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Pectine Development in Scorpion Embryos and First and Second Instars

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Pectine development in scorpion embryos and first and second instars

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Summary

The pectines are complex sensory organs that extend from the ventral surface of the anterior opisthosoma (mesosoma) in all extant scorpions and nearly all the fossil scorpions that have been examined. The pectines are synapomorphic for the Order Scorpiones. In this investigation, the scanning electron microscope (SEM) was used to study the development of the pectines in representatives from five scorpion families. In the more basal families (e.g., Vaejovidae) with apoikogenic development, the pectines start to develop early with enlargement of the limb buds on the third opisthosomal segment. The primordial pectines become elongate lobes attached to the ventro-lateral surface of the mesosoma. A series of striations develop on the pectinal surface, and these striations eventually become a row of teeth at the posterior margin of the pectines. As they develop, the pectines temporarily form the ventral surface of the fourth opisthosomal segment. The distal ends of the pectines separate from the fourth segment and become mobile, attached only at their proximal ends. This is the state of pectinal development as spiracles (indicators of terrestrialization) and sternites become evident on the ventral surface of mesosomal segments. The pectinal peg sensilla start development much earlier than the other sensory structures on the scorpion. The peg sensilla first appear in the embryos as small knobs on the ventral surface of the pectinal teeth. A tiny opening develops in the center of each knob, and nerve fibers can be seen extending toward the central nervous system. The early development of the sensilla and some first-instar choice experiments suggest the pectines may be functional in advanced embryos and first instars. The walls of the primordial knob-like sensilla gradually elongate so the second instar has many peg sensilla on the ventral surface of each pectinal tooth. In the more derived scorpion species with katoikogenic development (*Hadogenes paucidens,* Hemiscorpiidae; *Pandinus imperator*, Scorpionidae), the pectines start development in the embryos, but the early steps were not observed as in the more basal families. Pectinal formation appears to be accelerated with some early steps reduced or eliminated. In embryos of *H. paucidens* and *P. imperator*, the pectines are separated from the ventral surface of opisthosomal segment 4 and attached only at their proximal ends as the spiracles and sternites appear on the ventral surface of mesosomal segments. Spiracle and sternite development is preceded by the appearance of sturdy, transitory setae (bristles) on the ventral body surface. These setae have no apparent function in the embryos, but would seem to be advantageous in an aquatic environment with prey and predators beneath the scorpion.

Introduction

In the basal scorpion families (e.g., Buthidae, Vaejovidae, Caraboctonidae; Coddington et al., 2004), the embryos develop within the maternal ovariuterine tubules with nutrients obtained by passage through the epidermis. This mode of development was termed apoikogenic (Greek, 'away from home') by Laurie (1896a, b). In the more derived scorpion familes (e.g., Hemiscorpiidae, Scorpionidae), the maternal tubules and embryo development are modified for delivery of nutrients to the embryo's mouth and digestive tract (Polis & Sissom, 1990; Farley, 1999, 2001d; Volschenk et al., 2008; Warburg, 2010). Each embryo develops within a diverticulum of the ovariuterine tubules (katoikogenic development; Greek 'at home'; Laurie, 1896a, b). Authors as early as 1853 recognized that there are two major differences in the organization of the female reproductive tract of scorpions (Warburg, 2010), and within these two groups numerous other distinctions are subsequently described (Farley, 2001d; Volschenk et al., 2008; Warburg, 2010).

The earlier light microscopic studies of scorpion development were reviewed by Anderson (1973), Polis & Sissom (1990), and Farley (1999, 2001d). The scanning electron microscope (SEM) was used to examine the changing features of embryos of some species with apoikogenic development (Farley, 1996, 1998, 1999, 2001a-d, 2005, 2008, 2010). The investigation with SEM is herein extended with a focus mainly on the comparative development of pectines in four species with apoikogenic development and two species with katoikogenic development. The former four species are: *Smeringurus mesaensis* (Vaejovidae), *Cen-*

truroides vittatus (Buthidae), *Hadrurus arizonensis* (Caraboctonidae), and *Superstitionia donensis* (Superstitioniidae). The two species with katoikogenic development are *Hadogenes paucidens* (Hemiscorpiidae) and *Pandinus imperator* (Scorpionidae).

For those scorpions with apoikogenic development, each embryo is enclosed in a thin, transparent and fibrous membrane (amnion) and a thicker cellular membrane, the serosa (Francke, 1982; Farley 1999, 2001d). The oocytes commonly have much yolk, and the embryos enlarge within the maternal tubules. Nutrients must pass from the maternal hemolymph through the ovariuterine wall, serosa, amnion and embryo epidermis (Toolson, 1985). These membranes are retained until parturition in most species with apoikogenic development (Francke, 1982).

There has apparently been strong selective pressure for effective delivery of nutrients to scorpion embryos developing *in utero*. Scorpion embryos are much alike after the first molt, but there are substantial phylogenetic differences during embryogenesis as adaptations for embryo nourishment (Laurie, 1896a, b; Polis & Sissom, 1990; Farley, 1999, 2001d; Warburg, 2010). This developmental heterochrony (Mathew, 1959a) is further demonstrated in the present investigation.

In species with katoikogenic development, the oocytes have little yolk since oral feeding begins early. The amnion and serosa may be bypassed, reduced or replaced with others during development in more derived scorpions (Laurie, 1896c; Matthew, 1956, 1960; Farley, 1996, 1998, 1999, 2001d). In embryos of *Heterometrus scaber* (Scorpionidae; katoikogenic development), the embryos are enclosed sequentially in an embryonal capsule, trophamnion and second embryonal membrane, but the origin of these membranes differs from that of the amnion and serosa in species with apoikogenic development (Mathew, 1956). Embryos with katoikogenic development commonly have thin-walled, bilateral swellings (dorso-lateral processes) on their mesosomal segments (Mathew, 1956; Farley, 1999, 2001d). These dorso-lateral processes just above the heart are presumed to facilitate gas exchange with maternal hemolymph.

In most scorpions with katoikogenic development, each diverticulum with a confined embryo has an elongate appendix at its distal end (Mathew, 1956; Polis & Sissom, 1990; Farley, 1999, 2001d; Warburg, 2010). Maternal nutrients are apparently absorbed into the appendix and temporarily stored there until passage to the embryo mouth. Contractions of the embryo pharyngeal muscles are thought to provide the force for movement of nutrient fluid into and through the appendix channels to the embryo mouth and digestive tract (Mathew, 1948, 1956; Polis & Sissom, 1990). The

embryo chelicerae are commonly specialized to enclose the proximal end (teat) of the appendix.

In embryos with apoikogenic development, the pectines start formation very early with enlargement of the limb buds on the third opisthosomal segment while the limb buds disappear on most of the other opisthosomal segments (Farley, 2001a). A changing sequence of features takes place as the pectines gradually become a mobile and complex sensory organ (Swoveland 1978; Foelix & Müller-Vorholt, 1983; Gaffin & Brownell, 1992, 1997a, b, 2001; Brownell, 2001; Farley, 2001b-d, 2005, 2008, 2010; Gaffin, 2001, 2010; Kladt et al., 2007; Wolf, 2008).

As shown herein, the mesosoma enlarges rapidly in embryos with oral feeding, since the mesosoma has most of the digestive tract and digestive gland (Mathew, 1956; Hjelle, 1990; Farley, 1999, 2001d). The pectines appear also to undergo accelerated development compared with the more gradual process in embryos with apoikogenic development. In spite of apparent acceleration and deletion of steps in katoikogenic development, the resulting pectines eventually have similar structure and function in species from basal and derived scorpion families (Swoveland, 1978; Hjelle, 1990; Polis & Sissom, 1990).

Materials and Methods

Specimens of *Smeringurus* (formerly *Paruroctonus*) *mesaensis* (Vaejovidae) were collected at night in the Mohave Desert near Palm Springs and Indio, California. Specimens of *Centruroides vittatus* (Buthidae), *Hadrurus arizonensis* (Caraboctonidae)*, Superstitionia donensis* (Superstitioniidae), *Hadogenes paucidens* (Hemiscorpiidae) and *Pandinus imperator* (Scorpionidae) were purchased from suppliers (Strictly Reptiles, Hollywood, FL; Glades Herp Farm, Bushnell, FL; Hatari Invertebrates, Portal, AZ and Phoenix Exotica, Phoenix, AZ). The scorpions were maintained in the laboratory in terraria with cardboard shelters and a continuous supply of water and crickets. For the species of this investigation, references and information about taxonomy and other aspects of their biology are provided by Polis and Sissom (1990), Sissom (1990), Stockwell (1992), Fet et al. (2000), Soleglad and Fet (2003) and Coddington et al. (2004).

Microscissors and forceps were used to remove embryos from the ovariuterine tubules and surrounding membranes and expose tissues within the embryos. The serosa is easily removed by dissection, but the thin and transparent amnion is often very close to the body surface and difficult to remove without damage to the embryo. For species with katoikogenic development, the embryonic membrane was left in place or removed if some membrane was still present in more advanced embryos. Fine pins were sometimes used to hold the

dissections in place during initial application of glutaraldehyde. The physiological saline used for all dissections was a modification of a saline developed by Bowerman (1976). It contained the following (g/l). NaCl, 14.0; KCl, 0.7; MgCl$_2$, 0.17; CaCl$_2$, 0.9, Na$_2$SO$_4$, 0.7; NaHCO$_3$, 0.13; trehalose, 1.5; sucrose, 3.4; glucose, 1.8.

Tissues for electron microscopy were fixed (12−24 hr, 23−25°C) with 4% glutaraldehyde in 0.05 M cacodylate buffer (Lane, Harrison & Bowerman, 1981). The tissues were washed in 0.05 M cacodylate buffer and postfixed (2−4 days, 23−25°C) in 1% OsO$_4$ in 0.05 M cacodylate buffer. Tissues were dehydrated in acetone, critical-point dried (Balzers, CDD020) and sputter-coated with gold/palladium. Tissues were examined at 10−15 kv with a Philips XL-30 scanning electron microscope.

The age of the females and duration of pregnancy were unknown at the time of acquisition, so the embryo stages herein described are not attributed to specific times during or after gestation. The reported gestation periods range from 2−18 months (Polis & Sissom, 1990), but the rate is variable and dependent on environmental conditions (Polis & McCormick, 1986; Polis & Sissom, 1990).

Results

Apoikogenic Development

Smeringurus mesaensis (Vaejovidae)

More is known about the details of pectine development in this species than any other scorpion. The early stages of embryogenesis were described by (Farley, 1996, 1998, 1999, 2001a-d), and changes in the pectines were sometimes included. The main features of pectine development in this species are provided here to show the sequence that appears to be typical for apoikogenic development. This also gives a basis for comparison with other species examined in this investigation.

Figure 1 shows a ventral view of a scorpion embryo at a stage when the metasoma has nearly all its five segments and has started a ventral flexure. Limb buds in the prosoma and mesoma are prominent, and as reported earlier (Farley, 2001a) the limb buds on the third opisthosomal segment are larger than the others in the opisthosoma. The third-segment limb buds continue to increase in length and extend dorso-laterally as primordial pectines as the limb buds disappear on most of the other opisthosomal segments. The prosomal limb buds in Figure 1 are still partially covered with amnion, but the pedipalps have started to divide at their distal tip, and the chelicerae are widely separated from each other on either side of the stomodeum (mouth). The two

chelicerae will eventually come together dorsal to the mouth (e.g., Fig. 12, 14; Farley, 1998, 1999, 2001b-d).

Figure 2 is at a more advanced stage with the pectine as an elongate structure that extends dorso-laterally from the ventral surface of the third opis-thosomal segment. The pectine appears to be attached to the body wall for most of its length. It is fairly uniform in width except for a rounded tip, and it has a smooth surface without the transverse striations that eventually appear (Figs. 3, 4, 7, 13). At this stage, a rostrum (labrum) is evident just anterior to the mouth, and the chelicerae are still far apart on either side of the mouth. The pedipalps are divided distally; legs 1−4 are becoming segmented. The metasoma is sharply flexed ventrally and apparently has all five segments with a tapered telson at its tip. Other examples (*Smeringurus mesaensis*) of pectines at this stage are shown in Farley (1998, 2001a).

Especially to be noted in Figure 2, the segments and appendages in all three body regions (prosoma, meso-soma, metasoma) are undergoing substantial development. This is also evident in the other examples of apoikogenic development in this investigation (Figs. 5−7, 12, 14). This is in contrast to the species with katoikogenic development (Figs. 18, 20, 28, 29) where the mesosoma is especially large and prominent in comparison with the prosoma and metasoma.

The elongate pectines initially have no transverse striations (Fig. 2; Farley, 2001a), but striations eventually appear. The striations begin first at the distal end of the pectines so the pectines look like paddles at this stage. Figure 3 is a ventro-posterior view of an embryo (supine) with paddle-like pectines. In this view, opisthosomal segments 1−8 are clearly visible. The first opisthosomal segment is much reduced in size, and the third opisthosomal segment is clearly the source of the pectines. The pectines slightly overlap the ventral surface of opisthosomal segment 4 at this stage; the overlap becomes more substantial in later stages (Figs. 5−7, 21). The prosomal legs are segmented. Other examples (*Smeringurus mesaensis)* of pectines at this paddle-like stage are provided in Farley (1998, 2001a, d).

In a later stage, there are transverse striations for almost the entire length of the pectines. The pectines are narrow in width, ribbon-like and joined with the ventro-lateral body wall. An example of this stage is shown in Figure 7 (*Centruroides vittatus)* and in Farley (2001c, d) for *Smeringurus mesaensis*.

In Figure 4, a longitudinal groove (asterisks) divides the pectine into anterior and posterior regions. The anterior region is still joined with the lateral wall of the mesosoma while the posterior pectinal edge is starting to separate slightly from the body wall. In later instars, the anterior region will have numerous mechanoreceptors and will contain nerve fibers extending toward the

central nervous system from the thousands of peg sensilla on the ventral surface of the pectinal teeth (Figs. 8, 9, 11, 16; Foelix & Müller-Vorholt, 1983; Brownell, 2001; Gaffin, 2001; Gaffin & Brownell, 1992, 1997a, b, 2001; Kladt et al., 2007; Wolf, 2008). The striations at the posterior margin of the pectine in Figure 4 continue indentation and separation of teeth (Figs. 5, 6). A longitudinal pectinal groove is also present in Figures 13, 21 and 22.

In Figure 5 the pectines are oval in shape and have distinct anterior and posterior regions. They are still attached to the ventral surface of opisthosomal segment 4. The transverse striations at the posterior margin have not yet formed separate teeth. At this stage, there are small lobes (coxapophyses 1, 2) medial to legs 1 and 2. These lobes gradually extend anteriorly and form the ventral wall of the preoral tube (Fig. 14; Farley, 1999, 2001d. 2005, 2008).

In Figure 5 the legs (L1-L4) are segmented, and there is a sternum on the ventral surface of the prosoma. Opisthosomal segment 1 is no longer evident externally. Segment 2 is evident but has not yet started to form the paired lobes of the genital operculum that can be seen in later stages (Figs. 14, 15, 21−23, 32). Other examples of this oval-shaped stage of pectinal development are shown in Farley (1999, 2001b-d, 2005).

In Figure 6, the thin and transparent amnion was not removed, but the surface features beneath it can be seen. The pectines now have teeth at their posterior edges, and spiracles are evident near the posterior margin of the developing sternites. At the stage of spiracle appearance (Farley, 2005), the pectines are attached to the body wall only at their proximal end. In Figure 6 the distal pectine ends have probably separated from the ventral surface of opisthosomal segment 4 but are still held there by the amnion. Other examples of embryo pectines at this stage can be seen in Figures 22, 32, and Farley (2001b, c, 2005).

Farley (2001c) provides some information about the development of pectinal peg sensilla in embryos of this species. The sequence is like that shown in Figures 8, 9 and 16 for other species. Small, pale knobs appear on the ventral surface of the pectine teeth. An opening forms in the center of each knob as the wall around the opening increases in height. The pore becomes oval-shaped, apparently the beginning of the elongate slit that will appear later at the distal end of the fully developed sensory peg (Fig. 11).

Centruroides vittatus (Buthidae)

Figure 7 is a ventral view of an embryo mesosoma of this species. Evident are ribbon-like pectines with transverse striations. The pectines are elongate and fairly uniform in width except for tapering at the proximal and distal ends. The pectines are an integral part of the body wall, forming part of the ventral surface of opisthosomal segment 4. A slight indication of opisthosomal segment 1 can still be seen. Opisthosomal segment 2 has two small ridges, not yet starting to form the bilateral flaps of the genital operculum (Figs. 14, 15, 21−23, 32).

In Figure 7, the earlier limb buds have disappeared from opisthosoma segments 4−8. There may be an initial indication of spiracles (Sp) on some segments; the spiracles appear to be medial to the more lateral location of the earlier limb buds. The distal ends of the pectines will eventually separate from the ventral surface of opisthosomal segment 4, and spiracles will subsequently appear on this segment (Farley, 2005).

The early stages in the formation of pectinal peg sensilla are shown in Figure 8. On the ventral surface of the pectinal teeth, these primordial chemoreceptors begin as small, pale knobs on the cuticle surface. A small opening develops in the center of the knob. These receptors are developing well before any others on the scorpion body. The presence of a central pore raises the possibility that even here in the embryo, these receptors may be sensitive to chemical stimuli. The cuticular walls of these sensilla increase in height, and the central pore apparently lengthens to become an elongate slit (Fig. 11; Swoveland, 1978; Foelix & Müller-Vorholt, 1983; Gaffin & Brownell, 2001; Wolf, 2008; Gaffin, 2010).

Figure 9 provides further support for the hypothesis that these early knob-like sensilla on the pectinal teeth may be functional in advanced embryos and first instars, well before formation of the final structure of the peg sensilla in the first molt (Fig. 11). In Figure 9, the central core of the embryo pectine appears to be mainly yolk, but the ventral part of each tooth has a narrow band with nerve fibers that extend from the peg sensilla toward the central nervous system. Some sensilla at this stage are only associated internally with a small cellular growth (Fig. 10), presumably the nerve and supporting cells that eventually give rise to long nerve fibers to the central nervous system. The cuticle of the pectine is underlain by a layer of hypodermal cells, which presumably is the source of the sensory nerves and supporting cells.

The peg sensilla appear to be fully formed and functional in the second instar (Fig. 11). They are very dense on the ventral surface of each pectinal tooth, separated only by 2−5 μm in the example of Figure 11. The pectines are 2−3 μm in length with what appears to be a flexible base and a distal slit that allows chemical stimuli to reach the sensory dendrites inside the sensilla (Foelix & Müller-Vorholt, 1983; Gaffin & Brownell, 2001; Wolf, 2008; Gaffin, 2010). In the embryo sensilla of Figure 9, it appears that a single nerve fiber extends inward from each sensillum with its central pore. In Figure 11, the pegs have a faint striated appearance as though there are numerous dendrites exposed to the elongate distal opening, as in the adult peg sensilla

(Foelix & Müller-Vorholt, 1983; Gaffin & Brownell, 2001; Wolf, 2008; Gaffin, 2010).

Hadrurus arizonensis (Caraboctonidae)

As in earlier examples (Figs. 2−4, 7), an elongate pectine can be seen at the ventro-lateral wall of the mesosoma in the embryo of Figure 12. Some transverse striations are also present. The chelicerae are anterior to the mouth, and the pedipalps are segmented and divided distally. The metasoma has all five segments, a tapered telson and is sharply flexed ventrally. This embryo is unusual in having very large tergites on the segments of the mesosoma. These may facilitate gas exchange as hypothesized for the dorso-lateral processes in embryos with katoikogenic development (Figs. 18, 20, 28−30; Mathew, 1956; Farley, 1999). The legs are slender and tapered distally (digitigrade) in contrast to the plantigrade legs of second and later instars (Jeram, 2001; Farley, 2005). The plantigrade form is thought to be an adaptation for terrestrial locomotion (Jeram, 2001).

The pectine in Figure 13 is at about the same stage as the embryo in Figure 12, but is shown here at higher magnification. The slender, ribbon-like pectine has transverse striations, and an elongate groove (asterisk) has started to separate the pectine into anterior and posterior regions. The anterior part of the pectine is an integral part of the body wall while the posterior edge of the pectine appears to be slightly separated. The striations anterior to the groove are decreasing while those posterior to the groove are prominent and will continue as separations of the pectinal teeth. Embryo pectines with a longitudinal groove are shown in Figures 4, 21 and 22. In Farley (1999, Fig. 69; 2001c, Fig. 6), the pectinal groove is evident but not labeled or correctly described for embryos of *Smeringurus mesaensis*

Superstitionia donensis (Superstitioniidae)

The adults of this species are very small compared to the other scorpions of this investigation. The early stages of development were not examined, but a ventral view of a first instar is shown in Figure 14. This first instar of *S. donensis* looks much like the first instars of other species with apoikogenic development (Farley, 2005, 2008). The coxapophyses of the first and second legs are slender, rounded structures that extend well forward to form the ventral wall of the preoral tube. After the first molt, the second instar coxapophyses are broad and flat (Farley, 2005, 2008) as in the adult (Farley, 1999). The genital operculum in Figure 14 is a distinct structure consisting of bilateral flaps. There is a sternum in the ventral prosoma, and the pedipalps are segmented and divided distally. The chelicerae are anterior to the mouth. Spiracles are usually prominent in first instar scorpions (Farley, 2005, 2008). Their deve-

lopment may be delayed in this species since only one can be seen in opisthosomal segment 5, and/or possibly the magnification is not sufficient to show the spiracles.

Figure 14 shows that the pectines of this species are unusual in having only 6 teeth in each pectine. This is in comparison with 26 teeth per pectine in Figure 6 (*Smeringurus mesaensis*), 21−24 teeth for each pectine in Figure 7 (*Centruroides vittatus*), 14−17 teeth in Figure 13 (*Hadrurus arizonensis*), 17 teeth in Figure 23 (*Hadogenes paucidens*) and 14 teeth in Figure 32 (*Pandinus imperator*). Swoveland (1978) also noted six teeth per pectine for *Superstitionia donensis* in his comparison of pectine structure in ten adult scorpion species of North America. In that study, the tooth (dentes) number ranged from 6 to 38 per pectine.

