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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*

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Abstract

Three species of acanthocephalans are described from fishes caught in the Pacific coast off eastern Vietnam and from amphibians in the midlands in 2016: (1) *Acanthocephalus parallelcementglandatus* Amin, Heckmann, Ha, 2014 (Echinorhynchidae), described from 1 male specimen is now fully described from males and females collected from 2 species of amphibians, the similar frog *Hylarana attigua* Inger, Orlov, Darevsky and the odorous frog *Odorrana* sp. Fei, Ye, Huang (Ranidae) in Huong Thuy, Hue City and Chu Yang Sin Park, central Vietnam, respectively, as well as from the needlefish *Tylosurus* sp. Cocco (Belonidae) in Binh Thuân in the Pacific South. The allotype female is designated. *Neoechinorhynchus* (*N.*) *pennahia* Amin, Ha, Ha, 2011 described from 1 female specimen is now fully described from males and females collected from the Toli shad (Chinese herring), *Tenualosa toli* (Valenciennes) (Clupeidae) in the Pacific north coast off Haiphong. The allotype male is designated. One specimen of *Neoechinorhynchus* (*Neoechinorhynchus*) *longnucleatus* Amin, Ha, Ha, 2011 is also reported from the common ponyfish, *Leiognathus equulus* (Forssskål) (Leiognathidae) in the Pacific south coast of Nha Trang and its ecology briefly discussed.

Keywords

Descriptions, *Acanthocephalus parallelcementglandatus*, *Neoechinorhynchus pennahia*, *N. longnucleatus*, Vietnam, amphibians, fish

Introduction

Most of the recent taxonomic work on the Acanthocephala from Vietnam was reported by the Amin-Heckmann-Ha team since 2000. A number of acanthocephalan species from freshwater fish, amphibians, reptiles, birds, and mammals were previously described in Vietnam by Amin and Ha (2008) and Amin *et al*. (2000; 2004; 2008a, b, c). Additionally, 11 species of acanthocephalans were collected from marine fish off the eastern seaboard of Vietnam in Halong Bay in 2008 and 2009. Of these, six new species of *Neoechinorhynchus* Stiles and Hassall 1905, one new species of *Heterosentis* Van Cleave, 1931, and two new species of *Rhadinorhynchus* Lühe 1911 were described (Amin *et al*. 2011a, b, c). Four other species of Echinorhynchid acanthocephalans from marine fishes in Halong Bay were described by Amin and Ha (2011) and 5 other new species from fishes and amphibians of 8 collected host species were described by Amin *et al*. (2014). Three other species of *Rhadinorhynchus* and one species of *Gorgorhynchus* were previously reported from marine fishes in Vietnam; see Arthur and Te (2006).

Fifteen species of acanthocephalans in 5 families were more recently collected from fishes in the Pacific and amphibians in central Vietnam in 2016 and 2017. In the present

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report, we describe males and females of 1 echinorhynchid species and one neoechinorhynchid species, and report the presence of another neoechinorhynchid species from a new host and locality in Vietnam.

Materials and Methods

Collections

Two species of amphibians and 3 species of fish were collected and examined for parasites. The two amphibian species included 10 specimens of the similar frog *Hylarana attigua* Inger, Orlov, Darevsky collected in Huong Thuy, Hue City, central Vietnam (16°28´00″,107°34´45″E) in September, 2016 (5 were infected), and 2 specimens of the odorous frog *Odorrana* sp. Fei, Ye, Huang (Ranidae) collected in Chu Yang Sin National Park, central Vietnam (12°52´37″N,108°26´17″E) in October, 2012 (1 was infected). One specimen of needlefish *Tylosurus* sp. Cocco (Belonidae) was collected off Binh Thuân in the Pacific South (10°56″N108°6E). Thirteen specimens of the Toli shad (Chinese herring), *Tenualosa toli* (Valenciennes) (Clupeidae) were collected off the Pacific north coast off Hai Phong (20°51´54.5″N106°41´01.8″E) (5 were infected). One specimen of the common ponyfish, *Leiognathus equulus* (Forssskål) (Leiognathidae) was collected off the Pacific south coast of Nha Trang (12°15´N109°11´E).

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. When there was sufficient supply of specimen, some were reserved for SEM studies.

Methods

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 hr each), and cleared in 100% xylene then in 50% Canada balsam and 50% xylene (24 hr each). Whole worms were then mounted in Canada balsam. Measurements are in micrometers, 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 drawing were created by using a Ken-A-Vision micro-projector (Ward's Biological Supply Co., Rochester, N.Y.) which uses cool quartz iodine 150W illumination. Color-coded objectives, 10X, 20X, and 43X lenses, are used. Images of stained whole mounted specimens were projected vertically on 300 series Bristol draft paper (Starthmore, Westfield, Massachusetts), then traced and inked with India ink. Projected images were identical to the actual specimens being projected. The completed line drawings were subsequently scanned at 600 pixels on a USB and subsequently downloaded on a computer.

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

SEM (Scanning Electron Microscopy)

Four to six specimens that had been fixed and stored in 70% ethanol were processed for SEM following standard methods (Lee, 1992). These included critical point drying (CPD) 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 minutes using a Polaron #3500 sputter coater (Quorum (Q150 TES) www.quorumtech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in an FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon) Scanning Electron Microscope with digital images obtained in the Nanolab software system (FEI, Hillsboro, Oregon) 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 GSE detector.

X-ray microanalysis (XEDs), EDAX (Energy Dispersive Analysis for X-Ray)

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 analyzer (FEI, Hillsboro, Oregon). 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, Oregon) was used. Data was 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 (Ga) ion source (GIS) is used for the LIMS (Liquid Ion Metal Source) part of the process. The hooks of the acanthocephalans were centered on the SEM stage and cross sectioned using a probe current between 0.2nA and 2.1 nA according to the rate at which the area is cut. The time of cutting is based on the nature and sensitivity of the tissue. Following the initial cut, the sample also goes through a milling process to obtain a smooth surface. The cut was then analyzed 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 then transferred to a USB for future use. The intensity of the GIS was variable according to the nature of the material being cut.

Materials examined

Acanthocephalus parallelcemenglandatus **Amin, Heckmann, Ha, 2014**

This species was described from one male obtained from one of 15 examined walking catfish *Clarias batrachus* (Linn.) (Claridae) collected from the Ma River, Ben En National Park, Thanh Hoa Province (19°37″N, 105°31E) in April, 2010 (Figs. 15–18 in Amin *et al*., 2014). Our present collection includes 47 specimens (10 males, 37 females) from 5 infected of 10 examined specimens of the similar frog *Hylarana attigua* Inger, Orlov, Darevsky and 7 specimens (3 males, 4 females) from one infected of 2 examined odorous frogs *Odorrana* sp. Fei, Ye, Huang (Ranidae) in Huong Thuy, Hue City, and Chu Yang Sin Park, central Vietnam, respectively, as well as one adult female worm with ovarian balls from one examined needlefish *Tylosurus* sp. Cocco (Belonidae) in Binh Thuân in the Pacific South. The similar frog is a stream breeding amphibian that inhabits the subtropical and tropical moist lowland and montane forests and streams in Laos and Vietnam (van Dijk and Stuart, 2004). The odorous frog inhabits streams and surrounding forests of China, Thailand, and Vietnam (Fei, 1999). These amphibians may be the natural hosts of this acanthocephalan with the fish being accidental hosts. The finding of viable adult acanthocephalans into fishes in associated streams may explain the cross infectivity between amphibians and fish.

