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Cardicola polynemi sp. n. (TREMATODA: APOROCOTYLIDAE) FROM GILLS OF MARINE THREADFIN FISH, Eleutheronema tetradactylum (Shaw, 1804) IN THE VISAKHAPATNAM COAST, BAY OF BENGAL, INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author MG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author APV managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

A new species of *Cardicola* Short, 1953 (n= 27) was obtained from the gills of 16 infected marine threadfin fish, *Eleutheronema tetradactylum* Shaw, 1804 of Visakhapatnam coast, India between July 2005 and June 2007. The new species, *Cardicola polynemi* is characterized by possessing the body which is eight times longer than wide with a vestigial oral sucker, alveolar testis, multilobular ovary, straight posterior caeca, median opening of female genital pore, and extension of body spines to anterior and posterior extremities of the body.

Keywords: Aporocoylidae; Cardicola polynemi; Eleutheronema tetradactylum; Visakhapatnam coast; Bay of Bengal.

1. BACKGROUND

Blood flukes (Trematoda: Schistosomatoidea) are the aquatic parasites parasitizing the blood vascular system (habitually the lumen of the heart) of their definitive hosts. There are three families of blood flukes infecting different ultimate vertebrate host

lineages i.e., Schistosomatoidea (mostly parasitize birds and terrestrial mammals); Spirorchiidae (mostly parasitize reptiles) and Aporocotylidae (majority parasitize the fish). The blood flukes of Aporocotylidae, also known as Fish blood flukes (FBFs) are generally hermaphroditic in nature with two-host life-cycle i.e, asexual reproduction in

invertebrate hosts followed by direct penetration through the skin of the definitive host. Freshwater gastropods, marine terebellid polychaetes and bivalves serve as intermediate hosts for approactlyids of Actinopterygii and Chondrichthyan fishes while gastropods and polychaetes serve as intermediate hosts for Schistosomes and Spirorchiids [1,2,3,4]. Cribb and Bray [5] stressed on the higher species diversity of Aporocotylidae which are still awaiting discovery. Genus Cardicola is considered to be the richest, 45 species have been described so far from the 14 Actinopterygii fish families [6]. There are many reports of Cardicola spp. from the fishes all over the world [7-31]. Hutson et al. [4] recorded Cardicola spp. for the first time from a mammal (Dugong dugong). Polynemid fishes or thread fin fishes serve as very good hosts for a number of parasites [32-36]. But, so far there are no reports of Cardicola spp. from these hosts. In the present study, the description of a new blood fluke of the genus Cardicola from the polynemid fishes of Visakhapatnam coast is described.

2. MATERIALS AND METHODS

2.1 Study Site

Marine threadfin fish of the family Polynemidae, *Eleutheronema tetradactylum* (Shaw) (n= 490) were collected from boat seine and trawlers of fishing harbour, fish landing centers and fish markets in and around Visakhapatnam coast (17.67°'N & 83.32°'E), Bay of Bengal between July 2005 and June 2007 for a period of two years. The fishes were dead at the time of the collection.

2.2 Sample Collection and Identification of the Parasite

The morphometric and meristic data of the hosts were documented on exclusive data sheets, along with their length, weight and sex and continuum of metazoan parasites. Various external surfaces i.e., fins, body surface, buccal cavity and gills were screened for ectoparasites and internal organs such as stomach, intestine, muscles and heart were screened for endoparasites. However, Cardicola spp. were found attached to the gill filaments. Alienated gills were carefully placed in a saline solution, then teased and contents were washed and observed under a stereozoom microscope. Standard protocols were followed to preserve the collected specimens in an ideal fixative, A.F.A (Alcohol- 85%, Formalin- 5%, Glacial acetic acid- 10%) for 24 hours for the permanent slide preparation and further stained with alum carmine followed by dehydration with a graded series of alcohols (70%, 80%, 95%, 100%), xylene clearing and lastly mounted on a glass slide with Canada balsam or DPX mount. Parasites were observed, identified and photographed with Nikon microscope at various (10X, 30X and 40X) magnifications and scale was specified consequently. Figures were drawn with the aid of Reiss camera lucida using 4X, 10X, 25X and 40X magnifications. All measurements were given in micrometres (μ m), unless otherwise stated. Holotypes and paratypes of the parasites will be deposited in Zoological survey of India (ZSI), Kolkata.

