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ENDOHELMINTH PARASITES OF SOME MIDWATER AND BENTHOPELAGIC STOMIIFORM FISHES FROM THE NORTHERN GULF OF MEXICO

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ABSTRACT: Mesopelagic fishes represent significant ecological links between mesozooplankton and the larger pelagic squids, fishes, and marine mammals. As such, these fishes also play a significant role as intermediate or paratenic hosts for parasites that require a crustacean intermediate host and mature in marine mammals or pelagic fishes. We examined a total of 208 individuals representing 5 species of Sternoptychidae and 88 individuals representing 2 species of Phosichthyidae from 20 locations in the northern Gulf of Mexico (nGOM). Six of the 7 species we examined are mesopelagic and one species was benthopelagic. We found the larval stages of *Anisakis brevispiculata*, *Anisakis typica*, *Hysterothylacium fortalezae* (all Nematoda: Ascaridoidea); *Bolbosoma* sp. (Acanthocephala: Polymorphidae); and Tetraphyllidea (Cestoda) plus an immature specimen of *Brachyphallus* sp. (Digenea). Molecular sequencing was used to identify the ascaridoids and *Bolbosoma* sp. and to confirm the identification of 3 host sternoptychid species. The mesopelagic fishes hosted *Anisakis brevispiculata* (that matures in pygmy and dwarf sperm whales) and *Hysterothylacium fortalezae* (that matures in pelagic fishes, primarily mackerels), whereas the benthopelagic species was parasitized by *Anisakis typica* (that matures in dolphins). We suggest this pattern of infection indicates a pelagic life-cycle for *Anisakis brevispiculata* and *Hysterothylacium fortalezae* and a demersal life-cycle for *Anisakis typica*. Our study represents the first published sequences from the nGOM for the fishes *Argyrolepeus aculeatus*, *Maurolicus weitzmani*, and *Polyipnus clarus* and the first molecular identification of larval ascaridoids from mesopelagic fishes in the nGOM.

KEYWORDS: *Anisakis*, *Hysterothylacium*, marine mammals, Phosichthyidae, Sternoptychidae

INTRODUCTION

Mesopelagic fishes represent significant ecological links between mesozooplankton and the larger pelagic squids, fishes, and marine mammals (e.g., Pauly et al. 1998, Choy et al. 2013, Young et al. 2015). Members of the orders Stomiiformes and Myctophiformes account for the vast majority of such fishes (Bernal et al. 2015). Most parasitological studies of midwater fishes have revealed larval or juvenile stages of helminths that mature in large pelagic fishes and marine mammals rather than adult helminths (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Mateu et al. 2015). Typically, larval or juvenile stages of trematodes (that mature in fishes), cestodes (that mature in elasmobranchs), acanthocephalans (that mature in fishes and marine mammals), and nematodes, especially ascaridoids, have been reported (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Klimpel et al. 2006, 2010, Mateu et al. 2015).

The life-cycle of marine ascaridoids involves a crustacean first intermediate host and a vertebrate final host. Typically, members of the Raphidascarididae mature in teleosts, whereas those of Anisakidae mature in marine mammals. The crustacean first intermediate host is generally a copepod, but euphysiids serve as important intermediate hosts for species of *Anisakis* (see Smith and Wooten 1978). Larval ascaridoids commonly infect fishes and invertebrates that serve as paratenic, or transfer hosts (hosts that are not required for the development of the parasite). The use of these paratenic hosts acts as an adaptation to overcome the challenges of reaching appropriate final hosts in oceanic environments (Marcogliese 1995), and has led to the utility

of ascaridoids as biological tags for fishes (Mattiucci et al. 2008) and trophic interactions (Palm and Klimpel 2008). The use of paratenic hosts also has public health implications for certain fisheries products because of the zoonotic (can be passed from animal to human) potential of some ascaridoids, especially those that mature in marine mammals (species of *Anisakis* and *Pseudoterranova*; see Sakanari and McKerron 1989), but also some that mature in fishes (Overstreet and Meyer 1981, Overstreet 2012).

