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REDESCRIPTION OF *PALLISENTIS* (*BREVITRITOSPINUS*) *INDICA* (ACANTHOCEPHALA: QUADRIGYRIDAE) FROM *CHANNA PUNCTATUS* BLOCH & SCHNEIDER (CHANNIDAE) IN ALIGARH, INDIA WITH NEW UNDERSTANDINGS OF OLD STRUCTURES

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ABSTRACT: *Pallisentis indica* Mital and Lal, 1976 was originally described from *Channa gachua* Hamilton (Channidae) in Kali Nadi River, Aligarh, India. The parasite was later placed in the subgenus *Brevitritospinus* Amin, Heckmann, Ha, Luc, and Doanh, 2000. Our collection from the spotted snakehead *Channa punctatus* Bloch & Schneider in another locality of the same stream in Aligarh produced many specimens with variable traits, revealing new structures that have never before been described in the Acanthocephala, especially relating to the ducted trunk spines. The proboscis has 4 circles of 10 hooks each, with hooks in the anterior 2 circles being considerably larger than those in the posterior 2 circles. Y-shaped trunk spines are ducted in 2 regions separated by a spineless zone. The anterior collar spines are in complete rings of 9–17 circles of crowded spines and the larger posterior trunk spines are in 1 (posterior) to 41 (anterior) circles extending to level of cement glands in males posteriorly. Considerable variations from the original description and new structures are reported for the first time.

A detailed taxonomic treatment of the acanthocephalan genus *Pallisentis* Van Cleave, 1928 by Amin et al. (2000) has finally resolved the taxonomic challenges posed by this complex group of 26 species of acanthocephalans. Conflicting taxonomic arrangements were resolved by creating 3 subgenera based on the difference in the size of proboscis hooks in subsequent circles, the size of cement glands, and the number of their giant nuclei. In their classification, Amin et al. (2000) placed *Pallisentis indica* in the subgenus *Brevitritospinus* for having "proboscis hooks in circle 3 about half as long as hooks in circle 2, and cement gland usually with few giant nuclei." Later, Amin and Taraschewski (2003) updated the treatment of Amin et al. (2000) by adding 3 more species.

In the original description, Mital and Lal (1976) described *P. indica* from *Channa gachua* Hamilton but not from *Channa punctatus* Bloch & Schneider, which was the host of our specimens. They examined both fish species from the same waters but described another species of *Pallisentis*, *Pallisentis croftoni* Mital and Lal, 1976 from *C. punctatus*. We found our specimens in *C. punctatus* in contiguous waters. The host relationship alone may confuse the identity of these acanthocephalan species, as many of the acanthocephalan morphological characteristics appear to overlap (see table 1 of Mital and Lal, 1976). However, the key by Amin et al. (2000) clearly distinguishes between the 2 species. In the present treatment, we redescribe *P. indica* and explore the much wider and variable range of observed traits and report new structures and understandings for the first time.

MATERIALS AND METHODS

Live spotted snakehead, *C. punctatus*, (10–16 cm long) were purchased from the local fish market of Aligarh (latitude: 27°54′N, 78°05′E), North India. The fish was originally caught from the Kali Nadi River, a tributary of the Ganga (Ganges) River at Narora (28°11′48″N, 78°22′53″E) in the Bulandshahar District, Uttar Pradesh. The spotted snakehead is also found in Afghanistan, Pakistan, Sri Lanka, Nepal, Bangladesh, Myanmar, and China in swamps, ponds, and brackish water systems where they feed on insects and small fish (Hamilton, 1822; Pethiyagoda, 1991). The Kali Nadi River originates in the Doon Valley and passes through Saharanpur, Muzaffarnagar, and Bagpat districts before merging with Hindon River (at Barnava, Bagpat), which then merges with the Yamuna River (near Delhi) and, subsequently, joins the Ganga (Ganges) River which finally drains in the Bay of Bengal (Jain et al., 2007). In Aligarh, the Kali Nadi River forms the southwestern boundary of the Aligarh district. It is a small stream, particularly during the summer months, but it becomes a river of considerable dimensions during the rainy season (Kumar et al., 2005). The hosts of *P. indica* originally reported by Mital and Lal (1976) were also caught in the Kali Nadi at Khurja, 58 km away from Narora. The distance between Khurja and Aligarh is 51 km; 52 km between Narora and Aligarh. See Anonymous (2014) for a regional map of the waterways of the Aligarh District of Uttar Pradesh.

