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ORIGINAL RESEARCH ARTICLE

The morphology of the long forgotten *Pomphorhynchus kashmirensis* (Acanthocephala: Pomphorhynchidae) from freshwater fish in Kashmir using SEM, with notes on histopathology

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Abstract. *Pomphorhynchus kashmirensis* Kaw, 1941 is the first of 9 species of *Pomphorhynchus* Monticelli, 1905 described from freshwater fishes in the Jammu-Kashmir regions in the Northern Indian subcontinent. It was originally well described from *Nemachilus kashmirensis* Hora, 1922 (Balitoridae) in Kashmir but many of its features could not be adequately visualized or confirmed. The present collection from *Schizothorax plagiostomus* Heckel, 1838 enabled the elucidation of many morphological features and the description of others for the first time using SEM, e.g., proboscis, bulb, posterior proboscis hooks, trunk epidermal micropores, female gonopore, eggs, and bursa. The histopathological observations demonstrate the invasive effects of *P. kashmirensis* on host tissues, alterations and compression of intestinal villi, and granulocytosis.

Keywords: *Pomphorhynchus kashmirensis;* Acanthocephala; *Schizothorax plagiostomus;* Kashmir; India; SEM.

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Introduction

Of the 24 known species of *Pomphorhynchus* Monticelli, 1905, 9 species are reported in the Indian subcontinent that are only found in the Jammu and Kashmir area; 2 ecologically distinct regions (Amin et al., 2003, in part). *Pomphorhynchus kashmirensis* Kaw, 1941 was the first of the 9 Indian species described. Kaw's (1941) original description from *Nemachilus kashmirensis* Hora, 1922 (Balitoridae) in Kashmir was adequate but many features were not sufficiently observable in text or illustrations. These were subsequently made more readily visualized using SEM (this paper). Only one taxonomic treatment of this species was made after the original description by Fotedar and Dhar

(1977) who briefly reported *P. kashmirensis* as "agreeing with the known description" and illustrated it from the type host and from brown trout, *Salmo trutta fario* Linn, 1758 in Telhal and Harwan (Kashmir). This scarce reference to *P. kashmirensis* is rather unusual in light of the fact that this species appears to be a common acanthocephalan species in Kashmir. For example, Chishti and Peerzada (1998) reported on its prevalence and seasonal dynamics from 7 species of fish from Wular Lake, Kashmir. Dhar (1972) also reported 7 species of *Pomphorhynchus* from Jammu and Kashmir and distinguished between them based on proboscis armature. More recently, Yousuf et al. (2011) reported on the prevalence of 2 species of Acanthocephala (including *P. kashmirensis*), Trematoda, and Cestoda from water bodies in Kashmir Valley. The present collection from *Schizothorax plagiostomus* Heckel, 1838 was made available to further elucidate the morphology of *P. kashmirensis* showing more details not previously seen through light microscopy. No additional specimens were available to do a thorough internal anatomical revision.

Materials and methods

Three sampling sites were selected in the River Jehlum, and in 3 three different lakes for the collection of *Pomphorynchus* material. The River Jehlum sites included (1) Qamarwari (34^{o} 05' 35.9" N - 74^{o} 46'45.4" E; Elevation = 1569m), (2) Shadipora (34^{0} 10' 59.8" N - 074^{0} 40' 53.7" E; Elevation = 1575m), and (3) Hajan (34^{0} 17' 50.6" N - 34^{0} 17' 50.6" N; Elevation = 1588m). The lakes' sites included (1) Telbal, (2) Anchar Ghat, and (3) Manasbal Ghat in (1) Dal, (2) Anchar and (3) Manasbal lakes, repectively. All the specimens of *Pomphorynchus* spp. were only found in Shadipora and Hajan between June 2010 and March 2011. The rest of the sites did not yield any *Pomphorynchus* material. The *P. kasmirensis* material studied by SEM and reported herein was collected from *S. plagiostomus* in the Jehlum River on June 10, 2010. An additional specimen from the same host species attached to host tissue collected on March 17, 2011 in the Sind River was submitted for histopathological study. Specimens were fixed in 70% ethanol and shipped to our Arizona facility for study.

