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SHORT COMMUNICATION

On a new species of *Neoechinorhynchus* Hamann, 1892 (Neoechinorhynchinae Travassos, 1926) from *Eleutheronema tetradactylum* (Shaw, 1804) from Digha coast, West Bengal, India

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Abstract *Neoechinorhynchus* (*Neoechinorhynchus*) *dighaensis* n. sp. is described from the intestine of the fourfinger threadfin fish *Eleutheronema tetradactylum* (Shaw, 1804) from the Digha coast, West Bengal, India. Females were larger than males. In the male dorso-ventral lacunar canals well developed. Middle hooks in perfect circle, smaller than anterior hooks. Posterior hooks slightly smaller than middle hooks. Lateral anterior proboscis hooks larger than the others in same circles. Lateral posterior proboscis hooks equal to others in same circles. Lemnisci equal, longer reaching to testes in male. Male reproductive system usually occupies half of the total body length. Eggs were elliptical in shape with concentric membrane. Cement gland long, containing seven giant nuclei.

Keywords Fish endoparasites · Brackish water · SEM · Bay of Bengal

Introduction

Majority of the acanthocephalan (62.7%) species mainly infecting aquatic hosts (Kennedy 2006). *Neoechinorhynchus* is a freshwater acanthocephalan genus, mostly reported from various freshwater worldwide but few literatures also available on marine water fishes (Linton 1932; Podder 1937; Tripathi 1956; Chandra et al. 1984; Gupta and Fatma 1987; Gupta and Kumar 1987). Of the 109 nominal species described as belonging to *Neoechinorhynchus*, Amin (2002) considered only 48 valid species. The diversity of marine *Neoechinorhynchus* is incomplete especially in India.

Diseases caused by metazoan parasites are very common throughout the world and particularly in the tropic countries. Fishes serve as definitive or intermediate hosts in the life cycle of metazoan parasites. They affect fishes by lowering their body weights, reproduction and high mortality. *Eleutheronema tetradactylum* (Shaw 1804) is commonly known as Indian salmon or fourfinger threadfin fish is having a wide range of salinity tolerance, thus an amphidromous marine species migrating to brackish and freshwater (Riede 2004). The knowledge on the endoparasites of these fish species are very much essential due to hosts amphidromous habitat, possibly the evolution of parasites to adopt a wide range of salinity tolerance. Gudivada and Vankara (2010) and Gudivada et al. (2012) reported that Threadfin fishes, *Polydactylus sextarius* and *E. tetradactylum* were plagued with a number of metazoan parasites of different phyla such as monogeneans, digeneans, nematodes, acanthocephalans, copepods and isopods. Interestingly, Gudivada et al. (2012) also reported that the diversity of ectoparasites in *E. tetradactylum* equal to endoparasites.

In this study Parasites from the intestine of brackish water threadfin fish, *Eleutheronema tetradactylum* were collected from Digha, coast, West Bengal, India and identified to be a new species which is described here. Newly collected parasites were described in detail using light and scanning electron microscopy (SEM).

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Materials and methods

Specimen collection

Marine and brackish water fishes were examined for the infection of acanthocephalan parasites, which were collected from Digha coast, West Bengal. Fishes were identified with the help of FAO species identification sheets, Western Indian Ocean. *Eleutheronema tetradactylum* was found to be infected with *Neoechinorhynchus*. Live specimens of *Neoechinorhynchus* were collected from the intestine of host and kept in normal saline. Parasites fixed in AFA fixative {Alcohol (50%): formalin: acetic acid (100: 6: 2.5)}. Specimens stained with acetoalum carmine, dehydrated in ascending grades of ethanol (30, 50, 70, 90% and absolute ethanol), cleared in xylene and mounted in DPX. Figures were drawn with Camera Lucida, attached to Phase Contrast Microscope (Olympus CX-41). Measurements of parasites were taken with the aid of calibrated ocular micrometer. All measurements are given in micrometer, unless otherwise noted. The parasites were identified using conventional morphological criteria by Amin (2002) and literature available (Amin and Muzzall 2009; Gudivada et al. 2010). Specimens were deposited in Gangetic Plane Regional Centre, Zoological Survey of India (ZSI), Patna, India and also in Zoobank. Paratypes were deposited in the "Helminthology lab", Department of Zoology, University of Lucknow, U.P., India.

Scanning Electron Microscopy

Acanthocephalan parasites were fixed in 2.5% glutaraldehyde for 2–4 h at 4 °C, washed with phosphate buffer (0.1 M, pH 7.2) for 3 times at the interval of 15 min. Then post-fixation was done with 1% osmium tetraoxide for 2 h at 4 °C and then washed in 0.1 M phosphate buffer for 3 times each of 15 min at 4 °C to remove the unreacted fixative. Samples were mounted on to the stubs with adhesive tape (carbon), the sample was kept in desiccators for overnight and coated with palladium sputter coater and viewed with a scanning electron microscope (JEOL 6490 LV).

