The morphology and histopathology of Nephridiacanthus major *(Acanthocephala: Oligacanthorhynchidae) from hedgehogs in Iran*

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The morphology and histopathology of *Nephridiacanthus major* (Acanthocephala: Oligacanthorhynchidae) from hedgehogs in Iran

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Abstract The morphology of *Nephridiacanthus major* (Bremser 1811 in Westrumb 1821) Golvan, 1962 collected from the long-eared hedgehog *Hemiechinus auritus* (Gmelin 1770) and the Eastern European hedgehog *Erinaceus concolor* Martin, 1838 (Erinaceidae) is described using SEM for the first time. This acanthocephalan was previously described from hedgehogs in Europe, Asia, and Africa. Measurements of specimens from Iran, Bulgaria, Germany, Central Asia, Morocco, and Egypt show considerable variations in the size of the trunk, proboscis, proboscis hooks and receptacle, and eggs. The SEM studies add new perspectives to its morphology. Features observed for the first time include the near terminal position and shape of the female gonopore and orifice, among others. Histopathological studies for this species are reported for the first time. Tissue sections show extensive damage near the proboscis with hemorrhaging and formation of collagenous connective tissue, compression of the intestinal mucosa, obstruction of intestinal lumen, and extensive necrosis of host epithelial tissue.

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Introduction

Samples of *Nephridiacanthus major* (Bremser 1811 in Westrumb 1821) from the Central Asian and Middle Eastern long-eared hedgehog *Hemiechinus auritus* (Gmelin 1770) and the Eastern European hedgehog *Erinaceus concolor* Martin, 1838 (Erinaceidae) from Iran were made available for study. Specimens of *N. major* were reported and described from these and other species of hedgehogs from "Europe" (Porta 1908), Germany (Meyer 1931, 1933), Morocco (Dollfus 1951), Central Asia (Petrochenko 1958; Hoklova 1986), Egypt (Nelson and Ward 1966), and Bulgaria (Genov 1984). A complete set of measurements was lacking in some of these reports, e.g., Porta (1908) and Dollfus (1951). Ecological information and prevalence rates of *N. major* were also reported from various species of hedgehogs from additional locations in Tadzhikistan (Gafurov and Isakov 1970), Lebanon (Schmidt 1972), Sicily (Giannetto et al. 1993), Italy (Poglayen et al. 2003), Nigeria (Kaikabo et al. 2006), Turkey (Cirak et al. 2010), and Mongolia (Tinnin et al. 2011). The Iranian population of *N. major* and its histopathology in host tissue are described for the first time.

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Materials and methods

One specimen of *E. concolor* was collected from Nashtarood (36°44′15.65″N, 51°01′32.25″E) in July 2011 and another from Tonekabon (36°50′31.71″N, 50°48′37.69″E) in August 2011. The first hedgehog weighed 620 g and was not infected, but the younger specimen (304 g) had only two worms (22.0 and 8.0 mm long). One male *H. auritus* was collected from Shahrood (36°25′04.76″N, 54°58′25.48″E) in June 2010; it was infected with 35 worms. Some of the latter worms were reserved for histopathological and SEM studies, and 13 specimens were examined microscopically for diagnostic and descriptive purposes.

For microscopical examination, worms were placed in cold water for a few hours or until the proboscis was fully extended, then fixed in cold 70 % ethanol. Worms were then punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4 % hydrochloric acid in 70 % ethanol, dehydrated in ascending concentrations of ethanol (24 h each), and cleared in graduated concentrations of terpineol in 100 % ethanol to 100 % terpineol, then 50 % terpineol in 50 % Canada balsam (24 h each). Whole worms were then mounted in Canada balsam. All measurements are in micrometers unless otherwise noted. Total length measurements do not include the proboscis, neck, or bursa. Width measurements are those of maximum width. The range of length and width measurements is followed by mean values in parentheses. Specimens are deposited in the Harold W. Manter Laboratory collection at the University of Nebraska State Museum, Lincoln, Nebraska, USA.

For SEM, samples of *N. major* that had been alcohol–formalin–acetic acid (AFA) fixed and stored in 70 % ethanol were processed following standard methods (Lee 1992) that included critical point drying in sample baskets and mounted on SEM sample mounts using conductive double-sided carbon tape. Samples were then gold coated for 3 min using a Polaron E3500 sputter coater establishing an approximate thickness of 20 nm. 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; Keinan 2002) were employed for the examination of the infected host intestinal tissue. Samples of *N. major* embedded in host tissue that have been AFA fixed and 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 μm, placed on glass slides and stained with Harris hematoxylin and eosin, 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.