The fish were transported to the lab in water containers. They were paralyzed by cervical dislocation, immediately dissected, the stomach and intestine were cut open in a petri dish containing 0.74% NaCl (Advance Scientific and Chemical, Inc., Fort Lauderdale, Florida), and the live worms were carefully retrieved manually. The thoroughly washed worms were fixed in 70% ethanol for 24 hr. They were then stained with liquid borax carmine for 6 hr, destained with 0.5% HCl in 70% ethanol, dehydrated through an ascending series of ethanol, cleared in xylene, and whole mounted in DPX (DPX is a mixture of distyrene, a plasticizer, dissolved in toluene-xylene and used as a synthetic resin mounting media that replaces xylene-balsam). Measurements taken by ocular micrometer are in micrometers unless otherwise noted; the range is followed by the mean values in parentheses. Width measurements represent maximum width; trunk length does not include proboscis, neck, or bursa. Line drawings were created by using a Ken-A-Vision microprojector

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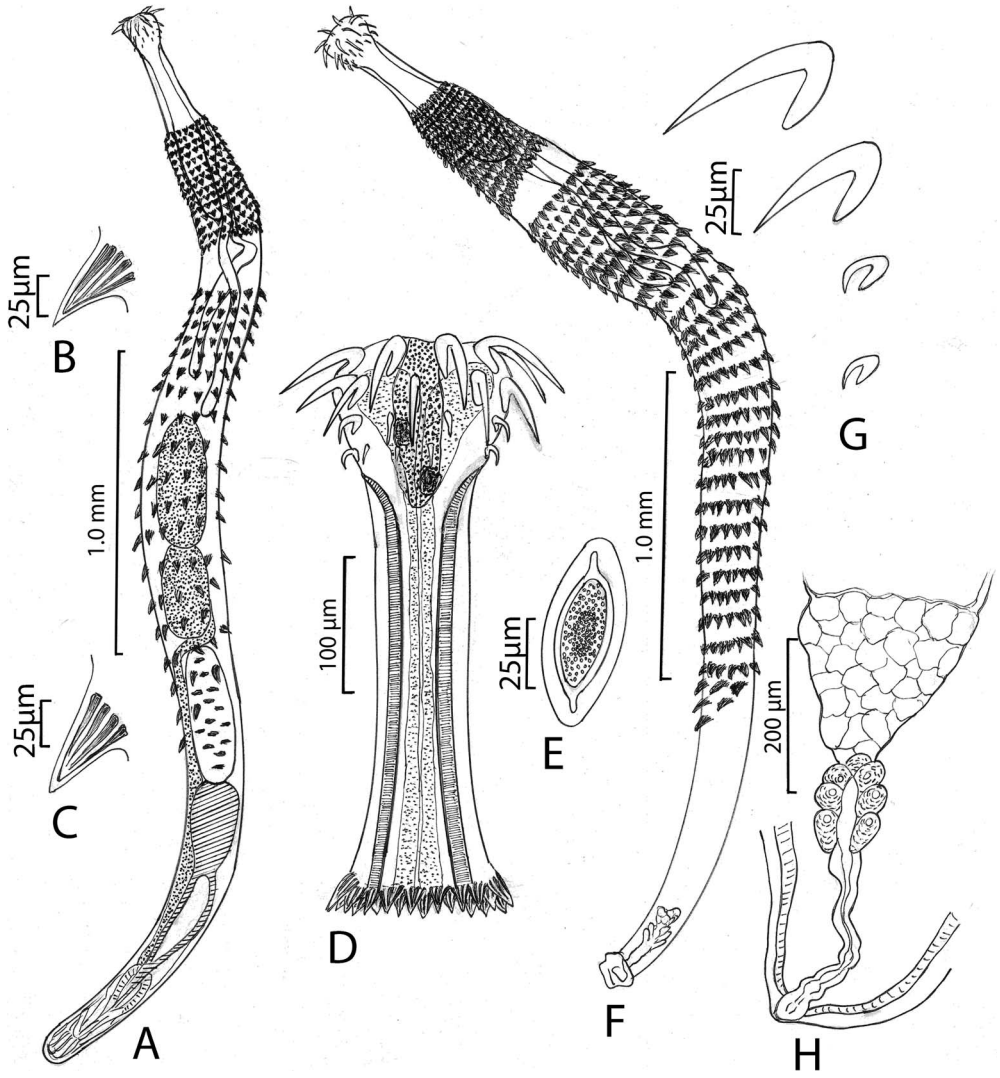

