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DOI 10.2478/helm-2018-0045

HELMINTHOLOGIA, 56, 1: 75 – 80, 2019

Research Note

Is *Amphiorchis* (Digenea: Spirorchiidae) an exclusive parasite of sea turtles?

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Article info

Received November 14, 2018
Accepted December 12, 2018

Summary

The side-necked turtle *Hydromedusa tectifera* commonly inhabits the tributary streams of the Rio de La Plata and occasionally is found in brackish waters within the estuary of the Rio de La Plata. Few studies have been conducted on its parasitic fauna, especially in Argentina. In the present work *Amphiorchis* sp. is registered for the first time in a freshwater turtle, expanding the knowledge about the specificity of the genus that until now was considered inhabiting only marine turtles.
Keywords: *Amphiorchis*; Argentina; Diplostomida; Freshwater turtles; *Hydromedusa tectifera*; Platyhelminthes; Sea Turtles; Spirorchiidae; South America; Trematoda

Introduction

Spirorchiid trematodes are parasites of freshwater and marine turtles inhabiting the circulatory and lymphatic system. The infection by members of this family may resulted in severe tissue damage in the form of inflamatory granulomatous reactions due eggs deposition in the bloodstream and accumulated in various tissues can lead the animal to death (Santoro et al., 2017). Among Spirorchiidae, *Amphiorchis* Price, 1934 is composed of seven valid species parasites exclusively of sea turtles. Species if this genus have been parasitizing heart, lung and circulatory system of green turtle *Chelonia mydas* L. and hawksbill turtle *Eretmochelys imbricata* L. (see Table 1).

Life cycles of spirorchiid are known for freshwater species (i.e. Turner and Corkum, 1977), whereas little is known for marine species of *Amphiorchis* having only one record for turtles in captivity in which gasteropods (Vermetidae) act has intermediate hosts from which the cercariae are released and actively infect their definitive host, the sea turtle *Caretta caretta* (Cribb et al., 2017).

In the present work *Amphiorchis* sp. is registered for the first time in a freshwater turtle, the side-necked turtle *Hydromedusa tectifera* Cope, 1870 in Argentina, expanding the knowledge about the specificity of the genus that to date has been considered inhabiting only marine turtles.

Material and Methods

In November 2017, a carcass of a road-killed side-necked turtle with evidence of multiple shell fractures found on a road near Martin stream (34°55'42"S, 58°03'30"W, datum: WGS84), Buenos Aires province, Argentina. The turtle was placed in a recipient containing 10 % formalin solution for further analysis in the laboratory. The viscera were observed under the stereomicroscope (Leica M60®) in search of cysts or lesions in the tissue and then with the help of tweezers and scissors the content was examined in search of parasites. The parasites found were collected and preserved in 70 % ethanol.

Digeneans were stained with hydrochloric carmine, dehydrated in a graded ethanol series, cleared in eugenol, and mounted in natural Canada balsam for their morphological study using a polarizing

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Table 1. Hosts and localities of *Amphiorchis* species reported in the literature.

Host	Species	Locality	Reference
Chelonia mydas	*A. amphiorchis*	USA	Price (1934)
	A. indicus	India	Gupta and Mehrotra (1981)
		Brazil	Werneck and Silva (2013)
	A. solus	India	Simha and Chattopadhyaya (1970)
		Costa Rica	Santoro *et al*. (2006; 2007)
		Brazil	Werneck *et al*. (2008); Werneck and Medeiros (2016)
	A. stacyi	USA	Werneck and Greiner (2018)
Eretmochelys imbricata	*A. amphiorchis*	Puerto Rico	Fischthal and Acholonu (1976)
	A. caborojoensis	Puerto Rico	Fischthal and Acholonu (1976); Dyer *et al*. (1995)
		Brazil	Werneck *et al*. (2008); Dutra *et al*. (2012)
	A. indicum	India	Simha and Chattopadhyaya (1978)
	A. lateralis	Japan	Oguro (1938)
Hydromedusa tectifera	*Amphiorchis* sp.	Argentina	Present report

microscope (Olympus BX51®). The drawings were made with the aid of a camera lucida. Measurements are given in micrometers unless otherwise indicated. Additionally, photographs were taken with a Q-Imaging Go-3 digital camera. Specimens were deposited in the Helminthological Collection of the Museo de La Plata MLP-He 7505. The analyzed host was deposited in the Herpetological Collection of the Museo de La Plata, number R-6514. The field study was carried out under permission issued by Dirección de Flora y Fauna of the Buenos Aires Province (Disp. 69/2016). The turtle were cared in accordance with the Guidelines for use of live amphibians and reptiles in field and laboratory research.