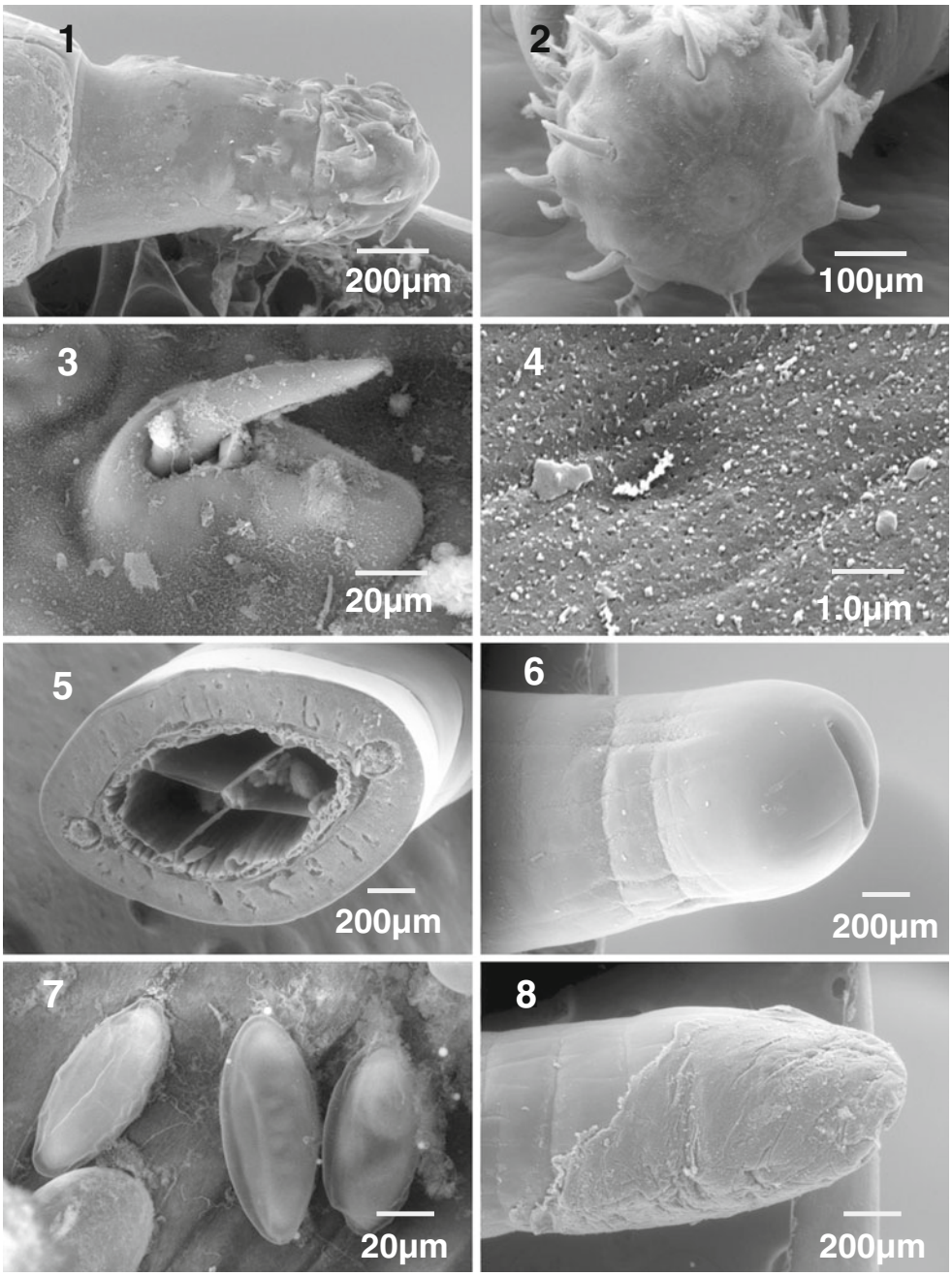
Results and discussion

N. major (Bremser 1811 in Westrumb 1821) Golvan, 1962 [=*Echinorhynchus major* Bremser 1811 in Westrumb 1821; *Gigantorhynchus major* (Porta 1908); *Nephridiorhynchus major* (Bremser 1811 in Westrumb 1821) Meyer 1932] is perhaps one of the earliest species of acanthocephalans described. It is commonly reported from various species of hedgehogs in Europe, Asia, and Africa. A small population of this acanthocephalan collected from *H. auritus* and *E. concolor* in Iran is described below then compared with collections of *N. major* described from elsewhere in the world.

