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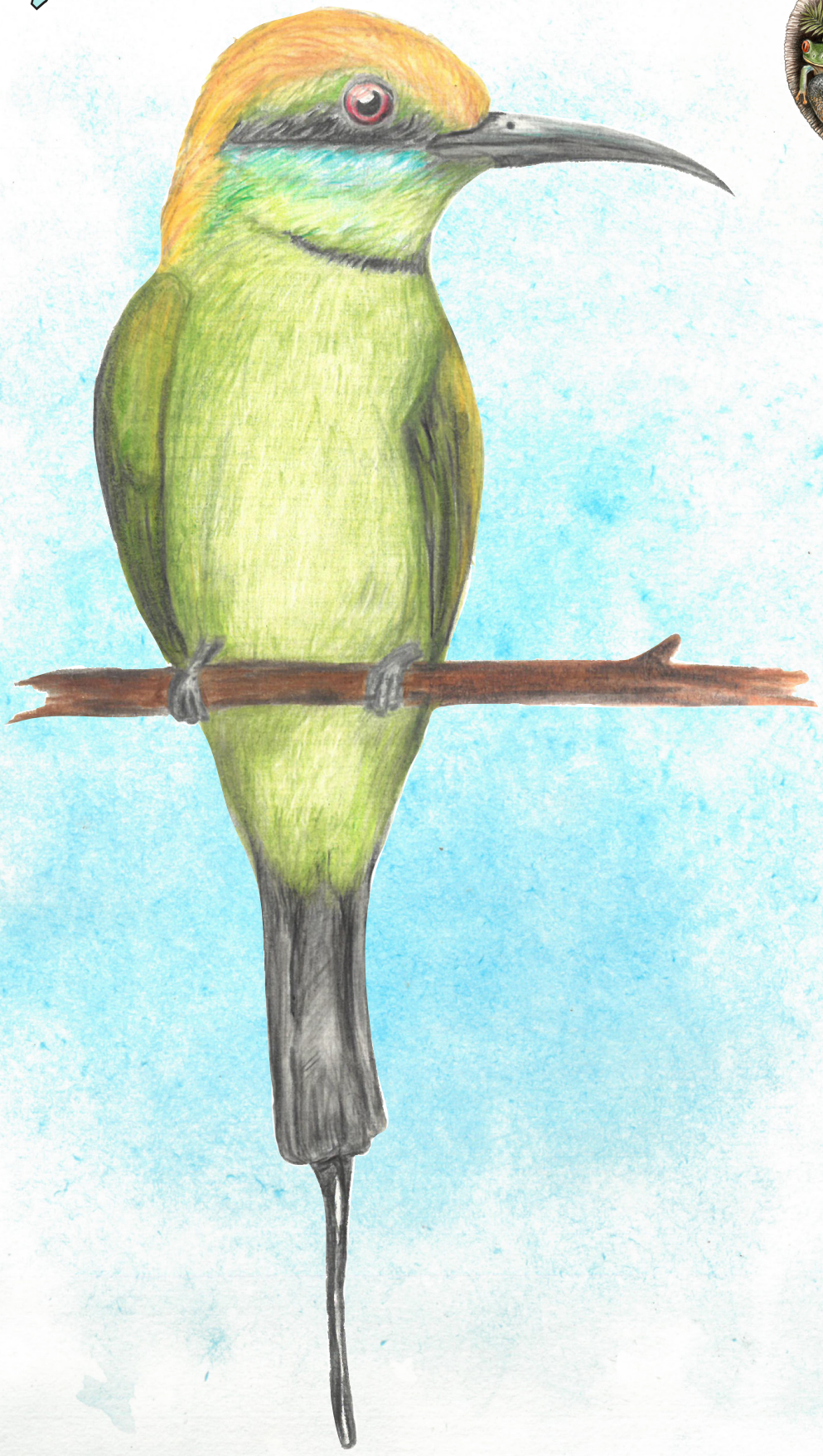

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Cover: Green Bee-eater with colour pencils and watercolor wash by Elakshi Mahika Molur.

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COMMUNICATION

Redefining *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933 from two freshwater fishes of Channidae family of Hooghly District, West Bengal, India

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Abstract: The genus *Pallisentis* has been mostly found among the freshwater fishes of Channidae family. *Pallisentis ophiocephali* is characterized with some unique characters of proboscis, hooks, and spines. However, initial description is a little sketchy and yet not updated. The present study communicates the detail morphology of an acanthocephalan species obtained from two host fishes of Channidae family—*Channa striatus* and *Channa punctatus*—during a one-year survey from different places of Hooghly District, West Bengal. The parasite is examined under light microscope as well as under scanning electron microscope. Unique four hook circles with different sizes, collar, and trunk spines, male and female genital organs are described for taxonomic study. Scanning electron microscopic study also provides the detailed information about the surface topography including longitudinal muscle, retractor muscles, and posterior ends. Comparing the studied specimen with other closely related species, the present acanthocephalan specimen has been identified as *P. ophiocephali* from *Channa striatus*. The retractable nature of proboscis has also been studied from the live specimens with the help of light microscope.

