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Research Note

Report of *Neospirochis schistosomatoides* Price 1934 (Digenea: Spirorchiidae) infecting a Green Turtle, *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae), from Brazil

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Summary

We report the occurrence of *Neospirochis schistosomatoides* Price 1934 in a juvenile green turtle (*Chelonia mydas* L.1758) from the Brazilian coast. This species has been reported only in green turtles from the USA, Bermuda and Australia. Only two entire specimens and parts of six worms were found in the host's heart. The collection, identification and morphometric data of intact specimens of *N. schistosomatoides* are rare and restricted to the original description. This paper presents the first report of *N. schistosomatoides* in green turtles from the western South Atlantic Ocean.

Keywords: Brazil; *Chelonia mydas*; Digenea; Green turtle; *Neospirochis schistosomatoides*; Spirorchiidae

Introduction

The genus *Neospirochis* was erected by Price 1934 (type species *N. schistosomatoides* Price 1934), and corresponds to one of ten genera included in the Family Spirorchiidae that are exclusive to sea turtles (Smith, 1997; Platt, 2002).

Only two species are currently accepted in this genus: *N. schistosomatoides* with the first report in a green turtle (*Chelonia mydas* L. 1758) maintained in the National Zoo, Washington, D.C. (Price, 1934) and *Neospirochis pricei* Manter & Larson, 1950, described originally in loggerhead turtles (*Caretta caretta* L. 1758) from Florida – USA (Manter & Larson, 1950).

This note describes the occurrence of *N. schistosomatoides* collected from a juvenile green turtle found on the coast of Brazil.

Materials and Methods

In October 2013, a *C. mydas* (47.7 cm curved carapace length and 7.0 kg) was found at Cacimbas Beach (northern limit 39° 41'

33.4752°S and 19° 20' 18.6354°W and Southern limit: 39° 48' 39.5784° S and 19° 38' 30.9114°W) Linhares, Espírito Santo, Brazil. This animal died in a rehabilitation tank and was kept frozen until necropsy. The heart was examined using the method described by Snyder and Clopton (2005) and simplified by Werneck *et al.* (2006). Only two entire specimen of *N. schistosomatoides* and parts of others six were found. The helminth were fixed in alcohol-formalin-acetic acid solution, stained with carmine and cleared with eugenol. The morphometric data were determined using a microscope Nikon Eclipse 80i and the software NIS – Elements – BR. The helminths collected were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC number 36739) Rio de Janeiro, Brazil. Parasite analysis was authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1).

A taxonomic key by Platt (2002) and the original descriptions of the two species (Price, 1934 and Manter & Larson, 1950) were used for morphological and morphometric comparisons (Table 1).

Results and Discussion

Description (Fig. 1 and 2): Body elongated and slender and filiform; oral sucker terminal and small; esophagus long and sinuous followed by caecal bifurcation; caeca fuse near the testicular region and continue as a single caecum to the posterior region of the body (Fig. 2D). Testis single, large with appearance of a coiled tube occupying the area from near the caecal union to ovary (Fig. 2B). Cirrus sac small, occupying the lateral side of the uterus. Ovary coiled posteriorly (Fig. 2A); Mehlis' gland posterior to ovary; Small vitelline follicles occupying the region from caecal bifurcation to the ovary; Vitelline reservoir oval near the posterior end of the body (Fig. 2C). Excretory vesicle "Y"- shaped. Eggs rounded without lateral processes (Fig. 2E).

Previous records: Blood vessels in *C. mydas* from USA (Price, 1934), Bermuda (Rand & Wiles, 1985), Australia (Gordon *et al.*, 1998) and heart from Brazil (Present report)

Fig. 1. *Neospororchis schistosomatoides* Price 1934 (Digenea: Spirorchidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil, ventral view. Bar= 1 mm

Remarks

The genus *Neospororchis* was created to house the species *N. schistosomatoides*, collected from the blood vessels of *C. mydas* by Price (1934). Subsequently Manter and Larson (1950) described *N. pricei* in *C. caretta* from Florida. These are the only two species described in the genus.

The eggs of *Neospororchis* spp. are responsible for causing damage to sea turtles (Rand & Wiles, 1985; Santoro *et al.*, 2007; Stacy *et al.*, 2010; Chen *et al.*, 2012). These reports were based on generic identification using the characteristics of the egg shape (i.e., rounded eggs – see Wolke *et al.*, 1982).