Taxonomic keys and specific literature were used for morphological identification and morphometric comparisons, respectively (Price, 1934; Oguro, 1938; Simha & Chattopadhyaya, 1970, 1978; Fischthal & Acholonu, 1976; Gupta & Mehrotra, 1981; Platt, 2002; Werneck & Greiner, 2018). Specimens here found were determined by comparison with available voucher specimens collected by one of the authors of this manuscript (M. Werneck). Those specimens included *A. caborojoensis* (Helminthological Collection of the Biosciences Institute (CHIBB), numbers: 1392, 1406 and 6211), *A. solus* (CHIBB, numbers: 4044 and 7843), *A. indicus* (CHIBB, numbers: 4046, 4048, 4991 and 4995) and *A. stacyi* (U.S. National Parasite Collection, number: 1482618) (Werneck et al. 2008, 2011; Dutra et al. 2012; Werneck & Silva 2013; Werneck & Medeiros 2016; Werneck & Greiner 2018).

Results

Two trematode specimens identified as "*Amphiorchis* sp." were recovered from the duodenum and are described below.

Spirorchiidae Stunkard, 1921
Amphiorchis Price, 1934
Amphiorchis sp. (measures based on a single intact specimen) (Figs. 1, 2)

Body thin and elongate 1.37 mm long by 166 wide, posterior end rounded; oral sucker terminal and large, 61 long by 48 wide; ventral sucker not observed; esophagus 440 long, sinuous, bifurcating in two caeca just posterior to the beginning of the vitelline follicles; left caeca (745) longer than right caeca (679); testes in tandem, large with few lobes, oval in shape, occupying the intracaecal area; anterior testis 94 long by 69 wide, between caecal bifurcation and external seminal vesicle, posterior testis 115 long by 66 wide, located between the ovary and end of caeca; ovary with few lobes, 94 long by 60 wide, occupying the area between the testes, closer to the posterior testis; external seminal vesicle small and oval-shaped, 37 long by 57 wide, just posterior to the anterior testis; cirrus sac between the testes, enclosing an internal seminal vesicle, prostatic cells and cirrus; genital pore diestral to the ovary; vitellarium formed by large rounded follicles occupying both the intracaecal and extracaecal areas, the fields start before the caecal bifurcation extending to near the end of body and are

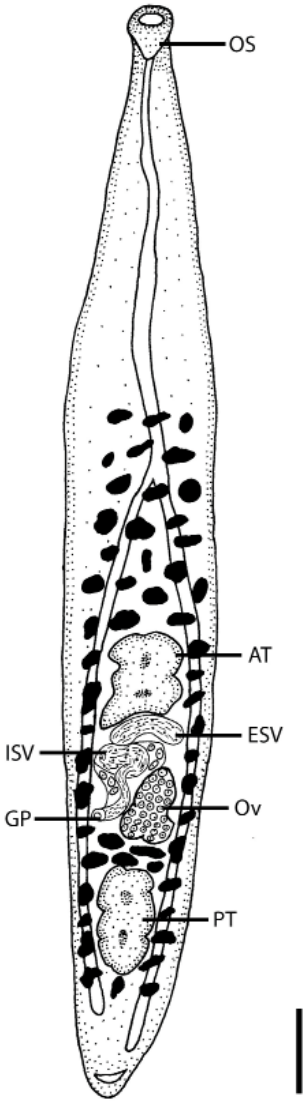

Fig. 1. Schematic illustration of *Amphiorchis* sp. **OS:** Oral sucker, **AT:** Anterior testis, **ESV:** External seminal vesicle, **ISV:** Internal seminal vesicle, **OV:** Ovary, **GP:** Genital pore, **PT:** Posterior testis. Scale bar 50µm.

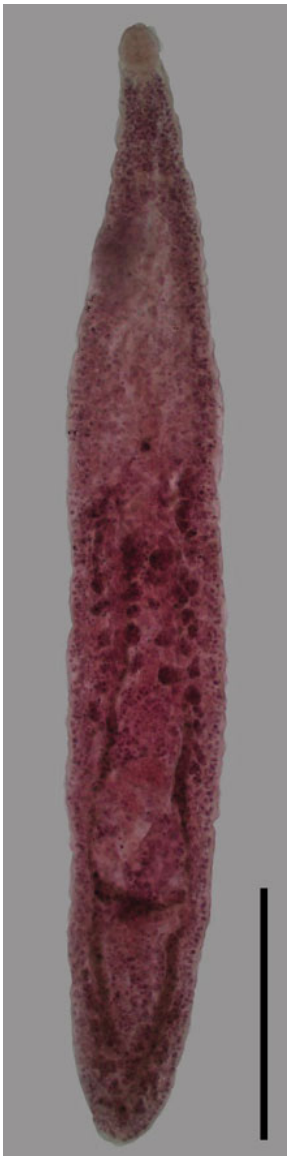

Fig. 2. Microphotograph of *Amphiorchis* sp. Scale bar 200 µm.