Six of these worms were processed for SEM study and other specimens were studied microscopically to provide a full descriptive account of both sexes following. A new allotype female is designated from *H. attigua* since no females were previously encountered. Line drawings are only provided for females since those of males have already been included in Amin *et al*. (2014). Microscopical pictures, SEM images, X-ray scans, complete measurements, and descriptive accounts of both sexes are provided herein for the first comprehensive description of the species.

Neoechinorhynchus **(*Neoechinorhynchus) pennahia*** **Amin, Ha, Ha, 2011**

Neoechinorhynchus pennahia was described from one female specimen collected from the only infected silver croaker, *Pennahia argentata* (Houttuyen) (Sciaenidae) obtained in July, 2008 in the north Pacific at Cat Ba Island, Halong Bay off Haiphong. The silver croaker is a benthopelagic temperate fish that inhabits the coastal waters of the Northwest Pacific and feeds on zooplankton, various invertebrates, and small fin fishes (Trewavas, 1977). In the present investigation, 40 specimens of *N. pennahia* were collected from 5 of 13 Toli shads (Chinese herring), *T. toli* (Clupeidae), examined in April, 2016 off the Pacific north coast also off Haiphong. The Toli shad inhabits fast-flowing, turbid estuaries and adjacent coastal waters of the northwest Pacific (Rainboth, 1996) and feeds on large zooplankton (Vidthayanon, 2005). This is a new host record in a second family of fishes that appears to include the more common hosts of this acanthocephalan. A few specimens were used for SEM studies and the remaining specimens are described below.

The following description is an expanded version of the one reported by Amin *et al*. (2011b) which described only one female. We are describing males for the first time and covering a wider range of variations in female characteristics as more females have become available. We are including figures of males and new figure of eggs and of a female reproductive system; the one in the original description (Fig. 18) was incomplete and exceptionally short (460 long in a 3.12 mm long female) compared to those observed in our new material. SEM images, complete measurements, and descriptive accounts of both sexes are provided in the first comprehensive description of the species. A new allotype male and a new subgenus, *Neoechinorhynchus*, are designated.

Results

Description of *Acanthocephalus parallelcemenglandatus* Amin, Heckmann, Ha, 2014 (Echinorhynchidae) (Figs 1–18, and figures 15–18 of male in Amin et al., 2014)

General: With characters of the genus *Acanthocephalus*. Worms arched ventrad, small to medium, cylindrical, thick, elongate, widest anteriorly (Figs. 1, 6). Body wall often thicker dorsally than ventrally (Fig. 1). Trunk and shared structures considerably larger in females than in males. Epidermis with many electron dense micropores in proboscis hooks (Fig. 10) and throughout epidermal surface of trunk associated with internal crypts and vary in diameter and distribution in different trunk regions (Fig. 13). Proboscis of moderate length, cylindrical with

Table I. X-ray scans for hooks of *Acanthocephalus parallelcementglandatus* from *Hylarana attigua**

	Hook (overall)	Hook base Edge	Hook base Center	Hook middle Edge	Hook middle Center	Hook tip Edge	Hook tip Center
Phosphorus (P)	14.90	7.21	15.89	3.27	9.78	0.96	0.87
Sulphur (S)	0.19	12.61	0.77	16.59	8.96	11.96	15.39
Calcium (C)	34.71	13.90	35.28	6.64	22.11	2.02	2.04

*Common elements for protoplasm (C,H,O, N) not listed, as well as coating and cutting elements (Pd, Au, Ga). Three chemical elements are listed by weight percent for area (wt %). (See figures 19 and 20) nearly parallel sides and no apparent apical structure (Figs. 2, 7, 8). Proboscis hooks curved posteriorly (Fig. 9), with prominent core extending to roots and thinner but marked cortical layer (Figs. 11, 12), larger and more numerous in females than males but with similar number of longitudinal rows in both sexes. No dorso-ventral differentiation in length of hooks or relatively shorter roots (Table I). Anterior and posterior-most hooks smallest; hook no. 3 longest and heaviest; thickness of hooks corresponding to length of blades (Figs. 5, 7, 9). Roots simple, about two thirds length of blades, directed posteriorly (Fig. 5). Neck prominent with 2 lateral sensory pores (Fig. 7). Proboscis receptacle about twice as long as proboscis, with double walls but outer wall not continuous posteriorly (Fig. 2). Receptacle with two nucleated cells at it outer posterior end and prominent lanceolate cephalic ganglion at its base. Lemnisci equal, digitiform, markedly longer than receptacle (Fig. 2).

Males (based on 9 mature adults with sperm): Trunk 6.12–8.70 (7.16) mm long by 1.00–1.42 (1.25) mm wide anteriorly. Proboscis 416–468 (434) long by 270–395 (331) wide posteriorly armed with 16–21 (18.3) longitudinal rows of 5 hooks each. Neck 104–224 (187) long dorsally by 364–478 (423) wide posteriorly. Proboscis receptacle 647–936 (803) long by 239–322 (284) wide posteriorly. Cephalic ganglion 156–239 (211) long by 73–104 (94) wide. Lemnisci 0.87–1.30 (1.14) mm long by 0.11–0.37 (0.24) mm wide. Reproductive system in posterior half of trunk; testes equal, contiguous, near post-equatorial. Anterior testis 0.62–1.12 (0.83) mm long by 0.45–0.67 (0.50) mm wide. Posterior testis 0.67–1.20 (0.84) mm long by 0.37–0.75 (0.51) mm wide. Four tubular, parallel, compact, multinucleated cement glands in 2 tight clusters each draining into one joint duct. Anterior-most cement gland longest, often bent anteriorly and reaching posterior testis, 604–875 (767) long by 175–239 (200) wide. Shortest cement gland more posterior 468–625 (519) long by 125–208 (161) wide. Cement gland ducts 800–1,075 (906) long by 114–175 (143) wide and 936–1,075 (970) long by 135–200 (172) wide. Common sperm duct 884 long by 146 wide, usually obscured by cement glands. Saefftigen’s pouch 0.88–1.14 (1.06) long by 0.21–0.42 (0.34) wide anteriorly, overlapping cement gland ducts. Bursa round, 676–675 (675) long by 697–750 (722) wide with ovoid sensory disks (Figs. 14, 15). Gonopore terminal.

Females (based on 20 mostly gravid specimens with eggs and ovarian balls): Trunk 10.25–22.50 (15.98) mm long by 1.02–2.12 (1.59) mm wide anteriorly. Proboscis 489–697 (587) long by 385–450 (400) wide posteriorly with 16–19 (18.3) rows (as in males) of 5–7 (5.8) hooks each (more than in males); 67% of specimens with 19 hook rows and 50% with 5/6 hooks each. Two specimens with 7/7 hooks per row and one specimens with 5/5. Neck 177–270 (218) long dorsally by 395–582 (507) wide posteriorly. Proboscis receptacle 925–1,350 (1.090) long by 260–425 (346) wide. Cephalic ganglion 187–281 (236) long by 73–250 (134) wide. Lemnisci 1.20–1.98 (1.63) mm long by 0.12–0.37 (0.23) wide. Reproductive system 1.04–1.51 (1.28) mm long (8% of trunk length) with subterminal gonopore (Figs. 4, 16, 17). Vagina prominent 125–166 (144) long. Uterus 572–780 (684) long with highly muscular posterior wall and few anterior uterine glands. Uterine bell 385–572 (459) long. Eggs ovoid elongate 67–92 (76) long by 22–27 (25) in diameter, with extensive fibrous coat and unremarkable polar prolongation of fertilization membrane (Figs. 3, 18).