Despite the potential human health risk associated with some ascaridoids, the Gulf of Mexico (GOM) is poorly characterized with respect to the intermediate and paratenic host use patterns of those nematodes. Kuhn et al. (2011) modeled the geographic range of 9 species of *Anisakis* but did not include data for the GOM presumably because species of *Anisakis* were reported under junior synonyms, incomplete larval names, misidentifications, or they lacked molecular data (e.g., Gunter and Overstreet 1974). Cavallero et al. (2011) surveyed stranded cetacean hosts from the eastern GOM (eGOM), southeastern U.S. Atlantic coast, and Caribbean Sea and reported the presence of 7 species of *Anisakis*, *Pseudoterranova ceticola*, and larvae of *Contracaecum multipapillatum* (exclusively from inshore populations of the common bottlenose dolphin, *Tursiops truncatus*). However, the highly migratory nature of most marine cetaceans and the varied geographic stranding localities examined by Cavallero et al. (2011) do not provide a clear picture of the richness of *Anisakis* species in the northern GOM (nGOM).

The purpose of this study was to document larval ascaridoids as well as other endohelminths in stomiiform fishes

that were incidentally caught in bottom trawl surveys along the nGOM outer continental shelf and continental slope. We examined 5 mesopelagic species (the Atlantic Sliver Hatchetfish, *Argyrolepecus aculeatus*; the Silvery Hatchetfish, *Argyrolepecus sladeni*; the Atlantic Pearlsides, *Maurollicus weitzmani*; the Slope Hatchetfish, *Polyipnus clarus* (all Sternoptychidae); and the Stareye Lightfish, *Pollichthys maui* (Phosichthyidae)) and one benthopelagic species (the Rendezvous Fish, *Polymetme corythaeola* (Phosichthyidae)) for helminths. Morphological identification of larval ascaridoids to species level is generally problematic (e.g., Zhu et al. 1998, Mattiucci and Nascetti 2006, Mattiucci et al. 2014); therefore, we molecularly genotyped the 28S and internal transcribed spacer region (ITS; = ITS1, 5.8S, and ITS2) ribosomal DNA (rDNA) regions to confirm identifications.

MATERIALS AND METHODS

Sample collection

Representative stomiiform fishes were collected from the nGOM by bottom trawl as a part of the NOAA–Fisheries Southeast Small Pelagic Survey in 2011–2012. Trawls were towed for 30 min and depths (m) at the end of trawl were recorded. Following capture of fishes, we identified and froze them at -20°C while on ship. We examined 208 individuals representing 5 species of Sternoptychidae and 88 individuals representing 2 species of Phosichthyidae from 20 locations (Figure 1A). In the laboratory, hosts were defrosted at $1-3^{\circ}\text{C}$, identifications confirmed using the keys developed by McEachran and Fechhelm (1998), measured (nearest mm SL), weighed (nearest 0.01 g), and sexed. The visceral organs and gastrointestinal tract of all

fish were placed in a small amount of physiological saline and examined for helminths under a stereomicroscope. An initial subsample of 20 individuals each of *Maurollicus weitzmani*, *Pollichthys maui*, *Polyipnus clarus*, and *Polymetme corythaeola*, and all specimens of *Argyrolepecus aculeatus* and *Argyrolepecus sladeni*, were examined for larval helminths in the musculature by candling. Candling was conducted by putting fillets between two glass slides and examining them by stereomicroscope; however, no larvae were found in the musculature of any of the fishes examined, and the process was discontinued. Parasite specimens were fixed in 70% ethanol for morphological and molecular analyses. Nematodes were cleared in 95 parts 70% ethanol + 5 parts glycerol for morphological examination. A single acanthocephalan and a single digenean were stained, cleared, and mounted according to the procedures in Andres and Overstreet (2013). Voucher specimens were deposited in the Gulf Coast Research Laboratory Museum (GCRLM), Ocean Springs, Mississippi (see Table 1 for accession numbers).

Ecological parasitology terms follow those defined by Bush et al. (1997): prevalence as the percentage of fish infected and mean intensity of infection as the mean number of parasites per infected fish. The 95% confidence interval for prevalence was calculated using the Clopper–Pearson exact CI (exactci) package found in the PropCIs library of R v. 2.15.2 (R Core Team, 2012).