Keywords: Acanthocephalan, *Channa punctatus*, *Channa striatus*, retractable proboscis.

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Author contributions: Authors have the equal contributions to do the entire research work.

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INTRODUCTION

Species of the genus *Pallisentis* has been mostly found among the freshwater fishes of Channidae family. The genus *Pallisentis* has been considered in the family of Quadrigyridae Van Cleave (1920) in the subfamily Pallisentinae Van Cleave (1928) (Harada 1935; Yamaguti 1963). Amin et al. (2000) revised the characters of the genus *Pallisentis* under the subgenus *Demidueterospinus*. Thapar (1930)& Baylis (1933) described the species *Pallisentis ophiocephali.* According to Thapar (1930), *Farzandia* is a new genus, whereas, Baylis (1933) considered the genus as synonymous to *Pallisentis*. The type species *P. ophiocephali* was first reported from fresh water fish *Channa marulius* in different places of India (Andhra Pradesh, Odisha, Uttar Pradesh). Soota & Bhattacharya (1982) revised the validity of the species in the genus *Pallisentis* from India. Later Gupta et al. (2012) mentioned two host fishes (family: Channaidae) of the genus *Pallisentis* from Bareilly, Uttar Pradesh. Gupta et al. (2015) described the ultrastructure of another species, *P. punctati*, from Bareilly, Uttar Pradesh from the host *C. punctatus.* The present study has been carried out to understand the structural details of proboscis, hooks, spines, and genital parts from light microscopic as well as from scanning electron microscopic study and also to study the retractable nature of unique thorny proboscis from the live specimens.

MATERIALS AND METHODS

In order to study the prevalence of intestinal acanthocephalan infection among different freshwater fishes from July 2014–August 2015, 50 specimens of *Channa punctatus* (average length 15.4 cm and weight 190 g) and 47 specimens of *Channa striatus* (average length 18.7 cm and weight 320 g) were examined in Chandannagore (22.86^{0}N & 88.36^{0}E) of Hooghly District, West Bengal (Figure 1).

The fish samples were identified after Dey (1996), Talwar & Jhingran (1991). The classification of fishes was done following the keys of Jayaram (1999). White tape-like parasites were found tightly anchored through the proboscis in the intestinal wall of the host fishes. The specimens were carefully removed with a fine brush. Then they were collected and preserved following the methods of Soota (1980). All the parasites were washed by shaking thoroughly in physiological saline and transferred to 70% alcohol for their use in light microscope (LM) study. Pictures were taken with the help of Labomed CXL microscope. Measurements (based on 20 specimens) were recorded in millimetres and represented by mean followed by ranges. Schematic illustrations were drawn using the software CorelDraw 12, a vector graphics editor by Corel Corporation. For scanning electron microscopic study, specimens were preserved in 4% glutaraldehyde. Then they were transferred to 30%, 50%, and 70% alcohol respectively. After that the specimens were passed through a mixture of absolute alcohol and amyl acetate in 3:1, 1:1 for half an hour and then in 1:3 ratio for two hours. Lastly, they were transferred into 100% amyl acetate. After Critical Point Drying (CPD) the specimens were coated with gold and photos were taken under scanning electron microscope (Hitachi; S-530).

RESULTS

The present acanthocephalan species were collected from the intestinal wall of the host fishes. Among the collected specimens of the parasite, 20 male and 25 female were identified after studying under a light microscope. The species showed the structural details under light and scanning electron microscopic study.

General body structure: White in colour, cylindrical and 5.9–19.4 mm in length (Figure 2A; Image 1A). The body is divided into three regions: proboscis, short neck, and elongated trunk region. Proboscis; with recurved hooks, looks globular just erecting from proboscis receptacle and it seems subglobular when fully erected (Figure 2C; Image 1C,3C). Proboscis receptacle is sac like; cylindrical, single walled, and measures 0.39 (0.38–0.42) mm x 0.104 (0.10–0.12) mm (Image 1B). Two unequal, ribbon like, coiled lemnisci are present within the body cavity. They measure 1.562 (1.553–1.575) mm and 1.861 (1.857–1.920) mm (Image 1B). Anterior and middle part of the body is marked by several circlets of characteristics collar spines and body or trunk spines.

Proboscis hooks: The proboscis is armed with four circles (H1, H2, H3, and H4) of recurved hooks and each circlet contains eight hooks (Image 3C). The hooks are similar in shape but different in size (Figure 2F). The hooks are deeply rooted in the proboscis wall and without any striations or grooves. Hooks are dagger shaped (Figure 2G; Image 3D). Initial 0.020–0.028 mm of the hook is projected upwards and the rest bends outwardly. Each hook consists of a backwardly projected blade; a horizontally directed root and a handle embedded into the proboscis wall (Figure 2G; Image 3D).

