

HELMINTHOLOGIA, 54, 4: 363 - 368, 2017

Research Note

Loggerhead turtle, *Caretta caretta* (Linnaeus, 1758) (Testudines, Cheloniidae), as a new host of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchidae) and associated lesions to spirorchid eggs

M. R. WERNECK^{1*}, C. NUNES², H. JERDY³, E. C. Q. CARVALHO³

¹BW Veterinary Consulting, Rua Ponciano Eugênio Duarte number 203, Centro, Ubatuba, SP, Zip code (CEP): 11680-000, Brazil.
*E-mail: maxrwerneck@gmail.com; ²CTA Serviços em Meio Ambiente, Rua Saturnino Rangel Mauro 283, Pontal de Camburi, Vitória, Zip code (CEP): 29062-030, Espírito Santo State, Brazil; ³North Fluminense State University – Darcy Ribeiro (UENF), Campos dos Goytacazes, Zip code (CEP): 28013-602, Rio de Janeiro State, Brazil

Article info

Received June 20, 2017
Accepted August 14, 2017

Summary

The present note describes the occurrence of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchidae) in an adult loggerhead turtle, *Caretta caretta* (Linnaeus, 1758), found on the coast of the state of Rio de Janeiro, Brazil. Pathological changes due to spirorchid eggs (type 1 and 3) were found in gastrointestinal system, endocrine system, and heart. This parasite has previously been described in the green turtle, *Chelonia mydas* (Linnaeus, 1758), from Pakistan, Brazil and Costa Rica as well as in the hawksbill sea turtle, *Eretmochelys imbricata* (Linnaeus, 1766), in Brazil. This note reports the loggerhead turtle as a new host for *M. indicum*.

Keywords: *Caretta caretta*; Cheloniidae; Loggerhead turtle, *Monticellius indicum*; Spirorchidae; spirorchid lesions

Introduction

Monticellius indicum Mehra, 1939 is a parasite of the family Spirorchidae that was originally described based on a single specimen found in the heart of a green turtle, *Chelonia mydas* (Linnaeus, 1758), in Pakistan (Mehra, 1939). Unfortunately, little is known about this species, as only 11 specimens have been studied morphologically (Mehra, 1939; Santoro *et al.* 2006, 2009; Werneck *et al.*, 2008; Werneck & Silva, 2015; Werneck *et al.*, 2015). To date, only four papers detailed on morphological features of *M. indicum* on the basis of specimens collected in green turtles from Pakistan (Mehra, 1939), Brazil (Werneck *et al.*, 2008) and Costa Rica (Santoro *et al.*, 2009) as well as a hawksbill turtle, *Eretmochelys imbricata* (Linnaeus, 1766), in Brazil (Werneck *et al.*, 2015). The loggerhead turtle, *Caretta caretta* (Linnaeus, 1758), is a known host for members of Spirorchidae represented by the genera *Carettacola*, *Hapalotrema*, *Neospororchis*, *Neocaballerotrema*, and *Shobanatrema shobanae* however the last two are considered

genera inquirenta (Smith, 1997). This note describes the first occurrence of *M. indicum* in an adult loggerhead turtle.

In October 2016, an adult female loggerhead turtle measuring 116 cm of curvilinear carapace length (CCL) and weighing 100 Kg was found stranded dead on the beach in the municipality of Guapimirim (Lat.: 22° 42' 32.832" S; long: 43° 5' 25.908" W) in the state of Rio de Janeiro, Brazil.

At necroscopy, no gross lesions were observed externally. The simplified method proposed by Werneck *et al.* (2006) was employed, which consists of an analysis of the heart, lungs, kidneys, spleen and liver as well as a washing of the body. A single specimen of *M. indicum* was found in the heart. The parasite was placed in a Petri dish, fixed in a 70 % alcohol solution, stained with carmine and cleared with eugenol. Morphometric data of the cardiovascular fluke were determined with the aid of a Nikon Eclipse 80i microscope (Kurobane Nikon Co., Ltd., Otawara, Tochigi, Japan) using the NIS Elements BR software program. Analyses of the parasite were authorized by federal licenses for activities with scientific pur-

* – corresponding author

poses (SISBIO 30600-1 and 9329-1). The helminth was deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC 38389) in the State of Rio de Janeiro, Brazil.

