DE GRUYTER

DOI 10.1515/helmin-2015-0027

HELMINTHOLOGIA, 52, 2: 148 – 154, 2015

Research Note

Pathological changes by *Hapalotrema postorchis* Rao 1976 (Digenea: Spirorchiidae) in a green turtle *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil

M. R. WERNECK[1], G. B. SOUZA[2], B. C. BERGER[2], A. TRAZZI[2], R. B. RIBEIRO[3], M. A. SILVA[3], H. J. LEANDRO[3], E. C. Q. CARVALHO[3]

[1]BW Consultoria Veterinária, Rua Ponciano Eugênio Duarte n.º 203, Centro, Ubatuba, SP, CEP 11680 – 000, Brazil, E-mail: *max@bwvet.com.br*; [2]CTA – Serviços em Meio Ambiente, Rua Saturnino Rangel Mauro 283, Pontal de Camburi, Vitória, ES, CEP 29062 – 030, Brazil; [3]Laboratório de Morfologia e Patologia Animal (LMPA), Setor de Patologia Animal (SPA), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000 – Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro, CEP 28013 – 602, Brazil

Article info

Received October 21, 2014
Accepted December 9, 2014

Summary

This note reports the occurrence of *Hapalotrema postorchis* Rao 1976 collected from an aortic aneurysm in a green turtle (*Chelonia mydas* Linnaeus 1758) found on the coast of Brazil. Besides the vascular lesion, granulomatous reactions with foreign – body giant cells were found surrounding the parasite eggs in the heart, intestines, liver, pancreas, spleen, brain and kidneys. This paper presents the first report of *H. postorchis* and accompanying lesions in a green sea turtle from the western South Atlantic Ocean.

Keywords: Aneurism; *Chelonia mydas*; *Hapalotrema postorchis*; sea turtles; Spirorchiidae; spirorchiids lesions

Introduction

The genus *Hapalotrema* is one of 10 genera of parasites from the family Spirorchiidae that are exclusive to sea turtles (Smith, 1997; Platt, 2002). Looss (1899) erected the genus (type species *Hapalotrema constrictum* Looss 1899) based on specimens collected from *Thalassochelys corticata* (*Caretta caretta* Linnaeus 1758) in Egypt (see Platt & Blair, 1996). There are currently four species accepted for this genus: *H. synorchis* Luhman, 1935, *H. mehrai* Rao, 1976, *H. mistroides* (Monticelli, 1896) and *H. postorchis* Rao, 1976 (Platt & Blair, 1998).

This note describes the occurrence of *H. postorchis* collected from an aortic aneurysm and tissue lesions in a juvenile green turtle (*Chelonia mydas* Linnaeus 1758) found on the coast of Brazil.

Materials and Methods

A moribund green sea turtle was found on the beach on São Mateus in the state of Espírito Santo, Brazil (18°45'18.33"S, 39º44'51.10"W) in February 2014, measuring 35.4 cm in curvilinear carapace length and weighing 3.2 kg. The turtle was emaciated and lethargic and died before treatment. The animal was kept refrigerated and necropsy was performed 24 hours after death.

Tissue samples of the aorta (aneurysm), heart, small intestine, liver, pancreas, spleen, brain and kidneys were collected for histological analysis. The samples were fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned (5 μm) and stained with hematoxylin and eosin (HE) for subsequent microscopic analysis. The nodules in the intestines, pancreas and spleen were dissected with the aid of needles and forceps. Spirorchiids eggs were analyzed under a stereomicroscope and morphologically classified following the method described by Wolke *et al*. (1982). The eggs were then mounted on temporary slides and measured using an optical microscope and an image analysis program (ImageJ, National Institutes of Health).

Specimens of *H. postorchis* were washed in buffer (Cacodylate 0.1M, pH 7.4), dried using a 030 Critical Point Dryer, dehydrated in a graded ethanol series (70 to 100 °GL) for twenty minutes during each step, dried in critical – point drying chamber, coated with palladium and examined. The specimens were placed in an SDC 050 Sputter Coater and observed using a scanning electron microscope (Zeiss DSM – 962) with an accelerating voltage of 15 Kvolts.

The parasites were placed in saline solution and fixed in 70 % alcohol, stained with carmine and cleared with eugenol. The morphometric data were determined with the aid of an image analysis program (ImageJ, National Institutes of Health). Drawings were made with the aid of a drawing tube. The specimens were depo-

sited in the Helminth Collection of the Institute of Biosciences of the Paulista State University, state of São Paulo, Brazil (CHIBB 7458).