Molecular analyses

Genomic DNA was extracted from a middle portion of 36 individual ascaridoids, a section of the posterior portion from the acanthocephalan, and a piece of muscle from a single individual of each sternoptychid species (with the exception of *Argyrolepecus sladeni*; $n = 4$) using Qiagen DNAeasy tissue kit (Qiagen, Inc., Valencia, CA, USA) following the instructions provided. For ascaridoids, DNA fragments of ca. 1,020 base pairs (bp) long for the 28S ribosomal DNA (rDNA) gene and 800 bp long comprising the ITS rDNA gene were amplified from the extracted DNA by polymerase chain reaction (PCR) following the procedures of Nadler et al. (2000) and Zhu et al. (1998), respectively. For the acanthocephalan, a 668 bp partial portion of the mitochondrial cytochrome c oxidase 1 (*cox1*) was amplified following García–Varela and Nadler (2006) and for sternoptychids, ca. 640 bp partial portion of the *cox1* was amplified following Ward et al. (2005). See Table 2 for the list of primers used and their corresponding references. The resulting PCR products were excised from PCR gel using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia,

TABLE 1. Gulf Coast Research Laboratory museum (GCRLM) and GenBank accession numbers for vouchers treated in this study.

Taxon	GenBank Accession No.			
	GCRLM No.	ITS	28S	cox1
Platyhelminthes				
<i>Brachyphallus</i> sp.	06551	-	-	-
Acanthocephala				
<i>Bolbosoma</i> sp.	06552	-	-	KX098556
Nematoda				
<i>Anisakis brevispiculata</i>	06553–5	KX098557–9	KX098560	-
<i>Anisakis typica</i>	06556	KX098561	KX098562	-
<i>Hysterothylacium fortalezae</i>	06557	KX098563	KX098564	-
Chordata				
<i>Argyrolepecus aculeatus</i>	36551	-	-	KX098553
<i>Maurolicus weitzmani</i>	36552	-	-	KX098554
<i>Polyipnus clarus</i>	36553	-	-	KX098555

FIGURE 1. Sampling localities in the northern Gulf of Mexico. A. Localities of sternoptychids (triangles), phosichthyids (squares), or both (circles) from 2011 (filled symbols) and (2012) (open symbols). B. Locations from which *Anisakis brevispiculata* were collected. C. Locations from which *Anisakis typica* (diamonds) and *Hysterothylacium fortalezae* (stars) were collected. D. Locations from which tetraphyllideans (pentagons), *Bolbosoma* sp. (+), and *Brachyphallus* sp. (white "X" overlaid a pentagon) were collected. Bathymetric contour lines represent 50 m, 100 m, 200 m, 300 m, 500 m, and 1,000 m.

CA, USA) following kit instructions. They then were cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, CA, USA), ethanol-precipitated, and processed on an ABI 3130 Genetic Analyzer™.

Sequences used for pairwise comparison were aligned using MAFFT version 6.611b (Katoh et al. 2005) with 1,000 cycles of iterative refinement and the *genafpair* algorithm.

TABLE 2. PCR primers used for the various taxonomic groups and regions in this study.

Group	Region	Primer	Direction	Annealing Temp.	Reference
Acanthocephala	cox1	5'–AGTTCTAATCATAARGATATYGG–3'	Forward	40°C	Folmer et al. 1994
		5'–TAAACTTCAGGGTGACCAAAAAATCA–3'	Reverse		
Ascaridoidea	28S	391: 5'–AGCGGAGGAAAAGAACTAA–3'	Forward	55°C	Nadler et al. (2000)
		501: 5'–TCGGAAGGAACCAGCTA–CTA–3'	Reverse		
	ITS	NC5: 5'–GTAGGTGAACCTGCGGAAGGATCATT–3'	Forward	60°C	Zhu et al. (1998)
		NC2: 5'–TTAGTTTCTTTCTCCGCT–3'	Reverse		
Sternopychidae	cox1	FishF1: 5'–TCAACCAACCACAAAAGACATTGGCAC–3'	Forward	54°C	Ward et al. (2005)
		FishF2: 5'–ACTTCAGGGTGACCGAAGAATCAGAA–3'	Reverse		