Description of the Iranian specimens

General With characters of the genus *Nephridiacanthus* Meyer 1931. Long robust worms, somewhat flattened laterally, with faint pseudosegmentation and dorsal and ventral longitudinal lacunar canals. Body wall with prominent giant nuclei; nuclei long, slender, little-branched or shorter, thick with lateral or dendritic branches pending reproductive state. Proboscis claviform anteriorly (Fig. 1) with 12 near longitudinal rows of four hooks each and prominent apical organ traceable externally (Fig. 2). Hooks progressively decrease in size posteriorly (Fig. 1). Anterior hooks in elevated base (Fig. 3) with robust roots directed anteriorly having posterior knob; smaller posterior hooks with lateral roots. Neck long (Fig. 1) with two elevated lateral sensory papillae anteriorly. Trunk with micropores throughout (Fig. 4) and thick radial layer with two major lacunar canals (Fig. 5). Proboscis receptacle two to three times as long as proboscis with thick dorsal wall; ventral wall thick posteriorly and thin anteriorly with prominent round cephalic ganglion in between. Lemnisci long, slender, somewhat subequal, with central canal and usually with nine and ten prominent ovoid nuclei in shorter and longer lemnisci, respectively. Bouquet-like protonephridia at anterior end of uterine bell.

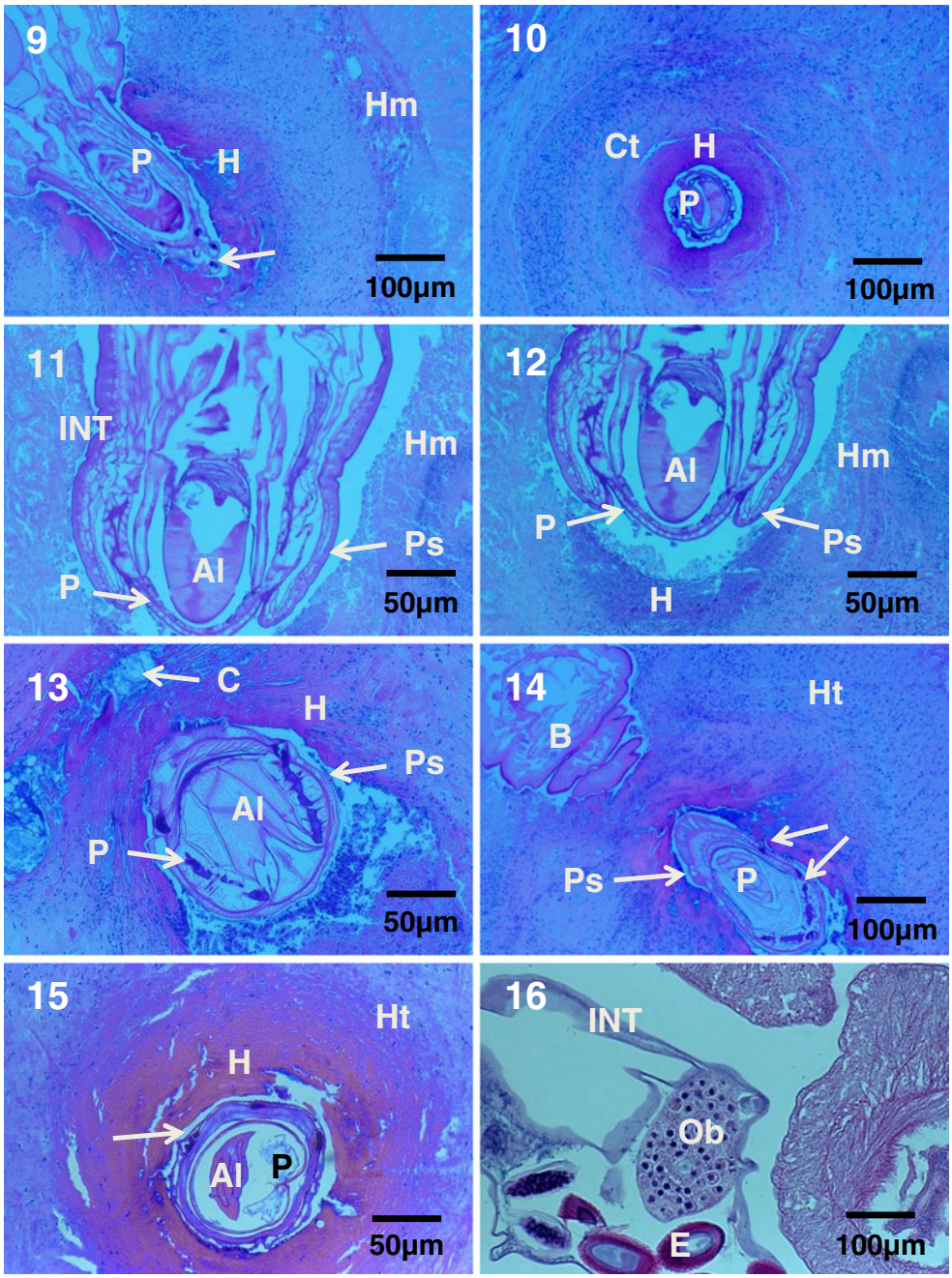
Males (based on seven adults) Trunk 18.87–85.75 (40.77) mm long by 1.00–3.17 (1.94)mm wide. Proboscis 478–572 (523) long by 395–468 (430) wide anteriorly. Length of hooks from anterior 107–120 (113), 80–107 (96), 65–80 (73), 55–70 (60). Neck 260–416 (347) long by 364–416 (388) wide posteriorly. Proboscis receptacle 0.94–1.61 (1.27)mm long by 0.36–0.57 (0.50)mm wide. Short lemniscus 3.42–14.50 (6.38)mm long by 0.17–0.55 (0.33)mm



