longer than wide, 606-1,230 (963±220, 15) long by 307-529 (398±54.3, 15) wide. Gravid proglottids not seen. Testes 67-107 (89±13.1, 12) in number, 6-14 (10±2.1, 13) in postvaginal field, oval, 15-43 (29±7.2, 13, 39) long by 36-66 (51±8.4, 13, 39) wide, arranged in 4-5 columns, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus sac and uterus. Genital pore lateral, 43-66% of proglottid length from posterior end; irregularly alternating. Cirrus sac oval, 108-166 (133±16.7, 13) long by 27-59 (41±8.4, 13) wide, extending 28-49% of proglottid width into the proglottid, containing slightly coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, located at posterior end of proglottid, 152-366 (254±59.5, 13) long by 172-321 (243±35.9, 13) wide; posterior margin of ovarian bridge 51-67% from posterior margin of ovary. Vagina narrow, extending

anteriorly from ootype region along medial line of proglottid, then curving laterally across anterior margin of cirrus sac, opening anterior to cirrus sac into genital pore. Uterus extending into the anterior 2/5 of proglottid. Vitellaria follicular, 8-16 (12±2.2, 13, 39) long by 16-39 (26±5.6, 13, 39) wide; in 2 lateral bands extending into field of testes; extending from posterior margin of proglottid to anterior of proglottid, stopping short of anterior margin of testes field, extending laterally into the field of testes, uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* St. Andrew Bay, Gulf of Mexico, Florida, U.S.A.

*Additional localities:* Indian Pass, Gulf of Mexico, Florida, U.S.A.; Horn Island, Gulf of Mexico, Mississippi, U.S.A.

*Site of infection:* Spiral intestine.

*Prevalence:* 5 of 20 blacktip sharks.

*Specimens deposited:* Holotype (USNPC No. 0000) and 7 paratype specimens (USNPC Nos. 0000-0000); 6 paratype specimens (LRP Nos. 0000-0000); 1 paratype specimen (HWML No. 0000); scolex mounted for SEM and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

*Remarks*

*Phoreiobothrium* n. sp. 4 is readily distinguished from *P. exceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the posterior border of the anterior loculus of each bothridium, which distinguishes it from *P. pectinatum*. Additionally, *P.* n. sp. 4 possesses an abaxial prong on each hook, which *P. manirei* and *P. exceptum* lack. *Phoreiobothrium* n. sp. 4 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum*, *P. pectinatum*, *P. tiburonis*, and *P. lewinense* (14-18 vs. 3, 5, 6, 6-7, 8-13, 9-11 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (14-18 vs. 25-29, 23-31, 25-30 respectively).

*Phoreiobothrium* n. sp. 4 has a longer cirrus sac than both *P. perilocrocodilus* (108-166 vs. 90-105). *Phoreiobothrium* n. sp. 4 possesses more testes than *P. anticaporum* and *P. perilocrocodilus* (67-107 vs. 36-54, 36-49 respectively), and fewer testes than *P. lewinense* (67-107 vs. 173). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4-5 vs. 6-7, 7-8, 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus,* (4-5 vs. 2, 2 respectively). *Phoreiobothrium* n. sp. 4 has a wider ovary than *P. robertsoni*, *P. perilocrocodilus*, and *P. anticaporum* (172-121 vs. 105-160, 53-73, 80-123 respectively).

*Phoreiobothrium* n. sp. 4 can be distinguished from *P.* n. sp. 1, 2, and 3 by having a narrower vagina than *P.* n. sp. 1, and by its vagina crossing the cirrus sac

unlike *P.* n. sp. 2. It can be distinguished from *P.* n. sp. 3 based on *P.* n. sp. 3's unique scolex morphology.

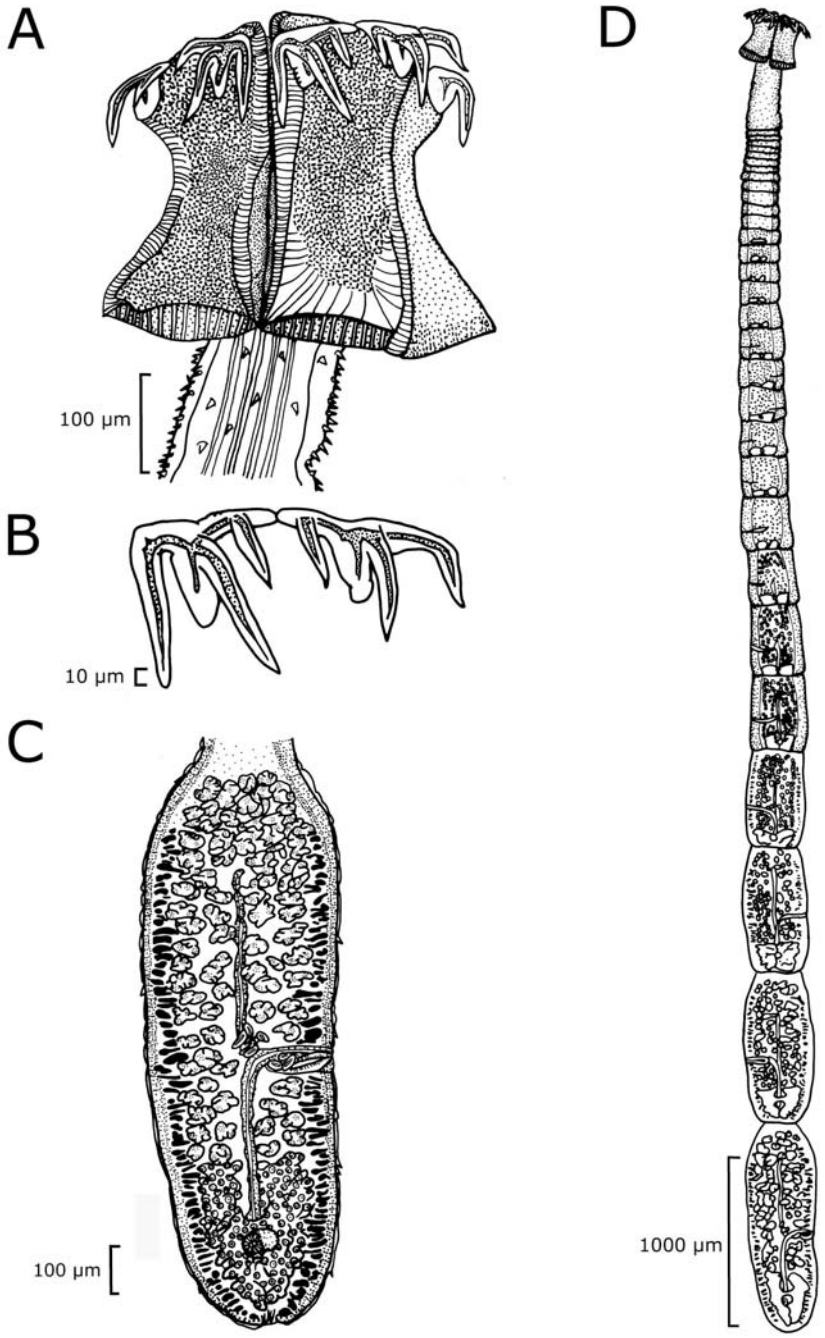


**Figure 9.** Line drawings of *Phoreiobothrium* n. sp. 4. **(A)** Scolex . **(B)** Hooks. **(C)** Mature proglottid. **(D)** Whole worm.