FIGURE 1. Line drawings of specimens of *Pallisentis indica* from *Channa punctatus* in India. (**A**) An adult male. (**B**, **C**) The Y-shaped collar and a trunk spine, respectively, in profile drawn to same scale. (**D**) The proboscis and neck of the specimen in A. (**E**) A fully developed egg. (**F**) A young female specimen drawn to the same scale as the male in A with a copulation plug at its posterior end. (**G**) One row of proboscis hooks and roots from specimen in A. (**H**) Female reproductive system.

(Ward's Biological Supply Co., Rochester, New York) which uses cool quartz iodine 150W illumination. Color-coded objectives, ×10, ×20, and ×43 lenses, are used. Images of stained, whole-mounted specimens are projected vertically on 300 series Bristol draft paper (Starthmore, Westfield, Massachusetts), then traced and inked with India ink. Projected images are identical to the actual specimens being projected. The completed line drawings are then scanned at 600 pixels on a universal serial bus (USB) and subsequently downloaded on a computer.

Voucher specimens were deposited at the University of Nebraska's State Museum Harold W. Manter Laboratory (HWML) collection in Lincoln, Nebraska.

No type or voucher specimens of *P. indica* were available for study, and the original manuscript by Mital and Lal (1976) was void of any reference as to where the type material was deposited, if at all. Communications with active researchers at the Universities where Mital and Lal worked in Khurja and Meerut, India failed to produce any reference specimens or information on their whereabouts.

For scanning electron microscope (SEM) studies, 11 specimens previously fixed in 70% ethanol were placed in critical point drying baskets and dehydrated using an ethanol series of 95% and 100% for at least 10 min per soak followed by critical point drying (Lee, 1992). Samples were gold coated at 80-nm thickness and observed under a SEM XL30 ESEMFEG (FEI, Hillsboro, Oregon). Digital images of the structures were obtained using digital imaging software. For studies of the spine structure (Fig. 3C), specimens were cut with plastic and diamond knives.

The cutting of spines was accomplished with a gallium beam using the FEI Helios Dual Beam Electron Microscope (FEI). Spines were positioned at the eccentric position of the stage and cut using a 30-Kv gallium ion gun operating at 2.8 nA. A cross-sectional pattern was used followed by a cleaning cross-section to provide a clean-cut surface to the image. Images were of the cut surface using a 5-Kv electron beam at 0.17 nA followed by X-ray spectrum analysis using a 15-Kv electron beam. Results were stored with a USB.