Taxonomic keys for genera (Platt, 2002) and species (Platt and Blair, 1998) were used for the characterization of the specimens. Morphological and morphometric data from studies by Rao (1976), Dailey *et al*. (1993), Cribb and Gordon (1998) and Werneck *et al*. (2014) were used for purposes of comparison (Table 1).

Table 1. Morphometric data of *Hapalotrema postorchis* Rao 1976 (Digenea: Spirorchiidae) from marine turtles. Data are presented as range (mean) and measurements in millimeters (mm) or micrometers (µm) as indicated

	Rao (1976)	Dailey *et al*. (1993)	Cribb and Gordon (1998)	Werneck et al. (2014)	Present report
Host	*C. mydas*	*C. mydas*	*C. mydas*	*E. imbricata*	*C. mydas*
Site	Heart	Heart	Aortas	Heart	Heart
	1	10	8	1	6 (3 measured)
Locality	India	Hawaii	Australia	Brazil	Brazil
Body length (mm)	12.7	10.3 – 12.8 (11.6)	5.8 – 10.0 (8.3)	6.8	14.8 – 21.9 (19.4)
Body width (mm)	1.8	1.0 – 1.8 (1.3)	0.7 – 1.1 (0.9)	0.6	0.920 – 1.6 (1.3)*
Oral sucker length (µm)	360	230 – 330 (250)	186 – 199 (193)	202	145 – 511 (365)
Oral sucker width (µm)	330	240 – 360 (275)	180 – 244 (211)	195	168 – 436 (322)
Ventral sucker length (µm)	710	420 – 910 (714)	334 – 488 (424)	307	295 – 861 (624)
Ventral sucker width (µm)	620		321 – 449 (396)	303	355 – 786 (571)
Esophagus length (µm)	870	810 – 870 (832)	385 – 616 (489)	695	911 – 1,374 (1,192)
No. testes anterior to ovary		7 – 11	6 – 7 (7)	8	6 – 8 (7)
No. testes posterior to ovary		6 – 9	8 – 8 (8)	8	6 – 8
Total testes		15 – 18 (17)	14 – 15 (15)	16	13 – 16
Testes length (µm)		430 – 620 (496)		121– 279 (170)	213 – 789 (444)
Testes width (µm)		280 – 420 (372)		161 – 304 (245)	295 – 847 (586)
Cirrus sac length (µm)	290	710 – 900 (840)	225 – 315 (280)	144 – 289	385 – 764 (637)
Cirrus sac width (µm)	83	240 – 310 (285)	71 – 112 (89)	97 – 136	112 – 228 (165)
Extenal seminal vesicle length (µm)		480 – 670 (590)		119	271 – 577 (457)
Extenal seminal vesicle width (µm)		250 – 380 (310)		231	436 – 775 (619)
Ovary length (µm)	620	710 – 870 (775)	321 – 629 (497)	401 – 420	557 – 883 (769)
Ovary width (µm)	460	470 – 680 (618)	411 – 488 (449)	283 – 293	391 – 754 (573)
Eggs length (µm)	330	140 – 330 (168)		–	150 – 216 (184)+
Eggs width (µm)	41	29 – 41 (32)		–	26 – 45 (35)

*On the genital pore level, +without including polar process

Results and Discussion

During necropsy dark nodules measuring approximately 1 mm in diameter were found on the serous surface of the small (Fig. 1) and large intestines, pancreas and spleen. An aortic aneurysm measuring approximately 0.5 cm in diameter (Fig. 2) was found near the heart, which, when opened, revealed a yellowish caseous material adhered to the wall of the artery containing six non fixed specimens of *H. postorchis* (Figs. 3 and 4). The nodules in the intestines, pancreas and spleen contained amber – colored eggs with an elongated shape and a filament at each extremity. Egg size ranged from 136 to 272 µm (mean: 199 µm) in length and 35 to 55 µm (mean: 43 µm) in width. The eggs were classified as Type I (see Wolke *et al*., 1982).

The histopathological analysis of the liver, kidneys, spleen and brain tissue revealed foreign – body giant cell granuloma surrounding the clusters of parasite eggs. Clusters of eggs were also found in the submucosa tunica of the small intestine and the pancreas, causing a granulomatous response (Fig. 5). The aortic aneurysm exhibited thinning of the arterial tunica media and parasite eggs in the arterial lumen, with a moderate giant cell granulomatous inflammatory response (Fig. 6).

