J. Parasitol., 103(5), 2017, pp. 458–470

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

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ABSTRACT: *Acanthogyrus* (*Acanthosentis*) *kashmirensis* n. sp. is described from recently collected acanthocephalan specimens in the Jhelum River in northern Kashmir that are conspecific with *Neoechinorhynchus kashmirensis* Fotedar and Dhar, 1977 originally described in a Ph.D. thesis in 1972 from 4 species of cyprinid fishes: *Tor tor* Hamilton, *Bangana diplostoma* (Heckel) (syn. *Labeo diplostoma* Heckel), *Labeo rohita* Hamilton, and *Ptychobarbus* sp. Steindachner. The poor unpublished diagnosis was followed by 1 uninformative abstract in a scientific meeting in 1977. The acanthocephalan was later designated as invalid because of the lack of a formal published description and absence of information on deposited type or voucher specimens. Recent collections of specimens of the same species were made from 2 other cyprinid species of cyprinid fishes, *Schizothorax plagiostomus* Heckel and *Schizothorax labiatus* (McClelland) from the Sandran River, a tributary of the Jhelum River, in southern Kashmir. It is now possible to provide a full description of these specimens and reassign them in the subgenus *Acanthosentis* Verma and Datta, 1929 based on the finding of circles of vestigial spines at the anterior end of the trunk of male and female specimens. These vestigial spines are barely visible and easy to miss with optical microscopy. The new species is also characterized by having (1) a para-receptacle structure in males and females, (2) unique double Saefftigen's pouches, (3) large round single-nucleated cells in the proboscis, and (4) the lemnisci being either equal or distinctly unequal with no intermediate states. A key to the species of *Acanthosentis* of the Indian subcontinent is provided. Histopathological sections show extensive damage to the host intestine with subsequent blood loss, cell necrosis, and attempted encapsulation. Results of the energy dispersive X-ray analysis (EDAX) study show hollow hooks high in sulfur but with limited calcium ions. Hooks of most acanthocephalans studied with X-ray scans are solid with high calcium and low sulfur ions.

Acanthogyrus (*Acanthosentis*) *kashmirensis* n. sp. (referred to as *Acanthosentis kashmirensis*) was first described as *Neoechinorhynchus kashmirensis* in the unpublished Ph.D. thesis of Dhar (1972) from 4 species of cyprinid fishes in the Jhelum River, northern Kashmir. The description was so inadequate that the species was barely recognizable. It was later reported only once as a new species, *N. kashmirensis* Fotedar and Dhar, 1977. This account also applies to a few species of *Neoechinorhynchus* Stiles and Hassall, 1905 as well as *Acanthosentis* Verma and Datta, 1929. Golvan (1994) recognized this species under *Neoechinorhynchus*, but Amin (2002) declared it invalid because it "was only reported in an uninformative abstract of a meeting; no formal description was provided." Amin (2002) further qualified invalid species as "Species published in abstracts are considered invalid when only the species name is mentioned without a description or when a given description is so uninformative as to render the species unrecognizable. Such abstracts usually do not indicate if type or voucher specimens were deposited or are available for examination."

To date, no other systematic accounts of the name *N. kashmirensis* have been reported except for listings in online global indexing sites. Our recent collections from other cyprinid species of fish in separate but connected streams, the Sandran River and the Lidder Stream, in southern Kashmir (Anonymous, 2013) provide ample information to justify the assigning of this species to the subgenus *Acanthosentis* and to document a complete morphological description. New and unusual features and histopathology are also described and a key to the species of *Acanthosentis* in the Indian subcontinent is included. X-ray microanalysis (XEDS) has been used for the study of the chemical element composition of parasite structures in conjunction with electron microscopy especially of the hooks of the acanthocephalan proboscis (Heckmann et al., 2012; M. D. Standing and R. A. Heckmann, unpubl. data). This method was used in the present research to analyze the chemical differences of the anterior and posterior proboscis hooks.

MATERIALS AND METHODS

Sample collection

An initial 86 and 109 specimens of 2 cyprinid species of fish, the snow trout, *Schizothorax plagiostomus* Heckel, and the Kunar snow trout, *Schizothorax labiatus* (McClelland), respectively, were gill-netted in 3 locations (U1–U3) of southern Kashmir's Sandran River, a southwestern tributary of the Jhelum River, by fishermen in late spring (April–May) 2015. An additional 200 fishes of the same 2 species as well as of the Sattar snowtrout, *Schizothorax curvifrons* Heckel, and the Chirruh snowtrout, *Schizothorax esocinus* Heckel, were also examined in the Lidder Stream, another nearby tributary of the Jhelum River, in October 2015 and May 2016, bringing the total number of fishes examined to 395 (Table I). The altitude and coordinates of the 3 sites of the Sandran River were as follows: U1: 1,865 m (33°32′17″N, 75°14′26″E); U2: 1,819 m (33°33′26″N, 75°14′40″E); and U3: 1,798 m (33°33′52″N, 75°13′34″E). The Sandran River is a perennial stream fed by a glacier from Peer Panchal Mountain and the Verinag Spring. It originates near Nandmarg pass in the

Received 16 February 2017; revised 8 May 2017; accepted 5 June 2017.
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DOI: 10.1645/17-27

TABLE I. Infection indices of *Acanthogyrus* (*Acanthosentis*) *kashmirensis* collected from 4 fish species of genus *Schizothorax* in the Sandran River, southern Kashmir.

Fish species examined	No. fish examined	No. fish infected	Prevalence (%)	No. parasites collected	Maximum worms/host	Mean/host
Schizothorax plagiostomus	140	30	21	75	2	0.54
Schizothorax labiatus	135	18	13	37	2	0.27
Schizothorax curvifrons	60	6	10	18	3	0.30
Schizothorax esocinus	61	11	18	22	2	0.36
Total	395	65	16	152	3	0.38

south eastern border areas of Kashmir Valley and flows to the northwest where it empties into the larger 774-km-long Jhelum River (Anonymous, 2013) where Dhar (1972) collected his original *N. kashmirensis* material.

Parasitological examination

Fish were examined for parasites that were collected from the incised digestive tract by using a dissecting scope. Acanthocephalans were placed in 70% ethanol to process for microscopy and scanning electron microscopy (SEM). Specimens embedded in host tissue were collected separately and fixed in 10% formalin for histological studies. In total, 395 fishes from the Sandran River and the Lidder Stream, tributaries of the Jhelum River in southern Kashmir, were examined, and parasites were collected from 65 individuals (Table I).

Microscopical examination

Specimens were placed in water overnight or until fully extended and then fixed in 70% ethanol. 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 and 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. Voucher specimens were deposited in the University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection in Lincoln, Nebraska.

SEM methods

Samples of parasites that had been fixed and stored in 70% ethanol were processed following standard methods (Lee, 1992) that included critical point drying in sample baskets and mounting on SEM sample mounts (stubs) by using conductive double-sided carbon tape. Samples were coated with gold and palladium for 3 min by 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 a Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon) Scanning electron microscope digital images were obtained in the NanoLab software system (FEI) and then transferred to a Universal Serial Bus (USB) for future reference. Images were taken at various magnifications. Samples were received under low-vacuum conditions by using 10 kV, spot size 2, 0.7 Torr using a gaseous secondary electron detector.

XEDS

Standard methods were used for preparation similar to the SEM procedure. Specimens were examined and positioned with the above-mentioned SEM instrument that was equipped with a Phoenix energy-dispersive X-ray analyzer (FEI). 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) 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 the hooks

A dual-beam scanning electron microscope with a gallium ion source (GIS) is used for the liquid ion metal source (LIMS) part of the process. The proboscis hooks are sectioned using a probe current between 0.2 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 is then analyzed for chemical ions with an electron beam (tungsten) to obtain an X-ray spectrum. Results are stored with the attached imaging software and then transferred to a USB for future use. The intensity of the GIS is variable due to the nature of the material being cut.