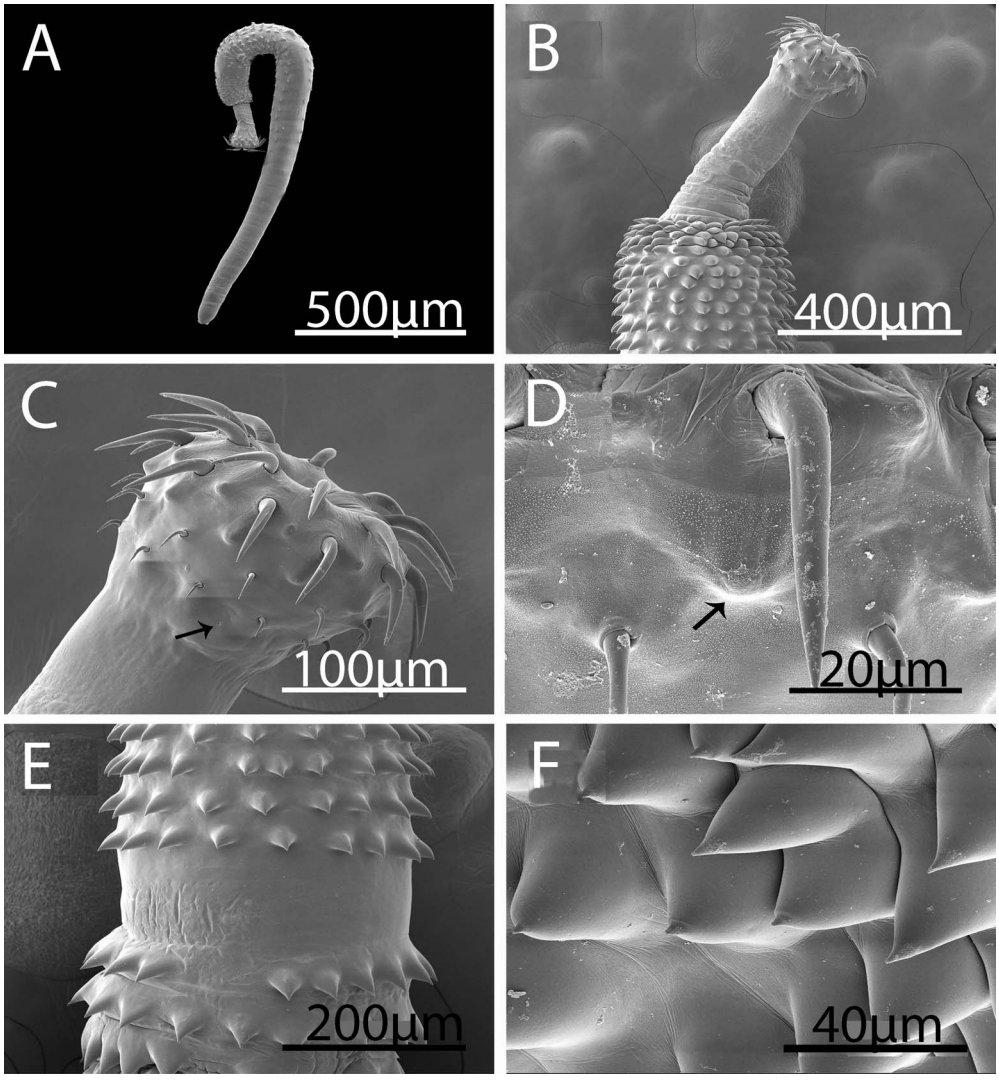

FIGURE 2. Scanning electron micrographs of specimens of *Pallisentis indica* from *Channa punctatus* in India. (**A**) A whole mount of a male specimen. (**B**) The anterior end of another male specimen depicting the long neck and the packed anterior field of collar spines. (**C**) A high magnification of the proboscis in specimen showing 1 of the 2 small sensory pores (arrow). (**D**) A different perspective of the same proboscis in C showing a high magnification of one of the unusual bumps (arrow) characteristic of this species. The distribution of these bumps can be seen in perspective in C. (**E**) The trunk of another specimen showing the spine-free zone separating the anterior collar spines from the larger posterior spines. (**F**) A high magnification of a few trunk spines showing their conical pointed posterior tip.

RESULTS

About 60 specimens and pieces of *P. indica* were collected from the intestines of spotted snakehead, *C. punctatus*, purchased from the local fish market in Aligarh, which were caught in the Kali Nadi River near Narora, Bulandshahar District, Uttar Pradesh, India. About 45 specimens were whole mounted but 30 suitable specimens (20 males and 10 females) were selected for microscopical study. The remaining specimens were used for SEM.

