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Redescription and molecular analysis of *Pallisentis* (*Pallisentis*) *nandai* Sarkar, 1953 (Acanthocephala: Quadrigyridae) in India

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Research Paper

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Acanthocephala; *Pallisentis nandai*; India; redescription; new features; molecular profile

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Abstract

Pallisentis (*Pallisentis*) *nandai* Sarkar, 1953 is a freshwater fish parasite restricted to the Indian subcontinent in the Ganga River and its tributaries. It was described from the leaffish, *Nandus nandus* (Hamilton) from the Ganga River delta at Calcutta. We recovered variant specimens from the same host species from the Ganga near its headwaters at Bijnor about 1500 km away. Our specimens were clearly identifiable as *P. nandai* but varied considerably from those in the original description, especially in the size of proboscis hooks, receptacle and lemnisci. The original description was incomplete (missing line drawings of female trunk and reproductive system, male trunk, complete proboscis, hooks and hook roots) and inaccurate (proboscis, hooks, receptacle wall), and some measurements were lumped together for both sexes. We provide a complete description and include new morphological information including the first description of para-receptacle structure in the genus *Pallisentis* Van Cleave, 1928, scanning electron microscopy and microscope images, molecular analysis, and energy dispersive X-ray analysis (EDXA) of hooks and spines of our specimens for the first time. Additional details of proboscis hook roots, trunk spines, micropores and micropore distribution are described. The unique metal composition of hooks (EDXA) demonstrated a considerably high but variable level of sulphur and negligible level of calcium in collar and trunk spines and hook tips, but a higher level of sulphur and calcium at the hook basal arch than at the hook tip and edge. A comparison with the EDXA pattern of another species of *Pallisentis*, *P. İndica* Mital & Lal, 1976, were considerably different. The phylogenetic position of *P. nandai* within Eoacanthocephala was generated to assess the molecular characterization based on 18S and ITS1-5.8S-ITS2 ribosomal DNA sequences. Maximum likelihood and Bayesian inference analyses placed *P. nandai* in a clade with other *Pallisentis* species under the family Quadrigyridae. This is the first report based on molecular evidence for *P. nandai*.

Introduction

Pallisentis (*Pallisentis*) *nandai* Sarkar (1953) was placed in the subgenus *Pallisentis* Van Cleave, 1928, *sensu stricto* established by Amin *et al.* (2000). The subgenus is characterized by proboscis hooks gradually declining in size posteriorly and by a long cement gland with many giant nuclei. Two other subgenera had different proboscis hook and cement gland patterns (Amin *et al.*, 2000). The distribution of *P. nandai* appears to extend throughout the length of the Ganga River and its tributaries from its northern sources in China to its lower delta in eastern India and Bangladesh. Its description from *Nandus nandus* (Hamilton) in the Ganga delta from a Calcutta fish market (Sarkar, 1953) was repeated with some variations by other observers, including Soota & Bhattacharya (1982), Bhattacharya (2007) and Naidu (2012). Since its description from *N. nandus* (Nandidae), *P. nandai* has also been reported from the tank goby, *Glossogobius giuris* (Hamilton) (Gobiidae), from other tributaries of the Ganga in Uttar Pradesh (UP), India, and from many tributaries of the Ganga in the Bangladesh delta (see Naidu, 2012 for references). Our finding of *P. nandai* in Bijnor near the northern reaches of the Ganga adds new geographical dimensions and morphological and descriptive information that expand our understanding of this interesting acanthocephalan. Scanning electron microscopy (SEM), energy-dispersive X-ray analysis, micropore and related studies, and DNA analysis expand the body of knowledge about *P. nandai* in particular and of the genus *Pallisentis* Van Cleave, 1928 in general.

In India, the validity of many species of acanthocephalans is questionable because descriptions over the past few decades have been based on morphological observations lacking detailed descriptive information and illustrations, type specimens were not deposited in recognized museums and previously published data were ignored by the authors (Tadros, 1966; Mital & Lal, 1976; Pichelin & Cribb, 2001; Gupta *et al.*, 2015a; Gautam *et al.*, 2017). Molecular data are very scarce for Indian acanthocephalans. We supplement our study with

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Table 1. Collections of *Pallisentis nandai* made from *Nandus nandus* in the Ganga River at Bairaj, Bijnor, in 2019.

Date	Fish examined	Fish infected	Prevalence	Specimens collected	Mean/host
March 8	5	1	20%	12	2.4
April 5	10	8	80%	20	2.0
April 18	20	11	55%	37	1.85
April 27	8	0	–	0	–
May 10	15	0	–	0	–
Total	58	20	34%	69	1.2

the molecular profile of *P. nandai*. Thirty sequences are available for the genus *Pallisentis* in the GenBank database. In all, 24 sequences are available for the 18S ribosomal RNA (rRNA), one for the internal transcribed spacer (ITS) 1 region and three for the ITS1-5.8S-ITS2 cluster, while two sequences are available for the 28S rRNA gene, which demonstrates the scarcity of molecular data for the species of *Pallisentis* in comparison to the species diversity available (Amin, 2013). In the present study, we inferred the phylogenetic relationship of *P. nandai* based on the 18S and ITS1-5.8S-ITS2 rRNA gene sequences within the genus *Pallisentis* (Quadrigyridae) in the class Eoacanthocephala.

Materials and methods

Collections

We collected 69 worms from the livers of 20 of 58 examined fish between March and April 2019 in the Ganga River at Bairaj, Bijnor (29°01′N, 77°45′E) in the state of UP, India (table 1). The fish were obtained from local fishermen in a small fish market in Bairaj. Of the 49 extended specimens (not all specimens were extended) that were used, 23 were processed for microscopical studies, 16 were used for SEM, gallium-cut hooks and energy dispersive X-ray analysis (EDXA), five were used for molecular studies and five remain in the Omar Mohamed Amin (OMA) collection. Specimens were deposited in the University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection, Lincoln, Nebraska, USA. Freshly collected specimens were extended in water until proboscides everted, then fixed in 70% ethanol for transport to our Arizona, USA laboratory for processing and further studies. Twenty contorted specimens collected from dead fish on April 5 were discarded.

Methods for microscopical studies

Worms were punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4% hydrochloric acid in 70% ethanol, dehydrated in ascending concentrations of ethanol (24 h each), and cleared in 100% xylene then in 50% Canada balsam and 50% xylene (24 h each). Whole worms were then mounted in Canada balsam. Measurements are in micrometres, unless otherwise noted; the range is followed by the mean values between parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck or bursa.

Line drawings were created using a Ken-A-Vision microprojector (Ward's Biological Supply Co., Rochester, NY, USA), which uses cool quartz iodine 150 W illumination with 10×, 20× and 43× objective lenses. Images of stained whole-mounted specimens were projected vertically on 300 series Bristol draft paper (Starthmore, Westfield, MA, USA), then traced and inked with India ink. Projected images were identical to the actual specimens being projected. Microscope images were created using 10× and 40× objective lenses of a BH2 light Olympus microscope (Olympus Optical Co., Osachi-shibamiya, Okaya, Nagano, Japan) attached to an AmScope 1000 video camera (United Scope LLC, dba AmScope, Irvine, CA, USA), linked to an ASUS laptop equipped with HDMI high-definition multimedia interface system (Taiwan-USA, Fremont, CA, USA). Images from the microscope are transferred from the laptop to a USB and stored for subsequent processing on a computer. Forty two images were made to create the figures.

SEM

Specimens fixed and stored in 70% ethanol were processed for SEM following standard methods (Lee, 1992). These included critical-point drying in sample baskets and mounting on SEM sample mounts (stubs) using conductive double-sided carbon tape. Samples were coated with gold and palladium for 3 min using a Polaron #3500 sputter coater (Quorum (Q150 TES); www.quorum-tech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in an FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, OR, USA) scanning electron microscope, with digital images obtained in the Nanolab software system (FEI, Hillsboro, OR, USA) and then transferred to a USB for future reference. Samples were received under low vacuum conditions using 10 KV, spot size 2, 0.7 Torr using a Gas Sensitive Electrochemical (GSE) detector.

EDXA

Standard methods were used for preparation similar to the SEM procedure. Specimens were examined and positioned with the above SEM instrument, which was equipped with a Phoenix energy-dispersive X-ray analyser (FEI, Hillsboro, OR, USA). X-ray spot analysis and live scan analysis were performed at 16 Kv with a spot size of 5, and results were recorded on charts and stored with digital imaging software attached to a computer. The TEAM (Texture and Elemental Analytical Microscopy) software system (FEI, Hillsboro, OR, USA) was used. Data were stored in a USB for future analysis. The data included weight percent and atom percent of the detected elements following correction factors.

Ion sectioning of hooks

A dual-beam SEM with a gallium ion source (GIS) is used for the LIMS (Liquid Ion Metal Source) part of the process. The hooks of

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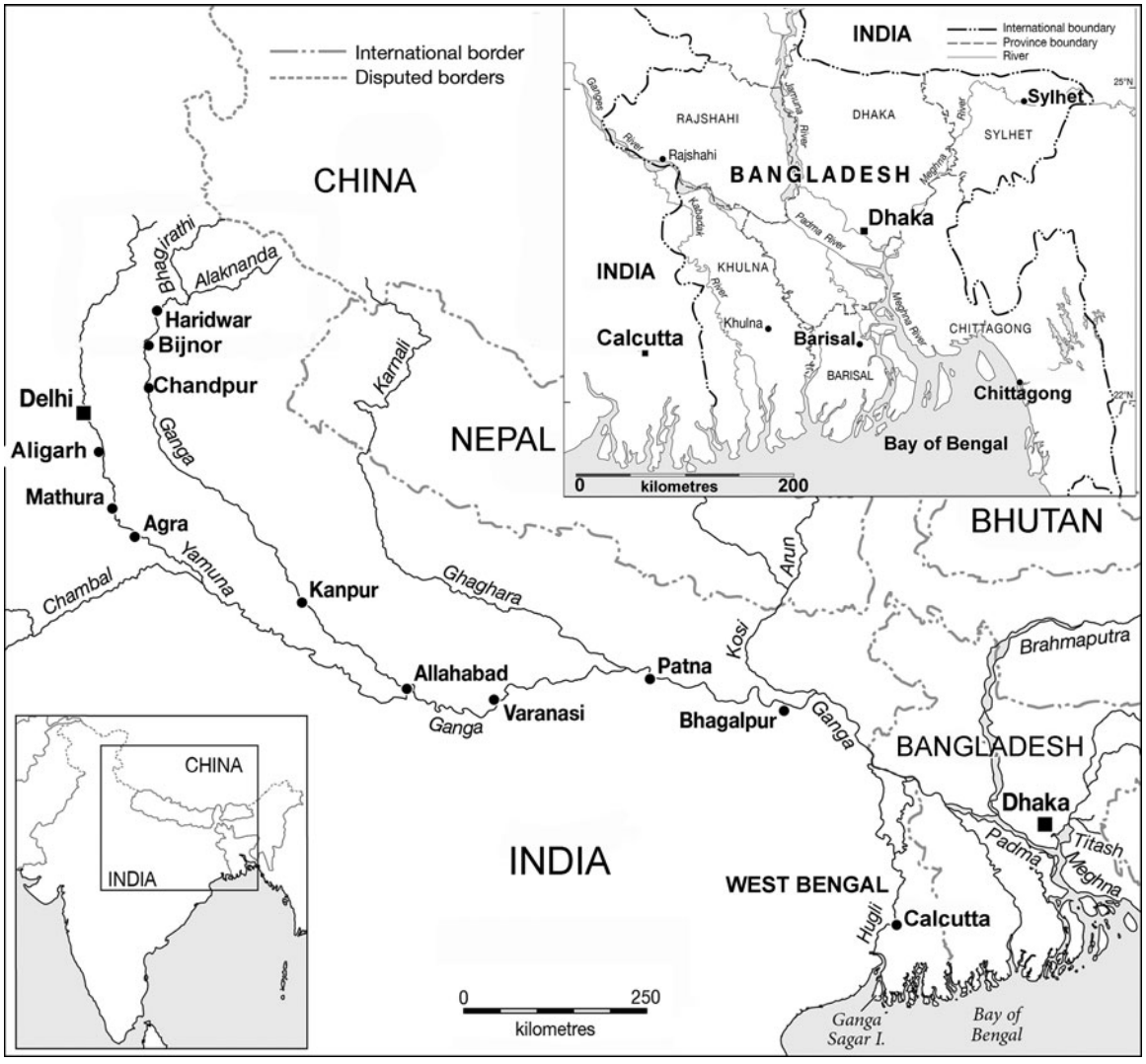

Fig. 1. Map of collecting localities of *Pallisentis nandai* in the Ganga River at Bijnor, Aligarh, Chandpur and Calcutta (India), and Sylhet, Dhaka, Barisal and Chittagong (Bangladesh).

the acanthocephalans were centred on the SEM stage and cross-sectioned using a probe current between 0.2 nA and 2.1 nA according to the rate at which the area was cut. The time of cutting was based on the nature and sensitivity of the tissue. Following the initial cut, the sample also underwent a milling process to obtain a smooth surface. The cut was then analysed with X-ray at the tip, middle and base of hooks for chemical ions with an electron beam (Tungsten) to obtain an X-ray spectrum. Results were stored with the attached imaging software. The intensity of the GIS was variable according to the nature of the material being cut.

