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Helminth Parasites of the Juvenile Hawksbill Turtle *Eretmochelys imbricata* (Testudines: Cheloniidae) in Brazil

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ABSTRACT: The helminth fauna of 31 juvenile specimens of *Eretmochelys imbricata* from the Brazilian coast was examined. Seventeen individuals were infected with helminths (54.8%). The helminths found were: *Diaschistorchis pandus*, *Cricocephalus albus*, *Metacetabulum invaginatum*, *Pronocephalus obliquus* (Pronocephalidae), *Cymatocarpus solearis* (Brachycoeliidae), *Styphlotrema solitaria* (Styphlotrematidae), *Carettacola stunkardi*, *Amphiorchis caborojoensis* (Spirorchiidae), *Orchidasma amphiorchis* (Telorchiidae), and *Anisakis* nematode larvae. This report is the first analysis of parasite communities in this host.

The Hawksbill turtle, *Eretmochelys imbricata* Linnaeus 1766, has a worldwide distribution and uses coastal tropical and subtropical waters for feeding and breeding grounds (Spotila, 2004). This species is currently classified as critically endangered; it is on the list of threatened species published by the Brazilian Ministry of the Environment (MMA, 2003) and is protected by federal law. In Brazil, this species is often found in the states of Bahia, Sergipe, and Rio Grande do Norte, where it uses beaches for egg laying (Marcovaldi et al., 2007). It is also seen in feeding grounds in the states of São Paulo and Ceará (Gallo et al., 2006).

Approximately 50 species of helminths distributed among 10 families are recognized as parasites of *E. imbricata* (Dyer et al., 1995). However, little is known on the helminth diversity of this species in Brazil, for which *Cricocephalus albus*, *Amphiorchis caborojoensis*, *Carettacola stunkardi*, *Styphlotrema solitaria*, *Hapalotrema postorchis*, and *Monticellius indicum* have been reported (Werneck et al., 2008, 2014, 2015; Werneck and Silva, 2012; Fernandes and Kohn, 2014). Most of these helminths occur in the gastrointestinal tract and associated organs. However, spirorchiids such as *H. postorchis* and *M. indicum* occur in the circulatory system where their eggs can cause significant pathology (Santoro et al., 2007).

Studies on parasite communities from sea turtles have been conducted with *Lepidochelys olivacea* (De León et al., 1996), *Caretta caretta* (Aznar et al., 1998; Valente et al., 2009; Santoro et al., 2010, Gracan et al., 2012), and *Chelonia mydas* (Santoro et al., 2006). However, until the date of this work there is no analysis of *E. imbricata* parasite communities available in the literature. The goal of the present study was to report the composition of the helminth parasite fauna of *E. imbricata* on the coast of Brazil.

This study was conducted on juvenile individuals of *E. imbricata* (n = 31) from 2005 to 2010 found in Ceará (2), Bahia (12), and São Paulo (17) states; mean curvilinear carapace length (CCL) was 40.6 ± 8.7 cm (30.5–65.5 cm) and mean weight was 6.7 ± 5.0 kg (2.0–25.0 kg). All hosts were found dead on the beach or had died in a rehabilitation center; in the latter case we only used animals which had died less than 72 hr prior to examination.

For the parasitological analysis of the digestive tract, the organs (esophagus, stomach, small intestine, and large intestine) were examined. The contents were cleansed and concentrated with sieves (mesh sizes: 0.3 mm and 0.150 mm) and examined under a stereomicroscope. All individuals from São Paulo State were analyzed by the method described by Snyder and Clopton (2005) and simplified by Werneck et al. (2006), recovering the helminths in a sedimentation flask. Trematode specimens were fixed in alcohol-formalin-acetic acid, stained with chlorhydric carmine, and cleared with eugenol. Nematodes were cleared with lactophenol and analyzed using a computerized system for image analyses (QWin Lite 3.1, Leica, Wetzlar, Germany).

Prevalence, mean infection intensity, and mean abundance values, determined according to Bush et al. (1997), were calculated in the Quantitative Parasitology Program (QP 3.0, Reiczigel and Rózsa, 2005). The 95% confidence intervals (CI) of prevalence were calculated by Sterne's exact method and for mean intensity and mean abundance using bootstrapping with 2,000 replications. Comparing among previously published works, we calculated the species richness with and without Spirorchiids (family Spirorchiidae).

