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THE FUNCTIONAL MORPHOLOGY OF THE PUTATIVE INJECTING APPARATUS OF *SPELEONECTES TANUMEKES* (REMIPEDIA)

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A B S T R A C T

Ever since the first individuals of the class Remipedia were discovered, it has been suggested that these animals are capable of injecting their prey with a venomous or digestive substance using the fang on their maxillules. In this study, we investigate the functional morphology of the putative injecting apparatus of *Speleonectes tanumekes*. Serial sectioning of specimens shows a pair of well-developed glands in the anterior part of the trunk. Spatial interpretations of serial sections suggest that extensions of the glandular cells collectively continue as ducts that lead to the distal segments of the maxillules where a complex of apodemal muscles, associated with a bulbous reservoir, serve to facilitate injection. Different components of the supposedly injectable substance appear to be separated throughout the apparatus.

Introduction

The remipede *Speleonectes epilimnius* Yager and Carpenter, 1999 is capable of stabbing other organisms with its maxillules and frequently does so when overcoming prey (Carpenter, 1999). This capability has long been assumed characteristic for remipedes in general. It has also been suggested that these animals have the capability of injecting their prey with a venomous or digestive substance while stabbing (Carpenter, 1999; Felgenhauer et al., 1992; Schram and Lewis, 1989). Morphological evidence that led to this assumption includes an apical pore on the fang of the maxillule, a reservoir within distal segments of the maxillule, a cephalic gland, and a connecting duct (Boxshall, 1997; Felgenhauer et al., 1992; Schram and Lewis, 1989; Yager, 1991). However, Schram and Lewis (1981) were previously unable to find specific musculature that would facilitate injection of a substance, as is usually present in arthropods capable of injecting substances (see below). These authors suggested that bulging of cuticular muscles upon the flexion of the maxillule could serve as injecting facilitator, but the pinnate orientation of the muscle fibers in the maxillule makes this unlikely (Boxshall, 1997).

Although uncommon among crustaceans, injecting substances into other organisms is common among many other species of arthropods. Musculature that facilitates injection is present in a variety of arrangements among taxa that posses apparatuses for injection. Often this musculature is part of the gland producing the injected substance. Many arachnids, for example the black widow spider *Latrodectus mactans* (Fabricius, 1775), have spiraling musculature surrounding the venom glands (Felgenhauer, 1999; Foelix, 1982). The venom glands of the chilopod *Scolopendra cingulata* (Latreille, 1829) contain both circular and radial gland musculature, whereas other chilopods lack this radial musculature and only have circular muscles associated with their venom glands (Lewis, 1981). However, cuticular musculature is also known to facilitate injections. The telson in many scorpions, for example, has a pair of venom glands located between the cuticle and cuticular musculature. Upon contraction, the glands are compressed between the muscles and the cuticle, thereby facilitating the injection (Hjelle, 1990). Among crustaceans, branchiuran taxa typically have an injecting apparatus associated with a pre-oral spine (Gresty et al., 1993; Swanepoel and Avenant-Oldewage, 1992). However, the exact arrangement of musculature that facilitates injection for these species is not known. In addition, caprellid amphipods carry a spine on their second gnathopod that is often referred to as a ''poison tooth'' (Caine, 1980; Guerra-García et al., 2002; Krapp-Schickel and Takeuchi, 2005; Krapp-Schickel and Vader, 1998; McCain, 1965). Whether this structure actually produces poisonous or venomous substances, or is capable of injecting such substances remains unclear (Guerra-García and Krapp-Schickel, personal communication).

In this study, we examined specimens of *Speleonectes tanumekes*, Koenemann, Iliffe and Van der Ham, 2003 to elucidate the internal morphology of the putative injecting apparatus in this remipede.

Material and Methods

Divers collected specimens of *Speleonectes tanumekes* on 15 January 2004, in Basil Minns Blue Hole, Great Exuma Island, Exuma Cays, The Bahamas (23°28′N, 75°45′W), at a depth of 33-43 m. Four specimens were fixed in Trump's fixative (glutaraldehyde and formalin in a mixture of sodium cacodylate buffer), rinsed in 0.2 M sodium cacodylate, postfixed in 2% osmium tetroxide and dehydrated through a graded series of ethanol and acetone, after which they were infiltrated and embedded in Spurr's low viscosity resin (Spurr, 1969). Thin sections for transmission electron microscopy (TEM) were obtained using a Diatome (Diatome®, USA) diamond knife, and were sequentially stained using aqueous uranyl acetate and lead citrate and examined on a Hitachi 7000 TEM. Semi-thin sections were prepared by the same procedures described for thin sections, but sections were stained in methylene blue borax at 50°C for one minute, and examined on a Nikon Eclipse E400 compound light microscope equipped with a Nikon Coolpix 990 digital camera. One specimen examined with the scanning electron microscope (SEM) was preserved in Trump's fixative, rinsed in 0.2 M sodium cacodylate, postfixed in 2% osmium tetroxide and dehydrated through a graded series of ethanol and acetone. It was

