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New Features of *Neoechinorhynchus* (*Neoechinorhynchus*) *dimorphospinus* (Acanthocephala: Neoechinorhynchidae) from Recent Collections in the Arabian Gulf Using SEM, with Notes on Histopathology

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ABSTRACT: *Neoechinorhynchus dimorphospinus* Amin and Sey, 1996, was described from 4 species of marine fishes in the Arabian Gulf off Kuwait. Specimens of *N. dimorphospinus* have been reported only twice since then from marine and freshwater fishes also from the Arabian Gulf and brackish waters associated with it. The 2 latter reports offered no new descriptive accounts. SEM studies based on recent collections of *N. dimorphospinus* from Klunzinger's mullets, *Liza klunzingeri* (Day, 1888) (Mugiliformes) (new host record), revealed many new features that were not possible to include in the original description using optical microscopy. These new features include an apical organ in the proboscis, a broad prominent collar at the anterior end of the trunk, prominent longitudinal grooves on proboscis hooks, a pair of sensory structures in the neck, the subterminal position of the female gonopore with a minute lipless vulva, micropores on the trunk not showing differentiation in various trunk regions, and eggs in different stages of development. There is extensive damage to the host intestine due to invading specimens of *N. dimorphospinus*. Worms destroy the epithelial lining of *L. klunzingeri* and migrate through the thin smooth muscle wall of the intestine into the host body cavity. Extensive host hemorrhaging, tissue necrosis, villi compression, and loss of host epithelial cells appears to be the typical pathology in intestinal sites occupied by this worm.

KEY WORDS: *Neoechinorhynchus dimorphospinus*, Acanthocephala, Arabian Gulf, SEM revision, histopathology.

Neoechinorhynchus dimorphospinus Amin and Sey, 1996, was first reported from 1 of 3 specimens of *Liza* (= *Chelon*) *macrolepis* (Smith, 1846) (Perciformes: Mugillidae) by Amin et al. (1984). The 4 female specimens collected were recognized as belonging to a new species but were considered inadequate for a formal description; nevertheless, they were measured. The species was subsequently described by Amin and Sey (1996) from 4 species of marine fish in 4 different families: *Allanetta* (=*Atherinomorus*) *forskalii* (Ruppell, 1838) (Atherinidae), *Dorosoma* (= *Nematolosa*) *nasus* (Bloch, 1795) (Clupeidae), *L. macrolepis* (Mugilidae), and *Pseudorhombus arsius* (Hamilton, 1822) (Bothidae) (= Paralechthyidae) in the same marine waters of the Arabian Gulf off the coast of Kuwait.

Since its original description, specimens of *N. dimorphospinus* have been reported only twice. Abdul-Rahman (1999; unpublished thesis, University of Basrah, Basrah, Iraq) reported it from the freshwater mullet, *Liza abu* (Heckel, 1843), in the Garmat Ali River, which drains into the Hammar Marsh of the Shatt Al-Arab drainage system, and Bannai (2002; unpublished thesis, University of Basrah, Basrah, Iraq) reported it from the marine fish *Liza* (= *Chelon*) *subviridis* (Valenciennes, 1836) in the northwest Arabian Gulf. The brief descriptions and measurements included in the latter 2 reports were similar to those in the original description and added nothing new to our knowledge of this parasite. Based on new collections of *N. dimorphospinus* from Klunzinger's mullet, *Liza klunzingeri* (Day, 1888), we herein present new information about its morphological features based on SEM studies, as well as new information on the host-parasite interface as observed from histopathological sections.

MATERIALS AND METHODS

A total of 60 specimens (33 males and 27 females) of *N. dimorphospinus* were collected from 5 of 8 examined Klunzinger's mullet ranging from 9.5 to 17.5 cm in total length and 14 to 64 g in weight, and all were collected from the mouth of the Shatt Al-Arab Estuary near Fao City, Basrah, Iraq (48°50′E, 29°32′N) during July 2011. The 5 infected hosts had 7–16 (mean of 12) parasites each. Live