Molecular methods

Genomic DNA was isolated from two specimens fixed in ethanol using a DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The 18S ribosomal DNA (rDNA) region V2-V8 was amplified using primers Worm A (5′-GCGAATGGCTCATTAAATCAG-3′); 1270R (5′-CCGTCAATTCCTTTAAGT-3′) (Littlewood & Olson, 2001) and 930F (5′-GCATGGAATAATGGAATAGG-3′); Worm B (5′-CTTGTTACGACTTTTACTTCC-3′) (Littlewood & Olson, 2001) and ITS1-5.8S-ITS2 by primers BD1 (5′-GTCGTAACAA GGTTTCCGTA-3′); BD2 (5′-TATGCTTAAATTCAGCGGGT-3′) (Luton *et al.*, 1992) and D1 (5′-AGGAATTCCTGGTAAGT GCAAG-3′); (5′-CGTTACTGAGGGAATCCTGGT-3′) (Galazzo *et al.*, 2002). The amplification reaction was performed as follows: denaturation at 95°C for 3 min for 40 cycles of 94°C for 40 s, 55°C for 45 s, 72°C for 1 min and termination at 72°C for 10 min. The polymerase chain reaction (PCR) products were checked on 1% agarose gel and purified with the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was done with a Big Dye Terminator version 3.1 cycle sequencing kit in an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) using the above-mentioned primers.

Contiguous sequences of 18S and ITS1-5.8S-ITS2 rDNA sequences of *P. nandai* were assembled with MEGA7 (Kumar *et al.*, 2016) and submitted to the GenBank database. To assess the phylogenetic relationship of *P. nandai* within *Pallisentis* in the class Eoacanthocephala, the newly generated sequences with other species data available on the GenBank database were aligned together using the program ClustalW (Thompson *et al.*, 1994),

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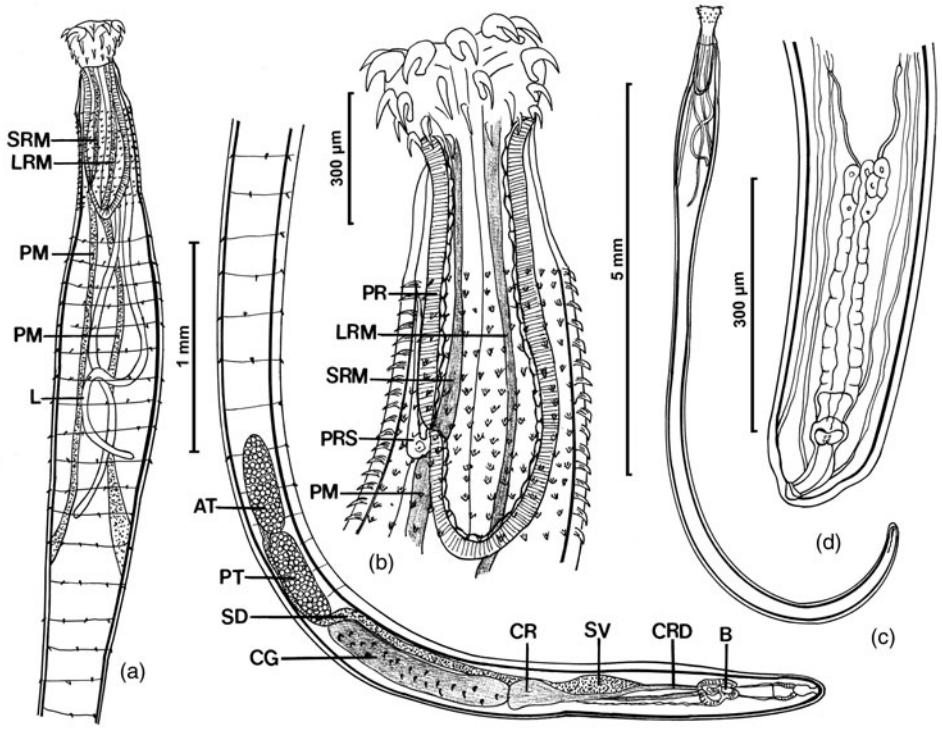

Fig. 2. Line drawings of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) A male specimen (in two sections). Note the distribution of trunk spines along the transverse lacunar canals characteristic of this species. (b) The anterior end of a female specimen. Note the inner receptacle lining. (c) A female. (d) Female reproductive system. Note the underdeveloped vulva, bent vagina, thick uterine wall, very thin uterine bell and the four long para-vaginal ligaments. Abbreviations: AT, anterior testis; B, bursa; CG, cement gland; CR, cement reservoir; CRD, cement reservoir duct; L, Lemniscus; LRM, long retractor muscle; PM, protractor muscle; PT, posterior testis; SD, sperm duct; SRM, short retractor muscle; SV, sperm vesicle; LRM, long retractor muscle; PM, protractor muscle; PR, proboscis receptacle; PRS, para-receptacle structure; SRM, short retractor muscle.

which is implemented in MEGA7. For molecular phylogeny, 18S and ITS1-5.8S-ITS2 sequences of species of Archiacanthocephala and Eoacanthocephala were included. Maximum likelihood (ML) analysis was performed by MEGA7 with 1000 bootstrap replicates. A Bayesian inference (BI) phylogenetic tree was generated by Topali 2.5 (Milne *et al.*, 2009), in which four independent Markov Chain Mont Carlo (MCMCMC) runs with every 100th tree saved and 'burn-in' was set to 25%. The evolutionary model GTR + G + I for both ML and BI analyses was estimated with the program jModelTest v2.1.10 (Darriba *et al.*, 2012) using corrected Akaike Information Criterion. Genetic distances (uncorrected *p*-distance) were estimated with MEGA7.

Results

The known distribution of *P. nandai* in the Gangetic Asian leaf-fish *N. nandus* appears to be limited to the Ganga River and its tributaries in India and Bangladesh (fig. 1; Naidu, 2012). The fish host is native to South Asia and Indochina (Pakistan to Thailand). It is common in slow-moving or stagnant bodies of water, including ponds, lakes, ditches and flooded fields, and feeds on aquatic insects and fish (Bhuiyan, 1964; Hossain *et al.*, 1992; Rainboth, 1996). There have been no reports of *P. nandai* within the natural range of distribution of *N. nandus* outside of India and Bangladesh in Bhutan, Cambodia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Thailand and Vietnam (Anonymous, 2019).

We provide a complete description of *P. nandai* and include new morphological information, SEM and microscope images, molecular analysis and EDXA of hooks of our specimens for the first time. We also report for the first time the presence of para-receptacle structure (PRS) in any member of the genus *Pallisentis*; all previous records were from acanthocephalans in the genera *Neoechinorhynchus* Stiles & Hassall, 1905 and *Acanthogyrus* (*Acanthosentis*) Verma & Datta, 1929. Additional details of proboscis hook roots, trunk spines, micropores and micropore distribution are described. The original description was incomplete and inaccurate, and some measurements were lumped together for both sexes.

The following morphological description is based on the microscopical examination of 23 specimens (11 males, 12 females) and others used in the SEM studies. These specimens were collected from the livers of 20 of 58 examined leaffish, *N. nandus*, between March and April 2019 in the Ganga River at Bairaj, Bijnour, UP, India (table 1). The liver has been reported as a site of infection for some other adult acanthocephalans of the genus *Pallisentis*.

Morphological description of our specimens from Bairaj

Pallisentis nandai Sarkar, 1953

General (figs 2–8). With characters of the family Quadrigyridae, genus *Pallisentis* and subgenus *Pallisentis* as diagnosed by Amin *et al.* (2000). Shared structures and spine counts larger and more numerous in females than in males (tables 2 and 3).

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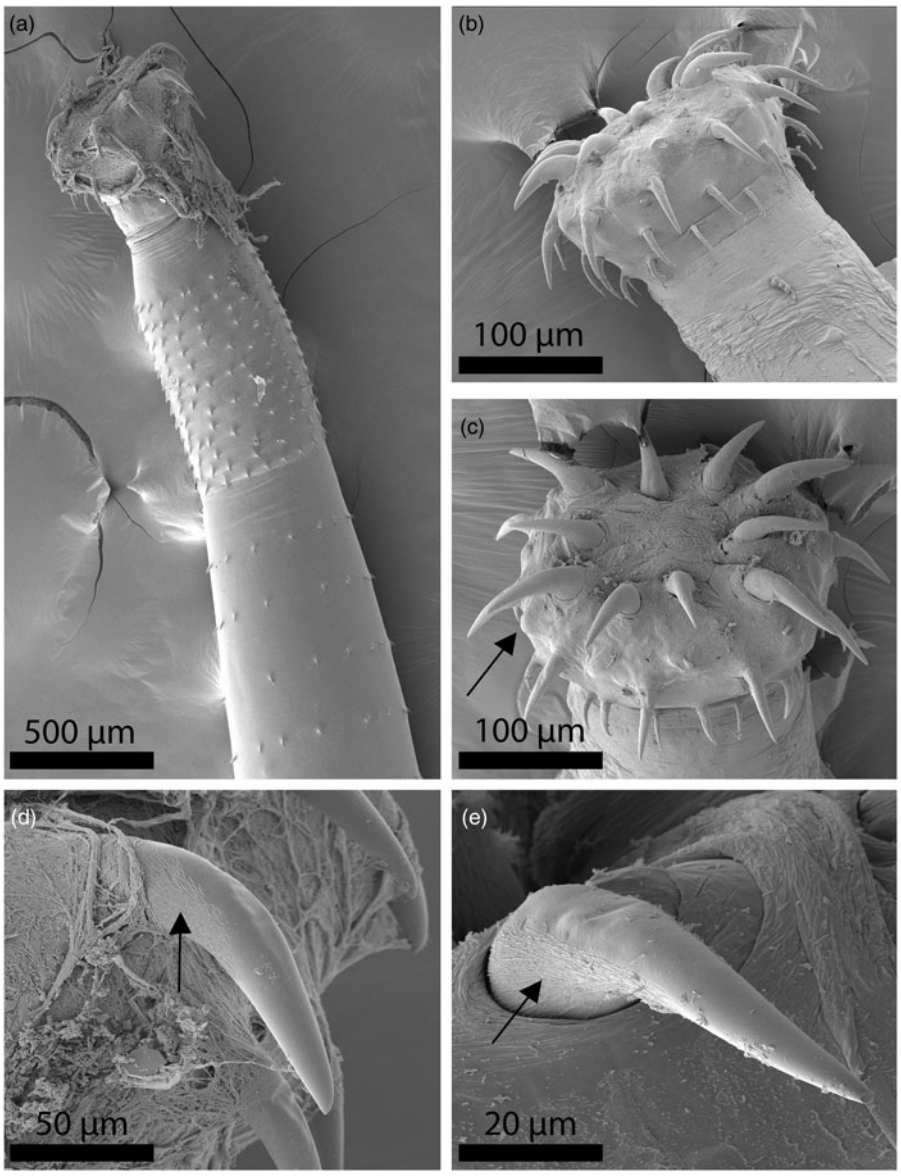

Fig. 3. SEM of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) Anterior part of a male specimen showing the proboscis, neck, close circles of collar spines starting posterior to anterior trunk and the more widely spaced trunk spines. (b) A profile of the proboscis and neck. Note the massive size of the apical hooks. (c) A near apical view of a proboscis showing the ten hook rows and the inter-hook pumps (arrow). (d) A lateral view of an apical hook. Note the elevated streaks at its base (arrow). (e) A near frontal view of another apical hook demonstrating a different perspective of the basal elevated streaks (arrow).

Trunk spinose, curved ventral mostly posteriorly, small, slender, cylindrical with anterior swelling more prominent in females than in males (figs 2a, c and 6a). Body wall even dorsoventrally, with electron dense micropores with different diameters and distributions in different trunk regions (fig. 5c, d), and up to three large hypodermal giant nuclei in occasional females (fig. 7d). Transverse lacunar canals connect two major lateral longitudinal canals at regular intervals, creating appearance of segmentation (fig. 7c). Trunk with slim triangular spines in two zones separated by a spine-free zone (figs 2a and 3a). Anterior collar spines (fig. 4c, d) heavily strengthened by two or three internal support rods of equal length to dermal spines (fig. 7a). Posterior trunk spines (fig. 4e, f) with single much longer and deeply embedded support rod each (fig. 7b). Collar spines in crowded complete circles closely set, beginning slightly posterior to anterior end of trunk and extending posteriorly to level of posterior end of receptacle in both sexes (figs 2b and 6a). Trunk spines in complete circles aligned with transverse lacunar canals at regular intervals widening posteriorly to level of male reproductive system (from anterior testis to mid-cement gland) (fig. 2a) and to level of anterior end to female reproductive system. All spines larger in females than males, with collar spines longer posteriorly than anteriorly and trunk spines smaller and more widely spaced posteriorly than anteriorly (table 3). Proboscis truncated, wider anteriorly and triangulating posteriorly into neck; with ten rows of four hooks each and unusual protruding bumps between larger hooks (fig. 3b, c). Hooks most robust anteriorly, gradually smaller and more slender posteriorly (figs 3d, e and 4a), with solid core and moderate cortical layer (fig. 4b), and with simple posteriorly directed and slightly curved roots, shorter than blades (table 2). Roots of apical and subapical hooks most robust; those of middle and posterior hooks slender (fig. 6c–f). Proboscis receptacle single-walled, about four times as long as proboscis, with dissimilar dorsoventral walls lined with undulating cell layer, and with prominent round cephalic ganglion near its base. Dorsal receptacle wall pinched close to posterior end at point of attachment of shorter retractor muscle and of insertion of anterior limb of PRS (fig. 6b). Other retractor muscle longer, extending along whole internal ventral wall of receptacle to its posterior end (figs 2b and 6a, b). Two sets of protractor muscles emerging from two posterior sites of