Neck: A short neck, 0.19–0.29 mm, is present in

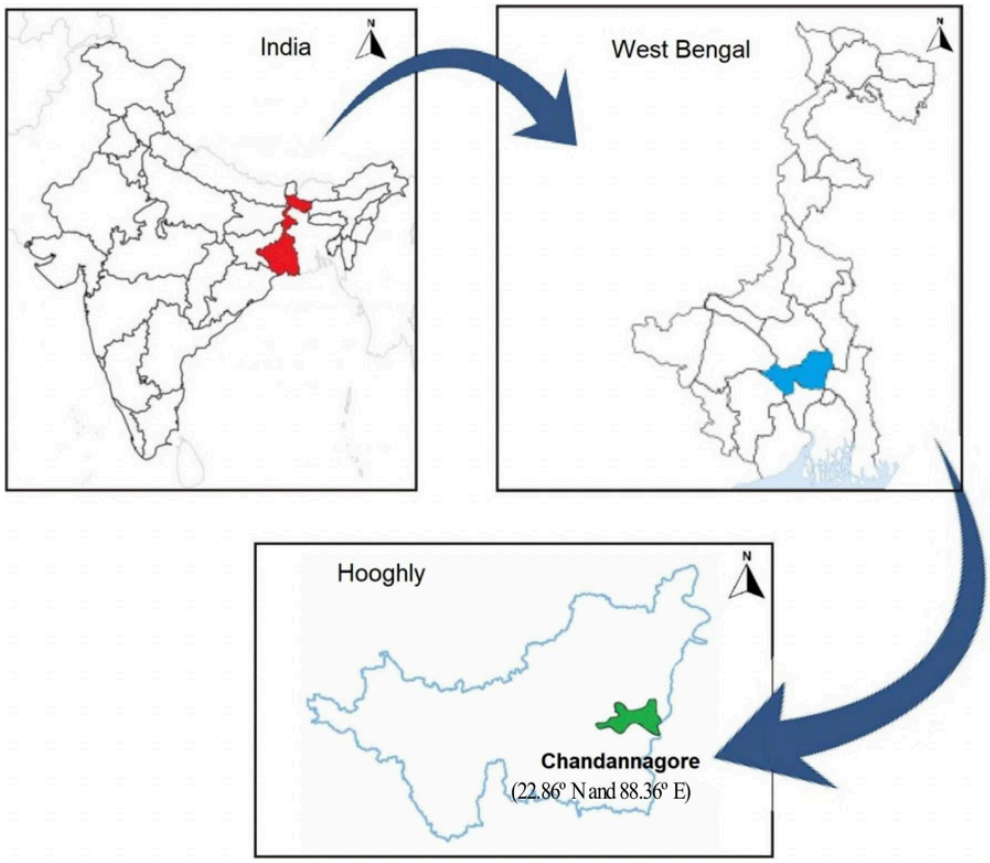

Figure 1. Survey area of of *Pallisentis ophiocephali* (Thapar, 1930) Baylis (1933) Chandannagore (22.86°N & 88.36°E) in Hooghly, West Bengal, India.

between the proboscis and the body. The retractor muscles are seen in the neck region (Image 3B). The parasite retracts the proboscis along with the neck into the proboscis receptacle.

Collar spines: Behind the neck, there are 13–16 circlets of collar spines and measure 0.027 (0.026–0.030) mm (Figure 2A,H). Collar spines are peepal leaf shaped structure, from the base it tapers gradually but at the distal end it tapers abruptly and ends in a tip. The base of the spine is 0.044 mm in average. The middle portion of the spine bulges outwardly and forms convex surface (Figure 2H; Image 3E,F).

Collar spines zone is followed by a spine free zone of 0.11–0.165 mm and body spines zone.

Body or trunk spines: The spine is pointed and projected downwards from a strong rounded base. Body is lined with longitudinal striations. Cement gland is single and syncytial with four–five nuclei. Distance between two spines within a row is 0.075 (0.072–0.078) mm. Distance between two rows is 0.25 (0.244–0.252) mm. Striations are of 0.075 (0.072–0.076) mm distance from each other (Figure 2I; Image 3G,H).

Male (Based on 20 specimens): The male is short and measures 6.1 (5.9–8.2) x 0.32 (0.28–0.33) mm (Figure 2C). Proboscis is 0.16 (0.16–0.18) x 0.21 (0.19–0.23) mm. Hooks in first–fourth circle are in the measure of 0.065 (0.064–0.067) mm, 0.06 (0.059–0.062) mm, 0.044 (0.042–0.047) mm, and 0.032 (0.031–0.034) mm respectively. Neck is 0.203 (0.19–0.21) x 0.209 (0.207–0.21) mm. Collar spines are in 13–14transverse circles, each with 16–17 spines. The length of the collar spines is 0.023 (0.022–0.024) mm. Trunk spines are in 26–29 circles. Numbers of trunk spines range from 14–15 in each circle. The length of the spines is 0.034 (0.032–0.036) mm. Testes are equal and measures 0.621 (0.616–0.643) mm. From each testis a vas deferens emerges and associates with cement gland, cement reservoir, and joins bursa. The bursa measures 0.181 (0.176–0.192) mm in length (Image 1G).