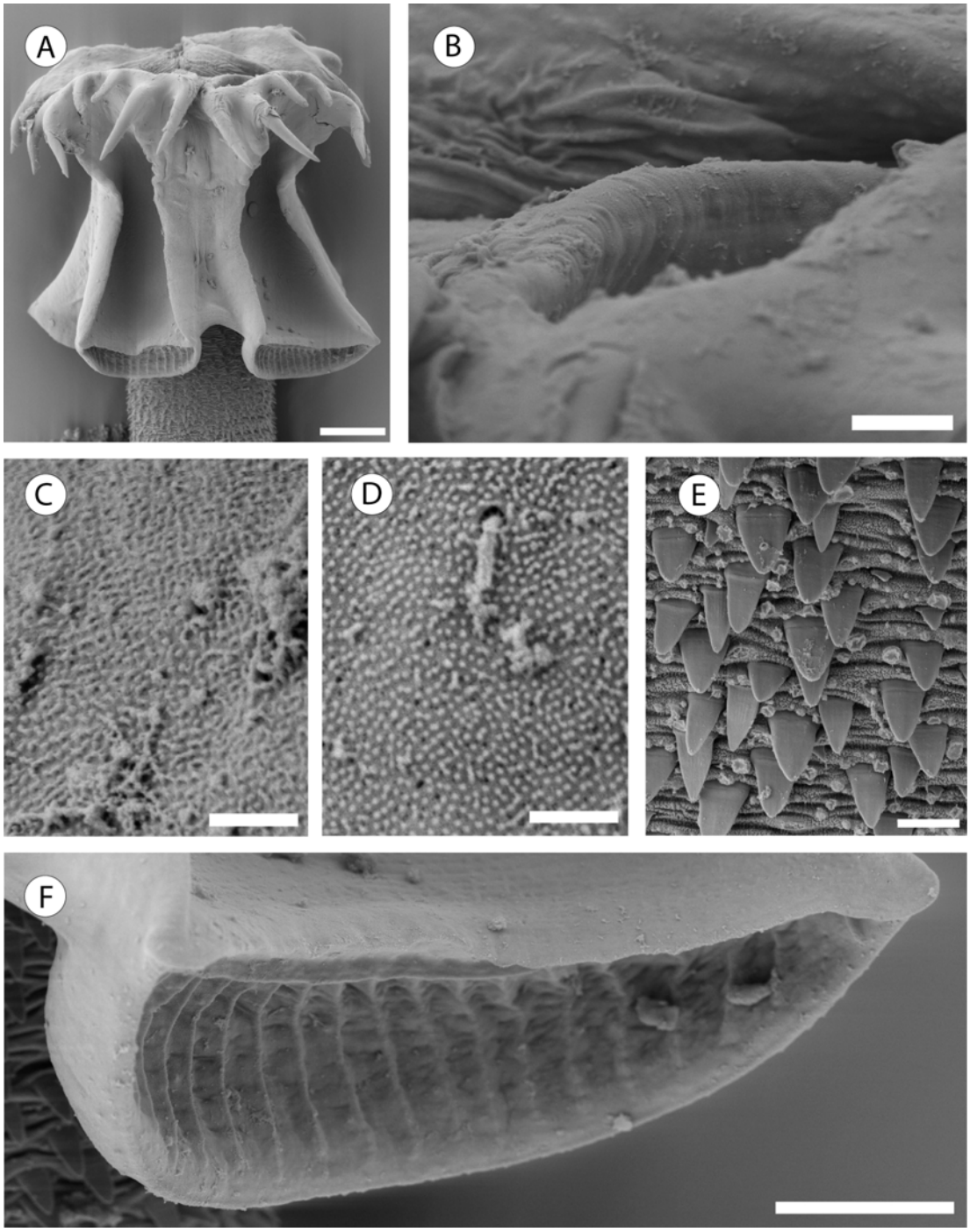


**Figure 10.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 4. **(A)** Scolex; scale bar 50 μm. **(B)** Enlarged view of anterior loculus; scale bar 10 μm. **(C)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(D)** Enlarged view of proximal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(F)** Enlarged view of sublocculi; scale bar 20 μm.

***Phoreiobothrium* n. sp. 5**

(Figs. 11-12)

*Description:* Based on 20 whole mounts and 1 scolex mounted for SEM. Worms euapolytic, 6.6-14.7 mm long (9.1±2.7, 20), 32-52 (39±5.3, 20) total proglottids; greatest width 326-442 (375±36, 20), generally at level of last mature proglottid. Scolex 319-380 (348±16, 20) long by 283-358 (329±22, 15) wide, consisting of scolex proper with 4 rectangular bothridia. Bothridia 285-370 (316±23, 18) long by 155-226 (188±23, 17) wide, each with one pair of tri-pronged hooks, anterior prehook loculus, posthook region divided into an anterior and posterior loculus. Anterior prehook region 26-49 (40±8, 5) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 26-44 (35±6, 17) long divided into 13-18 (15±1, 17) subloculi; subloculi 9-16 (13±2, 18) wide. Boundary between anterior and posterior loculi possesses muscular double ledge. Hooks tri-pronged with blunt talon embedded in musculature of scolex; talon of medial hook longer and thicker than that of lateral hook; prongs and talon hollow. Bases of medial and lateral hooks spaced apart slightly; accessory piece lacking. Hook surfaces covered with thin layer of tissue. Basal prongs greater than half length of axial prongs; axial and abaxial prong approximately equal length, abaxial prong conspicuously more extended toward horizontal axis on lateral hook than on medial hook. Lateral hook lengths: A 48-78 (63±12, 6), B 42-89 (61±15, 7), C 59-73 (68±5, 7), D 80-106 (91±12, 4), E 28-49 (38±6, 16), F 31-47 (37±4, 18). Medial hook lengths: A' 57-83 (69±8, 19), B' 65-92

(81±7, 19), C' 54-86 (74±10, 18), D' 66-132 (93±21, 18), E' 33-57 (44±7, 19), F' 34-61 (46±6, 19).

Distal and proximal bothridial surfaces and covered with short filitriches interspersed with cilia (Fig. 12C, D). Cephalic peduncle covered with long filitriches and bladelike spinitriches and oriented with points directed posteriorly (Fig. 12B). Boundary between anterior and posterior loculi possesses muscular double ledge (Fig. 12E).

Proglottids acraspedote. Immature proglottids 32-52 (35±4.5, 20) in number, initially wider than long, becoming longer than wide with maturation, last immature proglottid 398-727 (530±76, 19) long by 304-439 (354±36, 19) wide. Mature proglottids 2-6 (3±1.3, 20) in number, longer than wide, last mature proglottid 916-1,570 (1,239±161, 20) long by 297-442 (369±42, 20) wide. Gravid proglottids not seen. Testes 70-105 (83±9, 20) in number, 8-14 (11±2, 20) in postvaginal field, oval, 23-46 (32±5, 20, 60) long by 37-71 (50±8.3, 20, 60) wide, arranged in 4-5 columns, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus sac and uterus. Genital pore lateral, 37-56% of proglottid length from posterior end; irregularly alternating. Cirrus sac oval, 125-179 (145±14, 20) long by 47-87 (67±13, 20) wide, extending 36-49% of proglottid width into proglottid, containing coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, located at posterior end of proglottid, 241-439 (323±49, 20) long by 181-281 (227±20, 20) wide; posterior margin of

ovarian bridge 48-74% from posterior margin of ovary. Vagina narrow, extending anteriorly from ootype region along medial line of proglottid, then curving laterally around anterior margin of cirrus sac, opening anterior to cirrus sac into genital pore. Uterus extends into anterior 1/5 of proglottid. Vitellaria follicular, 9-35 (14±4, 20, 60) long by 15-55 (28±7.6, 20, 60) wide; in 2 lateral bands extending from posterior margin of proglottid to anterior margin of testes, not extending laterally into the field of testes, uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* St. Andrew Bay, Gulf of Mexico, Florida, U.S.A.

*Additional localities:* Indian Pass, Gulf of Mexico, Florida, U.S.A.

*Prevalence:* 6 of 20 blacktip sharks.

*Site of infection:* Spiral intestine.