3. RESULTS

Cardicola polynemi n.sp. (Figs. 1, 1a, 1b, 2, 2a)

Class: Tremtoda

Family: Aporocotylidae Genus: *Cardicola* Short, 1953

Description (based on 10 specimens):

Shape of the adult: Body elongate, flattened with tapering anterior and blunt posterior ends. Body measures $1604-1657 \times 158-210$.

Tegument: Spines on ventral margin of body arranged in about many short rows perpendicular to edge of body, rows of spines extending around body margin without interruption, group of spines representing a row. Spines at anterior tip of body and posterior margin of body are long and extend beyond the surface of body. Spines delicate, elongated with sharp, curved tips, approximately of same shape and size regardless of position, posterior spiny row varying in length from 7-10.

Anterior oral sucker: Oral sucker vestigial and does not bear spines, obovate, delimited posteriorly by fine membrane. Mouth opening small, sub terminal and ventral 15-18 from anterior end, surrounded by vestigial oral sucker.

Oesophagus: Oesophagus straight, narrow near mouth, becoming gradually wider, surrounded by oesophageal glands and measures 500-520 in length.

Intestine: Intestine 'H' shaped with two caeca extending anteriorly and two posteriorly from the junction of oesophagus. Caeca unequal, posterior caeca longer than anterior pair. Anterior caeca measure 250-260 in length. Posterior caeca sinuous and measures 660-680 in length.

Testis: Testis single, alveolar, surrounded by definite membrane, measuring 620-640, bounded anteriorly by

small group of vitelline follicles, posteriorly by ovary and laterally by posterior intestinal caeca.

Vas deferens: Vas deferens well-developed, arises from a point near middle of posterior border of testis, crosses ovary ventrally and coiled on itself. Before opening to exterior, it becomes constricted into short, narrow tube, then expanding into a small, bulbous terminal portion which opens through ventral male genital pore situated on small papilla near right margin of the body.

Ovary: Ovary more or less multilobular with posteriorly directed limbs, lying immediately posterior to testis, ends of posterior intestinal caeca touching anterior margin of ovary. Ovary measures 150-170 in diameter. Oviduct starts from posterior border of ovary, extends towards right margin of body, bending sharply on itself to enter ootype, surrounded by Mehlis gland.

Uterus: Uterus postovarian, extends from ootype posteriorly a short distance, then anteriorly with two loops to about level of posterior margin of ovary, then turns posteriorly to open through female genital pore. Female genital pore median, to the left of male genital pore.

Vitellaria: Vitellaria consist of small follicles extending throughout most of the body, from slightly beyond the anterior intestinal caeca, to region just behind ovary. Main vitelline duct traceable in some specimens from mid region of oesophagus posteriorly, ventral to testis, vas deferens, ovary and oviduct, to junction with oviduct near ootype.

Type and only host: Marine threadfin fish, *Eleutheronema tetradactylum* Shaw, 1804

Type locality: Visakhapatnam coast (17.67° N & 83.32°'E), Andhra Pradesh, Bay of Bengal, India

Site in host: Gill filaments

No. of hosts examined: 490

No. of hosts infected. 16

Worm burden, 27

Prevalence of infection: 3.26%;

Mean intensity: 1.69

Etymology. The species is named after the family name of the host, Polynemidae.