Figs. 1–8 SEM of specimens of *N. major* from hedgehogs in Iran. *1* The claviform proboscis and long neck of a female specimen. *2* The apical surface of the proboscis showing the round outline of the apical organ. *3* An anterior hook emerging from an elevated base. *4* Surface of trunk epidermis showing micropores. *5* A section of body wall showing a thick radial layer. *6* The posterior end of a female showing the slit-like orifice of the gonopore. *7* Eggs. *8* The posterior end of a female covered with a copulatory cap

wide. Long lemniscus 3.90–15.0 (6.97)mm long by 0.20–0.50 (0.33)mm wide. Longest lemnisci in 20.75-mm-long male having third lemniscus 2.12 mm long by 0.20 mm wide. Reproductive system post-equatorial; testes elongate, equal, usually not contiguous; posterior testis usually separated from cement glands. Anterior testis 1.37–6.50 (3.44) mm long by 0.27–1.50 (0.79)mm wide. Posterior testis 1.32–6.50 (3.36)mm long by 0.35–1.20 (0.71)mm wide. Cement glands in compact longitudinal chain of four pairs, each with one large round nucleus, 0.57–2.25 (1.22)mm long by 0.37–1.25 (0.69)mm wide, but occasionally not fully developed or measurable in some mature worms. Saefftigen's pouch alongside common sperm duct and common cement gland duct, 1.14–4.00 (2.53)mm long by 0.26–0.82 (0.55)mm wide. Gonopore terminal. Bursa retracted in all specimens.

Females (based on one juvenile and five adults) 6.25–126.25 (85.00)mm long by 0.67–3.77 (2.06)mm wide. Proboscis 416–562 (473) long by 332–520 (447) wide anteriorly. Length of hooks from anterior 104–135 (121), 104–112 (106), 62–94 (83), 47–83 (65). Neck 291–592 (485) long by 364–530 (433) wide posteriorly. Proboscis receptacle 1.12–1.77 (1.43)mm long by 0.35–0.80 (0.44)mm wide.



Figs. 9–16 The histopathology and light microscopy sections of *N. major* from hedgehogs in Iran. *9*, *10* The proboscis (*P*) of a specimen invading the host mucosal (*Hm*) lining with hooks (*arrow*) inserted into the tissue causing extensive hemorrhaging with granulocytes and red blood cells surrounding the proboscis (*P*) with surrounding hemorrhagic (*H*) tissue and a layer of connective tissue (*Ct*). *11* A longitudinal section of a partially everted proboscis (*P*) with a prominent proboscis sheath (*Ps*) and outer worm integument (*INT*). The host mucosal layer (*Hm*) is visible as well as the proboscis alveolar lobes (*Al*). *12* Another aspect of Fig. 11 showing the host damage due to the invasive parasite with blood loss and hemorrhaging (*H*) note host mucosal lining (*Hm*), proboscis (*P*), proboscis sheath (*Ps*), and alveolar lobes (*Al*). *13* A higher magnification of proboscis cross section (*P*) inserted into the host mucosa with hemorrhaging (*H*); the deep crypts of the mucosa layer (*C*) are visible next to the host submucosa. Note the outer proboscis sheath (*Ps*) and alveolar lobes (*Al*). *14* A single parasite cut twice while migrating through the host tissue (*Ht*). Note the proboscis (*P*) with sheath (*Ps*) and hooks (*arrows*) is at the bottom right while the body of the parasite (*B*) is at the top left with an island of host connective tissue between the two parts of the parasite. *15* A cross section of the proboscis (*P*) in Fig. 14 is displayed with alveolar lobes (*AL*), hooks (*arrow*), hemorrhaging (*H*), and host mucosal tissue (*Ht*). *16* A section of a female *N. major* in the host intestine (*left*) with eggs (*E*) ovarian balls (*Ob*), and parasite integument (*INT*)