REDESCRIPTION

Pallisentis (*Brevitritospinus*) *indica* Mital and Lal, 1976

(Figs. 1–3)

Diagnosis: With characters of the genus *Pallisentis* and the subgenus *Brevitritospinus* as diagnosed by Amin et al. (2000). Small, cylindrical, spinose worms with trunk almost 3 times larger in females than in males and other structures nearly twice as large (Figs. 1A, F, 2A). Body wall with micropores of different diameter and distribution in different regions (Fig. 1D); giant hypodermal nuclei not observed. Trunk with Y-shaped trunk spines in 2 zones. Anterior set of small, crowded collar spines in complete circles and a posterior set of larger, more-widely spaced trunk spines extending posteriorly to level of cement gland in males and a corresponding distance in females (Fig. 1A, F). Collar and trunk spines separated by a spine-free zone (Figs. 1A, F, 2E); all larger dorsally than ventrally. Trunk spines larger and more numerous in larger female specimens than in smaller male specimens. Trunk spines becoming smaller and more-widely spaced posteriorly, and fewer in posterior circles. Posterior-most spines are very few and only on ventral side (Fig. 1A, F). Spines (Fig. 2F) ducted (Fig. 3A, B), with complex ribs (Fig. 1B, C), narrow posterior tip (Fig. 2F), and peripheral channels (Fig. 3C) connected to micropores. Proboscis truncated, widest anteriorly, and triangulating posteriorly into a long, prominent neck (Figs. 1D, 2B, C). Proboscis with conspicuous apical organ bearing at least 2 complex giant nuclei and rooted hooks in 4 circles of 10 hooks each (Fig. 1D). Hooks in 2 anterior circles longest, with hooks in apical circle slightly longer than hooks in second circle. Hooks in next 2 circles considerably shorter, with hooks in fourth circle being shortest. Hooks in anterior 2 circles slightly arched