Specimens deposited. Holotype (ZDAU-2007/CVL/GM/CP) and one paratype (ZDAU-2007/CVL/GM/CP-I) deposited in the Museum of Zoology Department, Andhra University (ZDAU), INDIA: Andhra Pradesh, Visakhapatnam coast, Andhra University Campus, Coll. Mani G, 15.viii.2007 will be collected and submitted to Zoological Survey of India (ZSI), West Bengal.

Remarks

The genus Cardicola was proposed by Short [10] with Psettarium cardiocolum Manter, 1947 as type-taxon (now accepted as Cardicola cardiocolus [7,10] from the hearts of jolthead porgy, Calamus bajonado (Bloch et Schneider) of Gulf of Mexico, USA. According to [6], nearly 45 species were reported under the genus of which only 36 can be considered as valid and the remaining 9 are either synonymized under malformed suffix, superseded combinations or lapsus. The present species concurs well with the characters of Cardicola, by the possession of transverse rows of small ventro-lateral spines, an H- shaped intestine with anterior caeca shorter than posterior pair, a large intercaecal single testis, separate genital pores, a median ovary, postovarian uterus and a follicular vitellarium. So far, there are no reports of Cardicola from polynemid fishes. Chaulioleptos haewardii reported by Nolan and Cribb [18] is the first blood fluke described from the Polynemidae in the world. Chaulioleptos differs from the present species by the possession of two testes. Present species is distinguished from its congeners, by possessing the combination of a body 8 times longer than wide, vestigial oral sucker, testis, multilobular ovary, alveolar straight posterior caeca, median opening of female genital pore, and extension of body spines at anterior and posterior extremities of the body. Present species resembles Cardicola chaetodontis Yamaguti, [11] in body shape, presence of vestigial oral sucker, position of male genital aperture and follicular vitellaria. It differs from C. chaetodontis in having an equal anterior caecum, posteriorly directed limbs of ovary, median opening of female genitalia, anterior extension of vitellaria, arrangement of body spines in posterior and anterior region of body and different host species. The above differentiating features justify the erection of a new species named as Cardicola polynemi.

Table 1. List of valid species of Cardicola Short, 1953 with their host, Infectivity site and Geographical locality along with C.polynemi n.sp.

Sl. no.	Species name	Host	Infectivity site	Geographical locality	References
1	Cardicola cardiocolum [7,10]	Calamus bajonado (type- host)	Heart	Gulf of Mexico (Type-locality)	Manter [7] Short [10]
2	C. laruei [10]	Cynoscion arenarius (type-host)	Heart	Northern Gulf of Mexico (Type-locality) Eastern Gulf of Mexico, Tampa Bay, USA	Short [9,10] McVay et al. [28]
		Cynoscion nebulosus	Washings of gut	Northern Gulf of Mexico (Type-locality) USA	Short [10]
			Heart	Eastern Gulf of Mexico, Tampa Bay, USA	McVay et al. [28] McVay et al. [28]
			Heart	Northwestern Altantic ocean, Florida, USA	, []
3	C. coridodacis [7]	Odax pullus (type-host)	Heart	Southwest Pacific Ocean, New Zealand (Type-locality)	Manter [8]
4	C. whitteni [8]	Nemadactylus macropterus	Heart	Southwest Pacific Ocean, New Zealand (Type-locality)	Manter [8]
5	C. chaetodontis [11]	Chaetodon miliaris (type- host) C. aureofasciatus,	Gill, Heart Heart, gill, and blood vessels of intestine Heart, gill, and blood	Central Pacific Ocean, USA (type- locality) Southwest Pacific Ocean, Lizard island, Australia;	Yamaguti [11] Nolan and Cribb [19]
		C. citrinelius,	vessels of intestine Heart, gill, and blood	Southwest Pacific Ocean, French Polynesia;	Nolan and Cribb
		C. flavirostris,	vessels of intestine	Southwest Pacific Ocean, Heron Island, Australia;	Nolan and Cribb [19]
		C. lineolatus,	Heart, gill, and blood vessels of intestine	Southwest Pacific Ocean, New Caledonia; Southwest Pacific Ocean, Moorea Island,	Nolan and Cribb [19] Nolan and Cribb
		C. reticulatus,	Heart, gill, and blood vessels of intestine Heart, gill, and blood vessels of intestine	French Polynesia; Southwest Pacific Ocean, Heron Island, Australia;	[19] Nolan and Cribb [19]
		C. ulietensis,	heart, gill, and blood	Southwest Pacific Ocean, Palau; Southwest Pacific Ocean, Heron Island,	Nolan and Cribb