*Specimens deposited:* Holotype (USNPC No. 0000) and 8 paratypes (USNPC No. 0000-0000); 7 paratypes (LRP Nos. 0000-0000); 4 paratypes (HWML Nos. 0000-0000); scolex mounted for SEM and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

*Remarks*

*Phoreiobothrium* n. sp. 5 is readily distinguished from *P. exceceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the posterior border of the anterior loculus of each bothridium, which distinguishes it from *P. pectinatum*. Additionally, *P.* n. sp. 5 possesses an abaxial prong on each hook, which *P. manirei* and *P. exceptum* lack. *Phoreiobothrium* n. sp. 5 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum*, *P. pectinatum*, and *P. tiburonis* (13-18 vs. 3, 5, 6, 6-7, 8-13, 9-11 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (13-18 vs. 25-29, 23-31, 25-30 respectively).

*Phoreiobothrium* n. sp. 5 possesses a longer cirrus sac than *P. tiburonis* (125-179 vs. 70-108). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4 vs. 6-7, 7-8, 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus* (4 vs. 2, 2 respectively). *Phoreiobothrium* n. sp. 5 can be readily distinguished from the species previously described in this thesis in that it has a narrower vagina than *P*. n. sp. 1. Additionally, and unlike any of the four previously describes species, the vitellaria of *P.* n. sp. 5 extend to the extreme anterior border of testes in mature proglottids; these vitellaria also do not extend laterally into the field of testes.

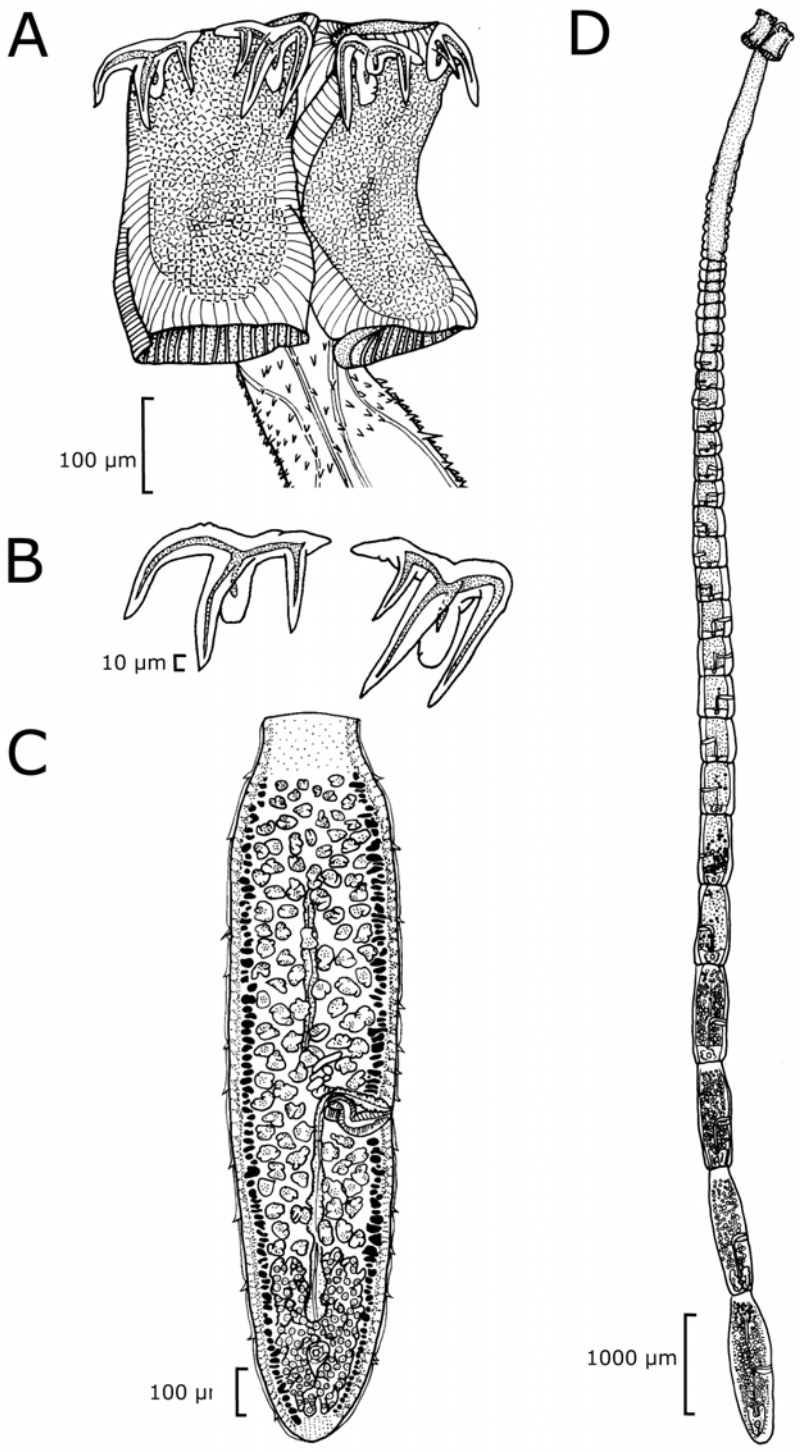


**Figure 11.** Line drawings of *Phoreiobothrium* n. sp. 5. **(A)** Scolex. **(B)** Hooks. **(C)** Mature proglottid. **(D)** Whole worm.