For SEM, samples of *P. kashmirensis* that had been AFA-fixed and stored in 70% ethanol were processed following standard methods (Lee, 1992) that included critical point drying (CPD) in sample baskets and mounted on SEM sample mounts using conductive double sided carbon tape. Samples were then gold coated for 3 minutes using a Polaron E3500 sputter coater establishing an approximate thickness of 20nm. Samples were then placed in a FEI XL30 ESEM FEG under low vacuum conditions. Samples were imaged using 10 KV, spot size 3 at 0.7 torr using the GSE detector. Permanent records were obtained with a digital camera at various magnifications.

For histopathological sections, standard methods (Galigher and Kozloff, 1971; Kiernan, 2002) were employed for the examination of the infected host intestinal tissue. Specimens that have been stored in 70% ethanol were transferred to 10% buffered formalin (v/v). The infected host tissue was dehydrated and blocked in paraffin. The blocks were sectioned at 4-6 micrometers (μ), placed on glass slides and stained with Harris hematoxylin and eosin (H & E) and then viewed with an LSM laser (Carl Zeiss, Thornwoood, New York) equipped compound light microscope. Representative pictures were taken with an attached digital camera at various magnifications and stored in a memory disk for future reference. H & E is a standard stain for viewing pathological tissue while Mallory's trichrome is used for viewing specific cells characteristic of tissue inflammation.

Results and discussion

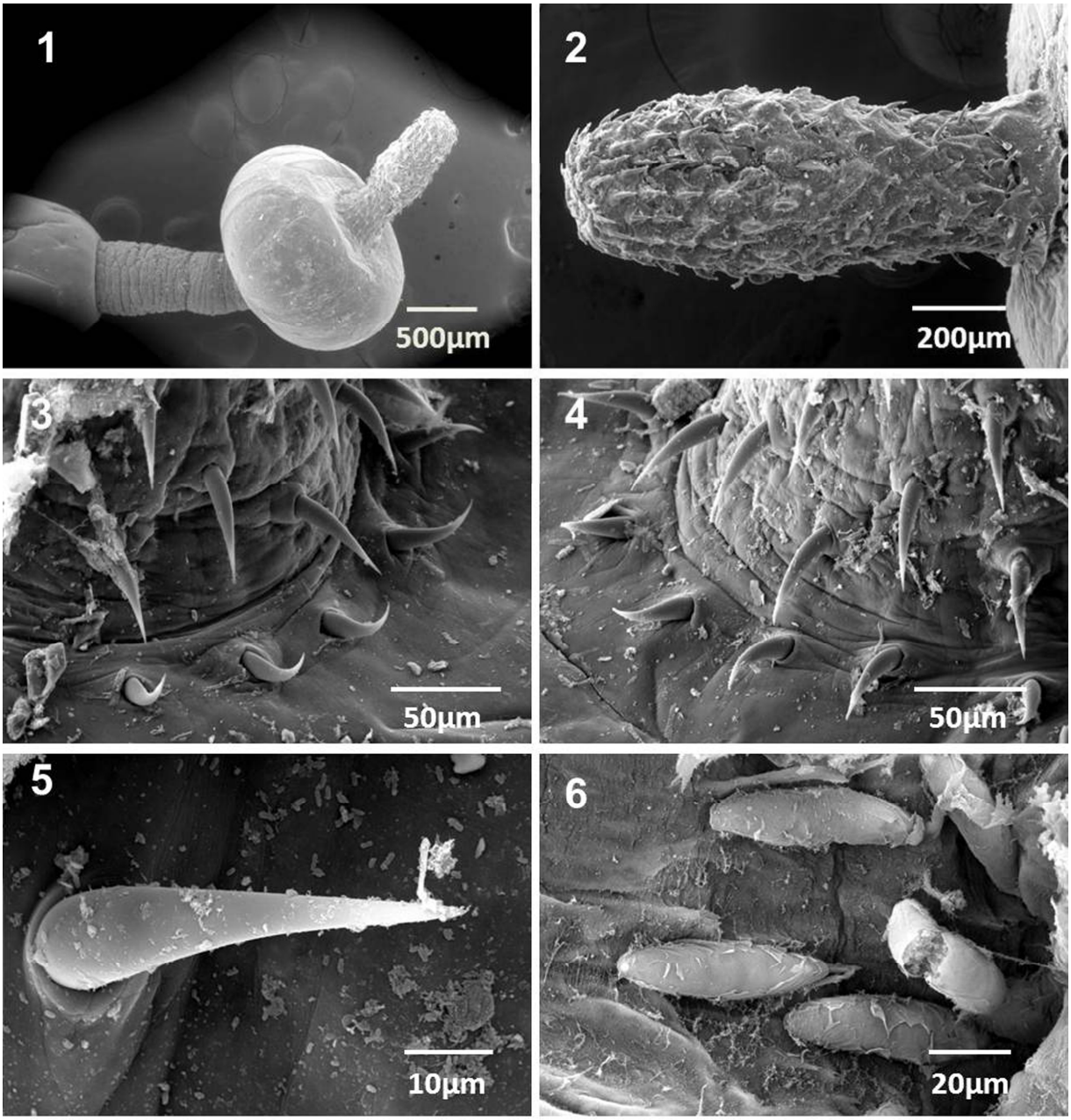
Amin et al. (2003) recognized 23 species of *Pomphorhynchus* from Europe, the Middle East, Asia (India, China, Armenia), and North and South America. A 24th species, *Pomphorhynchus moyanoi* Olmos and Habit, 2007, was later described from Chile. The 9 Indian species are all found in Jammu and Kashmir rivers and lakes (Amin et al., 2003). They are *Pomphorhynchus dubious* Kaw, 1941, *Pomphorhynchus kashmirensis* Kaw, 1941, *Pomphorhynchus bullocki* Gupta and Lata, 1968,