RESULTS

Host data

Of the 5 midwater species collected, *Polyipnus clarus* was the most common (n = 156 from 11 stations) followed by the phosichthyid *Pollichthys maui* (n = 35 from 3 stations) whereas 53 individuals of the benthopelagic *Polymetme corythaeola* were collected from 4 stations. Individuals of *Argyropelecus aculeatus* and *Polymetme corythaeola* were collected from the deepest depths as well as the largest depth range (see Table 3). Individuals of both phosichthyid species tended to be longer and heavier than the sternopychid species (Table 3). The *cox1* sequence of *Argyropelecus aculeatus* and *Maurollicus weitzmani* was identical to that of the unpublished sequence of *Argyropelecus aculeatus* (KF929623) collected from the slope water off Cape Hatteras and the sequence of *Maurollicus weitzmani* (KJ190037) from the Mid–Atlantic Bight (Davis et al. 2014), respectively. The *cox1* sequence of *Polyipnus clarus* differed from that of the *Polyipnus clarus* sequence FJ918932 collected from the slope water of southern New England (DeVaney 2008) by a single purine transition.

Genetic identification

Three ascaridoid species were found; *Anisakis brevispicu-*

lata (Anisakidae), *Anisakis typica*, and *Hysterothylacium fortalezae* (Raphidascarididae). Of the 20 individuals of *Anisakis brevispiculata* sequenced, one 28S genotype and 3 ITS genotypes were found. All differences in those genotypes occurred at position 177 in the ITS2, with 15 individuals having a guanine; 4 individuals having an adenine, and one individual having an ambiguous purine. The most common ITS genotype (KX098557) was identical to the sequence of *Anisakis brevispiculata* AY826719 of Cavallero et al. (2011). No intraspecific variation occurred in either gene region of the 6 individuals of *Anisakis typica*. The ITS sequences we generated for *Anisakis typica* were identical to EU327688 (Iñiguez et al. 2009) and JQ912690 (Mattiucci et al. 2014) for *Anisakis typica*, but they differed from sequence AY826724 by Cavallero et al. (2011) that had 3 single bp indels in the ITS1 at positions 125, 131, and 161. No intraspecific variation occurred in either gene region of the 11 individuals of *Hysterothylacium fortalezae*. The 28S sequences we generated for *Hysterothylacium fortalezae* differed from the GenBank sequence U94760 (provided by RMO to Nadler and Hudspeth 1998) by a single bp indel at position 436.

The *cox1* sequence generated from the acanthocephalan was 88% similar to sequence JQ040303 for *Bolbosoma balaenae* (Gregori et al. 2012); 66% similar to sequence JX442190 for *Bolbosoma* sp. (García–Varela et al. 2013); and 65% similar to sequence JX442189 for *Bolbosoma turbinella* (García–Varela et al. 2013). The *cox1* sequences of the latter 2 species were 84% similar, suggesting that the acanthocephalan we collected was a species of *Bolbosoma* (Polymorphidae). Morphologically, the proboscis of our specimen was not everted; however, it did pose a funnel–shaped bulb in the foretrunk.

Parasitological data

The prevalence of helminths was relatively low (Table 4). No individual of *Argyropelecus sladeni* was infected with any helminth. Individuals of *Anisakis brevispiculata* were identified from all other midwater fish with the

TABLE 3. Host information for the species examined in this study. SL = Standard length in mm; g = grams; m = depth; Stations = number of stations that hosts were obtained from; + = number of stations that had parasitized fish.

Taxonomic group	n	SL range	g	m	Stations	
					n	+
Phosichthyidae						
<i>Pollichthys maui</i>	35	61–154	1.00–17.79	396–419	3	3
<i>Polymetme corythaeola</i>	53	91–184	3.08–36.87	76–468	5	3
Sternopychidae						
<i>Argyropelecus aculeatus</i>	16	45–66	1.18–9.13	76–468	4	2
<i>Argyropelecus sladeni</i>	6	25–43	0.51–2.83	336	1	0
<i>Maurollicus weitzmani</i>	30	30–47	0.36–1.21	102–396	5	4
<i>Polyipnus clarus</i>	156	21–57	0.33–3.85	66–430	11	4