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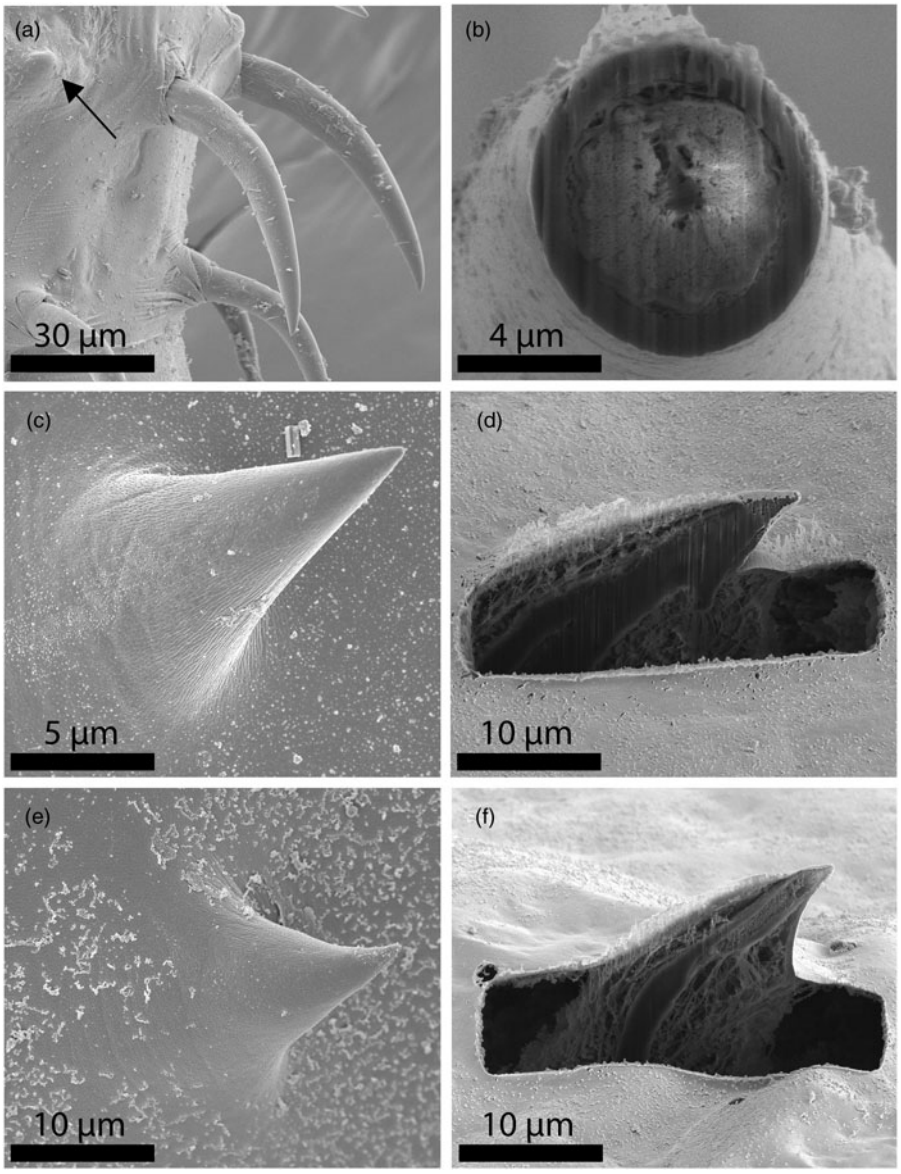

Fig. 4. SEM of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) A lateral view of sub-apical hooks with apparent inter hook pumps. (b) A gallium cut of an anterior hook showing its thick cortical layer and its almost solid core depicting its unique metal composition. (c) A collar spine. (d) A gallium-cut section of a collar spine. (e) A trunk spine. (f) A gallium-cut trunk spine. Note the difference in the degree of strength of the internal support rods.

receptacle, where long and short retractor muscles attach internally to receptacle wall, fan out to attach to body wall posteriorly (fig. 2a). Lemnisci unequal, long, extending well posterior to receptacle (fig. 2a). Gonopore terminal in males and ventro-terminal in females.

Males. (Based on 11 mature adults with sperm.) See tables 2 and 3 for measurements and counts of anatomical structures and spines. Reproductive system in posterior half of trunk. Testes contiguous elliptical elongate, with anterior testis slightly longer than posterior testis. Sperm ducts drain each testis dorsally, turning ventral as they unite just anterior to cement gland then joining into large thin-walled common seminal vesicle (fig. 2a). Cement gland as long as both testes with many crescent shaped giant nuclei and ducted connection to pear-shaped cement reservoir. Cement reservoir with two long and narrow ducts surrounding seminal vesicle to join base of penis posteriorly (figs 2a and 7e, f). Bursa not observed.

Females. (Based on 12 adults with ovarian balls.) See tables 2 and 3 for measurements and counts of anatomical structures and spines. Gonopore ventro-terminal with thick-lipped vulva. Vagina bent, with well-developed sphincter and two pairs of paravaginal ligaments extending anteriorly past uterine bell (fig. 2d and arrows in figs 5e, f and 8a). Uterus somewhat short, thick-walled, with two bands of long muscles. Uterine bell thin-walled, funnel-shaped, unattached to body wall, with few gland cells at base (fig. 8b). Eggs not seen.

Taxonomic summary

Type and present host. The leaffish, *N. nandus* (Hamilton).

Type locality. The Ganga River at a Calcutta fish market (22.5726°N, 88.3639°E).

Present locality. The Ganga River at Bairaj, Bijnor (29°01′N, 77°45′E), UP, India.

Site of infection. Liver.

Materials deposited. HWML collection number 216362 (male and female voucher specimens on three slides).

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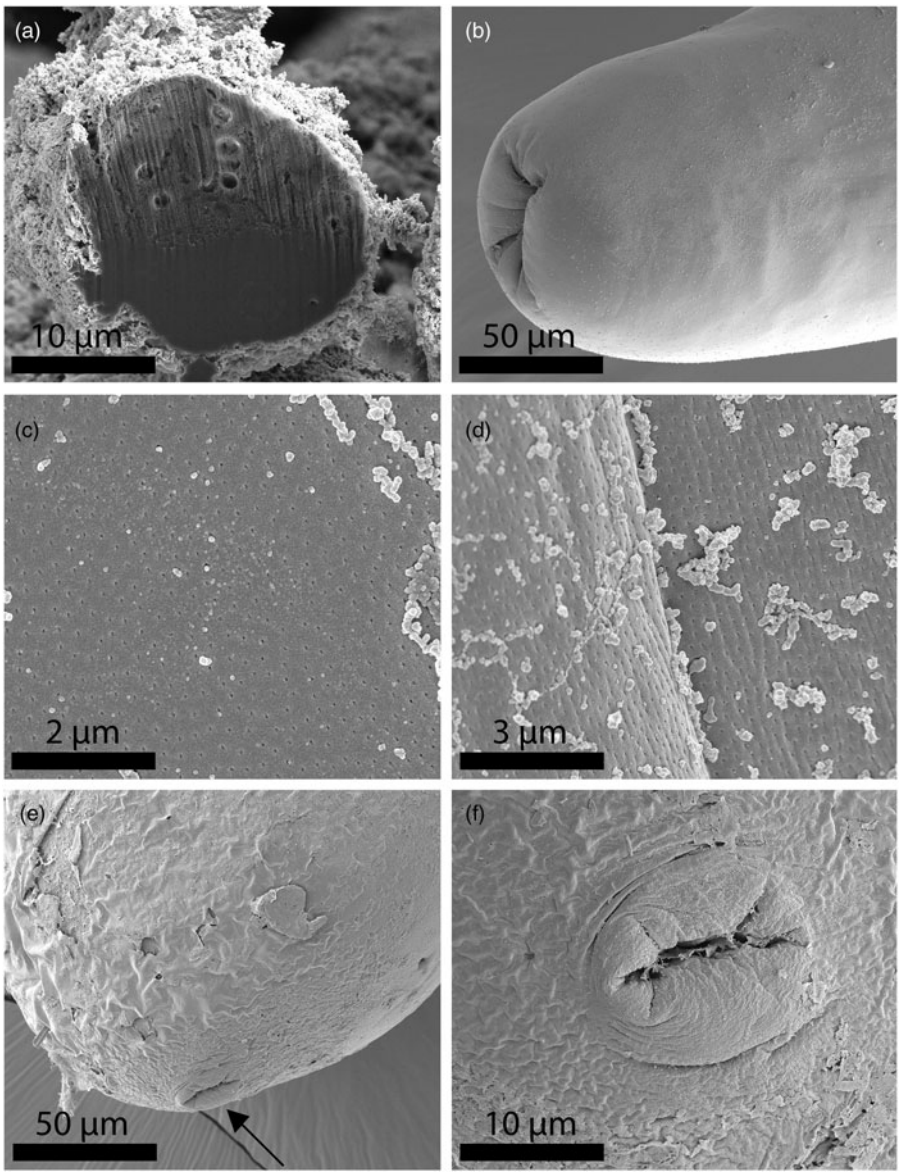

Fig. 5. SEM of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) A high magnification of the posterior circle of collar spines and the anterior circles of trunk spines. (b) The posterior end of a male specimen showing the terminal gonopore with unseen invaginated bursa. (c) Micropores at mid-trunk. (d) Micropores at anterior trunk. (e) The posterior end of a female specimen showing the sub-ventral position of the gonopore (arrow). (f) The orifice of the gonopore of a female specimen.

Representative DNA sequence. The 18S rDNA sequence of *P. nandai* was deposited in GenBank under the accession numbers MW164853 (1775 bp) and MW164854 (1770 bp), while the ITS1-5.8S-ITS2 region was deposited in GenBank under the accession numbers MW1825515 (785 bp) and MW1825514 (783 bp).

Remarks

The description of *P. nandai* has not been revised since its original description by Sarkar (1953), which was repeated with some variations by other observers, including Soota & Bhattacharya (1982), Bhattacharya (2007) and Naidu (2012). Since its description from *N. nandus*, *P. nandai* has also been reported from the tank goby, *G. giuris* (Hamilton) (Gobiidae), from other tributaries of the Ganga in UP, India, and from many tributaries of the Ganga in the Bangladesh delta (see Naidu, 2012 for references). For all practical purposes, the 66-year-old description by Sarkar (1953) remains the only source of taxonomic information about *P. nandai*. Our collection of the same acanthocephalan from the same host species in the same river system, albeit about 1500 km away from Calcutta in Bairaj, Bijnor, UP, revealed the extent of the incompleteness of the original description to which we added new information about unreported morphological structures and results of recent technologies such as SEM, gallium cuts, not to mention molecular analysis. We present an updated version of a comprehensive description of that species.

The morphological structures not included in the original description include line drawings of whole males and females, complete proboscis, hooks and roots, female reproductive system, collar and trunk spines, and detail of the proboscis receptacle and associated retractor muscles that are related to the discovery of the PRS not known at that time. We supplement this absent qualitative description with our detailed systematic treatment, line drawings, SEM and microscope images.

The EDXA and the molecular study add new dimensions not available to Sarkar in 1953. Comparative measurements (table 2) show that the size of hooks, receptacle, lemnisci in males and

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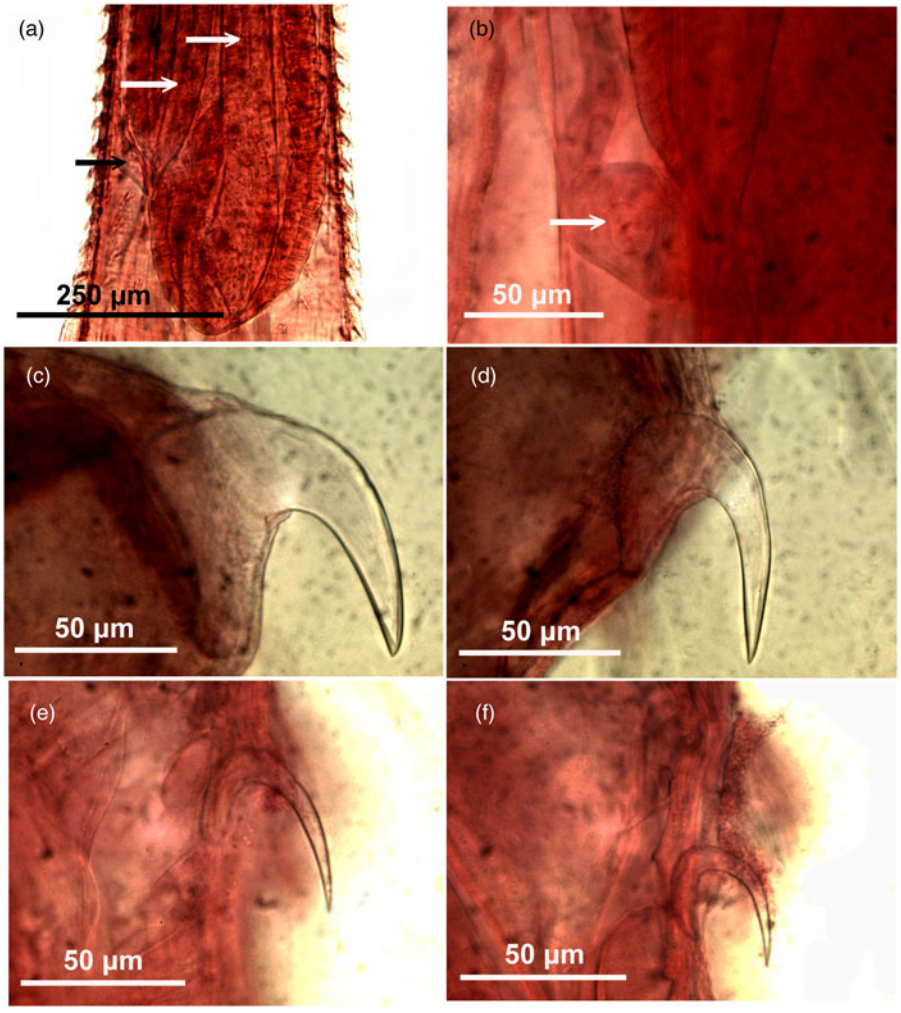

Fig. 6. Microscope images of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) The anterior end of a female specimen. Note the para-receptacle structure (PRS) (black arrow) inserted at juncture of the short retractor muscle (left white arrow) with the receptacle wall compared to the long retractor muscle (right white arrow). (b) A higher magnification of the nucleated PRS (white arrow) showing its insertion (opposite the junction of the short retractor muscle) and its anterior and posterior limbs. (c) Large anterior proboscis hook with robust root. (d) Second proboscis hook with broad root. (e) Third proboscis hook with more slender and shorter root. (f) Posterior proboscis hook with slender root. All hooks and roots are of female specimens.

females, as well as size of testes and trunk spines in females was markedly larger in our specimens from Bairaj than those of Sarkar (1953) from Calcutta. This is an interesting finding considering the fact that compared populations were collected from the same host species and from the same river system. Collection localities were, however, about 1500 km apart – one at the northern headwaters and the other near the southern delta of the Ganga.