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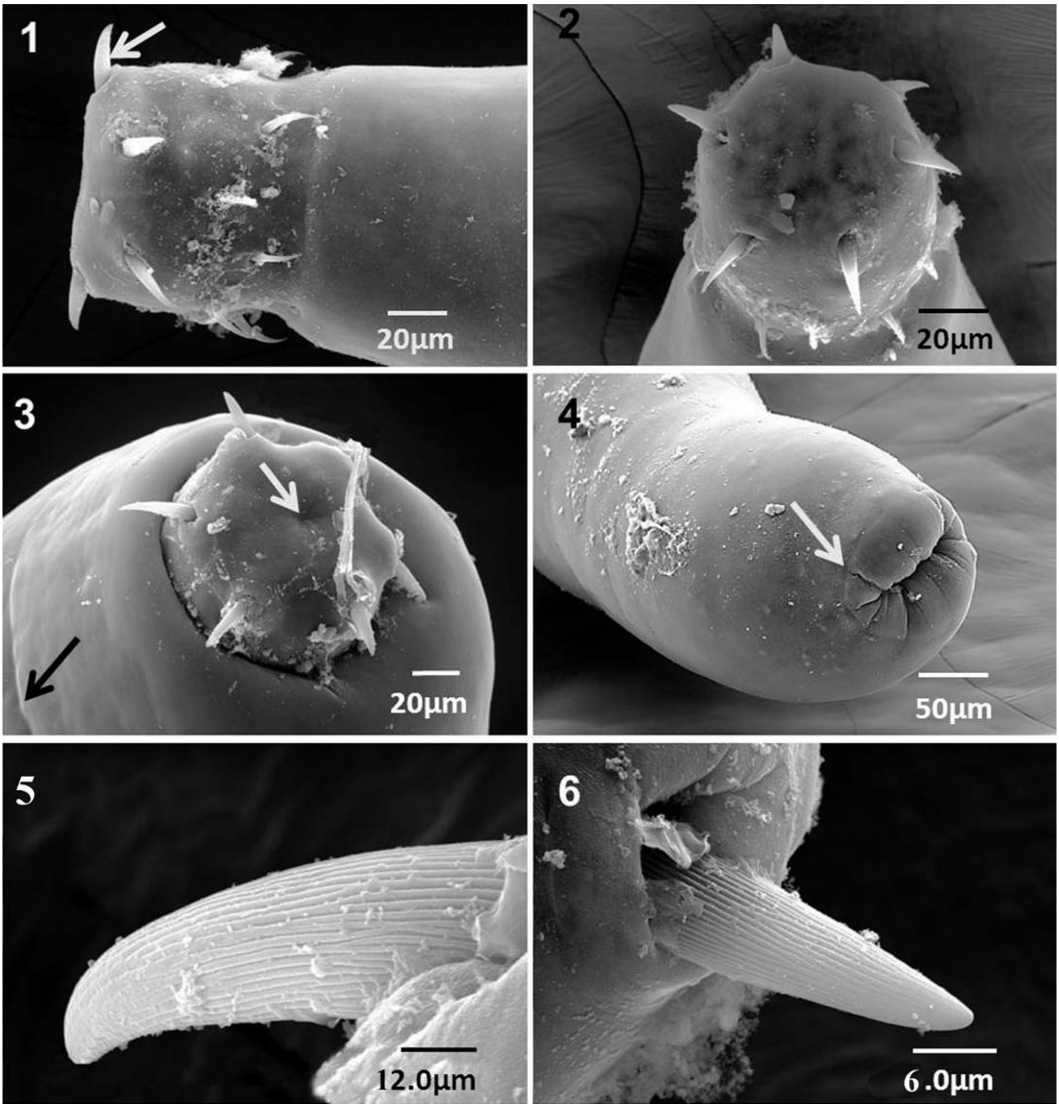
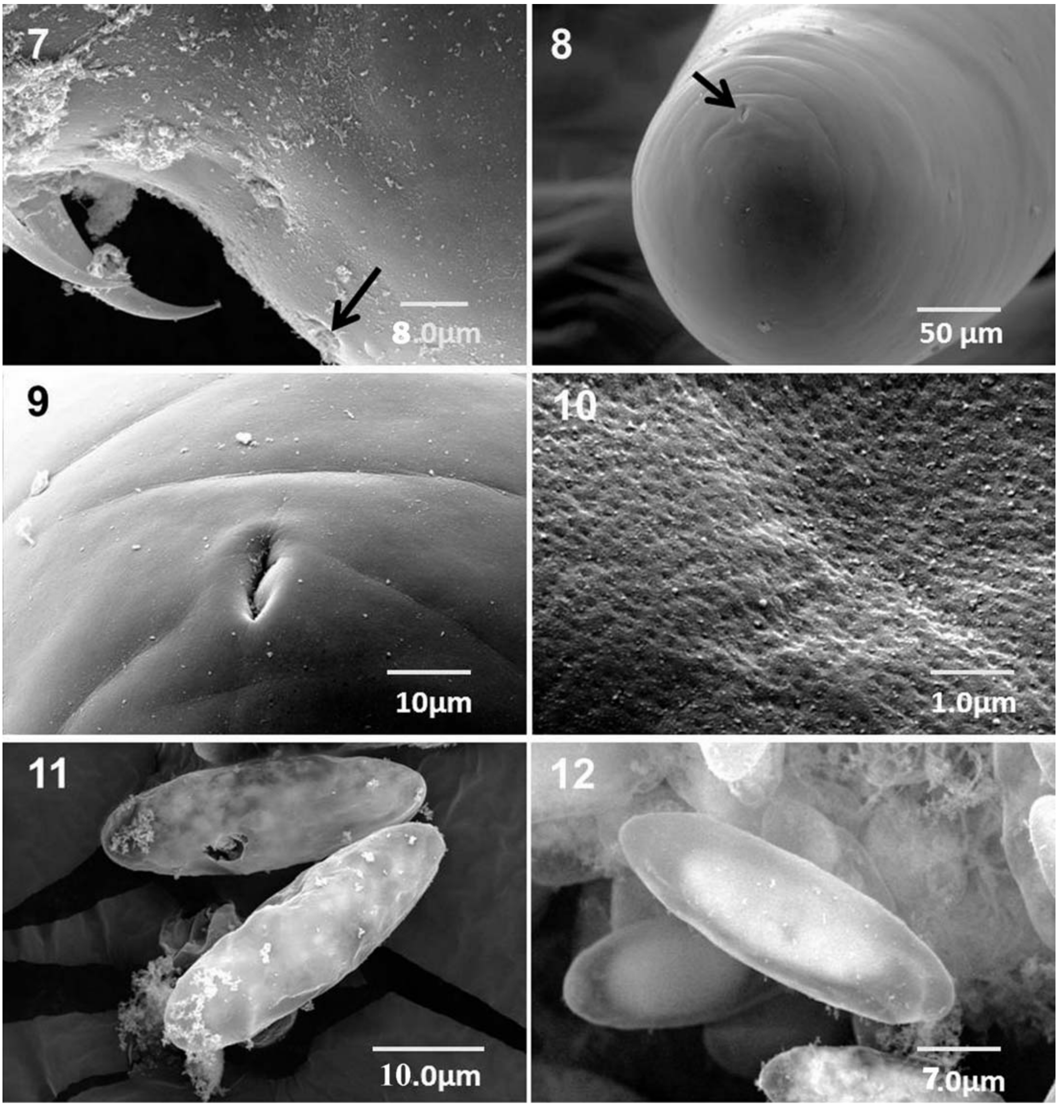