Tissue samples from the adrenal gland, brain, esophagus, stomach, gall bladder, heart, kidneys, liver, lungs, muscle, ovary, pancreas, spleen, small intestine, skin, thyroid gland, salt gland, eye and were fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned (5 µm) and stained with hematoxylin and eosin (HE) for subsequent analysis using an optical microscope. Parasite eggs were classified based on a previously described methodology (Wolke *et al.*, 1982).

Type 1 eggs are characterized as being from the genera *Learedius*, *Monticellius*, *Amphiorchis* and *Hapalotrema*. Type 3 eggs are characterized as being round and without tapering and are characteristic of the genus *Neospiorchis* (Wolke *et al.*, 1982). For parasite identification, the taxonomic key for genera proposed by Platt (2002), the original description (Mehra, 1939), the redescription by Santoro *et al.* (2009) and comments by Werneck *et al.* (2008 and 2015) were used for both morphological (Fig. 1) and morphometric comparisons (Table 1). Severity of histopathological lesions for each tissue followed the methods of Flint *et al.* (2010) modified by Santoro *et al.* (2017)

Description of parasite:

Parasite with anterior and posterior extremities tapered, oral sucker terminal, esophagus relatively long and somewhat sinuous, cecal bifurcation in anterior third of body and anterior to acetabulum; acetabulum nearly as large as width of parasite and positioned in anterior third of body, presenting small spines on its edge (Fig. 1A); testes with irregular shape, greater in width than length, totaling six, occupying area after acetabulum and external seminal vesicle; external seminal vesicle positioned transverse to body; cirrus sac relatively long and slightly sinuous, the trajectory of which runs to the posterior region of the body, following dorsally in area of ovary; ovary in posterior region of body near genital pore; excreting vesicle "Y" shaped; only one elongated egg, exhibiting lateral processes, process on one side has oval structure at end and other process small (Fig. 1B).

Host: *Caretta caretta* (Linnaeus, 1758)

Locality and collection date: municipality of Guapimirim (Lat.: 22° 42' 32.832" S; long: 43° 5' 25.908" W) state of Rio de Janeiro, Brazil, on October 22, 2016.