Histology

Infected host tissue was fixed in 10% buffered formalin. After dehydration and blocking, the specimens were processed using standard methods (Bancroft and Gamble, 2001; Kiernan, 2002). The paraffin-blocked tissue was sectioned at 4–6 μm, placed on glass slides, and stained with hematoxylin and eosin (H&E). Additional sections were stained with Mallory's trichrome to emphasize pathological responses to the parasite (Galigher and Kozloff, 1971). The prepared glass slides were viewed with an LSM laser (Carl Zeiss, Thornwood, New York) equipped compound light microscope, and representative pictures were taken at varying magnifications with a digital camera. H&E is a standard stain for tissue, whereas Mallory's trichrome helps differentiate granular tissue typical of parasite invasion. The histopathological sections (see Fig. 6) were selected from a much

larger collection of sections on 85 glass slides in R.A.H.'s collection.

RESULTS

Examined fish were lightly infected with acanthocephalans. Initially, 12 (14%) of 86 examined individuals of *S. plagiostomus* were infected with 23 specimens of *A. kashmirensis* in all 3 collecting sites of the Sandran River, a tributary of the larger Jhelum River. Corresponding figures for *S. labiatus* were 6 (6%) of 109 examined fish were infected with 20 acanthocephalans. An additional number of specimens were collected from the same two hosts as well as from *S. curvifrons* and *S. esocinus* in the Lidder Stream, another nearby tributary of the Jhelum River, in October 2015 and May 2016, bringing the total number of parasites collected to 152 obtained from 65 infected of 395 examined fishes (Table I).

Initial specimens were collected in the Sandran River, southern Kashmir, a river that is connected to the Jhelum River from which the worms were originally described by Dhar (1972) from 4 other species of cyprinid fishes—*Tor tor* Hamilton, *Bangana diplostoma* (Heckel) (syn. *Labeo diplostoma* Heckel), *Labeo rohita* Hamilton, and *Ptychobarbus* sp. Steindachner—in northern Kashmir. All 8 species of fish are native to Kashmir and northeastern India, but they are also known to inhabit rivers and tributaries throughout Himalaya extending to Afghanistan, Bangladesh, Pakistan, Nepal, Ladkah, Sri Lanka, and Tibet (China), with zooplankton being a primary food item in their diet (Talwar and Jhingran, 1991; Kullander et al., 1999). Worms were comparable to those of Dhar (1972) in hook measurements and similarities in the morphology of males, even though our specimens were almost twice as large as those reported by Dhar (1972), who did not detect the anterior trunk circles of vestigial spines.

DESCRIPTION

Acanthogyrus (*Acanthosentis*) *kashmirensis* n. sp.

(Figs. 1–6)

General: With characters of the genus *Acanthogyrus* Thapar, 1927 and the subgenus *Acanthosentis* as diagnosed by Amin (2005). Shared structures markedly larger in females than in males. Trunk medium in length, cylindrical but stubby with many micropores (Fig. 2G) of diverse diameter and distribution in different trunk regions, and 8–11 dorsal and 2–4 ventral prominent lobulated/amoeboid/branched giant nuclei (Fig. 1A, B). Anterior trunk with 3–12 circles of 14–28 vestigial spines each (Figs. 2H, 3A, B), extending posteriorly to level of about half length of proboscis receptacle (Fig. 1A, C). Spines volcano like with empty core and porous cortical layer continuous with trunk wall and micropores (Fig. 3A, B); usually slightly fewer and more widely spaced posteriorly (Fig. 2A). Proboscis small, angulate, slightly longer than wide, with 3 circles of 6 rooted hooks each and few mononucleated large cells (Fig. 1C) and sensory pit (Fig. 2C). Hooks not in perfect circles but alternate in position in same circle. Anterior hooks (Fig. 2D) arched diagonally, robust, markedly larger than other more slender hooks. Other hooks project more posteriorly. Middle hooks slightly longer than posterior hooks (Fig. 2E) and closer together. No dorsoventral differentiation. All hooks hollow with only cortical and subcortical layers (Fig. 2F). Hook roots spoon shaped, shorter than blades, with anterior manubria (Fig. 1D). Proboscis receptacle single walled, more than twice as long as proboscis, with large cephalic ganglion at its base. Para-receptacle structure evident, contiguous with ventral side of receptacle of both sexes (Fig. 1C). Lemnisci elongate lanceolate, either equal or unequal (no intermediate form), with narrowing rounded posterior end, loose in body cavity with no fibrous connectives to body wall or clearly distinguishable giant nuclei (Fig. 1A, B). Gonopore terminal in both sexes but occasionally near terminal to subterminal in females (Figs. 1E, 3E).

Males (based on 9 whole mounted sexually mature males with sperm, Fig. 1A, and 5 specimens studied by SEM): Trunk 5.00–11.12 (7.28) mm long by 0.72–1.75 (1.20) mm wide at middle. Body wall with 8–10 (8.7) dorsal and 2–3 (2.4) ventral giant nuclei. Proboscis 112–225 (158) long by 122–175 (138) wide. Anterior hooks 52–70 (59) long. Middle and posterior hooks 40–50 (46) and 32–42 (36) long, respectively. Proboscis receptacle 250–541 (401) long by 100–212 (164) wide. Equal lemnisci 1.90–2.91 (2.41) mm long by 0.20–0.31 (0.25) mm wide. Unequal lemnisci: shorter lemnisci 1.25–3.62 (2.40) mm long by 0.13–0.45 (0.24) mm wide; longer lemnisci 1.50–3.87 (2.67) mm long by 0.23–0.45 (0.28) mm wide. Testes post-equatorial, contiguous, usually with straight sides and posterior ventral prolongation especially of posterior testis (Fig. 1) and rarely eviscerated (Fig. 3C). Anterior testis 520–1,500 (1,021) long by 437–1,000 (748) wide, larger than posterior testis 499–1,320 (905) long by 426–1,229 (705) wide. Common sperm duct in 3 segments: anterior segment elliptoid 416–625 (520) long by 104–250 (177) wide, middle segment short and broader anteriorly 239–375 (307) long by 104–150 (202) wide anteriorly, posterior segment longest, club shaped 489–1,000 (758) long by 135–325 (178) wide anteriorly. Cement gland spheroid 416–850 (679) long by 343–600 (423) wide, with 4–6 large but barely discernible giant nuclei. Cement duct enlarged anteriorly but runs along with posterior limb of common sperm duct between 2 Saefftigen's pouches. Ventral Saefftigen's pouch shorter, more anterior 416–1,120 (698) long by 104–275 (179) wide. Dorsal Saefftigen's pouch longer and more slender and posterior, 478–1,250 (827) long by 135–175 (144) wide (Fig. 1A). Bursa muscular but without ornamentation of sensory pits (Fig. 3D) 239–551 (395) long by 312–624 (468) wide.

Females (based on 17 whole mounted adult females with ovarian balls and eggs, Fig. 1B, and 6 specimens, studied by SEM): Trunk 6.75–13.87 (9.28) mm long by 0.95–1.90 (1.32) mm wide anteriorly. Body wall with 8–11 (9.6) dorsal and 2–4 (2.6) ventral giant nuclei. Proboscis 150–187 (166) long by 130–170 (148) wide. Anterior hooks 55–80 (66) long. middle and posterior hooks 40–55 (58) and 34–50 (40) long, respectively. Proboscis receptacle 375–550 (444) long by 146–250 (211) wide. Equal lemnisci 1.95–2.80 (249) mm long by 0.22–0.25 (0.24) mm. Unequal lemnisci: shorter lemnisci 1.59–3.37 (2.45) mm long by 0.20–0.29 (0.23) mm wide, longer lemnisci 2.05–3.75 (3.10) mm long by 0.24–0.37 (0.28) mm wide. Reproductive system occasionally winding, 0.83–1. 35 (1.12) mm long; 10–17% (13%) of trunk length, with simple globular vaginal bulb, long uterus differentiated in 4 distinct parts, and uterine bell attached to ventral body wall, with very few (3 or 4) spheroid uterine bell cells (Fig. 1E). Gonopore subterminal (Figs. 1E, 3E). Eggs elliptoid with mild knob-like