Figures 1–6. SEM of *Neoechinorhynchus* (*Neoechinorhynchus*) *dimorphospinus* from Liza *klunzingeri*. **1.** The proboscis of a male specimen showing the deeply embedded longer lateral hook (arrow) and the adjacent anterior trunk collar. **2.** An apical view of another proboscis showing the darker sphere marking the position of the apical organ. **3.** Anterior end of another male showing a partially retracted apical organ (white arrow) and the posterior edge of the collar (black arrow). **4.** The anterior end of a worm with a retracted proboscis showing the demarcation line of the posterior edge of the trunk collar (arrow). **5.** A longer anterior lateral proboscis hook showing the grooves characteristic of this species. **6.** A ribbed hook from the posterior circle of the same proboscis used in Fig. 5.

specimens were kept in tap water for a few hr until the proboscides were evaginated, and worms were then fixed in 70% ethanol. Specimens were stained in Erlich's heamatoxylin, destained in 2% HCl in 70% ethanol, dehydrated in ascending concentrations of ethanol, cleared in methyl salicylate, and mounted in Canada balsam for optical examination and identification. Other specimens were stained in Mayer's acid carmine, destained in 4% hydrochloric acid in 70% ethanol, dehydrated in ascending concentrations of ethanol (70%, 80%, 90% twice, 100%), cleared in 100% xylene, and then further cleared in 50% Canada balsam and 50% xylene, each step for 24 hr. Whole worms were then mounted in Canada balsam.