The Spearman correlation test was used to check the relationship of CCL with mean abundance and species richness. We also compared the parasite communities of *E. imbricata* from the Brazilian Northeast (Bahia State) and Southeast (São Paulo State). The *Z*-test was used to compare the prevalence and the Mann–Whitney test was used to compare species richness, intensity of infection, abundance, and CCL data. Records from the state of Ceará were removed from this analysis because only 2 animals were analyzed.

The helminths collected during the study were deposited in the Coleção Helmintológica do Instituto de Biociências (CHIBB) of the Universidade Estadual Paulista, Botucatu, São Paulo State, Brazil (accession numbers 1389–1398, 1405–1410, 2656–2657, 6194–6217). All collections were authorized by federal licenses for activities with scientific purposes (Sistema de Autorizacão e Informação em Biodiversidade [SISBIO] 12421-1 and 12431-2) of the Brazilian environmental agency (ICMBIO-IBAMA).

Among the 31 Hawksbill turtles (5 males and 26 females) examined in the present study, 17 were positive for helminths (54.8%). A total of 464 helminths were collected from these hosts, representing 9 trematode species and larval nematodes of the genus *Anisakis*. The helminths found were *Diaschistorchis pandus*, *Cricocephalus albus*, *Metacetabulum invaginatum*, *Pronocephalus obliquus* (Pronocephalidae), *Cymatocarpus solearis* (Brachycoeliidae), *Styphlotrema solitaria* (Styphlotrematidae), *Carettacola stunkardi*, *Amphiorchis caborojoensis* (Spirorchiidae), *Orchidasma amphiorchis* (Telorchiidae), and *Anisakis* nematode larvae (Table I). Parasite species richness was 1.38 (95% CI = 1.06–1.79) without spirorchiids species and 1.47 (95% CI = 1.12–1.88) with spirorchiids species. The mean abundance was 13.8 (95% CI = 6–31.2) and the mean intensity was 25.1 (95% CI = 12.3–50.9). The most-prevalent helminth was *C. albus* (19.3%) followed by *D. pandus*, *O. amphiorchis*, and larvae of *Anisakis* (all with 9.6%). The greatest infection intensity was exhibited by *S. solitaria* (50.5) followed by *C. albus* (36.0 ± 17.3), *C. solearis* (21), and *O. amphiorchis* (20.0) (Table I).

In the present study, the hosts were juveniles (based on their small size and weight) and therefore recently recruited from open-sea environments. They were likely newcomers to the coast, encountering for the first time infective stages of parasites not present in oceanic waters (as proposed by Aznar et al., 1998 and Valente et al., 2009). Their short period of inshore

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TABLE I. Prevalence, mean intensity, and mean abundance of helminth parasites of *Eretmochelys imbricata* (n = 31) from Brazil.

Helminths	Number of helminths	Infected hosts	Prevalence % (95% CI)	Mean abundance (95% CI)	Mean intensity (95% CI)	Site
Pronocephalidae						
Diaschistorchis pandus	23	3	9.7 (0.7–25.5)	0.742 (0.0645–2.25)	7.67 (2–10.7)	SI
Cricocephalus albus	216	7	22.6 (10.8–40.2)	7.68 (2.31–19.8)	34 (13.9–72.4)	ESO, STO, SI, LI
Metacetabulum invaginatum	18	1	3.2 (0.2–17.2)	0.581 (0–1.74)	18	SI
Pronocephalus obliquus	1	1	3.2 (0.2–17.2)	0.0323 (0–0.0968)	1	STO
Brachycoeliidae						
Cymatocarpus solearis	21	1	3.2 (0.2–17.2)	0.677 (0–2.03)	21	STO
Styphlotrematidae						
Styphlotrema solitaria	101	2	6.5 (1.1–20.7)	3.26 (0–9.77)	50.5 (35–50.5)	STO, LI
Spirorchiidae*						
Carettacola stunkardi	2	1	5.9 (0.3–28.7)	0.118 (0–0.353)	2	LIV, BW
Amphiorchis caborojoensis	6	2	11.8 (2.1–35.0)	0.353 (0–1.29)	3 (1–3)	BW
Telorchiidae						
Orchidasma amphiorchis	63	3	9.7 (0.7–25.5)	1.94 (0.0645–7.92)	20 (2–34.7)	LI
Nematoda						
Anisakis larvae	13	3	9.7 (0.7–25.5)	0.419 (0.0323–1.66)	4.33 (1–7.33)	LIV