Higher magnification of the region of the genital operculum (Fig. 15) shows that the gonopore (GP) is forming in the midline between the two flaps of the operculum. Just posterior to this in the basal plate (basal piece) of the pectines there is another small opening. This may be a transitory ancestral gonopore, labeled here as GP'. A similar structure was seen in advanced embryos of *Centruroides vittatus* (Farley, 2005).

A further indication of somewhat delayed development in this species is evident in Figure 16, the ventral surface of two pectinal teeth in a first instar. The peg sensilla are relatively few in number and at an early stage of development compared with sensory pegs on the pectinal teeth of embryos of *Centruroides vittatus* (Figs. 8, 9; Farley, 2005). Though somewhat delayed, the sequence of development of the peg sensilla in *S. donensis* is like that in *C. vittatus* and other scorpion species (Fig. 16; Swoveland, 1978; Farley, 2001c), i.e., a knob-like swelling in the ventral cuticle of the teeth and eventually a small pore in the center of the knob.

Katoikogenic development

Hadogenes paucidens (Hemiscorpiidae)

An external view of a diverticulum (D) is shown in Figure 17. The diverticulum is an outgrowth of the ovariuterine tubules (OT). Some very small diverticulae (D') are also evident as tubular outgrowths in this figure. Inside the large diverticulum is an embryo with its head oriented toward the distal end of the diverticulum and the proximal end of the appendix (A). Thus oriented, maternal nutrients can enter the mouth as a result of rhythmic contractions of the embryo pharyngeal muscles (Mathew, 1948, 1956). Some information is provided about appendix development (Mathew, 1948, 1956, 1959b) and structure (Subburam & Gopalakrishna Reddy, 1989).

The embryo of Figure 18 is at about the same stage as the embryo inside the diverticulum of Figure 17. For Figure 18, the outer wall of the diverticulum was opened

so the embryo could be seen. The embryo is covered with an embryonic membrane which was torn in some places during the dissection (asterisks). The mesosoma is very large and advanced in development as compared with the prosoma and metasoma. There are no prominent limb buds on the ventral mesosoma although small ones may not be seen because of the covering membrane. Limb buds for the pedipalps and legs 1−4 are present, and there are large dorso-lateral processes on the segments of the mesosoma. These processes are thin-walled and filled with fluid (Mathew, 1956; Farley, 1999), and they readily collapse during preparation for microscopy. An appendix is present at the anterior end of the embryo.

Figure 19 is a higher magnification of a torn embryonic membrane as in Figure 18. The tear during dissection enables a view of the external (EM) and internal (EM') surfaces of the membrane. This appears to be mainly a layer of cells held together by fibrous material. As the embryo enlarges, the embryonic membrane does not enlarge with it, so gradually the dorsal portions of the embryo grow beyond the membrane. There may be little or no membrane present in more advanced embryos.

A later stage of an embryo is shown in Figure 20. Again, the outer wall of the diverticulum was opened and removed so the embryo could be seen. The appendages and part of the prosoma are no longer covered by embryonic membrane. The pedipalps are divided distally, and segment demarcations can be seen in the legs. No prominent limb buds are evident in the ventral mesosoma although small ones may not be seen because of the covering of the embryonic membrane. The large dorsal processes have collapsed as usual during preparation for microscopy. The mesosoma is very large and disproportionate in comparison to the prosoma and slender metasoma.

Figure 21 shows the earliest example of pectines seen in this species. Although the mesosoma is very large in the embryos (Figs. 18, 20), the developing pectines are not also increased in size. In the species with apoikogenic development, the early pectines are a prominent part of the mesosoma (Figs. 1−7, 12, 13). The early pectines of *H. paucidens* are very small in comparison with the large size of the mesosoma, and the initial stages of pectine formation may be short in duration so they are missed with the procedures used herein.

In this species, the pectines apparently originate as two small flaps from opisthosomal segment 3 without the prolonged earlier stages that are seen in more basal species with apoikogenic development (Figs. 1−7, 12, 13). Those early stages may be temporarily displayed during pectine formation in this species, but those stages if present are not prominent. The pectines of Figure 21 are joined with the ventral surface of opisthosomal

segment 4 as occurs in embryos with apoikogenic development (Figs. 5, 7). A groove is starting to form along the length of this early pectine of *H. paucidens*, also as seen in embryos with apoikogenic development (Figs. 4, 13). The groove divides the pectine into anterior and posterior regions (Fig. 21). The posterior region has the outline of teeth, but the teeth are not yet separated from each other. The groove is deeper in the more advanced embryo of Figure 22.

The bilateral lobes of the genital operculum are starting to appear in Figure 21, and there are sturdy setae (bristles, B) forming on the ventral surface of opisthosomal segment 4. These setae are shown in more detail in Figures 23−25. Such setae are also present in the embryos of another species with katoikogenic development (*Pandinus imperator*, Scorpionidae; Fig. 31), but they are not seen in any of the species with apoikogenic development examined so far.

The embryo of Figure 22 is slightly more advanced than the embryo of Figure 21. The pectines have separated from the ventral surface of opisthosomal segment 4, and there is a prominent groove (asterisks) along the length of each pectine. The teeth are partially separated from each other. The lobes of the genital operculum are larger than those in Figure 21.

The pectines in Figure 23 are more advanced than those in Figures 21 and 22. In Figure 23, the pectines are attached only at their proximal ends so the distal ends can move freely. Legs 3 and 4 were removed from this embryo, as is commonly done, so the structures in the anterior mesosoma can be seen. The bilateral flaps of the genital operculum are prominent, and the sternum can be seen at the ventral surface of the prosoma. Sturdy, transitory setae (bristles, B) are present on the ventral surface of the pectines and the ventral opisthosoma. As occurs in embryos with apoikogenic development (Farley, 2005, 2008), spiracles (Sp) become evident on the ventral surface of opisthosomal segment 4 after the pectines are separated from the ventral surface of that segment. Also evident in Figure 23 is a faint crease (Ste) indicating the posterior edge of the developing sternite of opisthosomal segment 4.

The embryos of Figures 24 and 25 are at about the same stage as Figure 23. The pectines are attached to the ventral body wall only at their proximal ends. Spiracles and sternites are starting to appear on the ventral mesosoma. In embryos with apoikogenic development (Farley, 2005, 2008), the pectines are also advanced and forming peg sensilla at the time when spiracles and sternites start to appear on the ventral opisthosoma.

In Figure 24, epithelial invaginations can be seen for spiracles and book lung tissue (Farley, 2008, 2010) in opisthosomal segments 4−6. In this species and *Pandinus imperator* (Fig. 31), the transitory setae (bristles, B) appear on the ventral mesosoma before spiracles and sternites start to form. As evident in Figures 24 and

25, the setae are aligned in a pattern that outlines the location of the lateral and posterior margins of the future sternites. A faint crease indicating the pos- terior edge of the sternites (Ste) is evident in these figures.

The ventral surfaces of opisthosomal segments 5 and 6 are shown at higher magnification in Figure 25. The short, sturdy setae (bristles, B) are pointed at their tip, and would seem to be effective for defense and predation at the ventral surface of the individual, possibly an ancestor. The lateral edges of the sternites are not yet indicated by creases, but the setae show where the sternite lateral edges will eventually appear.

Figure 26 shows the ventral surface of the prosoma of an embryo at about the same stage as in Figures 23−25. The appendix has been removed, but the chelicerae are highly modified for grasping the proximal end (teat) of the appendix so that nutrients can be delivered to the mouth in response to rhythmic pharyngeal contractions (Mathew, 1948, 1956). Rhythmic contractions (50−60/min.) of the pharyngeal muscles were seen in dissected embryos of this species.

In Figure 26, the preoral tube (Farley, 1999, 2001d, 2005, 2008) is starting to form surrounding the mouth. The rostrum (labrum) forms the dorsal wall of the tube. The medial surfaces of the pedipalp coxae form the lateral walls of the preoral tube. The cuticle of these medial coxal surfaces have a specialized texture that may macerate food and help secure the proximal appendix. The coxapophyses of the first and second leg are enlarged lobes at this stage. They are starting to extend forward and will eventually form the ventral wall of the preoral tube. A more advanced stage of formation of the preoral tube is shown in the first instar of Figure 14 (*Superstitionia donensis*) and the second instars of *Centruroides vittatus* and *C. gracilis* (Farley, 2005, 2008).

The tip of the pedipalps is bifurcated in the embryo of Figure 26; evident are the features of a claw that will have fixed and movable fingers. On the pedipalps and legs, there are small transitory setae (bristles, B) like those on the ventral surface of the mesosoma (Figs. 21, 23−25). These setae disappear, and many sensilla are present on the body surface after the first molt (Farley, 2005, 2008).

The metasoma in Figure 27 was removed from an embryo at a later stage of development than in Figures 18−26. The metasoma has five segments as in the instars, but the telson is only a small lobe and not yet tapered. The fifth metasomal segment has two dorsal lobes that increase surface area of the segment and may facilitate gas exchange with maternal hemolymph as is hypothesized for the dorso-lateral processes on mesosomal segments (Mathew, 1956). The metasoma of embryos with apoikogenic development is commonly flexed ventrally during development (Figs. 1, 2, 12, Farley, 1998, 1999, 2001a-d, 2005). The flexed

metasoma was often removed in the present investigation so the ventral mesosoma could be examined with the SEM. In embryos in this investigation with katoikogenic development, the metasoma lengthens with little flexure relative to the mesosoma (Figs. 18, 20, 27−29).

The metasomal segments in Figure 27 have short, sturdy setae (bristles, B) on their ventral surface much like those on the embryo mesosoma (Figs. 21, 23−25) and appendages (Fig. 26). The cuticle of the metasomal segments in Figure 27 lacks specialization for the dorsal flexure of the metasoma that begins as newborn scorpions continue development on their mother's back (Farley, 2005). In the first molt, the metasomal segments are highly sculptured with lateral pivot points for dorsal flexure and a cuticle shape that prevents ventral flexure (Bowerman, 1972a, b; Root, 1990; Farley, 2005).

Pandinus imperator (Scorpionidae)

For the embryo of Figure 28, the outer wall of the appendix was opened, and the embryo was removed. The embryonic membrane was absent or dissected away so the embryo surface could be examined with the SEM. As in embryos of *Hadogenes paucidens* (Figs. 18, 20), the mesosoma is large and developed compared to the prosoma and metasoma. When early embryos are removed from the diverticulum and examined with the dissecting microscope, the digestive tract can be seen extending posteriorly from the mouth before any other organs and appendages appear. An appendix forms very early; its proximal end leads directly to the anterior end of the digestive tract when the embryo is only an elongate tube of cells with a digestive tract inside (Mathew, 1956). That basic and early structure is still evident in Figure 28 except that the mesosomal segments have large dorso-lateral processes.

No segments can be discerned in the prosoma and metasoma of Figure 28, but the mesosomal segments are distinct with the large dorso-lateral lobes on each segment. No prominent limb buds are evident on the ventral surface of the mesosoma, though some small wrinkles or lobes may be indicative of limb buds. There is no apparent indication of pectinal formation.

The embryo of Figure 29 was removed from the diverticulum and is more advanced than the embryo in Figure 28. Some prosomal appendages are evident in Figure 29, but there is still no indication of chelicerae or pectines. Apparently, the chelicerae will differentiate from the lobe of tissue (Lo) in the anterior part of the prosoma. There are no prominent limb buds in the ventral mesosoma, though there are some small lobes that may be indicative of limb buds (Fig. 30). Collapsed dorso-lateral processes are present on the mesosomal segments, and some faint indications of segments can be discerned in the metasoma. As in the embryo of Figure

28, the appendix leads directly to the prosoma where there is apparently a mouth and anterior digestive tract.

The ventral mesosoma of the embryo of Figure 29 is shown at higher magnification in Figure 30. The genital operculum and pectines will eventually form just posterior to the fourth leg (Fig. 32), but the tissues in this region in Figure 30 still show little indication of the structures that will form. Some small lobes are labeled in the ventral mesoma as possible traces of limb buds (LB).

Figure 31 shows mesosomal segments 6 and 7 of an embryo more advanced than those in Figures 28−30. The features in Figure 31 look much like those at a similar stage in embryos of *Hadogenes paucidens* (Figs. 23−25) where spiracles and sternites are starting to appear on the ventral surface. The prominent setae (bristles, B) in Figure 31 appear to precede the formation of the sternites. They are in a pattern that outlines the flap-like form of the sternites before the sternites appear. The asterisks indicate setae just inside the initial creases (Ste) that are the first indication of sternite formation. The setae in Figure 31 are about 130 μm in length while those of *H. paucidens* are about 80 μm in Figure 25.

The initial steps in formation of the genital operculum and pectines were not seen in this species, but these structures are evident in a more advanced stage in Figure 32. The setae (bristles, B) at this stage appear to be deteriorating. They are much less prominent than in Figure 31, although the flap-like sternites are more distinct on the ventral surface of the mesosomal segments. The pectines are attached only at their proximal ends, and they have distinct anterior and posterior regions with a row of teeth at the posterior edge. Some invaginations that appear to be primordial spiracles are labeled in the figure.

Discussion

Heterochrony; appendix

These studies with the SEM provide further evidence of heterochrony (Mathew, 1959a) in the development of scorpion embryos. The embryos of different families and species are much alike after the first molt, but the timing and sequence of development is substantially modified *in utero*. In embryos with apoikogenic development there is anterior-to-posterior development with early formation of prosomal appendages and more delayed appearance of mesosomal and metasomal segments and structures (Figs. 1−3, 5−7, 12, 14).

In the more derived species examined herein with katoikogenic development, the early mesosoma is disproportionately large (Figs. 18, 20, 28, 29), and the development of prosomal appendages and metasoma is delayed. The mouth and digestive tract are formed very early (Mathew, 1956). The chelicerae form late in species with apoikogenic development (Figs. 1, 2, 14) and early in species with katoikogenic development (Fig. 26) where the chelicerae are used for grasping the appendix (Fig. 26, Mathew, 1956). The appendix is formed very early in the latter embryos (Mathew, 1948, 1956, 1959b), and was present in all the stages herein for embryos of *Hadogenes paucidens* (Figs. 17, 18, 20) and *Pandinus imperator* (Figs. 28, 29).

The appendix is entirely maternal in origin, developing from large follicular cells (cell plug; Pflugfelder, 1930; Mathew, 1948, 1956, 1959b) just distal to the ovum at the apex of the diverticulum. These cells proliferate inward and surround the early embryo with the cup-shaped embryonal capsule (Mathew, 1956). The plug cells also proliferate outward and become a slender, elongate mass of cells, the primordial appendix. The cells at the central axis of the appendix are much longer and appear to secrete and degenerate (holocrine secretion), giving rise to the central conducting tubules of the appendix (Mathew, 1948; Subburam & Gopalakrishna Reddy, 1989). The cells of the embryonal capsule and those more lateral in the appendix become somewhat polygonal or rectangular in shape. They also secrete and degenerate, but the cells in the outer layers of the appendix form small chambers or lacunae that apparently store nutrient fluid for transfer to the central conducting tubules and embryo (Mathew, 1948, 1956).

Embryo membranes; dorso-lateral processes

Relatively less yolk is present in oocytes of the vaejovid *Smeringurus mesaensis*, and a follicular placenta provides a means for nutrients to reach the embryo without passing through the thick serosa (Farley, 1996, 1998, 1999, 2001d). The placenta consists of a bun-shaped mass of cells that directly abuts the side of the embryo and apparently delivers nutrients to it. The maternal ovariuterine tubules with attached trophic layer provide a network with substantial absorptive surface area intermingled among the large digestive glands in the maternal mesosoma.

The buthid *Lychas tricarinatus* is another apoikogenic species with embryo modifications that bypass the amnion and serosa (Mathew, 1960; Farley, 2001d). The embryo mesosoma has a hollow dorsal stalk and globular body that apparently transfers nutrients from the maternal ovariuterine wall directly to the digestive tract of the embryo.

Laurie (1896c) described *Opistophthalmus* species (Scorpionidae) with a hollow outgrowth from the anterior end of the embryo. Rather than ingesting nutrients as occurs in other scorpionids with katoikogenic development, the nutrients apparently pass from the maternal ovariuterus to the embryo through the hollow protrusion. On one species, a tubular structure extends like a proboscis from a region just anterior to the

mouth. In another species, a protrusion arises from the dorsal carapace behind the median eyes.

For this and earlier studies of scorpion embryos with apoikogenic development, the cellular serosa is easily removed by dissection. There are differences among the species, but the amnion usually adheres close to the embryo body and must be carefully pulled away without damaging the embryo. The amnion was left intact on the mesosoma of Figure 6, and there are fragments of amnion in other figures (e.g., Figs. 1−3, 5, 7, 12).

The amnion and serosa arise from the embryo blasoderm in embryos with apoikogenic development (Laurie, 1890; Brauer, 1894, 1895; Anderson, 1973; Abd-el Wahab, 1951; Francke, 1982). In embryos of *Heterometrus scaber* (katoikogenic development), there is a sequence of membranes labeled by Mathew (1956) as embryonal capsule, trophamnion and second embryonal membrane. The embryonic capsule is thought to originate from follicle cells. The trophamnion results from proliferation of polar body cells, and the second embryonal membrane is derived from follicle cells.

In embryos of *Liocheles australasiae* (Pflugfelder, 1930, as *Hormurus*) (Hemiscorpiidae) and *Heterometrus scaber* (Mathew, 1956), the second embryonal membrane (embryo envelope) is a cellular layer that continues as the embryo increases in size. This appears to be similar to the cellular membrane (Fig. 19) left intact for some embryos herein with katoikogenic development (Figs. 18, 20). In this investigation, the outer covering for the embryos is labeled simply as embryonic membrane (EM), since preceding coverings and developmental stages of this membrane were not examined.

The dorso-lateral processes are a very distinctive feature of embryos with katoikogenic development (Figs. 18, 20, 28−30). Examination with the light microscope (Mathew, 1956; Rosin & Shulov, 1962) and SEM (Farley, 1999) show the dorso-lateral processes are fluid-filled bulges with very thin cuticle. They are formed on mesosomal segments, probably as an adaptation for gas exchange with maternal hemolymph. Mathew (1956) suggested that as the embryo enlarges, the dorso-lateral processes increase the surface area for gas exchange for the larger volume of the embryo. Their reduction just before and/or after birth supports the notion that they are an embryo adaptation (Mathew, 1956; Rosin & Shulov, 1962). The very large mesosomal tergites of *Hadrurus arizonensis* (Fig. 12) may be a similar modification for embryo sustenance.

Development of pectines

Some aspects of pectinal development were included in earlier SEM studies of the embryos of *Smeringurus mesaensis* (Farley, 1998, 1999, 2001a-d),

Centruroides vittatus (Farley, 2005), and *Centruroides gracilis* (Farley, 2008). The present investigation extends the earlier observations with emphasis herein on the step-by-step formation of pectines in embryos with apoikogenic development. For these embryos, pectine formation takes place for weeks or months so the intermediate steps are seen as the embryos of numerous females are examined. For embryos with katoikogenic development in this study, pectinal development appears to be relatively rapid, and some early steps may be reduced or omitted.

In his comprehensive and very helpful histological study of embryo development in *Heterometrus scaber* (Scorpionidae), Mathew (1956) provides little information about the formation of the pectines. The present study is an overview of the process in species from two more derived families with katoikogenic development, but tissue sections (probably for transmission electron microscopy) are needed during the short time interval and small space (Figs. 29, 30) where pectines form in the anterior mesosoma.

In embryos with apoikogenic development, the first main step of pectinal development is the enlargement of the limb buds on the third opisthosomal segment as limb buds are reduced on other mesosomal segments (Fig. 1; Farley, 2001a). The segment 3 limb buds apparently grow dorso-laterally along the body surface so that a second major step is an elongate and narrow pectine with a smooth surface (Fig. 2; Farley, 1998, 2001a). The pectines appear to be joined closely with the body surface. Third: the distal ends of the pectines develop transverse striations so the pectines have a paddle-like appearance with a smooth surface at the proximal half of the pectine (Fig. 3, Farley, 1998, 2001a, d). Fourth: there are transverse striations nearly the entire length of the pectines. The pectines are elongate and slender with a ribbon-like appearance, and apparently they are still an integral part of the body wall (Figs. 7; Farley, 2001c, d). The pectines form part of the ventral surface of the fourth opisthosomal segment.

In the preceding steps, the transverse striations suggest use of the pectines as paddles for swimming, a stabilizer for the elongate opisthosoma in water and/or striated gills with increased surface area for gas exchange in an aquatic environment. In the fifth step of pectinal development, a change occurs that results in an important characteristic of all extant and nearly all fossil scorpions (Kjellesvig-Waering, 1986), a row of teeth at the posterior margin of the pectines.

Step five: a crease forms along the length of the pectine, dividing it into anterior and posterior regions. The transverse striations disappear from the anterior part while those at the posterior part deepen and become the separations between teeth at the posterior edge of the pectines (Figs. 4, 13; Farley, 2001c). The result is the sixth stage of development, a somewhat oval or tri-

angular pectine with a distinct anterior region and a row of striations at the posterior edge. The pectines in the embryos still form the ventral surface of the fourth opisthosomal segment (Figs. 5, Farley, 2001c, 2005). Seventh: the distal ends of the pectines separate from the ventral surface of opisthosomal segment four. The pectines are attached only at their proximal ends (Figs. 6, 14; Farley, 2001c, 2005). Teeth rather than striations are evident at the posterior pectinal margin.