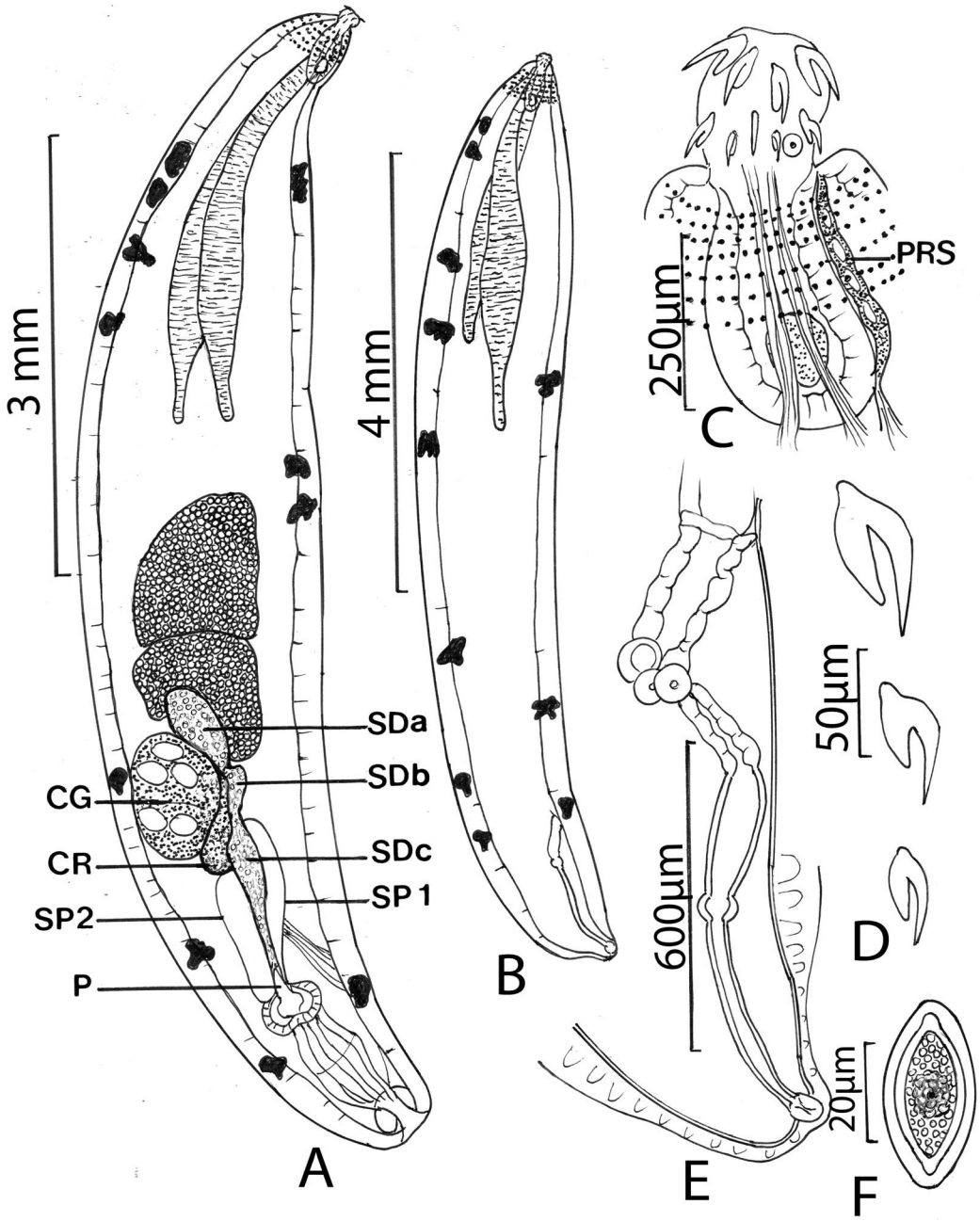

FIGURE 1. Specimens of *Acanthogyrus* (*Acanthosentis*) *kashmirensis* collected from *Schizothorax plagiostomus* and *Schizothorax labiatus* in the Sandran River, southern Kashmir, showing anterior circles of vestigial trunk spines. (**A**) Male with equal lemnisci and 7 dorsal and 4 ventral subdermal giant nuclei. Note the 3-part sperm duct (SDa-SDc), the 2 Saefftigen's pouches (SP1 and SP2), the cement gland (CG) with 5 giant nuclei, the adjacent cement reservoir (CR), and the penis (P). (**B**) Female with unequal lemnisci and 8 dorsal and 3 ventral subdermal giant nuclei. (**C**) Proboscis and the receptacle of a female specimen showing the para-receptacle structure (PRS) and the large mononucleated cell in the proboscis. Vestigial trunk spines are less prominent than they appear. (**D**) Anterior, middle, and posterior hooks with manubriated roots on the proboscis of the female specimen shown in C. (**E**) Reproductive system of the female specimen in B. Note the simple vaginal bulb and the 4-part uterus. (**F**) Egg. Note the ellipsoid shape with the mild knob-like polar extension of fertilization membrane.

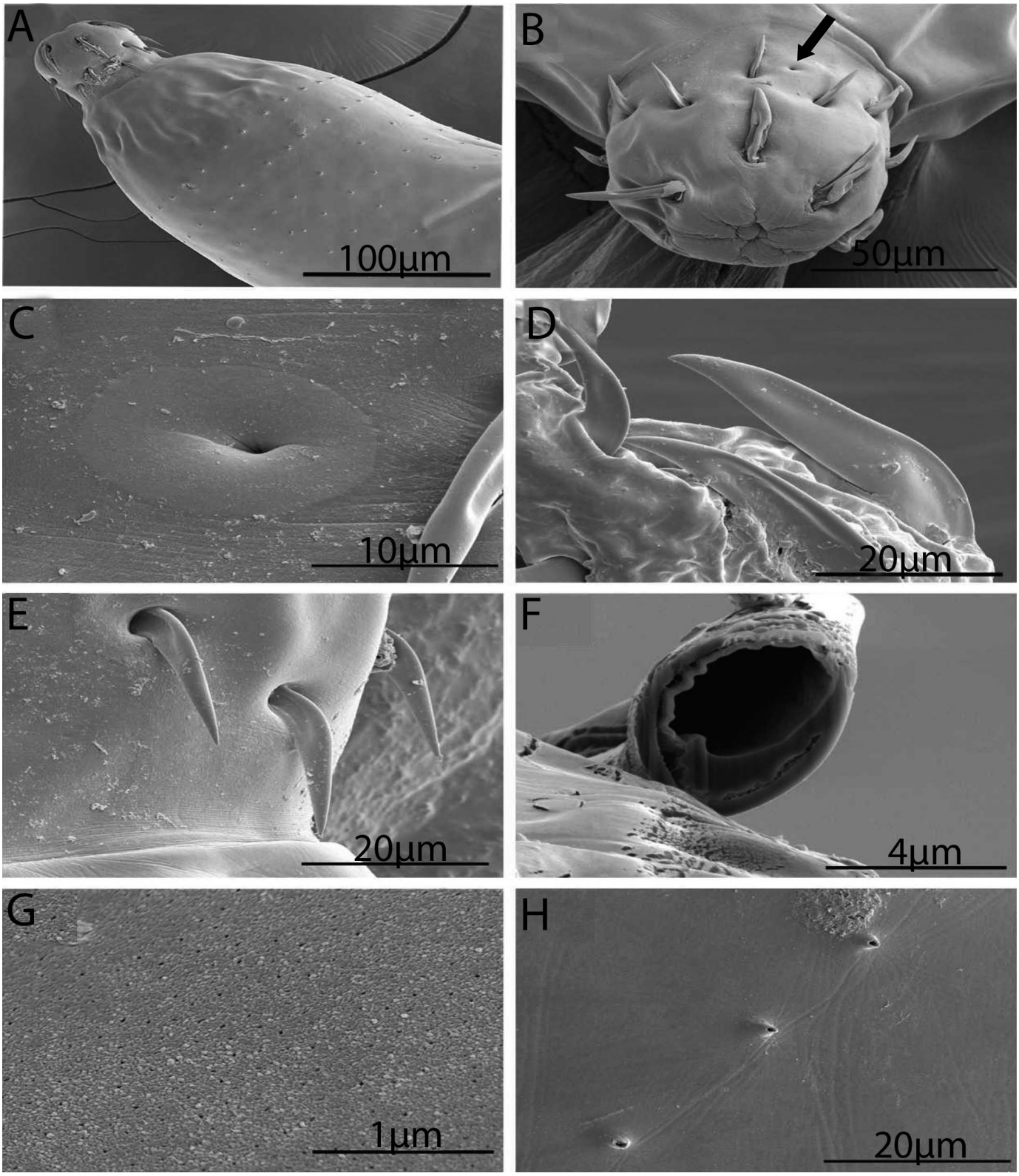

FIGURE 2. Scanning electron micrograph of *Acanthosentis kashmirensis* from the intestine of *Schizothorax plagiostomus* in Kashmir. (**A**) Anterior end of a female specimen showing the circles of vestigial spines. (**B**) Near apical view of the proboscis of the specimens in A showing the sensory pore at its base (arrow). (**C**) Enlarged view of the sensory pore. (**D**) Profile of an anterior and middle hooks. (**E**) Posterior hooks. (**F**) Section through an anterior hook showing its hollow core. (**G**) Micropores of the anterior trunk. Micropores in other trunk regions have different diameter and distribution. (**H**) Part of a circle of trunk spines.