For SEM studies, a few specimens previously fixed in 70% ethanol were placed in critical-point drying baskets and dehydrated using ethanol series of 95% and 100% for at



Figures 7–12. SEM of *Neoechinorhynchus* (*Neoechinorhynchus*) *dimorphospinus* from *Liza klunzingeri*. **7.** Posterior proboscis hooks and a sensory pit (arrow) of the proboscis of a male specimen. **8.** The posterior end of a female showing the subterminal position and size of the gonopore (arrow). **9.** A higher magnification of a female gonopore showing its orientation and lack of lips or any specialized structures. **10.** The evenly distributed micropores on the trunk of a specimen. **11.** Young eggs. **12.** A well-developed embryonated egg.

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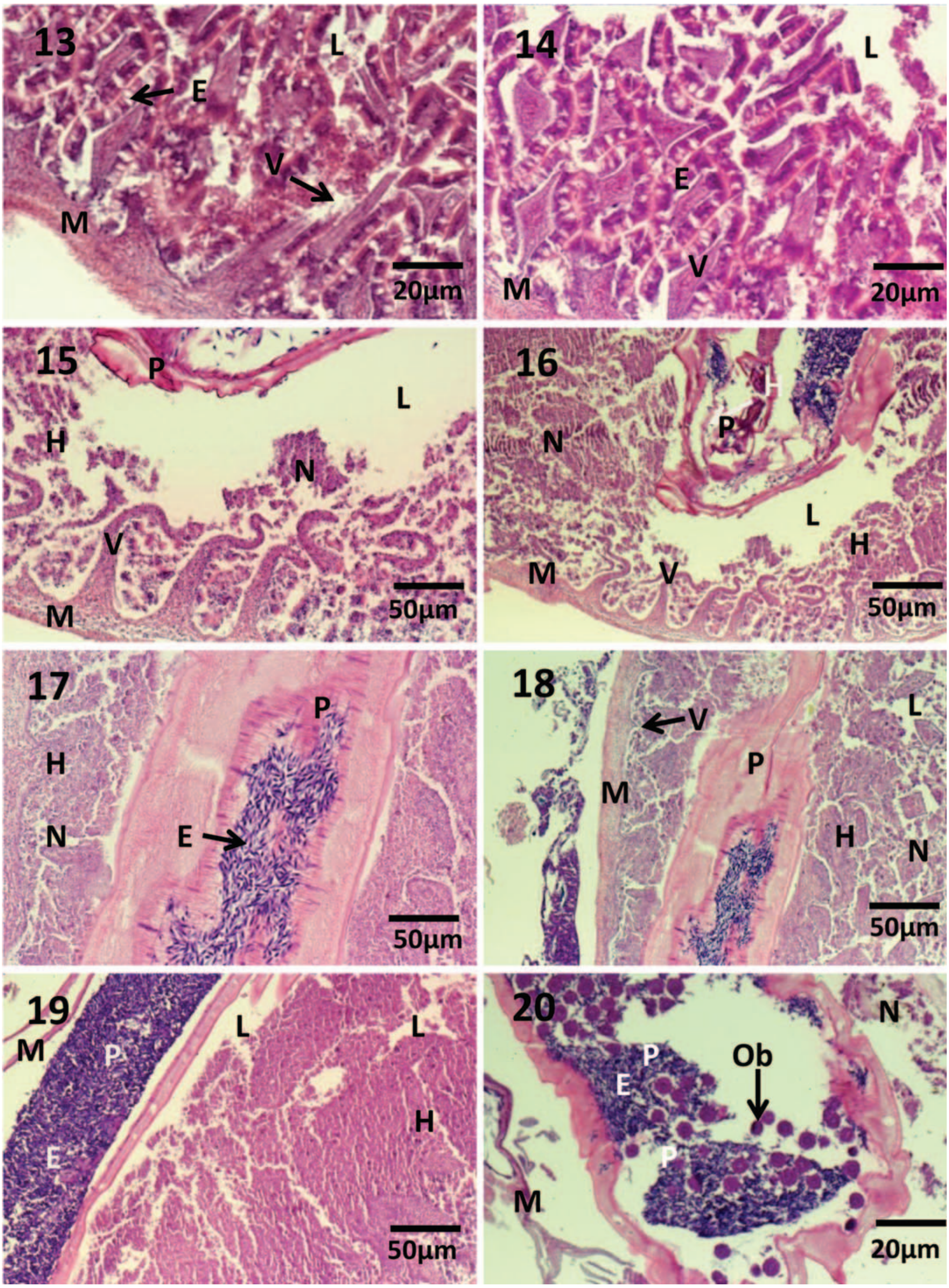
lumen (L) compressing the villi (V), causing extensive hemorrhaging (H) with granulocytes present and tissue necrosis (N). M is the thin outer smooth muscle layer, muscularis externa. **17.** A female specimen of *N. dimorphospinus* (P) with eggs (E) occupying most of the host intestinal lumen with subsequent compression of the epithelium and hemorrhaging (H) and necrosis (N). **18.** Similar to figure 17 with better view of damaged villi (V) due to the worm (P) impacting the thin outer muscular layer (M) with subsequent hemorrhaging (H) and tissue necrosis (N). **19.** A female parasite (P) with eggs (E) is impacting the thin muscle layer (M) within the host lumen (L) with extensive "laking" of blood (H). 20. A female parasite (P) with eggs (E) and ovarian balls (Ob) next to the muscularis externa (M) of the host intestine with extensive host tissue necrosis (N).