Site of infection: Heart

Prevalence and intensity of infection: one parasite in one host

Voucher specimens deposited: CHIOC number 38389

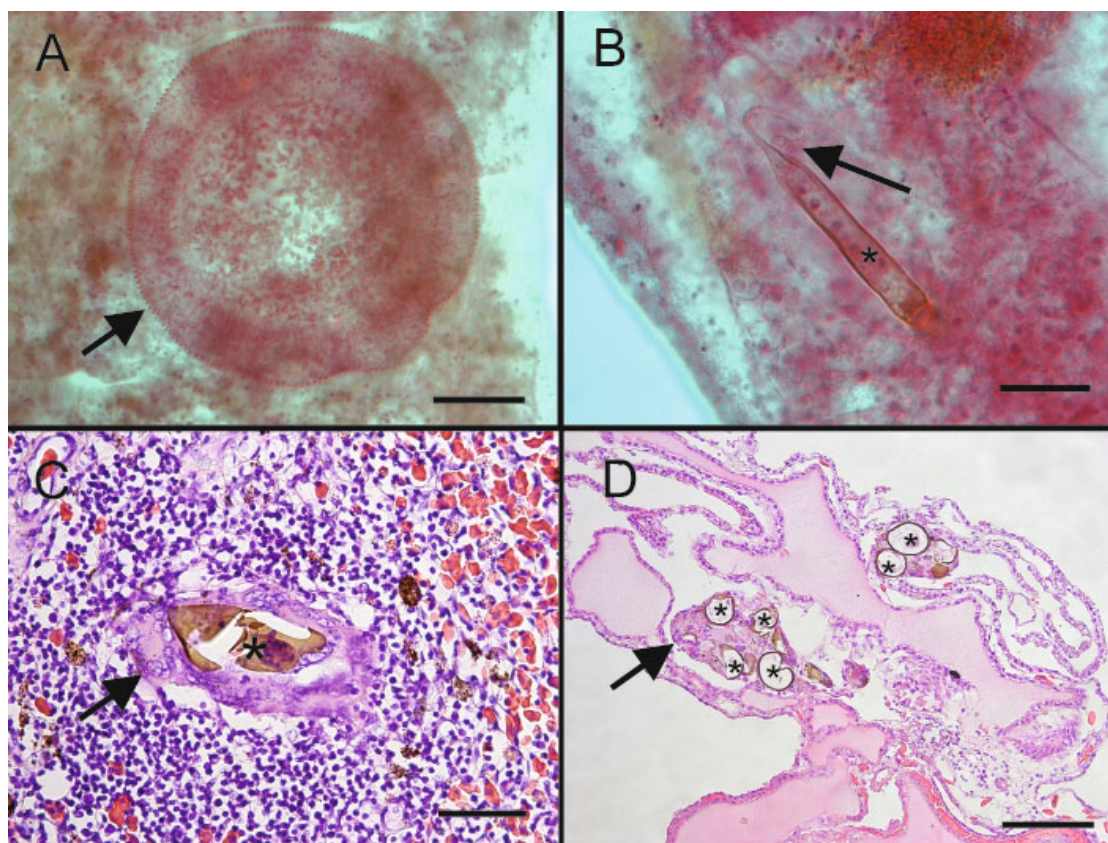


Fig. 1 *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchidae) found in *Caretta caretta* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil (A) Detail of spines on edge of ventral sucker (scale bar = 100 µm); (B) egg in uterus with one oval lateral process (black arrow) (scale bar = 50 µm). C – Spleen – Type 1 egg (asterisk) in center of periarteriolar lymphoid tissue surrounded by multinucleated giant cell (arrow) (scale bar = 50 µm). D – Thyroid gland – clusters of type 1 eggs (asterisk) in cross-sectional cut surrounded by multinucleated giant cells between thyroid follicles (arrow) (scale bar = 100 µm).

Table 1. Morphometric data of *Monticellius indicus* Mehra, 1939 (Digenea: Spirochidae), from marine turtles. Data presented as mean (range) are in micrometers.

Host	Mehra (1939)	Werneck et al. (2008)	Santoro et al. (2009)	Werneck et al. (2015)	Present Report
Locality	<i>C. mydas</i> Pakistan	<i>C. mydas</i> Brazil	<i>C. mydas</i> Costa Rica	<i>E. imbricata</i> Brazil	<i>C. caretta</i> Brazil
Site of infection	Heart	Heart	Heart ventricle	Heart	Heart
Number of parasites	1	3	6	1	1
Body length	3,000	4,535.7 (3,665 – 5,646)	3,098 (2,259 – 4,284)	3,260	5,149
Body width	400	390 (358 – 432)	351 (214 – 571)	450	753
Oral sucker length	144	132 (86 – 185)	162 (112 – 206)	160	202
Oral sucker width	148	172 (144 – 194)	127.5 (82 – 194)	120	180
Esophagus length	240	418 (338 – 513)	421 (309 – 586)	460	452
Esophagus width	–	–	–	30	152
Ventral sucker length	256*	239 (164 – 318)	241 (184 – 326)	210	401
Ventral sucker width	–	165 (313 – 194)	229 (122 – 326)	190	392
Cirrus sac length	720	713 (517 – 868)	728 (612 – 1,020)	710	834
Cirrus sac width	75	76 (66 – 94.1)	62 (52 – 74)	130	89
Ovary length	400	474 (450 – 717)	402 (306 – 541)	530	574
Ovary width	160	162 (122 – 220)	135 (74 – 235)	230	244

