

Redescription of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) from the Heart of Green Sea Turtles (*Chelonia mydas*) in Costa Rica

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Abstract: *Monticellius indicum* Mehra, 1939, is redescribed from the heart of green sea turtles (*Chelonia mydas*) at Tortuguero National Park, Caribbean coast of Costa Rica. Of the 40 green turtles examined, only 5 (12.5%) were infected with a mean intensity of 1.6. Our specimens are consistent with the original description based on a single fluke from the heart of the same host species in the Arabian Sea, Pakistan, but our redescription provides an unreported range of variation while adding new information about acetabulum structure and egg morphology.

INTRODUCTION

Cardiovascular infections by digenetic trematodes belonging to Spirorchiidae Stunkard, 1921, have been recorded worldwide from 4 sea turtles including black (*Chelonia mydas agassizii*), green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*) and loggerhead (*Caretta caretta*) [1, 2]. Smith [1] reported a total of 18 species from the cardiovascular system of green turtles, but a few of those were considered synonyms and to date the validity of some taxa is questionable [3]. Several of those genera or species descriptions were based only on a single specimen. This is the case for the only 2 known species of the *Monticellius*: *M. similis*, Price, 1934 and *M. indicum*, Mehra, 1939. Since their description from green turtles, neither species has ever been recorded again. Only recently 3 specimens of *M. indicum* have been collected from the green turtles of Brazil [4]. In this paper, *M. indicum* is redescribed on the basis of specimens collected from the heart of *C. mydas* at Tortuguero National Park, Costa Rica. New features and the range of variation for this species are recorded.

MATERIALS AND METHODOLOGY

From June to September 2003 and 2004, the heart and great vessels of 40 nesting green turtles (curved carapace length ranged from 92 to 111 cm) found dead on the beach of Tortuguero National Park, on the northeast Caribbean coast of Costa Rica were examined for parasites following the methods described by Greiner *et al.* [5]. Specimens were placed in tap water and refrigerated overnight, fixed in AFA (alcohol-formalin-acetic acid), stained in Mayer's acid carmine, mounted in Canada balsam, and studied by light microscopy. Measurements are reported in micrometers with the mean followed by the range in parentheses. Forebody is

the distance from anterior end to anterior edge of acetabulum. Prevalence and intensity are reported according to the definitions by Bush *et al.* [6]. Figures were drawn with the aid of a camera lucida. Specimens were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska State Museum, Lincoln, Nebraska, U.S.A.; United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A.; and Colección Helminológica de Costa Rica (CHCR), San José, Costa Rica.

RESULTS

Eight specimens of *M. indicum* were collected from the heart of 5 of the 40 green turtles examined for parasites. Our description is based on 6 mature specimens. Complete morphometric measurements are listed in Table 1 along with those by Mehra [7] and Werneck *et al.* [4].

Taxonomic Description

Monticellius indicum Mehra, 1939 (Fig. 1(1-3)).

Body thin, narrow and elongated with rounded extremes. Tegument unspined. Body 3,098 (2,295-4,284) long by 351 (214-571) wide at the midbody level. Forebody 1,142 (836-1,632) long. Oral sucker subterminal, protrusible, cup shaped, 162 (112-206) long by 127.5 (82-194) wide. Prepharynx and pharynx absent. Esophagus 421 (309-586) long, surrounded by glandular cells anteriorly to cecal bifurcation. Esophagus bifurcates far anterior to acetabulum, occupies 13.8% (11-15.5%) of body. Ceca extends close to posterior end of the body. Acetabulum circular, 241 (184-326) long by 229 (122-326) wide, pedunculated, located in anterior third of the body, between cecal bifurcation and anterior testis, armed with a single circle of minute margined spines. Acetabulum spines (n = 12) 11 (7-12) long. Testes 5-6, lobed, (n = 12) 133 (74-268) long by 167 (64-286) wide, with irregular margins forming a single linear row in intercecal space between posterior margin of acetabulum and anterior margin of external seminal vesicle, occupy 26% (20.6-35.7%) of body. External seminal vesicle elongated 178

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Table 1. Measurements of *Monticellius indicum* Mehra, 1939 from Green Turtles, *Chelonia mydas**