The PRS

The PRS is reported here in a species of *Pallisentis* for the first time in an unusual arrangement where, as its insertion point into the receptacle, it is associated with the posterior attachment site of the shorter retractor muscles to the receptacle. Like species of *Neoechinorhynchus* and *Acanthosentis*, *P. nandai* also has a weak single-walled proboscis receptacle. We have examined specimens of four other species of *Pallisentis* in OMA's personal collection. Only specimens of one of them – *Pallisentis indica* Mital & Lal, 1976 – had a PRS similar to that described in *P. nandai*. This brings the total number of species of *Pallisentis* with PRS to two. Specimens of the other three species that we examined that did not have PRS are *Pallisentis* (*Pallisentis*) *celatus* (Van Cleave, 1928) Baylis, 1933, *Pallisentis* (*Brevitritospinus*) *vietnamensis* Amin, Heckmann, Ha, Luc, Donah (2000) and *Pallisentis* (*Pallisentis*) *rexus* Wongkham & Whitfield (1999).

EDXA

The unique metal composition of hooks (EDXA) demonstrated a considerably high but variable level of sulphur and negligible level of calcium in collar and trunk spines (table 4 and figs 9 and 10) and hook tips (table 6 and fig. 12), but a higher level of sulphur and calcium at the hook basal arch area (table 5 and fig. 11) than at the hook tip and edge. A comparison with the EDXA pattern of another species of *Pallisentis*, *P. indica* Mital & Lal, 1976, was considerably different (table 7).

Micropores

The electron-dense micropores present throughout the epidermal surface of the trunk of *P. nandai* are described. They have been found in various regions of the trunk in different diameters and distributions (fig. 5c, d).

Molecular results

Two identical sequences for the 18S and ITS1-5.8S-ITS2 region were generated from isolates of *P. nandai* collected from *N. nandus* in India. Phylogenetic relations estimated by ML and BI methods resulted in consensus trees with identical topologies (figs 13 and 14). The phylogenetic relationships of the *Pallisentis* species were very similar to the phylogenetic

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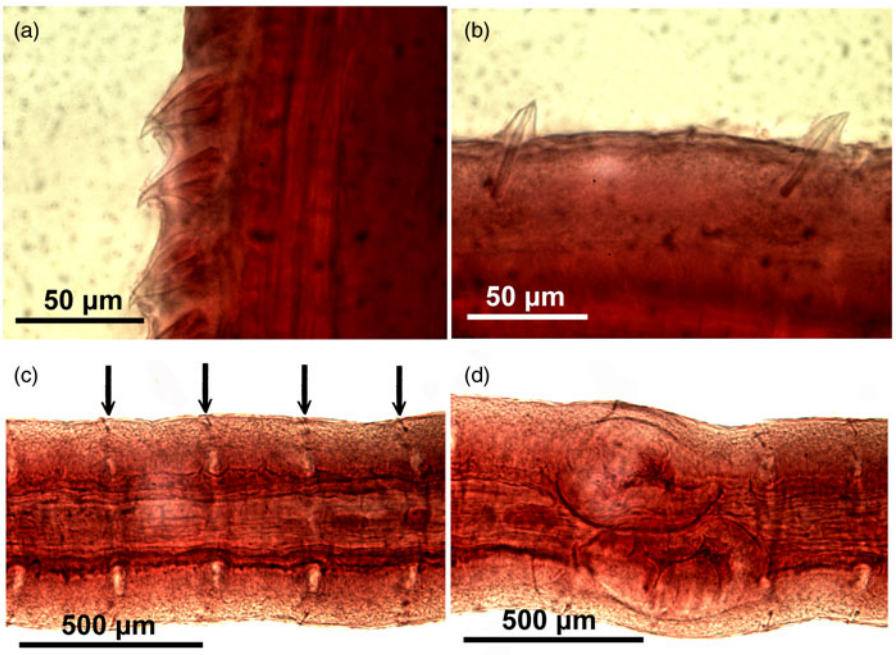

Fig. 7. Microscope images of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) Collar spines of a female specimen. Note the short multi-lobed rod supports. (b) Trunk spines of a female specimen. Note the long single rod support. (c) A mid-trunk segment of a female specimen showing the two heavily stained lateral lacunar canals and a few transverse lacunar canals aligned with trunk spines (arrows). (d) Two giant hypodermal giant nuclei in one female specimen. (e) The posterior part of cement gland with crescent-shaped giant nuclei and the anterior end of the cement gland reservoir (arrow). (f) The posterior part of the male reproductive system showing the posterior half of the cement gland reservoir (right) and its two ducts (white arrows), and the seminal vesicle (black arrow).

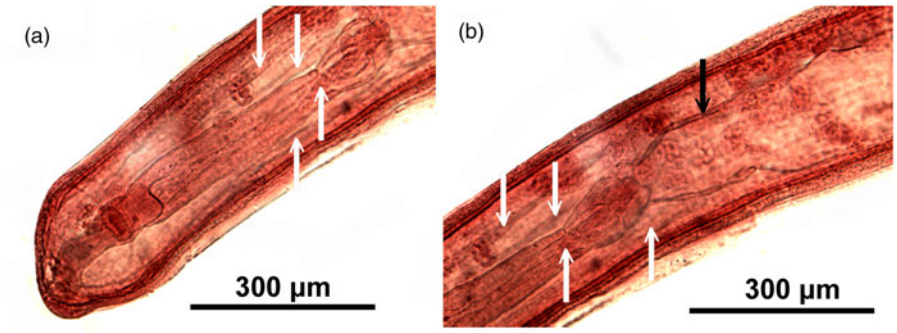

Fig. 8. Microscope images of specimens of *Pallisentis nandai* from *Nandus nandus* collected in the Ganga River at Bairaj, Bijnor, state of Uttar Pradesh, India. (a) The posterior part of a female reproductive system showing the curved vagina, moderately developed sphincter and the thick-walled uterus. Note the four paravaginal ligaments (white arrows). (b) The distal part of the female reproductive system shown in (a), showing the funnel-shaped, thin-walled, unattached uterine bell (black arrow) and the anterior extension of the four paravaginal ligaments.

relationship obtained in previous studies (e.g. Chaudhary *et al.*, 2019) within the Eoacanthocephala, which included members of the Quadrigyridae and the Neoechinorhynchidae, The 18S and ITS1-5.8S-ITS2 region of rDNA dataset alignment includes sequences of acanthocephalans representing species of the Quadrigyridae, Neoechinorhynchidae, Moniliformidae and Qligacanthorhynchidae (table 8). 18S sequences obtained from the isolates of *P. nandai* were placed in a well-supported clade (89/0.91) with other species of *Pallisentis* reported from India (fig. 13). The clade formed by the members of the *Pallisentis* was found to be sister of a clade including members of *Acanthosentis* and *Neoechinorhynchus*, with good support (fig. 13). Within *Pallisentis*, the phylogenetic analyses showed that *P. nandai* was closest to *P. indica*, a species from *Channa punctata* (fig. 13). Intra-generic pairwise differences based on partial 18S rDNA sequence between *P. nandai* with *P. indica*, *Pallisentis* sp. BR-2017 and *Pallisentis* sp. 2 NKG-2016 were 0.56%, 0.86% and 1.02%, respectively. The present phylogenetic analysis based on the ITS1-5.8S-ITS2 data set predicts that *P. nandai* forms a clade with *Pallisentis nagpurensis* and *P. indica*

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Table 2. Morphometric comparisons between our specimens of *Pallisentis nandai* from *Nandus nandus* in the Ganga River at Bairaj, Bijnor, Uttar Pradesh, India, and those originally described by Sarkar (1953) from the same host in the Ganga River at, Calcutta, West Bengal.

Source	Present paper	Sarkar, 1953[a]
Locality	Ganga River at Bairaj, Bijnor	Ganga River at Calcutta
Sample size	11 MM, 12 FF	13 MM, 9 FF
Males		
Trunk L × W (mm)	5.25–9.74 (6.90) × 0.40–0.60 (0.51)[b]	5.60–9.90 × 0.37–0.63
Proboscis L × W	218–281 (242) × 218–322 (264)	170–480 × 190–320[a]
Hook rows × H/row	10 × 4	8–10 × 4
Hook 1 L × root L	**75–112 (96)/55–87 (69)**[c]	90 × _
Hook 2 L × root L	57–87 (78)/30–62 (49)	80–90 × _
Hook 3 L × root L	45–62 (55)/25–37 (32)	60 × _
Hook 4 L × root L	**30–47 (40)/20–30 (26)**	30 × _
Collar spines	12–16 circles × 16–20/circle (L = 25–37)	13–14 × 16–20 (L = 29)
Trunk spines	24–35 × 2–18/circle (L = 25–40)	28–34 (L = 18)
Receptacle L × W	**728–1,010 (856) × 208–312 (253)**	440–840 × 120–250[a]
Lemnisci L × W (mm)	**0.92–2.12 (1.81) × 0.05–0.08 (0.07)**	0.72–1.30 × 0.04[a]
	2.08–2.50 (2.34) × 0.06–0.08 (0.07)	1.10–1.90 × 0.04[a]
Ant. testis L × W	**489–842 (622) × 150–208 (188)**	430–560 × 140–160
Post. testis L × W	**437–832 (606) × 156–220 (184)**	370–530 × 140–160
Cement gland L × W (mm)	0.99–1.51 (1.18) × 0.13–0.17 (0.16)	0.77–1.40 × 0.14–0.22
Cement gland giant nuclei	18–29 (24)	23–25
Cement reservoir L × W	325–541 (454) × 122–198 (168)	290–480 × 120–210
Seminal vesicle L × W	416–550 (477) × 58–104 (76)	240–890 × 130–900 (?)
Females		
Trunk L × W (mm)	7.12–13.12 (10.48) × 0.48–0.72 (0.55)	6.30–10.40 × 0.35–0.56
Proboscis' L × W	198–260 (233) × 198–343 (289)	170–480 (?) × 190–320[a]
Hook rows × H/row	10 × 4	8–10 × 4
Hook 1 L × root L	**90–125 (104)/62–85 (74)**	93 × _
Hook 2 L × root L	**76–97 (84)/42–57 (51)**	80 × _
Hook 3 L × root L	**55–67 (61)/33–42 (36)**	60 × _
Hook 4 L × root L	**42–50 (44)/25–32 (29)**	33 × _
Collar spines	**14–16 circles × 18–24/circle (L = 27–37)**	13–16 × 18–20 (L = 29)
Trunk spines	**64–74 × 4–18/circle (L = 17–37)**	44–55 × 16–20 (L = 18)
Receptacle L × W	**863–1,041 (915) × 229–302 (257)**	440–840 × 120–250[a]
Lemnisci L × W (mm)	**2.08–2.44 (2.26) × 0.06–0.06 (0.06)**	0.72–1.30 × 0.04[a]
	2.50–2.91 (2.77) × 0.05–0.07 (0.06)	1.10–1.90 × 0.04[a]
Reproductive system L	439–801 (653); 6–7% of trunk	–
Eggs L × W	–	–

L: length, W: width, H/row: hooks/row.
[a]Sarkar's (1953) measurements of proboscis, receptacle and lemnisci in male and female specimens were the same.
[b]Range (mean) in μm unless otherwise stated.
[c]Measurements and numbers in bold represent markedly higher figures in our specimens compared to those in the original description.

with good bootstrap support (99/1), both reported from India (fig. 14). The clade formed by the members of the *Acanthosentis* and *Neoechinorhynchus* was found to be sister of a clade formed by species of *Pallisentis*.