* Features only for São Paulo State hosts. Abbreviations: CI = confidence intervals: ESO =esophagus; STO = stomach; SI = small intestine; LI = large intestine; BW = body wash; LIV = liver.

residence would have limited their opportunities for parasite acquisition (e.g., Santoro et al., 2006; Valente et al., 2009).

Turtles from the Northeast region had CCL smaller than those from the Southeast region ($P \leq 0.001$); however, all infection-related parameters (total number of parasites, species richness and number of species, prevalence, mean abundance, and mean intensity) were similar for animals from both regions (Table II). No relationship was observed between CCL and parasite abundance ($rs = 0.248$; $P = 0.192$) or richness ($rs = 0.249$; $P = 0.190$).

Nine species of parasites were found in turtles from the Southeast region. Spirorchiids (i.e., *A. caborojoensis* and *C. stunkardi*) were only found from this region (turtles from the Northeast were not examined for spirorchiids), along with 6 trematode species (*D. pandus*, *C. albus*, *P. obliquus*, *C. solearis*, *S. solitaria*, and *O. amphiorchis*) and larval *Anisakis*. In the Northeast, only 2 species of trematodes (i.e., *M. invaginatum* and *D. pandus*) were found.

No gross lesions associated with parasites were observed except in 2 hosts from the state of São Paulo in which spirorchiids were found. Dark nodules 1–2 mm in diameter were observed in the small and large intestine and, when opened and examined under a stereomicroscope, revealed the presence of spirorchiid eggs (Werneck et al., 2008). Chronic inflammatory reactions against the eggs were noted in the hosts' small intestines, lungs, hearts, and livers (Dutra et al., 2012).

Studies on parasite communities from sea turtles have been conducted with *Lepidochelys olivacea* in Mexico (De León et al., 1996), *Caretta caretta* in the Mediterranean (Aznar et al., 1998; Santoro et al., 2010, Gracan et al., 2012) and the Atlantic Ocean (Valente et al., 2009), and *Chelonia mydas* in the Caribbean (Santoro et al., 2006). However, there has been no such analysis for parasites of *E. imbricata*.

Among the helminths studied, only *P. obliquus* has not previously been reported parasitizing *E. imbricata* anywhere in its range. *Cricocephalus albus*, *C. stunkardi*, *A. caborojoensis*, and *S. solitaria* have previously been reported in *E. imbricata* on the Brazilian Coast (Werneck et al., 2008, Werneck and Silva, 2012; Fernandes and Kohn, 2014). Also, *C. albus* and *C. stunkardi* have been reported from *C. mydas* in the same region (Werneck et al., 2013; Fernandes and Kohn, 2014). All other parasite species found are here reported for the first time from *E. imbricata* in the southwestern Atlantic.

In this study 9 species of digenetic trematodes and 1 nematode larva were identified. These parasites are known from marine turtles, as observed in other studies (De Leon et al., 1996; Aznar et al., 1998; Santoro et al., 2006, 2010; Valente et al., 2009; Gracan et al., 2012).

TABLE II. Features of helminth infection in *Eretmochelys imbricata* from 2 localities in Brazil (São Paulo State in Southeast and Bahia State in Northeast).

	Southeast	Northeast	Statistics
Number of host	17	12	—
Number of total parasites	409	43	—
Number of species	9	3	—
Prevalence (%)	52.9	50.0	$Z = 0.218$; $P = 0,827$
Mean species richness*	1.00 ± 0.28 (0–3.0)	0.50 ± 0.15 (0–1.0)	$U = 162.00$; $P = 0.437$
Mean abundance*	24.06 ± 9.56 (0–134.0)	3.58 ± 1.73 (0–18.0)	$U = 162.50$; $P = 0.451$
Mean intensity of infection*	45.44 ± 14.97 (1.0–134.0)	7.17 ± 2.84 (1.0–18.0)	$U = 33.50$; $P = 0.099$
CCL (cm)*	44.61 ± 1.91 (34.0–65.5)	33.50 ± 0.58 (30.0–36.0)	$U = 87.00$; $P \leq 0.001$

* All variables are represented as mean ± standard error (range).