Figures 13–20. Uninfected (13, 14) and infected (15–20) intestinal tissue of *L. klunzingeri* with specimens of *Neoechinorhynchus dimorphospinus*. V = villus, E = epithelial lining with prominent goblet cells, M = muscularis externa (outer smooth muscle coat), L= lumen. **14.** Similar to Fig. 13 with more prominent goblet cells (E) attached to the villus (V). M = muscularis externa, L= intestinal lumen. **15.** Invasion of host intestine by *N. dimorphospinus* showing naked villi (V), hemorrhaging (H), and necrotic tissue (N) within the host intestinal lumen (L). **16.** Parasitized (P) host tissue within the host

least 10 min per soak, followed by critical point drying (Lee, 1992). Samples were mounted on SEM sample mounts, gold/palladium coated, and observed with a scanning electron microscope (XL30 ESEMFEG; FEI, Hillsboro, Oregon).

For histopathological sections, standard methods (Galigher and Kozloff, 1971; Kiernan, 2002) were employed for the examination of the infected host intestinal tissue. Samples of *N. dimorphospinus* embedded in host tissue were fixed in 10% formalin and transferred to 10% buffered formalin (v/v). The infected host tissue was then dehydrated and blocked in paraffin. Blocks were sectioned at 4–6 μm using a Leica Model RM225 microtome, placed on glass slides, stained with Harris hematoxylin and eosin, and viewed with an LSM laser (laser scanning compound microscope, Carl Zeiss LSM1, Thornwood, New York). Representative pictures were taken with an attached digital camera at various magnifications and images stored for future reference. Fish classification followed Carpenter et al. (1997) and was verified using Froese and Pauly (2013).

RESULTS AND DISCUSSION

The recovery of *N. dimorphospinus* from marine fishes of 4 different families in 4 orders, as well as from a freshwater fish species, suggests that this is a highly diverse and adaptable acanthocephalan that likely infects a wider assortment of fish species and whose range may extend further inland into Iraq as well as into additional marine fishes beyond the Arabian Gulf. Only additional collections into those areas will reveal the extent of its distribution, which must also be regulated by the distribution of its currently unidentified crustacean intermediate host(s).