The genus *Hapalotrema* currently has four species: *H. synorchis* (syn. *H. orientalis*) *H. mehrai* (syns. *H. dorsopora* and *H. pambanensis*) *H. mistroides* (syn. *H. lossi*) and *H. postorchis* (Platt & Blair, 1998). A list of *Hapalotrema* species, hosts, geographic locations and references is presented in Table 2.

Hapalotrema postorchis was first described by Rao (1976) based on ten specimens found in the heart of *C. mydas* in Pamban in the Gulf of Mannar (southern India). This parasite was subsequently found in the same host in USA (Dailey *et al*., 1993; Stacy *et al*., 2010), Australia (Platt & Blair, 1998; Gordon *et al*., 1998; Cribb &

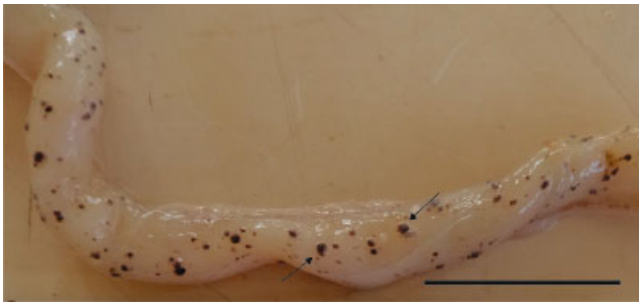

Fig 1. Nodular lesions (arrows) on small intestine serosa caused by *Hapalotrema postorchis* Rao, 1976 eggs; Bar = 3.0 cm

Gordon, 1998), Costa Rica (Santoro *et al*., 2007), Taiwan (Chen *et al*., 2012) and Brazil (present report) as well as in *E. imbricata* in Brazil (Werneck *et al*., 2014). This paper reports the first occurrence of *H. postorchis* in *C. mydas* from the western South Atlantic Ocean

Gordon *et al*. (1998) report the occurrence of an aneurysm associated with the presence of parasites in 96 stranded *C. mydas* from southeastern Queensland, Australia, and the authors reported the occurrence of *H. postorchis* in the left and right aorta.

Santoro *et al*. (2006) report *H. postorchis* in 20 % (8/47) of nesting green sea turtles in Costa Rica, with a mean infection intensity of 19.9 ± 45.4 (range: 1 to 132 specimens). Santoro *et al*. (2007) report the association between *H. postorchis* and cases of aneurysm in two sea turtles (4.2 %), large vessel arteritis, thromboses and hemorrhagic lesions in eight sea turtles (17.0 %) as well as mural endocarditis and vasculitis in one sea turtle (2.1 %). The same authors also report thickening of the arterial wall in cases of aneurysm, with the formation of yellowish, cream – colored plaques where *H. postorchis* acetabular structures were deeply rooted in the vessel walls.

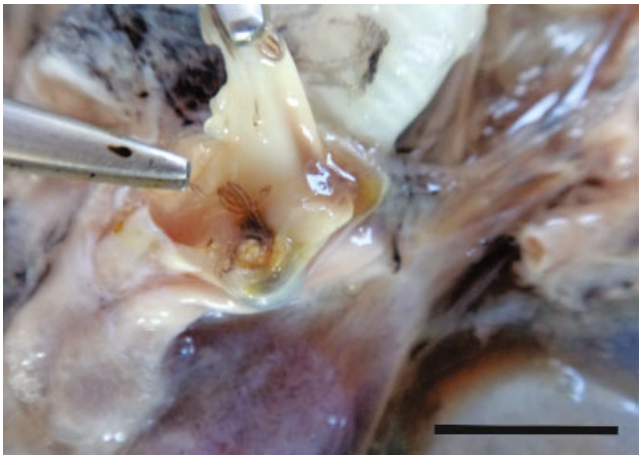

Fig 3. *Hapalotrema postorchis* Rao, 1976 specimens found in aneurism; Bar = 1.5 cm

Analyzing 50 individuals of *C. mydas* in the state of Florida (USA), Stacy *et al*. (2010) report the occurrence of *H. postorchis* in only one individual (2.0 %), which exhibited thickening of the aortic wall associated with chronic inflammation. Analyzing 30 individuals of *C. mydas* stranded on the shore of Taiwan between 2007 and 2010, Chen *et al*. (2012) found spirorchidiasis in 21 turtles (70 %) and adult parasites in 14 (44 %), the majority of which (85 %) were found in the heart and large blood vessels, with *H. postorchis* present in 46 % (6/13). The inflammatory response to spirorchiid eggs consisted of lymphocytes, macrophages and giant cells generally surrounding one to three eggs, except in the spleen, where the number of eggs ranged from 10 to 30.