In the eighth stage of pectinal formation, peg sensilla start to appear on the ventral surface of the pectinal teeth (Figs. 8, 16, Farley, 2001c). The sensilla appear first as small knobs. A small opening develops in the center of each knob, and the walls of the sensilla apparently lengthen so a peg-like structure with a distal groove is formed. Nerve fibers can be seen extending from the sensilla toward the central nervous system (Figs. 9, 10, Farley, 2005). The ninth stage of pectinal development occurs in the first molt: the peg sensilla and other sensilla on the anterior pectine appear to be fully formed and functional (Fig. 11; Farley, 2005, 2008). Similar observations about the formation of peg sensilla were reported by Swoveland (1978) in his study of adult pectine structure in 10 scorpion species of North America. He included some comparative information about the pectines of immature scorpions.

For embryos with katoikogenic development, the initial steps (1−4 above) of pectine formation are still not clear. Step 5 (elongate crease) can be seen in Figures 21 and 22. Step 6 (oval or triangular shape, striations at posterior margin) is also evident in Figures 21 and 22. The distal ends of the pectines are separated from the ventral surface of opisthosomal segment 4 (step 7 above) in Figures 22, 23 and 32, and the posterior pectinal striations become separate teeth.

In the present investigation, the formation of the peg sensilla (steps 8 and 9 above) was not fully examined in embryos with katoikogenic development. The few SEM photos available for peg sensilla formation in embryos of *Pandinus imperator* (Farley, unpublished observations) suggest the process is similar to that for embryos with apoikogenic development (Figs. 8, 9, 16; Farley, 2001c).

Stages 1−4 above were not seen in embryos with katoikogenic development in the present study, nor were they a part of Mathew's (1956) brief description of pectine development in embryos of *Heteromerus scaber*. In that species, Mathew reports that the mesosomal limb buds do not become prominent and project from the body surface. There appears to be invaginations at the limb bud site, and these invaginations give rise to the small initial buds of the pectines.

In Figures 18 and 20 there is no indication of prominent mesosomal limb buds in embryos of *Hadogenes paucidens,* although small limb buds could be concealed by the embryonic membrane still present.

In Figure 30, some invaginations and possible small limb buds are evident in the ventral view of the mesosoma of *Pandinus imperator*. Whatever the initial steps in pectine formation for embryos of *H. paucidens* and *P. imperator*, the result (Figs. 21−23, 32) is pectines much like those in advanced embryos and first instars with apoikogenic development (Figs. 6, 14; Farley, 2001c, 2005, 2008).

Pectines are a distinctive feature of scorpions (Fet et al., 2000; Kjellesvig-Waering, 1986; Sissom, 1990; Stockwell, 1992; Dunlop, 2010). Their beginning very early in the scorpion embryo (Figs. 1−5; Farley, 1998, 2001a) suggests an early separation of the scorpion clade. The basic shape and structure of the pectines is present in the embryos well before indications of terrestrialization, i.e., spiracles and book lungs in the ventral mesosoma (Figs. 1−5, 24, 32; Farley, 2005, 2008). This raises the possibility that the early stages (1−4 above) of pectine development in the basal scorpion families are an indication of some ancestral role for the pectines in an aquatic environment.

Transitory setae in the embryos

The sturdy setae on the embryos may be another indication of ancestral existence in an aquatic habitat. The setae are formed on the ventral surfaces of the mesosoma (Figs. 21, 23−25, 31), the appendages (Fig. 26) and metasoma (Fig. 27). They appear before and at the same time as the first appearance of spiracles and sternites (i.e., indications of terrestrialization), and then the setae disappear (Fig. 32). The setae have no apparent function in the embryo, but their structure and location suggests an ancestral function especially in an aquatic environment with prey and predators beneath the scorpion.

The ventral flexure of the metasoma during embryogenesis and a shift in position of the openings in the telson (Farley, 2001a), provide further evidence of the importance of activity beneath the scorpion before terrestrialization and dorsal flexure of the metasoma and sting. The mouth is initially directed ventrally in the embryo with enlarged coxal lobes (coxapophyses, coxal endites) that could assist with delivery of food to the mouth (Farley, 1999, 2001a, b, d, 2005, 2008). As reviewed earlier (Farley, 2005), these lobes gradually extend forward and become the ventral wall of the preoral tube.

Pectine variability and function

There is substantial variability in pectine structure among fossil scorpions (Kjellesvig-Waering, 1986). There may be slender filaments at the posterior pectine margin or larger and more bulbous teeth. There are also phylogenetic differences in the number of teeth and in

the length, width and shape of the pectines. No pectines are evident in the fossils of the Silurian scorpion *Proscorpius osborni* (Dunlop et al., 2008).

Located on the anterior opisthosoma, the pectines are not a weight-bearing appendage necessary for locomotion as are the prosomal legs. As suggested from the changes in developmental morphology described herein (e.g., Figs. 1−6), it is reasonable that the pectines had a diversity of morphologies and functions in the long evolutionary history of scorpions. It is also reasonable that these non-locomotory appendages may have been lost in some fossil species.

A diversity of pectinal morphologies is evident in extant scorpions (Swoveland, 1978; Hjelle, 1990; Fet & Brownell, 1998). There are often gender differences in pectinal structure, with males having longer pectines and more teeth. The variable features of pectines and their sensilla are commonly used as taxonomic characters (e.g., Lourenço & Bastos, 1983; Navidpour & Lowe, 2009; Botero-Trujillo & Noriega, 2011). In the 10 scorpion species he examined, Swoveland (1978) observed that the general structure of the pectines is similar, but closer examination with SEM shows differences in the number and shape of the teeth and in the structure of the peg sensilla and other receptors. The pectines appear to be adaptable structures, but information is needed about their morphological diversity in relation to scorpion behavior, ecology and evolution.

The size of the pectines and the number of peg sensilla per tooth (dens) increases as the scorpions mature (Swoveland, 1978). In that investigation, the number of peg sensilla per tooth varied from 82 to 1600 depending on species. The peg sensilla on the ventral surface of the pectinal teeth are mainly chemoreceptors (Gaffin & Brownell, 1992, 1997a, b; Brownell, 2001; Gaffin & Brownell, 2001; Gaffin & Walvoord, 2004; Knowlton & Gaffin, 2010; Gaffin, 2010), but each sensillum has at least one mechanoreceptor at its base (Wolf, 2008). The high density of the peg sensilla and their neural projection to the central nervous system apparently provides a detailed chemical/physical image of the substrate (Gaffin & Brownell, 1992, 1997a, b, 2001; Brownell, 1998, 2001; Gaffin, 2001, 2010; Wolf, 2008).

There are also chemo- and mechanoreceptors on the pectine lamellae (Swoveland, 1978; Kladt et al., 2007). The mechanoreceptors here probably inform the scorpion about obstacles on the substrate and the position of the pectines relative to the body. A diversity of behavioral experiments show the pectines are involved in mate detection and courtship and the detection of prey, predators, substrate texture and vibrations (Cloudsley-Thompson, 1955; Carthy, 1966; Krapf, 1986; Hjelle, 1990; Root, 1990; Farley, 1999; Tallarovic et al., 2000; Benton, 2001; Melville et al., 2003; Steinmetz et al., 2004; Mineo & Del Claro, 2006).

As reviewed by Farley (2005), there are reports of behavioral differences in scorpion first instars (pronymphs); the differences may be related to their apoikogenic or katoikogenic development (Williams, 1969). The latter tend to be stronger and more active, possibly as a result of better nutrition *in utero* with the special adaptations for oral feeding. The first instars do not feed while continuing development on their mother's back; these instars rely entirely on stored maternal nutrients.

The first instars from apoikogenic development are commonly weak and inactive (Williams, 1969; Shulov et al., 1960) while first instars of katoikogenic species (e.g., *Pandinus imperator*) have been used in choice experiments that require considerable mobility as well as chemical sensitivity (Vannini et al., 1978; Vannini & Ugolini, 1980; Mahsberg, 1990, 2001). Functioning pectines may be the basis for this sensitivity since embryos and first instars have developing peg sensilla with nerve fibers leading toward the central nervous system (Figs. 8−10, 16; Farley, 2001c, 2005, 2008).

Acknowledgments

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Figure 1: Initial indication of pectine formation: embryo with enlargement of the limb buds on the third opisthosomal segment (O3). *Smeringurus mesaensis* (Vaejovidae), supine, ventro-lateral view. SEM. At this stage, all the appendages of the prosoma (Pr) can be identified, and there are transitory limb buds in the anterior opisthosoma (M, mesosoma). These will gradually disappear except for the limb buds on the second (primodial genital operculum) and third opisthosomal segment. The third-segment limb buds continue to enlarge as the pectines. The metasoma (Mt) has 4 segments and a ventral flexion. The bilobed tip of the metasoma appears to be an area of growth producing the final segment and telson. Extending along the length of the embryo is an elongate neural groove (NG), the likely anlage for the ventral nerve cord. 1−4, prosomal segments with attached legs L1−L4; asterisk, stomodeum (mouth); Ch, chelicera; PCL, precheliceral lobe; O1, opisthosomal segment 1; Pd, pedipalp. Scale, 200 μm.

Figure 2: Elongate pectinal lobe (P) extends laterally from opisthosomal segment 3 (O3) in embryo of *Smeringurus mesaensis* (supine). Ventro-lateral view, SEM. At this stage the primordial pectine has a smooth surface and is attached to the ventro-lateral body surface of the mesosoma (M). The other mesosomal limb buds can barely be discerned among the fragments of membrane (amnion). At this stage the metasoma (Mt) is sharply flexed ventrally and has a slighly tapered telson (T). In the prosoma (Pr), the legs (L1−L4) are segmented, the pedipalps (Pd) are divided distally and the chelicerae (Ch) are small lobes on either side of the stomodeum (asterisk). The latter is preceded by the single lobe of the rostrum (R) or labrum. 1−4, segments with attached legs L1−L4. NG, neural groove; O1−O3, opisthosomal segments 1−3; Pd', prosomal segment with attached pedipalps. Scale, 200 μm.
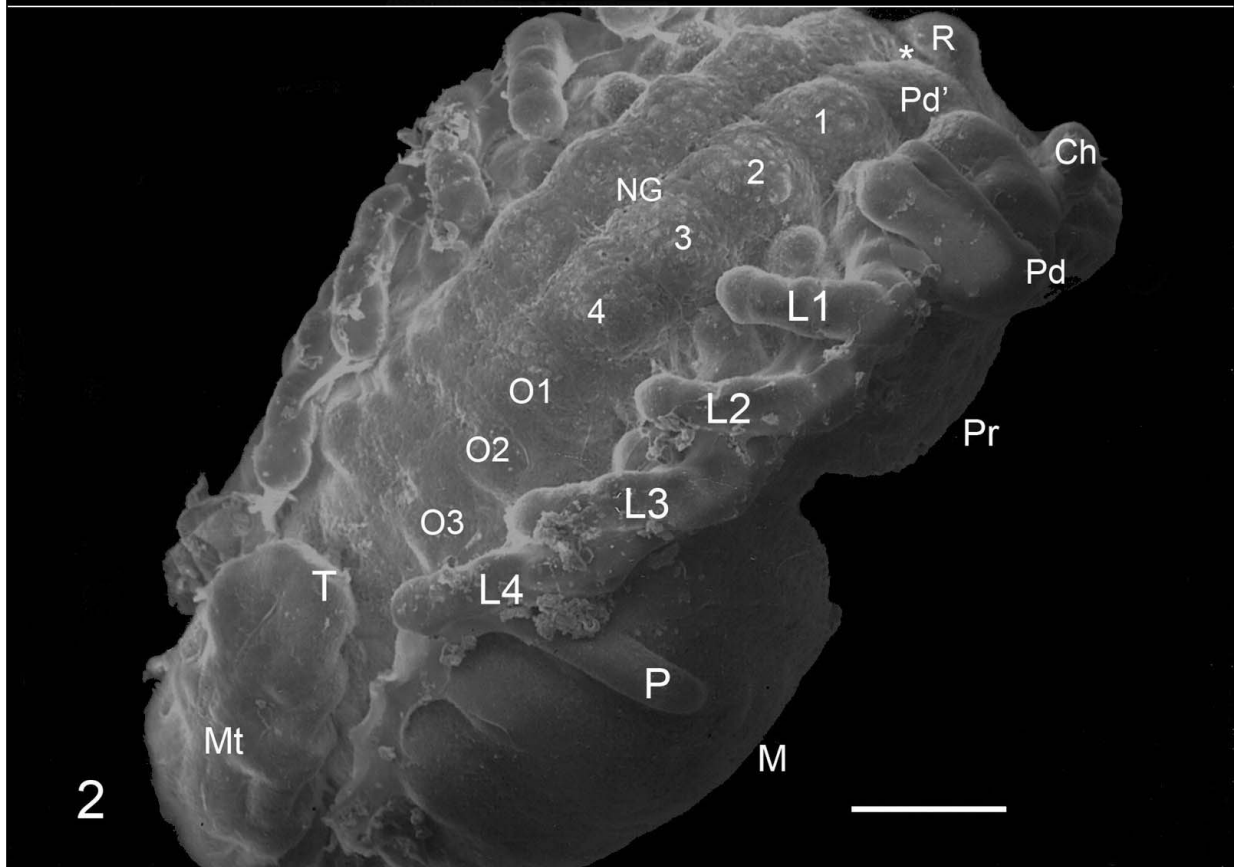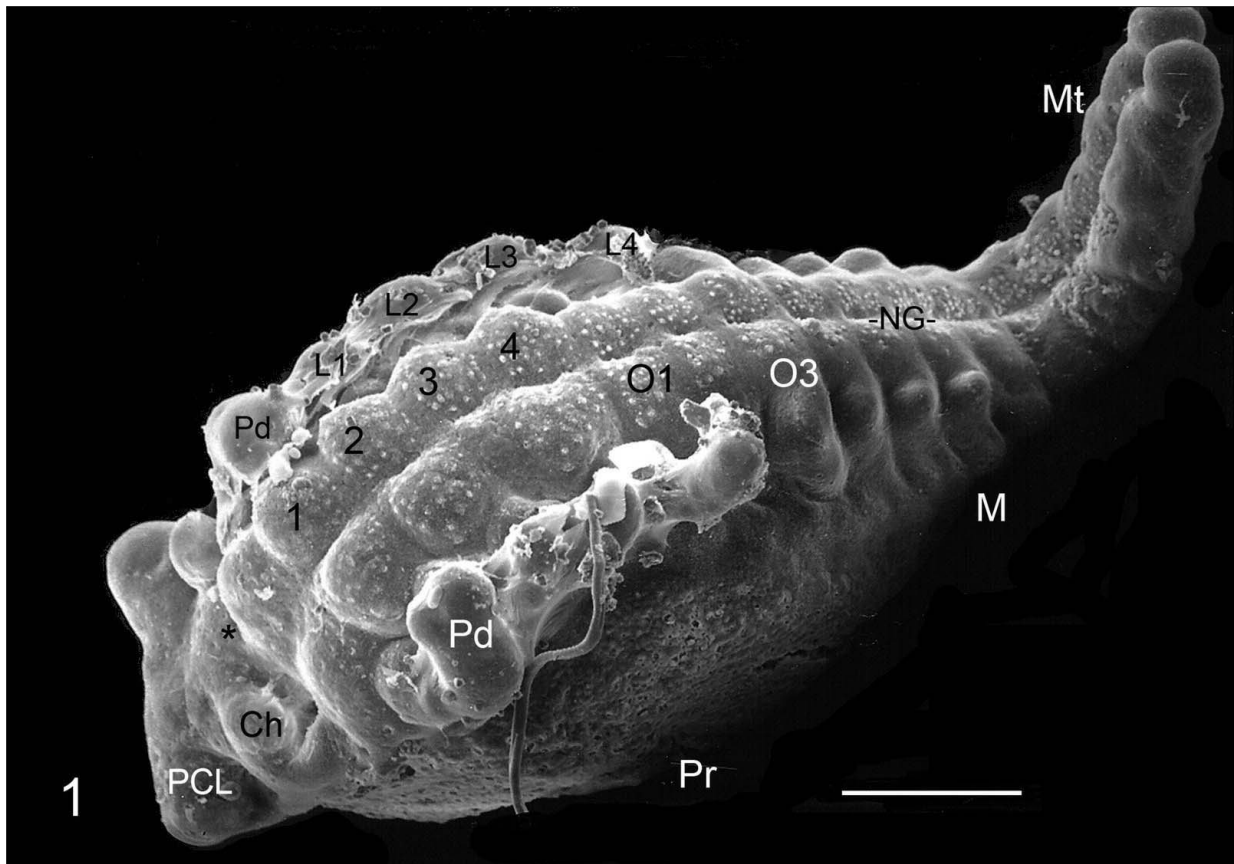
Figure 3: Ventro-posterior view of embryo *Smeringurus mesaensis* (supine) with early paddle-shaped pectines (P). SEM. Opisthosomal segments 1−8 (O1−O8) can be discerned. The pectines are developing from opisthosomal segment 3. The genital operculum will eventually develop from opisthosomal segment 2 (Figs. 14, 15, 21−23, 32), and opisthosomal segment 1 will disappear (Figs. 5, 14, 15, 22, 23). The pectines overlap somewhat on the ventral surface of segment 4, and this overlap increases as the pectines increase in width (Figs. 6, 7). The surface of the pectines is smooth at their proximal ends but somewhat wider and with transverse striations at the distal end (asterisk). This gives each pectine a paddle shape at this stage of development. The amnion membrane (Am) was partially removed by dissection, but portions of it still remain on the surface of the embryo. 1−4, prosomal segments associated with legs 1−4; L3, L4, legs 3, 4. Scale, 200 µm.

Figure 4: An elongate groove (asterisks) has begun to divide each pectine (P) into anterior and posterior regions. Embryo, *Smeringurus mesaensis*, supine, lateral view. SEM. Each primordial pectine with a smooth surface (Fig. 2) develops transverse striations for nearly all its length and becomes an elongate, ribbon-like structure attached to the ventro-lateral wall of the mesosoma (Figs. 7, 13; Farley, 1999, 2001d). The pectinal region anterior to the longitudinal groove will eventually have sensilla (Kladt et al., 2007) and will contain nerve fibers (Figs. 9, 10) from the thousands of sensory pegs (Figs. 8−11, 16) that form on the ventral surface of the teeth at the posterior margin of the pectines. L4, leg 4. Scale, 100 µm.