Female (Based on 25 specimens): Females are larger than males. It measures 17.1 (16.7–19.4) x 0.54 (0.52–0.60) mm (Figure 2D). Proboscis measures 0.18 (0.17–0.20) x 0.21 (0.19–0.23) mm. Proboscis hooks measure 0.079 (0.077–0.080) mm, 0.067 (0.065–0.072)

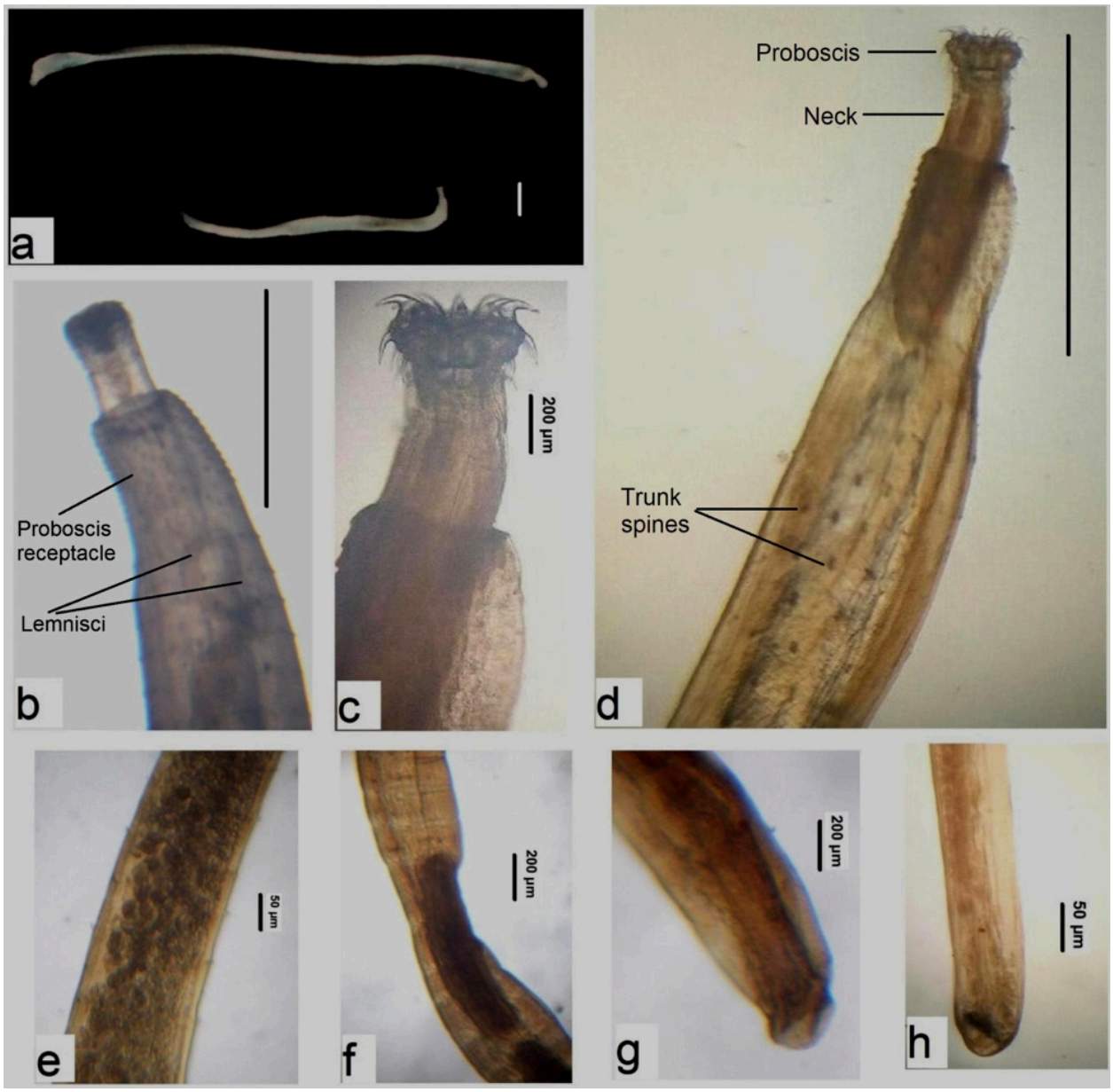

Image 1. Light microscopic pictures of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A—Adult specimens | B—Anterior region of male | C—Proboscis | D—Anterior region of female | E—Middle portion of female | F—Middle portion of male | G—Posterior region of male | H—Posterior region of female. (Bar = 1 mm). © Prabir Banerjee.