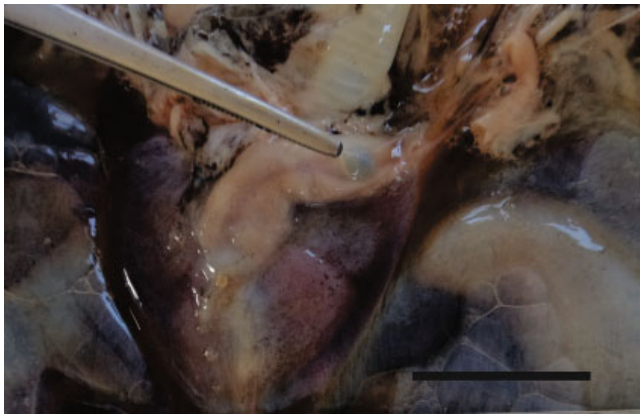

Fig 2. Aneurysm in aorta in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil; Bar = 3.0 cm

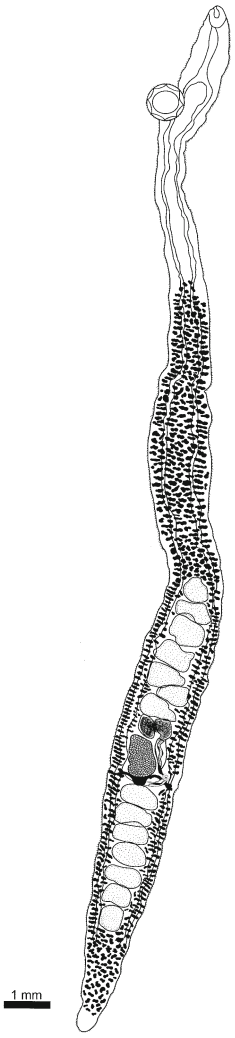

Fig 4. *Hapalotrema postorchis* Rao, 1976 (Digenea: Spirorchiidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil, ventral view

Table 2. *Hapalotrema* species reported in sea turtles

Host	Species	Locality	Reference
Caretta caretta	*Hapalotrema mistroides*	Italy	Monticelli (1896)
		Egypt	Looss (1899); Looss (1902)
		Australia	Platt & Blair (1998)
		USA	Stacy et al. (2010); Greiner (2013)
	Hapalotrema synorchis	Florida	Luhman (1935)
		Australia	Platt & Blair (1998)
Chelonia mydas	*Hapalotrema mehrai*	India	Rao (1976); Gupta & Mehrotra (1981).
		USA	Dailey et al. (1993); Dailey & Morris (1995); Graczyk et al. (1995); Aguirre et al. (1998); Stacy et al. (2010).
		Australia	Platt & Blair (1998); Crib & Gordon (1998); Gordon et al. (1998).
		Taiwan	Chen et al. (2012)
	Hapalotrema mistroides		Gohar (1934), Gohar (1935),
	Hapalotrema postorchis	India	Rao (1976)
		USA	Dailey et al. (1993)
		Australia	Platt & Blair (1998); Crib & Gordon (1998); Gordon et al (1998).
		Costa Rica	Santoro et al. (2006)
		Taiwan	Chen et al. (2012)
		Brazil	Present report
Eretmochelys imbricata	*Hapalotrema mehrai*	Australia	Platt & Blair (1998)
	Hapalotrema postorchis	Brazil	Werneck et al. (2014)
	Hapalotrema synorchis	Puerto Rico	Fichthal & Acholonu (1976)