Character	This Study; n = 6	Mehra, 1939; n = 1	Werneck <i>et al.</i> , 2008; n = 3
Geographical Locality	Caribbean Sea, Tortuguero (Costa Rica)	Arabian Sea, Karachi (Pakistan)	Ubatuba, São Paulo State (Brazil)
Site	Heart	Heart Ventricle	Heart
Body length	3,098 (2,295-4,284)	3,000	4,535.7 (3,666-5,646.6)
Body width	351 (214-571)	400	390.1 (358.7-432.7)
Oral sucker length	162 (112-206)	144	132.2 (86.3-185.4)
Oral sucker width	127.5 (82-194)	148	172.5 (1,44.5-1,94.7)
Acetabulum length	241 (184-326)	256 (diameter)	239.3 (164-318.4)
Acetabulum width	229 (122-326)	-	165.1 (131-194.4)
Acetabulum spine length	11 (7-12), n = 12	-	-
Esophagus length	421 (309-586)	240	418.8 (338-513.1)
Testes number	5-6, n = 5	5	5
Testes length	133 (74-268), n = 12	160-176 (diameter range)	179.8 (110.9-227)
Testes width	167 (64-286), n = 12	-	187.5 (134.7-276.1)
External seminal vesicle length	178 (136-222)	190	-
External seminal vesicle width	85 (70-111)	60	-
Cirrus sac length	728 (612-1,020)	720	713.4 (517.8-868.8)
Cirrus sac width	62 (52-74)	75	76.4 (66.8-94.1)
Ovary length	402 (306-541)	400	474.3 (450-517.7)
Ovary width	135 (74-235)	160	162.8 (122.8-220.9)
Egg length	174 (168-180), n = 2	162	-
Egg width	22, n = 2	21	-
Forebody	1,142 (836-1,632)	1,056	-
Distance From:			
Acetabulum to posterior end	1,773 (1,326-2,550)	1,950	-
Anterior testis to anterior end	1,442 (1,102-1,938)	-	-
Posterior testis to posterior end	862 (734-1,061)	1,040	-
External seminal vesicle to posterior end	829 (638-1,020)	910	-
Ovary to posterior end	316 (205-479)	390	-
Genital pore to posterior end	260 (198-420)	360	-
Vitelline reservoir to posterior end	255 (148-408)	180	-

*Measurements are reported in micrometers with the mean followed by the range in parentheses.