External seminal vesicle length	190	–	178 (136 – 222)	110	300
External seminal vesicle width	60	–	85 (70 – 111)	50	24
Number of testis	5	5	5–6 (5)	5	6
Testis length	(160–176) *	179 (110 – 227)	133 (74 – 268) <i>n</i> = 12	216 (180–260)	222.6 (192–293) <i>n</i> = 6
Testis width	–	187 (134 – 276)	167 (64 – 286) <i>n</i> = 12	238 (220–280)	303.5 (203–407) <i>n</i> = 6
Egg length	162	–	174 (168 – 180) <i>n</i> = 2	135 (120–150) <i>n</i> = 2	206; <i>n</i> =1
Egg width	21	–	22; <i>n</i> = 2	30; <i>n</i> = 2	18; <i>n</i> =1
Distance from					
Acetabulum to posterior end	1,950	–	1,773 (1,326 – 2,550)	1,930	3,225
Anterior testis to anterior end	–	–	1,442 (1,102 – 1,938)	1,470	2,333
Posterior testis to posterior end	1,040	–	862 (734 – 1,061)	540	1,260
External seminal vesicle to posterior end	910	–	829 (638 – 1,020)	470	1,183
Ovary to posterior end	390	–	316 (205 – 479)	390	620
Genital pore to posterior end	360	–	260 (198 – 420)	240	416
Vitelline reservoir to posterior end	180	–	255 (148 – 408)	320	555

*Diameter

The histopathological analysis revealed the presence of spirorchiid eggs. Type 3 eggs were found in mucosa of the small intestine, large intestine, stomach, cerebral arterioles, choroid of ocular globe, pancreas, spleen, adrenal gland and heart. Type 1 eggs were found in small clusters of two eggs and individualized in the spleen (Fig. 1C) and heart. Small clusters (two to six eggs) were found between thyroid follicles (Fig. 1D). In all organs, type 1 and 3 eggs from the parasite were surrounded by multinucleated giant cells, forming small granulomas. In all tissues granulomas had less than five spirorchiid eggs, biggest granulomas were found in thyroid with 3 – 4 spirorchiid eggs.

Remarks

To date, only 11 specimens of *M. indicum* have been described in the literature, 10 of which were found in *C. mydas* in Pakistan, Brazil and Costa Rica (Mehar, 1939; Santoro *et al.*, 2006, 2009; Werneck *et al.*, 2008; Werneck & Silva 2015) and one was found in *E. imbricata* in Brazil (Werneck *et al.*, 2015). Thus, the present report represents the twelfth specimen described and the first found in *C. caretta*.

The specimen analyzed had a thin body, with intestinal ceca not fused posteriorly and six irregularly-shaped testes located anterior to the ovary between the external seminal vesicle and the acetabulum; moreover, small spines were found on the acetabulum. These characteristics are in agreement with descriptions of the species using the identification key proposed by Platt (2002) as well as all previous descriptions published on the species (Mehra, 1939; Werneck *et al.*, 2008; Santoro *et al.*, 2009; Werneck *et al.*, 2015).

The morphometric analysis and comparison with previously published data on the parasite (Table 1) revealed that the specimen described herein has a larger width of the body, esophagus and ovary, larger length of the external seminal vesicle, larger width of the acetabulum and greater distances between the posterior region of the body and the acetabulum, anterior testicle, external seminal vesicle, ovary and vitelline reservoir. However, such differences were believed to be due to individual variations among specimens.