mm, 0.052 (0.048–0.053) mm, and 0.037 (0.032–0.044) mm respectively in the four successive circlets. The neck measured 0.282 (0.280–0.293) x 0.206 (0.202–0.21) mm. Collar spines are arranged in 14–16 circles. Each circle of collar spines is layered with 16–17 spines. Collar spines are 0.027 (0.026–0.030) mm in length. Numbers of circles of body spines range from 57–60 and, there are 14–15 spines in each circle. Trunk spines are 0.044 (0.042–0.046) mm in length. Whole body cavity of mature worm is filled with large number of eggs (Figure 2J; Image 1H). Eggs are 0.053 (0.051–0.062) x0.022 (0.021–0.033) mm and ovarian balls are 0.107(0.105–0.113) x 0.051(0.050–0.054) mm (Image 1E). The posterior region is rounded with a small gonopore (Figure 2E & Image 1H).

Observations on retractable proboscis

The inward and outward movement of retractable proboscis from the proboscis receptacle was observed on ten live specimens. The time of stretching in and out of the proboscis for each step was also calculated. The total process can be divided into three consecutive steps. First step is the emergence of the proboscis from

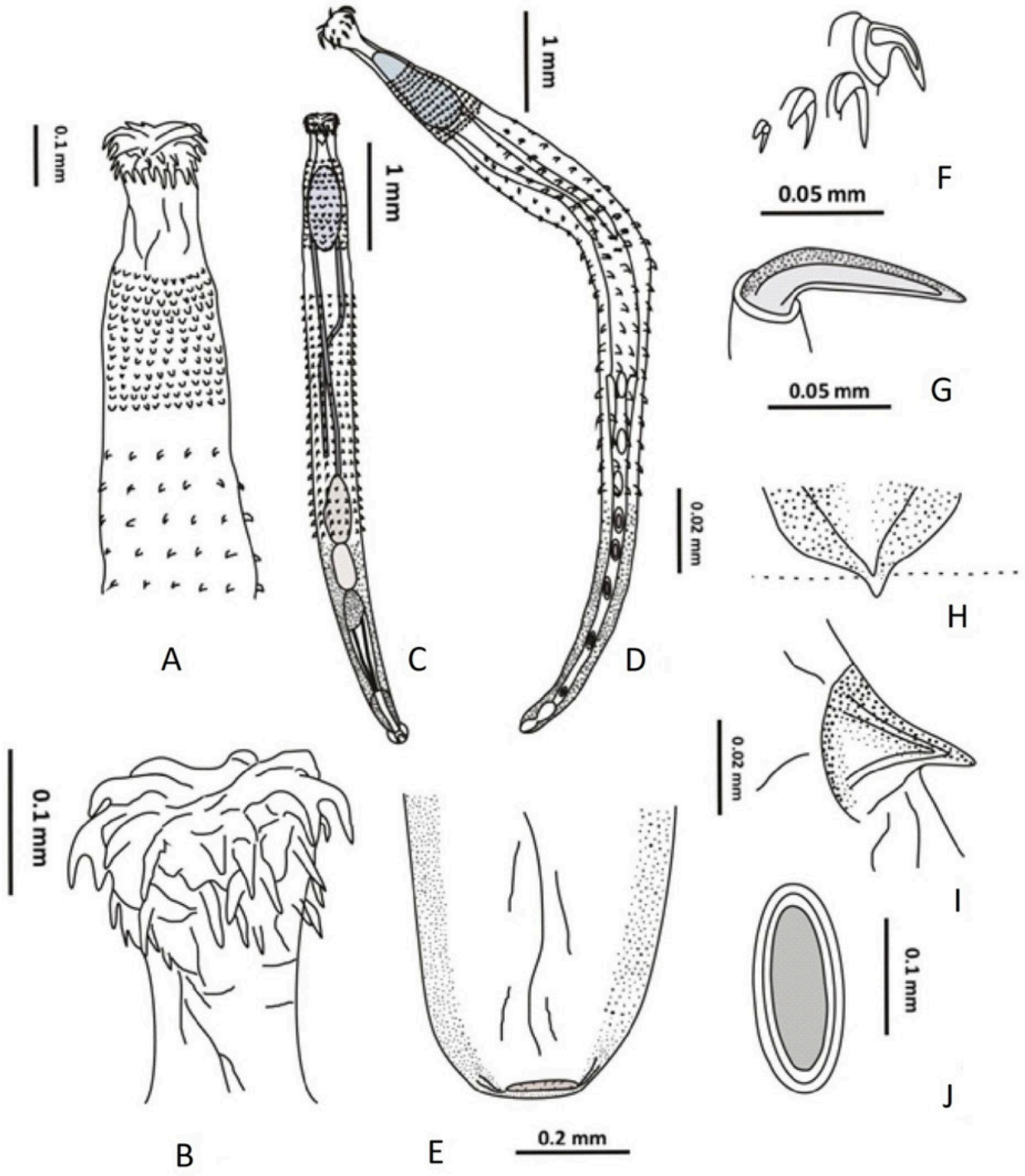

Figure 2. Schematic illustrations of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A—Anterior region | B—proboscis| C—Male specimen | D—Female specimen | E—Posterior region of female | F—Different types of proboscis hooks | G—Proboscis hook | H—Collar spine | I—Body spine | J—Egg.