Taxonomic summary

Type hosts. Walking catfish *Clarias batrachus* (Linn.) (Clariidae) of holotype male (Amin *et al*., 2014). The similar frog *Hylarana attigua* Inger, Orlov, Darevsky (Ranidae) of allotype female (this paper).
Other hosts. The odorous frog *Odorrana* sp. Fei, Ye, Huang (Ranidae) and the needlefish *Tylosurus* sp. Cocco (Belonidae).
Type localities. The Ma River, Ben En National Park, Thanh Hóa Province (19°37´N,105°31´E) for the male holotype (Amin *et al*., 2014) and Huong Thuy, Hue City, central Vietnam (16°28´00″,107°34´45″E) for the female allotype.
Other localities. Chu Yang Sin National Park, central Vietnam (12°52´37″N,108°26´17″) and the Pacific south at Binh Thuân (10°56´N, 108°6´E).
Type specimens. HWML collection no. 49917 (holotype male) (Amin *et al*., 2014), HWML collection no. 139365 (new allotype female), and HWML collection no. 139366 (paratype males and females).

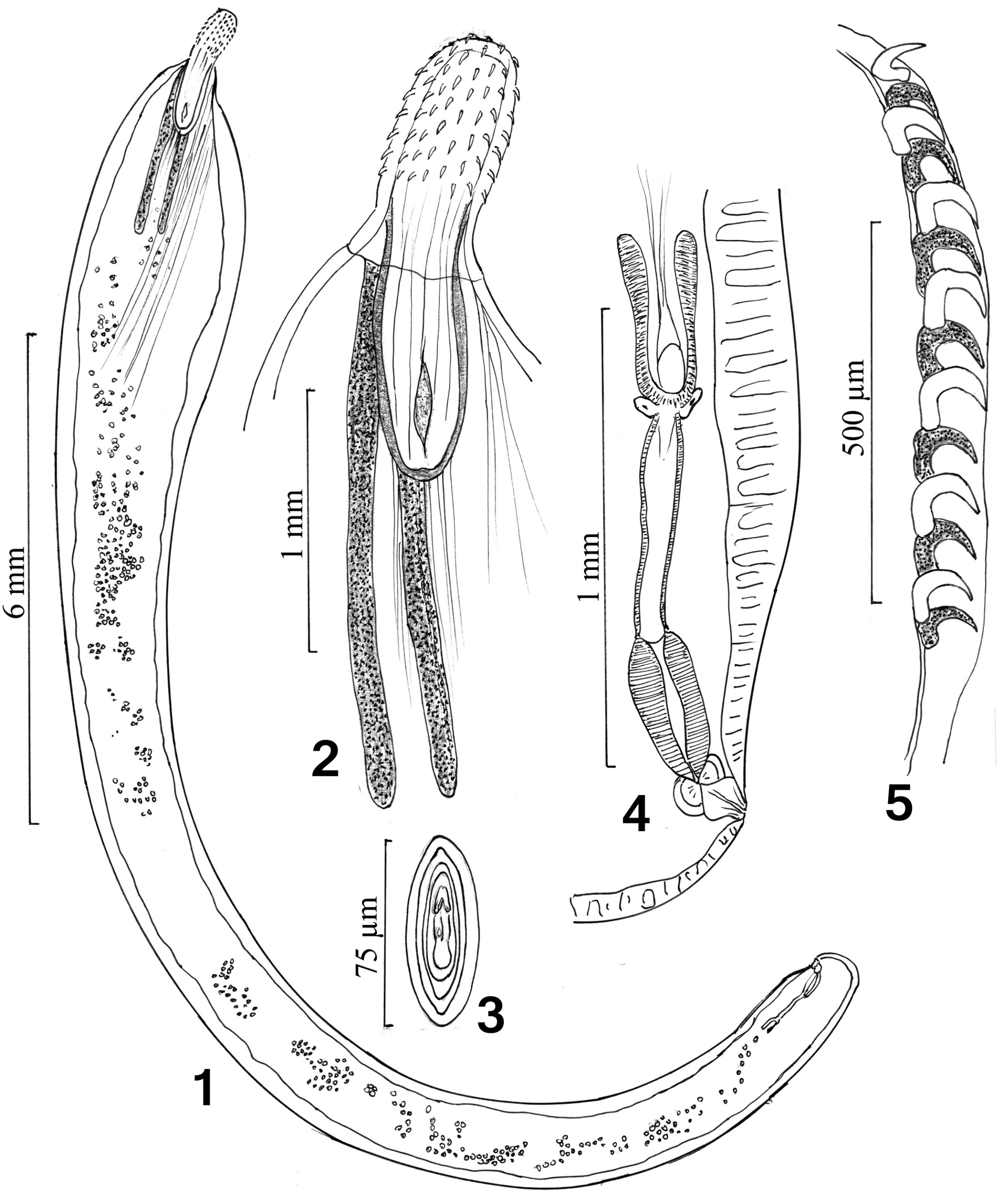
Remarks

The present collection of acanthocephalans from Vietnam provides a great opportunity to expand our knowledge of taxa previously described from only one or very few specimens. The previous description of *A. parallelcementglandatus* from one male specimen in a different host and locality by Amin *et al*. (2014) is a case in a point. The complete description above provides a range of variation in the male morphometric characteristics and a description of females for the first time. That description provides new information about the lack of apical structure on the proboscis, the presence of 2 sensory pores on the neck and of sensory structures on the bursa. The presence of micropores on the proboscis hooks suggests that hooks are also involved in nutrient absorption like the trunk of practically all acanthocephalans including *Neoechinorhynchus pennahia*; see following. The micropores have been shown to vary in diameter and distribution in various body regions in proportion to their rate of absorption of nutrients through the body wall (Amin *et al*. 2009).

Description of *Neoechinorhynchus* (*Neoechinorhynchus) pennahia* Amin, Ha, Ha, 2011