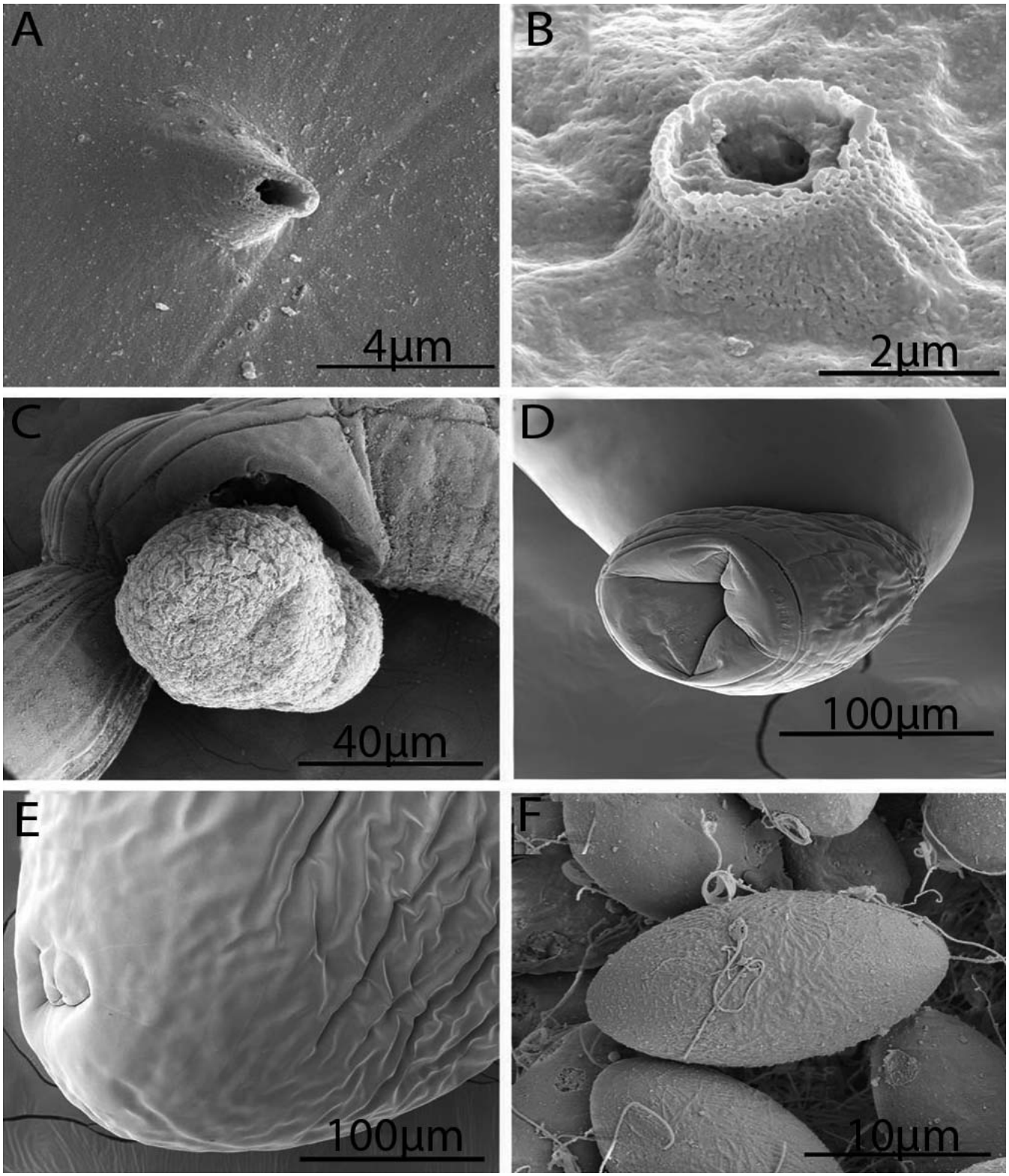

FIGURE 3. Scanning electronic micrograph of *Acanthogyrus* (*Acanthosentis*) *kashmirensis* from the intestine of *Schizothorax plagiostomus* in Kashmir. (**A**) Close-up of a trunk spine showing its shape and hollow center. (**B**) Higher magnification of another trunk spine showing its typical volcano-top shape, hollow center, and the association of its cortical layer with the trunk and its micropores. (**C**) Posterior end of a male specimen with naturally eviscerated testes. (**D**) Plain muscular bursa of a male specimen. (**E**) Subterminal female gonopore showing its 2 lips. (**F**) Egg.

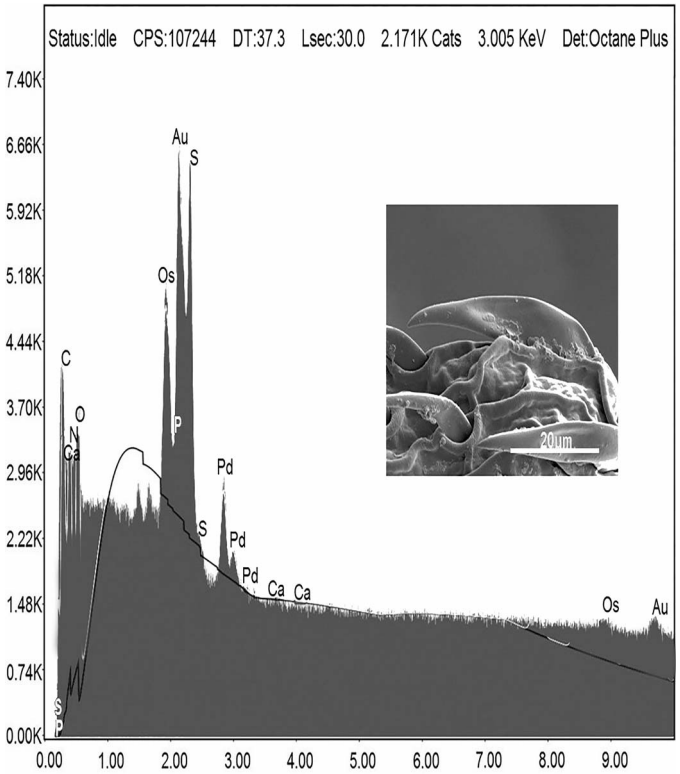

FIGURE 4. Element analysis of anterior hooks of *Acanthogyrus* (*Acanthosentis*) *kashmirensis*.

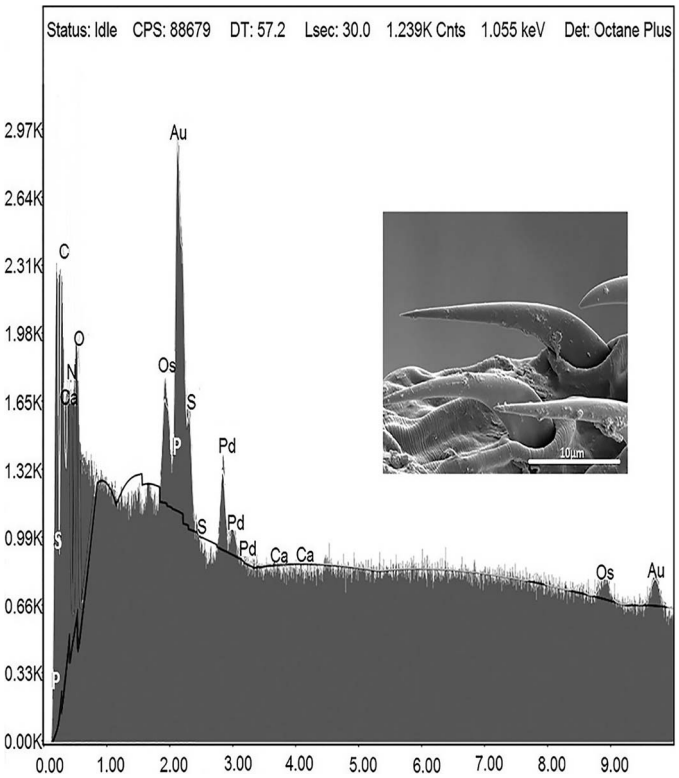

FIGURE 5. Element analysis of posterior hooks of *Acanthogyrus* (*Acanthosentis*) *kashmirensis*.

polar extension of fertilization membrane, 30–37 (33) long by 10–15 (14) wide (Figs. 1F, 3F).