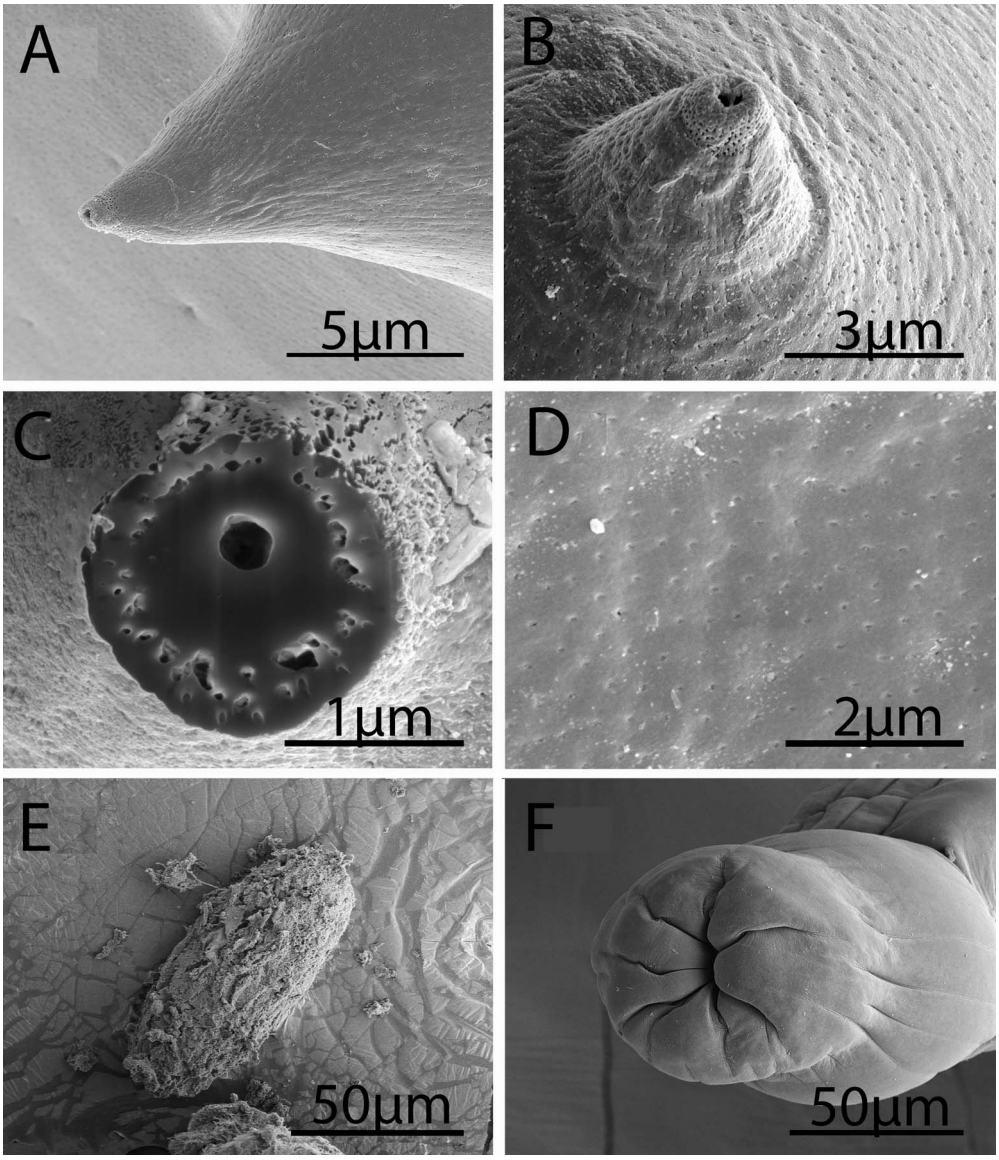

FIGURE 3. Scanning electron micrographs of specimens of *Pallisentis indica* from *Channa punctatus* in India. (**A**) A lateral view of an anterior collar spine showing the ducted tip. (**B**) An apical view of a similar ducted spine showing the continuation of its cuticular surface and micropores with those of the trunk surface. (**C**) A gallium-cut spine showing the central canal and the peripheral pores that appear to be continuous with the cuticular micropores. (**D**) Micropores on the cuticular surface of the mid-trunk of a specimen. (**E**) An egg. (**F**) The invaginated bursa of a male specimen.

and oriented somewhat laterally. Smallest hooks in posterior 2 circles sharply bent posteriorly. Hook roots simple, directed posteriorly, shorter than blades (Fig. 1G). Space between hooks in second circle, and between those hooks and hooks of third circle, with protruding bumps appearing like subcutaneous incipient hooks (Fig. 2C, D). One pair of sensory pores just posterior to posterior circle of hooks (Fig. 2C, arrow). Neck long, robust, half as long as receptacle. Proboscis receptacle single-walled, about 5 times as long as proboscis, widest posteriorly (Figs. 1D, 2B), with prominent ovoid cephalic ganglion at posterior end. Receptacle extending posteriorly to between levels of middle of collar spines and anterior circles of trunk spines. Lemnisci unequal, club-shaped, longer than receptacle, extending to level of anterior testis and corresponding distance in females, with small oval giant nuclei. Gonopore terminal in males (Fig. 1A), latero-terminal in females (Fig. 1H).

Males (based on 20 adult specimens examined microscopically and 5 using SEM): Trunk 1.55–4.75 (3.03) mm long by 0.17–0.35 (0.25) mm wide. Collar spines in 12–17 (15) complete circles, each with 12–22 (16) spines measuring 18–42 (30) long by 12–25 (18) wide at base. Spineless area 37–162 (ventrally–dorsally) (86) long. Trunk spines in 15–27 (19) circles, each with 10–16 (13) spines anteriorly measuring 30–70 (43) long by 20–62 (32) wide at base. Posterior-most circle with 1 ventral spine. Proboscis 92–150 (114) long by 130–162 (145) wide anteriorly. Length of proboscis hooks from anterior 65–82 (76), 57–75 (68), 23–32 (28), 15–25 (20). Neck 187–395 (272) long by 87–156 (114) posteriorly. Proboscis receptacle 450–700 (553) long by 75–140 (101) wide. Shorter lemnisci 350–915 (547) long by 27–40 (34) wide posteriorly. Longer lemnisci 425–1,260 (789) long by 25–45 (36) wide posteriorly. Reproductive system in posterior 3/5 to 3/4 of trunk; testes oblong, contiguous (Fig. 1A). Anterior testis 291–884 (473) long by 73–218 (112) wide. Posterior testis shorter: 208–520 (373) long by 82–239 (119) wide. Cement gland continuous with and somewhat smaller than posterior testis: 156–520 (324) long by 83–239 (124) wide containing 9–18 (13) giant nuclei. Cement reservoir