ventral to the caeca; not eggs observed.
New host: *Hydromedusa tectifera* Cope, 1870
New locality: Martin Stream (34°55'42" S, 58°03'30" W, datum: WGS84), Buenos Aires, Argentina
Location: duodenum
Although we have observed significant differences with the seven previously identified species of the genus (see discussion below) we prefer to maintain a more conservative description regarding a possible new species, for the following reasons: I) few specimens found, which does not guarantee an adequate analysis in the description of a new species, although some species have been described based on few specimens before, and some others are still completely lost scientifically (e.g. *A. lateralis*, *A. indicum*); II) it was not possible performs the genetic analysis of the present specimens due to the previous fixation of the host, although genetic analyses of *Amphiorchis* species are scarce and only few 28S ribosomal RNA sequences of undetermined *Amphiorchis* spp. exist in GenBank (see Cribb *et al.*, 2016).
In this way we prefer to identify the two specimens found only at the genus level and being described in this note as *Amphiorchis* sp.

Remarks
Amphiorchis sp. differs from *A. amphiorchis*, *A. caborojoensis*, *A. indicus* and *A. lateralis* by the presence of a longer esophagus occupying the first third of body. Also, it can be distinguished by *A. amphiorchis*, *A. caborojoensis* and *A. solus* by having lobed testes. Additionally, differs from *A. solus* and *A. caborojoensis* for not having a caeca loop and a constriction at level of acetabulum.

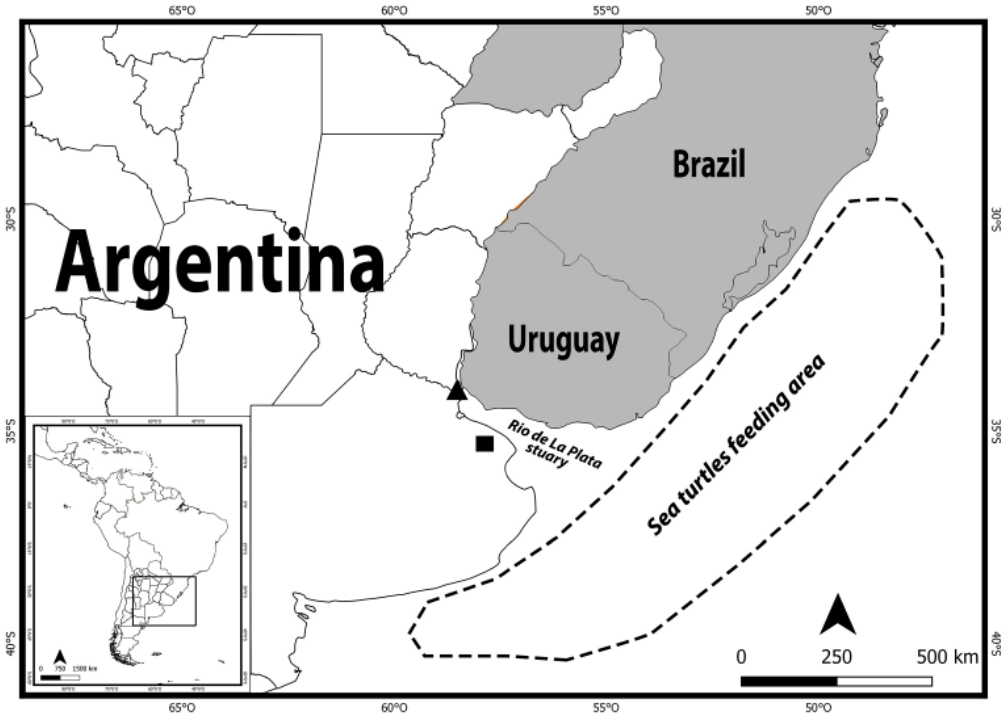

Fig. 3. South America map showing the new record of *Amphiorchis* sp. (black square), the record of *Dermochelys coriacea* within the estuary of the Rio de la Plata (black triangle), the estuary of the Rio de la Plata, and the feeding area of the marine turtles.