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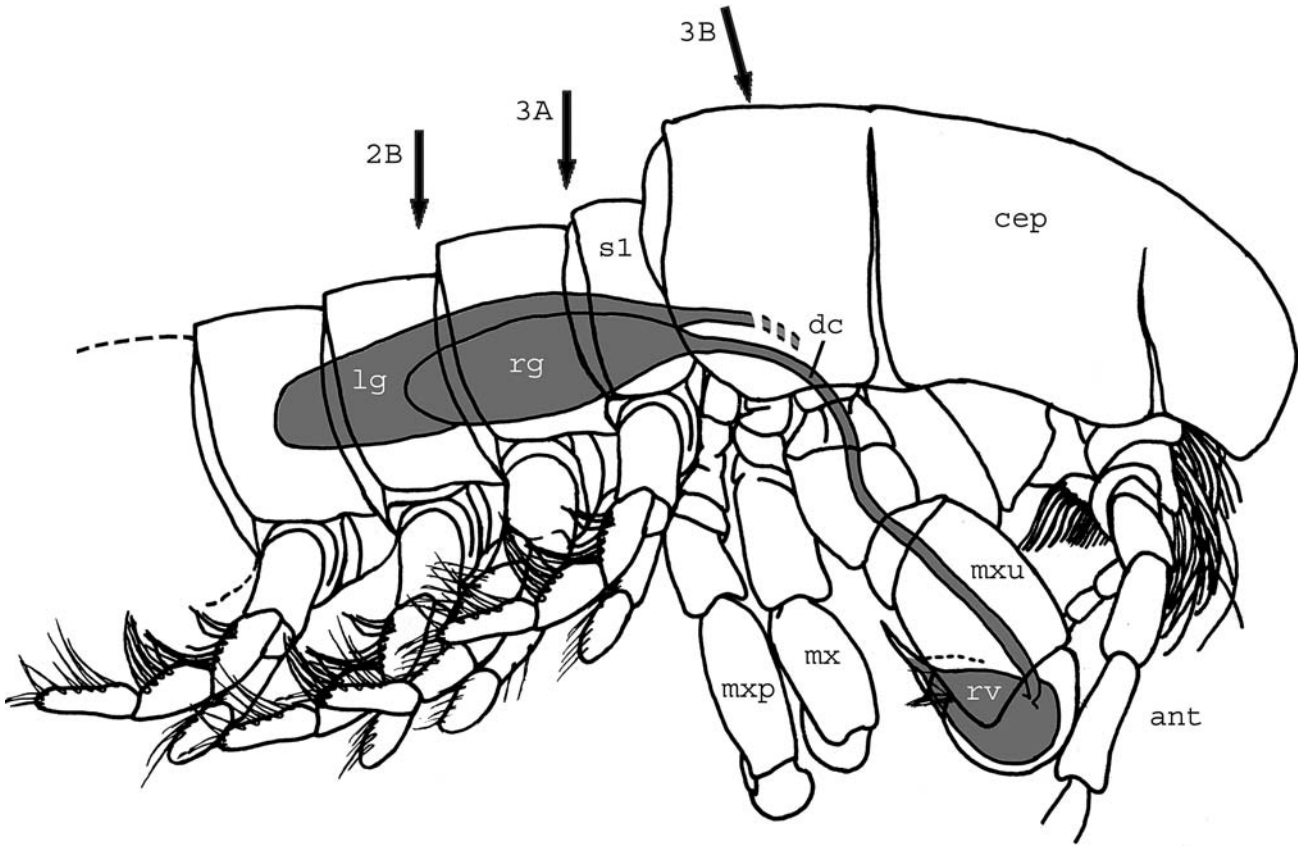

Fig. 1. Schematic sagittal overview of the anterior region of *Speleonectes tanumekes*. The dotted duct of the left gland indicates where the duct descends into the left maxillule. Arrows indicate locations of cross-sections. ant = antennule; cep = cephalon; dc = duct; lg = left gland; mx = maxilla; mxp = maxilliped; mxu = maxillule; rg = right gland; rv = reservoir; s1 = first trunk somite.

subsequently critical-point dried, mounted on stubs and sputter-coated with gold-palladium, and examined with a Hitachi S-3000N SEM at an accelerating voltage of 15 kV.