Furthermore, pairwise intra-generic differences based on ITS1-5.8S-ITS2 sequence between *P. nandai* with *P. nagpurensis* and isolates of *P. indica* were 0.20% and 0.26%, respectively. In addition, *Neoechinorhynchus brentnickoli* and three unidentified

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Table 3. Distribution and length of trunk spines of 11 male and 12 female specimens of *Pallisentis nandai* collected from *Nandus nandus* in the Ganga River at Bairaj, Bijnor, Uttar Pradesh, India.

Males				Females			
Spine circles	Spines/ circle	Spine length (μm)	Distance between circles (μm)	Spine circles	Spines/ circle	Spine length (μm)	Distance between circles (μm)
Collar spines							
12–16 (14)	16–20 (18)[a]	Ant. 25–32 (27)	13–25 (19)	14–16 (15)	16–24 (20)	Ant. 27–37 (32)	12–25 (17)
		Post. 30–37 (33)				Post. 32–37 (35)	
Trunk spines							
24–35 (29)	Ant. 10–18 (13)	Ant. 30–40 (35)	Ant. 35–135 (85)	64–74 (70)	Ant. 10–18 (14)	Ant. 27–37 (33)	Ant. 83–125 (101)
	Post. 2–6 (4)	Post. 25–32 (28)	Post. 87–250 (131)		Post. 4–8 (7)	Post. 17–35 (28)	Post. 104–198 (148)

[a]Range (mean).

Table 4. Chemical composition of the gallium-cut intact spines of *Pallisentis nandai*.

	Collar spines		Trunk spines	
Element[a]	WM	Cut	WM	Cut
Sodium (Na)	2.95[b]	**0.04**[c]	3.24	**0.32**
Magnesium (Mg)	0.67	**0.37**	0.69	**0.30**
Phosphorus (P)	0.14	**1.29**	0.09	**0.81**
Sulphur (S)	2.40	**23.17**	1.67	**12.77**
Calcium (Ca)	0.11	**0.41**	0.13	**0.40**

[a]The common elements for protoplasm (C, N, O) and those for sample processing (Au, Pd, Ga) are omitted.
[b]Given in weight%.
[c]Figures in bold are used in corresponding EDXA spectra.

species of *Neoechinorhynchus* clustered as a sister group with strong support (fig. 14).

Discussion

Completeness of life cycle

All our female specimens were gravid with only ovarian balls. Eggs were not found. Reported specimens did not reach sufficient sexual maturity in the liver, where they have been found, to produce eggs. This unusual site of infection opens questions about completing the life cycle with specimens trapped in the liver without egg production and access to the external environment. Sarkar (1953) did not find eggs either; all his specimens were collected from 'the alimentary canal and liver' of 16 infected fishes from a Calcutta fish market in June 1952. The question of completing the life cycle remains an open question, especially in light of the reporting of *P. nandai* from the many collecting sites along the Ganga River (fig. 1). Only occasional references were made to intestinal sites of infection – for example, Parveen & Sultana (2014) collected four specimens of *P. nandai* from the liver, one from the stomach and one from the intestine of *N. nandus* in Bangladesh. Ahmed (1981) also collected specimens of *P. nandai* from the liver, intestine and mesenteries of *N. nandus*. Alam & Alam (2014) found specimens only in the liver of *Oreochromis nilotiocus* (Lin.), as in our case, but Naidu (2012) collected specimens only from the intestine of *N. nandus* and *G. giuris* (Hamilton). It appears that only intestinal sites support reproductive infrapopulations contributing to the continuation of the life cycle of that species. Worms in the liver do not produce eggs.

Morphological variability

The marked differences in the size of proboscis hooks, receptacle, lemnisci, testes and female spines between our specimens and those described by Sarkar (1953) still fall within the range of intraspecific variations not related to host species. Our specimens and those of Sarkar (1953) were collected from the headwaters and the lower delta of the Ganga River, respectively, about 1500 km apart. Intraspecific variability related to geographical factors has been repeatedly demonstrated in other species of Acanthocephala. The observed differences in our *Rhadinorhynchus trachuri* Harada, 1935 (Rhadinorhynchidae) specimens also appear to represent intraspecific variations among Asian and American geographical populations, which may be affected by changes in feeding behaviour (Amin, 2019), as has been previously demonstrated in the comparable case of *Mediorhynchus papillosus* Van Cleave, 1916 (Gigantorhynchidae) by Amin & Dailey (1998). Amin & Dailey (1998) studied key taxonomic characteristics in various geographical populations of *M. papillosus*, which has a wide range of distribution in at least 73 species of birds outside of North and South America, in Asia from Taiwan to the east into China, many of the former Soviet Republics and to Eastern Europe to the west. Amin & Dailey (1998) compared measurements of specimens from two species of birds in Maryland, one from Colorado (their study material), six from Taiwan, two from Yakutia, Trans-Baikal, three from Lower Yansi River basin, 45 from the Volga basin and Oren Byreg, seven from Ukraine, seven from Bulgaria, three from China and one from Brazil, and demonstrated a distinct geographically based variability, especially in the size of proboscis and its armature, neck, receptacle and testes, which appeared related to geographical restrictions, intermediate and definitive host specificity and distribution, and host feeding behaviour. 'The U.S. population from Colorado and the Taiwanese population (were shown to be) at the opposite ends of the spectrum' by Amin & Dailey (1998) who dismissed the possibility of elevating them to a specific status. The population variants of *P. nandai*, like those of *R. trachuri*, are comparable to the east–west-intraspecific clinal variants of *M. papillosus* and could have

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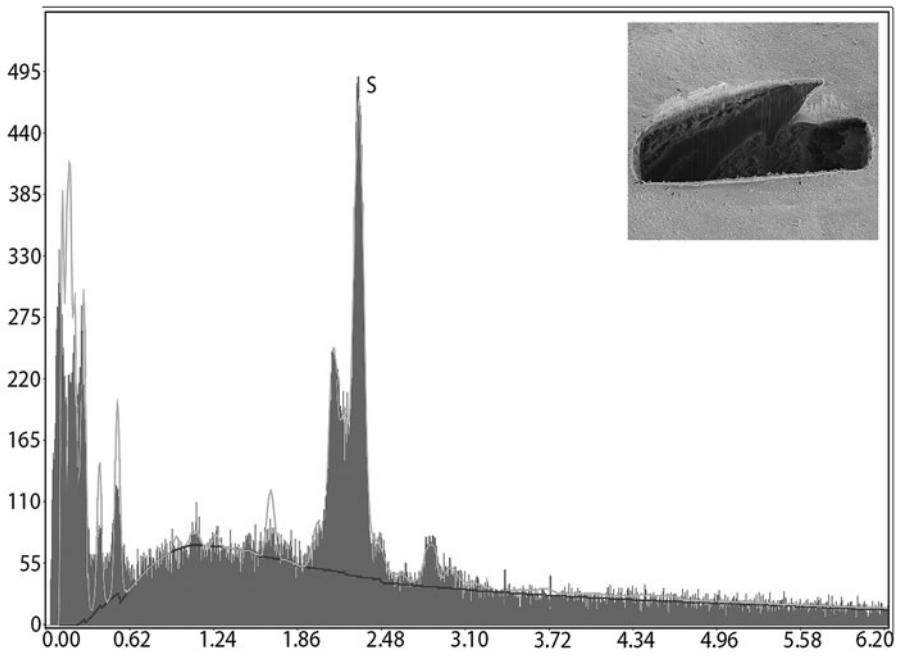

Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	19.82	36.83	41.38	56.58	0.0677
N	16.61	26.47	16.63	36.24	0.0478
O	10.71	14.94	13.19	53.52	0.0347
Na	0.04	0.04	99.99	0.47	0.0003
Ga	1.00	0.32	54.72	6.19	0.0075
Mg	0.37	0.34	63.77	5.78	0.0026
P	1.29	0.93	26.28	17.45	0.0107
Au	20.67	2.34	10.07	115.48	0.1559
S	23.17	16.13	5.94	291.23	0.1878
Cl	0.38	0.24	89.79	3.86	0.0028
Pd	5.41	1.14	27.11	26.65	0.0351
K	0.12	0.07	99.99	0.96	0.0009
Ca	0.41	0.23	76.88	2.89	0.0033

Fig. 9. Energy-dispersive X-ray spectrum of gallium-cut collar spines showing high level of sulphur. The X-ray data present the elemental analysis of the spine (see boldfaced figures of collar spines in table 4). Insert: SEM of a gallium-cut collar spine.

been considered as distinct species, but this notion is dismissed here also for the same reasons.

The PRS

The PRS inserts anteriorly in the body wall near the neck and posteriorly at the posterior end of the receptacle. The presence of the PRS in eoacanthocephalan species with a weak single proboscis receptacle wall was first demonstrated in *Neoechinorhynchus* (*N.*) *qatarensis* Amin, Saoud, Alkuwari, 2002 by Amin *et al.* (2002). It had since been reported in other eoacanthocephalan species of *Neoechinorhynchus* Stiles & Hassall, 1905 and *Acanthogyrus* (*Acanthosentis*) Verma & Datta, 1929 by Amin (2005) and Amin *et al.* (2011a, 2019a, b, c). In the description of the PRS, Amin *et al.* (2002, 2007) proposed that it may regulate the hydrostatic pressure in the receptacle to facilitate the retraction and eversion of the proboscis. The PRS is here reported for the first time in two members of the genus *Pallisentis*: *P. nandai* and *P. indica*.

Micropores

The micropores of *P. nandai*, like those reported from other species of the Acanthocephala, are associated with internal crypts and vary in diameter and distribution in different trunk regions corresponding with differential absorption of nutrients. Micropores have been reported in a large number of acanthocephalan species (Heckmann *et al.*, 2013) and in a few more since, and demonstrated the tunnelling from the cuticular surface into the internal crypts by Transmission Electron Microscope (TEM). Amin *et al.* (2009) summarized the structural–functional relationship of the micropores in various acanthocephalan species, including *Rhadinorhynchus ornatus* Van Cleave, 1918, *Polymorphus minutus* (Goeze, 1782) Lühe, 1911, *Moniliformis* (Bremser, 1811) Travassos (1915), *Macracanthorhynchus*

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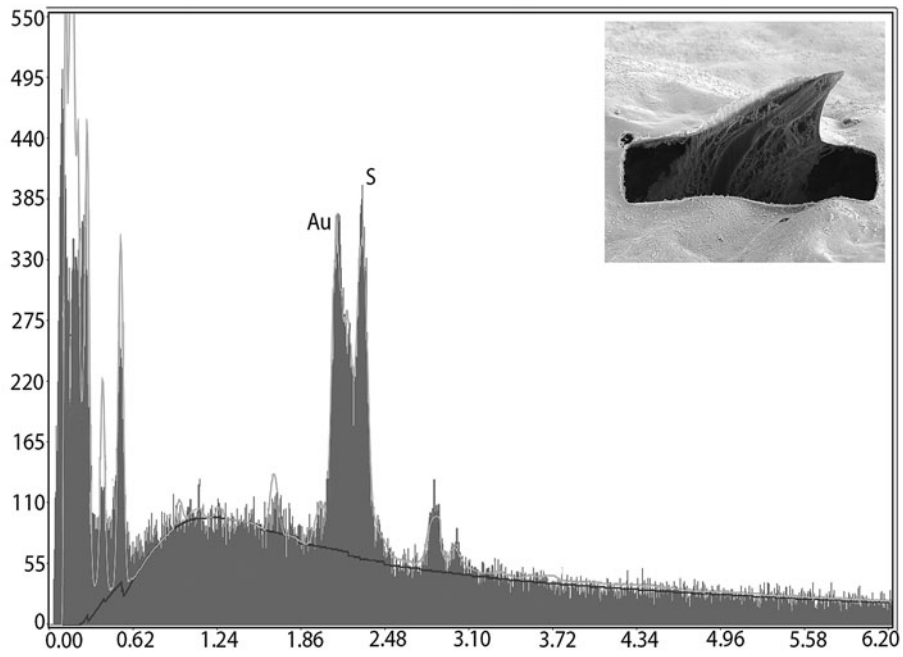

Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	18.32	34.68	35.09	87.39	0.0836
N	17.24	27.97	16.67	54.11	0.0571
O	15.53	22.06	10.94	103.25	0.0535
Na	0.32	0.31	69.65	4.18	0.0018
Ga	0.66	0.21	51.88	4.94	0.0048
Mg	0.30	0.28	64.18	5.76	0.0021
P	0.81	0.60	55.73	13.76	0.0067
Au	27.34	3.15	9.43	188.47	0.2033
S	12.77	9.05	6.67	197.95	0.1020
Cl	0.11	0.07	99.99	1.36	0.0008
Pd	6.08	1.30	24.30	37.94	0.0399
K	0.14	0.08	99.99	1.46	0.0011
Ca	0.40	0.23	82.86	3.54	0.0033

Fig. 10. Energy-dispersive X-ray spectrum of gallium-cut trunk spines showing high level of sulphur. The X-ray data present the elemental analysis of the spine (see boldfaced figures of trunk spines in table 4). Insert: SEM of a gallium-cut trunk spine.

Table 5. Chemical composition of the longitudinal gallium-cut hook of *Pallisentis nandai*.