exception of *Maurolicus weitzmani*. *Anisakis typica* was the only helminth found to infect *Polymetme corythaeola*, and was found to parasitize that host only. *Maurolicus weitzmani* had the highest prevalence of *Hysterothylacium fortalezae* because a single location in the western GOM (wGOM) had 5 infected individuals. The site of infection for individuals of *Anisakis brevispiculata* was in the mesenteric lining associated with the gonads, with the exception of 2 individuals in a single *Polypinus clarus*, where one individual occurred near the gonads and the other near the liver. Likewise, all individuals of *Anisakis typica* occurred in the mesenteric lining around the gonads, even in the single fish with an intensity of 2. All individuals of *Hysterothylacium fortalezae* were found in the mesenteric lining of the liver. None of the fish examined from the 2011 eGOM locations (east of Mobile Bay, AL; see Figure 1A) were infected with ascaridoids, whereas all of the 2012 sampling locations occurred in the eGOM, and only the southernmost location exhibited uninfected fish. All three ascaridoid species were found across the nGOM (Figure 1B–C). The depth range of fish infected with ascaridoids was 66–468 m for *Anisakis brevispiculata*, 98–468 m for *A. typica*, and 212–419 m for *Hysterothylacium fortalezae*, and all but the shallowest two locations (both of which occurred over the Texas shelf) occurred in > 200 m of depth.

The mean intensity (Table 4) for all helminths, with the exception of larval tetraphyllideans (Cestoda) from *Polypinus clarus*, was between 1.0–1.2. Tetraphyllideans were not identified past order, but were found in only mesopelagic fishes from the eGOM (Figure 1D) ranging from a depth of 102–419 m. The single specimen of *Bolbosoma* sp. was found in the proximal portion of the intestinal tract of *Maurolicus weitzmani* from the wGOM (Figure 1D) at a depth of 186 m. A single immature (based on possessing adult characters but not being mature) specimen of *Brachyphallus* sp. (Digenea: Hemiuridae) infected the stomach of *Polypinus clarus* from the eGOM (Figure 1D) at a depth of 419 m.

DISCUSSION

We provide the first molecular identification of anisakids

and a species of *Bolbosoma* from fish in the nGOM, the first parasitological survey of 4 sternoptychid species and 2 phosichthyid species in the nGOM, and provide the first *cox1* sequences for sternoptychids from the GOM. We found the larval stages of 5 helminth taxa (*Anisakis brevispiculata*, *Anisakis typica*, *Hysterothylacium fortalezae*, *Bolbosoma* sp., and Tetraphyllidea) and an immature species of *Brachyphallus*. The presence of generalist parasite taxa in the hosts that we examined is similar to the findings of previous authors who examined midwater fishes (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Klimpel et al. 2007, 2008, 2010, Mateu et al. 2015). We found an overall low prevalence and mean intensity of all parasitic taxa across all host species examined. *Polypinus clarus* had the largest number of recovered helminth taxa (4), but it also had the highest sample size (156). *Argyropelecus sladeni* was not infected with any helminths, but only 6 individuals were examined, and, consequently, we do not include it in the following discussion.

All midwater species were parasitized by *Hysterothylacium fortalezae*, with *Maurolicus weitzmani* having the highest prevalence of infection (20%) for any host–parasite pair. *Hysterothylacium fortalezae* primarily parasitizes pelagic, piscivorous fishes (e.g., species of *Scomberomorus* as well as the Leatherjacket, *Oligoplites saurus*; Deardorff and Overstreet 1980), but those authors also reported adults from the Black Grouper, *Mycteroperca bonaci*. Our finding of *Hysterothylacium fortalezae* parasitizing midwater fishes rather than the benthopelagic *Polymetme corythaeola* is likely indicative of those fishes acquiring an infection while feeding on epipelagic crustaceans. Deardorff and Overstreet (1981) described larvae that they attributed to *Hysterothylacium fortalezae* from the viscera of coastal and offshore pelagic fishes (3 species of *Anchoa*, 2 species of *Peprilus*, and the Atlantic Cutlassfish, *Trichiurus lepturus*) but also from a penaeid shrimp and the structure-associated Black Sea Bass, *Centropristis striata* (as *C. melana*). However, the demersal species they found to be infected with *Hysterothylacium fortalezae* larvae have pelagic life–history stages that could have allowed those species to acquire their infection before settling. Most of the locations Overstreet and Deardorff (1981) sampled occurred in shal-

TABLE 4. Percent prevalence (P) with 95% confidence interval and mean intensity of infection (I; $\pm$ SE) for the parasitic taxa of the 4 mesopelagic (M) hosts and one benthopelagic (B) host.