Results

Systematics

Systematics are based on Amin (2002) and Bhattacharya, (2007)
Phylum Acanthocephala
Class Eoacanthocephala Van Cleave 1948
Order Gyracanthocephala Van Cleave 1936
Family Neoechinorhynchidae Van Cleave 1919
Subfamily Neoechinorhynchinae Travassos 1926
Genus *Neoechinorhynchus* Hamann, 1892
Species *Neoechinorhynchus dighaensis* n. sp.

Specimens studied

Four males, five females. Canada balsam slides and 1 female for SEM.

Holotype male registration No. ZSI/GPRC, IV-4359(a) and Alotype female Registration No. ZSI/GPRC, IV-4359 (b). Paratypes: Males-LU/Z/2018/01, LU/Z/2018/02, LU/Z/2018/03, Females-LU/Z/2018/04, LU/Z/2018/05, LU/Z/2018/06.

Description

Males were creamy white in color. Total body length of males 4.50–4.82 mm long by 0.32–0.69 mm wide, a maximum width in middle of the body. Body wall of males with well-developed reticular lacunar system (Fig. 1b). Trunk cylindrical, aspinose, fusiform curved ventrally, widest in middle two-third, attenuating posteriorly (Fig. 1a, b), 4.42–4.71 mm long by 0.32–0.69 mm. Neck rudimentary. Proboscis globular, short spheroid slightly longer than wide, rounded anteriorly (Fig. 1c), 70–80 long, 60–70 wide. Proboscis with the very short neck. Apical organ apparently contractile occasionally extending to full length

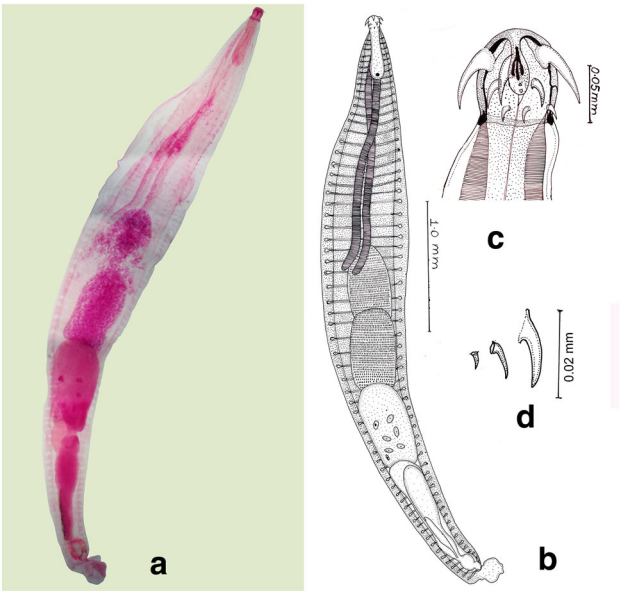

Fig. 1 Male *Neoechinorhynchus dighaensis* n. sp. of *Eleutheronema tetradactylum* from Digha coast, West Bengal: **a** light microphotograph of male, **b** Line drawings of male specimen, **c** Enlarged view showing proboscis, **d** Enlarged view of proboscis hooks

of proboscis. Proboscis hooks in three circles of six hooks in each (Fig. 3). All hooks rooted (Fig. 1d). Lateral anterior proboscis hooks larger than the others in the same circle hooks. Hooks in anterior circle powerful, with well-developed roots. Length of proboscis hooks in anterior circle 50–52 (lateral anterior). Middle hooks perfect circle, smaller than anterior hooks, 43–44 (middle anterior), middle circle hooks twice of the third circle, pointed with small roots; 30 long (Fig. 1d). Posterior hooks slightly smaller than middle hooks. Middle and posterior hooks with discoid bases (Fig. 1d).Hooks of basal circle similar, spiniform, with very small roots; 13 long. Proboscis receptacle receptacle single-walled, 270–390 long by 70–110 wide. Lemnisci equal elongate, much longer than receptacle, reaching to anterior testes, 1.40–1.93 mm long, 50 wide. Reproductive system usually occupies half of the body length. Testis oval, partially overlapping, contiguous almost equal in size, measuring anterior testis 450–640 long by 150–340 wide while posterior testis 450–650 long by 150–350 wide. Cement gland 360–720 long by 150–360 wide containing seven giant nuclei. Cement gland reservoir 430–439 long by 110–117 wide. Saefftigen's pouch oval–shaped with blunt end; 150–550 long and 50–90 wide posteriorly. Bursa was almost oval in shape measuring 47–50 long by 46–50 wide.