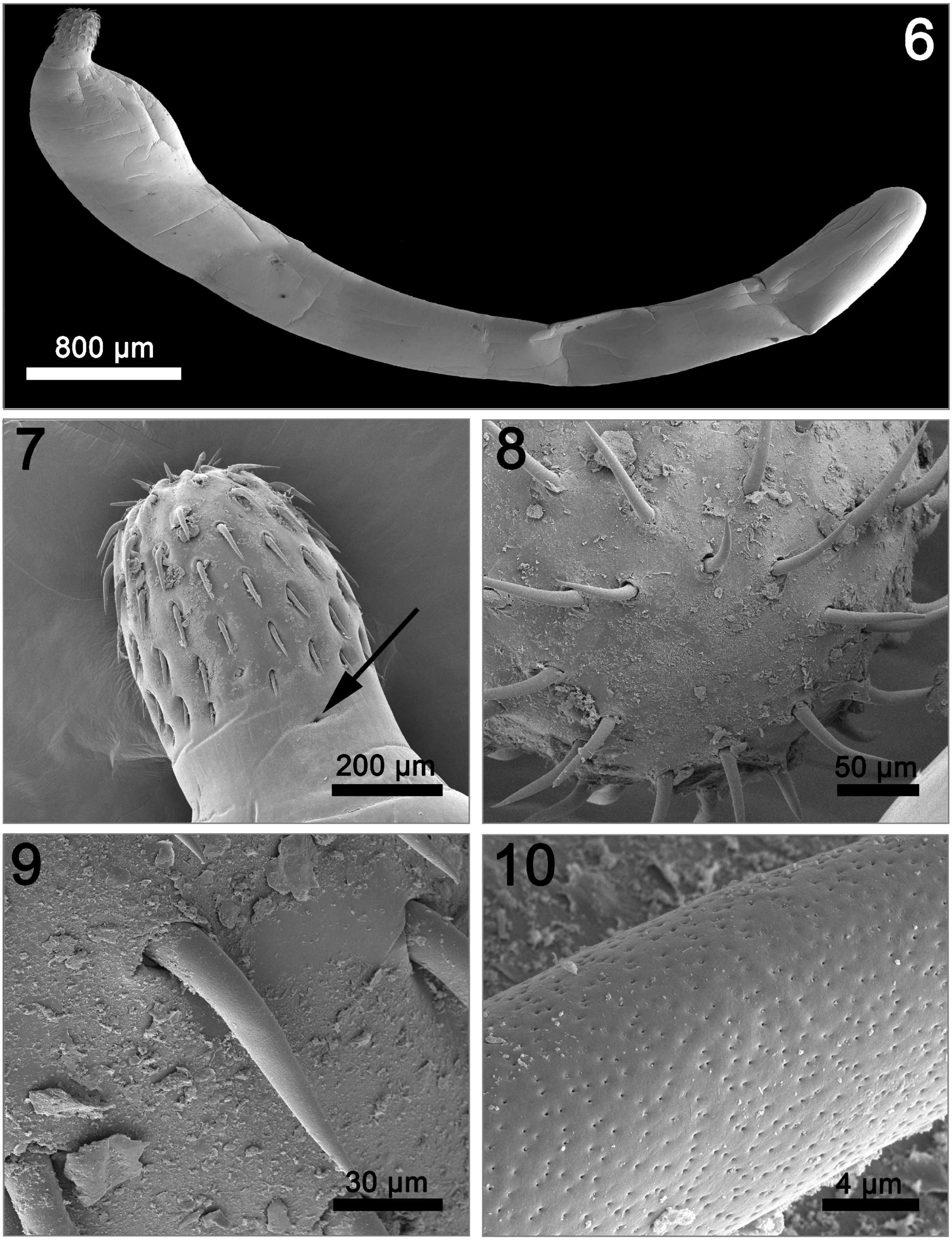
(Figs. 21-32 and Figs. 15–18 of female in Amin et al., 2011)

General: Neoechinorhynchidae. With characters of the genus *Neoechinorhynchus* and the subgenus *Neoechinorhynchus* as

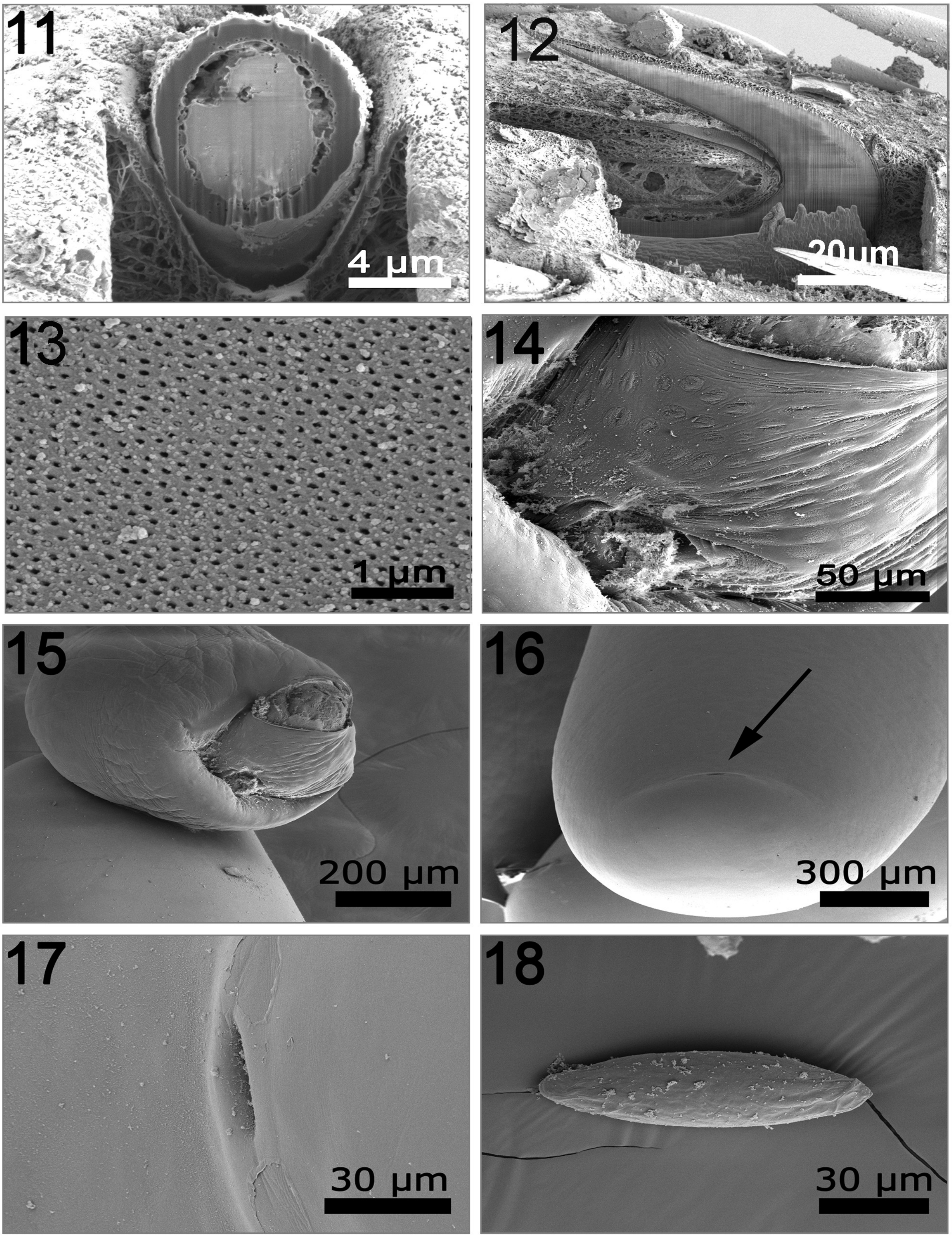


Figs. 1-5. Line drawings of female specimens of *Acanthocephalus parallelcementglandatus* collected from the similar frog *Hylarana attigua* in central Vietnam. **1.** – Allotype female; **2.** – The proboscis, receptacle, and lemnisci of allotype female in Fig. 1; **3.** – A ripe egg. **4.** – Female reproductive system; **5.** – Two adjacent rows of proboscis hooks showing size transition and roots

described by Amin (2002). All common structures relatively larger in females than in males. Trunk cylindrical, slightly curved and relatively enlarged anteriorly, with 4–6 dorsal and 1–3 ventral giant nuclei (Fig. 21). Epidermis with many micropores associated with internal crypts and vary in diameter and distribution in different trunk and other locations (Fig. 29). Proboscis about as long as wide (Figs. 22, 25) with prominent apical organ (Fig. 26) occasionally reaching neck and connecting with cephalic ganglion via apical sensory fibers (Fig. 22). Anterior and middle hooks about equal and relatively larger than posterior hooks (Figs. 22, 26). All hooks with prominent vacuolated core and thin cortical layer (Fig. 28),