proboscis receptacle and the recorded time was 27.3 (27.12–27.42) seconds (Image 2A–E). After emerging, the fully stretched proboscis paused for 32.52 (32.48–32.58) seconds (Image 2F). Lastly, the species took 12.08 (11.90–12.11) seconds for the inward movement, i.e., retraction was much faster than eversion. The total time for the completion of the whole process was 71.90 (71.84–72.33) seconds. The repetition of the whole process began after a pause of 19.20 (15.55–22.73) seconds.

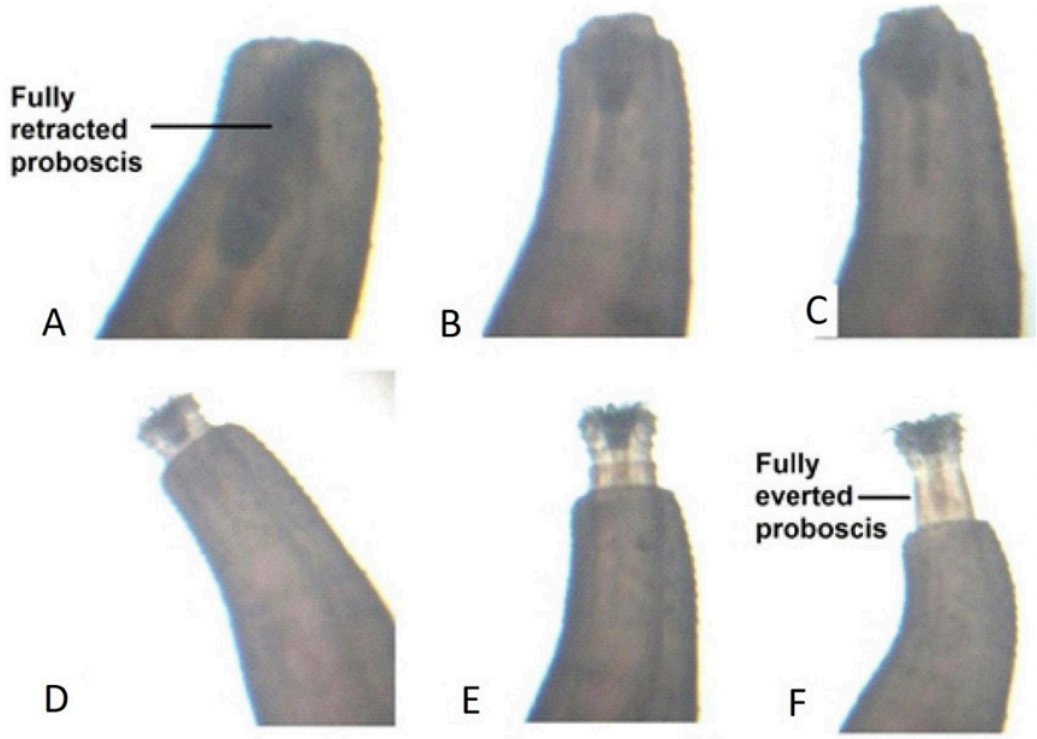

Image 2. Light microscopic pictures of retractable proboscis of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933 in different stages: A—Fully retracted proboscis | B–E—Outward movement of the proboscis | F—Fully stretched proboscis. © Prabir Banerjee.

Type host: *Channa marulius* (Hamilton, 1822).

Other host from different genera: *Nandus nandus* (Hamilton), *Grihiria* (*Cirrhina*) *cupla*, *Ompokpabda* (Hamilton), *Xenentodon cancila* (Hamilton), *Rana trigrina* (Daudin).

Other hosts in this study: *Channa punctatus* (Bloch, 1793) and *Channa striatus* (Bloch, 1793).

Distribution: Andhra Pradesh, Uttar Pradesh, Odisha, West Bengal of India.

Site of infection: Mostly in stomach, in few cases in proximal intestine.

Present collection Locality: Chandannagore (22.86 N & 88.36 E), Hooghly, West Bengal.