Taxonomic summary

Type host: Snow trout, *Schizothorax plagiostomus* Heckel (Cyprinidae).

Other hosts: Kunar snow trout, *Schizothorax labiatus* (McClelland); Sattar snowtrout, *Schizothorax curvifrons* Heckel; and Chirruh snowtrout, *Schizothorax esocinus* Heckel (Cyprinidae).

Other hosts reported by Dhar (1972): Tor tor Hamilton, *Bangana diplostoma* (Heckel) (syn. *Labeo diplostoma* Heckel), *Labeo rohita* Hamilton, *Ptychobarbus* sp. Steindachner (Cyprinidae).

Specimens: University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection nos. 101845 and 101846.

Type locality: The Sandran River, a tributary of the Jhelum River, southern Kashmir in 3 sites: U1: altitude of 1,865 m (33°32′17″N, 75°14′26″E); U2: 1,819 m (33°33′26″N, 75°14′40″E); and U3: 1,798 m (33°33′52″N, 75°13′34″E).

Another locality: Lidder Stream, another tributary of the Jhelum River.

Other localities: The Jhelum River, northern Kashmir (see Dhar, 1972).

Site of infection: Intestine.

Etymology: The specific name "*kashmirensis*" used in the original description by Dhar (1972) is retained. The subgeneric name *Acanthosentis* is used upon the discovery of circles of vestigial trunk spines missed earlier by other observers.

Remarks

Interspecific comparisons: Acanthosentis kashmirensis is the only species of all the 45 known valid species of *Acanthosentis* (44 species were reported and keyed out by Amin, 2005) that has vestigial spines. In addition, of the 23 species of *Acanthosentis* known from the Indian subcontinent (see following key), *A. kashmirensis* is closest to *Acanthosentis gobindi* Chowhan, Gupta and Khera, 1987 and *Acanthosentis putitorae* Chowhan, Gupta and Khera, 1988 in having a few circles of anterior trunk spines, among other features. Both species were collected from cyprinids in streams associated with the Sutlej River of Nagal, in the Punjab region of northwest India, an unrelated river system to that from which *A. kashmirensis* was collected. In these 2 species, the proboscis hooks have much shorter roots and no manubria, unlike those of *A. kashmirensis*. Their lemnisci have prominent nuclei and extend posteriorly past anterior testis. In *A. kashmirensis*, with considerably larger pre-testicular space, the lemnisci are distant from the anterior testis and have no distinguishable giant nuclei. Specimens of *A. gobindi* are considerably smaller (males 3.32–4.38 and females 4.16–8.13 mm long) than those of *A. kashmirensis* (reaching 10.25 mm in males and 13.87 mm in females). They have 7–8 circles of developed spines and anterior proboscis hooks are at the same level; those of *A. kashmirensis* alternate in position. Trunk spines of *A. gobindi* are described as "slightly curved ventrally and broad at the mid-region … and not extending beyond proboscis receptacle" (Chowhan et al., 1987). Specimens of *A. putitorae*, like those of *A. gobindi*, are very small (males 2.72–3.40 and females 5.08–6.04 mm long), and the size of all structures is

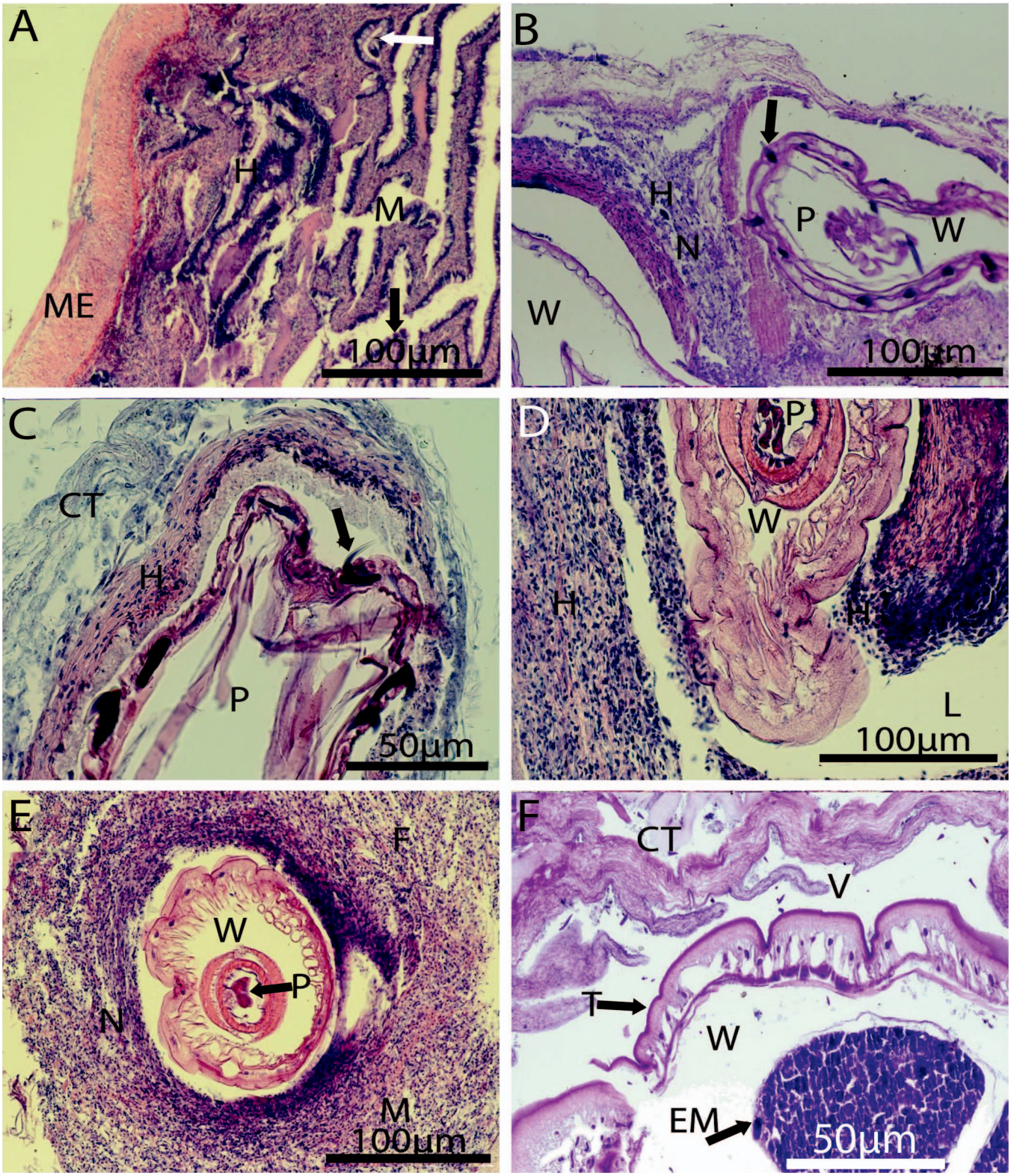

FIGURE 6. Histopathology of *Acanthogyrus* (*Acanthosentis*) *kashmirensis* in the intestine of *Schizothorax plagiostomus*: (**A**) Normal host intestine with prominent tissue layers. M = mucosa (black arrow); ME = muscularis externa; H = host intestine; arrow = goblet cells (white arrow). (**B**) Worm (W) attached to host intestinal tissue with surrounding cell necrosis (N) and hemorrhaging. Proboscis (P) visible with prominent hooks (arrow). (**C**). Proboscis (P) with prominent hooks (arrow) and surrounding blood loss (H) and necrosis. Note connective tissue (CT) with collagenous fibers around the damaged host tissue. (**D**) Worm (W) in the lumen (L) of the host with a cross section of the proboscis (P) visible. Note hemorrhaging (H) around the invading worm due to capillary destruction of the host intestine. (**E**) Cross section of a worm (W) and proboscis (arrow) within the host intestinal mucosa (M). Note proboscis (P), host tissue necrosis (N), and collagenous fibers (F) surrounding the worm. (**F**) Parasite (W) with egg mass (EM, arrow) and tegument (T, arrow). Note the damaged villi (V) and extensive connective tissue invasion (CT) in the area of parasite attachment.