Two egg types (types 1 and 3) were found in the tissues in the present study. In all tissues the eggs were surrounded by multinucleated giant cells, forming granulomas with less than 5 eggs, this infection were classified as mild (Santoro *et al.*, 2017). The injuries were compatible with previous findings in the same host (Wolke *et al.*, 1982; Stacy *et al.*, 2010) in the United States and the present paper is one of the first reports outside the USA. Few eggs from the family Spirorchiidae were found in the affected organs, suggesting incidental infection [see Appendix 1 in Stacy *et al.* (2010)]. Thus, the tissue injuries were mild, although a brain embolism can have serious consequences. Granulomas formed by eggs have been described in different organs in *C. caretta* (Wolke *et al.*, 1982; Jacobson *et al.*, 2006; Santoro *et al.*, 2017; Stacy *et al.*, 2010). However, the occurrence of eggs in the choroid of the

ocular globe in the present study is a new location for eggs of the family Spirorchiidae.

Wolke *et al.* (1982) described eggs from the genus *Monticellius* as type 1, with the following characteristics: "...yellow-brown, elongate and bore two processes, one of which was frequently hooked..." In the present study, only one egg was found in the uterus and exhibited one of the processes terminating in an oval structure (Fig. 1B), which is the same structure described previously by Santoro *et al.* (2009), but not by Mehra (1939), who described *M. indicum* eggs as "large with narrow, somewhat curved or hook-shaped prolongations at both ends". Considering the re-description performed by Santoro *et al.* (2009) and the present report, we believe that this characteristic could be used in the differential diagnosis of *M. indicum* eggs.

In the present study, only a single specimen of *M. indicum* was found. Although the tissues were examined for parasites, no other cardiovascular fluke was detected. In the case of the genus *Neosporichis*, however, the parasites are quite small and may be associated with small blood vessels, which could hinder their collection (Stacy *et al.*, 2010; Werneck *et al.*, 2016).

In Brazil, little is known regarding helminth fauna in *C. caretta* (Werneck *et al.*, 2008; Werneck & Silva, 2016). There are only reports of one aspidogastriid (*Lophotaspis vallei*), five digeneans (*Calycodes anthos*, *Rhytidodes gelatinosus*, *Pronocephalus trigonocephalus*, *Pyelosomum renicapite* and *Orchidasma amphiorchis*) and two nematodes (*Sulcascaaris sulcata* and *Kathlania leptura*). Thus, the present note broadens knowledge on the helminth fauna of this host on both the local and global levels.

Acknowledgments

The authors wish to express their special thanks to Dr. Paula Baldassin for her suggestions for improving the manuscript. Biological samples were obtained through the "Phase 2" beach monitoring project in the state of Rio de Janeiro, Brazil, which is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA), for the exploration of oil and gas by Petrobras at the Santos Basin pre-salt province.

References

- FLINT, M., PATTERSON-KANE, J.C., LIMPUS, C.J., LILLS, P.C. (2010): Health surveillance of stranded green turtles in southern Queensland, Australia (2006 - 2009): an epidemiological analysis of causes of disease and mortality. *EcoHealth*, 7: 135 – 145
- JACOBSON, E. R., HOMER, B. L., STACY, B. A., GREINER, E. C., SZABO, N. J., CHRISMAN, C. L., ORIGGI, F., COBERLEY, S., FOLEY, A. M., LANDSBERG, J. H., FLEWELLING, L., EWING, R. Y., MORETTI, R., SCHAF, S., ROSE, C., MADER, D. R., HARMAN, G. R., MANIRE, C. A., METTEE, N. S., MIZISIN, A. P., SHELTON, G. D. (2006): Neurological disease in wild loggerhead turtles (*Caretta caretta*). *Dis. Aquat. Org* 70: 139 – 154. DOI: 10.3354/dao070139