In addition, in the present specimens the vitellaria occupies both intracaecal and extracaecal fields in almost entire body, whereas in *A. amphiorchis* it is extracaecal after the posterior testicle (Price, 1934). *Amphiorchis* sp. differs from *A. lateralis* because caeca ends asymmetrically whereas they end symmetrically in *A. lateralis* (Oguro, 1938). The caeca extends beyond the testes in *Amphiorchis* sp. whereas they end immediately after the posterior testicle in *A. solus* (Simha & Chattopadhyaya, 1970). Present specimens differs from *A. caborojoensis* by having a considerable distance between the second testis and the end of body, meanwhile in *A. caborojoensis* it is very close to the posterior end (Fischthal & Acholonu, 1976). *Amphiorchis* sp. possess the genital pore lateral to the ovary, and the caeca end in an asymmetrical position, in contrast in *A. indicum* the genital pore is posterior to the ovary and the caeca end symmetrically (Simha & Chattopadhyaya, 1978). Despite *Amphiorchis* sp. shares the same asymmetry in the length of the caeca than *A. indicus*, the former has less dense vitellaria and have not so voluminous posterior testis. The testes in *Amphiorchis* sp. are located in the posterior half of body, whereas in *A. indicus* the testes are located near to the caecal bifurcation and the anterior testis is clearly located in the anterior half of body (Mehrotra, 1973; Gupta & Mehrotra, 1981). Finally, *Amphiorchis* sp. differs from *A. stacyi* because the vitelline follicles begin at the level of the caecal bifurcation and they are smaller than those in *A. stacyi*. Also, in present specimens the caeca are asymmetric, whereas in *A. stacyi* they are symmetric (Werneck & Greiner, 2018).

Discussion

Considering that the genus *Amphiorchis* had only been registered for sea turtles since its description by Price (1934) (see table 1), the finding of these specimens in a freshwater turtle is very striking. The Martin stream flows to the Rio de La Plata, which forms a large estuary (see Fig. 3) with mixtures of fresh and salt waters and flows into the sea at the north of Buenos Aires province, which is a path of constant exchange between the two environments (Guerrero, 1998). There are records of sea turtles in the rivers that flow into the Rio de la Plata (i.e. *Dermochelys coriacea* (Vandelli), and also there are records of *H. tectifera* specimens in estuarial environments with marine influence areas near Buenos Aires province coast (Bona *et al.*, 2009; Carman *et al.*, 2011). This point of contact could be one of the ways in which marine parasite species diverged in freshwater species, gradually adapting to the physical and chemical changes that occur in this transition, differentiating and conquering empty niches.

The presence of *Amphiorchis* sp. in *H. tectifera* represents the first record of the genus in a species of freshwater turtle and extends the geographic range since it was not registered in any marine species near the Argentine coasts.

The features of specimens here describe would indicate the presence of a new species of *Amphiorchis*. However, it is necessary to found more and well preserved mature specimens for could be able to corroborate this hypothesis.

Amphiorchis sp. here found is smaller in size than other species in the genus. It could be explained by the size of the host, given that *H. tectifera* does not exceed 35 cm, whereas the sea turtles are bigger. It is known that there is a positive correlation between the body size of host and length of parasite (Poulin, 2007).

Although it is possible to hypothesize that the presence of *Amphiorchis* sp. in a freshwater turtle is accidental, the fact that the cercarie actively infects its definitive host and that, despite not having eggs, the specimens found were mature, it is feasible to propose that *H. tectifera* acts as definitive host of this *Amphiorchis* species. In *H. tectifera* there were reported four species of digeneans, *Cheloniodiplostomum testudinis* (Dubois, 1936) and *Cheloniodiplostomum argentinensis* Palumbo and Diaz, 2018 in Argentina, *Nematophila grandis* (Diesing, 1839) in Paraguay, and *Pseudotelorchis devincenzii* (Catto and Amato, 1993) and *Telorchis platensis* Mane-Garzon and Gil, 1961 in Uruguay (Fernandes & Kohn, 2014; Palumbo & Diaz, 2018). The presence of *Amphiorchis* sp. in *H. tectifera* represents the sixth digeneans species reported for this turtle species being the third in Argentina.

Ethical Approval

All animals were cared in accordance with the Guidelines for use of live amphibians and reptiles in field and laboratory research (Am. Soc. Ichth. and Herpt., 1987).

Conflict of Interest

Authors state no conflict of interest.

Acknowledgements

We would like to thank Leandro Alcalde (ILPLA) for providing the host. This research was partially funded by UNLP (N828 to JD).

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