correspondingly very small compared to that of *A. kashmirensis*. The trunk of specimens of *A. putitorae* is armed with 7–10 "rows" of developed spines with the "6 anterior rows forming complete circles and the posterior ones incomplete dorsally ... the size of spines decreases posteriorly." In *A. putitorae*, "The 2 lateral (proboscis) hooks of the anterior-most circle (are) placed somewhat posteriorly" and the middle and posterior hooks are of equal size (Chowhan et al., 1988), which is not the case in *A. kashmirensis*. Male specimens of *A. kashmirensis* are further distinguished by having a 3-segmented common sperm ducts and 2 Saefftigen's pouches each. These 2 features and the para-receptacle structure in *A. kashmirensis* were not noted in the texts or illustrations of the other 2 species. The para-receptacle structure was found in 2 other species of *Acanthosentis*, *Acanthosentis parareceptaclis* Amin, 2005 from Japan and *Acanthosentis barmeshoori* Amin, Gholami, Akhlaghi and Heckmann 2013 from Iran.

To put the above comparisons, among others, into context, we are providing below a key to the 23 valid species of *Acanthogyrus* (*Acanthosentis*) in the Indian subcontinent:

Key to species of the subgenus *Acanthosentis* in the Indian subcontinent

1. Worms with para-receptacle structure, segmented common sperm duct and 2 Saefftigen's pouches. Trunk spines vestigial and anterior proboscis hooks alternate ..... *A.* (*A.*) *kashmirensis* n. sp.
– Worms without para-receptacle structure. With one sperm duct and one Saefftigen's pouch. Trunk spines not vestigial and anterior proboscis hooks vary 2
2. Trunk spines covering whole body 3
– Trunk spines may be confined to anterior trunk 4
3. Trunk spines comb like. Proboscis hooks in perfect circles. Lemnisci enclosed in common sheath, fused terminally. No neck or Saefftigen's pouch. Female reproductive system about half as long as trunk .... *A.* (*A.*) *arii* Bilqees, 1971
– Trunk spines not comb like in 31–36 circles; 5–6 posterior-most spines closely set at posterior tip of female trunk. Proboscis hooks not in perfect circle; unequal. Lemnisci free, equal. With prominent neck and Saefftigen's pouch. Females reproductive system short ..... *A.* (*A.*) *golvani* Gupta & Jain, 1980
4. Hooks not gradually decreasing in length posteriorly or equal in middle and posterior circles or middle and anterior circles 5
– Hooks gradually decreasing in length posteriorly 9
5. Hooks in posterior circle larger than hooks in middle circle. Hook length from anterior 55–59, 37, 40–41 μm in males; 64–66, 38–43, 44–46 μm in females. Circles of spines covering whole trunk. Female gonopore subterminal; vagina without glands .... *A.* (*A.*) *heterospinus* Khan and Bilqees, 1990
– Middle and posterior or middle and anterior hooks equal 6
6. Middle and posterior hooks equal 7
– Middle and anterior hooks equal 8
7. Hook length from anterior 50, 40, 40 μm. Spines in 16–18 circles anteriorly. Lemnisci digitiform. Female gonopore subterminal; vagina with 1 pair of sacular vaginal glands .... *A.* (*A.*) *oligospinus* Anantaraman, 1980
– Hook length from anterior 33, 24–28, 24–28 μm in males; 32–36, 24–28, 24–28 μm in females. Spines in 20–22 circles, not covering whole trunk. Lemnisci balloon shaped. Female gonopore terminal with no vaginal glands ..... *A.* (*A.*) *bilaspurensis* Chowan, Gupta and Khera, 1987
8. Hook length from anterior 29, 29, 20 μm. Proboscis 100–180 × 100–120 μm. Trunk spines in 10–15 circles ..... *A.* (*A.*) *hilsai* Pal, 1963
– Hook length from anterior 20–31, 20–31, 15–20 μm. Proboscis 80–150 × 70–80 μm. Trunk spines in 22–26 circles ..... *A.* (*A.*) *bacailai* Verma, 1973
9. Vagina with 1 pair of prominent club-shaped vaginal glands attached distally. Female gonopore subterminal. Proboscis markedly longer than wide. Spines in circles covering whole trunk 10
– No vaginal glands present. Other characters vary ... 11
10. Hook length from anterior 48, 40–44, 28–36 μm. Uterus with prominent coil near uterine bell. Proboscis 185–190 × 110–125 μm in males, 175–205 × 125–162 μm in females. Giant hypodermal nuclei: 1 dorsal, 2 ventral ..... *A.* (*A.*) *thapari* Prasad, Sahay, Shambhunath, 1969
– Hook length from anterior 50, 30, 26 μm. Uterus not coiled. Proboscis 120 × 55 μm. Giant hypodermal nuclei 6–8: 4–6 dorsal, 2 ventral ..... *A.* (*A.*) *dattai* Podder, 1938
11. Spines covering entire or almost entire trunk 12
– Spines restricted to anterior half of trunk 14
12. Posteriormost spines on posterior tip of trunk of females. Proboscis 200 × 180 μm. Lemnisci miniscule, narrow, shorter than proboscis receptacle .... *A.* (*A.*) *shuklai* Agrawal and Singh, 1982
– Posteriormost spines near posterior tip of female trunk. Proboscis markedly smaller. Lemnisci normal, considerably longer than proboscis receptacle 13
13. Large worms; 9–10 mm long. Proboscis 127–133 × 127. Length of hooks from anterior 57–68, 38–53, 24–41 μm. Lemnisci much longer than receptacle which is 435–483 × 145 μm. Trunk spines in 42–44 regular circles. Saefftigen's pouch and neck present ..... *A.* (*A.*) *betwai* Tripathi, 1959
– Trunk 0.9–2.4 mm long. Proboscis ~100 × 50 μm. Hooks in 3 regular circles. Anterior hook ca. 50 μm. Lemnisci nearly equal, ca. 300 μm long, slightly longer than proboscis receptacle. Circles of spines unknown. Saefftigen's pouch and neck absent... *A.* (*A.*) *holospinus* Sen, 1938
14. Trunk spines in 7–10 circles. Anterior hooks not much larger than middle hooks. Giant nuclei 8–13 15
– Trunk spines in more than 10 circles. Anterior hooks and giant nuclei variable 16
15. Trunk spines in 7–8 circles anteriorly. Proboscis 120–130 × 120–130 μm in males, 150–160 × 120–140 μm in females. Length of hooks from anterior 57–61, 32–41, 28–40 μm in males, 66–74, 41–49, 37–45 μm in females. Giant nuclei 11–16: 10–12 dorsal, 3–4 ventral .... *A.* (*A.*) *gobindi* Chowan, Gupta & Khera 1987

– Trunk spines in 7–10 circles anteriorly. Proboscis 120 × 80–120 μm in males, 130 × 130 μm in females. Length of hooks from anterior 57–78, 41–58, 33–41 μm in males, 61–74, 41, 37–41 μm in females. Giant nuclei 8–11: 6–8 dorsal, 2–3 ventral .... *A.* (*A.*) *putitorae* Chowan, Gupta & Khera, 1988

16. Trunk spines in 12–14 circles. Proboscis as long as wide, 60–80 × 70–80 μm. Lemnisci balloon shaped. Female posterior end with papilla by genital aperture. Cement gland with 6–11 giant nuclei..... *A.* (*A.*) *seenghalae* Chowan, Gupta, Khera, 1988

– Circles of trunk spines variable. Proboscis not as long as wide. Lemnisci not balloon-shaped. Females without genital papillae. Cement gland with up to 8 giant nuclei..... 17