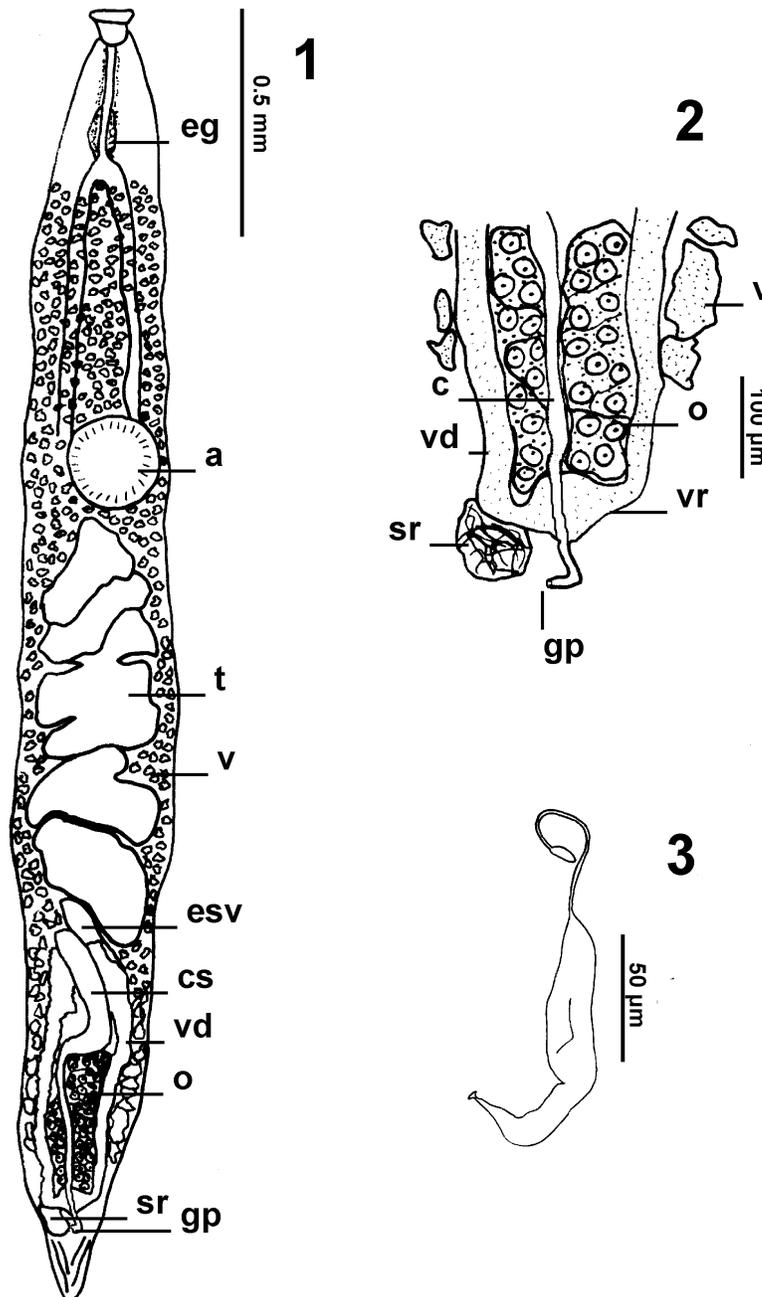


Fig. (1). *Monticellius indicum* adult specimen. **1.** Ventral view of the entire specimen. a, acetabulum; cs, cirrus sac; eg, esophageal glands; esv, external seminal vesicle; gp, genital pore; o, ovary; sr, seminal receptacle; t, testis; v, vitelline follicle; vd, vitelline duct. **2.** Terminal genitalia. c, cirrus; gp, genital pore; o, ovary; sr, seminal receptacle; v, vitelline follicle; vd, vitelline duct; vr, vitelline reservoir. **3.** Egg.

(136-222) long by 85 (70-111) wide, located between posterior testis and basal portion of cirrus sac. Cirrus sac well developed 728 (612-1,020) long by 62 (52-74) wide, S-shaped, with large internal seminal vesicle followed by ejaculatory duct and surrounded by prostatic cells. Genital pore median, at vitelline reservoir level. Ovary lobed, intercecal 402 (306-541) long by 135 (74-235) wide, located in

posterior third of the body, extending from basal part of cirrus sac to anterior margin of vitelline reservoir. Seminal receptacle dextral, posterior to ovary. Laurer's canal and Mehlis' gland not found. Vitelline follicles intra- and extracecal extending from cecal bifurcation to ovary. In pretesticular zone, vitelline follicles occupy entire field. Vitelline ducts arising close to external seminal vesicle area unite to

form a reservoir posterior to ovary. Uterus contains a single hook-shaped egg with unequal terminal bipolar processes. Eggs (n = 2) 174 (168-180) long by 22 wide. Polar processes are a tiny circular structure on one end and a large filamentous process with a conspicuous terminal thickening on the other end. Excretory vesicle Y-shaped with excretory pore sub-terminal.

Taxonomic Summary

Host

Green sea turtle, *Chelonia mydas* Linnaeus, 1758 (Testudines: Cheloniidae).

Locality and Collection Dates

Tortuguero National Park (10°32'27"N, 83°29'59"W - 10°21'17"N, 83°23'29"W), Limón Province, northeast Caribbean coast of Costa Rica. June to September 2003 and 2004.

Site of Infection

Heart.

Prevalence and Intensity of Infection

Five of 40 turtles sampled (12.5%). Mean intensity, 1.6; range, 1-2.

Specimens Deposited

HWML 48242; USNPC 97476, 97477, 97478; CHCR 278, 279, 280.

Additional Records

Chelonia mydas in the Arabian Sea, near the Kiamari coast at Karachi, Pakistan [7]; *C. mydas* in Ubatuba, on the north coastline region of São Paulo State, Brazil (June 2006 and June 2007) [4].

DISCUSSION

Price [8] erected the genus *Learedius* (Spirorchiidae) and described 2 new species, *L. learedi* and *L. similis*. Both species descriptions were based on the single specimen obtained from the circulatory system of a green turtle, which was died in the National Zoological Park of Washington (U.S.A.). Price [8] transferred *Distoma constrictum*, Leared, 1862 to *Learedius*, and proposed a new name, *L. europaeus*, for it. Mehra [7] described *Monticellius* to contain *M. indicum* on the basis of a single fluke collected from the heart ventricle of a green turtle caught in the Arabian Sea, Pakistan. In the same paper, he described *Learedius orientalis* and transferred *L. similis* to *Monticellius*. Finally, *Learedius lochooensis* was described by Takeuti [9]. However, Dyer *et al.* [10] and Inohuye-Rivera *et al.* [2] suggested that *L. learedi* and *L. orientalis* could be synonymous.