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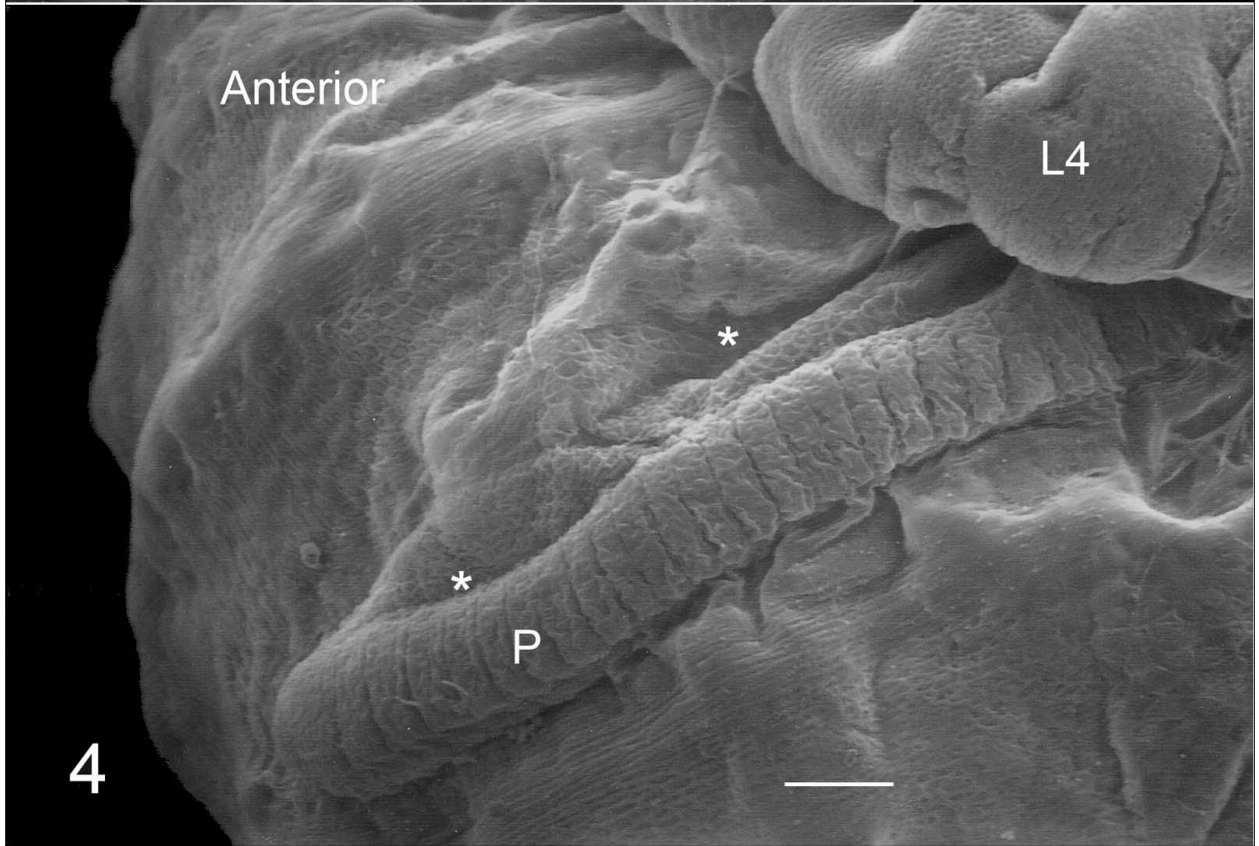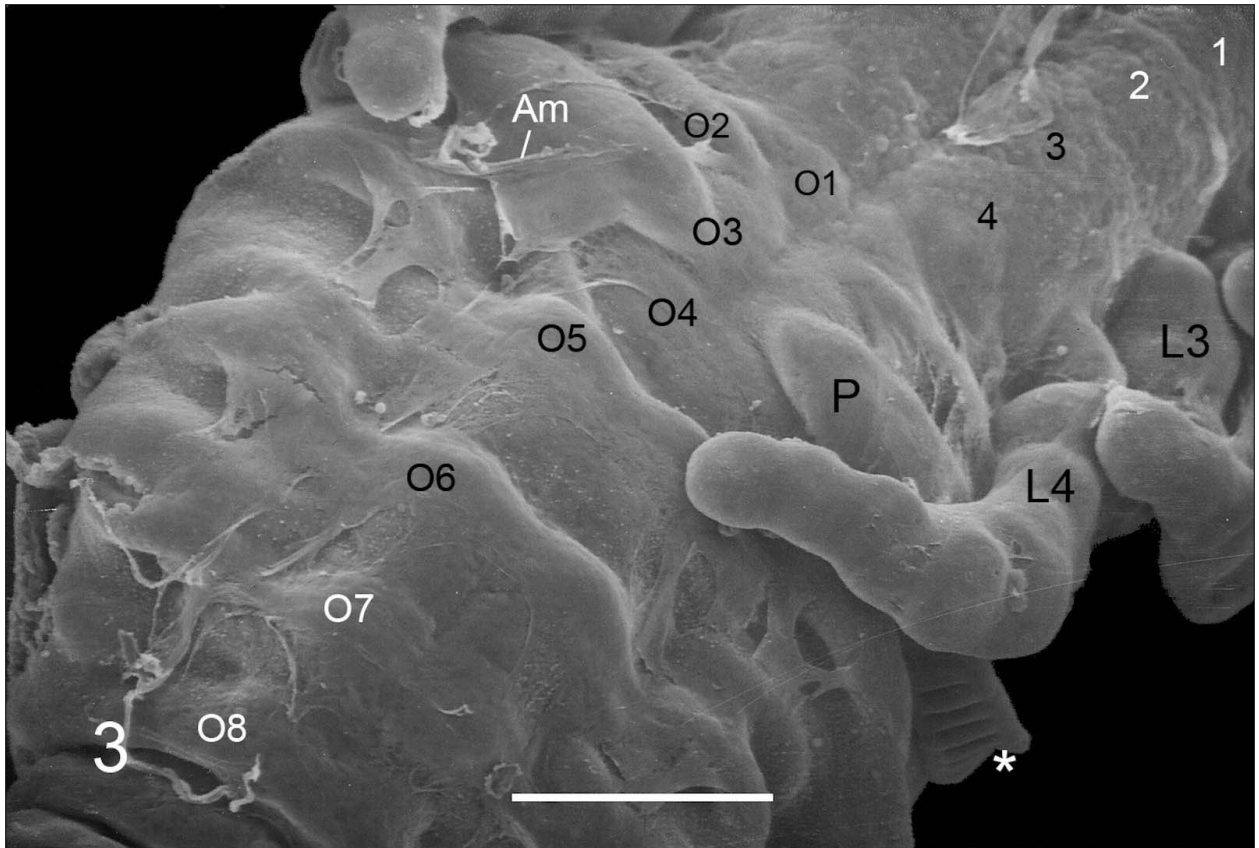
Figure 5: Oval-shaped pectines (P) with anterior and posterior regions clearly evident, and transverse striations (primordial teeth) at the posterior margin. Embryo, *Smeringurus mesaensis,* supine, ventral view. SEM. At this stage, the first opisthosomal segment is no longer evident; the second segment (O2) can clearly be distinguished but has not yet started to form the bilateral flaps of the genital operculum (Figs. 14, 15, 21−23, 32). In the prosoma, the legs (L1−L4) are segmented, and coxapophyses (Cx1, Cx2) are small lobes starting to form from the medial aspect of legs 1 and 2. These lobes gradually extend forward and form the ventral wall of the preoral tube that later surrounds the mouth (Fig. 14). Asterisk, stomodeum; O4, O5, opisthosomal segments 4, 5; St, sternum. Scale, 200 μm.

Figure 6: Pectines (P) well advanced in structure as the spiracles (Sp) and sternites (Ste) appear on the ventral mesosoma. Embryo, *Smeringurus mesaensis,* supine, ventral view. SEM. The thin, transparent amnion membrane was not removed from this example. As evident here, the membrane adheres very close to the body surface and is difficult to remove without damaging the embryo. Most mesosomal limb buds (Fig. 1) disappeared earlier, and the spiracles are close to the posterior edge of the developing sternites. The pectines have a row of teeth at their posterior margin. At this stage, the distal ends of the pectines are separated from the ventral surface of opisthosomal segment 4 (O4), but are still held in place by the amnion. O5−O7, opisthosomal segments 5−7; L4, leg 4. Scale, 0.5 mm.
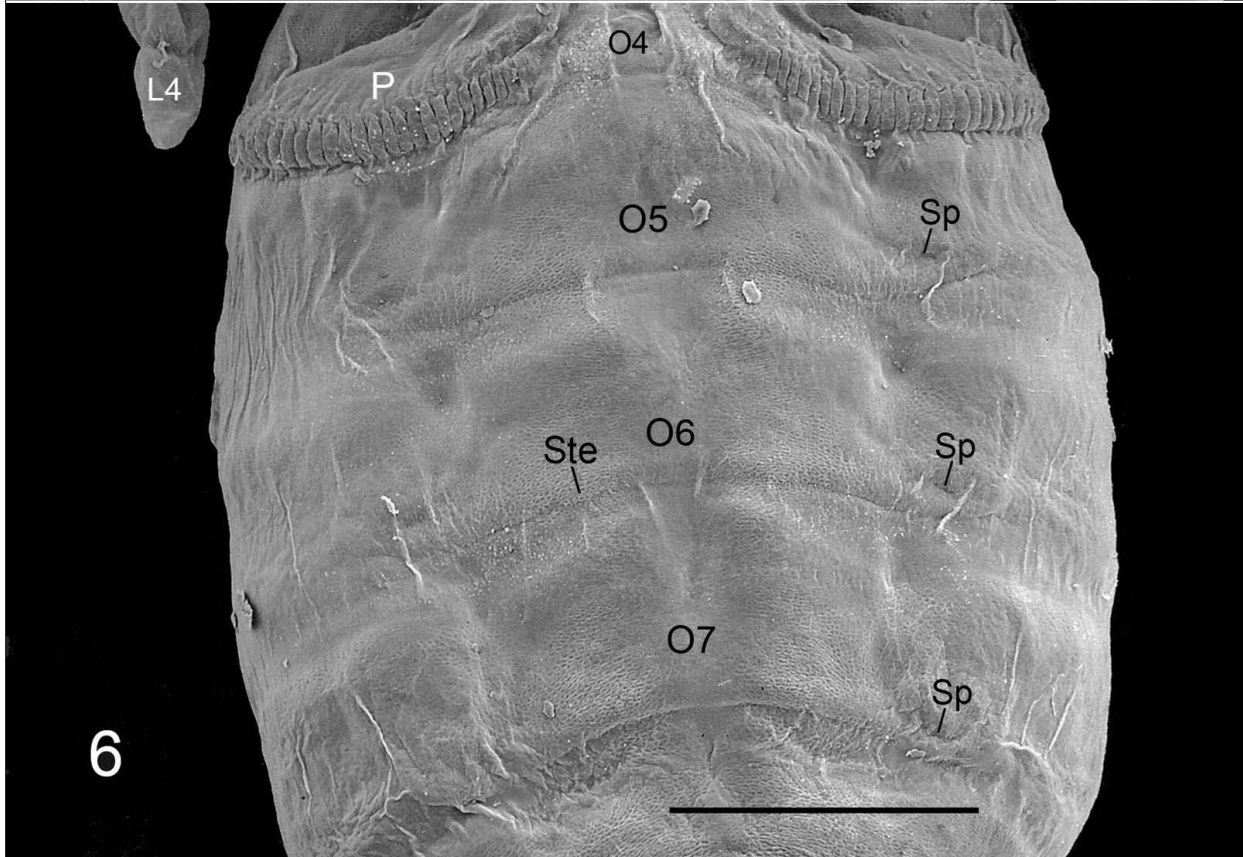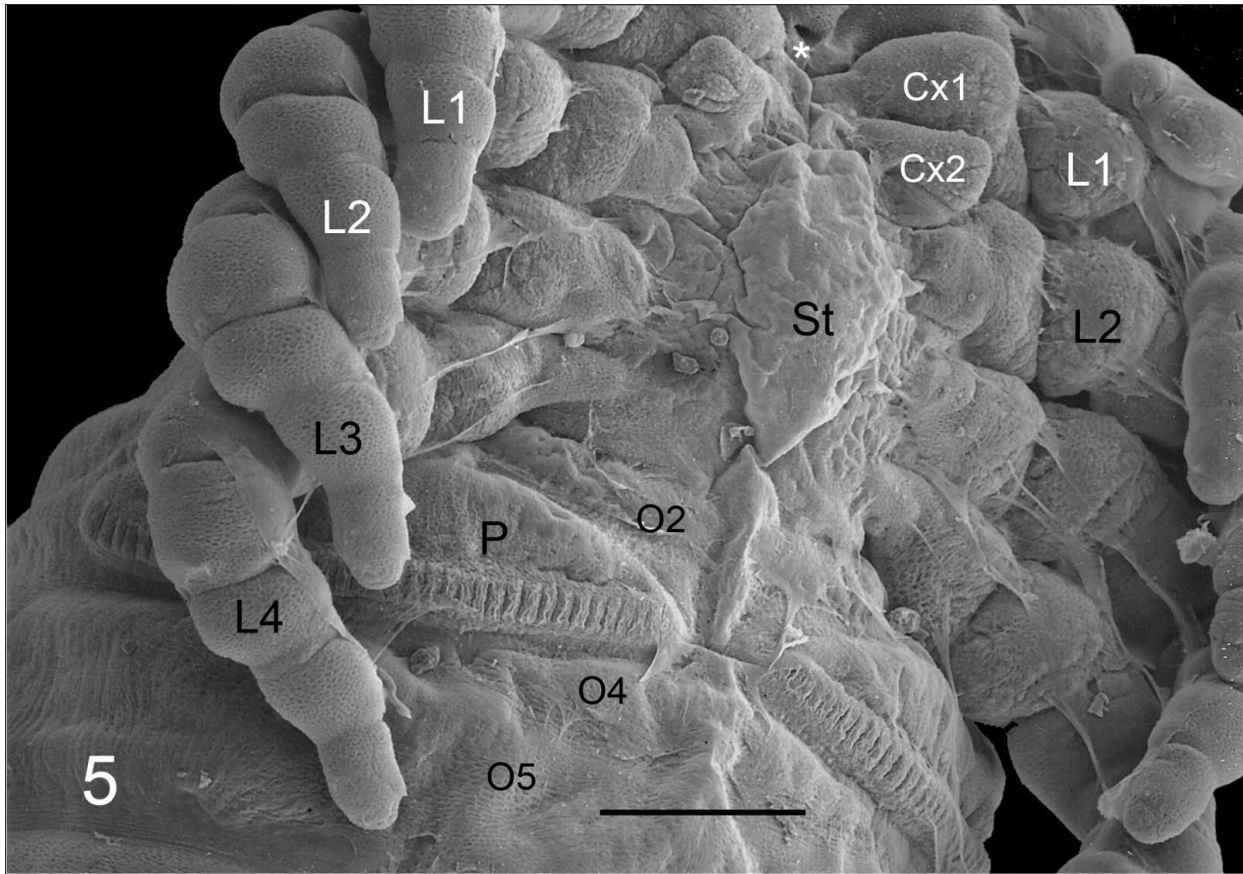
Figure 7: Ventral view of embryo of *Centruroides vittatus* (Buthidae, supine) at the stage where pectines (P) are elongate and ribbon-like with transverse striations. Ventral view, SEM. The legs were removed so mesosomal structures can be seen. The mesosoma (M) is swollen, apparently with maternal nutrients. Some primordial spiracles may be discerned on the ventral surface of some segments. Opisthosomal segments 1, 2 and 4−8 (O4−O8) are evident. The pectines apparently represent segment 3 (Figs. 1−3). Opisthosomal segment 1 is still evident, and segment 2 is starting to form lobes that continue development to become the genital operculum (Figs. 14, 15, 21−23, 32). L4, leg 4. Scale, 0.5 mm.

Figure 8: Developing sensory pegs (Pe, primordial sensilla) on the ventral surface of a tooth at the posterior edge of an embryo pectine. *Centruroides vittatus,* supine, SEM. In all the species examined so far, these sensilla start as small knobs (e.g., Fig. 16; Farley 2001c) that eventually develop a tiny opening in the center. The peg sensilla start to appear on the embryo pectines well before sensilla elsewhere on the body. The walls of these early sensilla increase in height and eventually become pegs with an elongate slit at the distal edge (Fig. 11). Scale, 5 μm.
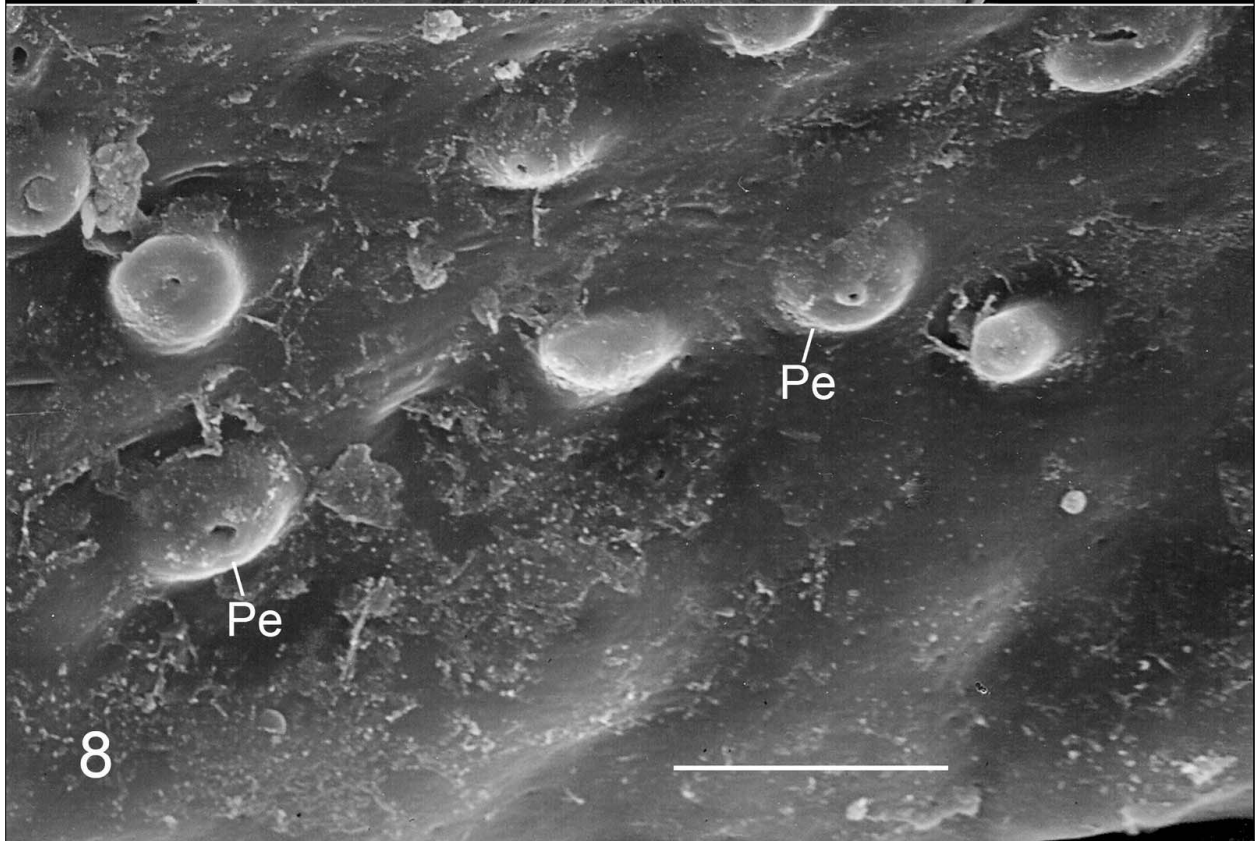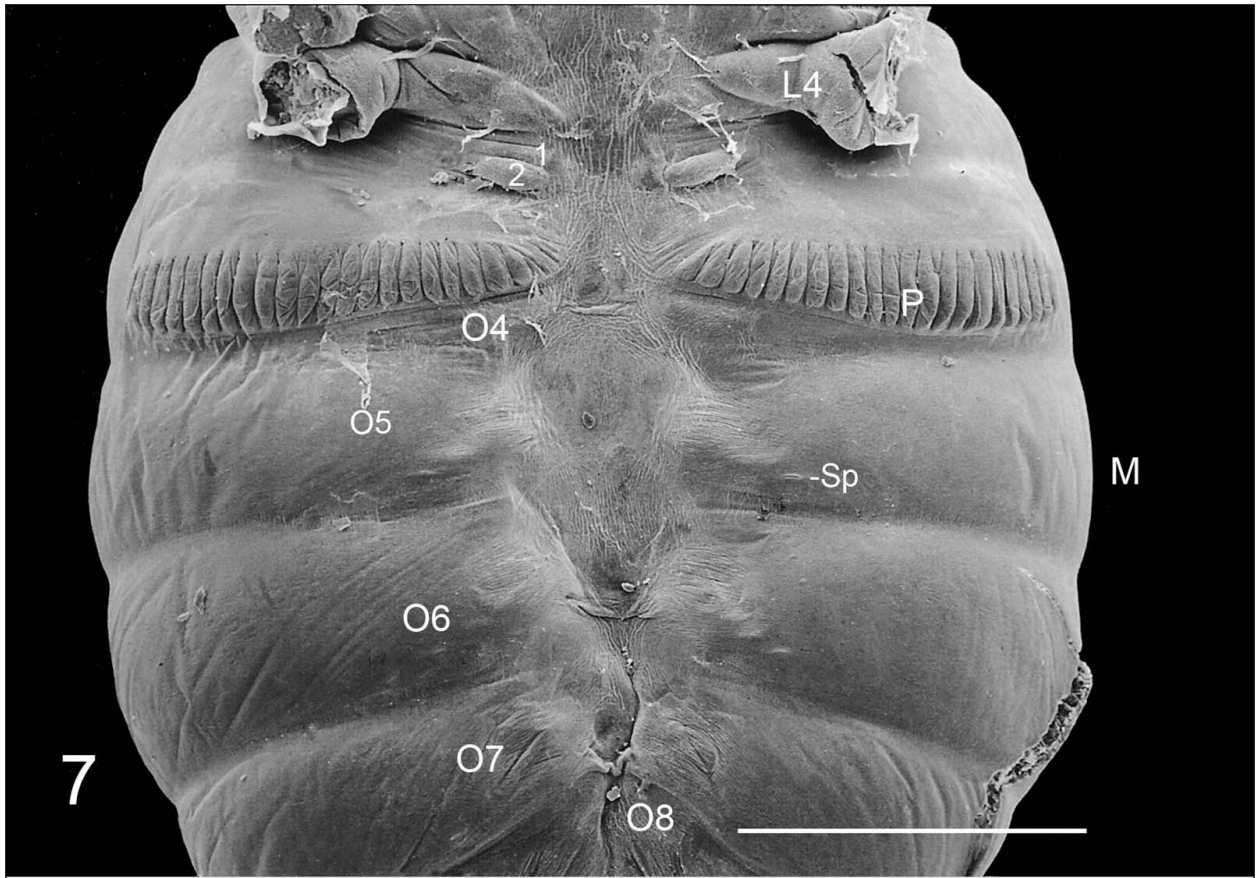
Figure 9: Nerve fibers (N) extending from the primordial peg sensilla (Pe) on the ventral surface of a pectinal tooth. *Centruroides vittatus,* embryo, prone. SEM. A small opening is evident at the tip of the sensillar knob. The sensillar walls increase in height and eventually become more elongate peg sensilla (Fig. 11) with a distal groove that allows passage of chemical stimuli to receptor dendrites inside the peg. The material in the central core (Co) of the pectine is probably yolk. Cu, cuticle. Scale, 10 µm.

Figure 10: Presumptive nerve (N) and supporting cells developing on the inner surface of primordial peg sensilla on the ventral surface of a pectinal tooth. *Centruroides vittatus*, embryo, prone. SEM. Nerve fibers to the central nervous system apparently start as cells of the hypodermis (H) beneath the cuticle. Extending from the ventral mesosoma, the pectines are large and accessible for studies on sensory nerve development. Scale, 10 µm.