17. Trunk with 23 circles of spines. Proboscis wider than long, 70 × 100 μm in males, 70–90 × 90–130 μm in females. Proboscis and prominent neck with thick cuticular layer ..... *A.* (*A.*) *giuris* Soota & Sen, 1956

– Circles of trunk spines variable. Proboscis longer than wide. Proboscis and unremarkable neck without thick cuticular layer 18

18. Lemnisci unequal..... 19

– Lemnisci equal or subequal..... 20

19. Males 10 mm long, females 12–13 mm. Trunk with 18–20 circles of spines. Proboscis cone shaped, broadest at base 150 × 120–160 μm. Proboscis hooks not decreasing much in size posteriorly. Hook length from anterior 55, 50, 40 μm in males, 60–61, 45–60, 30–35 μm in females..... *A.* (*A.*) *vancleavei* Gupta and Fatma, 1985

– Males 3–5 mm long; females 3–12 mm. Trunk with 14 circles of spines Proboscis ovoid, 138 × 115 μm. Posterior hooks decreasing abruptly in size; length of hooks from anterior 55, 48, 18 μm..... *A.* (*A.*) *circari* Podder, 1941

20. Proboscis hooks large, length from anterior 72, 54, 48 μm. Trunk spines in 20–31 circles.... *A.* (*A.*) *antespinus* Verma & Datta, 1929

– Proboscis hooks smaller; length from anterior <58, <39, <31, Trunk spines fewer, in 10–12 circles..... 21

21. Length of hooks from anterior 37–45, 32–39, 20–30 μm in males, 40–48, 35–39, 28–31 μm in females. Giant hypodermal nuclei many, 8–15: up to 9 dorsal and 6 ventral, in groups of 2–4 adjacent nuclei. Proboscis 60–150 × 70–100 μm in males, 80–140 × 40–80 μm in females. Cement gland small, 40–41 × 50–52 μm ..... *A.* (*A.*) *vittatusi* Verma, 1973

– Hook size variable. Giant hypodermal nuclei fewer, 2–8, not clustered in groups. Proboscis larger or smaller. Cement gland markedly larger..... 22

22. Length of hooks from anterior 35–45, 19–28, 15–25 μm in males, 31–40, 20–23, 15–20 μm in females. Giant hypodermal nuclei 2 or 3: 1–2 dorsal, 1 ventral. Proboscis 110–180 × 50–80 μm in males, 80–135 × 56–60 μm in females. Cement gland 130–360 × 100–310 μm ..... *A.* (*A.*) *cameroni* Gupta and Kajaji, 1969

– Length of hooks from anterior 41–45, 38, 31–36 μm. Giant hypodermal nuclei 6: 3 dorsal, 3 ventral. Proboscis larger 145–217 × 80–110 μm. Cement gland 940 × 550 μm ..... *A.* (*A.*) *indicus* Tripathi, 1956

Intraspecific comparisons: In his thesis, Dhar (1972) reported 4 specimens of *N. kashmirensis* from 2 individuals of *T. tor*, 6 specimens from 40 *L. diplostoma*, 4 specimens from 10 *Labeo rohita*, and 6 specimens from 10 *Ptychobarbus* sp. As with our reported material, cyprinid fish hosts do not seem to be heavily infected. This low intensity of infection may be related to (1) the pathogenicity to host tissue (see the histopathological discussion following); (2) occasional drying and flooding of the streams affecting the crustacean intermediate host population density; and (3) some fish hosts are migratory, e.g., *Tor tor*, found only in the summer in Kashmir (Dhar, 1972), making their contribution to the overall parasite population density variable, especially seasonally. The only descriptive statements that Dhar (1972) provided addressed (1) the proboscis (comparatively small), (2) the lemnisci being unequal in specimens from *B. diplostoma* (0.47–0.64 and 0.28–0.32 mm long) and equal in specimens from the 3 other fish species (0.32–1.10 mm long), and (3) the number of subcuticular nuclei being 3–6 dorsally and 1 ventrally. Dhar (1972) also measured the trunk (3.65–4.73 × 0.64–0.98 mm in males and 3.80–7.20 × 0.72–1.15 mm in females), the proboscis (78–100 long), and the proboscis hooks from anterior (30–38, 22–26, 18–26). The above-mentioned measurements encompassed all variations in specimens from all his 4 host species. All other structures including the proboscis width; the receptacle, testes and other male reproductive structures; and female reproductive system and eggs were not measured, and the sex differences in the size of measured structures were not provided. Inadequate and unrepresentative interpretation of the male reproductive system, proboscides, and hooks (roots were not shown) of specimens from 3 fish species were depicted in line drawings, but anterior trunk spines were not observed or reported.

Effects of host species: Compared to the account of Dhar (1972), our presented comprehensive description of *A. kashmirensis* is the first complete description of that species that greatly documents its diagnostic criteria and the full range of size variations of its various structures in both sexes. All structures in our specimens from *S. plagiostomus* and *S. labiatus* are larger than those reported by Dhar (1972) from his 4 other examined host species, and even counts of such features as the number of subcuticular nuclei are considerably greater (Table II). The effect of host species on the size of acanthocephalan parasites has been previously reported. See, for example, Amin and Redlin (1980) who described considerably larger specimens of *Echinorhynchus salmonis* Müller, 1784 from bloater, *Coregonus hoyi* Milner (Salmonidae), than from rainbow smelt, *Osmerus mordax* Mitchill (Osmeridae), collected from the same waters of Lake Michigan. Host species also affected corresponding differences in the size of proboscis, proboscis hooks, proboscis receptacle, lemnisci, testes, and cement glands as well as body form and differential growth rates in specimens from each host species. Linear regression analysis indicated that curves describing the growth pattern of these characters by worm length (age) were significantly different as a function of host species (Amin and Redlin, 1980). The larger worms recovered from *C. hoyi* invariably showed a higher regression coefficient compared to those from *O. mordax* in all characters. There is

TABLE II. Comparison between descriptive characters of *Acanthogyrus* (*Acanthosentis*) *kashmirensis* according to Dhar (1972) described as *Neoechinorhynchus kashmirensis* and as observed by us.

	Characters listed by Dhar (1972)	Characters observed by us (this study)
Hosts	*Tor tor*, *Bangana diplostoma*	*Schizothorax plagiostomus*, *Schizothorax labiatus*
(Cyprinidae)	*Labeo rohita, Ptychoharbus* sp.	*Schizothorax curvifrons*, *Schizothorax esocinus*
Locality	Jhelum River	Jhelem River at Sandran River and Lidder Stream
Trunk	Oblong, tapering at both ends*	Oblong, tapering at both ends
Male trunk (mm)	3.65–4.73 × 0.54–0.98	5.00–11.12 × 0.72–1.90
Female trunk (mm)	2.68–7.21 × 0.72–1.15†	6.75–13.87 × 0.95–1.90
Proboscis	78–110 × 62–80	112–225 × 122–175 (males); 150–187 × 130–170 (females)
Anterior hook length	30–38	52–70 (males); 55–80 (females)
Middle hook length	22–26	40–50 (males); 40–55 (females)
Basal hook length	18–26	32–42 (males); 34–50 (females)
Subcuticular nuclei	Dorsal 2–5, ventral 1	Dorsal 8–11, ventral 2–4
Lemnisci length (mm)	0.28–1.1 equal or distinctly unequal, broad, much longer than receptacle, not approaching anterior testis	1.25–3.60 equal or distinctly unequal, broad, much longer than receptacle, not approaching anterior testis
Male reproductive system	In posterior half of trunk	In posterior half of trunk
Testes	Post-equatorial, contiguous with straight sides and posterior ventral prolongation	Post-equatorial, contiguous with straight sides and posterior ventral prolongation
Cement gland	Spheroid with 3–6 giant nuclei	Spheroid with 4–6 giant nuclei
Cement reservoir	Prominent, just posterior to cement gland	Prominent, just posterior to cement gland
Female gonopore	Subterminal, adhering to ventral body wall	Subterminal, adhering to ventral body wall

* Qualitative characters in Dhar (1972) are shown in his plates XXXIII–XXXV.
† All measurements are in micrometers except when otherwise noted.

ample literature associating worm size with host species. Geography has also been shown to have an impact on worm size, but this is not relevant here because both our material and Dhar's material were collected from the same stream.