Host	Zone	Tetraphyllidea		<i>Anisakis brevispiculata</i>		<i>Anisakis typica</i>		<i>Hysterothylacium fortalezae</i>	
		P	I	P	I	P	I	P	I
<i>Pollichthys maui</i>	M	6 (0.7–19.2)	1	14 (4.8–30.3)	1	0	–	3 (0.1–14.9)	1
<i>Polymetme corythaeola</i>	B	0	–	0	–	9 (3.1–20.7)	1.2 $\pm$ 0.2	0	–
<i>Argyropelecus aculeatus</i>	M	0	–	13 (1.6–38.3)	1	0	–	6 (0.2–30.2)	1
<i>Maurolicus weitzmani</i>	M	5 (0.1–24.9)	1	0	–	0	–	20 (7.8–38.6)	1.2 $\pm$ 0.2
<i>Polypinus clarus</i>	M	2 (0.4–5.2)	7 $\pm$ 1.9	8 (4.0–13.1)	1.1 $\pm$ 0.1	0	–	1 (0.2–4.6)	1

low water where trophic interactions between demersal and pelagic organisms are more likely to have occurred.

Anisakis brevispiculata parasitized all midwater fish species with the exception of *Mycteroperca weitzmani* and was the most numerous of all examined nematodes. One individual each of *Argyropelecus aculeatus* and *Polypinus clarus* had a co-infection of *Hysterothylacium fortalezae* and *Anisakis brevispiculata*. Pygmy and dwarf sperm whales (Kogiidae) serve as the final hosts for *Anisakis brevispiculata* (Mattiucci et al. 2001, Cavallero et al. 2011) and feed primarily on squids and to a lesser degree on mesopelagic fishes (Pauly et al. 1998). However, Mattiucci et al. (2001) reported that the demersal *Merluccius merluccius* (Macrouridae) served as a paratenic host in the central–eastern Atlantic Ocean; demersal fishes have been known to feed on midwater fishes, especially along continental slopes and seamounts (Pusch et al. 2004, Gartner et al. 2008), and this is reflected in their parasite fauna (e.g., Klimpel et al. 2008, Palm and Klimpel 2008). Therefore, we believe that *Anisakis brevispiculata* has a pelagic life–cycle in the nGOM but that additional midwater fish (especially myctophids) and squid species from the GOM should be studied to confirm this.

Anisakis typica parasitized *Polymetme corythaeola*, the only benthopelagic species examined. Palm et al. (2008) suggested a pelagic life–cycle for *Anisakis typica* off Indonesia based on their finding of larvae in scombrid and carangid fishes, but our findings suggest a demersal cycle in the nGOM. Cavallero et al. (2011) found that *Anisakis typica* was the dominant anisakid in offshore delphinids that had stranded along the coasts of the GOM, southeastern U.S., and Caribbean Sea. Our finding of *Anisakis typica* in the benthopelagic species only was surprising, considering that the diets of most oceanic delphinids consist of squid and epipelagic and mesopelagic fishes (e.g., Fiedler et al. 1998, Pauly et al. 1998, Davis et al. 2002). Therefore, additional paratenic hosts are likely necessary to transfer *Anisakis typica* to its final hosts. Additional benthopelagic and demersal fishes along the outer continental shelf and slope should be examined to further establish the life–cycle patterns of *Anisakis typica* in the nGOM.

The current underrepresentation of parasitological data for species of *Anisakis* in the nGOM is likely an artifact of the bathymetry of the GOM. The continental shelf is shallow and wide across most of the commercial and recreational fishing grounds, and the majority of ichthyoparasitological examinations in the region for ascaridoids (e.g., Overstreet 1978, Deardorff and Overstreet 1980, 1981) have focused on host species that occur in coastal waters or over the continental shelf. Likely reasons why anisakids are not commonly encountered in fisheries products from the GOM are the lack of surveys using molecular tools, the importance that oceanic euphysiids may play in the life–cycles of species of *Anisakis* (Smith and Wooten 1978), and the diversity of

marine mammals associated with the bathymetric features (e.g., salt domes and diapirs) of the nGOM continental slope (Davis et al. 2002).