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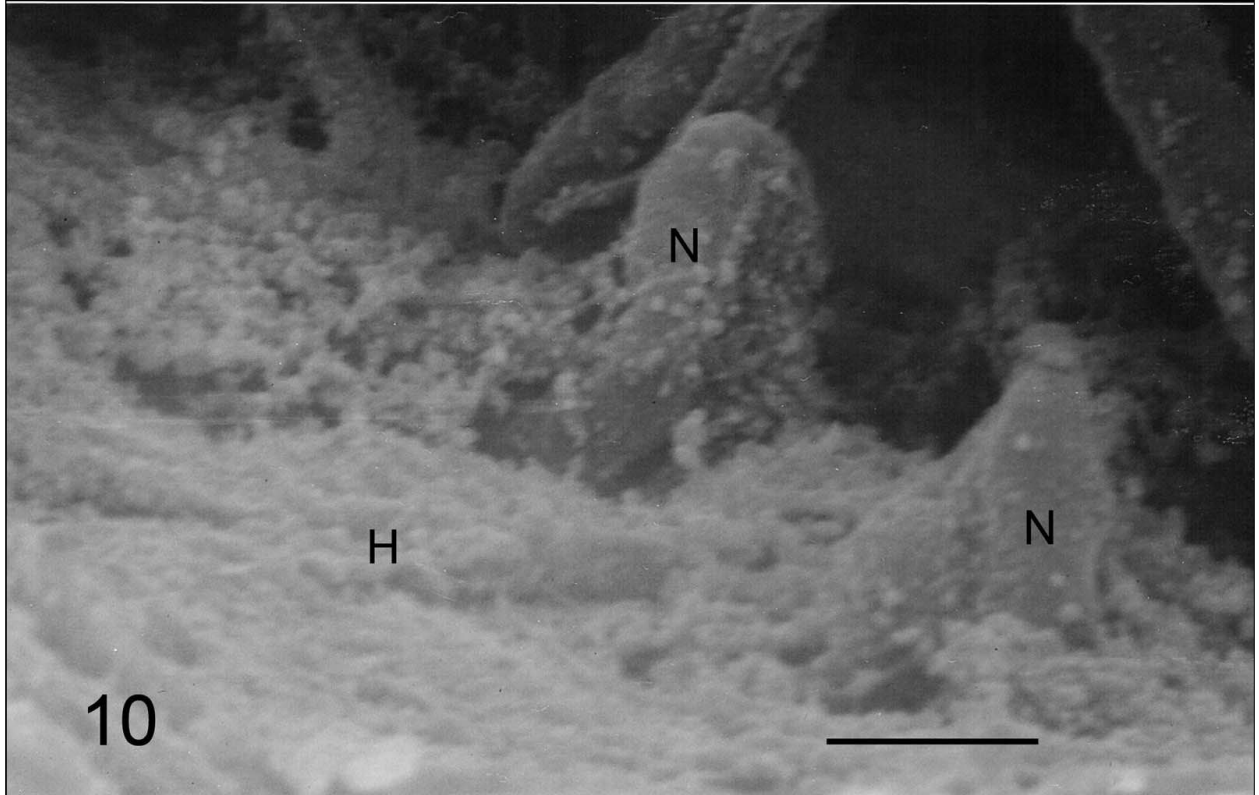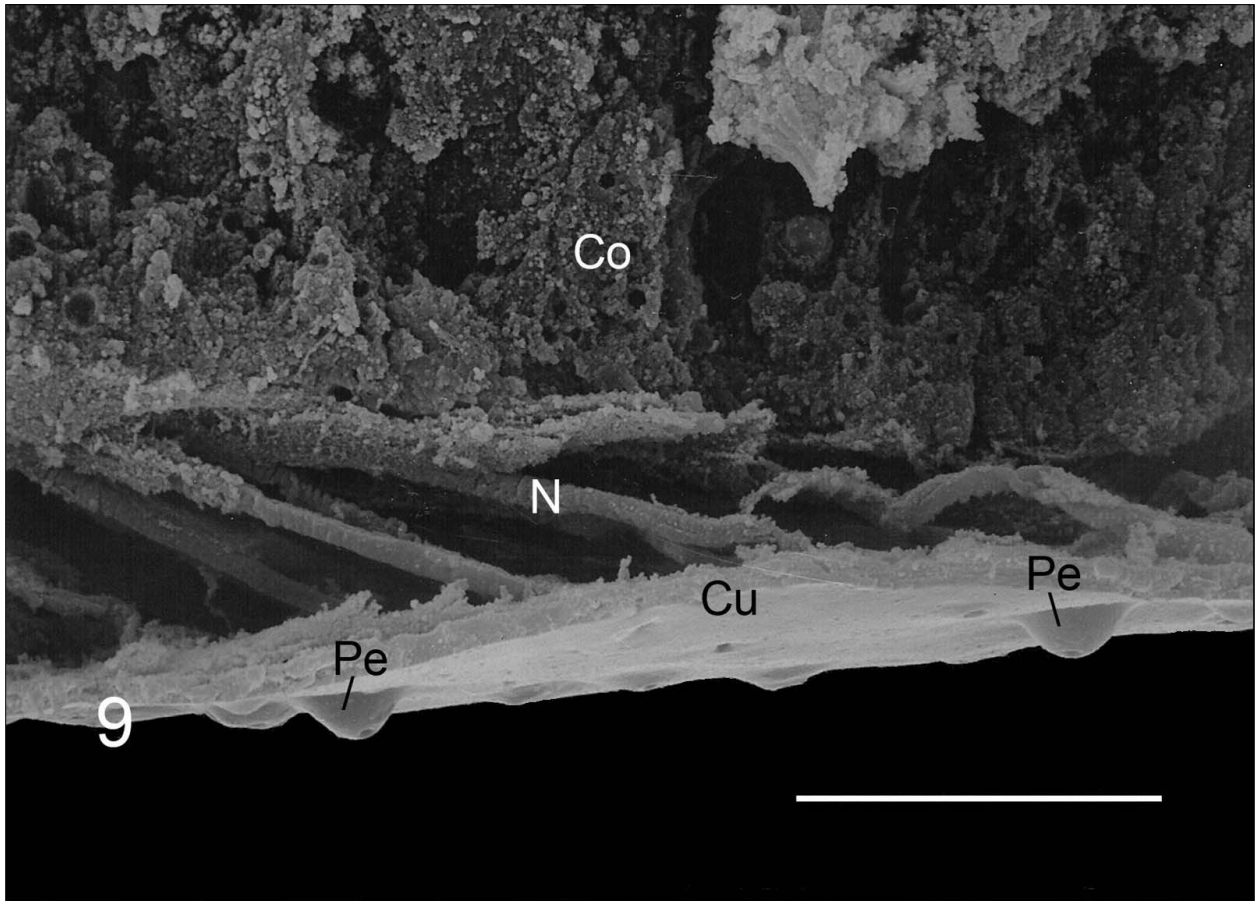
Figure 11: Peg sensilla (Pe) on the ventral surface of a pectinal tooth. *Centruroides vittatus*, second instar, supine. SEM. The sensilla have a flexible base and an elongate slit along their ventral surface. This slit is an opening that allows chemical stimuli to reach the sensory dendrites inside the peg (Wolf, 2008). Cu, cuticle. Scale, 5 μm.

Figure 12: Early stage of the pectine (P) in an embryo of *Hadrurus arizonensis* (Caraboctonidae). Lateral view, SEM. Striations are evident at the posterior edge of the pectine. The anterior pectinal edge is still joined to the body wall. The mesosoma (M) at this stage has transitory enlarged tergites (Te) which may be helpful for gas exchange with maternal hemolymph. The metasoma (Mt) has its final 5 segments and a distal tapered telson (T). The pectines are derived from the third opisthosomal segment (Figs. 1−3, 5−7), but overlap temporarily on the ventral surface of opisthosomal segment 4 (O4). 1−4, legs; Ch, chelicera; O8, opisthosomal segment 8; Pd, pedipalp. Scale, 1 mm.
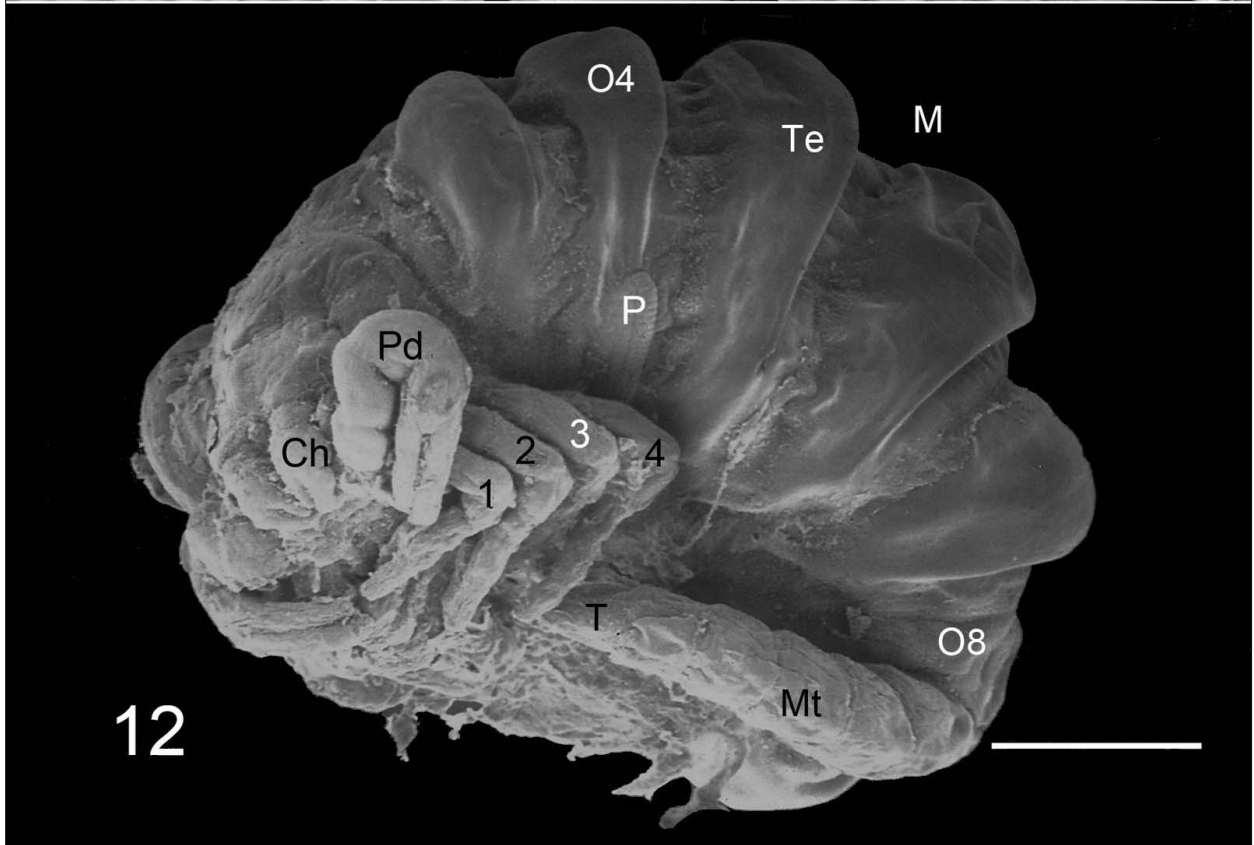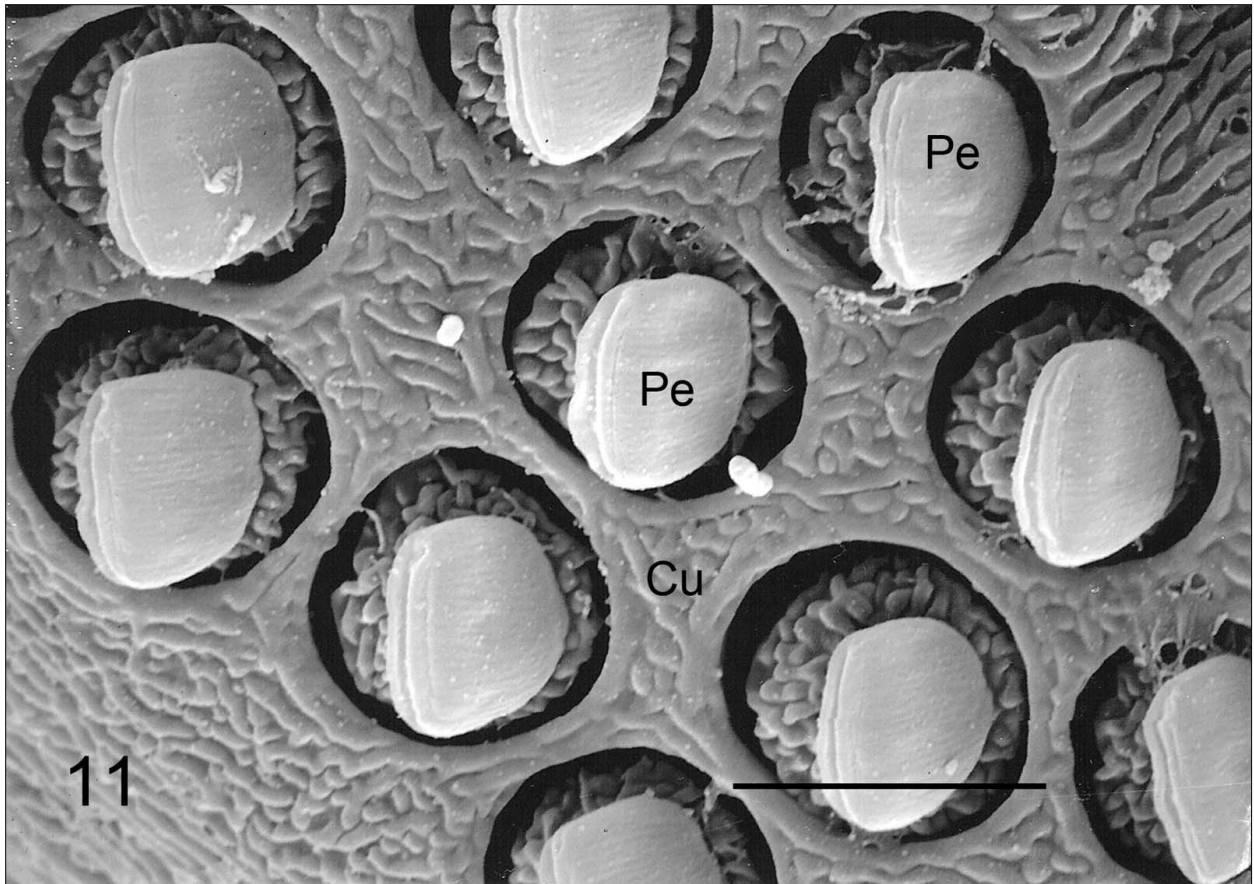
Figure 13: Higher magnification of an embryo pectine at about the same stage as in Figure 12. *Hadrurus arizonensis* (prone), lateral view. SEM. The pectine has a series of transverse striations, and a longitudinal groove (asterisk) that separates the pectine into anterior and posterior regions. The anterior striations are disappearing while the posterior ones continue as divisions between teeth. The anterior edge is joined to the body wall, while the posterior edge is slightly separated. L4, fourth leg. Scale, 200 μm.

Figure 14: Only 6 teeth in each pectine (P) of a first instar (supine) *Superstitionia donensis* (Superstitioniidae). Ventral view, SEM. The small number of teeth in each pectine of this species is in sharp contrast to the other species in this study in which tooth number is about 14−17 (Fig. 13), 17, (Fig. 23), 21 (Fig. 7) and 26 (Fig. 6). This first instar shows development that is very much like that for other species with apoikogenic development as described in earlier literature (Farley, 1999, 2001a-d, 2005, 2008). The pectines at this stage are mobile, attached only at their proximal ends. The coxapophyses (Cx1, Cx2) of the first and second legs (L1, L2) are rounded structures that form the ventral wall of the preoral tube. The body surface is smooth with little indication of setae and sensilla except for sensilla forming on the ventral surface of the pectine teeth (Fig. 16). The legs are tapered and digitigrade with distal claws not yet evident. Ch, chelicera; GO, genital operculum; L3, L4, legs 3, 4; O4−O8, opisthosomal segments 4−8; Pd, pedipalp. Scale, 0.5 mm.
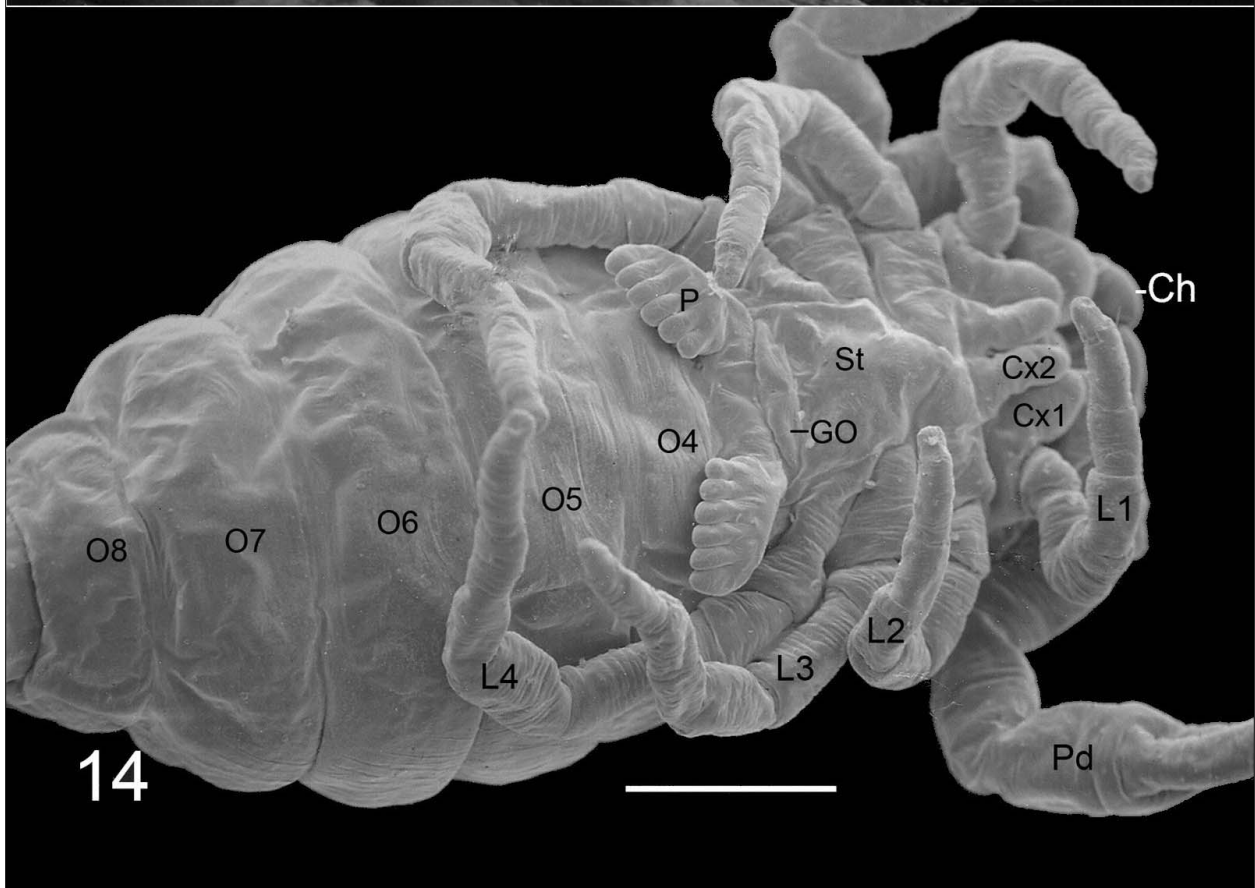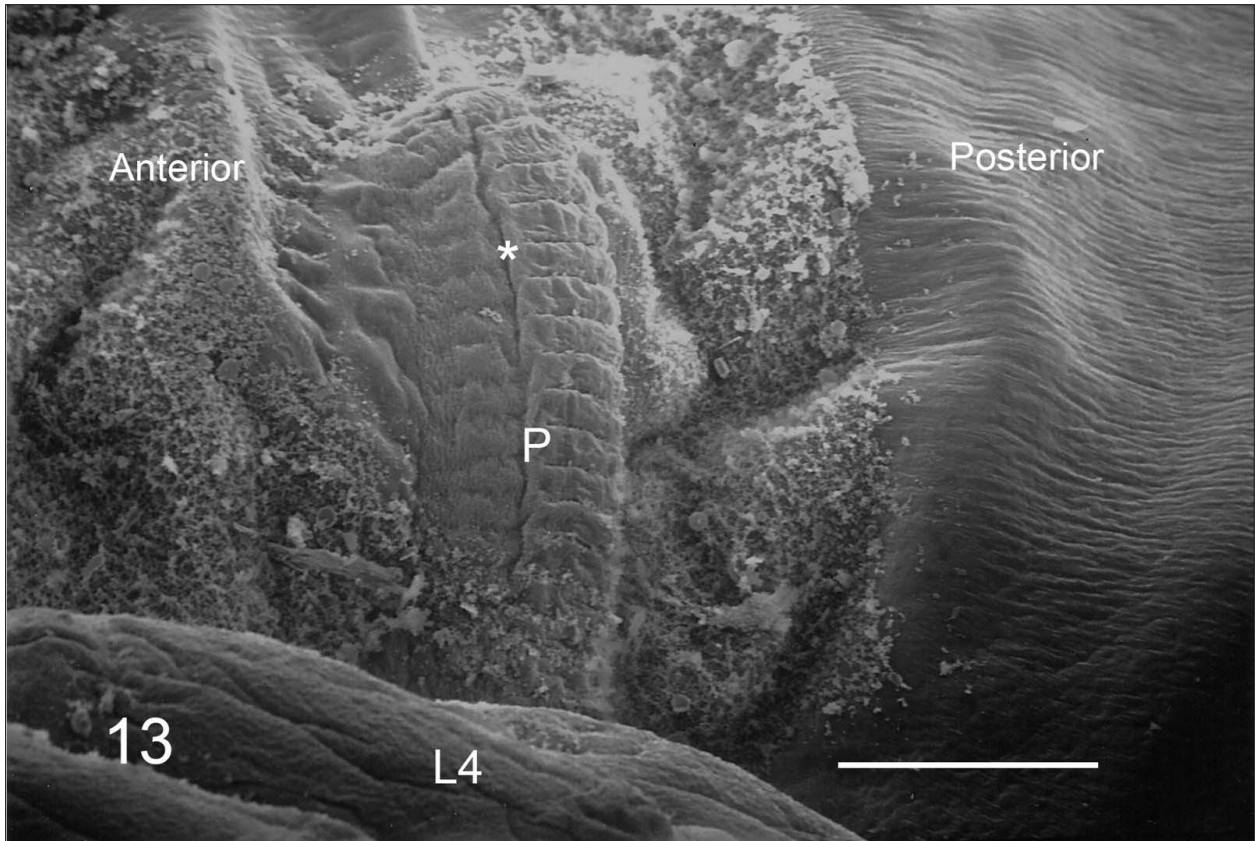
Figure 15: Development of the gonopore (Gp) between the paired flaps of the genital operculum (GO). First instar, *Superstitionia donensis,* supine. Ventral view, SEM. A small invagination in the basal plate (BP, basal piece) between the pectines (P) suggests a transitory ancestral gonopore (Gp') that will eventually disappear as the continuing gonopore (Gp) is formed. A similar transitional opening was seen in the pectinal basal plate of first instars of the buthid *Centruroides vittatus* (Farley, 2005). L4, leg 4; St, sternum. Scale, 100 μm.