Females were creamy white in color with annulation on body and larger than male with long ribbon like body, measuring 5.15–6.7 mm long by 0.65–0.69 mm wide, a clyndical, attenuating posterioly (Fig. 3a, b). Proboscis of females very short as compare to total body length, with rudimentary neck, measuring 110–110 long, 10–90 wide.

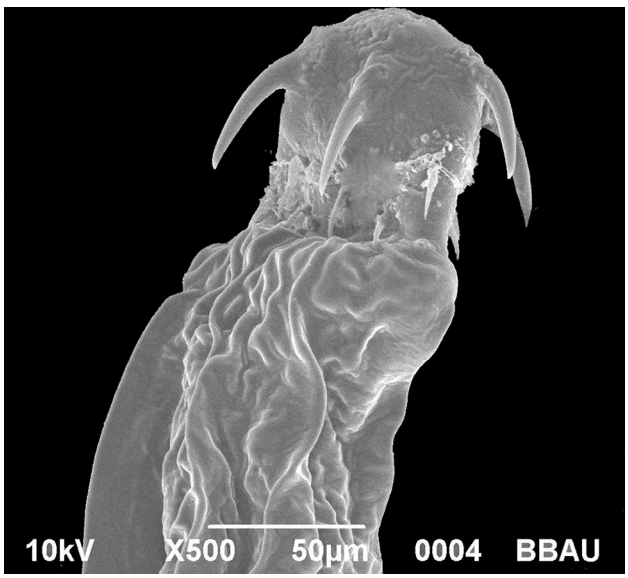

Fig. 3 The detailed proboscis hooks of Neoechinorhynchus dighaensis n. sp. of Eleutheronema tetradactylum from Digha coast, West Bengal

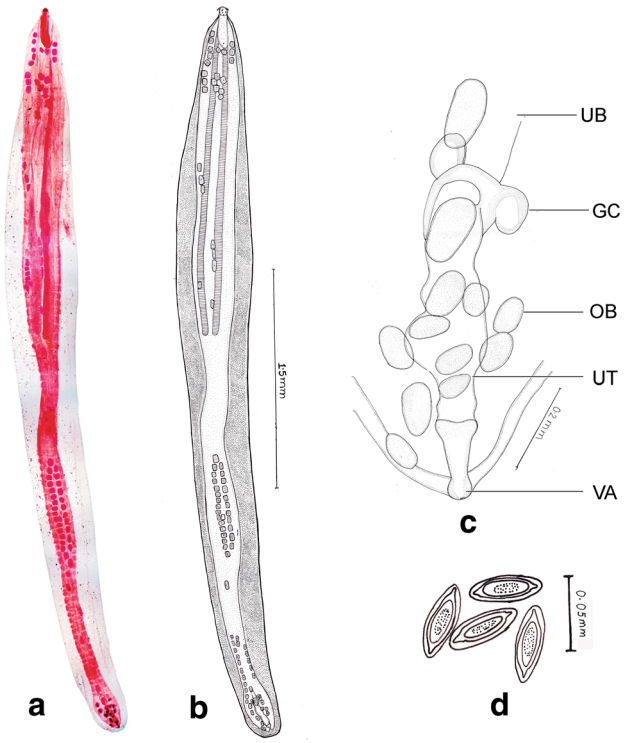

Fig. 2 Female *Neoechinorhynchus dighaensis* n. sp. of *Eleutheronema tetradactylum* from Digha coast, West Bengal: **a** light microphotograph of female, **b** Line drawings of female specimen, **c** Enlarged view of female reproductive system (UB-uterine bell, GC-guard cell, OB-ovarian ball, UT-uterus and VA-vagina), **d** Eggs

Hooks of anterior row are much larger than the other two rows. Proboscis receptacles of females thick, single layered, cylindrical, measuring 320–390 long by 110–130 wide. Lemensci in females long cylindrical equal in size, reaching to half of the body, 2.20–3.30 mm long, 70–170 wide. The body of females devoid of spines and lacunar system. Body wall composed of thick outermost layer of cuticle. Female reproductive system consists of ovarian bells, uterine bell, uterus and vagina (Fig. 3c). At the posterior end of the uterine bell, there are two guard cells, which serve the function of separating the mature from the immature ova. Uterine bell 100 long by 40 wide, gonopore terminal. Uterus 210 long and 50 wide. Female body filled with ovarian ball measuring 80–110 wide and 50–60 wide. Mature eggs are elliptical in shape with the concentric membrane, measuring 60 long, 30 wide at greatest width (Fig. 3d).