Results

The anterior trunk region of *Speleonectes tanumekes* contains two glands, which are unequal in length. In all the material examined, the left gland is almost a single somite longer than the right gland (Fig. 1). The esophagus is located between these two glands (Figs. 2B, 3A), and the esophagus connects to the midgut directly posterior to the right gland. The glandular cells span the length of the glands, and in the anterior portion of each gland, these cells become reduced in diameter and extend into the cephalon. These cellular extensions collectively form the ducts that lead from the glands through the cephalon and descend into the maxillules (Fig. 3B). A thin layer of circular musculature surrounds each gland (Fig. 4A-C). The secretory product in these glands consists of three components, an electron lucid component (Figs. 2A, 4A, 6B) and two electron dense components (Figs. 2A, 4F, 6B). One of these electron dense components is regular and rod-shaped (Fig. 4E, F), whereas the other is irregular and granular (Fig. 4B, F). The irregular electron dense component is often aggregated in spherical accumulations, which can differ distinctly in density (Fig. 4F). Glandular cells contain either the electron lucid component, the electron lucid component in combination with the regular electron dense component, or the electron lucid component in combination with the irregular electron dense component. The regular and irregular electron dense components are maintained in separate cells throughout the glands and cellular extensions (Figs. 4F, 7D). The nuclei of the glandular cells, which have distinct nucleoli, are commonly located at the posterior periphery of the gland. Large amounts of rough endoplasmatic reticulum (RER) are located in the vicinity of the nuclei of cells that contain electron dense components (Fig. 4B-D). The RER becomes less abundant anteriorly in the cell. The RER of cells containing the regular electron dense component is very distinctive in morphology as the cisternae form a network of round enclosures (Fig. 4D). The RER in cells containing the dense irregular component, on the other hand, form the typical parallel arrays of cisternae (Fig. 4B). It is noteworthy that between the cisternae that form the round enclosures, the electron dense component does not have the regular rod-shaped appearance as elsewhere in these cells (Fig. 4D, E). The cells containing the electron lucid component without either of the electron dense components have very little RER (Fig. 4A). Nevertheless, these cells have cellular extensions similar to cells containing the electron dense components. Large cephalic glands lay against the ducts (Fig. 3B) and even though these are similar in appearance, i.e., composed of very long cells that presumably contain a secretory product, they do not connect to the ducts, nor do they lead to the distal maxillulary segments.

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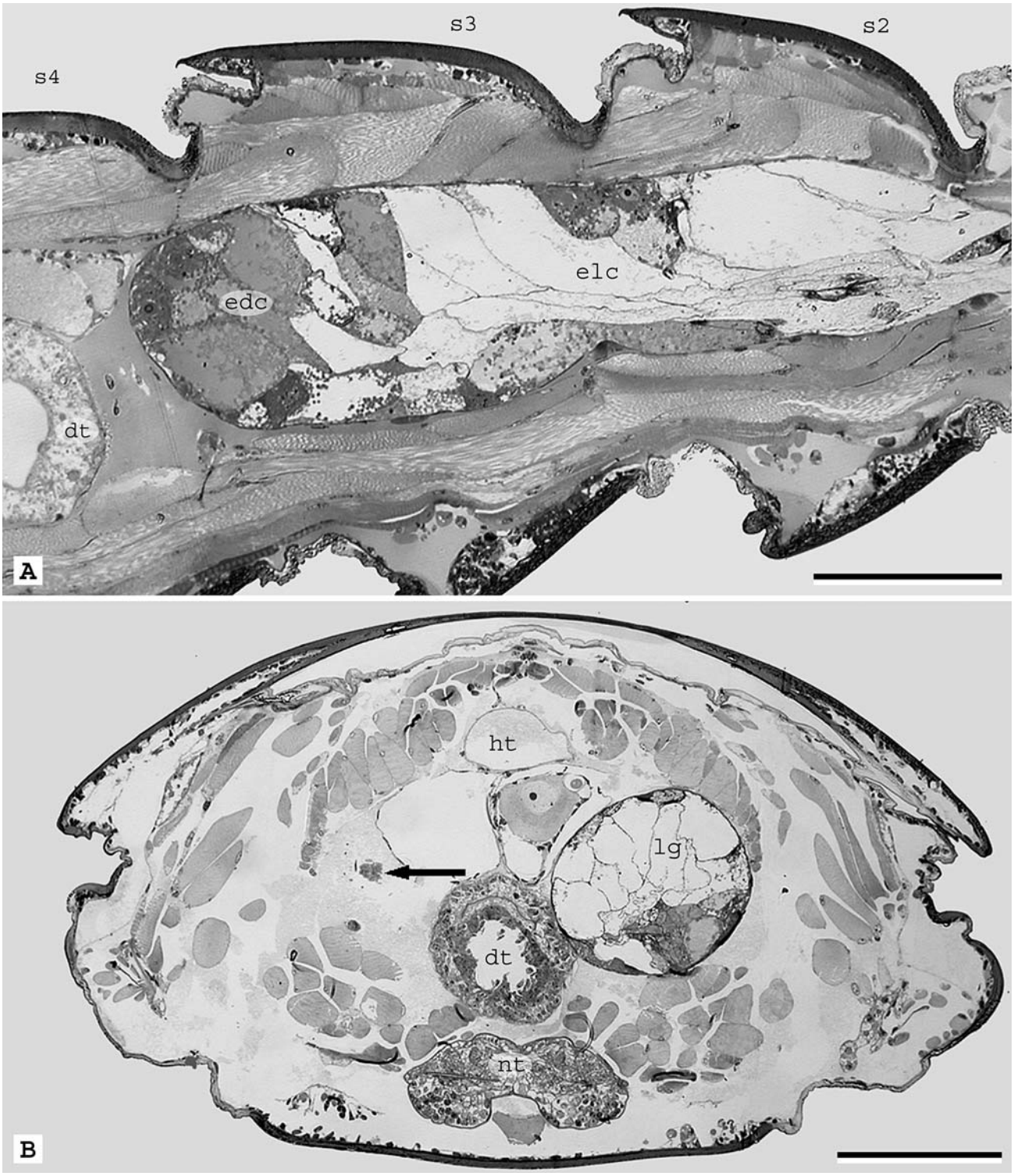

Fig. 2. A, sagittal section of anterior trunk somites 2, 3, and 4 through the left gland; B, cross-section of third trunk somite, black arrow indicate most posterior extension of right gland, scale bars indicates 0.2 mm. dt = digestive tract; edc = electron dense component; elc = electron lucid component; ht = heart; lg = left gland; nt = nervous tissue; s2 = second trunk somite; s3 = third trunk somite; s4 = fourth trunk somite.