The recently collected specimens from L. *klunzingeri* were similar to those originally described by Amin and Sey (1996). The SEM studies (Figs. 1–12), however, made it possible to document additional features not reported in the original description. The most characteristic feature about *N. dimorphospinus* is the distinctly larger size of the 2 lateral anterior hooks compared to the dorsal and ventral hooks in the same ring. These larger lateral hooks are invariably more anterior than the other hooks of the same ring and are directed vertical to the proboscis and not posteriorly as for all other hooks on the proboscis (Fig. 1, arrow; Fig. 2, below; Fig. 3, left). A similar pattern was also noted in *Neoechinorhynchus doryphorus* Van Cleave and Bangham, 1949, which was collected from a brackish water cyprinodont fish, *Jordanella floridae* Goode and Bean, 1879, in the Englewood area of Florida, U.S.A. (Van Cleave and Bangham, 1949).

New features observed in this study include the apical organ, which was observed at the anterior end of the proboscis as a dark sphere (Fig. 2) and as a pit when retracted (Fig. 3, arrow). In some acanthocephalans, e.g., *Polyacanthorhynchus kenyensis* Schmidt and Canaris, 1967 (Polyacanthorhynchidae), the apical organ is a well-developed, ducted, contractile, and conically shaped secretory structure (Amin and Dezfuli, 1995). Two distinct sensory pits exist on the neck just behind the posterior hooks (Fig. 7, arrow). The proboscis and unremarkable neck are marked off from the trunk with a prominent elevated cuticular thickening forming a collar or girdle at the anterior end of the trunk (Fig. 1; Fig. 3, black arrow; Fig. 4, arrow at retracted proboscis). Similar but narrower girdles are occasionally seen in other species of *Neoechinorhynchus* Stiles and Hassall, 1905, from the same region, e.g., *Neoechinorhynchus* (*Neoechinorhynchus*) *iraqensis* Amin, Al Sady, Mhaisen, and Bassat, 2001, from Iraq, or from elsewhere, e.g., *Neoechinorhynchus* (*Neoechinorhynchus*) *ascus* Amin, Ha, and Ha, 2011, and *Neoechinorhynchus* (*Neoechinorhynchus*) *longnucleatus* Amin, Ha, and Ha, 2011, from Vietnam. The significance of these girdles is not known. Additional newly observed features from the anterior end of worms include the ribbed pattern of proboscis hooks. Longitudinal grooves were demonstrated in anterior, middle, and posterior hooks alike (Figs. 5, 6). Ribbed proboscis hooks appear to be more common across higher taxa than the scarcity of their reporting suggests. In addition to their present reporting in *N. dimorphospinus* (Eoacanthocephala: Neoechinorhynchidae), ribbed hooks have also been reported in 3 other species in 2 other families of Palaeacanthocephala: *Dentitruncus truttae* Sinzar, 1955 (Illiosentidae), *Rhadinorhynchus ornatus* Van Cleave, 1918 (Rhadinorhynchidae), and *Leptorhynchoides polycristatus* Amin, Heckmann, Halajian, El-Naggar, Tavakol, 2013 (Rhadinorhynchidae). Dezfuli et al. (2008) and Amin et al. (2009) suggested that such hook striations may provide a more effective attachment to the host's intestine. The striations of the hooks of *D. truttae* appeared as small dents on the outer periphery of the hooks' outer striped layer (Dezfuli et al., 2008).

Other newly observed features include the position and morphology of the female gonopore. The gonopore position was not described in the original description but was shown to be terminal to slightly subterminal (Figs. 1, 3, Amin and Sey, 1996). Figure 8 shows it as decidedly subterminal and very small, and Fig. 9 shows that it is lipless and aligned along the longitudinal axis of the trunk. The trunk of *N. dimorphospinus* is porous and has many micropores that are evenly distributed throughout its length (Fig. 10). These peripheral canals are continuous with canalicular crypts, which appear to constitute a huge increase in external surface area and which are also implicated in nutrient uptake (see Whitfield 1979 and Amin et al. 2009 for a discussion of this topic). Accordingly, it would appear that nutrient uptake is similar along the length of the trunk and not variably performed in different regions of the trunk as has been shown for both *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911, and *Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911, by Amin et al. (2011) and Heckmann et al. (2011), respectively. Eggs at different stages of development are found in the female and vary from young (with multi-cellular granular-like clusters; Fig. 11) to more developed and larger embryonated eggs (Fig. 12). The eggs appeared more elongate than illustrated in the original description by Amin and Sey (1996, Fig. 4).