Taxonomic summary

Type host: *Eleutheronema tetradactylum* (Shaw, 1804)
Site of infection: Intestine
Type locality: Digha, West Bengal, India (21.6266°N, 87.5074°E)

Table 1 Comparative measurements of male and female *N. dimorphospinus* and *Neoechinorhynchus dighaensis* n. sp. (measurements are given in micrometer unless otherwise noted)

Measurements	*N. dimorphospinus*	*Neoechinorhynchus dighaensis* n. sp.
	Male	Male
Host	*Dowsoma nasus* (Bloch, 1795)	*Eleutheronema tetradactylum* (Shaw, 1804)
Habitat	Brackish water	Brackish water
Trunk	2.970–6.660 (4.430) × 330–660 (462) mm	4.42–4.71 × 0.32–0.69 mm
Proboscis	65–117 (82) × 91–130 (104)	70–80 × 60–70
Proboscis hooks		
1st	59–96 (79), 51–86 (others in same ring)	43–44. 50–52 (others in same ring)
2nd	30–49 (38)	30
3rd	23–36 (30)	13
Proboscis receptacle	325–585 (442) × 78–169 (121)	270–390 × 70–110
Lemnisci	1.300–1.950 (1.595) × 91–130 (108) mm	1.40–1.93 × 50 wide mm
Reproductive system	2/3 of the trunk	2/3 of the trunk
Anterior testis	338–1,300 (682) × 169–377 (273)	450–640 × 150–340
Posterior testis	377–1,040 (630) × 169–390 (243)	450–650 × 150–350
Cement gland	143–182(160) × 104–143 (126)	360–720 × 150–360
Cement reservoir	NM	430–439 × 110–117
Saefftigens pouch	312–585 (429) × 143–390 (221)	150–550 × 50–90
Bursa	195 X 169	47–50 × 46–50
	Female	Female
Trunk	3.135–15.411 (9.867) × 330–880 (641) mm	5.04–6.59 × 0.65–0.69 mm
Proboscis	86–117 (95) × 96–156 (122)	110 × 10–90
Proboscis receptacle	351–650 (483) × 91–195 (138)	320–390 × 110–130
Lemnisci	2.240–2.730 (2.596) × 143–195 (160) mm	2.20–3.30 × 0.070–0.170 mm
Uterine bell		100 × 40
Ovarian ball		11 × 09
Eggs	29–36 (32) × 9–13 (10)	60 × 30

Zoobank registration No. lsid:zoobank.org:pub: 04C49E29-463F-42D3-B865-11B1BB4274AF

Etymology: The new species is named as per the site of collection of the host and parasite.

Discussion

The newly collected specimens placed in the genus *Neoechinorhynchus* Hamann, 1892 because of the presence of three circles of six hooks each on the proboscis and a single layered proboscis sheath with ganglion at its base. Present parasites compared with eight species, *Neoechinorhynchus dimorphospinus* (Amin and Sey 1996); *Neoechinorhynchus curemai* (Noronha 1973); *Neoechinorhynchus pseudemydis* (Cable and Hopp 1954); *Neoechinorhynchus emydis* (Leidy 1851); *Neoechinorhynchus emyditoides* (Fisher 1960); *Neoechinorhynchus chelonos* (Schmidt, Esch and Gibbons, 1970); *Neoechinorhynchus constrictus* (Little and Hopkins 1968); *Neoechinorhynchus stunkardi* (Cable and Fisher 1961) in having elliptical shape eggs with concentric egg membranes. It further differs from above mentioned species except *N. dimorphospinus* in having lateral proboscis hooks unequal in size.

Length of posterior proboscis hooks of males and females *N. dimorphospinus* 23–36 and 26–40 respectively but in *Neoechinorhynchus dighaensis* n. sp. form 13 in males and 14 in females. Length of anterior lateral hooks of males and females in *N. dimorphospinus* is 59–96 and 59–100 respectively but in *Neoechinorhynchus dighaensis* n. sp., 50–52 in males and 40 in females; other anterior hooks 51–86 (66) in males, 51–92 (68) in females but in present form 30 in male and 27 mm in females. Proboscis 65–110 long, 91–130 wide in males, 86–110 long, 96–150 wide in females but in *N. dighaensis* n. sp. 70–110 long, 60–100 wide. Eggs were ovoid shape in *N. dimorphospinus* but in the new species eggs elliptical in shape and measuring 30–60. Cement gland nuclei seven in number. A table comparing the measurements of present species with *N. dimorphospinus* is given (Table 1). All these differences

are sufficient to describe the current specimens as a new species *Neoechinorhynchus dighaensis* n. sp.

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Author's contributions Neelam Kumari Gautam: Data collection, analysis, drawing of the figures and drafting the manuscript. Anil Mohapatra: Data collection, fish identification and revising the manuscript. A. M. Saxena: Conception and design of study. Revising the manuscript critically for important intellectual content.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

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