Pomphorhynchus jammuensis Fotedar and Dhar, 1977, *Pomphorhynchus kawi* Fotedar and Dhar, 1977, *Pomphorhynchus megacanthus* Fotedar and Dhar, 1977, *Pomphorhynchus orieni* Fotedar and Dhar, 1977, *Pomphorhynchus orientalis* Fotedar and Dhar, 1977, and *Pomphorhynchus tori* Fotedar and Dhar, 1977. Bhattacharya (2007) included a 10th species, *Pomphorhynchus bufonis* Fotedar, Duda and Raina, 1970 which Amin et al. (2003) declared invalid and unrecognizable. *Pomphorhynchus kawi* was considered recognizable and acceptable despite its poor and incomplete description (see Amin et al., 2003).

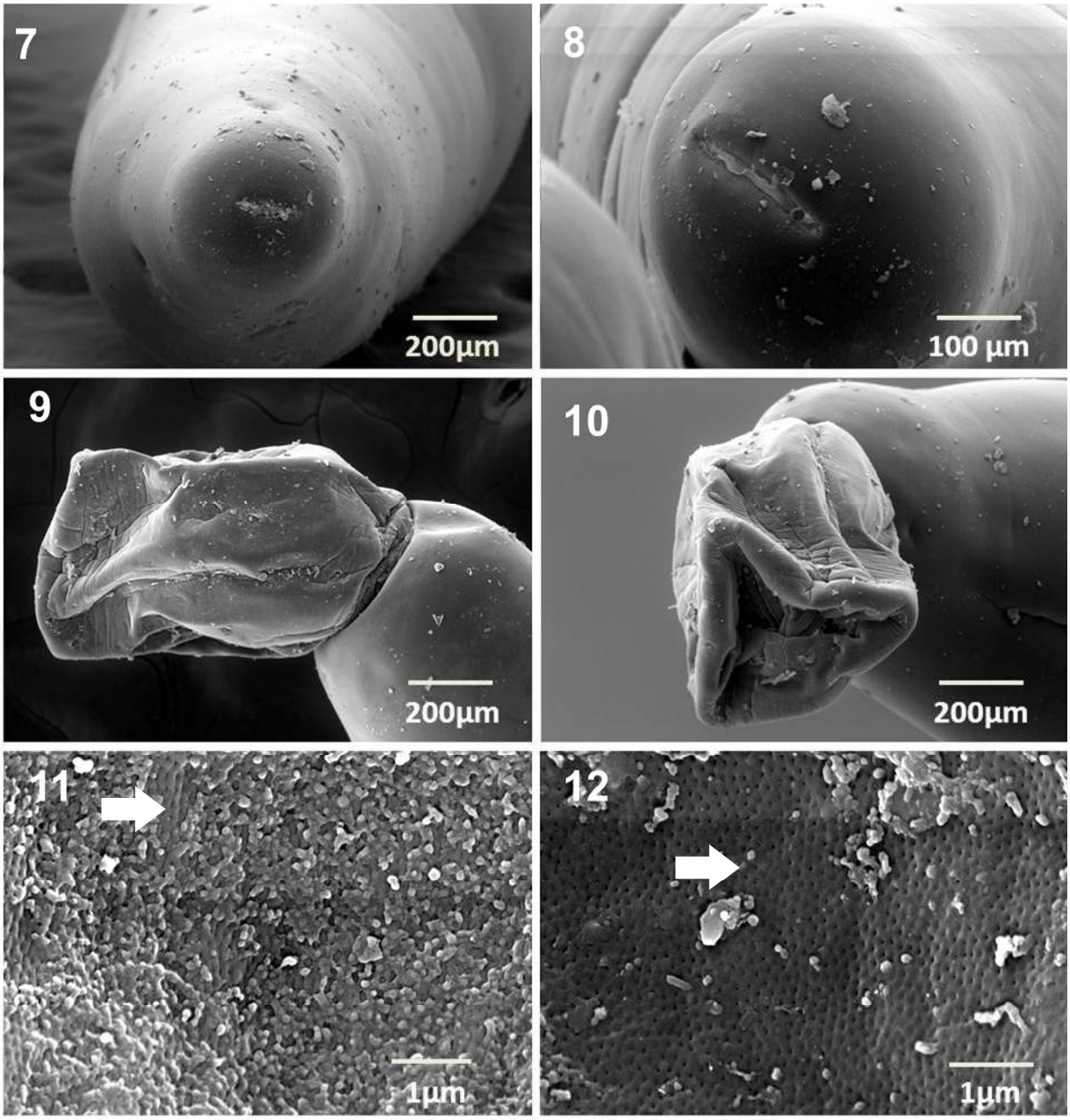
The new observations are best illustrated in figures 1-12. Figure 1 shows that the bulb is actually donut shaped and not round, pear-shaped, or ovoid as shown by Kaw (1941, figures 1, 5) or Fotedar and Dhar (1977, figures 1, 3). Figure 2 shows that the posterior half of the proboscis gradually constricts before it enlarges basally; it is not perfectly cylindrical as shown by Kaw (1941, figure 5) and Fotedar and Dhar (1977, figure 3) even though both authors depict a short epically truncated proboscis with a slight posterior constriction figures 2 and 5, respectively). The basal proboscis hooks were shown to be straight by Kaw (1941, figure 2) and Fotedar and Dhar (1977, figure 6). However, these hooks are actually curved and may be directed anteriorly or posteriorly (figures 3, 4); they are invariably found in a complete ring set off from all other hooks that are organized in regularly alternating longitudinal rows, as Kaw (1941) correctly mentioned. Posterior hooks appear