Sl. no.	Species name	Host	Infectivity site	Geographical locality	References
			vessels of intestine	Australia	[19]
		C. unimaculatus			Nolan and Cribb
					[19]
6	C. mugilis [11]	Mugil cephalus	Heart, blood vessels	Central Pacific Ocean, off Hawai, USA (type-locality)	Yamaguti [11]
7	C. brasiliensis [12]	Mugil platanus	Heart, kidney, liver, gill	Southwestern Altantic Ocean, Brazil	Knoff and Amato
8	C. forsteri [14]	Thunnus maccoyli	Heart	Southwest Pacific Ocean, Louth Island, South Australia	Cribb et al. [15]
		Thunnus thynnus	Heart	Northwestern Atlantic Ocean, Off cape North Carolina, USA	Bullard et al. [20]
			Heart, kidney, gill (Eggs)	Adriatic Sea, Island of Brač, Croatia	Mladineo and Tudor [16]
9	C. palmeri [21]	Pogonias cromis	Heart	Northern Gulf of Mexico, Mississippi Sound, USA (type locality)	Bullard and Overstreet [21]
10	C. currani [21]	Sciaenops ocellatus	Heart	Northern Gulf of Mexico, Mississippi Sound, USA (type locality)	Bullard and Overstreet [21]
11	C. coeptus [19]	Siganus punctatus	Heart, gill	Southwestern Pacific Ocean, Heron Island, Australia (type locality)	Nolan and Cribb [19]
		Siganus vulpinus	Heart, gill	Southwestern Pacific Ocean, Heron Island, Australia	Nolan and Cribb [19]
12	C. covacinae [19]	Siganus punctatus (type host)	Heart	Southwestern Pacific Ocean, Heron Island, Australia (type locality)	Nolan and Cribb [19]
13	C. bartolii [19]	Siganus lineatus	Heart, gill	Southwestern Pacific Ocean, Heron Island, Australia (type locality)	Nolan and Cribb [19]
		Siganus corallinus	Heart, gill	Southwestern Pacific Ocean, Heron Island, Australia	Nolan and Cribb [19]
14	C. watsonensis [19]	Siganus corallinus (type host)	Heart	Southwestern Pacific Ocean, Lizard Island, Australia (type locality)	Nolan and Cribb
15	C. lafii [19]	Siganus fuscescens (type host)	Heart	Southwestern Pacific Ocean, Lizard Island, Australia (type locality)	Nolan and Cribb [19]