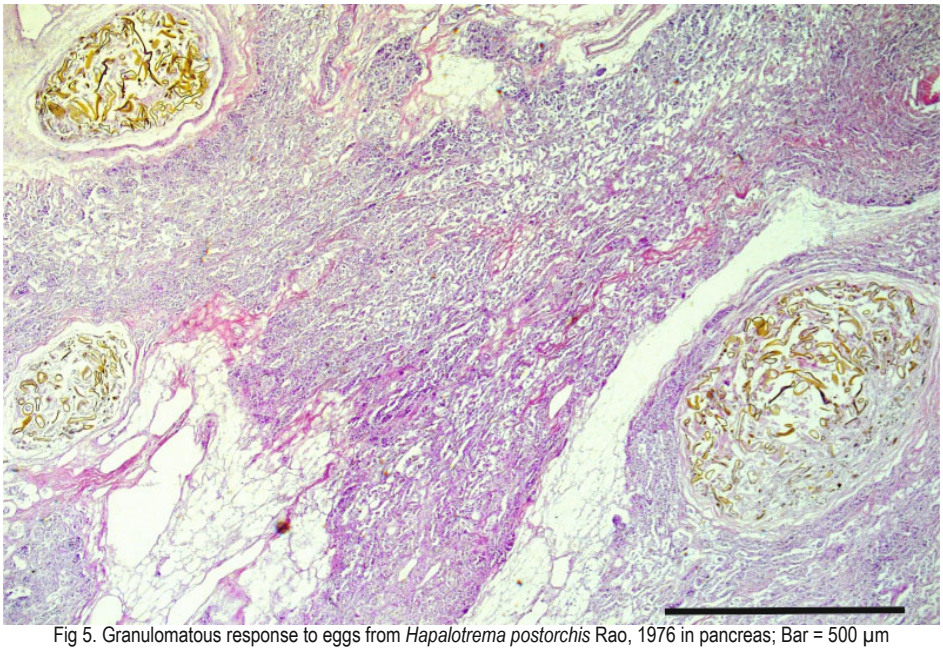

Fig 5. Granulomatous response to eggs from *Hapalotrema postorchis* Rao, 1976 in pancreas; Bar = 500 µm

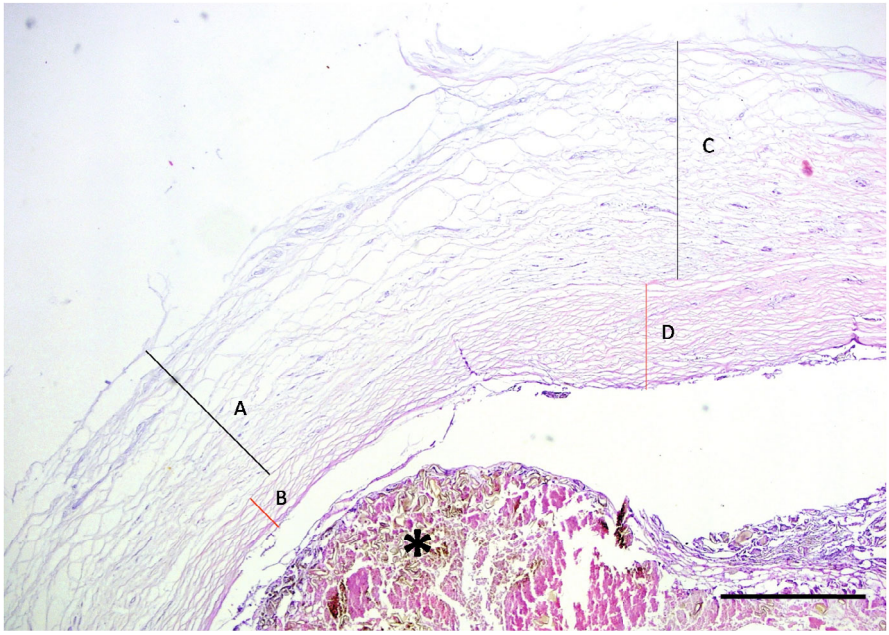

Fig 6. Arterial wall aneurysm (A = serosa artery tunica 602.4 µm in width; B = artery tunica media 139.6 µm in width) and normal artery wall (C = serosa artery tunica 816.7 µm in width; D = artery tunica media 359.7 µm in width). *Hapalotrema postorchis* eggs with moderate giant cell granulomatous inflammatory response (asterisk); Bar = 500 µm

Although the specimens analyzed in the present study had a larger total length, oral sucker, ventral sucker, testicles, ovary, external seminal vesicle and eggs width in comparison to previously published data (Table 1), the amount and arrangement of the testicles and positioning of the genital pore are compatible with *H. postorchis* (see Platt and Blair, 1998) and such variations are likely within the range of the species.

According to Wolke *et al*. (1982), Type I eggs are characteristic of the genera *Learedius, Monticellius* and *Hapalotrema*. Moreover, the measurements of the eggs from the specimens analyzed and those found in the tissues of the present study are compatible with *H. postorchis* (Table 1).