Proximally, in the fifth segment of both maxillules, the diameter of the entire duct decreases (Figs. 5A, 6A, 7C). Directly distal to this restriction, the cellular extensions form a bulbous reservoir filling the majority of the lumen of the distal maxillulary segments (Figs. 5A, 6A, B). Originating from the seventh segment and extending into the lumen of the fifth and sixth segments, a well-developed apodeme (Figs. 6B, 7A) extends along the ventral side of the reservoir (Fig. 5A, B). Two pairs of muscles attach to this apodeme, a dorsal-ventral pair and a lateral pair (Figs. 5B, 6B). The dorsal

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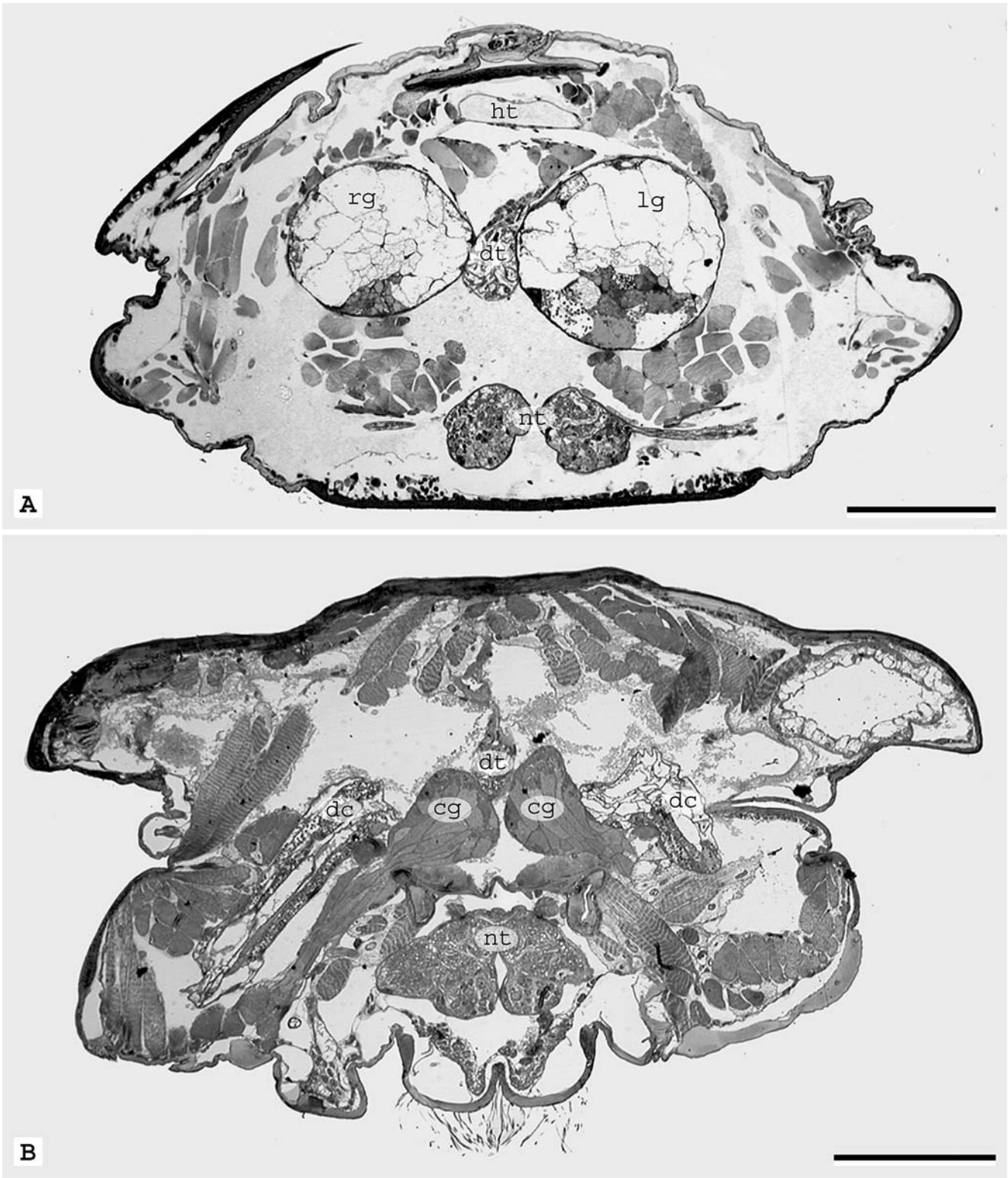