to be at least 40µ long, about as long as basal hooks (figures 3-5). Corresponding hook measurements were reported by Kaw (1941) to be 28µ and 41µ long. Basal and posterior hooks illustrated by Fotedar and Dhar (1977, figure 6) appear to maintain the same size proportion mentioned by Kaw (1941). The eggs were described only by Kaw (1941) as "spindle-shaped" and were illustrated with moderately constricted polar ends (figure 6). However, in reality, eggs in our material have no such constrictions (our figure 6); they just gradually taper to blunt ends. The position and shape of the female gonopore were not previously described except for the line drawings of whole females by Kaw (1941, figure 5) and Fotedar and Dhar (1977, figure 2) that suggest a near terminal position of the gonopore. Our specimens show that the female gonopore opens in a slit-like orifice that varies in location from terminal (figure 7) to near terminal (figure 8). The bursa was not described, discussed or illustrated by other observers. It is clearly terminal and tilts ventrad (figure 9) and bears no sensory structures or ornamentations (figure 10). The epidermal micropores (figures 11, 12) are reported here for the first time and are typical of those seen in many species of acanthocephalans. Their size and distribution appear to be related to the differential absorption of nutritional elements through the body wall (see Amin et al., 2009, p. 661). We hope that this work will entice more research into additional aspects of the anatomy, ecology and host parasite relationships of this very interesting species of Acanthocephala.