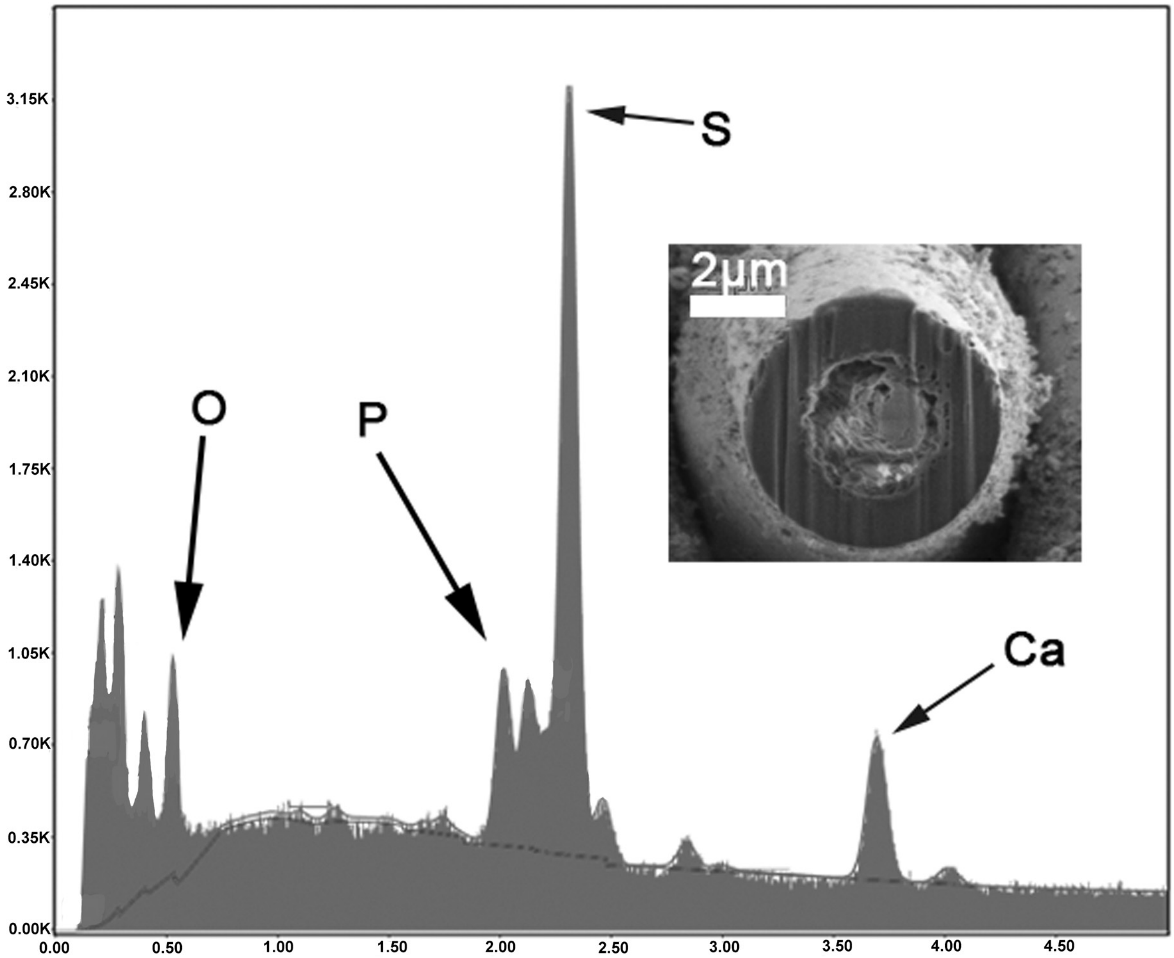


Figs. 6-10. SEM of specimens of *Acanthocephalus parallelcementglandatus* collected from the similar frog *Hylarana attigua* in central Vietnam. **6.** – A paratype female showing the typical body form; **7.** – A typical proboscis with nearly parallel sides showing one sensory pore (arrow) on the neck; **8.** – The apical end of a proboscis showing no sign of apical structure; **9.** – A typical hook at the middle of the proboscis; **10.** – A high magnification of a hook showing the unusual presence of micropores



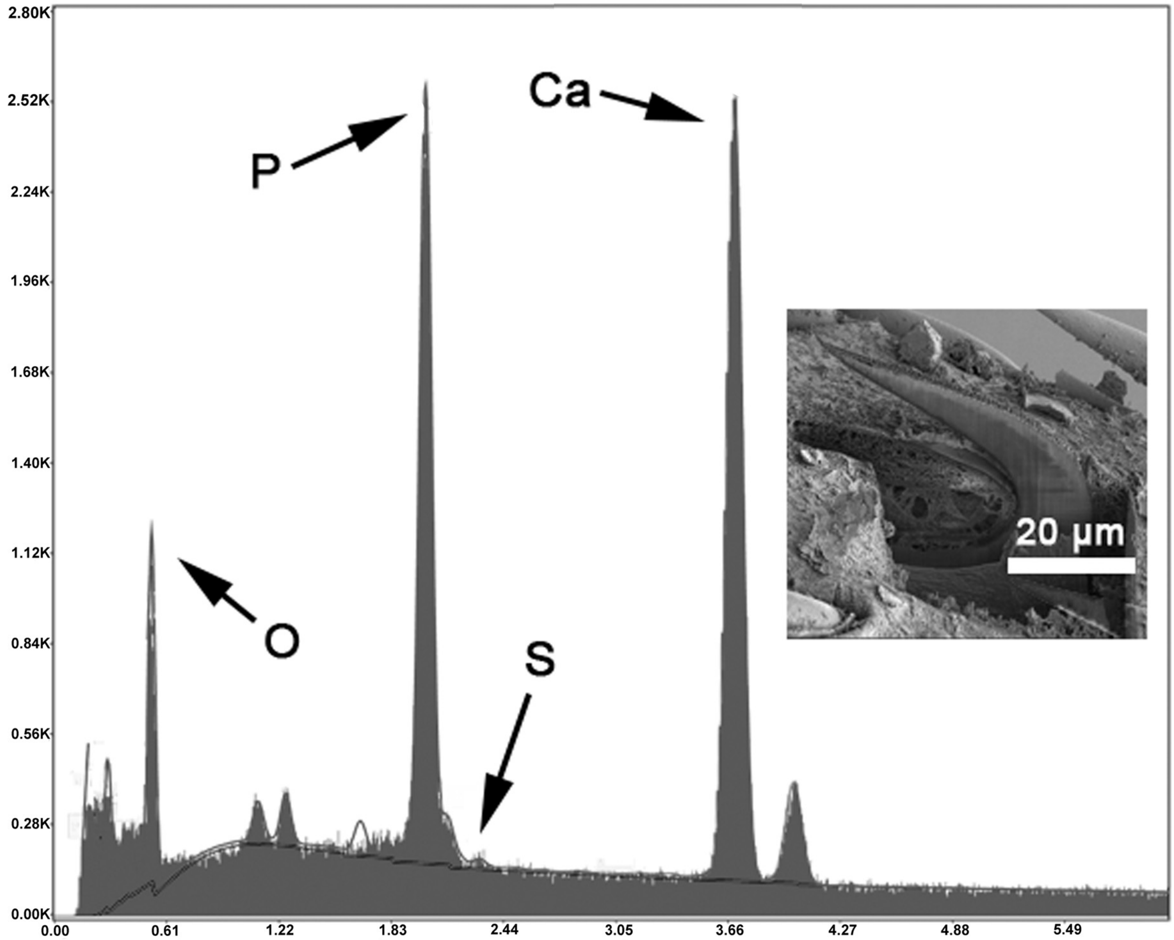
Figs. 11-18. SEM of specimens of *Acanthocephalus parallelcementglandatus* collected from the similar frog *Hylarana attigua* in central Vietnam. **11.** – A gallium cut cross section of a proboscis hook showing the prominent core and the relatively thick cortical layer. See Figures 19 and 20 Energy Disruptive X-ray Analysis of metals of hooks; **12.** – A gallium cut lateral section of a proboscis hook showing the solid core extending from the tip of the hook to the root; **13.** – Micropores at mid-trunk; **14.** – A magnified view of the area of sensory plates in the bursa shown in Figure 15; **15.** – A ventro-terminal aspect of a bursa from which the sensory plates are magnified in Figure 14; **16.** – The posterior end of a female showing the location of the small subterminal gonopore (arrow); **17.** – A high magnification of the female gonopore; **18.** – An egg