DISCUSSION

The present species has been collected from the wall of the intestinal tract of *Channa punctatus* and *Channa striatus*. Prevalence of the infection in two host fishes were recorded 26% and 21.2%, respectively. According to Soota & Bhattacharya (1982) and Bhattacharya (2007), members of the genus *Pallisentis* can be differentiated by the shape and size of the proboscis and hooks. The studied specimens are compared with *P. ophiocephali* (Thapar, 1930) Baylis, 1933, *P. colisai* (Sarkar, 1954) and *P. punctati* (Gupta et al. 2015). Comparing the present specimen with other closely related species, the present acanthocephalan species has been considered as *P. ophiocephali* as described by Thapar (1930) and later Baylis (1933) (Table 1). The species has been reported from Hooghly for the first time. In the description of type species, the geographical distribution was not specifically mentioned. Gupta et al. (2012) mentioned *Channa punctatus* as a host of *P. ophiocephali.* SEM studies reveal the detailed proboscis structure, arrangement of hooks and retractor muscles in the neck region. The scanning electron microscopic pictures also provide the surface structural details with high magnification that facilitates the understanding of the spatial relationships among surface structures and other minute organelle. Hooks and spines are some useful taxonomic tools for differentiating subgenera and species of acanthocephala. Morphometrically identical species generally have been examined and identified with the help of arrangements of hooks and spines. Moreover, some preliminary data has also been recorded from the retractable movements of the proboscis along with the required time span. The inward movement of the proboscis is comparatively faster than the outward movement. Further examinations are required to explore the untouched area of acanthocephalan biology.

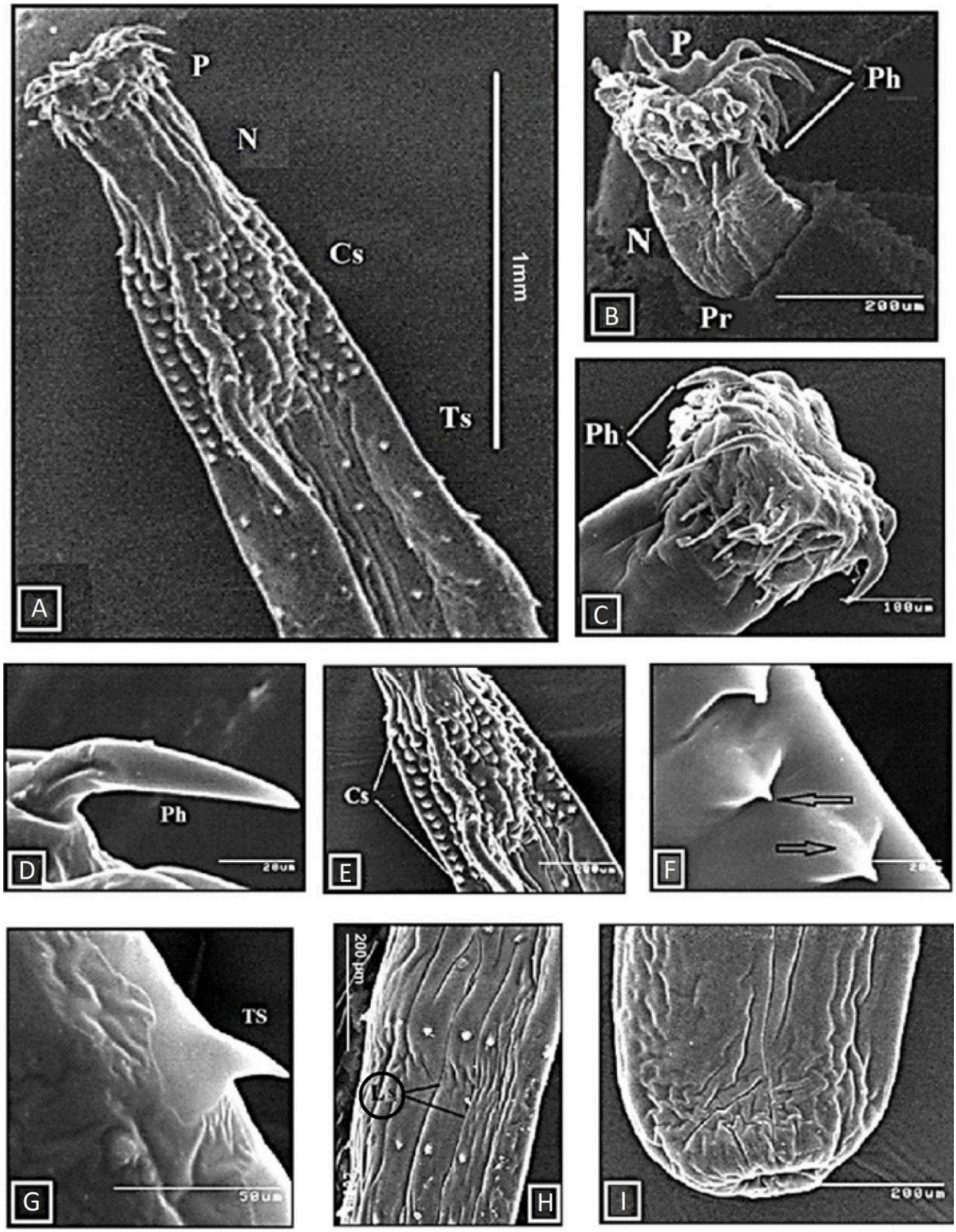

Image 3. Scanning Electron Microscopic pictures of female specimen of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A–C—Pictures showing the body with proboscis (*P*—Proboscis, *N*—Neck, *Cs*—Collar spine, *Bs*—Body spine, *Ph*—Proboscis hooks, *Pr*—Proboscis receptacle) | D—One proboscis hook | E–F—Collar spines (←) shows pointed tip and (→) indicates convex surface | G–H—Trunk spine(*Ts*—Trunk spine, *Ls*—Longitudinal striations) | I— Male posterior end (with retracted bursa). © Prabir Banerjee.