Figure 16: Primordial peg sensilla (Pe) on the ventral surface of two pectinal teeth. First instar, *Superstitionia donensis*, supine. Ventral view, SEM. The peg sensilla in this species are not as developed as the pectinal peg sensilla of the embryos of *Centruroides vittatus* in Figures 8 and 9. For these species and others examined so far (e.g., Farley, 2001c), the peg sensilla appear initially as small cuticular knobs with a pore in the center. There is usually little indication of sensilla elsewhere on the body until after the first molt. Note the smooth body surface and absence of setae and sensilla in the first instars of Figures 14 and 15 and Farley (2005, 2008). Scale, 20 μm.

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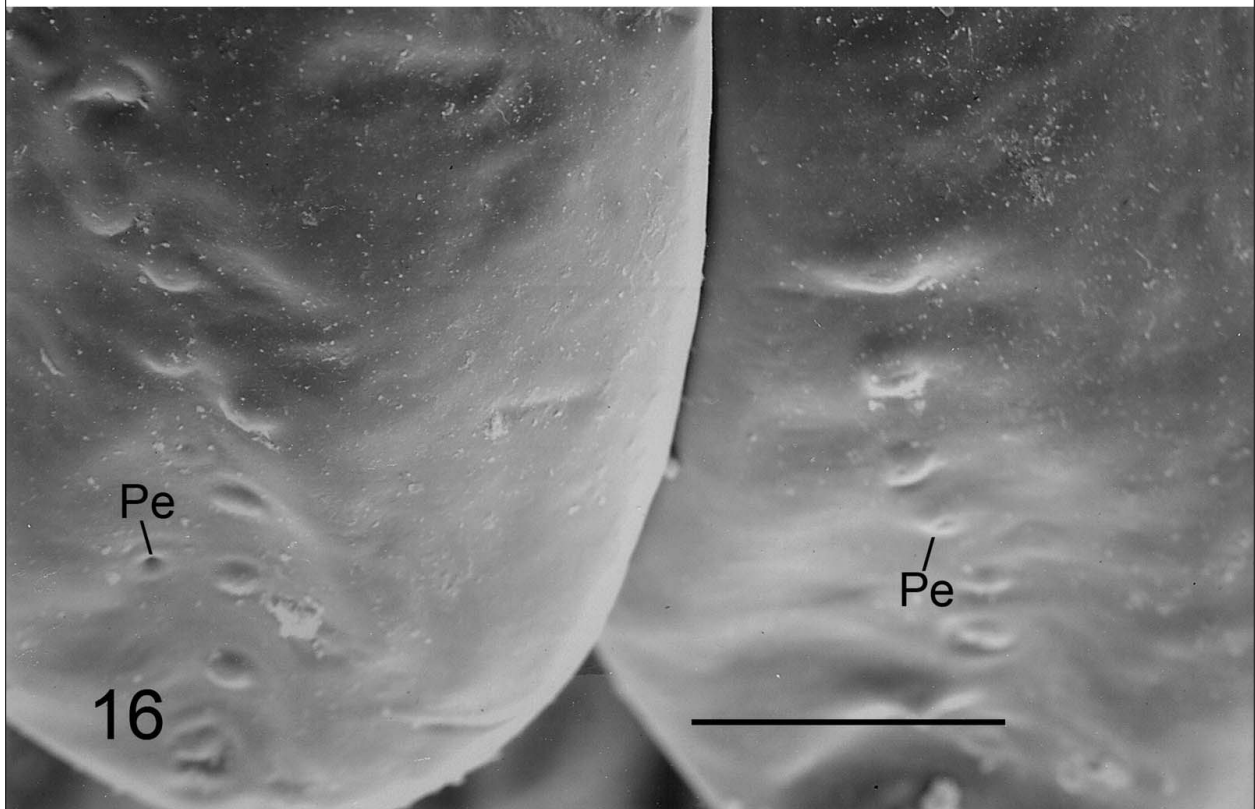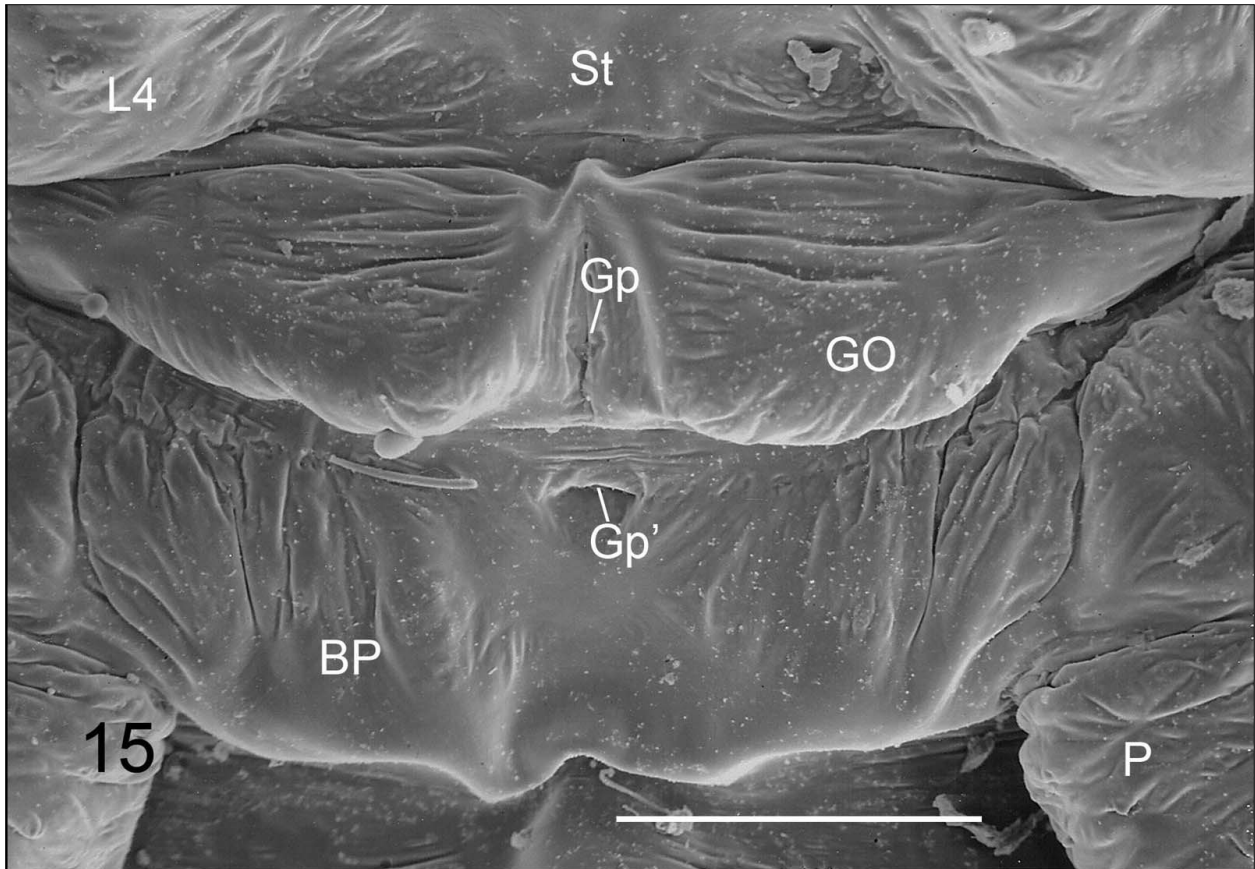
Figure 17: Lateral view of the ovariuterine tubule (OT), diverticulum (D) and appendix (A) at an early stage of embryo development in a species with katoikogenic development. *Hadogenes paucidens* (Hemiscorpiidae), SEM. The outer wall of the diverticulum forms a fibrous and cellular covering for the embryo developing inside. The head end of the embryo is at the proximal end of the appendix so nutrients temporarily stored in the appendix can be taken into the embryo digestive tract. Oral feeding of the embryo at an early stage of development is characteristic of scorpions with katoikogenic development. Some diverticulae (D') on the ovariuterine tubule are at an early stage. Scale, 1 mm.

Figure 18: Embryo with katoikogenic development at about the same stage as in Figure 17 but with the diverticulum wall (DW) opened and pulled posteriorly. *Hadogenes paucidens*, lateral view, SEM. The embryo body is enclosed in an embryonic membrane that was torn in some places (asterisks) during dissection. The mesosoma (M) with digestive tract and glands for oral feeding is greatly enlarged relative to the prosoma (Pr) and metasoma (Mt). There are no prominent limb buds evident on the ventral surface of the mesoma although the embryonic membrane prevents a clear view of what is there. Each segment of the mesoma has large swellings, dorso-lateral processes (DP) that may facilitate gas exchange with maternal hemolymph. These are apparently thin-walled and filled with fluid since they readily collapse during preparation for microscopy. 1−4, legs 1−4; A, appendix; Pd, pedipalp. Scale 1 mm.
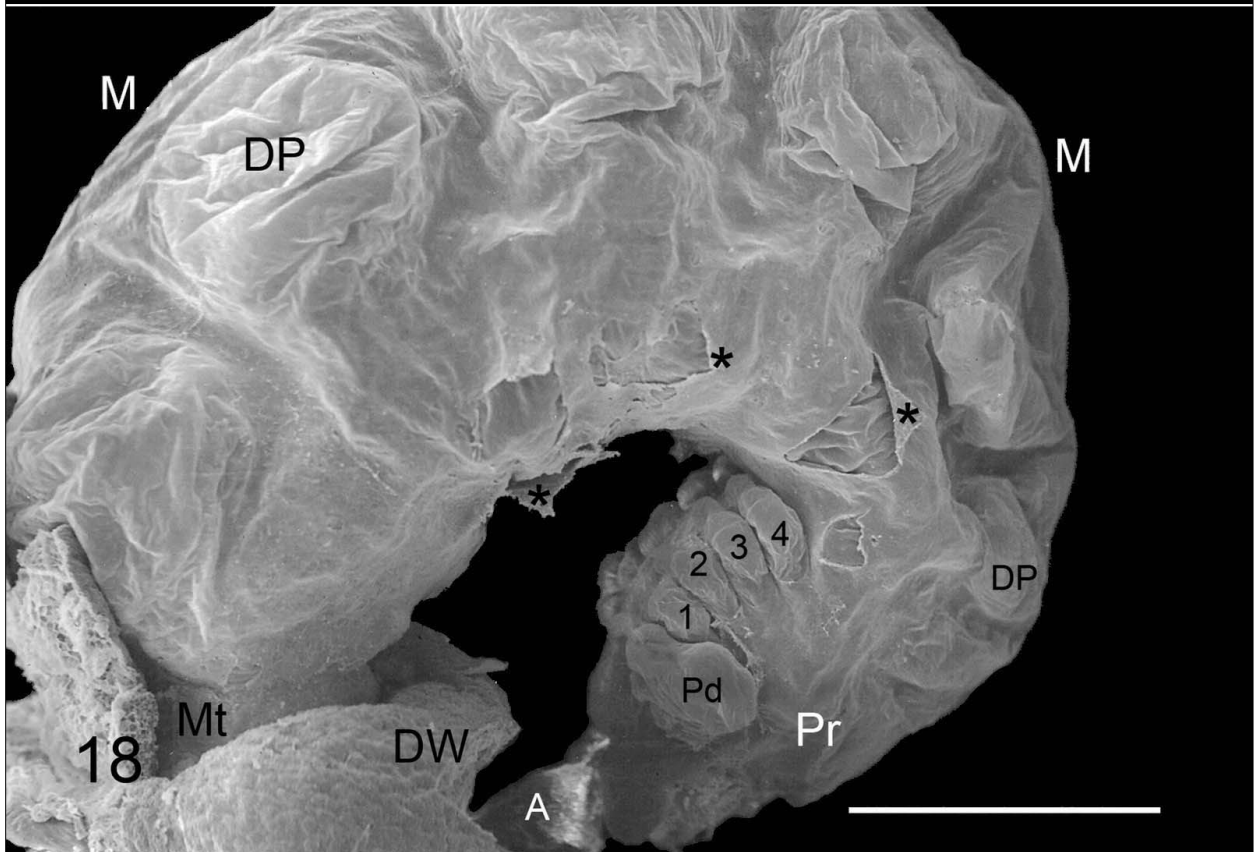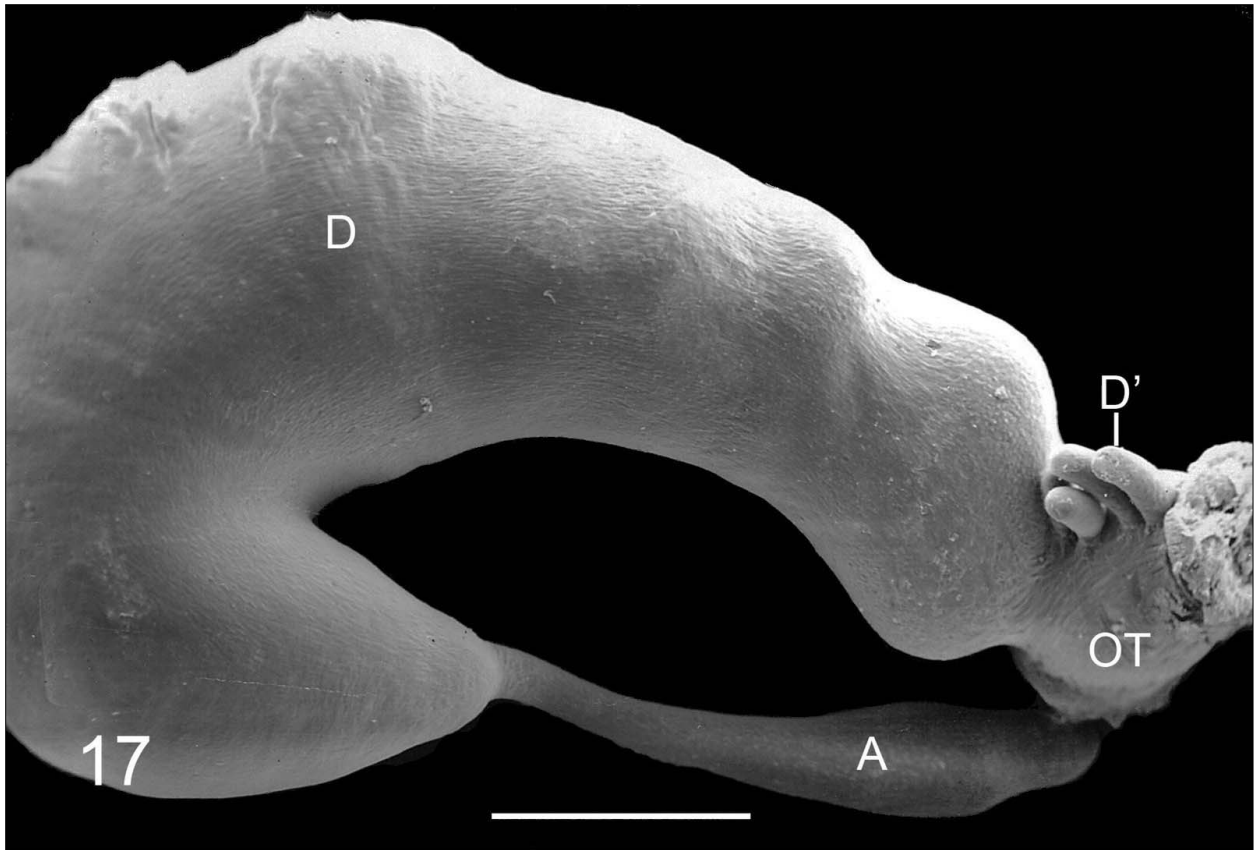
Figure 19: Higher magnification of the outer (EM) and inner (EM') surfaces of the embryonic membrane of an embryo like that in Figure 18. *Hadogenes paucidens*, lateral view, SEM. The membrane was torn during dissection as in Figure 18, enabling examination of the inner and outer membrane surfaces. The embryonic membrane is mainly a layer of cells (C) that may transfer nutrients to the embryo. With later development, the embryonic membrane only partially covers the enlarging body (Fig. 20). The membrane eventually disappears, or remaining portions were removed by dissection (Figs. 21−32). Scale, 50 μm.

Figure 20: Embryo body with katoikogenic development more advanced than the embryo in Figure 18. *Hadogenes paucidens*, ventro-lateral view, SEM. The mesosoma (M) is greatly enlarged relative to the prosoma (Pr) and metasoma (Mt). The cellular embryonic membrane (asterisks) covers most of the body except for the pedipalps (Pd) and legs (1−4). No prominent limb buds are evident on the ventral mesosoma although the embryonic membrane prevents a clear view of what is there. In the mesosoma, the large dorso-lateral processes (DP) collapse during preparation for microscopy as though they are only filled with fluid. The proximal end of the appendix (A) is attached to the prosoma where the mouth and anterior digestive tract are located. Scale, 1 mm.
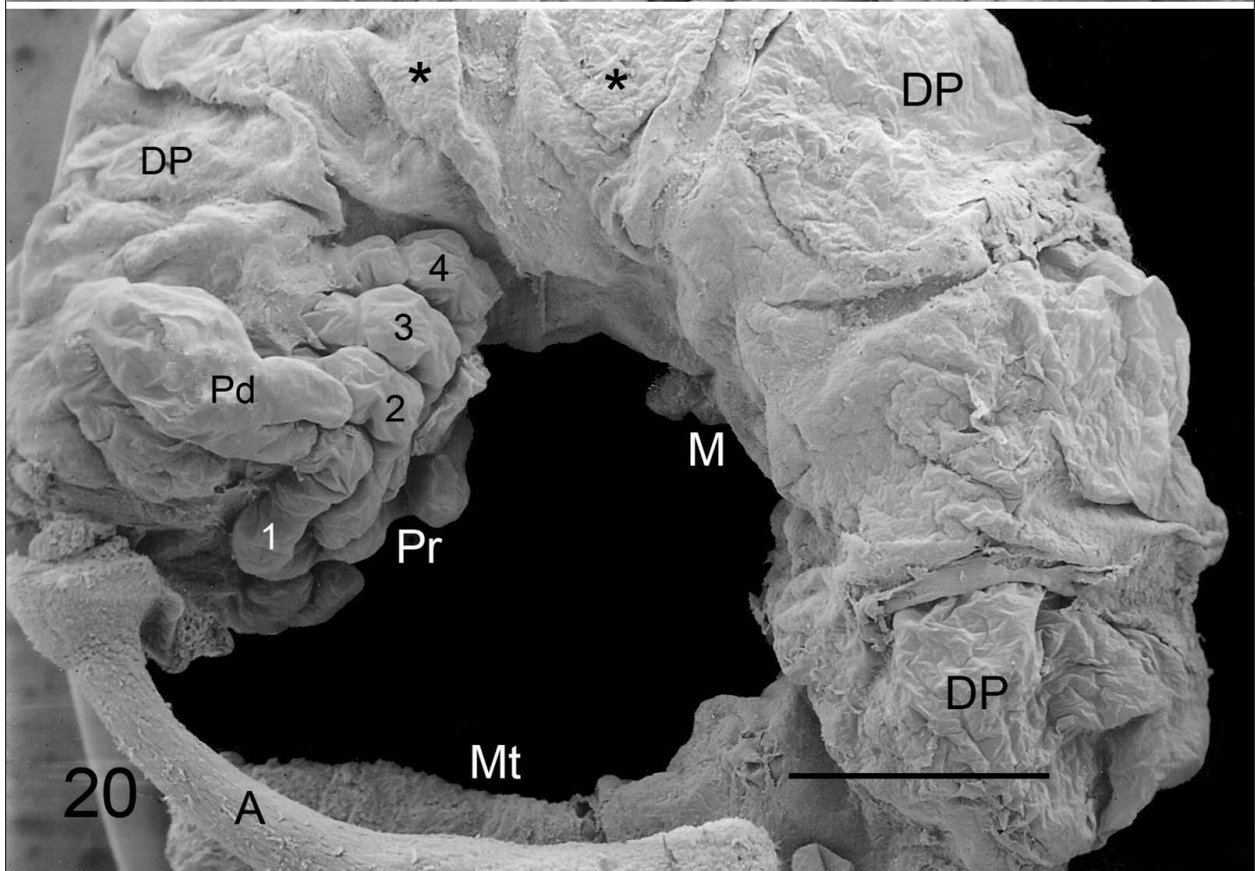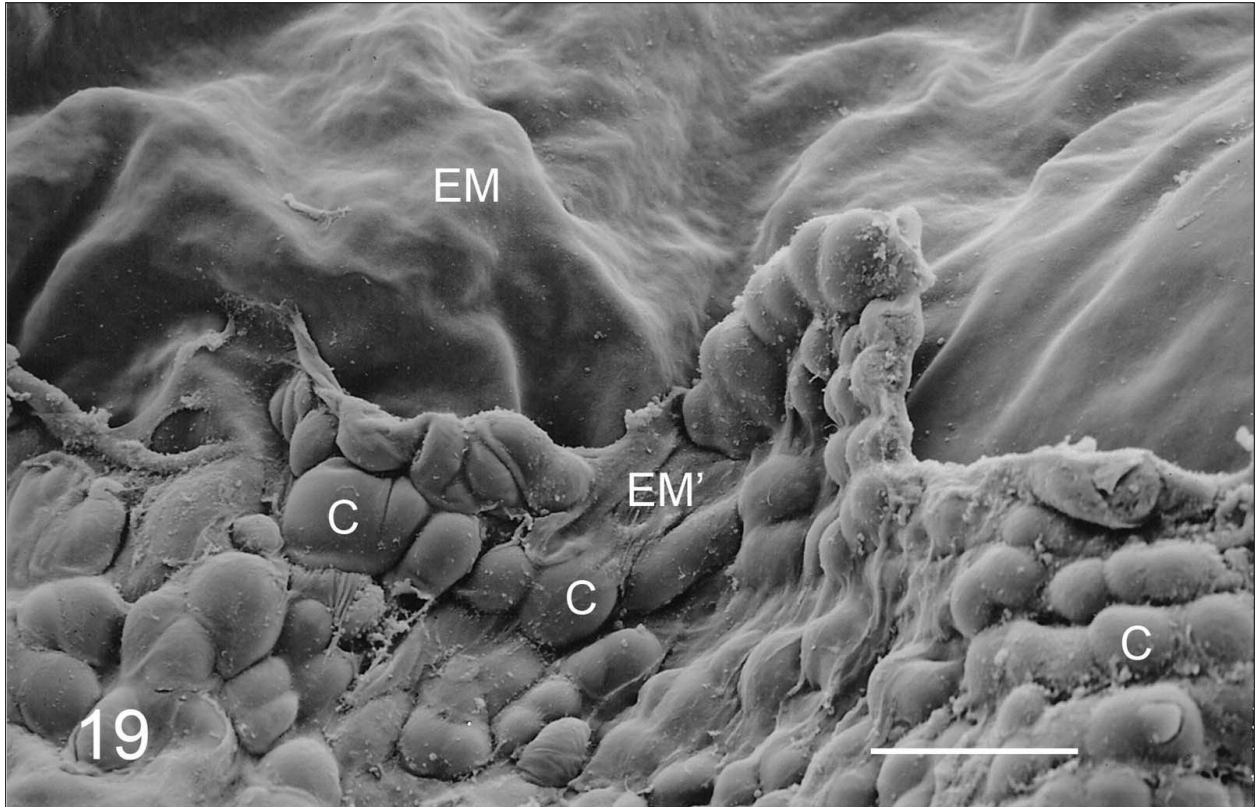
Figure 21: Pectine (P) in an embryo with katoikogenic development at a stage more advanced than in Figures 18 and 20. *Hadogenes paucidens*, supine, ventral view. SEM. For this and the following figures (Fig. 22−32), the embryonic membrane was absent, or remaining portions were removed by dissection. The small, flap-like pectine is still attached to the ventral surface of the fourth opisthosomal segment (O4) as occurs at a similar stage in embryos with apoikogenic development (Figs. 5, 7; Farley, 2001b, c, 2005). A groove (asterisk) is starting to form along the length of the pectine, separating it into anterior and posterior regions, also as seen in embryos with apoikogenic development (Figs. 4, 13). This groove is more prominent in the slightly more advanced embryo of Figure 22. The outline of teeth is evident at the posterior margin, but the teeth are not yet separated from each other. Sturdy, transitory setae (bristles, B) appear on the ventral mesosoma before and at the same time as spiracles and sternites (Figs. 23−25). The bilateral flaps of the genital operculum (GO) are starting to form. L4, leg 4. Scale, 200 µm.