and with simple posteriorly directed roots. Roots of anterior and middle hooks spoon-shaped, slightly shorter than blades; those of posterior hooks abbreviated and stubby (Fig. 22 and figure 17 in Amin *et al*., 2011). Neck long, longer than proboscis and wider at base, with paired sensory pores. Proboscis receptacle about 3 times as long as proboscis enveloped in thin



Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	14.04	23.81	11.29	255.58	0.0344
N	17.63	25.65	12.99	161.84	0.0232
O	19.82	25.24	11.3	464.84	0.032
Ga	0.02	0.01	83.8	1.09	0.0001
Mg	0.59	0.5	16.39	81.61	0.0038
Al	0.52	0.39	17.39	80.07	0.0038
Si	0.39	0.28	27.57	64.52	0.0032
P	9.78	6.43	3.32	1353.34	0.0836
Au	4.82	0.5	12.73	327.01	0.0468
S	8.96	5.7	3.77	1157.44	0.0752
Pd	1.32	0.25	31.16	71.25	0.0096
Ca	22.11	11.24	3.09	1676.95	0.1939

Fig. 19. Energy disruptive X-ray analysis of proboscis hook tip of *Acanthocephalus parallelcementglandatus* collected from the similar frog *Hylarana attigua* in central Vietnam. Note the high sulfur content

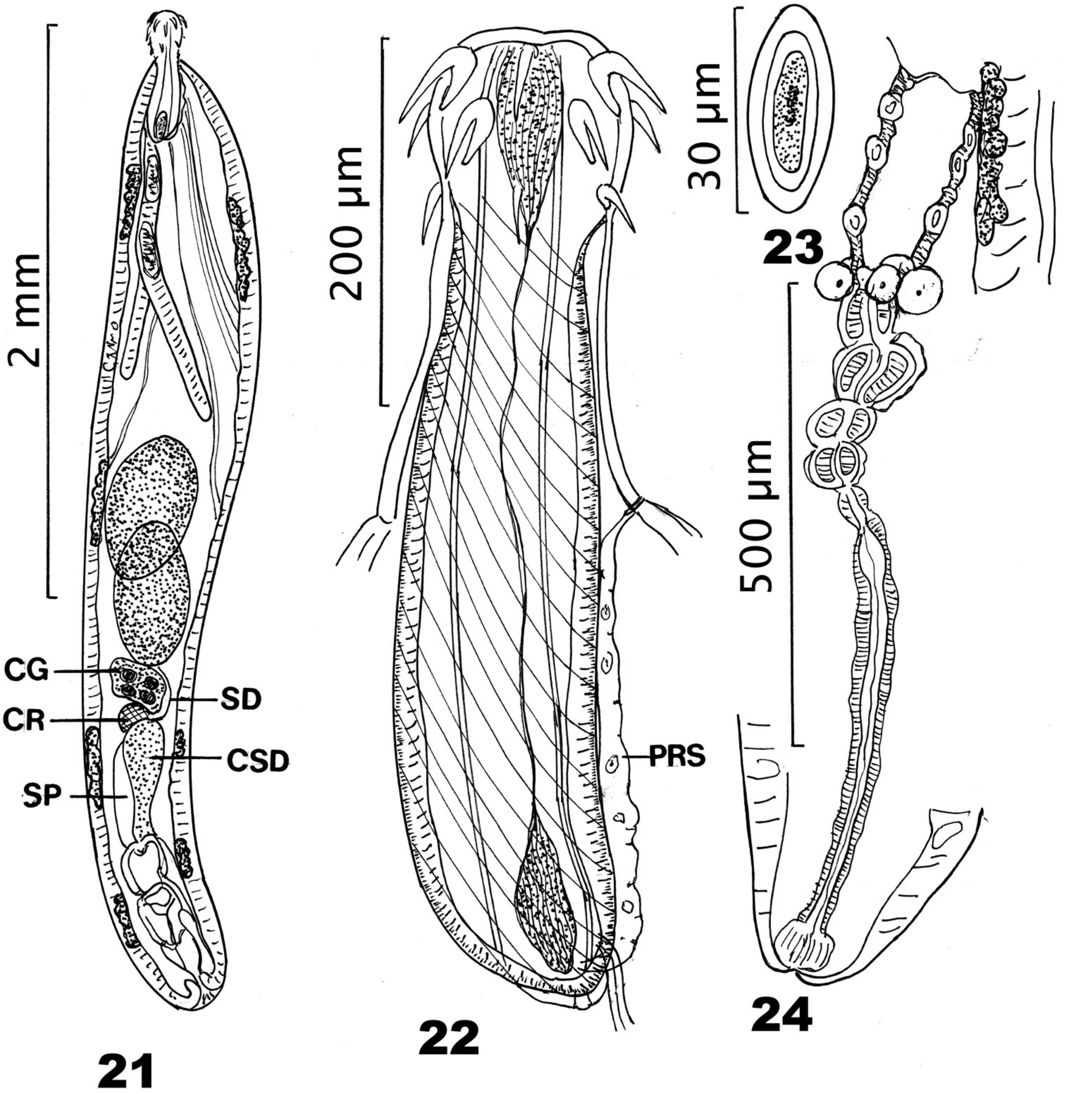


Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C	6.42	11.55	12.69	110.21	0.0187
N	15.61	24.06	14.13	107.06	0.0193
O	25.15	33.94	11.2	450.09	0.039
Ga	1.38	0.43	13.92	51.96	0.0085
Mg	0.82	0.73	13.88	85.2	0.0049
P	14.9	10.38	3.19	1630.85	0.1268
Au	0.72	0.08	24.95	52.61	0.0095
S	0.19	0.13	57.88	19.31	0.0016
Pd	0.1	0.02	56.34	4.49	0.0008
Ca	34.71	18.7	2.52	2155.59	0.3137

Fig. 20. Energy disruptive X-ray analysis of proboscis hook base of *Acanthocephalus parallelcementglandatus* collected from the similar frog *Hylarana attigua* in central Vietnam. Note the high Ca and P content