Some authors report the difficulty of finding intact specimens (possibly due to the long, slender body which results in the helminth breaking quite easily), moreover, in some situations the helminthes are deeply enmeshed in the tissues which makes it difficult or even impossible to obtain intact individuals (Stacy *et al.*, 2010; Chen *et al.*, 2012.). This difficulty is reflected in the lack of morphometric information which is currently restricted to the original descriptions (see Price, 1934; Manter & Larson, 1950).

Manter & Larson (1950) reported the difference between *N. pricei* and *N. schistosomatoides* as: (1) a shorter oesophagus; (2) more anterior extent of testis; (3) a longer, more slender, thicker-walled cirrus sac; (4) a short, inconspicuous vas deferens; (5) a distinct, tightly spiraled posterior portion of the uterus separated from an anterior sac-like portion; and (6) a ventral genital pore (this could be related to compression).

The specimens examined in the present study have a longer esophagus and as shorter and thicker cirrus sac than reported in the original description of *N. schistosomatoides*. The testis occupies a more posterior location in the body, and the uterus is more highly coiled. These characteristics are similar to the specimens of *N. schistosomatoides* described by Price (1934), but our specimens are larger than Price's, have a longer esophagus and have a similar sized oral sucker, and the uterine eggs (Table 1) The eggs found during the present study were clearly deformed (Fig. 2E) possibly caused by the host's freezing and/or due to sample processing.

In our specimens we could not observe the vas deferens or the lateral position of genital pore as reported by Price (1934), but the host analyzed in the present study had been frozen which could explain the morphological variation of the *N. schistosomatoides* collected.

An important point was the number of eggs inside the uterus. Price (1934) reported 7 – 15 eggs as compared to Manter & Larson (1950) who estimated the presence of 200 eggs in the uterus of *N. pricei*. Our specimens also had relatively few eggs in uterus similar to the original description of *N. schistosomatoides* (Fig. 2E). There are few reports of *N. schistosomatoides*, that is exclusive to green turtles and has a geographic distribution in: USA (Price, 1934), Australia (Gordon *et al.*, 1998), Bermuda (Rand & Wiles, 1985) and Brazil (present report).

This work presents the first report of *N. schistosomatoides* in a green turtle in the Brazilian coast. We also provide additional morphometric data of this parasite to augment the original description.

Fig 2. *Neospirochis schistosomatoides* Price 1934 (Digenea: Spirorchidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil (A) Coiled ovary (black arrow) in (scale bar= 100 µm); (B) Coiled testis (black arrow) (scale bar= 100 µm); (C) Vitelline reservoir (black arrow) with oval shape (scale bar= 100 µm); (D) Posterior caeca fusion (asterisk) (scale bar= 100 µm); (E) Uterus containing deformed eggs (scale bar= 50 µm)

Table 1. Morphometric data of *Neospororchis schistosomatoides* Price 1934 from *Chelonia mydas* (Testudines, Cheloniidae). Data are presented in micrometers unless otherwise indicated

Locality	Price (1934)	Present report
	USA	Brazil
Number of parasites	?	2 entire (and parts of 6 others)
Site of infection	Visceral blood vessels	Ventricle of the heart
Body length (mm)	7.45 – 9.5	10.046 – 10.552 (10.299 ± 0.357)
Body width	140 – 220	228 – 237 (233 ± 6.3)
Oral sucker (diameter)	32 – 40	38.8 – 39 (39 ± 0.1)
Esophagus length	595 – 680	605 – 766 (686 ± 113.5)
Esophagus width	-	62 – 106 (84 ± 30.8)
Cirrus sac length	-	263 – 428 (346 ± 83.2)
Cirrus sac width	-	28 – 43 (37 ± 6.4)
Testis width	-	66 – 162 (98 ± 29.9)
Ovary width	-	40 – 72 (61 ± 18.3)
Cecae width	-	29 – 59 (44 ± 11.2)
Yolk reservoir length	-	75 – 152 (113 ± 38.1)
Yolk reservoir width	-	53 – 105 (77 ± 26.2)
Egg length	44	19 – 46.3 (36.36 ± 6.04) n=30
Egg width	32	19 – 33 (24.1 ± 4.7) n=8
Distance From:		
Genital pore to posterior end (mm)	1.0 – 1.2	0.204 – 0.790 (0.584 ± 0.329.1)
Uterus to posterior end	500	815

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