Fig. 3. A, cross-section of second trunk somite.; B, slightly oblique cross-section of cephalon, showing ducts extending into the basis of maxillules, scale bars indicate 0.2 mm. cg = cephalic gland; dc = duct; dt = digestive tract; ht = heart; lg = left gland; nt = nervous tissue; rg = right gland.

muscle extends posteriorly around the reservoir where it attaches to cuticle, surrounding mostly the proximal part of the reservoir. This muscle appears to be closely positioned alongside the restriction between the duct and reservoir (Fig. 7C). The ventral muscle extends from the apodeme in the fifth segment into the fourth segment where it attaches to the dorsal cuticle of the fourth segment. Two lateral muscles surround the reservoir and attach to the cuticle on the anterior and

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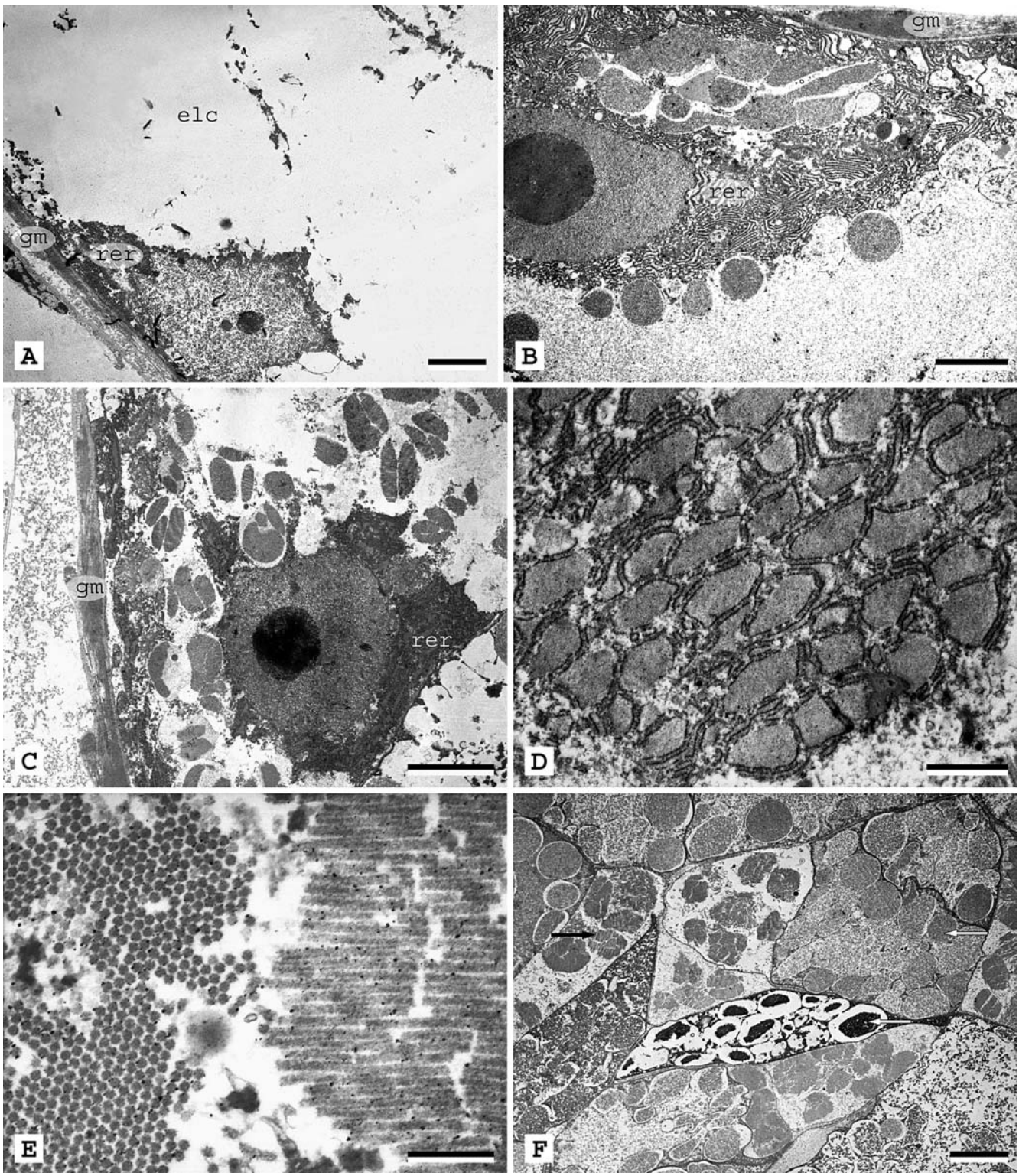

Fig. 4. A, nucleus of glandular cell containing only electron lucid component, scale bar indicates 5 μm; B, nucleus of glandular cell containing irregular electron dense component, note prominent nucleolus, scale bar indicates 5 μm; C, nucleus of glandular cell containing regular electron dense component, scale bar indicates 5 μm; D, rough endoplasmatic reticulum associated with the regular electron dense component, scale bar indicates 1 μm; E, regular electron dense components, scale bar indicates 0.5 μm; F, cross section of the anterior region of the gland, white arrows indicate irregular electron dense component, black arrow indicates regular electron dense component, scale bar indicates 5 μm. elc = electron lucid component; rer = rough endoplasmatic reticulum; gm = gland musculature.