In the GOM, the only species of *Bolbosoma* reported is *Bolbosoma vasculosum* from Blainville's beaked whale, *Mesoplodon densirostris*, (Salgado–Maldonado and Amin 2009). However, in the Caribbean Sea, *Bolbosoma vasculosum* has been reported from both the pygmy killer whale, *Feresa attenuata*, and the Atlantic spotted dolphin, *Stenella frontalis*; another species, *Bolbosoma capitatum*, has been reported from the short–finned pilot whale, *Globicephala macrorhynchus*, and an unidentified species of *Bolbosoma* infected the pygmy sperm whale, *Kogia breviceps* (see Mignucci–Giannoni et al. 1998). Unfortunately, the freezing and subsequent thawing of the single specimen that we attribute to *Bolbosoma* sp. was based on molecular sequencing of the *cox1* gene and did not allow for morphological identification to species level. Species of *Bolbosoma* are one of the two acanthocephalan genera known to parasitize marine mammals (Raga et al. 2009). Euphysiids and copepods act as first intermediate hosts (Hoberg et al. 1993, Gregori et al. 2012), and fishes act as paratenic or transport hosts (Raga et al. 2009).

Likewise, tetraphyllideans use crustacean and fish intermediate hosts, mature in elasmobranchs, and the larvae are commonly found in marine fishes (e.g., Jensen and Bullard 2009). We attempted to amplify the 28S from the extracted DNA of 3 individual larvae but were unsuccessful. Jensen and Bullard (2009) provided a key to 15 larval types that they identified but our specimens could not clearly be assigned to any of them, most likely because of the freezing and thawing of hosts. The only species of *Brachyphallus* reported from the GOM by Overstreet et al. (2009) is *Brachyphallus parvum* (as *Lecithochirium parvum*). *Brachyphallus parvum* has been reported from 27 teleost species in the GOM, 5 of which are pelagic. The life–cycle of the congener *Brachyphallus crenatus* involves an opisthobranch snail, calanoid copepods, matures in teleosts, and can include chaetognaths and ctenophores as paratenic hosts (Køie 1992); therefore, the finding of an immature species in *Polypinus clarus* is not necessarily unexpected.

Few other studies on the parasites of midwater fishes have included sternoptychids or phosichthyids as hosts. Gartner and Zwerner (1989) examined *Argyropelecus aculeatus* and *Sternoptyx diaphana* from the western North Atlantic; Klimpel et al. (2004, 2007) examined *Maurollicus muelleri* from the Norwegian Deep and Mid–Atlantic Ridge; and Bray and Gaevskaya (1993) provided a description of a monorchiid (Digenea) from *Polymetme corythaeola* from the eastern mid–Atlantic Ocean. Gartner and Zwerner (1989) found only cestode larvae in the 2 sternoptychids they examined. Klimpel et al. (2004, 2007) found parasite communities similar to ours, but they found 2 digenean species and the ascaridoid species *Anisakis simplex* (sensu stricto) and *Hystero-*

thylacium aduncum. We did not find *Anisakis simplex* (sensu stricto) in any of the fish we examined, but Cavallero et al. (2011) did find individuals in stranded delphinids, *Kogia breviceps*, and *Mesoplodon europaeus*. Cavallero et al. (2011) also found five additional species of *Anisakis* plus *Pseudoterranova ceticola*; therefore, some of the additional species of *Anisakis* they identified but were not collected by us likely occur in the nGOM.

Our results must also be accompanied with a few caveats. The first of which is that the midwater fishes treated in our study were collected incidentally with a bottom trawl. We do not believe that all of these fishes were collected near the bottom, but Gartner et al. (2008) reported large numbers of *Maurollicus weitzmani* and *Polypinus clarus* associated with the benthic boundary layer. This may explain why we were able to attain a sample size greater than 100 for *Polypinus clarus*. Our sample sizes were also relatively small for most species

examined (only *Polypinus clarus* and *Polymetme corythaeola* had $n > 50$); therefore, we elected to pool parasitological data for species across the nGOM rather than comparing infections by locations. Ross et al. (2010) found a homogeneous mesopelagic fish assemblage across the nGOM that would support our consideration. However, based on diet data for some sternoptychid species in the eGOM, the species composition of available prey at lower taxonomic levels spatially varies slightly (e.g., Hopkins and Baird 1985). The stomachs of most of the fishes we examined were either inverted, empty, or contained unrecognizable (digested) prey, and could not be used to reliably compare diets. To generate a more concise picture of the parasite communities of sternoptychids and phosichthyids and to generate a better understanding of the life-cycles of such parasites, fishes collected by midwater trawls should be examined.

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