According to Mehra [7], *Monticellius* differs from *Learedius* by having a smaller size and different body shape, a much shorter esophagus, the intestinal bifurcation closer to acetabulum, no intestinal caeca loops at their origin, different shape, number and arrangement of the testes, different shape, size and cirrus sac arrangement and different ovary shape. *Monticellius indicum* differs from *M. similis* by the absence of spines or verrucae, different oral sucker shape, different ratio of the size of the 2 suckers, smaller numbers and di-

verse arrangement of testes, and better developed vitellaria [7].

Our comparison with Mehra's [7] fluke was based on his original description because there is no evidence that he deposited the type material in any available collection. Our specimens have a longer esophagus; an acetabulum armed with a single circle of minute spines, not mentioned by Mehra [7], and lobed testes (5 in 2 specimens and 6 in 3 specimens). In a single fluke (USNPC 97477), the exact number was not ascertainable because the testes were strongly overlapped. Mehra [7] described the egg of *M. indicum* as "...large with narrow, somewhat curved or hook-shaped prolongations at both ends...". Probably the single egg observed [7] was in a poor condition or incompletely developed. From the 8 specimens collected, we observed a total of 7 eggs, but only 2 were in a good enough condition to be described. The eggs observed in our flukes have features different from all other spirorchiid eggs known to date, from the marine turtles. Despite these differences and the variation noted above, we prefer to place these specimens within *M. indicum* until more material is available for study, preferably including specimens from the type locality. Basic morphological data from the 3 specimens from Brazil [4] was similar to Mehra's [7] specimen except for the body length and esophagus features.

ACKNOWLEDGEMENTS

We wish to thank the Tortuguero Conservation Area and the Park Rangers for a logistic support and to facilitate the sampling on the beach. The manuscript was improved by comments and advices from Mr. John M. Kinsella (Helm West Laboratory, Missoula, Montana, U.S.A.). Research activities at the Tortuguero National Park were conducted under permission 091-2003-OFAU from Ministry of Environment and Energy and from the National System of Conservation Areas of Costa Rica.

REFERENCES

- [1] Smith JW. The blood flukes (Digenea: Sanguinicolidae and Spirorchiidae) of cold-blooded vertebrates: part. 2. *Helminthol Abstr* 1997; 66: 329-44.
- [2] Inohuye-Rivera RB, Cordero-Tapia A, Arellano-Blanco J, Gardner S. *Learedius learedi* Price, 1934 (Trematoda: Spirorchiidae), in black turtle (*Chelonia mydas agassizii*) hearts from Magdalena Bay, Baja California Sur, Mexico. *Comp Parasitol* 2004; 71: 37-41.
- [3] Platt TR. Family spirorchiidae stunkard 1921. In: Gibson DI, Jones A, Bray RA, Eds. *Keys to the Trematodes*, London: CAB International and The Natural History Museum 2002; Vol. 1, pp. 453-67.
- [4] Werneck MR, Gallo BMG, da Silva RJ. First report of *Monticellius indicum* Mehra, 1939 (Digenea: Spirorchiidae) infecting *Chelonia mydas* Linnaeus, 1758 (Testudines: Cheloniidae) from Brazil. *Braz J Biol* 2008; 68: 455-6.
- [5] Greiner EC, Forrester JJ, Jacobson ER. Helminths of mariculture-reared green turtles (*Chelonia mydas*) from Grand Cayman, British West Indies. *Proc Helminthol Soc Wash* 1980; 47: 142-4.
- [6] Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J Parasitol* 1997; 83: 575-83.
- [7] Mehra HR. 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* 1939; 9: 155-67.
- [8] Price W. New genera and species of blood flukes from a marine turtle, with a key to the genera of the family Spirorchiidae. *J Wash Acad Sci* 1934; 24: 132-41.

- [9] Takeuti E. New blood flukes of the family Spirorchidae from Japanese freshwater tortoise and marine turtles. *Jpn J Med Sci* 1942; 2: 161-74.
- [10] Dyer WG, Williams EH, Jr, Bunkley-Williams L. *Angiodictyum mooreae* n. sp. (Digenea: Microscaphidiidae) and other digeneans from an atlantic hawksbill turtle *Eretmochelys imbricata* from Puerto Rico. *J Aquat Anim Health* 1995; 7: 38-41.

Received: November 25, 2008

Revised: February 20, 2009

Accepted: February 25, 2009

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