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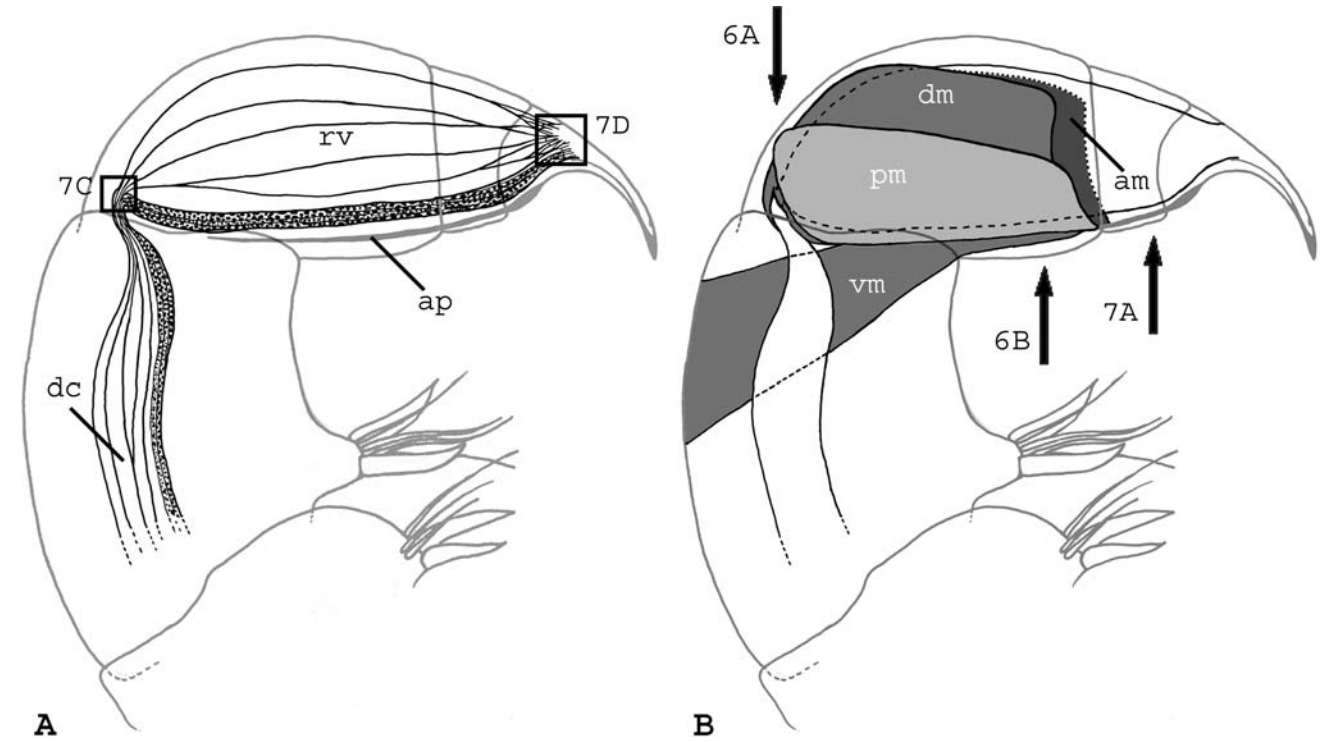

Fig. 5. A, schematic posterior view of maxillule indicating the position of the duct and the reservoir in this appendage, boxes indicate locations of sagittal sections; B, schematic posterior view of maxillule indicating the position of the four apodemal muscles surrounding the reservoir, arrows indicate locations of cross-sections. am = anterior apodemal muscle; ap = apodeme; dc = duct; dm = dorsal apodemal muscle; pm = posterior apodemal muscle; rv = reservoir; vm = ventral apodemal muscle.