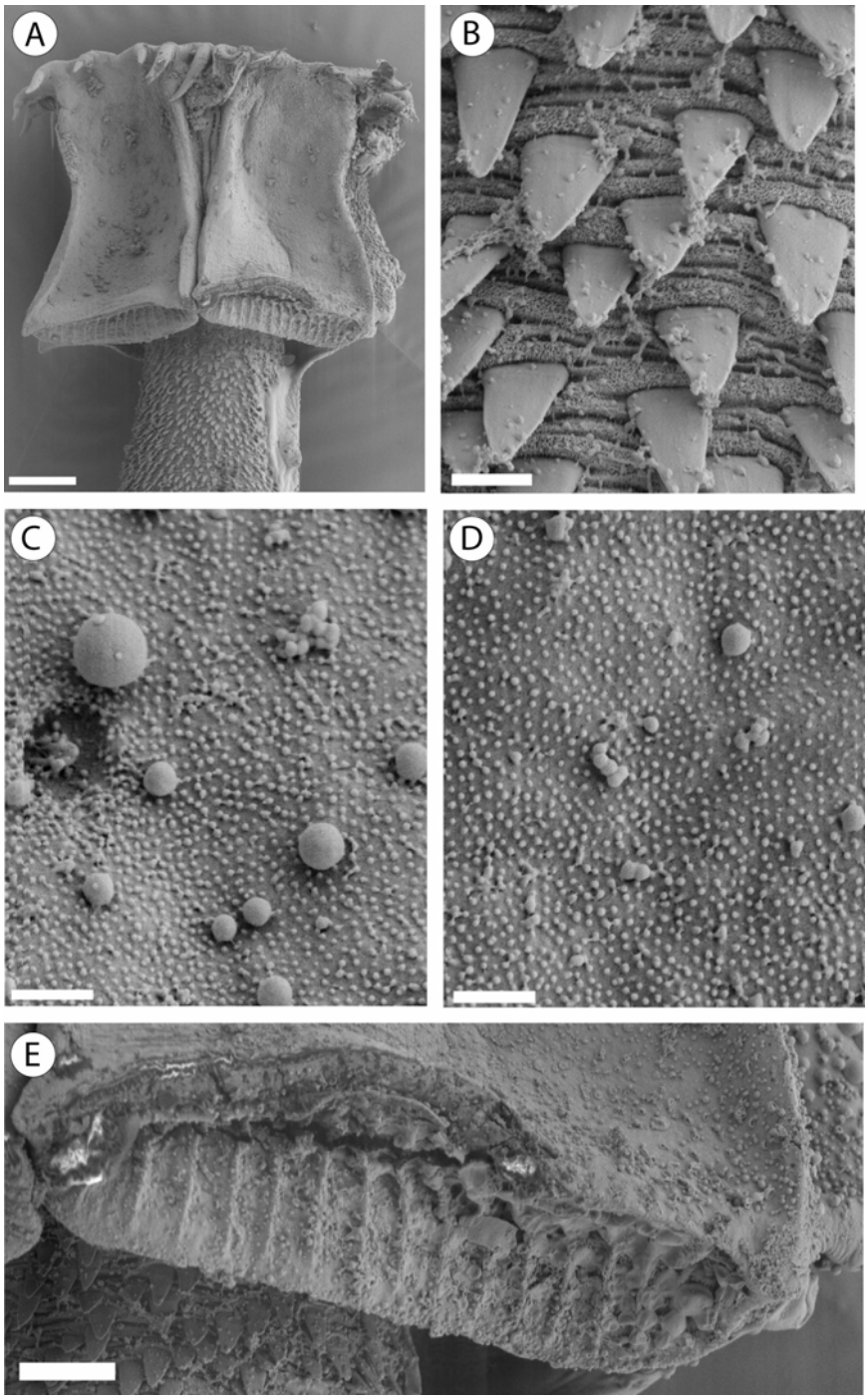


**Figure 12.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 5. **(A)** Scolex; scale bar 50 μm. **(B)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(C)** Enlarged view of subloculi; scale bar 20 μm. **(D)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of proximal bothridial surface; scale bar 1 μm.

***Phoreiobothrium* n. sp. 6**

(Figs. 13-14)

*Description*: Description based on 8 whole mounts and 1 scolex mounted for SEM. Worms euapolytic, 10.7-16.0 mm long (12.4±1.8, 8), 28-40 (35±4.7, 8) total proglottids; greatest width 312-470 (406±58.7, 8), generally at level of last mature proglottid. Scolex 295-339 (318±14, 8) long by 285-365 (318±32.8, 6) wide, consisting of scolex proper with 4 bothridia. Bothridia 227-294 (264±25.7, 8) long by 159-221 (190±21, 6) wide, each with one pair of tri-pronged hooks, anterior prehook region in form of loculus, and posthook region divided into an anterior and posterior loculus. Anterior prehook region 31-41 (36±3.6, 7) long; margin of prehook region lacking papillae and accessory sucker. Anterior loculus conspicuously longer than posterior loculus; posterior loculus 29-46 (39±5.4, 7) long divided into 14-16 (15±1, 6) subloculi; subloculi 10-14 (12±2, 6) wide. Boundary between anterior and posterior loculi possesses muscular double ledge. Hooks tri-pronged with blunt talon embedded in musculature of scolex; talon of medial hook longer and thicker than that of lateral hook; prongs and talon hollow. Bases of medial and lateral hooks in close proximity to one another, medial base longer and narrower than medial base; accessory piece lacking. Hook surfaces covered with thin layer of tissue. Axial prongs longest; basal prongs greater than 1/2 length of axial prongs; abaxial prong greater than 1/2 the length of axial prong, conspicuously more extended toward the horizontal axis on lateral hook than on medial hook. Lateral hook lengths: A 39-70 (59±11, 6), B 48-68 (57±7, 6), C 45-52 (48±2.9, 6), D 41-87 (71±16, 8, 16), E 30-49

(37±6.4, 7), F 19-40 (31±7, 7). Medial hook lengths: A' 50-80 (66±11, 8), B' 56-80 (69±8, 8), C' 42-66 (56±7, 8), D' 73-104 (91.1±10.7, 8), E' 42-54 (47±4, 8), F' 25-46 (38±6, 6).

Distal and proximal bothridial surface and covered with short filitriches (Fig. 12 C, D). Cephalic peduncle covered with short bladelike spinitriches oriented with points directed posteriorly (Fig. 12E). Boundary between anterior and posterior loculi possesses muscular double ledge (Fig. 12F).

Proglottids acraspedote. Immature proglottids 28-40 (31±4, 8) in number, initially wider than long, becoming longer than wide with maturity, last immature proglottid 622-1,096 (812±182, 8) long by 308-482 (373±64, 8) wide. Mature proglottids 2-5 (3.5±0.9, 8) in number, longer than wide, 1,372-2,174 (1,725±286, 8) long by 332-575 (417±81, 8) wide. Gravid proglottids not seen. Testes 63-107 (87±14, 8) in number, 8-18 (12±3, 8) in postvaginal field, oval, 25-67 (47±11, 8, 24) long by 37-73 (56±9.7, 8, 24) wide, arranged in 4-5 columns, extending from anterior margin of ovary to anterior extremity of proglottid, interrupted by cirrus sac and uterus. Genital pore lateral, 48-56% proglottid length from posterior end; irregularly alternating. Cirrus sac round, 132-211 (175±22.3, 8) long by 85-123 (106±15, 8) wide and extending 37-50% of proglottid width into proglottid, containing tightly coiled cirrus armed with small microtriches. Vas deferens coiled at anteromedian margin of cirrus sac.

Ovary H-shaped in dorsoventral view, located at posterior end of proglottid, 329-592 (464±77.4, 8) long by 170-372 (258±61.4, 8) wide; posterior margin of

ovarian bridge 46-66% from posterior margin of ovary. Vagina wide, extending anteriorly from ootype region along medial line of proglottid, then curving laterally around anterior margin of cirrus sac, opening anterior to cirrus sac into genital pore. Uterus extends into anterior 1/5 of proglottid. Vitellaria follicular, 11-27 (18±4, 8, 24) long by 21-66 (39±12, 8, 24) wide; in 2 lateral bands extending from posterior margin of proglottid to anterior margin of proglottid even with testes, not extending laterally into field of testes, uninterrupted by ovary. Excretory ducts lateral.

*Taxonomic summary*

*Type host: Carcharhinus limbatus*, blacktip shark (Carcharhinidae, Elasmobranchii).

*Additional hosts:* None.

*Type locality:* Indian Pass, Gulf of Mexico, Florida, U.S.A.

*Additional localities:* Crooked Island Bay, Gulf of Mexico, Mississippi, U.S.A.

*Prevalence:* 7 of 20 blacktip sharks.

*Site of infection:* Spiral intestine.

*Specimens deposited:* Holotype (USNPC No. 0000) and 3 paratypes (USNPC Nos. 0000-0000); 3 paratypes (LRP Nos. 0000-0000); 1 paratype (HWML No. 0000); scolex mounted for SEM and its voucher retained in the laboratory of Dr. Kirsten Jensen at the University of Kansas.

*Remarks*

*Phoreiobothrium* n. sp. 6 is readily distinguished from *P. exceceptum*, *P. perilocrocodilus*, *P. anticaporum*, *P. lewinense*, and *P. manirei* on the basis of the absence of pre-hook papillae. It is also lacking in papillae along the anterior border of the anterior loculus of each bothridium, which distinguishes it from *P. pectinatum*. Additionally, *P.* n. sp. 6 possesses an abaxial prong on each hook, which *P. manirei* and *P. exceptum* lack. *Phoreiobothrium* n. sp. 6 has more subloculi than *P. triloculatum*, *P. manirei*, *P. exceptum*, *P. pectinatum*, *P. tiburonis*, and *P. lewinense* (14-16 vs. 3, 5, 6, 6-7, 8-13, 9-11 respectively) and fewer subloculi than *P. robertsoni, P. blissorum,* and *P. lasium* (14-16 vs. 25-29, 23-31, 25-30 respectively).