Histopathology

Results of the histopathological studies of specimens of *N. dimorphospinus* interfacing with intestinal sites of *L. klunzingeri* displays marked pathological damage to the host tissue. Gross examination showed worms protruding outside the lumen of the infected intestine. These are relatively large worms, allowing the possibility of worms escaping into host body cavity. Figures 13 and 14 depict normal uninfected intestinal section. Figure 13 shows the thin intact intestinal layers. Note the thin smooth muscle layer (M). The columnar epithelial cells are intact on the villi of the intestine, including the goblet cells (Fig. 14). Worms cause extensive damage to the host intestine, especially to the mucosa, upon invasion. There is a loss of host epithelial cells resulting in naked villi (Figs. 15, 16). The invasive effects of *N. dimorphospinus* on the intestinal lining are very prominent. The armed proboscis of the worm displaces the normal mucosal lining of the host with subsequent hemorrhaging and host cell necrosis (Figs. 17, 18). The parasite body is surrounded by numerous the red blood cells and granulocytes typical of invaded host tissue. The presence of numerous migrating granulocytes is consistent with acute host tissue damage. Parasite presence compresses host villi, which become necrotic (Figs. 21, 22). An abundance of ovarian balls and eggs are seen in female worms (Figs. 20, 23–25). Invading worms not only damage the mucosal lining of the host, as demonstrated by compressed host villi and epithelial cell necrosis, but also damage subsequent layers of the host intestine (Figs. 20, 26). This progresses until worms destroy the outer smooth muscle (muscularis externa) and exit into the host body cavity (Figs. 27, 28). Proboscis hooks are visible (Fig. 26), and a female fertilization cone (Fig. 25) is visible. Worms appear to occupy most of the host lumen (Figs. 17, 18). The host appears to be unable to isolate/encapsulate the worm with collagenous connective tissue; thus it continues to damage and destroy the intestinal lining until it emerges into the body cavity (Figs. 27, 28). Hemorrhaging is prominent around specimens of *N. dimorphospinus* within the invaded tissue. Necrotic tissue is abundant in the area of the parasite presence. The invasive properties of *N. dimorphospinus* are well demonstrated in the figures presented.

We conclude that *N. dimorphospinus* infecting the intestinal lining of *L. klunzingeri* causes major damage to the host tissue layers. Due to the size of the worm, it can break through the intestinal tissue layers and reside in the body cavity of the host. Initial damage to the area of worm attachment includes destruction of the absorptive epithelial lining of the intestine and exposure of host capillary beds with subsequent hemorrhaging and macrophage migration. The end result is extensive cell necrosis and destruction of the absorptive surface of the intestine with the potential for host death as the parasite exits into the body cavity.