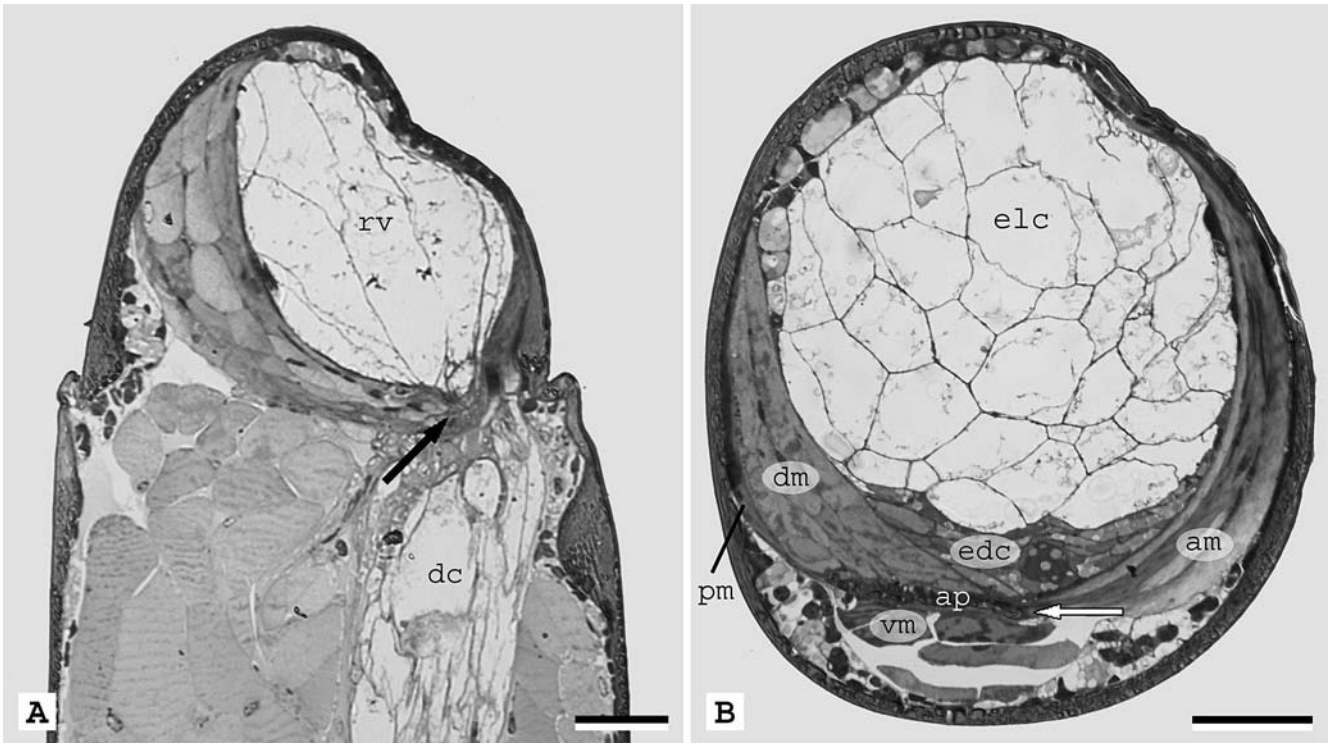

Fig. 6. A, cross section of fourth and fifth segments of the maxillule, black arrow indicates the restriction between duct and reservoir; B, cross section of fifth segment of the maxillule, white arrow indicates lateral insertion of anterior apodemal muscle to the apodeme, scale bars indicate 50 μm. am = anterior apodemal muscle; ap = apodeme; dc = duct; dm = dorsal apodemal muscle; edc = electron dense component; elc = electron lucid component; pm = posterior apodemal muscle; rv = reservoir; vm = ventral apodemal muscle.

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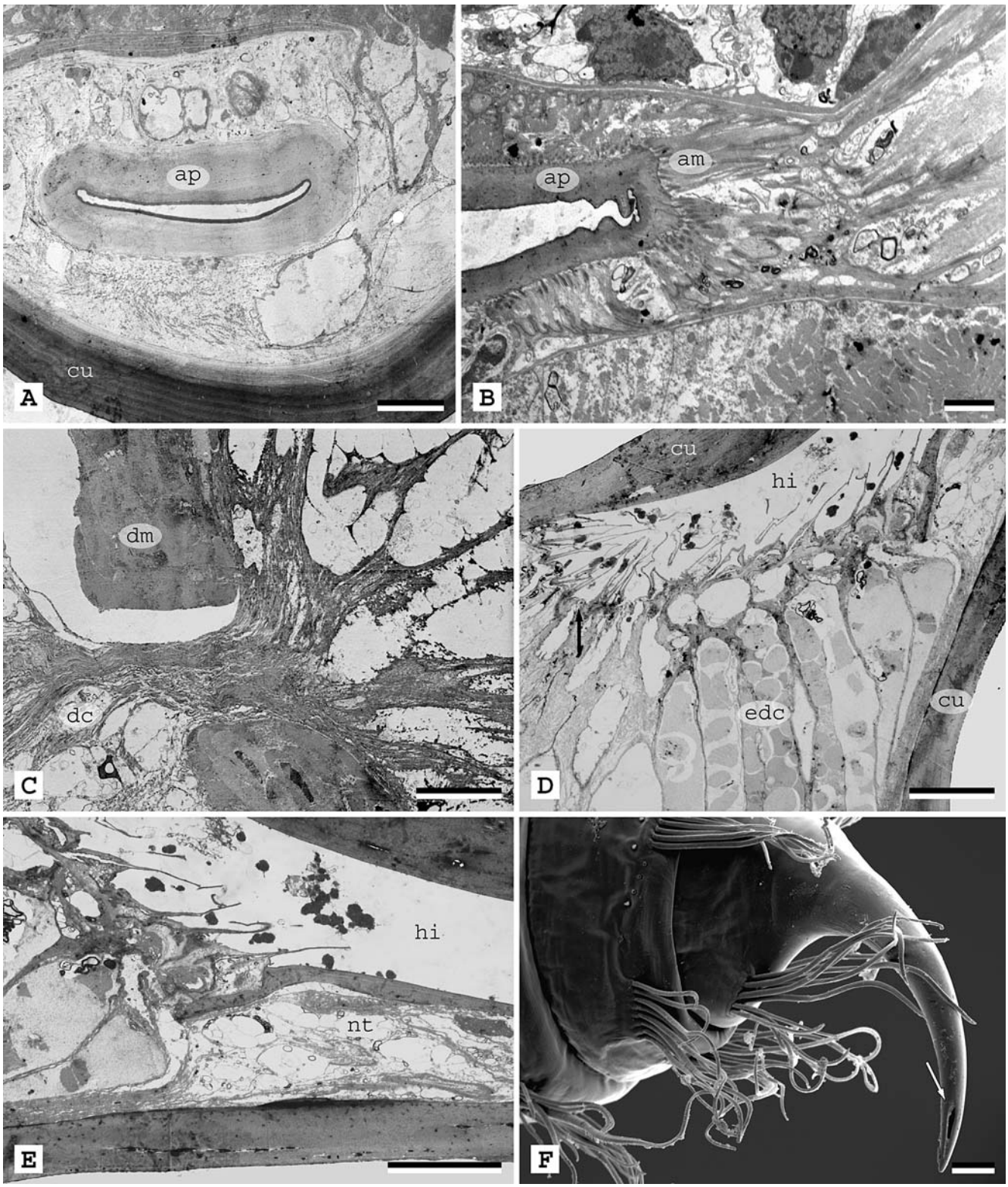