Additional differences between *P.* n. sp. 6 and previously described species include the possession of fewer testes than P. *lewinense* (63-107 vs. 173), and more testes than *P. perilocrocodilus* and *P. anticaporum* (63-107 vs. 36-49, 36-54 respectively). *Phoreiobothrium* n. sp. 6 has a wider ovary than *P. robertsoni*, *P. perilocrocodilus*, and *P. anticaporum* (170-372 vs. 105-160, 53-73, 80-123 respectively) and a longer ovary than *P. tiburonis* (329-592 vs. 155-243). It also has fewer columns of testes than *P. blissorum*, *P. lasium*, and *P. lewinense* (4 vs. 6-7, 7-8, 7-8 respectively), and more columns of testes than *P. anticaporum*, and *P. perilocrocodilus,* (4 vs. 2, 2 respectively). It can be distinguished from *P.* n. sp. 1, *P.* n. sp. 2, *P.* n. sp. 3, and *P.* n. sp. 4 in that the vitellaria of *P.* n. sp. 6 extend to the extreme anterior border of testes in mature proglottids; these vitellaria also do not extend laterally into the field of testes. *Phoreiobothrium* n. sp. 6 can be distinguished

from *P.* n. sp. 5 by its wider vagina, as well as its distinct scolex morphology—it is pinched posterior to the hooks whereas *Phoreiobothrium* n. sp. 5 has a much more rectangular scolex.

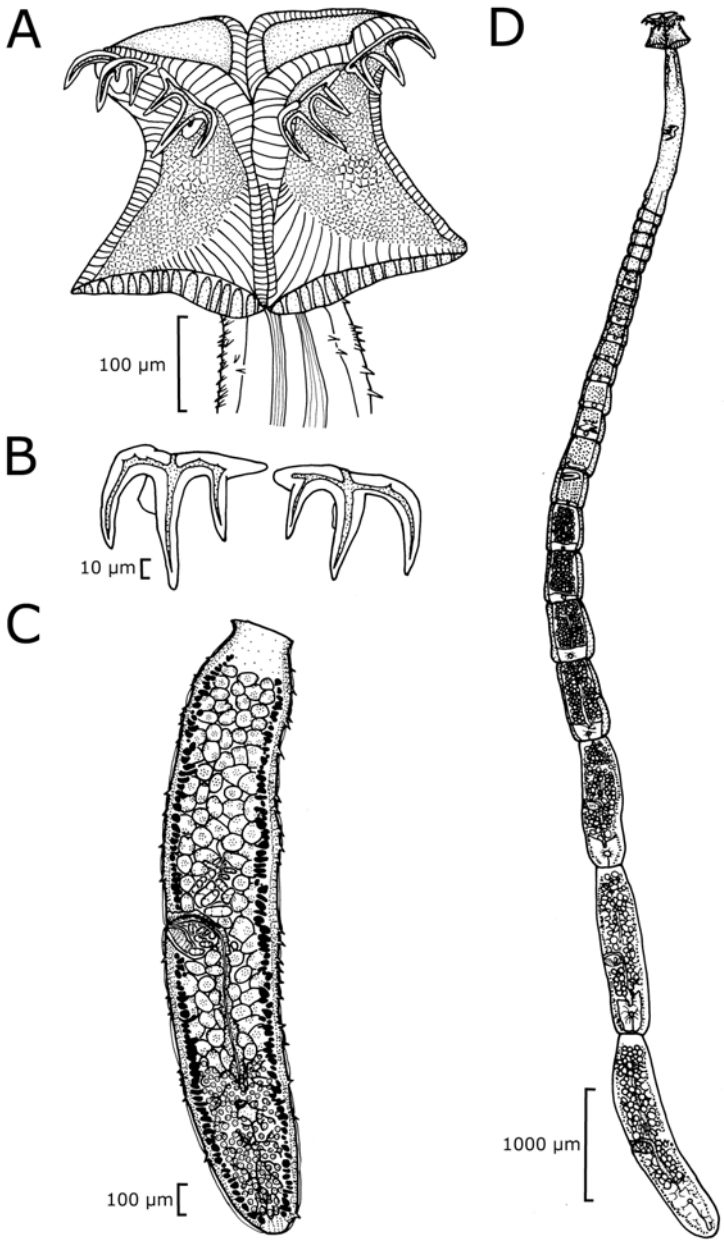


**Figure 13.** Line drawing of *Phoreiobothrium* n. sp. 6. **(A)** Scolex **(B)** Hooks **(C)** Mature proglottid **(D)** Whole worm.

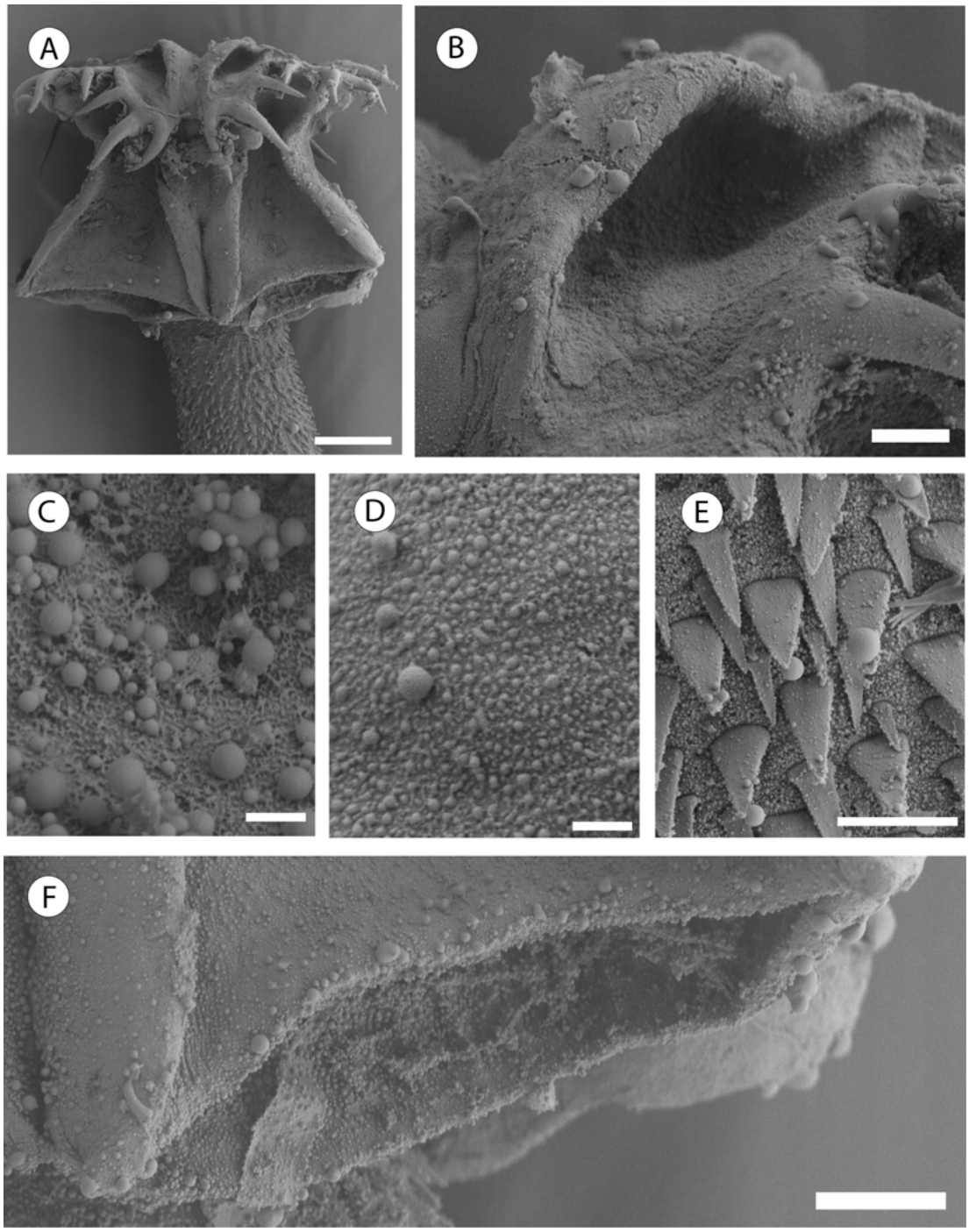


**Figure 14.** Scanning electron micrographs of *Phoreiobothrium* n. sp. 6. **(A)** Scolex; scale bar 50 μm. (**B)** Enlarged view of anterior loculus; scale bar 10 μm. **(C)** Enlarged view of distal bothridial surface; scale bar 1 μm. **(D)** Enlarged view of proximal bothridial surface; scale bar 1 μm. **(E)** Enlarged view of cephalic peduncle; scale bar 5 μm. **(F)** Enlarged view of sublocculi; scale bar 20 μm.