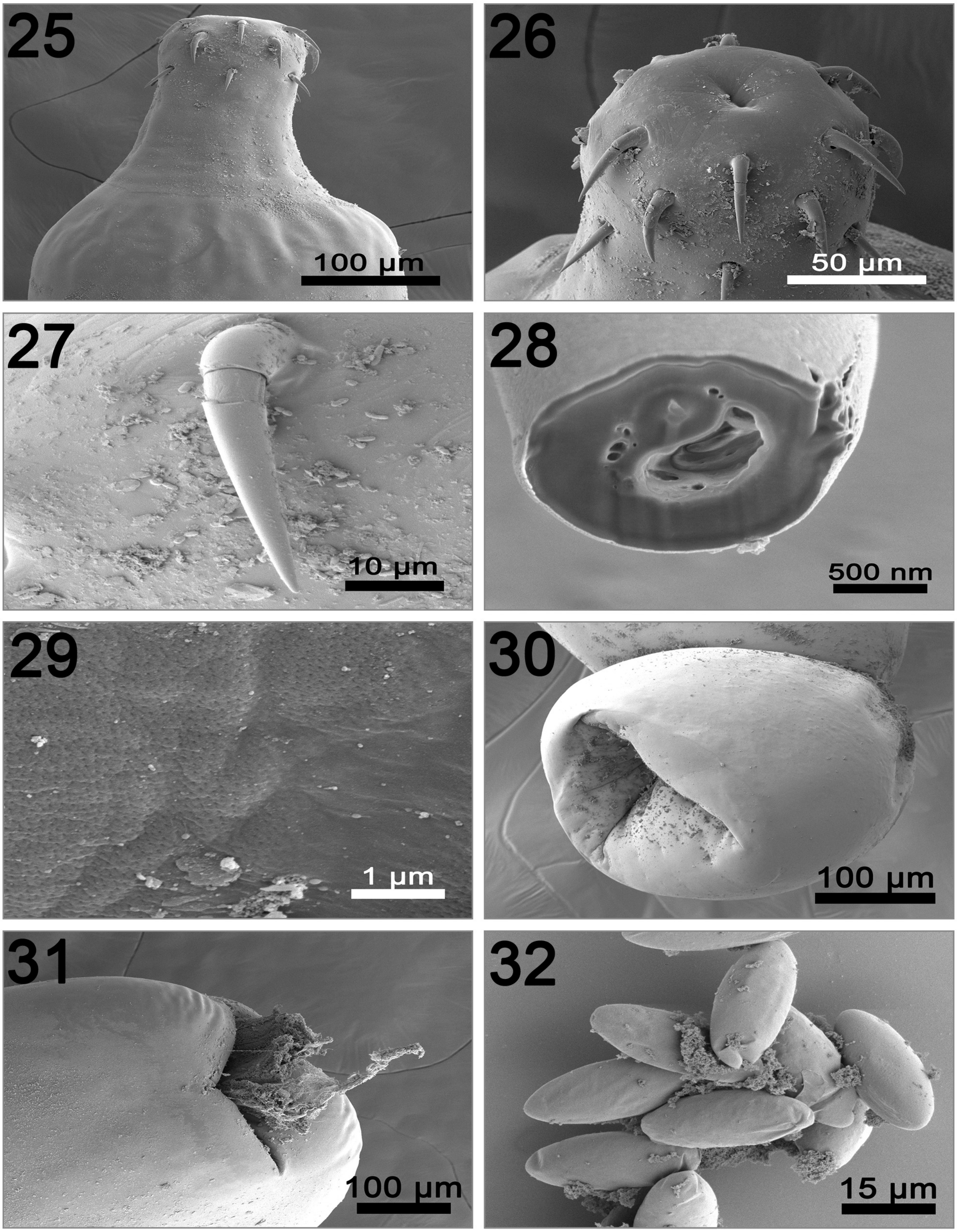
diagonal muscular layer, with large triangular cephalic ganglion at base connected to prominent apical organ via apical sensory nerve (Fig. 22), and associated para-receptacle structure (PRS) at least on one side. Lemnisci finger-like, subequal, considerably longer than receptacle, with 2 and 1 giant nuclei in longer and shorter lemniscus, respectively (Fig. 21).

Males (based on 4 mature specimens with sperm): Trunk 3.05–4.62 (3.81) mm long by 0.52–0.80 (0.71) mm wide anteriorly. Proboscis 115–125 (120) long by 112–125 (119) wide. Proboscis hooks from anterior 55–60 (57), 50–55 (53), 40–47 (44), respectively. Proboscis receptacle 255–572 (365) long by 92–156 (116) wide. Shorter lemniscus 0.88–1.14 (1.01) mm long by 0.09



Figs. 21-24. Line drawings of male (Figs 21, 22) and female (Figs 23, 24) specimens of *Neoechinorhynchus pennahia* collected from the Toli shad (Chinese herring), *Tenualosa toli* in the Pacific north coast of Vietnam off Haiphong. **21.** – Allotype male; note the unequal lemnisci, subcutaneous giant nuclei, and the labeled reproductive structures. CG: cement gland with 4 giant nuclei; CSD: common sperm duct; CR: cement reservoir; SD: sperm duct; SP: Saefftigen's pouch; **22.** – The proboscis, prominent neck, and receptacle. Note the large apical organ and the apical sensory cord connecting it with the basal cephalic ganglion, as well as the para-receptacle structure (PRS) and the spiral muscular layer enveloping the receptacle; **23.** – An egg with concentric shells; **24.** – The female reproductive system with simple vagina, relatively long uterus with beady muscular enlargements at distal end and the uterus attached to body wall ventrall

mm wide. Longer lemniscus 1.00–1.25 (1.13) long by 0.10–0.12 (0.11) mm wide. Reproductive system in posterior part of trunk with testes about equally long and overlapping; all other structures tightly connected. Anterior testis 416–1,120 (680) long by 281–475 (346) wide. Posterior testis 364–1,120 (660) long by 270–520 (340) wide. Cement gland 156–468 (299) long by 177–416 (266) wide, with 4 prominent giant nuclei. Cement reservoir triangulating posteriorly, 125–364 (215) long by 104–229 (152) wide. Sperm ducts turn around cement gland and reservoir emerging posteriorly as prominent common sperm duct,



Figs. 25-32. SEM of specimens of *Neoechinorhynchus pennahia* collected from the Toli shad (Chinese herring), *Tenualosa toli* in the Pacific north coast of Vietnam off Haiphong. **25.** – The proboscis, prominent neck, and anterior trunk of a male specimen; **26.** – The proboscis of another specimens showing in the invaginated apical end of the apical organ; **27.** – An anterior proboscis hook; note its curvature; **28.** – A gallium cut section of an anterior hook showing its prominent core and thin cortical layer; **29.** – Micropores at the mid-trunk; **30.** – The simple muscular bursa; **31.** – The position and shape of the female gonopore; **32.** – Eggs

384–884 (624) long by 114–187 (150) wide, adjacent to Seafftigen's pouch 374–728 (551) long by 114–156 (135) wide, almost as long as common sperm duct (Fig. 21). Bursa highly muscular with no prominent sensory structures (Fig. 30).