Element[a]	Hook arch area	Hook base area	Surrounding tissue
Sodium (Na)	**0.11**[b]	0.06	0.38
Magnesium (Mg)	**0.11**	0.06	0.12
Phosphorus (P)	**1.49**	1.26	1.15
Sulphur (S)	**17.25**	20.70	13.40
Potassium (K)	**0.25**	0.35	0.09
Calcium (Ca)	**29.68**	38.90	23.18

[a]The common elements for protoplasm (C, N, O) and those for sample processing (Au, Pd, Ga) are omitted.
[b]Given in WT%. Figures in bold are used in the corresponding EDXA spectrum.

hirudinaceus (Pallas, 1781) Travassos (1916, 1917) and *Sclerocollum rubrimaris* Schmidt & Paperna, 1978. Wright & Lumsden (1969) and Byram & Fisher (1973) reported that the peripheral canals of the micropores are continuous with canalicular crypts. These crypts appear to 'constitute a huge increase in external surface area … implicated in nutrient up take.' Whitfield (1979) estimated a 44-fold increase at a surface density of 15 invaginations per 1 μm^2 of *M. moniliformis* tegumental surface. The micropores and the peripheral canal connections to the canaliculi of the inner layer of the tegument of *Corynosoma strumosum* (Rudolphi, 1802) Lühe, 1904 from the Caspian seal *Pusa caspica* (Gmelin) in the Caspian Sea were demonstrated by transmission electron micrographs in Amin *et al.* (2011b).

EDXA

Results of the X-ray scans of the gallium-cut hooks (dual-beam SEM) of *P. nandai* show differential compositions and distributions of metals in different hook parts, with calcium and sulphur levels being considerably higher at the basal arch of hooks where tension and strength are paramount compared to the hook tip and edge where the level of sulphur was considerably higher

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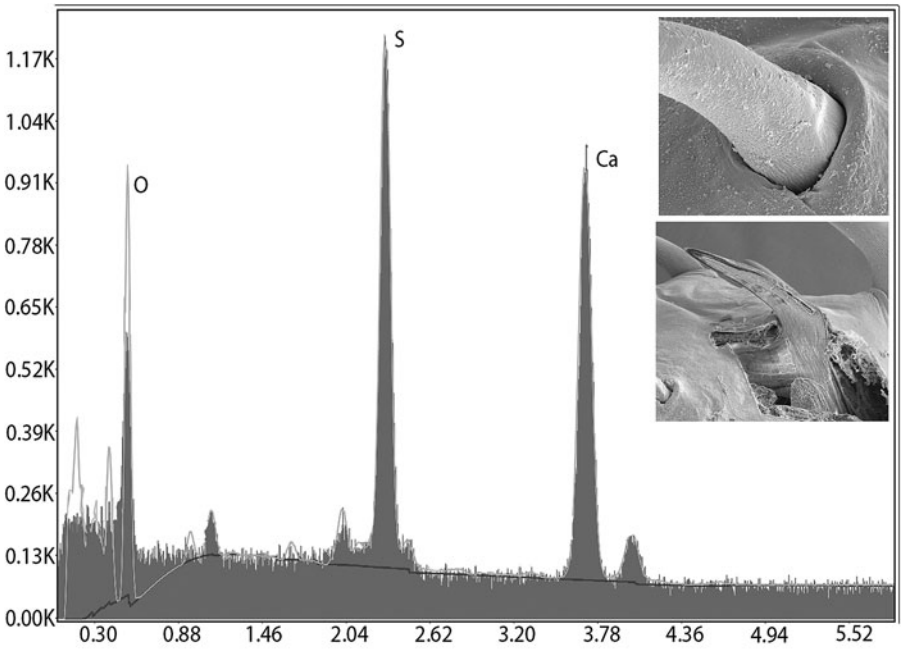
Table 6. Chemical composition of the gallium-cut and intact hooks for *Pallisentis nandai*.

Element[a]	Hook	Hook tip cut		Hook mid cut	
	Intact	Edge	Centre	Edge	Centre
Sodium (Na)	2.05[b]	**0.52**	0.02	0.13	0.00
Magnesium (Mg)	0.33	**0.04**	0.08	0.07	0.03
Phosphorus (P)	0.56	**0.75**	1.50	1.63	2.30
Sulphur (S)	10.91	**11.76**	27.09	20.82	27.08
Calcium (Ca)	8.94	**5.33**	36.26	23.25	44.91

[a]The common elements for protoplasm (C, N, O) and those for sample processing (Au, Pd, Ga) are omitted.
[b]Given in WT%.



Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	3.30	6.02	99.99	26.16	0.0099
N	17.28	27.02	12.86	75.36	0.0318
O	26.79	36.67	10.42	289.57	0.0603
Na	0.11	0.10	76.54	3.29	0.0006
Ga	2.19	0.69	13.19	37.81	0.0146
Mg	0.11	0.10	66.54	4.93	0.0007
P	1.49	1.05	11.57	64.44	0.0128
Au	1.37	0.15	25.46	25.89	0.0112
S	17.25	11.78	3.22	734.20	0.1538
Cl	0.01	0.01	99.99	0.33	0.0001
Pd	0.18	0.04	65.29	2.99	0.0013
K	0.25	0.14	60.91	7.32	0.0022
Ca	29.68	16.22	3.32	695.45	0.2600

Fig. 11. Energy-dispersive X-ray spectrum of gallium-cut hook arch showing high level of calcium and sulphur. The X-ray data present the elemental analysis of the hook (see boldfaced figures of hook arch in table 5). Insert: SEM of a hook arch and a gallium-cut hook.

(tables 5, 6 and figs 11 and 12). The chemical elements present in the hooks are typical for acanthocephalans (Heckmann *et al.*, 2007, 2012; Standing & Heckmann, 2014; Amin & Heckmann, 2017). Note the moderate outer layer (fig. 40) of the hook which relates to the sulphur content (tables 5 and 6) in the hook of *P nandai*, which is different than in other

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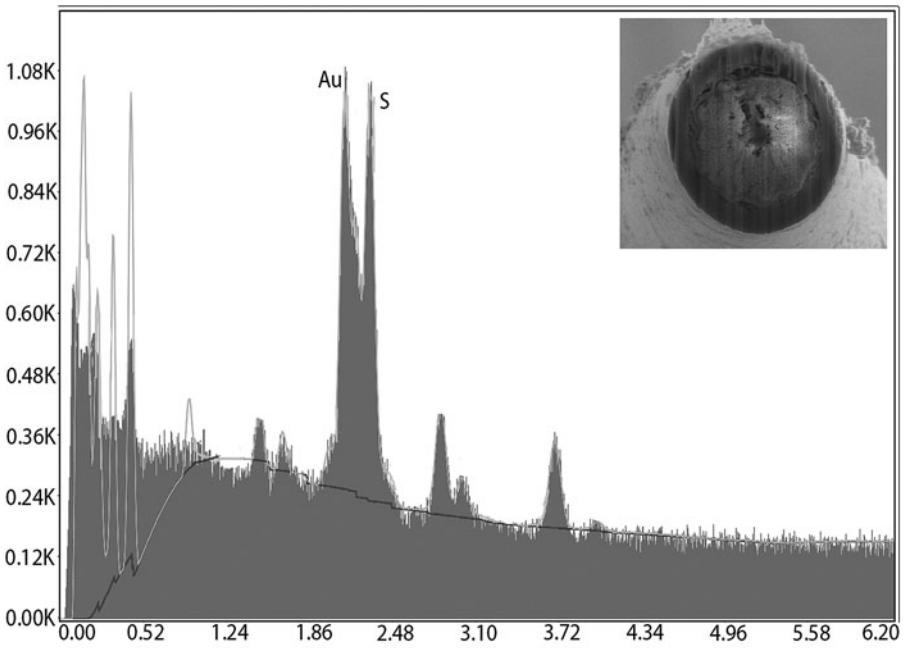

Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	9.88	19.78	99.99	134.29	0.0464
N	21.19	36.38	10.68	192.13	0.0731
O	16.42	24.69	9.56	288.97	0.0541
Na	0.52	0.55	61.86	18.86	0.0030
Ga	0.45	0.16	16.07	9.25	0.0032
Mg	0.04	0.04	83.77	2.24	0.0003
Al	1.06	0.94	17.10	58.49	0.0079
P	0.75	0.58	29.80	34.99	0.0062
Au	24.39	2.98	8.77	467.89	0.1822
S	11.76	8.82	5.72	509.24	0.0947
Cl	0.02	0.01	99.99	0.58	0.0001
Pd	8.15	1.84	11.77	142.98	0.0543
K	0.04	0.02	96.29	1.13	0.0003
Ca	5.33	3.20	12.73	130.87	0.0434

Fig. 12. Energy-dispersive X-ray spectrum of gallium-cut hook tip showing high level of sulphur and low calcium. The X-ray data present the elemental analysis of the hook (see boldfaced figures of hook tip in table 6). Insert: SEM of a cross-section of a gallium-cut hook.

Table 7. Chemical composition of the gallium-cut hook tip for two species of *Pallisentis*.

	P. indica	*P. nandai*
Element[a]	Hook tip	Hook tip
Magnesium (Mg)	**1.42**[b]	**0.04**
Phosphorus (P)	**12.04**	**0.75**
Sulphur (S)	**4.08**	**11.76**
Calcium (Ca)	**20.86**	**5.33**

[a]The common elements for protoplasm (C, N, O) and those for sample processing (Au, Pd, Ga) are omitted.
[b]Given in WT%. Figures in bold are used in corresponding EDXA spectra.

acanthocephalans (Amin & Heckmann, 2017; Amin *et al.*, 2017, 2018a; Ha *et al.*, 2018). The high sulphur content shows up in the outer edge of X-ray scans of hooks (tables 5 and 6; Amin *et al.*, 2018b). The hook centre in mid-cuts has a completely different chemical profile than the cortical layer (table 6). X-ray scans (EDXA) provide insight into the hardened components – for example, calcium and phosphorus – of acanthocephalan hooks. The EDXA appears to be species-specific, as in fingerprints. The hook tip of *P. indica* has a considerably higher level of phosphorus and calcium and lower levels of sulphur than the hook tip of *P. nandai* (table 7). EDXA is shown to have significant diagnostic value in acanthocephalan systematics – for example, *Moniliformis cryptosaudi* Amin, Heckmann, Sharifdini & Albayati, 2019 was erected based primarily on its EDXA pattern (Amin *et al.*, 2019d).

Molecular analysis

Molecular methods are an important tool for morphological identification, diversity and taxonomic relationships among acanthocephalan species (García-Varela *et al.*, 2017, 2019; Li *et al.*, 2018; Amin *et al.*, 2019a, b, c; Lisitsyna *et al.*, 2019; Menasria

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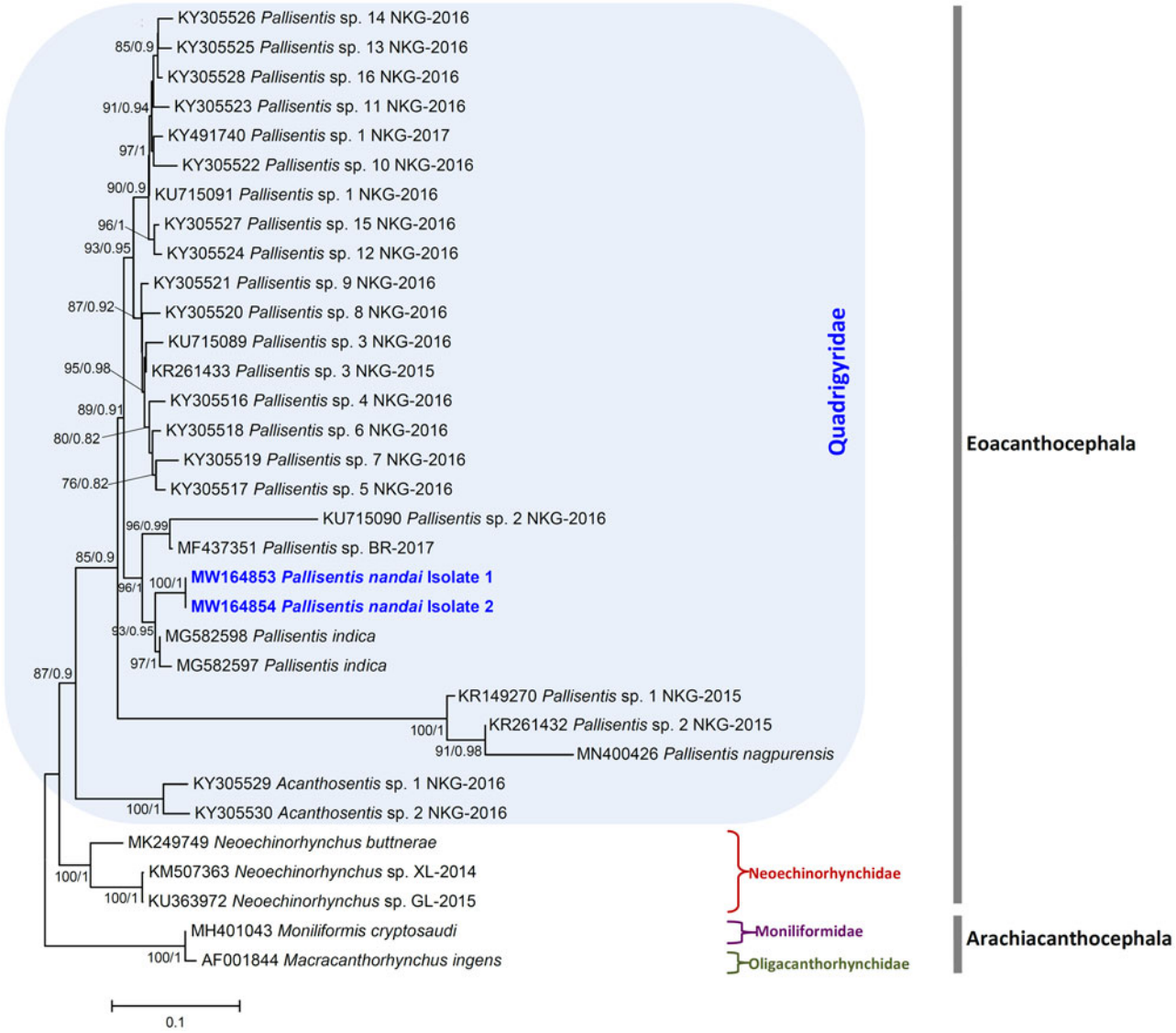

Fig. 13. Phylogenetic reconstruction based on the maximum likelihood (ML) and Bayesian inference (BI) analysis using 18S rDNA sequences of *Pallisentis nandai* and sequences of Acanthocephala deposited in GenBank. The numbers indicate values of bootstrap >70%. Numbers above branches indicate nodal support as ML and posterior probabilities from BI. Species of Archiacanthocephala are used as an outgroup. The scale bar indicates the expected number of substitutions per site.