Fig. 7. A, cross section of sixth segment of the maxillule, scale bar indicates 5 μm; B, lateral insertion of anterior apodemal muscle to the apodeme in fifth segment of the maxillule, scale bar indicates 2 μm; C, sagittal section of the restriction between duct and reservoir in the maxillule, scale bar indicates 10 μm; D, sagittal section of the fang region on seventh segment of the maxillule, scale bar indicates 10 μm; E, sagittal section of the fang indication the cuticular division between hollow interior and nervous tissue, scale bar indicates 10 μm; F, scanning electron micrograph of distal segments of maxillule, arrow indicates apical pore, scale bar indicates 25 μm. am = anterior apodemal muscle; ap = apodeme; cu = cuticle; dc = duct; dm = dorsal apodemal muscle; edc = electron dense component; hi = hollow interior; nt = nervous tissue.

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posterior side of the fifth segment (Fig. 5B). The lateral muscle surrounding the reservoir posteriorly also encapsulates the above mentioned dorsal muscle and is much thinner than the lateral muscle surrounding the reservoir anteriorly (Fig. 6B).

The cellular extensions terminate with long, thin valve-like structures (Fig. 7D), which are positioned in the hollow interior of the fang on the seventh segment. The hollow interior extends through the fang, leading to the apical pore (Fig. 7F). A layer of cuticle separates the hollow interior from nervous tissue that extends into the fang ventrally (Fig. 7E). No external sensilla could be observed on the surface of the fang (Fig. 7F).

Discussion

Without empirical evidence of transfer of substances from the glandular structures described above into a prey animal, we can only assume that these structures are capable of functioning as an injecting apparatus. We hypothesize that this injecting apparatus would function as follows. Both electron dense components found in the glandular cells are presumably synthesized by RER. Contractions of circular musculature surrounding the glands facilitate movement of the glandular contents into the reservoir. By contraction of the dorsal muscle and the two lateral muscles, the maxillulary apodeme is pressed against the reservoir presumably injecting its contents. At this time the various components of the injected substance are combined in the hollow interior of the fang. The ultrastructural arrangement of the terminal portion of the cellular extensions suggests that it functions similar to the cellular non-return valves described by Dass and Jangi (1978) of the chilopod *Scolopendra morsitans* L. The restriction between duct and reservoir prevents the contents of the reservoir from moving back into the duct during the injection. Contraction of the ventral muscle enlarges the volume of the reservoir after injection.

If indeed injected, the effect that this substance may have on the physiology of the prey is unclear. The regular electron dense component has a striking resemblance to hemocyanin (Magnum, personal communication), a commonly found respiratory pigment among arthropods. The presence of this component has also been observed in the putative injecting apparatus of *Speleonectes tulumensis* Yager 1987 (Felgenhauer, unpublished results). The question arises as to why these animals would inject an oxygen carrying respiratory pigment into their prey? Recent studies have demonstrated that sub-units of the hemocyanin molecule show phenoloxidase activity (Adachi et al., 2003; Adachi et al., 2005; Pless et al., 2003). We hypothesize that in addition to a hemocyanin-like component, another injected component initiates the conversion of the hemocyanin-like component into active phenoloxidase. By injecting a large amount of the hemocyanin-like component in combination with such a converting component, the injected substance could have a deleterious effect on the prey.

Morphological adaptations of appendages that aid crustacean predators in capture of their prey commonly result in raptorial structures, e.g., chelae, gnathopods, the second thoracopod of stomatopods (McLaughlin, 1980), and the first thoracopod of some anostracans (Rogers et al., 2006). The injecting apparatus as described in this study confirms a predatory strategy that is, among crustaceans, currently confined to the Remipedia.

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