Histopathology

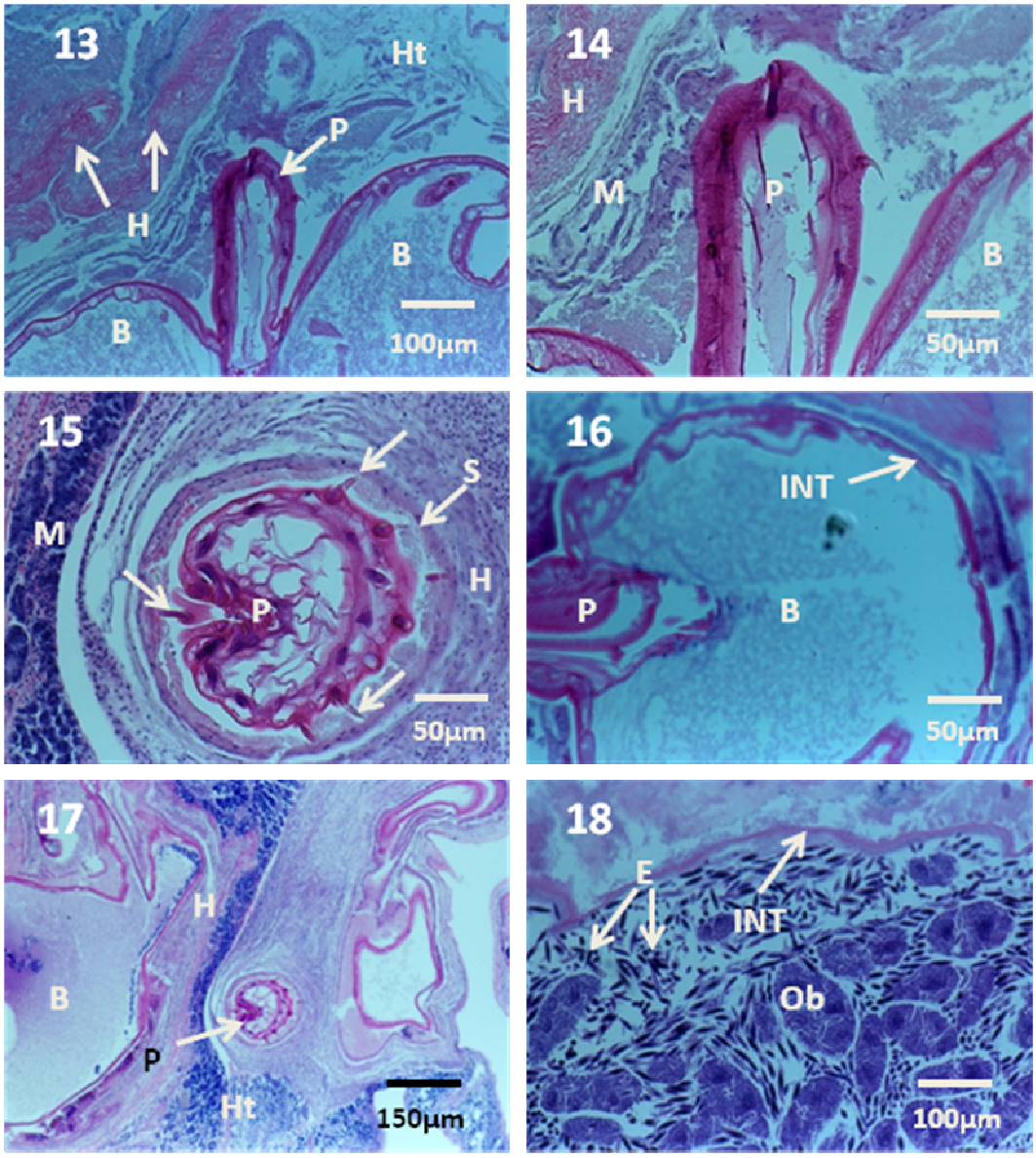
The histopathological observations demonstrate the invasive effects of *P. kashmirensis* on the intestinal tissues of *S. plagiostomus* (figures 13-18). The armed proboscis is inserted into host intestinal tissue displacing the mucosal lining with a prominent bulbous structure next to the inverted proboscis (figures 13, 14). Surrounding the proboscis are numerous red blood cells and granulocytestypical of hemorrhaging tissue. The presence of numerous granulocytes is consistent with acute host tissue damage (figure 14). Hooks are seen attached to the host epithelial lining (figure 15). Host villi are compressed and there is a loss of the typical columnar epithelium lining the villi. There is obstruction and blockage of the intestinal lumen due to the size of *P. kashmirensis* (figure 15). The prominent donut shaped bulb (figures 1, 15, 16) is visible and occupies extensive space in the host intestinal tissue. Figure 15 is a low magnification of the invaded tissue showing a cross section of the armed proboscis, and parts of the bulb of the acanthocephalan. There is blood loss and numerous granulocytes surrounding the worm. *Pomphorhynchus kashmirensis* appears to occupy most of the host intestinal lumen.