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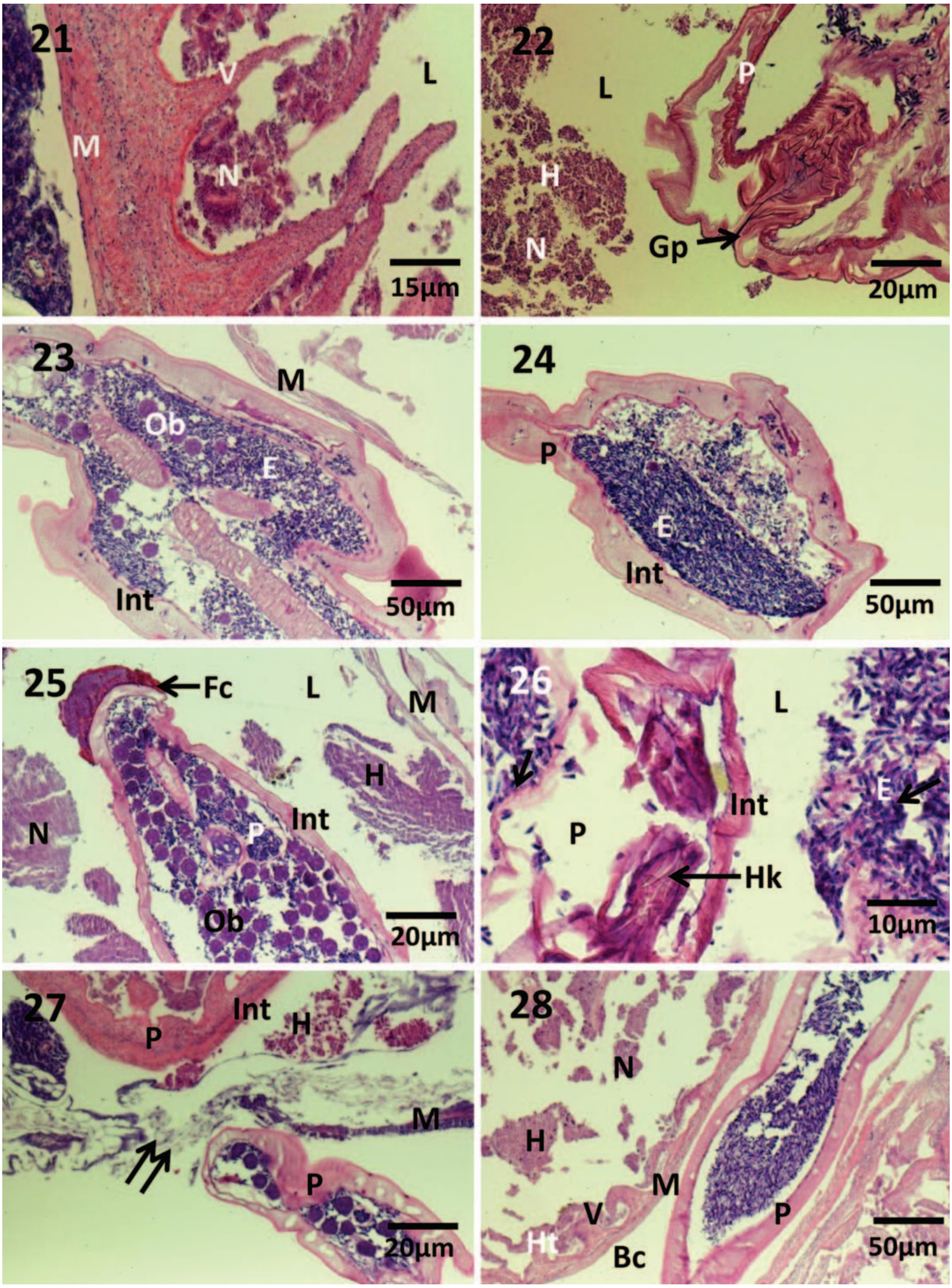

Figures 21–28. Infected intestinal tissue of *L. klunzingeri* with specimens of *Neoechinorhynchus dimorphospinus*. **21.** A high magnification of the host intestine depicting the damaged villi (V) and subsequent necrotic epithelial tissue (N) within the lumen (L). The outer smooth muscle, muscularis externa (M), is visible. **22.** The genital pore (Gp) is visible for a parasite (P) found in the lumen (L) of the host intestine. Note hemorrhaging (H) and necrosis (N). **23.** A longitudinal section of a female *N. dimorphospinus* (P) occupying the host lumen next to the intestinal muscularis externa (M) and enclosed within a

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thick muscular integument (Int). Eggs (E) and ovarian balls (Ob) are visible. **24.** A cross section of the gravid female specimen (P) with numerous eggs (E) in 2 compartments. The outer integument (Int) is labeled. **25.** A female parasite (P) with an outer integument (Int) filled with eggs and ovarian balls and with a prominent fertilization cone (Fc) occupying the host intestinal lumen (L) causing hemorrhaging (H) and displacement of the intestinal muscularis externa (M). **26.** An acanthocephalan (P) with proboscis hooks (Hk) visible with the outer tegument (Int) within the host lumen (L) of the intestine. Eggs (E) of a second parasite are visible (right). **27.** A parasite (P) is migrating through the host intestine (arrows) into the body cavity. The outer tegument (Int) of the parasite is visible and another parasite (P) with ovarian balls is seen on the outside (lower right). Note necrotic tissue (N). **28.** The worm (P) is outside the host intestine (Ht) in the body cavity (Bc). The lumen of the host tissue contains damaged villi (V) and a thin outer smooth muscle coat (M). Necrotic tissue (N) and hemorrhaging (H) visible within the lumen.