Table 1. Morphometric comparison of *Pallisentis ophiocephali* (Thapar, 1930) Baylis (1933) with other closely related species.

	***P. ophiocephali* (Thapar, 1930) Baylis, (1933) (Present species)**	***P. ophiocephali* (Thapar, 1930) Baylis, (1933) (Type species)**	***P. colisai* (Sarkar 1954)**	***P. punctati* (Gupta et al. 2015)**
Host	*Channa punctatus, Channa striatus*	*Channa marulius*	*Colisa fasciatus*	*Channa punctatus*
Location	Hooghly, West Bengal	Different places of India	Delhi	Bareilly, Uttar Pradesh
Size of body (L x W)	♂ 6.1 (5.9–8.2) x 0.32 (0.28–0.33) ♀ 17.1 (16.7–19.4) x 0.54 (0.52–0.60)	♂ 05.99 x 0.34 ♀ 14.3 x 0.495	♂ 4.13 x 0.39 ♀ 5.4–12.9 x 0.610	♂ (3.015–5.899) x (0.307–0.461) ♀ (5.472–14.791) x (0.461–0.820)
Size of proboscis (L x W)	♂ 0.16 (0.16–0.18) x 0.21 (0.19–0.23) ♀ 0.18 (0.17–0.20) x 0.21 (0.19–0.23)	♂ 0.14 x 0.22 ♀ 0.175 x 0.242	♂ 0.13 x .15 ♀ –	♂ (0.104–0.118) x (0.090–0.120) ♀ (0.126–0.180) x (0.140–0.198)
Length of neck (L x W)	♂ 0.203 (0.19– 0.21) x 0.209 (0.207–0.21) ♀0.282 (0.280–0.293) x 0.206 (0.202–0.21)	♂ 0.198 x 0.22 ♀ 0.308 x 0.22	♂ 0.26 x 0.17 ♀ –	♂ (0.190–0.255) x (0.108–0.118) ♀ (0.288–0.558) x (0.162–0.273)
Proboscis hooks length H1	♂ 0.065 (0.064–0.067), ♀ 0.079 (0.077– 0.080)	0.076–0.085	0.08 x 0.007	♂ 0.057 , ♀ 0.073
H2	♂ 0.06 (0.059–0.062), ♀ 0.067 (0.065–0.072)	0.068–0.076	0.07	♂ 0.054 , ♀ 0.063
H3	♂ 0.044 (0.042–0.047), ♀ 0.052 (0.048– 0.053)	0.051	0.03	♂ 0.021 , ♀0.025
H4	♂ 0.032 (0.031–0.034), ♀ 0.037 (0.032–0.044)	0.034–0.0425	0.026	♂ 0.018 , ♀ 0.018
Lemnisci	1.861 (1.857–1.920)	1.925	2.2 x 0.05	–
No. of collar spines	♂ 13–14 x 16–17 ♀ 14–16 x 16–17	♂ 11–13 x 14–16 ♀ 13–14 x 14–16	♂16 x 14–16, ♀–	♂ 14, ♀ 22
Collar spines length	♂ 0.023 (0.022–0.024) x (0.016–0.018), ♀ 0.027 (0.026–0.03) x 0.023 (0.022–0.025)	–	–	♂ (0.021–0.028) x (0.010–0.014) ♀ (0.025–0.046) x (0.010–0.025)
No. of body spines	♂ 26–29 x 14–15 ♀ 57–60 x 14–15	♂ 28–34, ♀ 60–65	♂22 x 12–16, ♀67	♂ 12, ♀ 14–18
Body spines length	♂ 0.034 (0.032–0.036) x 0.013 (0.010–0.016) ♀ 0.044 (0.042–0.046) x 0.016 (0.014–0.022)	–	–	♂ (0.021–0.028) x (0.010–0.018) ♀ (0.036–0.057) x (0.014–0.025)
Testis	0.621 (0.616–0.643)	0.605–0.66	(1) 0.39 x 0.17 (2) 0.35 x 0.17	(0.374–0.684) x (0.133–0.216)
Egg	0.053 (0.051–0.062) x 0.022 (0.021–0.033)	0.068 x 0.025	–	(0.028–0.061) x (0.010–0.025)
Ovarian balls	0.107 (0.105–0.113) x 0.051 (0.50–0.054)	–	–	(0.039–0.064) x (0.025–0.054)

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