**Cestode Fauna of *Carcharhinus limbatus* in the Gulf of Mexico**

Currently, *Carcharhinus limbatus* parasite records in the literature encompass 34 species in three orders—Trypanorhyncha, Tetraphyllidea, and Cathetocephalida.

While *Carcharhinus limbatus* has been previously examined for cestode parasites, no such survey has been done to list all cestodes of blacktip sharks from a single locality. Table 6 shows a comparison of previous records from the literature with the cestodes recovered from Gulf of Mexico blacktip sharks during the course of this study.

Of the 34 cestode species previously recorded from *C. limbatus*, 25 are members of the order Trypanorhyncha; however, four of these (*Pterobothrium acanthotruncatum*, *Pterobothrium heteracanthum*, *Otobothrium cysticum*, and *Synbothrium filicolle*) have only been recovered in immature stages from blacktip sharks (Palm, 2004), and so will not be discussed further. Palm and Overstreet (2000) reported *Callitetrarhynchus* cf. *gracilis*, *Heteronybelinia estigmena, Otobothrium insigne*, *Nybelinia lingualis*, *Nybelinia* cf. *bisculatus*, *Callitetrarhynchus gracilis,* and *Grillota perelica* from *C. limbatus* in the Gulf of Mexico. This study adds one trypanorhynch cestode to *C. limbatus's* record list, both for the locality and the host species, a species of eutetrarhynchid that could not be more specifically identified.

**Table 2.** A comparison of previous cestode records **(A)** from *Carcharhinus limbatus* with findings from this study **(B)**. New parasite records are indicated in bold.

**A**

| Cestodes previously identified in the literature | Source |
|---|---|
| **Trypanorhyncha** | |
| *Tentacularia coryphaenae* Bosc, 1802 | Palm (2004) |
| *Nyebelinia* cf. *bisulcata* (Linton, 1889) | Palm (2004) |
| *Nyebelinia indica* Chandra, 1986 | Palm (2004) |
| *Nyebelinia lingualis* Cuvier, 1817 | Palm (2004) |
| *Heteronyebelinia estigmena* (Dollfus, 1960) | Palm (2004) |
| *Heteronybelinia overstreeti* Palm, 2004 | Palm (2004) |
| *Heteronybelinia robusta* (Linton, 1890) | Palm (2004) |
| *Pseudogrillota perelica* (Shuler, 1938) | Palm (2004) |
| *Dasyrynchus variouncinatus* (Pintner, 1913) | Palm (2004) |
| *Dasyrhynchus giganteus* (Diesing, 1850) | Carvajal et al. (1976) |
| *Dasyrhynchus pacificus* Robinson, 1959 | Palm (2004) |
| *Callitetrarhynchus gracilis* (Rudolphi, 1819) | Palm (2004) |
| *Floriceps saccinatus* Cuvier, 1817 | Palm (2004) |
| *Pseudogrillotia perelica (*Shuler, 1938) | Palm and Overstreet (2000) |
| *Poecilancistrium caryophyllum* (Diesing, 1850) | Palm (2004) |
| *Proemotobothrium southwelli* Beveridge and Campbell, 2001 | Palm (2004) |
| *Otobothrium australe* Palm, 2004 | Palm (2004) |
| *Otobothrium insigne* Linton, 1905 | Palm (2004) |
| *Otobothrium minutum* Suphapradha, 1955 | Palm (2004) |
| *Otobothrium penetrans* Linton, 1907 | Palm (2004) |
| *Otobothrium crenacolle* Linton, 1890 | Linton (1924) |
| *Otobothrium cysticum* (Mayer, 1842) | Palm (2004) |
| *Pterobothrium acanthotruncatum* Escalante and Carvajal, 1984 | Palm (2004) |
| *Pterobothrium heteracanthum* Diesing, 1850 | Palm (2004)* |
| *Pterobothrium filicolle* (Linton, 1924) | Linton (1924) |
| **Tetraphyllidea** | |
| *Platybothrium* sp. | Healy (2002) |
| *Phoreiobothrium lasium* Linton, 1889 | Linton (1924) |
| *Crossobothrium angustum* (Linton, 1889) | Linton (1924) |
| *Disculiceps pileatus* (Linton, 1891) | Linton (1924) |
| **Cathetocephalidea** | |
| *Cathetocephalus australis* Schmidt and Beveridge, 1990 | Schmidt and Beveridge (1990) |

*Not confirmed

**B**

| Cestodes from the Gulf of Mexico |
|---|
| **Trypanorhyncha** |
| *Callitetrarhynchus* cf. *gracilis* |
| **Eutetrarhynchidae sp.** |
| *Heteronybelinia estigmena* |
| *Otobothrium* sp. |
| **Tetraphyllidea** |
| ***Anthobothrium* sp. 1** |
| ***Anthobothrium* sp. 2** |
| ***Paraorygmatobothrium* sp. 1** |
| ***Paraorygmatobothrium* sp. 2** |
| ***Phoreiobothrium n.* sp. 1** |
| ***Phoreiobothrium n.* sp. 2** |
| ***Phoreiobothrium n.* sp. 3** |
| ***Phoreiobothrium n.* sp. 4** |
| ***Phoreiobothrium n.* sp. 5** |
| ***Phoreiobothrium n.* sp. 6** |
| *Disculiceps pileatus* |

As has already been discussed, this study adds additional tetraphyllidean records to those that currently exist for *C. limbatus*. Worms of the genus *Platybothrium* Linton, 1890 were collected from the Northern Territory, Australia (Healy, 2002), but no reports exist for the Gulf of Mexico and members of this genus were not collected in this study. *Phoreiobothrium lasium* has also been reported, but in a recent paper by Caira et al. (2005), the status of this multiple-host parasite is called into question, and it is instead suggested that each host species hosts its own species of *P. lasium*-type. However, as was discussed in the previous section, *C. limbatus* was discovered to host not one, but six new species of *Phoreiobothrium*. New host records were also recovered for *Anthobothrium* Carus, 1863 (two species) and *Paraorygmatobothrium* Carus, 1863 (two species). Images of a selection of cestodes recovered from *Carcharhinus limbatus* are shown in Figure 15.

An unusual tetraphyllidean recovered in this study was first recorded from *C. limbatus* in Woods Hole in 1924 (Linton)--*Disculiceps pileatus* (Linton, 1924) Joyeux and Baer, 1936. Caira and collaborators (1999) suggest its close relationship to *Cathetocephalus* on the basis of morphological evidence. It has a Tetraphyllidea-like strobila, but a strange scolex that lacks bothridia (Fig. 15F) (Caira et al., 1999).