The impact of parasitosis by members of the family Spirorchiidae results in damage due to the depositing of eggs in the blood stream and consequent embolism and accumulation in tissues, causing inflammatory lesions (Santoro *et al*., 2007; Stacy *et al*., 2010; Chen *et al*., 2012) that can contribute to or even cause the death of the host (Gordon *et al*., 1998).

The occurrence of members of the family Spirorchiidae on the Brazilian coast has been reported in *C. mydas* (Werneck *et al*., 2013) and *E. imbricata* (Werneck *et al*., 2008; Dutra *et al*., 2012; Werneck *et al*., 2014; Werneck *et al*., 2015).However, the analysis of the impact of parasitosis on the sea turtle in the region has been limited.

Fig 7. Scanning electron microscopy showing tegument spines (arrow) on anterior end of *Hapalotrema postorchis* Rao, 1976 (Digenea: Spirorchiidae). Bar = 200 µm

Analyzing 11 juveniles *C. mydas* found in the city of Ubatuba on the coast of the state of São Paulo (Brazil), Werneck *et al*. (2006) report dark nodules measuring 1 to 2 mm in diameter containing spirorchiid eggs on the serous surface of the large and small intestines in only one host (10 %). Werneck *et al*. (2008) also report the occurrence of dark nodules measuring 1 to 2 mm in diameter in the small and large intestine of a *E. imbricata* juvenile found in the state of São Paulo, which, when opened and examined under a stereomicroscope, exhibited spirorchiid eggs. Dutra *et al*. (2012) report chronic inflammatory reactions due to spirorchiid eggs in the small intestine, lungs, heart and liver in another *E. imbricata* juvenile found on the coast of the state of São Paulo. Goldberg *et al*. (2013) report lesions caused by spirorchiid eggs in the lungs of a specimen of *C. mydas* found in the city of Florianópolis (state of Santa Catarina). Lesions caused by spirorchiid eggs have also been found in the liver of the same host in the state of Espírito Santo (Werneck *et al*., 2015a).

Hapalotrema postorchis is a relatively large parasite that seems to have a predisposition for occupying arterial spaces, as evidenced in the majority of studies (Gordon *et al*., 1998; Santoro *et al*., 2007; Stacy *et al*., 2010; present report). Moreover, this parasite has spines covering the entire tegument (Fig.7). It is possible that the constant mechanical trauma caused by these spines leads to continuous irritation of the vessel walls and induces an inflammatory response (arteritis), which, when associated with blood pressure, predispose the host to aneurysm.

The histopathological analysis of all tissues examined in the present study revealed the occurrence of granuloma, with eggs surrounded by macrophages and giant cells. This finding is in agreement with data reported in previous studies conducted in different parts of the world (Gordon *et al*., 1998; Santoro *et al*., 2007; Stacy *et al*., 2010; Chen *et al*., 2012).

Spirorchiids appear to be an important cause of debility (Stacy *et al*., 2010) and death in sea turtles (Gordon *et al*., 1998). Despite the evident tissue damage caused by *H. postorchis* eggs in the present study, it was not possible to determine that death was caused by spirorchidiasis. However, parasitosis is believed to have been an important debilitating factor that contributed to the stranding and death of the host.

Acknowledgements

The authors wish to express their special thanks to Dr. Thomas Platt for help in the *Hapalotrema* distribution and Dr. Paula Baldassin for critical reading and suggestions to improve the manuscript and Dr. João Almeida for SEM images. Biological samples were obtained through the Beach Monitoring Project of the state of Espírito Santo and northern portion of the Campos Basin, which is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA). Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600 – 1 and 9329 – 1).

References

Aguirre, A., Apraker, A., Balazs, T.R., Zimmerman, G. H. (1998): Spirorchiidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. *J. Wildl. dis.*, 34(1): 91 – 98. DOI: 10.7589/0090-3558-34.1.91

Chen, H., Kuo, R.J., Chang, T.C. , Hus, C.K., Bray, R.A., Cheng, I.J. (2012): fluke (Spirorchiidae) infections in sea turtles stranded on Taiwan: prevalence and pathology. *J. Parasitol.*, 98(2): 437 – 439. DOI: 10.1645/GE-2875.1

Cribb, T.H., Gordon, a.n. (1998): *Hapalotrema* (Digenea: Spirorchiidae) in the green turtle (*Chelonia mydas*) in Australia. *J. Parasitol.*, 84(2): 375 – 378