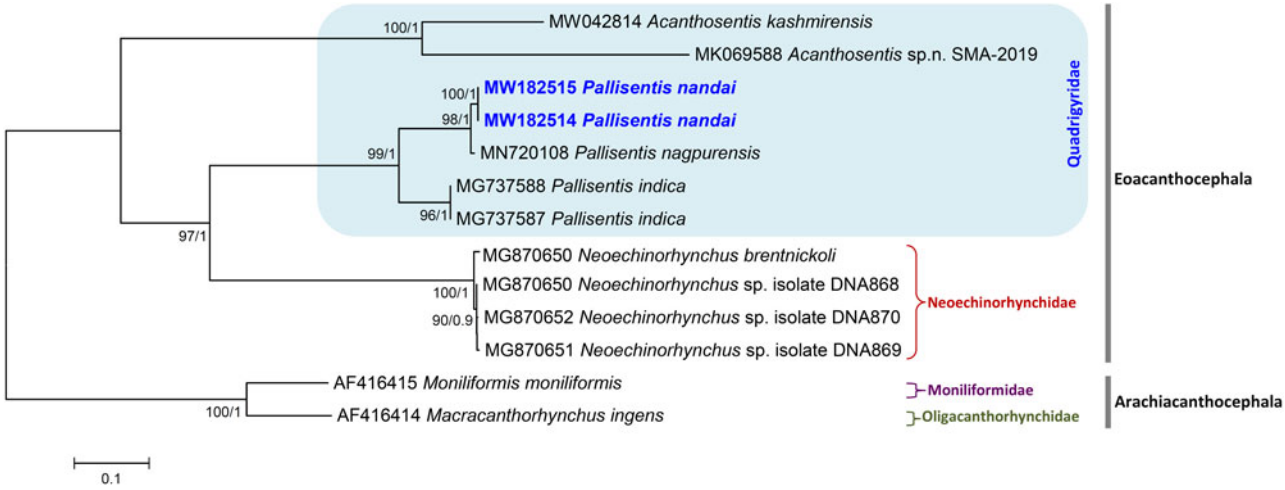

Fig. 14. Maximum likelihood (ML) and Bayesian inference (BI) tree for *Pallisentis nandai* based on ITS1-5.8S-ITS2 rDNA sequences. Numbers above branches indicate nodal support as ML analysis followed by bootstrap values of posterior probabilities from BI. Support values lower than 70% are not shown. The scale bar indicates the expected number of substitutions per site.

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Table 8. Acanthocephalan species included in the phylogenetic analysis with information on the host, locality and GenBank accession number.

Species	Host	Location	GenBank accession no.		References
			18S gene	ITS region	
Class: Eoacanthocephala					
Family: Quadrigyridae					
Genus: *Pallisentis* Van Cleave, 1928					
Pallisentis sp. 1 NKG-2015	HNA	India	KR149270	–	Gautam, 2015[a]
Pallisentis sp. 2 NKG-2015	HNA	India	KR261432	–	Gautam, 2015[a]
Pallisentis sp. 3 NKG-2015	HNA	India	KR261433	–	Gautam, 2015[a]
Pallisentis sp. 1 NKG-2016	*Channa striata*	India	KU715091	–	Gautam, 2016[a]
Pallisentis sp. 2 NKG-2016	*Channa punctata*	India	KU715090	–	Gautam, 2016[a]
Pallisentis sp. 3 NKG-2016	*Channa punctata*	India	KU715089	–	Gautam, 2016[a]
Pallisentis sp. 4 NKG-2016	HNA	India	KY305516	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 5 NKG-2016	HNA	India	KY305517	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 6 NKG-2016	HNA	India	KY305518	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 7 NKG-2016	HNA	India	KY305519	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 8 NKG-2016	HNA	India	KY305520	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 9 NKG-2016	HNA	India	KY305521	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 10 NKG-2016	HNA	India	KY305522	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 11 NKG-2016	HNA	India	KY305523	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 12 NKG-2016	*Channa striata*	India	KY305524	–	Gautam *et al*., 2020
Pallisentis sp. 13 NKG-2016	HNA	India	KY305525	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 14 NKG-2016	HNA	India	KY305526	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 15 NKG-2016	*Channa striata*	India	KY305527	–	Gautam *et al*., 2020
Pallisentis sp. 16 NKG-2016	HNA	India	KY305528	–	Gautam & Saxena, 2016[a]
Pallisentis sp. 1 NKG-2017	HNA	India	KY491740	–	Gautam, 2016[a]
Pallisentis sp. BR-2017	*Channa striata*	India	MF437351	–	Zimik & Roy, 2017[a]
***Pallisentis nandai* Isolate 1**	***Nandus nandus***	**India**	**MW164853**	**MW182515**	**Present study**
***Pallisentis nandai* Isolate 2**	***Nandus nandus***	**India**	**MW164854**	**MW182514**	**Present study**
Pallisentis indica ISL1	*Channa punctata*	India	MG582597	MG737588	Chaudhary *et al*., 2019
Pallisentis nandai ISL2	*Channa punctata*	India	MG582598	MG737587	Chaudhary *et al*., 2019
Pallisentis nagpurensis	*Channa striata*	India	MN400426	MN720108	Rana & Kaur, 2019[a]
Genus: *Acanthosentis* Verma & Datta, 1929					
Acanthosentis sp. 1 NKG-2016	*Mystus seenghala*	India	KY305529	–	Gautam *et al*., 2020
Acanthosentis sp. 2 NKG-2016	*Mystus seenghala*	India	KY305530	–	Gautam *et al*., 2020
Acanthosentis kashmirensis	*Schizothorax plagiostomus*	India	–	MW042814	Amin *et al*., 2017
Acanthosentis sp. n. SMA-2019	*Barbonymus schwanefeldii*	Malaysia	–	MK069588	Surzanne *et al*., 2019[a]
Family: Neoechinorhynchidaed					
Genus: *Neoechinorhynchus* Hamann, 1892					
Neoechinorhynchus buttnerae	*Gammarus pulex*	France	MK249749	–	Souza & Benavides, 2018[a]
Neoechinorhynchus sp. XL-2014	HNA	China	KM507363	–	Liu *et al*., 2014[a]
Neoechinorhynchus sp. GL-2015	*Capoeta aculeate*	Iran	KU363972	–	Adel & Dadar, 2016[a]
Neoechinorhynchus brentnickoli	*Dormitator latifrons*	Mexico	–	MG870697	Pinacho-Pinacho *et al*., 2018
Neoechinorhynchus sp. isolate DNA868	*Dormitator latifrons*	Mexico	–	MG870650	Pinacho-Pinacho *et al*., 2018

(*Continued*)

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Table 8. (*Continued.*)

Species	Host	Location	GenBank accession no.		References
			18S gene	ITS region	
Neoechinorhynchus sp. isolate DNA870	*Dormitator latifrons*	Mexico	–	MG870651	Pinacho-Pinacho *et al.*, 2018
Neoechinorhynchus sp. isolate DNA869	*Dormitator latifrons*	Mexico	–	MG870652	Pinacho-Pinacho *et al.*, 2018
Outgroups					
Class: Archiacanthocephala					
Family: Moniliformidae					
Genus: *Moniliformis* Travassos, 1915					
Moniliformis cryptosaudi	*Hemiechinus auritus*	Iraq	MH401043	–	Amin *et al.*, 2019d
Moniliformis	HNA	Mexico	–	AF416415	Garcia-Varela *et al.*, 2003[a]
Family: Oligacanthorhynchidae					
Genus: *Macracanthorhynchus* Travassos, 1917					
Macracanthorhynchus ingens	*Procyon lotor*	USA	AF001844	–	Near & Nadler, 1998
Macracanthorhynchus ingens	HNA	Mexico	–	AF416414	Garcia-Varela *et al.*, 2003[a]

[a]Unpublished sequences are available on the GenBank database. HNA, host name not available.
Species in bold sequenced during the present study.

et al., 2019; Sharifdini *et al.*, 2020). The phylogenetic relationships and systematic position of *Pallisentis* are poorly explored compared to other genera. Molecular data for nuclear genes have been provided only for few in comparison to the >30 species available of the genus (Amin, 2013). Our phylogenetic analyses obtained from two methods – ML and BI – predict the same topology sowing the isolates of *P. nandai* from a well-supported clade within the Eoacanthocephala. The genetic differences between *P. nandai* and other species of the *Pallisentis* support the morphological observations indicating its independent identity. Other species of *Pallisentis* described from India were added to the listing in Amin (2013): *Pallisentis channi* and *Pallisentis vinodai* by Gautam *et al.* (2015); *Pallisentis punctati* by Gupta *et al.* (2015b); *Pallisentis anandai* (as KR149270 *Pallisentis* sp. 1 NKG-2015) by Gautam *et al.* (2017); *Pallisentis lucknowensis*, *Pallisentis amini* (as KY305523 *Pallisentis* sp. 11 NKG-2016), *Pallisentis meyeri* (as KY305525 *Pallisentis* sp. 13 NKG-2016) and *Pallisentis unnaoensis* (as KY305528 *Pallisentis* sp. 16 NKG-2016) by Gautam *et al.* (2019) and *Pallisentis thapari* (as KY305524 *Pallisentis* sp. 12 NKG-2016 and KY305527 *Pallisentis* sp. 15 NKG-2016) by Gautam *et al.* (2020). However, the genetic divergence in the 18S rDNA sequences between these species and *P. nandai* was 1.11–1.14.

Moreover, the tree also showed that our sequences of *P. nandai* were placed separately from other species of *Pallisentis*. The strong support in both trees also indicated that all these species share a common ancestor. Due to the unavailability of ITS sequences of *Pallisentis* in GenBank for comparison, we did perform molecular analysis of *P. nandai* isolates with only *P. indica* and *P. nagpurensis*. However, one ITS sequence of *P. nagpurensis* is too short (MN423292; 245 bp) and, therefore, unreliable for phylogeny, which is why we did not include it in our analysis. Aside from this, no ITS sequences have been made available for other *Pallisentis* members to date, that can help better elucidate their phylogenetic affinities with each other.

In conclusion, it becomes clear that making additional genetic markers available, as well as the addition of more species of this genus, would be useful for better understanding their phylogenetic affinities.

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Conflicts of interest. None.

Ethical standards. The authors declare that they have observed all applicable ethical standards.

References

Ahmed ATA (1981) Helminth infection in freshwater fishes of Bangladesh. *Fish Pathology* **15**, 229–236.

Alam MN and Alam J (2014) A comparative study of endoparasite infestation of *Oreochromis niloticus* (Linnaeus, 1758) in polluted and non-polluted water bodies of Bangladesh. *International Journal of Fauna and Biological Studies* **1**, 4–9.

Amin OM (2005) Occurrence of the subgenus *Acanthosentis* Verma & Datta, 1929 (Acanthocephala; Quadrigyridae) in Japan, with the description of *Acanthogyrus (Acanthosentis) alternatspinus* sp. n. and *A.* (*A.*) *parareceptaclis* sp. n. from Lake Biwa drainage fishes and a key to species of the subgenus. *Systematic Parasitology* **60**, 125–137.

Amin OM (2013) Classification of the Acanthocephala. *Folia Parasitologica* **60**, 273–305.

Amin OM (2019) Redescription of *Rhadinorhynchus trachuri* Harada, 1935 (Acanthocephala: Rhadinorhynchidae) from marine fish in Vietnam and California with a discussion of its zoogeography. *Acta Parasitologica* **65**, 77–89.

Amin OM and Dailey MD (1998) Description of *Mediorhynchus papillosus* (Acanthocephala: Gigantorhynchidae) from a Colorado, U.S.A., population, with a discussion of morphology and geographical variability. *Journal of Helminthological Society Washington* **65**, 189–200.

https://doi.org/10.1017/S0022149X20001005 Published online by Cambridge University Press

Amin OM and Heckmann RA (2017) *Neoandracantha peruensis* n. gen. n. sp. (Acanthocephala: Polymorphidae) described from cystacanths infecting the ghost crab *Ocypode gaudichaudi* on the Peruvian coast. *Parasite* **24**, 40.

Amin OM, Heckmann RA, Ha NV, Luc PV and Doanh PN (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 OM, Saoud MFA and Alkuwari KSR (2002) *Neoechinorhynchus qatarensis* sp. n. (Acanthocephala: Neoechinorhynchidae) from the blue-barred flame parrot fish, *Scarus gobban* Forsskål, 1775, in Qatar waters of the Arabian Gulf. *Parasitology International* **51**, 171–196.