- MEHRA, H.R. (1939): New blood flukes of the family Spirorchiidae Stunkard (Trematoda) from the marine turtle *Chelone mydas* of the Arabian Sea with observations on the synonymy of certain genera and classification of the family. *Proc. Natl. Acad. Sci. India*, 9: 155 – 167
- PLATT, T.R. (2002): Family Spirorchiidae Stunkard. In: GIBSON, D. I., JONES, A., BRAY, R.A. (Eds) Keys to the Trematoda. Volume 1. London, UK: CABI Publishing, pp. 453 – 467
- SANTORO, M., GREINER, E.C., MORALES, J.A., RODRIGUEZ-ORTIZ, B. (2006): Digenetic Trematode Community In Nesting Green Sea Turtles (*Chelonia mydas*) From Tortuguero National Park, Costa Rica. *J. Parasitol.*, 92: 1202 – 1206. DOI: 10.1645/GE-866R.1
- SANTORO, M., GREINER, E.C., MORALES, J. A. RODRIGUES-ORTIZ, B. (2009): Redescription of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) from the heart of Green Sea Turtle (*Chelonia mydas*) in Costa Rica. *Open Parasitol J* 3: 4 – 8.
- SANTORO, M., NOCERA, F., IACCARINO, D., LAWTON, S.P. CERRNOE, A., DEGLI UBERTI, B., D'AMORE, M.D., AFFUSO, A., HOCHSCHEID, S., MAFUCCI, F., GALINERO, G. (2017): Pathology and molecular analysis of *Haplotrema mistroides* (Digenea: Spirorchiidae) infecting a Mediterranean loggerhead turtle *Caretta caretta*. *Dis. Aquat. Org.*, 124: 101 – 108. DOI: 10.3354/dao03117
- SMITH, J.W. (1997): The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: Part 2. *Helminthol. abstr.*, 66: 329 – 344
- STACY, B.A., FOLEY, A.M., GREINER, E., HERBST, L.H., BOLTON, A., KLEIN, P., MANIRE, C.A., JACOBSON, E.R. (2010): Spirorchidiasis 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., 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: 550 – 555. DOI: 10.1590/S0102-09352006000400016
- WERNECK, M.R., GALLO, B.G. SILVA, R.J. (2008): First report of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) infecting *Chelonia mydas* Linnaeus, 1758 (Testudines: Chelonidae) from Brazil. *Braz. J. Biol.*, 68: 455 – 456
- WERNECK, M. R., SILVA, R. J. (2016): Checklist of sea turtles endohelminths in neotropical region. *Helminthologia*, 53: 211 – 223. DOI: 10.1515/helmin-2016-0045.
- WERNECK, M.R., SILVA, R.J. (2015): Some helminth parasites of juvenile green turtles *Chelonia mydas* (Testudines, Cheloniidae) in Brazil. *J. Parasitol.*, 101(6): 713 – 716. DOI: 10.1645/15-780
- WERNECK, M.R., SOUZA, G.B., BERGER, B.C. (2016): Report of *Neosporichis schistosomatoides* Price 1934 (Digenea: Spirorchiidae) infecting a Green Turtle, *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae), from Brazil. *Helminthologia*, 53: 94 – 98. DOI: 10.1515/helmin-2015-0074
- WERNECK, M.R., SOUZA, V.R., TRAZI, A., BERGER, B. (2015): *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) in a Hawksbill Turtle, *Eretmochelys imbricata* Linnaeus 1766 (Testudines, Cheloniidae) from Brazil. *Comp. Parasitol.*, 82: 155 – 157. DOI: 10.1654/4716.1
- WERNECK, M.R., THOMAZINI, C., MORI, E., GONÇALVES, V., GALLO, B., SILVA, R.J. (2008): Gastrointestinal helminth parasites of Loggerhead turtle *Caretta caretta* Linnaeus 1758 (Testudines, Cheloniidae) in Brazil. *Panamjas*, 3: 351 – 354
- WOLKE, R.E., BROOKS, R., GEORGE, A. (1982): Spirorchidiasis in loggerhead turtles (*Caretta caretta*): pathology. *J. Wildl. Dis.*, 18: 175 – 185. DOI: 10.7589/0090-3558-18.2.175