Figure 22: Early stage in the development of pectines (P) in an embryo with katoikogenic development slightly more advanced than the embryo in Figure 21. *Hadogenes paucidens*, supine, ventral view. SEM. The pectines develop as small flaps, but some of the earliest stages were not seen like those in embryos with apoikogenic development (Figs. 1−3, 7). A crease (asterisks) extends along the length of each pectine and divides it into anterior and posterior regions. This also occurs in the developing pectines of embryos with apoikogenic development (Figs. 4, 13). Teeth are partially separated from each other at the posterior pectinal margin. The lobes of the genital operculum are larger and more advanced than those in Figure 21. O4, opisthosomal segment four; St, sternum. Scale, 200 µm.
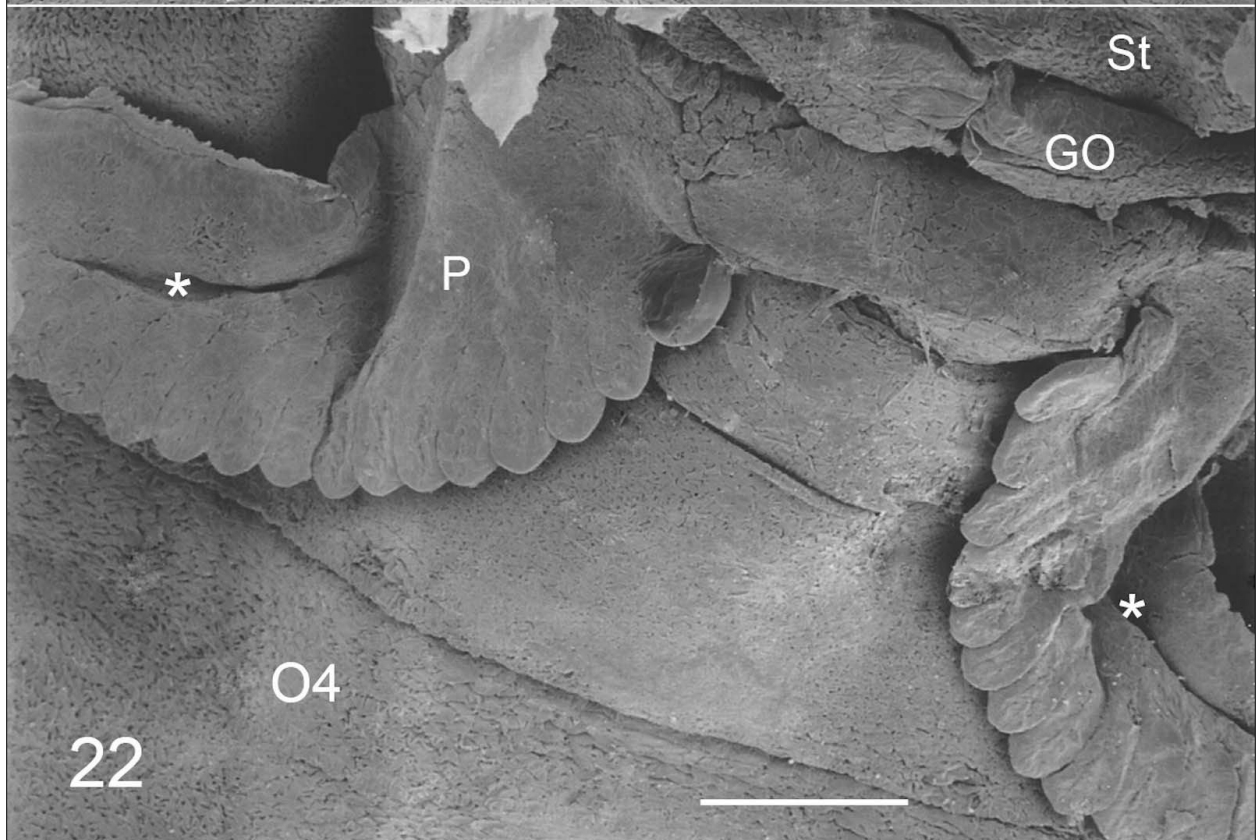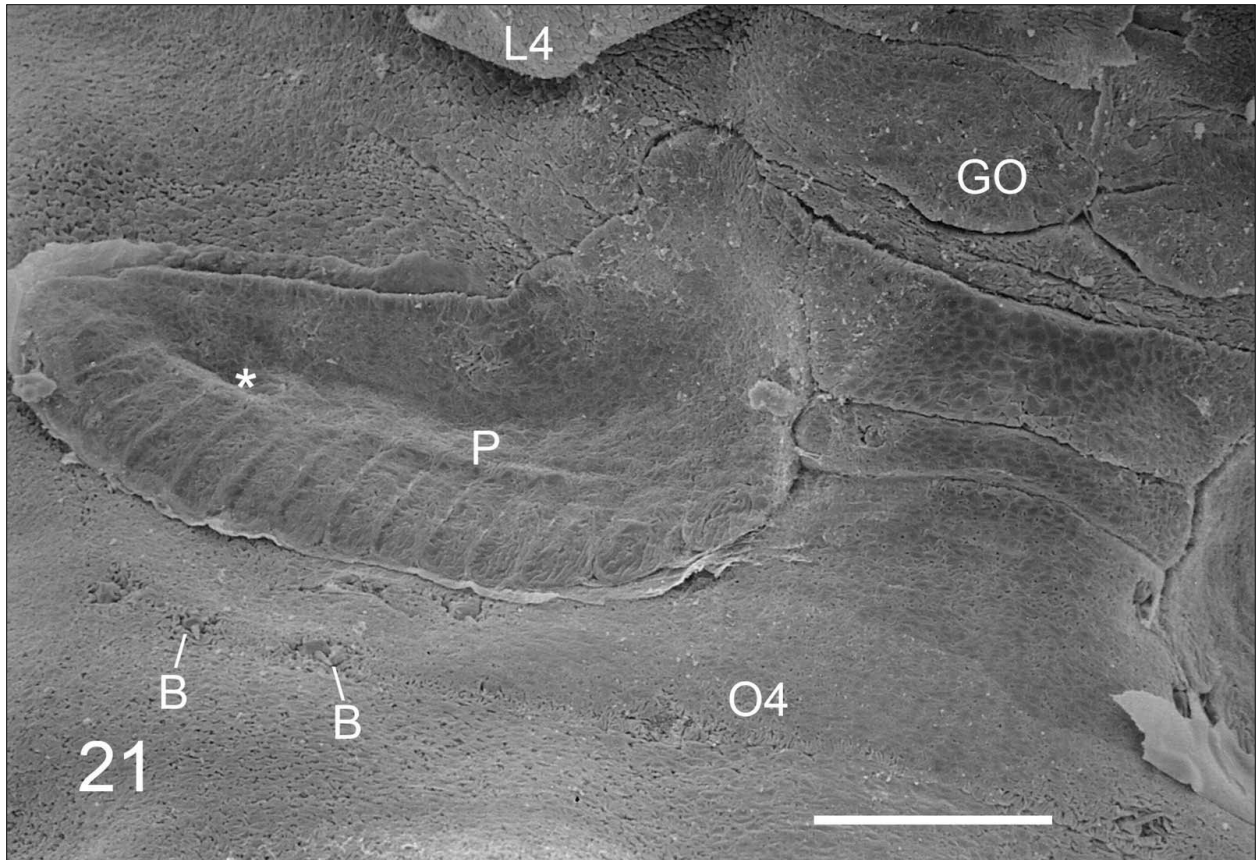
Figure 23: Pectines (P) on an embryo with katoikogenic development at a stage more advanced than in Figure 22. *Hadogenes paucidens*, supine, ventral view. SEM. The third and fourth legs (L3, L4) were removed by dissection. The pectines are mobile and attached only at their proximal ends. The bilateral flaps of the genital operculum (GO) are becoming prominent. Transitory setae (bristles, B) appear on the pectines and ventral mesosoma before and at the same time as spiracles (Sp) and sternites (Ste; Figs. 24, 25). O4, O5, opisthosomal segments 4 and 5. Scale, 500 μm.

Figure 24: Ventral view of mesosoma of an embryo with katoikogenic development at about the same stage as the embryo in Figure 23. *Hadogenes paucidens*, supine. SEM. Evident here are epithelial invaginations for spiracles (Sp) in opisthosomal segments 4−6 (O4−O6). The shape of the future sternites is outlined with rows of sturdy, transitory setae (bristles, B) as the posterior edges of the sternites (Ste) are starting to appear as faint creases. At this stage, the pectines (P) have a row of teeth at their posterior margin and are attached only at their proximal ends. L2−L4, legs 2−4; O7, opisthosomal segment 7. Scale, 1 mm.
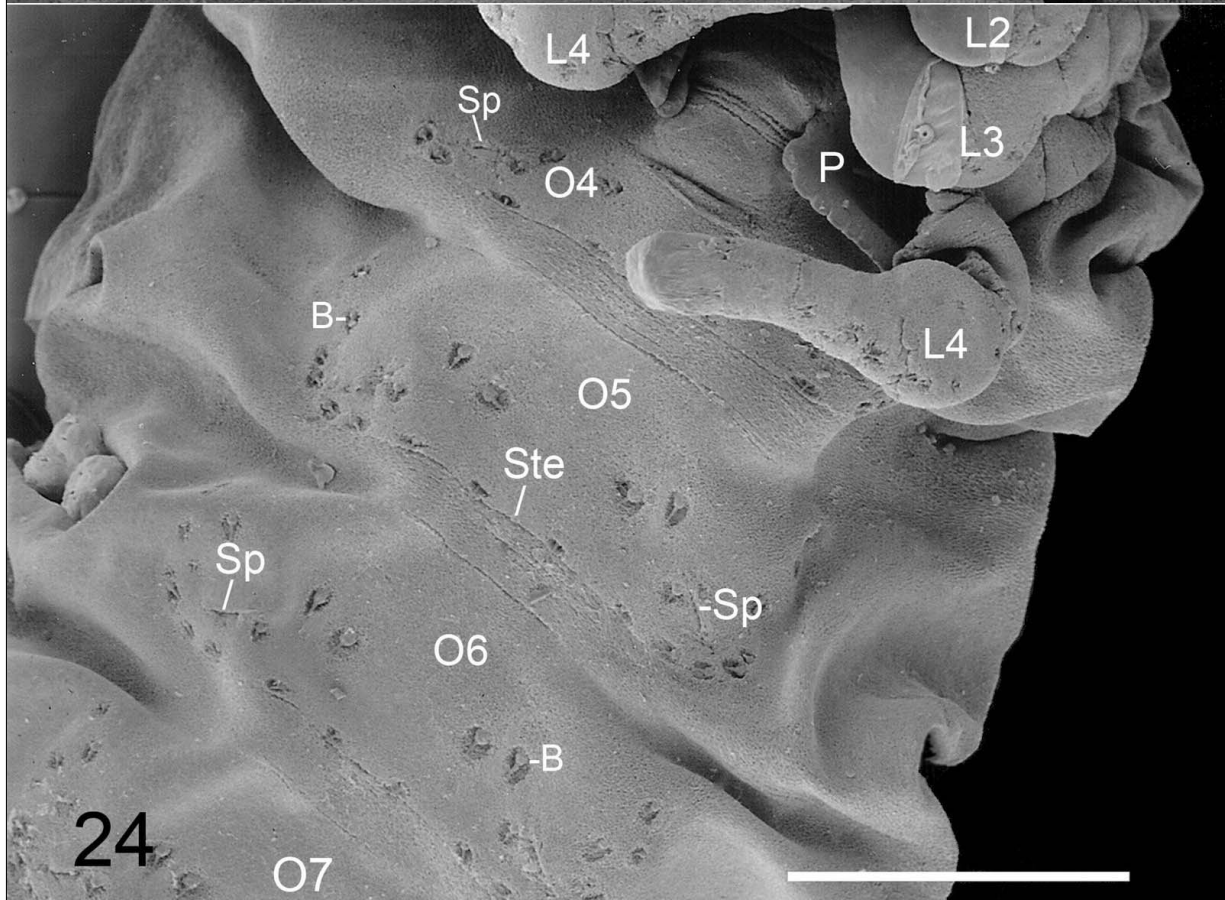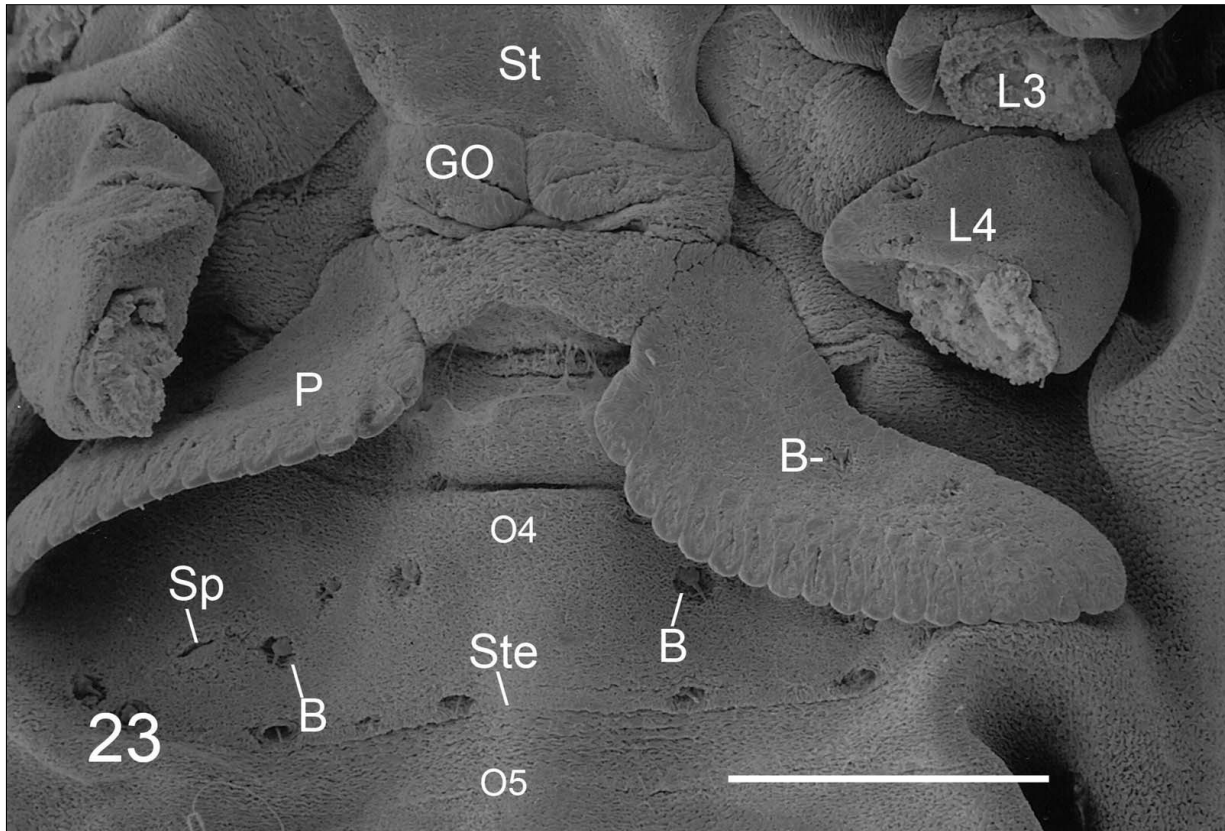
Figure 25: Higher magnification of opisthosomal segments 5 and 6 (O5, O6) of an embryo with katoikogenic development at about the same stage of development as in Figures 23 and 24. *Hadogenes paucidens*, supine, ventral view. SEM. Evident here are epithelial invaginations for spiracles (Sp). The shape of the future sternites is outlined with a row of sturdy, transitory setae (bristles, B) as the posterior edges of the sternites (Ste) are starting to appear as faint creases. Scale, 500 µm.