Dailey, M.D., Fast, M.L., Balazs, G.H. (1993): *Hapalotrema dorsopora* sp. n. (Trematoda: Spirorchiidae) from the heart of the green turtle (*Chelonia mydas*) with a redescription of *Hapalotrema postorchis*. *J. Helminthol. Soc. Wash.,* 60(1): 5 – 9

Dailey, M.D., Morris, R. (1995): Relation of parasites (Trematoda: Spirorchiidae) and their eggs to the occurrence of fibropapillomas in the green turtles (*Chelonia mydas*). *Can. J. Fish. Aquat. Sci.*, 52(Suppl. 1): 84 – 89

Dutra, G.H.P., Silva, A.N.E., Nascimento, C.L., Werneck, M.R. (2012): Lesões macroscópicas e histopatológicas da infecção por helmintos da Família Spirorchiidae em *Eretmochelys imbricata* Linnaeus 1758 (Testudines, Chelonidae): relato de um caso no litoral brasileiro. *Nat. Resour.*, 2(1): 83 – 89. DOI: 10.6008/ESS2237-9290.2012.001.0006

Fischthal, J.H., Acholonu, A.D. (1976): Some digenetic trematodes from Atlantic Hawksbill turtle E*retmochelys imbricata imbricata* (L.) from Puerto Rico. *J. Helminthol. Soc. Wash.*, 43: 174 – 185

Gohar, N. (1934): Liste des trematodes parasites et de leurs hôtes vertebrés signalés dans la vallée du Nil. *Ann. Parasitol. Hum. Comp.*, 12: 322 – 331

Gohar, N. (1935): Liste des trematodes parasites et de leurshôtes vertebrés signalésdans la vallée du Nil. *Ann. Parasitol. Hum. Comp.*, 13: 80 – 90

Goldberg, D.W., Stahelin, G.D., Cegoni, G.D., Wanderlinde, C.T., Paes e Lima, E., Medina, R.M., Ribeiro, R.B., da Silva, M.A., Carvalho, E.C.Q. (2013): Case report: Lung Spirorchidiasis in a Green Turtle (*Chelonia mydas*) in Southern Brazil. *Mar. Turtles Newslett.*, 139: 14 – 15

Gordon, A.N., Kelly, W.R., Cribb, T.H. (1998): Lesions caused by cardiovascular flukes (Digenea: Spirorchiidae) in stranded green turtles (*Chelonia mydas*). *Vet. Pathol.*, 35(1): 21 – 30

Graczyk, T.K., Aguirre, A A., Balazs, G H. (1995): Detection by ELISA of circulating anti – blood flukes (*Carettacola*, *Hapalotrema*, and *Learedius*) immunoglobulins in Hawaiian green turtles (*Chelonia mydas*). *J. Parasitol.*, 81: 416 – 421

Greiner, E.C. (2013): Parasites of marine turtles. In: Wyneken, J., Lohmann, K.J., Lutz, J.A. (Eds) *The biology of sea turtles.* Volume 3. USA: CRC Press, pp. 427– 446

Gupta, N.K., Mehrotra, V. (1981): On two blood flukes (trematoda) of the family Spirorchiidae Stunkard, 1921 from Indian marine turtles. *Acta Parasitol.*, 28(2): 11 – 20

Looss, A. (1899): Weitere Beiträge zur Kenntniss der Trematoden Fauna Aegyptens, Zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. *Zool. Jb-r. Abt. Syst.*, 12: 521 – 784

Looss, A. (1902): Ueber neue und bekannte Trematoden aus Seeschildkröten. Nebst Erörterungen zur Systematik und Nomencla-

tur. *Zool. Jb-r. Abt. Syst., Oekol. Geogr. Tiere*, 16: 411 – 894

Luhman, M. (1935): Two new trematodes from the loggerhead turtle (*Caretta caretta*). *J. Parasitol.*, 21: 274 – 276

Monticelli, F.S. (1896): Di unenatozoodella *Thalassochelys caretta* Linn. *Int. Mschr. Anat. Physiol. (Leipzig)*, 13: 141 – 172

Platt, T.R. (2002): Family Spirorchiidae Stunkard.In: Gibson, D.I., Jones, A., Bray, R.A. (Eds) *Keys to the Trematoda*. Volume 2. London, UK: CABI Publishing, pp. 453 – 467