Sl. no.	Species name	Host	Infectivity site	Geographical locality	References
16	C. milleri [19]	Lutjanus bohar (type host)	Heart	Southwestern Pacific Ocean, Lizard Island, Australia (type locality)	Nolan and Cribb [19]
		(type nost)		Island, Australia (type locality)	[19]
17	C. parilus [19]	Siganus fuscescens (type	Heart	Indian Ocean, Ningaloo Reef off	Nolan and Cribb
		host)		Western Australia (type locality)	[19]
18	C. tantabiddii [19]	Siganus fuscescens (type	Heart	Indian Ocean, Ningaloo Reef off Western	Nolan and Cribb
		host)		Australia (type locality)	[19]
19	C. ambrosioi [22]	Percophis brasiliensis	Liver, gill	Southwestern Atlantic Ocean, off Mar del	Braicovich et al.
		(type host)		Plata, Argentina (type locality)	[22]
20	C. aurata [23]	Sparus aurata (type host)	Gill	Mediterranean Sea, off Valencia, Spain	Holzer et al. [23]
				(type locality)	
21	C. orientalis [26]	Thunnus orientalis (type	Gill	Western Pacific Ocean, off Japan (type	Ogawa et al. (2010)
		host)		locality)	, ,
22	C. nonamo [28]	Phanerodon furcatus	Heart	Eastern Pacific Ocean, Monterey Bay,	Bullard (2010)
				California, USA (type locality)	
		Rhacochilus toxotes	Gill	Eastern Pacific Ocean, Naples Reef,	Bullard (2010)
				California, USA	
23	C. opisthorchis Ogawa, Ishimaru,	Thunnus orientalis (type	Heart	Western Pacific Ocean, off Japan (type	Ogawa et al. (2011)
	Shirakashi, Takami et Grabner,	host)		locality)	, ,
	2011				
24	C. parvus Bullard, Baker et de	Micropogonias undulates	Heart	Northwestern Atlantic Ocean, South	Bullard et al. [20]
	Buron, 2012	(type host)		Atlantic Bight (type locality)	
25	C. langeli [25]	Archosargus	Heart	Northern Gulf of Mexico, off Horn	Bullard [25]
		probatocephalus (type		Island, USA (type locality)	
		host)			
26	C. polynemi n.sp.	Eleuthronema	Gills	Visakhapatnam Coast, Bay of Bengal,	Present Study
		tetradcatylum (type-host)		India	

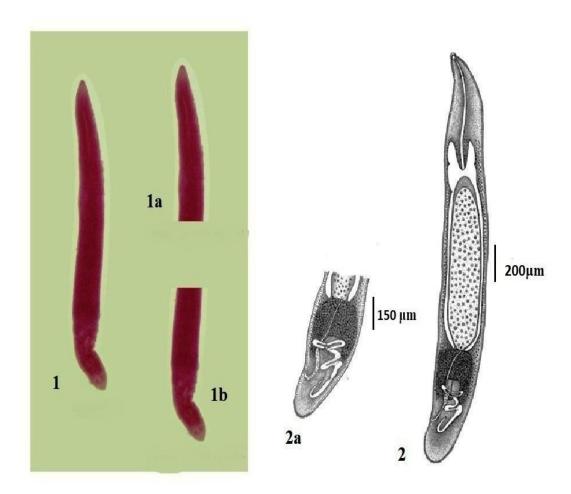


Fig. 1. Microphotograph of *Cardicola polynemi* n sp. Whole mount 40X; Fig. 1a. Anterior region 100x; Fig. 1b. Posterior region 100X; Fig. 2. Line diagram of *Cordicola polynemi* n sp.. Fig. 2a. Posterior region of *C. polynemi*

4. CONCLUSION

The present study declares that the Polynemid fishes of Visakhapatnam coast, Bay of Bengal has an extensive array of unidentified parasite biodiversity which has to be disclosed before the host species is lost due to anthropogenic pollution. The identification of a new species of blood fluke of the genus *Cardicola* for the first time from marine threadfin fish is an example set to perk up more strenuous parasitological surveys to expose the parasite diversity of this geographical location to the world.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

ETHICS

The ethics concerning the use of fish for this research were deemed unnecessary according to the Animal ethics committee (CPCSEA) as the fish was dead at the time of collection, edible and was easily available to carry out research. Meanwhile the number of fishes used for this research was regulated by the Institute (Andhra University).

SIGNIFICANCE STATEMENT

This study ascertains the fact that the parasitic community structure of the threadfins from Visakhapatnam coast, Bay of Bengal, Andhra Pradesh, India has many new species of metazoan parasites which still needs to be unveiled. This study will be immeasurably constructive to present a catalog on host-parasite association in the Bay of Bengal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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