contiguous with and about as large as cement gland and branching posteriorly into 2 ducts, 200–572 (348) long by 87–208 (131) wide. Anterior sperm duct adjacent to cement gland, 130–416 (280) long by 15–73 (51) wide. Posterior sperm duct alongside of cement reservoir, 300–364 (325) long by 32–104 (63) wide. Saefftigen's pouch drop-shaped anteriorly with gradually narrowing posterior tube-like section (Fig. 1A); inflated portion 212–525 (long) by 70–135 (96) wide anteriorly.

Females (based on 10 adult specimens examined microscopically and 6 specimens using SEM): Trunk 4.12–13.50 (8.11) mm long by 0.35–0.75 (0.58) mm wide. Collar spines in 9–17 (14) complete circles each with 20–30 (27) spines measuring 37–75 (57) long by 20–55 (37) wide at base. Spineless area 110–260 (ventrally–dorsally) (86) long. Trunk spines in 26–41 (33) circles each with 16–24 (13) spines anteriorly measuring 45–117 (77) long by 25–75 (48) wide at base. Posterior-most circle with 1 ventral spine (Fig.1F). Proboscis 150–260 (181) long by 190–343 (258) wide anteriorly. Length of proboscis hooks from anterior 85–110 (99), 75–95 (87), 28–40 (34), 18–30 (25). Neck 333–749 (522) long by 218–312 (263) posteriorly. Proboscis receptacle 640–1,200 (974) long by 130–220 (187) wide. Shorter lemnisci 1.40–1.80 (1.58) mm long by 0.05–0.10 (0.06) wide posteriorly. Longer lemnisci 2.70–3.48 (2.89) long by 0.07–0.10 (0.08) wide posteriorly. Reproductive system 468–624 (572) long with at least 8 prominent mononucleated uterine glands and a cone-shaped uterine bell of large reticulate matrix (Fig. 1H). Eggs fusiform, unsculptured, with thick outer shell and prolongation of fertilization membrane (Figs. 1E, 3E), 67–87 long by 30–40 wide.

Taxonomic summary

Host: Spotted snakehead *Channa punctatus* Bloch & J. G. Schneider (Channidae).

Type host: Channa gachua Hamilton (Channidae) (Mital and Lal, 1976).

Other host: Channa punctatus Bloch & J. G. Schneider (Channidae).

Site of infection: Intestine.

Type locality: "Kali Nadi near Khurja" (Mital and Lal, 1976).

Other locality: Kali Nadi River at Narora, Bulandshahar District, Uttar Pradesh (28°11′48″N, 78°22′53″E).

Materials deposited: Harold W. Manter Laboratory (HWML) collection no. 102007.

Remarks

Specimens of *P. indica* and *P. croftoni* described by Mital and Lal (1976) were not available for examination. The 2 species were distinguished in the key of Amin et al. (2000) by the shape of trunk spines; conical in *P. croftoni* and Y-shaped in *P. indica*. Additional distinguishing characters include the larger size eggs and the extension of trunk spines posteriorly up to the level cement glands in *P. indica* (Fig. 1A). In *P. croftoni*, the eggs are considerably smaller and trunk spines extend only to the anterior testis.

Our study provides new information on the structure of spines, the female reproductive system, the proboscis, and eggs, among others. It gives a variable qualitative account of practically all features, from the structure of the eggs to the position of the female gonopore, and expands the range of variation in sizes well beyond those reported in the original description by Mital and Lal (1976). Especially unique are the trunk spines, which are decidedly ducted with peripheral canals that appear to correspond to the body wall micropores. A ducted spine with a terminal opening suggests a spine with secretory or glandular function. It seems possible that trunk spines have additional functions other than just anchoring. This has not been previously reported, at least in species of *Pallisentis*; see for example Amin et al. (2000) and Amin and Taraschewski (2003). The fusiform eggs with polar prolongation of fertilization membrane were not originally described or illustrated, and are unusual for this group of acanthocephalans.