Figures 1-6. SEM of specimens of *Pomphorhynchus kashmirensis* from *Schizothorax plagiostomus.*1. Proboscis, bulb and neck of a female specimen. Note the donut-shaped bulb. 2. Proboscis of the same specimen in figure 1 showing its characteristic shape. 3, 4.Posterior and basal hooks of other specimens. Note the curved shapes of the basal hooks (anteriorly and posteriorly) and their similar size to that of posterior hooks. 5. A posterior hook longer than 40µ. 6. Fusiform eggs gradually tapering to blunt polar ends.



Figures 7-12. SEM of specimens of *Pomphorhynchus kashmirensis* from *Schizothorax plagiostomus.* 7, 8. Slit-shaped female gonopore orifice showing variation in their position from terminal to near terminal. 9. Lateral view of a bursa showing its terminal position and ventral tilt. 10. Face view of the same bursa in figure 9 showing the lack of sensory structures and ornamentation. 11,12. Epidermal micropores from the trunk of *P. kashmirensis* (arrows point to micropores).



Figures 13-18. Histopathology and light microscopy of *Pomphorhynchus kashmirensis* from *Schizothorax plagiostomus.* 13. Shows the proboscis (P) with the bulbous part (B) of the acanthocephalon invading the host tissue (Ht) causing extensive hemorrhaging (H) with numerous granulocytes and red blood cells. 14. Is a higher magnification of figure 13 showing prominent hooks on the proboscis (P) damaging the mucosal layer (M) of host tissue causing extensive hemorrhaging (H). The bulbous part (B) of the parasite is visible. 15. A cross section of the proboscis (P) with prominent hooks (arrows) and the proboscis sheath (S) causing hemorrhaging (H) of the host mucosal layer (M). 16. The donut-shaped bulbous part (B) (see figure 1) with the attached proboscis (P) and outer integument (INT). 17. A low magnification of 3 specimens of *P. kashmirensis* inhabiting a similar area of the piscine host (Ht). Note the sections through the bulbous (B) part of the parasites and the proboscis (P) of one of the 3 worms. Hemorrhaging (H) is prominent around the proboscis (P) and bulbous (B) parts of the worms. 18. A section through the body of a female *P. kashmirensis* showing numerous eggs (E), ovarian balls (Ob) and the integument (INT).

The host has generated a wall of collagenous connective tissue around the proboscis (figures 16 and 17). Figure 17 is a lowermagnification of the host tissue surrounding the proboscis of *P. kashmirensis.* Hooks of the everted proboscis are visible as well as the crypts of the host intestine. Hemorrhaging is prominent. The proboscis reaches to the host's multilayered muscularisexterna. Figure 18 shows a section of agravid female of *P. kashmirensis*within host tissue with numerous spindle shaped eggsand ovarian balls (figures 6, 18). Necrotic tissue is typical of the areas adjacent to the worms.

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References

Amin O.M., Abdullah S.M.A., Mhaisen F.T. 2003. Description of *Pomphorhynchus spindletruncatus* n. sp. (Acanthocephala: Pomphorhynchidae) from freshwater fishes in Northern Iraq, with the erection of a new pomphorhynchid genus, *Pyriproboscis* n.g., and keys to the genera of Pomphorhynchidae and the species of *Pomphorhynchus* Monticelli, 1905. Syst. Parasitol. 54:229-235.

Amin O.M., Heckmann R.A., Radwan N.A.E., Mantuano Anchudia J.S., Zambrano Alcivar M.A. 2009. Redescription of *Rhadinorhynchus ornatus* (Acanthocephala: Rhadinorhynchidae) from skip jack tuna, *Katsuwonus pelamis*, collected in the Pacific Ocean off South America, with special reference to new morphological features. J. Parasitol. 95:656-664.

Bhattacharya S.B. 2007. Handbook on Indian Acanthocephala. Zool. Surv. India, Kolkata, 255 pp.

Chishti M.Z., Peerzada. 1998. Host and seasonal occurrence of Acanthocephala in the fishes of Wular Lake. Oriental Sci. 31-38.

Dhar R.L. 1972. Studies on the Helminth Parasites of Fishes of Jammu & Kashmir. Ph.D. Thesis, University of Kashmir, India, 152 p.

Fotedar D.N., Dhar R.L. 1977. On five new species of the genus *Pomphorhynchus* Monticelli 1905 from freshwater fishes of Jammu and Kashmir, India. Ind. J. Zool. 5:15-29.

Galigher A.E., Kozloff E.N. 1971. Essentials of Practical Microtechnique, 2nd Edition. Lee and Febiger, Philadelphia, Pennsylvania, 531 pp.

Kaw B.L. 1941. Studies on the helminth parasites of Kashmir. Part I. Description of some new species of the genus *Pomphorhynchus* Monticelli (1905). Proc. Ind. Acad. Sci. 13:369-378.

Kiernan J.A. 2002. Histological and Histochemical Methods, Theory and Practice. Churchill Livingston, Edinburgh, U.K., 502 pp.

Lee R.E. 1992. Scanning electron microscopy and Xray microanalysis. Prentice Hall. Englewood Cliffs, New Jersey, 458 pp.

Yousuf R., Mir S.H., Shah A.W., Ganaie G.A. 2011. A comparative study of parasites infecting some selected fishes from the water bodies of Kashmir Valley. Ind. J. Fund. Appl. Life Sci. 1:150-153.