Platt, T.R., Blair, D. (1996): *Hapalotrema* Looss, 1899 (Digenea): Proposed Designation of *H. loossi* Price, 1934 As the type species. *Bull. Zool. Nomencl.*, 53: 89 – 91

Platt, T.R., Blair, D. (1998): Redescription of *Hapalotrema mistroides* (Monticelli, 1896) and *Hapalotrema synorchis* Luhman, 1935 (Digenea: Spirorchidae), with comments on other species in the genus. *J. Parasitol.*, 84: 594 – 600

Rao, S.L. (1976): On two new blood flukes (Trematoda, Spirorchidae) from the heart of *Chelone mydas* L. *Acta Parasitol.*, 24: 119 – 124

Santoro, M., Greiner, e.c., Morales, j.a., Rodrígues – Ortiz, b. (2006): Digenetic trematode community in nesting green sea turtles (*Chelonia mydas*) from Tortuguero Nacional Park, Costa Rica. *J. Parasitol.*, 92(6): 1202 – 1206. DOI: 10.1645/GE-866R.1

Santoro, M., Morales, J.A., Rodriguez – Ortiz, B. (2007): Spirorchiidiosis (Digenea: Spirorchiidae) and lesions associated with parasites in Caribbean Green turtles (*Chelonia mydas*). *Vet. Rec.*, 161: 482 – 486. DOI:10.1136/vr.161.14.482

Smith, J.W. (1997): The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold – blooded vertebrates: Part 2. *Helminthol. Abst.*, 66: 329 – 344

Stacy, B.A., Foley, a.m., Greiner, e., Herbst, l.h., Bolten, a., Klein, p., Manire, C.A., Jacobson, E.R. (2010): Spirorchiidiasis in stranded loggerhead *Caretta caretta* and green turtles *Chelonia mydas* in Florida (USA): host pathology and significance. *Dis. Aquat. Org.*, 89: 237 – 259. DOI: 10.3354/dao02195

Werneck, M.R., Baldassin, P., Dazeredo, F., Trazi, A., Berguer, B. (2014): The Hawksbill Sea Turtle *Eretmochelysimbricata* Linnaeus 1758 (Testudines, Cheloniidae) as new host of *Hapalotrema postorchis* Rao, 1976 (Digenea: Spirorchiidae). *Comp. Parasitol.*, 81: 75 – 78. DOI: 10.1654/4662.1

Werneck, M.R., Baldassin, P., Torres, F., Trazi, A., Berguer, B. (2013): Report of *Carettacola stunkardi* (Martin & Bamberger, 1952) Dailey, Fast & Balazs, 1991 (Digenea: Spirorchiidae) infecting Green Turtle *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae) in Brazil. *Braz. J. Biol.*, 73(3): 675 – 676. DOI: 10.1590/S1519-69842013000300030

Werneck, M.R., Binoti, E., Martins, I.V.F., Calais Junior, A., Gomes, M.C., Boeloni, J.N., Trazi, A., Berger, B. (2015a): Occurrence of *Rhytidodoides similis* Price, 1939 (Digenea: Rhytidodidae) and lesions due to spirorchiid eggs in a Green Turtle *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae) from Brazil. *Comp. Parasitol.*, 82(2) (In Press)

Werneck, M.R., Becker, J.H., Gallo, B.G., Silva, R.J. (2006): *Learedius learedi* Price 1934 (Digenea, Spirorchiidae) in *Chelonia mydas* Linnaeus 1758 (Testudines, Chelonidae) in Brazil: case report. *Arq. Bras. Med. Vet. Zootec.*, 58(4): 550 – 555. DOI: 10.1590/S0102-09352006000400016

Werneck, M.R., Gallo, B., Silva, R.J. (2008): Spirorchiids (Digenea: Spirorchiidae) infecting a Hawksbill sea turtle *Eretmochelys imbricata* (Linnaeus 1758) from Brazil. *Arq. Bras. Med. Vet. Zootec.*, 60: 663 – 666. DOI: 10.1590/S0102-09352008000300021

Werneck, M.R., Souza, V.R., Trazi, A., Berger, B. (2015): *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) in a Hawksbill Turtle, *Eretmochelys imbricata* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil. *Comp. Parasitol.* (In Press)

Wolke, R.E., Brooks, R., George, A. (1982): Spirorchidiasis in loggerhead sea turtles (*Caretta caretta*): pathology. *J Wildl. Dis.*, 18(2): 175 – 185. DOI: 10.7589/0090-3558-18.2.175