Other structures not originally described include the apical organ and giant nuclei in the proboscis, the hook roots, the nucleated lemnisci, the 2 sperm ducts, and the position of the gonopores. The extreme sexual dimorphism in the size of trunk and other structures, as well as in the counts of trunk spines, is also different and had not been observed by Mital and Lal (1976). The size of their worms was markedly smaller than ours. For example, the following are smaller measurements from the original description: trunk in females (4.5–7.7 mm long compared to 4.12–13.5, mean 8.11 mm in our specimens), proboscis in females (172–219 × 125–298 compared to 150–260 × 190–343 in our specimens), proboscis hooks from anterior in females (80–92, 76–84, 24–36, 24 compared to 85–110, 75–95, 28–40, 18–30 in our specimens) and in males (56–68, 52–60, 20–24, 16–20 compared to 65–82, 57–75, 23–32, 15–25 in our specimens), proboscis receptacle in females (737–769 × 109–141 compared to 640–1200 × 130–220 in our specimens), lemnisci in females (1.60–1.99 mm long compared to 1.40–3.48 mm long in our specimens), collar spines (24–44 in females, 24–32 in males compared to 37–75 and 18–42 in our specimens) and trunk spines (44–52 in females, 32–48 in males compared to 45–117 and 30–70 in our specimens), and eggs (24–54 × 12–36 compared to 67–87 × 30–40 in our specimens). The size of proboscis hooks and eggs are especially important species-specific diagnostic traits and reported differences could be confusing.

This confusion may, however, be resolved if the female specimens and their eggs examined by Mital and Lal (1976) might not have been fully developed. The number of collar and trunk spines in the original description was also comparatively lower than in our specimens, but detailed comparison could not be made because Mital and Lal (1976) did not keep separate counts of spine numbers in males vs. females. Their line drawings of certain structures such as proboscis hooks (showing no roots), trunk spines, Saefftigen's pouch, and female reproductive system (their figures 12, 14, 18, 19, respectively) were incorrect and unrepresentative. We have corrected these misrepresentations in our line drawings.

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LITERATURE CITED

Amin, O. M., R. A. Heckmann, N. V. Ha, P. V. Luc, and P. N. Doanh. 2000. Revision of the genus *Pallisentis* (Acanthocephala: Quadrigyridae) with the erection of three new subgenera, the description of *Pallisentis* (*Brevitritospinus*) *vietnamensis* subgen. et. sp. n., a key to species of *Pallisentis*, and the description of a new quadrigyrid genus, *Pararaosentis* gen. n. Comparative Parasitology **67:** 40–50.

Amin, O. M., and H. Taraschewski. 2003. Description of subadult *Pallisentis* (*Pallisentis*) *rexus* (Acanthocephala: Quadrigyridae) from the vertebrate intermediate host in Thailand with an examination of the species identity. Proceedings of the Biological Society of Washington **116:** 215–221.

Anonymous. 2014. India map atlas. Available at: http://www.indiamapatlas.com/uttarpradesh/rivers/uttar-pradesh-river-map.html. Accessed 18 June 2015.

Hamilton, F. 1822. An account of the fishes found in the river Ganges and its branches. A. Constable and Company, Edinburgh, U.K., 405 p.

Jain, S. K., P. K. Agarwal, and V. P. Singh, 2007. Hydrology and water resources of India. Springer, New York, New York, 349 p.

Kumar, P., R. D. Singh, and K. D. Sharma. 2005. Water resources of India. Current Science **89:** 794–811.

Lee, R. E. 1992. Scanning electron microscopy and x-ray microanalysis. Prentice Hall, Englewood Cliffs, New Jersey, 458 p.

Mital, R. P., and S. S. Lal. 1976. Two new acanthocephalan worms *Pallisentis croftoni* sp. nov. and *P. indica* sp. nov. (Family–Pallisentidae) from fresh-water fishes of the genus *Ophicephalus*. Indian Journal of Zoology **17:** 169–175.

Pethiyagoda, R., 1991. Freshwater fishes of Sri Lanka. Wildlife Heritage Trust of Sri Lanka, Colombo, Sri Lanka, 362 p.