Short lemniscus 4.65–8.37 (6.90)mm long by 0.15–0.34 (0.26)mm wide. Long lemniscus 5.30–8.75 (7.33)mm long by 0.16–0.38 (0.28)mm wide. Near terminal gonopore opens in a wide slit-like orifice (Fig. 6). Eggs ovoid with

thick sculptured membrane and a longitudinal seam (Fig. 7), 94–114 (107) long by 52–73 (61) wide. Distal end of vagina attached to body wall at posterior end of trunk with thick muscular connector. Copulatory cap occasionally seen on posterior end of some females (Fig. 8).

Remarks

The morphology and anatomy of our Iranian specimens fit within the full range of variations described for this species from various geographical regions but not identical to any described from one specific region alone. Geographical variability is clearly involved especially in important taxonomic traits. For example, eggs were markedly smaller than those from Iran in most European and Egyptian specimens. They measured 75×36 (Porta 1908 from "Europe"), 80–100×50 (Meyer 1931, 1933 from Germany), and 40–90×20–50 (Nelson and Ward 1966 from Egypt). They were comparable in size in Central Asian specimens being 110–116×61–65 (Petrochenko 1958) and 90–116×50–65 (Hoklova 1986), but considerably larger than those from Iranian specimens from Bulgaria, being 135–136×76–84 (Genov 1984). The same Bulgarian specimens also had markedly larger proboscis 690–698×493–513 and anterior proboscis hooks 128–130 in males and 140 in females, but smaller proboscis receptacle 800–965×360–372 (Genov 1984) than in our specimens. The longest hooks were reported from Central Asian specimens by Petrochenko (1958) with the anterior hooks reaching 116–157 in length. Similar geographical variations in taxonomic traits were noted in other acanthocephalan species, e.g., *Mediorhynchus papillosus* Van Cleave, 1916 across its geographical distribution from Maryland and Colorado in the USA through Europe and Asia to Taiwan in the east (Amin and Dailey 1998).

Histopathology

Figures 9 to 16 show the results of the histopathological study. Worms with a well-armed proboscis caused major damage to the host intestinal lining. Figure 9 shows the armed proboscis (arrow) invading the host tissue at the mucosal area of the intestine. There is extensive damage near the proboscis with hemorrhaging and formation of collagenous connective tissue. Many red blood cells and granulocytes are visible near the proboscis. Parts of the internal mucosal lining are visible with crypts crowded to one side. There are numerous connective tissue fibers surrounding the inserted proboscis whereby the host is attempting to isolate the invading parasite (Figs. 9, 10). Figure 10 is a cross section of the armed proboscis with visible hooks. It has extended into the muscle layer (muscularis externa) of the host intestine. Figures 11 and 12 represent the initial invasion of the acanthocephalan into the host intestine. The proboscis, proboscis receptacle, and the adjacent host tissue are visible. Figure 12 shows the host response at the interface with the proboscis exemplified by extensive hemorrhaging and presence of granulocytes. Dead, necrotic epithelial host tissue is visible in this area. Remnants of villi and host epithelial cells are visible. The acanthocephalan is attached to the host intestinal mucosa. There is a prominent compression of the intestinal wall with *N. major* obstructing the lumen of the intestine. Extensive necrosis of the host epithelial tissue is visible with the worm migrating through the outer intestinal lining towards the outer muscular layers (Figs. 9 and 11). A cross section of the proboscis of *N. major* is shown in Figs. 13 and 15. The peripheral hooks of the proboscis are visible as well as the surrounding necrotic tissue, red blood cells, and granulocytes. The host response is the formation of collagenous connective tissue attempting to isolate the parasite creating a visible cyst-like structure on the intestine which was observed by the third author during host dissection. Figure 15 shows both the trunk and the proboscis of *N. major*. The alveolar lobes of the proboscis and the lemniscus are visible (Figs. 13–15). Figure 14 shows a separation by host tissue between worm trunk and the proboscis. It is assumed that the parasite formed a loop as it invaded the host intestine. Note the proboscis hooks in Fig. 15 and the adjacent hemorrhaging and granulocytes. A section of a female worm is shown (Fig. 16) next to host epithelial tissue. The oval eggs and ovarian balls are represented. The invasive properties of *N. major* are well depicted by the figures displaying classic tissue pathology due to an invading parasite.

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