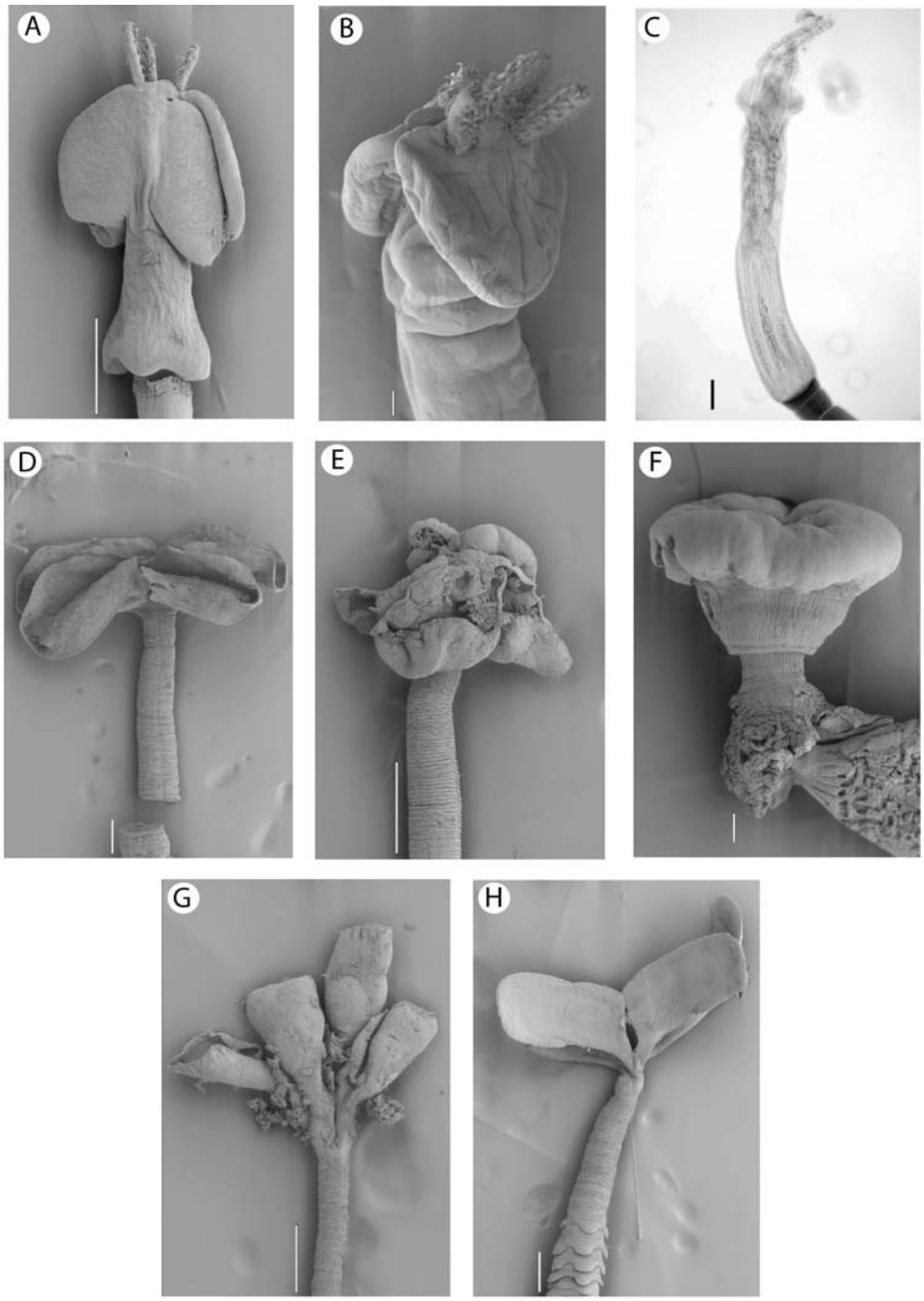


**Figure 15.** Images of cestodes of *Carcharhinus limbatus* from the Gulf of Mexico. A, B, D, E, F, G, and H are scanning electron micrographs; C is a light micrograph. Scale bars indicate 100 μm. **(A)** *Otobothrium* sp. **(B)** *Callitetrarhynchus* cf. *gracilis* **(C)** Unspecified eutetrarhynchid sp. **(D)** *Paraorygmatobothrium* sp. 1 **(E)** *Paraorygmatobothrium* sp. 2 **(F)** *Disculiceps piliatus* **(G)** *Anthobothrium* sp. 1 **(H)** *Anthobothrium* sp. 2.

**Discussion**

These findings increase the number of *Phoreiobothrium* species described from 17 to 23, expanding the host records for *Phoreiobothrium* to include *Carcharhinus limbatus*, and extending the geographic range of *Phoreiobothrium* to include coastal waters off Gulf Springs, Mississippi and Panama City, Florida. The findings of this survey of *Phoreiobothrium* cestodes of *Carcharhinus limbatus* also yielded an unprecedented number of congeners in a single host species. Previously, the highest reported number of *Phoreiobothrium* species was from *Rhizoprionodon acutus* (as "*Carcharias acutus*") collected from the Indian Ocean (see Table 1). This host had five species of *Phoreiobothrium* reported from it, all of which are currently considered *inquirendae* (see Caira et al., 2005), as was discussed earlier.

While the extraordinary diversity of *Phoreiobothrium* species found in *Carcharhinus limbatus* may be atypical, it indicates the likelihood that *Phoreiobothrium* diversity is quite underrepresented in the literature. There are currently 50 described species of sharks in the Family Carcharhinidae and nine in the Family Sphyrnidae. Of these, *Phoreiobothrium* species have been described from seven species in the Family Carcharhinidae and five species in the Family Sphyrnidae. This leaves 47 carcharhinids to examine for *Phoreiobothrium*, if infections are limited only to hosts in these families. Even if *Phoreiobothrium* species are limited to congeners of previous host records, this leaves 34 carcharhinid species and three sphyrnid species as potential hosts of *Phoreiobothrium*. Extrapolating from the hypothesis (conservative given the findings in this study) that

each species of shark in these families hosts one distinct species of *Phoreiobothrium*, and limiting the search to congeneric hosts, this means that there are 37 new species of *Phoreiobothrium* awaiting description.

**Patterns of Infection**

The original intent of this study was to examine the possibility of using cestodes of the genus *Phoreiobothrium* as biological tags for determining populations of blacktip sharks, *Carcharhinus limbatus*. Unfortunately, there were too many new species of *Phoreiobothrium* and too few *C. limbatus* specimens collected to allow for a robust statistical analysis of the patterns of infection. Still, some qualitative observations were made with regard to prevalence—use of intensity data was beyond the scope of this project due to the large number of species of *Phoreiobothrium* recovered.

Prevalence data are summarized in Table 3. Prevalences are separated by host sex, year of collection, and locality of collection. When host sex is considered, *P*. n. sp. 4 and *P*. n. sp. 5 have the most marked difference between the sexes. No males were infected with either species, whereas 41.7% (5 of 12) of females were infected with *P*. n. sp. 4 and 16.7% (2 of 12) of females were infected with *P*. n. sp. 5. Of the remaining four species, two infected males more often than females—*P*. n. sp. 1 infected 25% (2 of 8) of males and 16.7% (2 of 12) females, *P*. n. sp. 6 infected 37.5% (3 of 8) of males and 8.3% (1 of 12) of females. These differing infection rates may be due to sex differences in migration pattern—adult females return to the

same breeding grounds year after year, whereas males show less strict tendencies (Keeney et al., 2005). However, it may also be an artifact of the small and uneven sampling size.