Females (based on 13 adults with ovarian balls and eggs): Trunk 3.25–8.37 (5.93) mm long by 0.35–1.12 (0.67) mm wide anteriorly. Proboscis 132–137 (135) long by 125–132 (127) wide. Proboscis hooks from anterior 53–62 (57), 50–57 (54), 45–50 (47), respectively. Proboscis receptacle 262–520 (381) long by 95–165 (128) wide. Shorter lemniscus 0.84–1.20 (0.99) mm long by 0.08–0.11 (0.10) wide. Longer lemniscus 1.00–1.46 (1.21) mm long by 0.08–0.13 (0.10) wide. Reproductive system 0.87–1.14 (1.00) long [14–18 (16%) of trunk length], with near subterminal gonopore (Fig. 31), simple rounded vagina and prominent muscular bulbs along distal portion of long uterus (Fig. 24). Uterine bell attached to ventral body wall and with few uterine bell glands. Eggs ovoid with concentric shells (Figs. 23, 32), 28–35 (31) long by 6–10 (8) wide.

Taxonomic summary

Type host of holotype female: Silver croaker, *Pennahia argentata* (Houttuyen) (Scianidae).

Other host of allotype male: Toli shad, *Tenualosa toli* (Valenciennes) (Clupeidae).

Type locality of holotype female: Halong Bay at Cat Ba Island, Hai Phong (20°48´00″N106°59´59″E)

Other locality of allotype male: Pacific coast off Hai Phong (20°51´54.5″N106°41´01.8″E).

Site of infection: Intestine.

Type specimens: HWML Collection no. 49213 for holotype female (Amin *et al*., 2011b). Collection no. 139367 for allotype male and other paratypes on same slide.

Remarks

Neoechinorhynchus (*N.*) *pennahia* remains the only species of *Neoechinorhynchus* characterized by a combination of characters including its proboscis armature, long neck, sub-equal lemnisci, spiral muscle envelope around the receptacle, terminal gonopore, and the PRS. See Amin *et al*. (2011) for distinctions from other species with similar proboscis armature including *Neoechinorhynchus* (*Hebesoma*) *idahoensis* Amin and Heckmann, 1992, *Neoechinorhynchus* (*N.*) *notemigoni* Dechtiar, 1967, and *Neoechinorhynchus* (*N.*) *crassus* Van Cleave, 1919. Its recovery in large numbers from the Toli shad in the same area off Hai Phong where the single specimen from which the species was originally described from the silver croaker suggests that the shad may be the more natural host.

Neoechinorhynchus (*Neoechinorhynchus*) *longnucleatus* Amin, Ha, Ha, 2011

Neoechinorhynchus (*N.*) *longnucleatus* was described from 5 males and 7 females collected in May, 2009 from 2 spottail needlefish *Strongylura strongylura* (Van Hasselt) (Belonidae) from Halong Bay at Cat Ba Island in the north Pacific off Haiphong. In the present investigation, 1 gravid female of *N.* (*N.*) *longnucleatus* was collected from the only infected common ponyfish, , *L. equulus* (Leiognathidae) off the Pacific south coast of Nha Trang in October, 2016. This represents a new host record in fish from 2 different families and a new geographical record in 2 distant locations off the Pacific coast of Vietnam. This acanthocephalan may be more widespread than this initial distributional records may imply.

Discussion

The hooks of *A. parallelcementglandatus*

The results for the X-ray microanalysis of are given by Table I and figures 19 and 20. The common chemical element for living material (C, H, O, N) are present as well as the processing chemicals (Pd, Au, Ga). What is prominent is the amount of Sulfur in the hardened hooks (edge and tip, 16.59 and 15.39 wt. %). The other chemical elements are present (Ca, P) for the attachment structure. The sulfur rich thick outer layer high was clearly demonstrable (Fig. 11). The typical layers for acanthocephalan hooks are present especially the hardened outer layer.

The X-ray scans (XEDs) and gallium cuts (LIMs) for the hooks of *A. parallelcementglandatus* are similar to results this lab has obtained with other studies (Amin and Heckmann 2017; Heckmann *et al*. 2007, Heckmann *et al*. 2012). The hook layering is prominent with a high content of Sulfur in the hardened outer layer. This layer is probably due to disulphide bonds found in the outer calcium phosphate apatite similar to the enamel layer of mammalian teeth (Heckmann *et al*. 2012).

An appreciation for sex-related differences in shared structures such as proboscis armature was particularly interesting in light of the extreme variations observed. Also of great interest is establishing that the natural host for this acanthocephalan is a water breeding amphibian irrespective of the fact that the type host of the single described male specimen is a fish.

The para-receptacle structure (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 eoacanthocephalans with weak single proboscis receptacle wall was first demonstrated in *Neoechinorhynchus* (*N.*) *qatarensis* Amin, Saoud, Alkuwari, 2002 by Amin *et al*. (2002) and had since been reported in other species of *Neoechinorhynchus* Stiles and Hassall, 1905 and *Acanthogyrus* (*Acanthosentis*) Verma and Datta, 1029 reviewed in part in Amin *et al*, (2011b). 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 electron dense micropores present throughout epidermal surface of the trunk of *N. pennahia* , 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. We have reported micropores in a large number of acanthocephalan species summarized in Heckmann *et al*. (2013) and in a few more since, and demonstrated the tunneling from the cuticular surface into the internal crypts by TEM. Amin *et al*. (2009) gave a summary of the structural-functional relationship of the micropores. Wright and Lumsden (1969) and Byram and 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 µm² of *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 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*. (2011d).

The apical organ of *N. pennahia* and receptacle muscles

The interesting connection of the apical organ with the cephalic ganglion via the apical nerve cord in *N. pennahia* is similar to that observed by Dunagan and Schmitt (1995) in *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos, 1917 where the apical organ is served by a pair of nerves from the cerebral ganglion as well as by a duct from the sensory support cell (stützzelle). One function of the apical organ appears to be transduction of chemical sensory information (Dunagan and Schmitt, 1995). A similar but more prominent diagonal muscle layer enveloping the receptacle was observed in one other species of *Neoechinorhynchus*, *N.* (*Hebesoma*) *spiramuscularis* Amin, Heckmann, Ha, 2014, collected from the freshwater fish *Xenocypris davidi* Bleeker (Cyprinidae) in the Ma River in the Ben En National Park, Thanh Hóa Provinve, Vietnam (Amin *et al*., 2014). Clearly, the development of spiral muscular layer surrounding the receptacle in species of *Neoechinorhynchus* is not related to water salinity.

Micropores

The presence of micropores on the proboscis hooks of *N. parallelcemenglandatus* suggests that hooks are also involved in nutrient absorption like the trunk of practically all acanthocephalans. We have documented this phenomenon in 16 species of acanthocephalans (Heckmann *et al*., 2013) and a few more since. The functional aspects of micropores in a few other acanthocephalan species including *Rhadinorhynchus ornatus* Van Cleave, 1918, *Polymorphus minutus* (Goeze, 1782) Lühe, 1911, *Moniliformis moniliformis* (Bremser, 1811) Travassos (1915), *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos (1916, 1917), and *Sclerocollum rubrimaris* Schmidt and Paperna, 1978 were reviewed earlier by Amin *et al*. (2009). The micropore canals appear to be continuous with canalicular crypts that constitute a huge increase in external surface area implicated in nutrient up take (Amin *et al*., 2009). The description of *N. pennahia* documents its long neck, para-receptacle structure, long proboscis receptacle enveloped in thin diagonal muscular layer, and large lanceolate cephalic ganglion at base connected to prominent apical organ via apical sensory nerve.

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

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