Amin OM, Heckmann RA and Standing MD (2007) Structural-functional relationship of the para-receptacle structure in Acanthocephala. *Comparative Parasitology* **74**, 383–387.

Amin OM, Heckmann RA, Radwan NA, Mantuano JS and Alcivar MAZ (2009) Redescription of *Rhadinorhynchus ornatus* (Acanthocephala: Rhadinorhynchidae) from skipjack tuna, *Katsuwonus pelamis*, collected in the Pacific Ocean off South America, with special reference to new morphological features. *Journal of Parasitology* **95**, 656–664.

Amin OM, Ha NV and Ngo HD (2011a) First report of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from marine fish of the eastern seaboard of Vietnam, with the description of six species. *Parasite* **18**, 21–34.

Amin OM, Heckmann RA, Halajian A and El-Naggar AM (2011b) The morphology of a unique population of *Corynosoma strumosum* (Acanthocephala, Polymorphidae) from the Caspian seal, *Pusa caspica*, in the land-locked Caspian Sea using SEM, with special notes on histopathology. *Acta Parasitologica* **56**, 438–445.

Amin OM, Heckmann RA and Zargar UR (2017) Description of a new quadrigyrid acanthocephalan from Kashmir, with notes on metal analysis and histopathology, and a key to species of the subgenus *Acanthosentis* from the Indian subcontinent. *Journal of Parasitology* **103**, 458–470.

Amin OM, Heckmann RA, Ha NV and Pham DN (2018a) 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 OM, Heckmann RA and Ha NV (2018b) Descriptions of *Acanthocephalus parallelcementglandatus* (Echinorhynchidae) and *Neoechinorhynchus* (*N.*) *pennahia* (Neoechinorhynchidae) (Acanthocephala) from amphibians and fish in Central and Pacific coast of Vietnam, with notes on *N.* (*N.*) *longnucleatus*. *Acta Parasitologica* **63**, 572–585.

Amin OM, Chaudhary A, Heckmann R, Ha NV and Singh HS (2019a) Redescription and molecular analysis of *Neoechinorhynchus* (*Neoechinorhynchus*) *johnii* Yamaguti, 1939 (Acanthocephala: Neoechinorhynchidae) from the Pacific Ocean off Vietnam. *Parasite* **26**, 43.

Amin OM, Chaudhary A, Heckmann R, Ha NV and Singh HS (2019b) The morphological and molecular description of *Acanthogyrus* (*Acanthosentis*) *fusiformis* n. sp. (Acanthocephala: Quadrigyridae) from the catfish *Arius* sp. (ariidae) in the Pacific Ocean off Vietnam, with notes on zoogeography. *Acta Parasitologica* **64**, 779–796.

Amin OM, Sharifdini M, Heckmann RA and Ha NV (2019c) On three species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from the Pacific Ocean off Vietnam with the molecular description of *Neoechinorhynchus* (*N.*) *dimorphospinus* Amin and Sey, 1996. *Journal of Parasitology* **105**, 606–618.

Amin OM, Heckmann RA, Sharifdini M and Albayati NY (2019d) *Moniliformis cryptosaudi* n. sp. (Acanthocephala: Moniliformidae) from the long-eared hedgehog *Hemiechinus auritus* (Gmelin) (Erinaceidae) in Iraq; a case of incipient cryptic speciation related to *M. saudi* in Saudi Arabia. *Acta Parasitologica* **64**, 195–204.

Anonymous (2019) *Nandus nandus* was reported from 11 countries/islands. Available at https://www.fishbase.se/Country/CountryList.php?ID=10260&GenusName=Nandus&SpeciesName=nandus (accessed 12/2019).

Bhattacharya SB (2007) *Handbook on Indian Acanthocephala*. 255 pp. Kolkata, India, Zoological Survey of India.

Bhuiyan AL (1964) *Fishes of Dacca*. Publ. No. 13, 107–108 pp. Dacca, Asiatic Society of Pakistan.

Byram JE and Fisher FM Jr (1973) The absorptive surface of *Moniliformis dubius* (Acanthocephala). 1. Fine structure. *Tissue and Cell* **5**, 553–579.

Chaudhary A, Amin OM and Singh HS (2019) Molecular characterization and phylogenetic relationships of *Pallisentis* (*Brevitritospinus*) *indica* (Acanthocephala: Quadrigyridae), a parasite of the spotted snakehead (*Channa punctatus*). *Journal of Parasitology* **105**, 180–185.

Darriba D, Taboada GL, Doallo R and Posada D (2012) jmodeltest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772–772.

Galazzo D, Dayanandan S, Marcogliese DJ and McLaughlin JD (2002) Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA sequence data and comparisons with European congeners. *Canadian Journal of Zoology* **80**, 2207–2217.

García-Varela M, Mendoza-Garfias B, Choudhury A and Pérez-Ponce de León G (2017) Morphological and molecular data for a new species of *Pomphorhynchus* Monticelli, 1905 (Acanthocephala: Pomphorhynchidae) in the Mexican redhorse *Moxostoma austrinum* Bean (Cypriniformes: Catostomidae) in central Mexico. *Systematic Parasitology* **94**, 989–1006.

García-Varela M, Park JK, Hernández-Orts JS and Pinacho-Pinacho CD (2019) Morphological and molecular data on a new species of *Plagiorhynchus* Lühe, 1911 (Acanthocephala: Plagiorhynchidae) from the long-billed curlew (*Numenius americanus*) from northern Mexico. *Journal of Helminthology* **94**, e61.

Gautam NK, Upadhyay MK, Maurya R, Verma SK and Saxena AM (2017) Molecular and morphological study of a new species *Pallisentis anandai* n. sp. (Quadrigyridae, Van Cleave, 1920) of *Channa punctatus*. *Trends in Biosciences* **10**, 1540–1543.

Gautam NK, Misra PK and Saxena AM (2019) Four New Species of the Genus *Pallisentis* (Quadrigyridae, Van Cleave, 1920) from Freshwater Fish in Uttar Pradesh, India. *Acta Parasitologica* **64**, 71–85.

Gautam NK, Misra PK, Saxena AM and Monks S (2020) Description of *Pallisentis thapari* n. sp. and a re-description of *Acanthosentis seenghalae* (Acanthocephala, Quadrigyridae, Pallisentinae) using morphological and molecular data, with analysis on the validity of the sub-genera of *Pallisentis*. *Zootaxa* **4766**, 139–156.

Gupta N, Gupta DK and Singhal P (2015a) Description of *Pallisentis* (*Brevitritospinus*) *punctati* n. sp. (Acanthocephala: Quadrigyridae) from *Channa punctatus* in Bareilly, Uttar Pradesh, India. *Iranian Journal of Parasitology* **10**, 605–616.

Gupta R, Maurya R and Saxena AM (2015b) Two new species of the genus *Pallisentis* Van Cleave, 1928 (Acanthocephala: Quadrigyridae) from the intestine of *Channa punctatus* (Bloch, 1793) from the River Gomti at Lucknow, India. *Iranian Journal of Parasitology* **10**, 116–121.

Ha NV, Amin OM, Ngo HD and Heckmann RA (2018) Descriptions of acanthocephalans, *Cathayacanthus spinitruncatus* (Rhadinorhynchidae) male and *Pararhadinorhynchus magnus* n. sp. (Diplosentidae), from marine fish of Vietnam, with notes on *Heterosentis holospinus* (Arhythmacanthidae). *Parasite* **25**, 35.

Heckmann RA, Amin OM and Standing MD (2007) Chemical analysis of metals in Acanthocephalans using energy dispersive X-ray analysis (EDXA, XEDS) in conjunction with a scanning electron microscope (SEM). *Comparative Parasitology* **74**, 388–391.

Heckmann RA, Amin OM, Radwan NAE, Standing MD, Eggett DL and El Naggar AM (2012) Fine structure and energy dispersive X-ray analysis (EDXA) of the proboscis hooks of *Radinorynchus ornatus*, Van Cleave 1918 (Rhadinorynchidae: Acanthocephala). *Scientia Parasitologica* **13**, 37–43.

Heckmann RA, Amin OM and El-Naggar AM (2013) Micropores of Acanthocephala, a scanning electron microscopy study. *Scientia Parasitologica* **14**, 105–113.

Hossain MA, Parween S and Rahman MA (1992) Food and feeding habit of *Nandus nandus* (Hamilton-Buchanan). *The Rajshahi University Studies (Part-B)* **20**, 279–286.

Kumar S, Stecher G and Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Lee RE (1992) *Scanning electron microscopy and X-ray microanalysis*. 458 pp. Englewood Cliffs, New Jersey, Prentice Hall.

https://doi.org/10.1017/S0022149X20001005 Published online by Cambridge University Press

Li L, Chen HX and Yang Y (2018) Morphological and molecular study of *Neorhadinorhynchus nudus* (Harada, 1938) (Acanthocephala: Cavisomidae) from *Auxis thazard* Lacepede (Perciformes: Scombridae) in the South China Sea. *Acta Parasitologica* **63**, 479–485.

Lisitsyna OI, Kudlai O, Cribb TH and Smit NJ (2019) Three new species of acanthocephalans (Palaeacanthocephala) from marine fishes collected off the East Coast of South Africa. *Folia Parasitologica* **66**, 012.

Littlewood DTJ and Olson PD (2001) Small subunit rDNA and the phylum Platyhelminthes: signal, noise, conflict and compromise. pp. 262–278 *in* Littlewood DTJ and Bray RA (*Eds*) *Interrelationships of the Platyhelminthes*. London, UK, Taylor and Francis.

Luton K, Walker D and Blair D (1992) Comparison of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Molecular and Biochemical Parasitology* **56**, 323–328.

Menasria A, Barčák D, Kaouachi N, Bensouilah M, Scholz T and Hernández-Orts JS (2019) Redescription of *Acanthogyrus (Acanthosentis) maroccanus* (Dollfus, 1951) (Acanthocephala: Quadrigyridae), a parasite of the Algerian barb *Luciobarbus callensis* (Valenciennes) (Cyprinidae) in Algeria, and first molecular data. *Journal of Helminthology* **94**, e82.

Milne I, Lindner D, Bayer M, Husmeier D, Mcguire G, Marshall DF and Wright F (2009) TOPALiv2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics* **25**, 126–127.

Mital R P and Lal SS (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 Zootomy* **17**, 169–175.

Naidu KV (2012) *Fauna of India and adjacent countries-Acanthocephala*. 638 pp. Kolkata, India, Zoological Survey of India.

Parveen S and Sultana S (2014) Infestation of helminth parasites in Gangetic leaffish *Nandus nandus* (Hamilton, 1822). *Bangladesh Journal of Zoology* **42**, 183–190.

Pichelin S and Cribb TH (2001) The status of the Diplosentidae (Acanthocephala: Palaeacanthocephala) and a new family of acanthocephalans from Australian wrasses (Pisces: Labridae). *Folia Parasitologica* **48**, 289–303.

Pinacho-Pinacho CD, García-Varela M, Sereno-Uribe AL and de León GPP (2018) A hyper-diverse genus of acanthocephalans revealed by tree-based and non-tree-based species delimitation methods: ten cryptic species of *Neoechinorhynchus* in Middle American. *Molecular Phylogenetics and Evolution* **127**, 30–45.

Rainboth WJ (1996) *Fishes of the Cambodian Mekong*. 265 pp. FAO species identification field guide for fishery purposes, FAO, Rome.

Sarkar HL (1953) On a new Acanthocephala, *Pallisentis nandai*, from the fish *Nandus nandus* (Hamilton), with notes on other species of the genus. *Proceedings of the Zoological Society of Bengal* **6**, 139–147.

Sharifdini M, Amin OM and Heckmann RA (2020) The molecular profile of *Paratrajectura longcementglandatus* Amin, Heckmann Et Ali, 2018 (Acanthocephala: Transvenidae) from percid fishes in the marine waters of Iran and Iraq. *Helminthologia* **57**, 1–11.

Soota TD and Bhattacharya SB (1982) On the validity of the species of *Pallisentis* Van Cleave, 1928 (Acanthocephala: Pallisentidae) from the Indian Subcontinent. *Records of the Zoological Survey of India* **80**, 157–167.

Standing MD and Heckmann RA (2014) Features of Acanthocephalan hooks using dual beam preparation and XEDS phase maps. *Microscopy and Microanalysis Meeting*, Hartford, Connecticut, USA. Poster. Submission number 0383–00501.

Tadros G (1966) On three new Acanthocephala of the genera *Pallisentis* Van Cleave, *Saccosentis* gen. nov. and *Acanthocephalus koelreuther*, from fish. *Journal of Helminthology* **40**, 155–180.

Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Whitfield PJ (1979) *The biology of parasitism: an introduction to the study of associating organisms*. 277 pp. Baltimore, Maryland, University Park Press.

Wongkham W and Whitfield PJ (1999) *Pallisentis rexus* n. sp. (Eocanthocephala: Quadrigyridae) from snakehead fish, *Channa striata* Bloch, from Chiang Mai Basin. *Thailand. Thai Journal of Agricultural Sciences* **32**, 241–261.

Wright RD and Lumsden RD (1969) Ultrastructure of the tegumentary pore-canal system of the acanthocephalan *Moniliformis dubius*. *Journal of Parasitology* **55**, 993–1003.

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