Host species may have also had an impact on the size of lemnisci being equal or distinctly unequal in our material and the material of Dhar (1972). They were unequal only in specimens from *L. diplostoma*, but equal in specimens from Dhar's 3 other species of fish.

Variability: The range of variation in all characters studied is considerably wider than previously reported by Dhar (1972). Characters traditionally regarded as stable and taxonomically diagnostic are shown to vary considerably by worm sex and size, and by host species and geography (Amin and Redlin, 1980 and others quoted therein). The taxonomic implications are vast and include the possible description of extremely variable populations from different host species (and geographies) as distinct species. We do not believe that this is an issue in our present investigation as long as we acknowledge that host species does account for the extreme differences in the morphometric characterization of the studied populations of *A. kashmirensis* as reported herein.

TABLE III. Energy dispersive X-ray analysis (X-ray microanalysis) results for hooks on the proboscis of *Acanthogyrus* (*Acanthosentis*) *kashmirensis*.*

	Weight %	Atom %
Top Hook		
Phosphorus	0.27	0.25
Sulfur†	9.20	6.28
Calcium	0.15	0.08
Bottom Hook		
Phosphorus	0.16	0.10
Sulfur	4.05	2.50
Calcium	0.15	0.08

* Average of 2 scans.
† Sulfur seems to be the main element varying in weight percent for the top hook and bottom hook. It is important for hardening the hooks.

EDAX: This is one of the first reports of the use of a LIMS to cut and analyze the structure of a parasite. Gallium ions were used from a dual-beam SEM source (GIS) to cut the hooks of specimens of *A. kashmirensis*, thereby displaying their hollow nature. From previous studies using XEDS and EDAX (Heckmann et al., 2007, 2012), most acanthocephalan hooks have high levels of calcium and phosphorus with limited amounts of sulfur. The hooks of specimens of *A. kashmirensis* were, however, considerably higher in sulfur ions (Table III).

X-ray chemical spectrum analysis (XEDS, EDAX) has been used in the past to understand the chemical structure of acanthocephalans (Heckmann et al., 2007, 2012), especially the attachment structures. This method has also been used for analyzing nonliving materials (Alley et al., 2011) and the reasons for aquatic animal deaths (Heckmann, 2006). For this project, we were interested in the chemical elements present in unique hollow proboscis hooks of specimens of *A. kashmirensis* that had a high content of sulfur. The proboscis hooks were also cut with a gallium beam (Gallium ion metal source) to display the hollow nature of the hooks. Figures 4 and 5 and Tables III–V represent the results of the EDAX study of chemical elements for the scans of *A. kashmirensis*. Both the anterior and posterior hooks of the proboscis had a high content of sulfur, more than the calcium and phosphorus (Table III). Calcium and phosphorus usually represent the highest elemental composition for the hooks, especially the body or core of the hook. The lack of calcium and phosphorus may be one reason why the hooks are hollow. The worms had been coated with gold and palladium previous to the

TABLE IV. Results of the energy dispersive X-ray analysis study of chemical elements for the scans of anterior hooks of *Acanthogyrus* (*Acanthosentis*) *kashmirensis*. Abbreviations: Z = correction number; R = florescence; A = absorption.

Element*	Weight %	Atom %	Error %	Net intensity	K ratio	Z	R	A
Carbon (C) K	18.14	27.47	8.51	1,760.30	0.0718	1.1296	9.2470	0.3503
Nitrogen (N) K	30.36	39.42	9.83	1,757.16	0.0763	1.1025	0.9370	0.2278
Oxygen (O) K	23.85	27.11	10.01	2,245.09	0.0469	1.0788	0.9477	0.1823
Osmium (Os) M	4.96	0.47	7.67	1,067.89	0.0411	0.6183	1.3592	1.2465
Phosphorus (P) K	0.07	0.04	99.99	31.16	0.0006	0.9380	1.0046	0.8840
Gold (AU) M	12.55	1.16	6.77	2,366.66	0.1027	0.6154	1.3605	1.2710
Sulfur (S) K	6.53	3.71	4.23	2,745.19	0.0541	0.9560	1.0108	0.8645
Palladium (Pd) L	3.48	0.59	6.43	602.40	0.0245	0.7111	1.1971	0.9892
Calcium (Ca) K	0.05	0.02	57.42	13.18	0.0005	0.9199	1.0318	0.9293

* K, L, and M are the lengths of the spectra of these elements.

EDAX scan. Specimen preparation also requires osmication, which explains the presence of the element osmium. Carbon, hydrogen, and oxygen are common elements in living matter. For comparison, proboscis hooks from a previous study (Amin and Heckmann, 2012) of another species of *Acanthosentis* (*A. tilapiae*) were also cut with the gallium beam (dual-beam SEM) and scanned using EDAX for chemical elements. These hooks were not hollow and had a high content of calcium and phosphorus similar to most acanthocephalan hooks that have been studied.

Histopathology: Normal host intestinal tissue is displayed in Figure 6A. This figure shows the prominent layers of the host intestine: mucosa, submucosa, muscularis externa, and fibroserosa. The mucosa is lined with simple columnar epithelial cells and goblet cells (Fig. 6A). There is extensive hemorrhaging and necrosis of the epithelial lining of the host mucosa at attachment sites (Fig. 6B, C). The host generates collagenous connective tissue around the worm (Fig. 6C, D) in an attempt to isolate the parasite and arrest its movement. The worm is shown to migrate through the mucosal-submucosal layers to the outer smooth muscle, muscularis externa (Fig. 6D, E). The consequences of worm invasion included extensive cell necrosis, blood loss, and destruction of the absorbing epithelial surface of the mucosa (Fig. 6E, F). Compare Figure 6A to 6F to elucidate the loss of the normal columnar epithelial lining of the mucosa. Specimens of *A. kashmerinsis* obstruct the surface of the host mucosa depriving it of nutrient absorption (Fig. 6D, F). Within the host, worms continue to develop eggs and sperm (Fig. 6F). Numerous nucleated red blood cells and granulocytes are present where the worm attaches. The hemorrhaging is primarily due to capillary destruction of the host intestine. Compaction of villi is common where the worm attaches (Fig. 6E, F).

ACKNOWLEDGMENTS

The support and encouragement of Dr. M. Z. Chishti (Professor Emeritus and Coordinator DST, Center of Research for Development [CORD], University of Kashmir, Hazratbal) were indispensable for carrying out of research activities in Kashmir. We are grateful to Madison Elcye Giles (Bean Museum, Brigham Young University [BYU]) for expert help in the preparation and organization of plates and figures. We are also grateful to Michael Standing (BYU Electron Optics Center) for professional help with electron optics equipment. This work was supported by an institutional grant from the Institute of Parasitic Diseases, Scottsdale, Arizona.

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TABLE V. Results of the energy dispersive X-ray analysis study of chemical elements for the scans of posterior hooks of *Acanthogyrus* (*Acanthosentis*) *kashmirensis*. Abbreviations: Z = correction number; R = florescence; A = absorption.

Element*	Weight %	Atom %	Error %	Net intensity	K ratio	Z	R	A
Carbon (C) K	17.81	25.51	7.63	943.14	0.0939	1.1111	0.9352	0.4746
Nitrogen (N) K	34.85	42.80	9.93	940.07	0.0996	1.0841	0.9471	0.2636
Oxygen (O) K	26.74	28.76	10.31	1,002.06	0.0511	1.0605	0.9576	0.1802
Osmium (Os) M	2.57	0.23	10.18	226.67	0.0213	0.6071	1.3702	1.2498
Phosphorus (P) K	0.07	0.04	61.70	12.60	0.0006	0.9210	1.0126	0.8938
Gold (AU) M	12.77	1.12	6.86	976.38	0.1034	0.6041	1.3709	1.2950
Sulfur (S) K	1.83	0.98	7.18	314.04	0.0151	0.9385	1.0186	0.8766
Palladium (Pd) L	3.29	0.53	13.35	236.03	0.0235	0.6978	1.2055	1.0192
Calcium (Ca) K	0.07	0.03	59.06	7.28	0.0006	0.9025	1.0384	0.9457

* K, L, and M are the lengths of the spectra of these elements.

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