Factors such as food-specificity ocean movements (Santora et al., 2006), geographic distribution (Valente et al., 2009; Santoro et al., 2010; Gracan et al., 2012), and possibly phylogenetic variety (hypothesis by Valente et al., 2009) can influence the community of parasites. In this context individuals of *E. Imbricata*, like the other sea turtle species, experience great marine habitat change throughout their lives.

Chelonia mydas are omnivorous when juveniles and herbivorous when adults. *Caretta caretta* and *L. olivacea* are general carnivores feeding on fish, crustaceans, and molluscs, and *E. imbricata* eat invertebrates and sea sponges (Bjorndal, 1996). Dietary preferences must largely dictate the nature of helminth communities in sea turtle species, but relevant data are very limited for *E. imbricata*.

The analysis of turtles from 2 Brazilian coastal regions does not reveal a direct relationship between the CCL and parasites richness, prevalence, intensity of infection, and abundance (Table II). It seems that the community structure of parasites in *E. imbricata* is different from that seen in other sea turtles and that the host species ontogenetic changes do not directly reflect on its parasites populations (Aznar et al., 1998; Valente et al., 2009; Santoro et al., 2010; Gracan et al., 2012). In other studies, larger turtles are generally found to have more parasites, reflecting their longer residence time in inshore areas and their increasing food consumption as they grow (Aznar et al., 1998; Santoro et al., 2006; Valente et al., 2009). However, in our case, it is possible that this controversial data could be related to the number of turtle specimens analyzed in comparison with previous studies.

Anisakis larvae are often reported in marine invertebrates, with cetaceans as the definitive hosts (Valente et al., 2009). In Brazilian waters the genus *Anisakis* is found in fish, cetaceans, and *C. mydas* (Muniz-Pereira et al., 2009). The occurrence of nematode larvae in only 3 individual *E. imbricata* suggests accidental infection, as Santoro et al. (2010) has suggested for *C. caretta* in the Mediterranean. The occurrence of these larvae may reflect the low degree of host specificity of this helminth (Valente et al., 2009).

In this study 2 spirorchiid species were identified: *A. caborojoensis*, which has been reported from *E. imbricata* only in Puerto Rico (Fischtal and Acholonu, 1976) and Brazil (Werneck et al., 2008; Dutra et al., 2012); and *C. stunkardi*, which has also been reported in *C. mydas* in the United States (Martin and Bamberger, 1952), Panama (Caballero et al., 1955), and Brazil (Werneck et al., 2013) and from *E. imbricata* from Brazil (Werneck et al., 2008).

Most helminths reported here are not restricted to *E. imbricata* but can infect other sea turtles (Santoro et al., 2006). The same is true for helminths in *C. caretta* (Aznar et al., 1998; Valente et al., 2009; Santoro et al., 2010) and *L. olivacea* (De León et al., 1996) and half of the helminths found in *C. mydas* (Santoro et al., 2006). This suggests that different sea turtle species may overlap in dietary preferences as well as sharing similar marine environments.

Trematodes of the family Pronocephalidae likely encyst their metacercariae on sea grasses and other plants. The presence of pronocephalids in turtles demonstrates a trend toward herbivory (De León et al., 1996), as found in *C. mydas* (Santoro et al., 2006). In the present study, 4 trematode species found belong to this family. According to Bjorndal (1996), analysis of the digestive tract of *E. imbricata* reveals some herbivory, although a large portion of the gut contents may be invertebrates and sponges.

This paper reports the occurrence of helminths in juvenile sea turtles of the species *E. imbricata* from the coast of Brazil. These data represent the first analysis of component community in this host. The lack of previous studies on this species of turtle hampers a better understanding of parasitic aspects in these hosts and underscores the need for further investigations with a broader scope on the parasite fauna of this species.

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