**Table 3.** Prevalence of infection (expressed as percentage) of *Carcharhinus limbatus* by *Phoreiobothrium* by the six new species described in terms of host Sex **(A)**, year of collection **(B)**, state **(C)**, and detailed location **(D).** Presence or absence of *Phoreiobothrium* worms also noted.

| **A** | Host Sex | Present? | *P.* n. sp. 1 | *P.* n. sp. 2 | *P.* n. sp. 3 | *P.* n. sp. 4 | *P.* n. sp. 5 | *P.* n. sp. 6 |
|---|---|---|---|---|---|---|---|---|
| | Male (n=8) | yes | 25 | 12.5 | 12.5 | 0 | 0 | 37.5 |
| | Female (n=12) | yes | 16.7 | 16.7 | 8.3 | 41.7 | 16.7 | 8.3 |

| **B** | Year of Collection | Present? | *P.* n. sp. 1 | *P.* n. sp. 2 | *P.* n. sp. 3 | *P.* n. sp. 4 | *P.* n. sp. 5 | *P.* n. sp. 6 |
|---|---|---|---|---|---|---|---|---|
| | 2005 (n=3) | yes | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2006 (n=6) | yes | 0 | 0 | 33.3 | 16.7 | 16.7 | 16.7 |
| | 2007 (n=11) | yes | 36.4 | 27.3 | 0 | 36.4 | 9.1 | 27.3 |

| **C** | State | Present? | *P.* n. sp. 1 | *P.* n. sp. 2 | *P.* n. sp. 3 | *P.* n. sp. 4 | *P.* n. sp. 5 | *P.* n. sp. 6 |
|---|---|---|---|---|---|---|---|---|
| | Mississippi (n=6) | yes | 0 | 16.7 | 16.7 | 16.7 | 16.7 | 0 |
| | Florida (n=14) | yes | 28.6 | 14.3 | 14 | 28.6 | 35.7 | 35.7 |

When collection year is the variable by which prevalence is examined, another pattern emerges. Between 2005 and 2006, a sharp increase in the prevalence of infection of the species of *Phoreiobothrium* that have been described in this thesis occurred—from 0% (0 of 3) to 50% (3 of 6). In 2007, prevalence increased slightly to 63.6% (7 of 11)--this was the only year from which hosts were infected with species *P.* n. sp. 1 and *P.* n. sp. 2. Overstreet (2007) suggested Hurricane Katrina, which occurred in August 2005, as a possible phenomenon that explains temporal differences in levels of parasite prevalence. Many factors altered by Hurricane

Katrina were suggested as possible causes for differences in parasite prevalence before and after the storm (Overstreet, 2007); these include sediment perturbation which would disturb invertebrate intermediate host habitat, introduction of high salinity water into freshwater habitats which would kill off osmotically sensitive freshwater inhabitants and introduce marine inhabitants to new areas, and the release of toxicants from sediments and damage spills that could kill both parasites and hosts. However, it is unlikely that these hurricane-related factors contributed to higher post-Katrina prevalence of *Phoreiobothrium* species in *Carcharhinus limbatus.* It is far more likely that these data are an artifact of unequal sampling between localities from year to year—the Mississippi localities were sampled more heavily in 2005, and Florida localities were sampled more heavily in 2006 and 2007, suggesting Florida may simply have a higher prevalence of infection with *Phoreiobothrium* species.

These considerations introduce the last potential source of variation examined in this study– host specimen locality. The examination of biogeographic differences in parasite prevalence was the original intent of this study, but as with the above factors, only very general patterns are identifiable. All six species described in this thesis were recovered from Florida localities, whereas *P.* n. sp. 2, *P.* n. sp. 3, *P*. n. sp. 4, and P. n. sp. 5 were the only species recovered from Mississippi localities. The Mississippi hosts were parasitized by *P*. n. sp. 2, *P*. n. sp. 4, and *P*. n. sp. 5 at a rate of 16.7% (1 of 6) each, whereas Florida hosts were parasitized by these species with 14.3% (2 of 14), 14% (2 of 14), 28.6% (4 of 14), and 35.7% (5 of 14) respectively.

Two other species were recovered from the Florida hosts—28.6% (4 of 14) were infected with *P*. n. sp. 1 and 35.7% (5 of 14) were infected by *P*.n. sp. 6.

These data show three possible patterns of prevalence of *Phoreiobothrium* worms in *C. limbatus*: two potentially biogeographically distinct *C. limbatus* populations are defined by Florida's higher diversity of *Phoreiobothrium* species when compared to Mississippi (two additional species), a higher prevalence of tapeworm infection in female sharks when compared to male sharks, and an appreciable increase in prevalence of infection between 2005 and 2006. While it is tempting to make conclusions with regard to the effects of these factors, prudence stemming from limited host sampling precludes such statements.

**Future Directions**

There is a rich potential for further work to expand this study in the future; I envision this further research to focus on four areas: sampling size, geographic data, parasite diversity, and phylogeny. As has already been mentioned, the sampling size of this study was quite limited. In order to have a robust data series, ideally at least 20 sharks of approximately the same level of maturity would be collected at Gulf Springs, Mississippi and Panama City, Florida (to control for the suspected effect of shark age on the biology affecting infection prevalence). This would guarantee that worms with greater than 5% prevalence (as is the case with the species described in this study) would be detected. These specimens would then be examined not only for

prevalence of *Phoreiobothrium* species previously described, but intensity of each species of *Phoreiobothrium* would be described.

Once these original sample locations are examined for differences in prevalence of *Phoreiobothrium* species the next phase of the study would be to expand the study to include samples from within the Louisianan Province—for example, Texas—as well as biogeographic provinces beyond the Louisianan Province. Specimens of *Phoreiobothrium* have already been recovered from *C. limbatus* collected in the western Atlantic, the Caribbean Sea, and off the coast of Australia. These would be priority targets, since *Phoreiobothrium* is known from these regions, but ideally this study would continue to expand until the entire circumtropical range of the host has been sampled.

The last two ways to expand the study follow the trends currently emerging in investigations of parasite biogeography—assessment of a larger diversity of parasites beyond the genus *Phoreiobothrium*, and the incorporation of genetic data. The former trend, assessing a larger diversity of parasites, is the less practical and necessary of the two. With 40 species of cestodes currently reported from *C. limbatus—Phoreiobothrium* diversity alone consisting of the six species herein described—that incorporating a wider diversity of parasites may prove unnecessarily taxing in terms of added research hours. However, incorporating a few parasites from sites other than the spiral intestine and comparing their biogeographic variability to that of *Phoreiobothrium* species would prove an interesting study. Using a non-spiral-intestine-infecting parasite would have the added advantage of not being in

direct competition with spiral intestine cestodes. One such potential parasite is the digenean *Selachohemecus benzi*, which is a blood fluke that is likely host-specific (see Bullard et al., 2005). It would also be beneficial to examine a parasite with a life cycle less complex than that of a cestode—little is known about the life cycle of the tapeworm, and since the intermediate hosts are unknown, the effect of intermediate host availability on prevalence and intensity is unknown.

The second area to expand upon involves using genetic data to further inform hypotheses of population boundaries and coevolution. For host samples, this would mean taking tissue samples from all hosts collected and creating haplotype networks for populations from the different sampling areas and comparing them, expanding on Keeney and collaborators' previous work (2005, 2006). These data would then be compared to the findings of the parasite biogeography data. For *Phoreiobothrium* species, genetic data could be used to probe further into the phenomenon of the unusually high number of congeners present in *C. limbatus*, perhaps looking at questions of vicariance speciation within the group. It would also be useful to compare the phylogeny of the worms and their hosts in order to further elucidate questions of diversification in the genus *Phoreiobothrium*.

**Conclusion**

This study has resulted in the description of six new species of *Phoreiobothrium*, a new host record for *Phoreiobothrium*, and a new locality record for the genus, a list of cestodes parasitizing the spiral intestine of *Carcharhinus*

*limbatus* in the Gulf of Mexico, and a preliminary investigation using *Phoreiobothrium* cestodes to answer questions of *C. limbatus* stock differentiation. The tiny nibble of knowledge contained in these pages has served most of all as an appetizer for a possible lifetime hunger to probe deeper into these questions. Bon appétit.

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