Figure 26: Ventral view of embryo prosoma at about the same stage of development as Figures 23−25. *Hadogenes paucidens*, supine, SEM. The distal ends of the chelicerae are highly modified for grasping the proximal end (teat) of the appendix. The medial surfaces of the pedipalp coxae have a specialized texture that may macerate food and assist in securing the appendix teat. A rostrum (R, labrum) precedes the stomodeum (S, mouth). The ventral surfaces of the pedipalps (Pd) and legs (L3, L4) have setae (bristles, B) like those on the ventral mesosoma at this stage (Figs. 21, 23−25). The coxapophyses (Cx1, Cx2) of the first and second legs (L1, L2) are distinct lobes that gradually extend forward to form the ventral wall of the preoral tube (Fig. 14; Farley, 1998, 1999, 2001c, d, 2005, 2008). Scale, 500 µm.
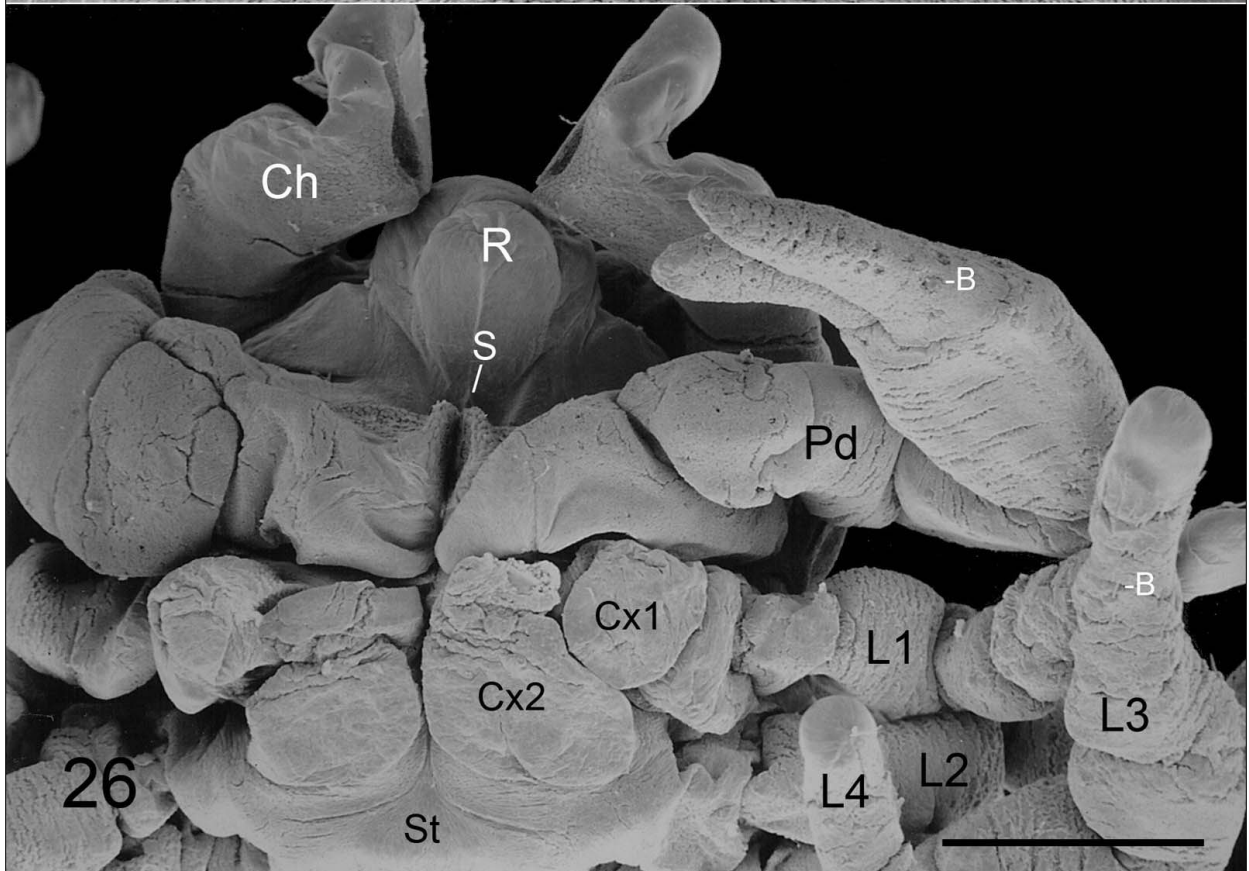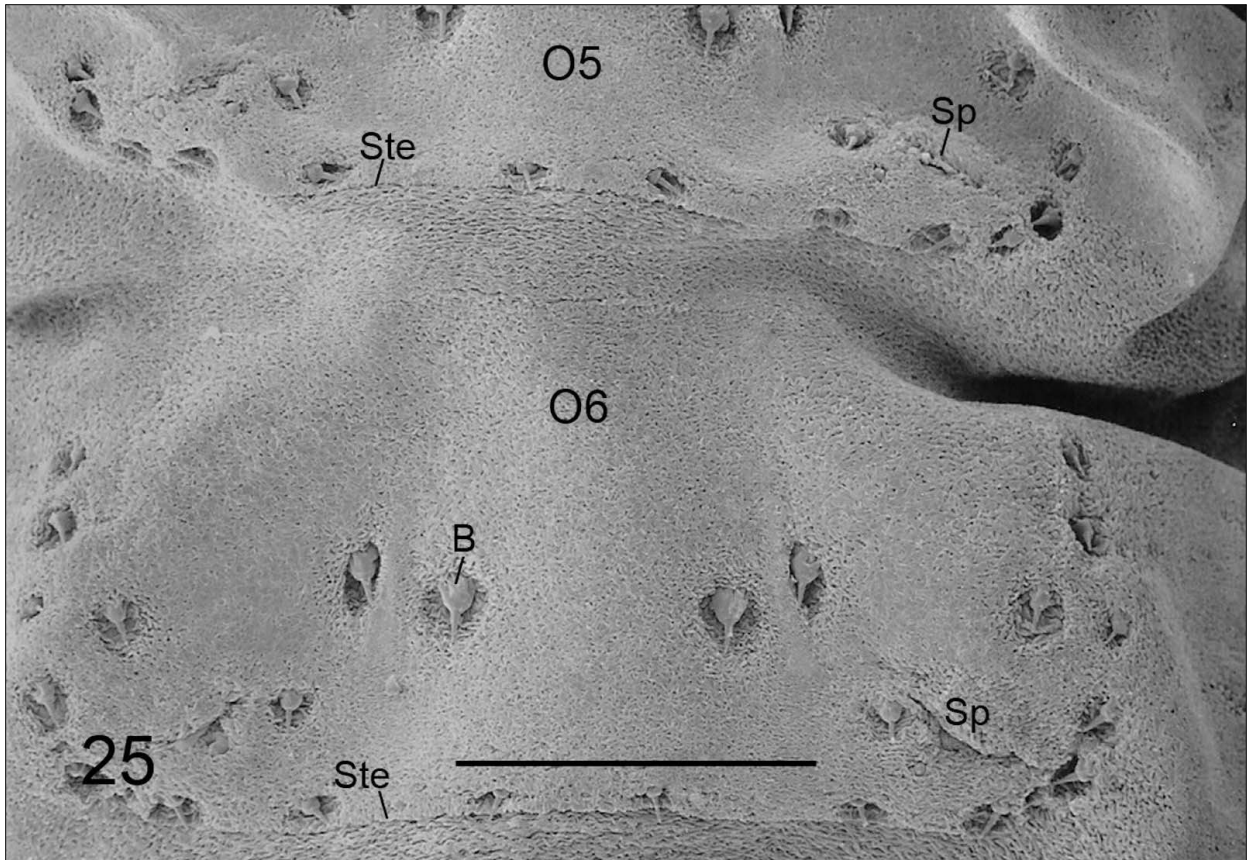
Figure 27: Lateral view of the distal end of the metasoma of an embryo at a later stage of development than Figures 18−26. *Hadogenes paucidens*, prone, SEM. The metasoma was removed from the embryo body. The metasoma now has all five segments and a primodial telson (T). The fifth metasomal segment (Mt5) has enlarged dorsal lobes that may assist with gas exchange with maternal hemolymph. The ventral surface of the segments has setae (bristles, B) like those on the ventral mesosoma (Figs. 21, 23−25) and appendages (Fig. 26). In the second and later instars, the metasomal segments and telson develop cuticular specializations that facilitate dorsal flexion and use of the sting (Bowerman, 1972a, b; Farley, 2005). X, debris. Scale, 500 μm.

Figure 28: Early embryo of *Pandinus imperator* (Scorpionidae), another species with katoikogenic development. Lateral view, SEM. The mesosoma (M) is very large compared with the prosoma (Pr) and metasoma (Mt). The mesosoma has large, thin-walled dorso-lateral processes (DP) that often collapse during preparation for microscopy. The appendix (A) leads directly to the anterior end of the digestive tract which develops very early (Mathew, 1956). No indication of pectinal development can be discerned. Limb buds are not prominent in the ventral mesosoma, but there are some faint ridges that may be indicative of limb buds. Scale, 1 mm. From Farley (1999), Scorpiones. Reprinted by permission of John Wiley & Sons, Inc.
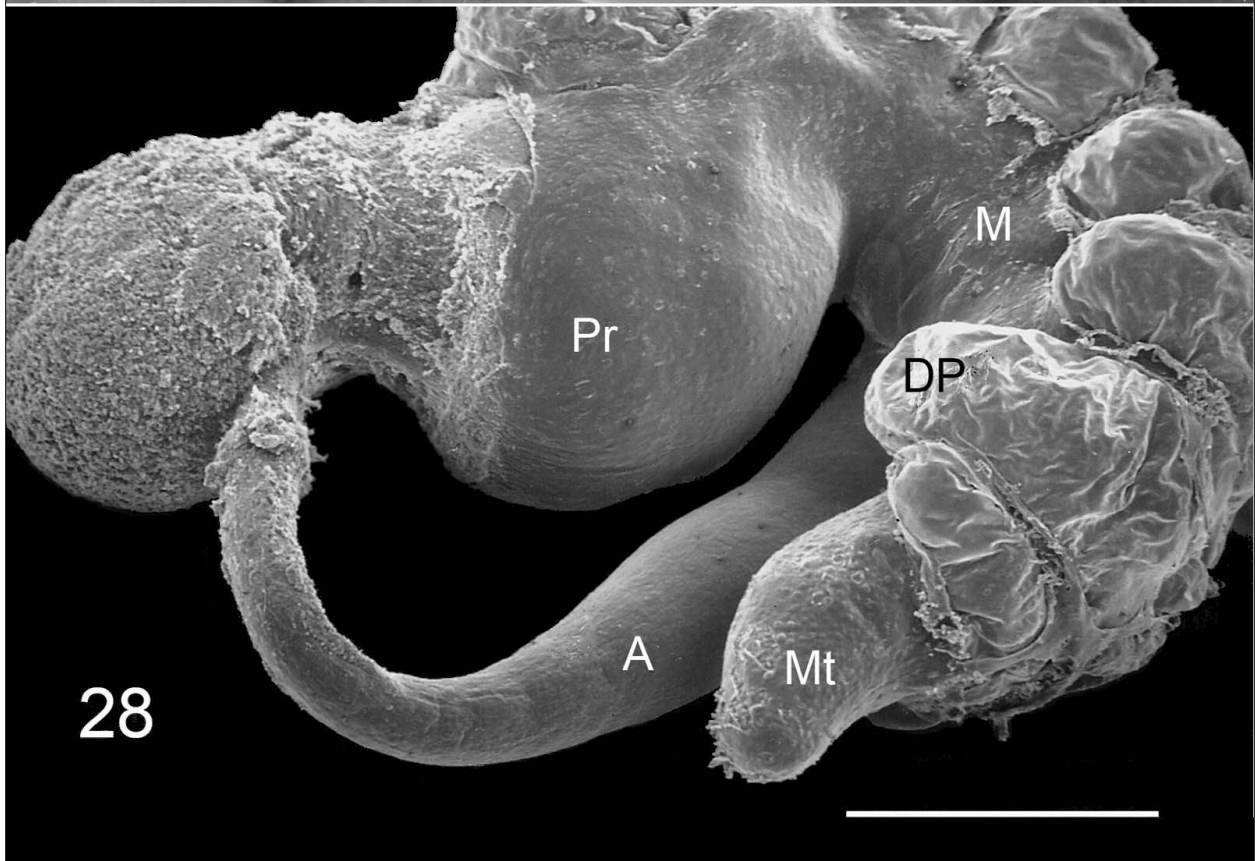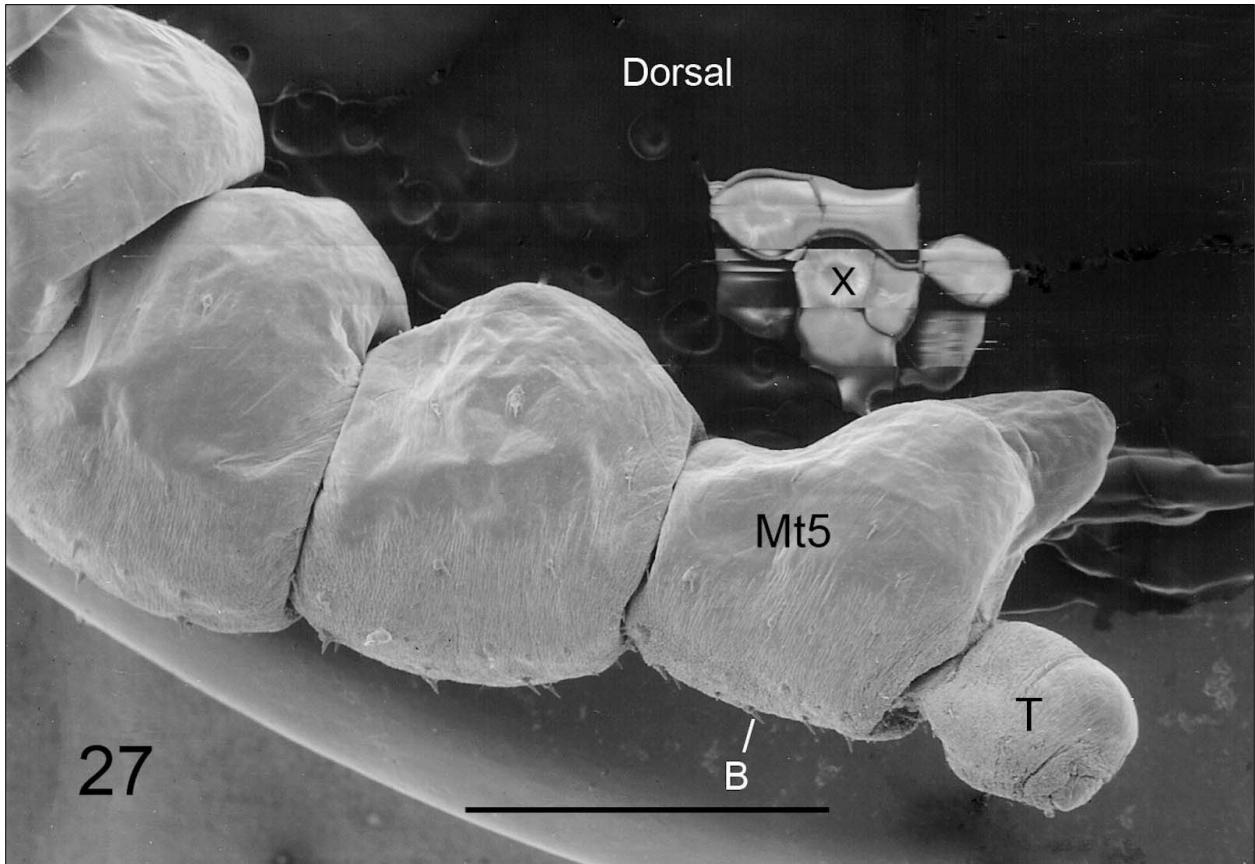
Figure 29: Ventral view of embryo of *Pandinus imperator* (supine) at a later stage of development than in Figure 28. SEM. The pedipalps (Pd) and legs (L) are starting to differentiate in the prosoma (Pr). Apparently the chelicerae will develop later from the large lobe (Lo) at the anterior prosoma. The appendix (A) leads directly to the anterior end of the digestive tract which develops very early (Mathew, 1956). The mesosoma (M) has large dorso-lateral processes (DP) that collapse during preparation for microscopy. The metasoma is narrow and elongate with faint demarcation of segments. No clear indication of pectinal development can be discerned. Limb buds are not prominent in the ventral mesosoma, but there are some lobes (Fig. 30) that may be indicative of limb buds. Scale, 1 mm. From Farley (2001d), Structure, Reproduction and Development. By permisson of Oxford University Press, Inc. http://www.oup.com. P. 32, Figure 2.12 from Ch. 2, 'Structure, Reproduction and Develoment' by Roger Farley from 'Scorpion Biology and Research' by Brownell & Polis. Free permission, author's own material.

Figure 30: Higher magnification of the mesosoma (M) of the same embryo as Figure 29. Ventral view, *Pandinus imperator*, supine. SEM. Even at this magnification no clear indication of pectinal development can be discerned. It is difficult to identify segment boundaries and limb buds, although some small lobes (LB) may be indicative of limb buds. The pectines will eventually develop (Fig. 32) posterior to the legs (L1−L4) of the prosoma (Pr). DP, dorso-lateral process; Mt, metasoma. Scale, 500 μm.
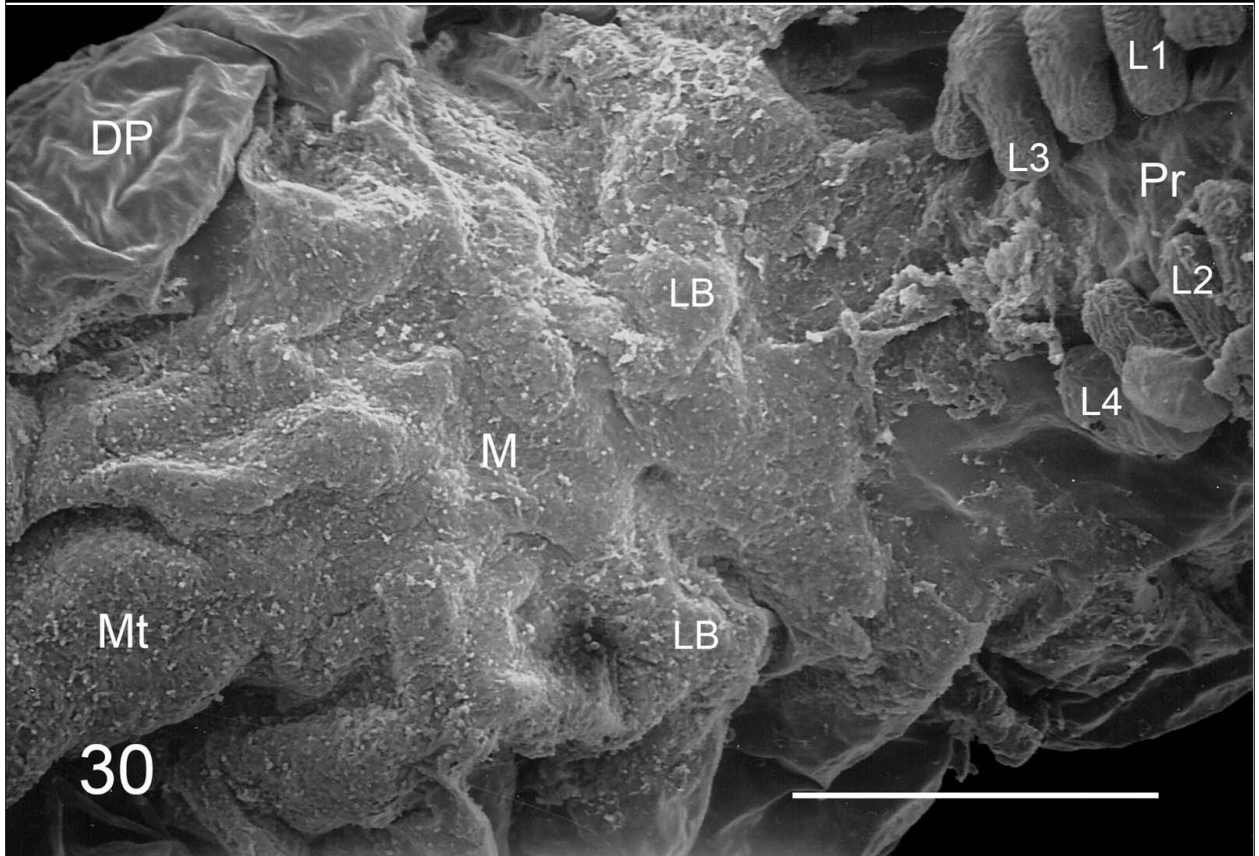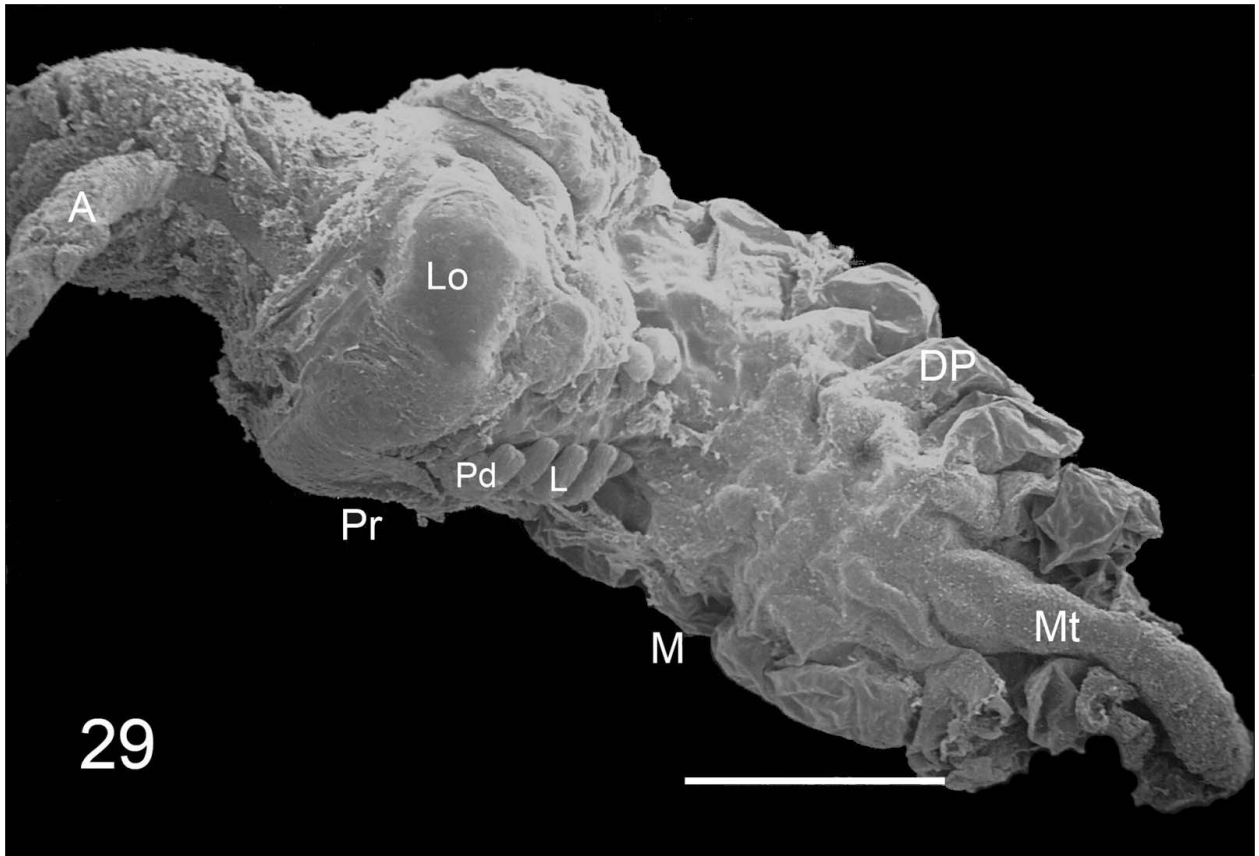
Figure 31: Ventral view of embryo of *Pandinus imperator* (supine) at a later stage of development than in Figures 28−30. SEM. At this stage, the pectines are fairly advanced in development as spiracles (Sp) and sternites (Ste) become evident here on the ventral surface of opisthosomal segments 6 and 7 (O6, O7). At the posterior edge of segment 6, small creases in the cuticle indicate the beginning of sternite formation. At the posterior edge of segment 7, a small flap-like sternite margin can be seen. As in embryos of *Hadogenes paucidens* (Figs. 21, 23−25), large and sturdy setae (bristles, B) form a pattern in the shape of the sternites before the sternites appear. The asterisks indicate setae that are just inside the creases of the lateral edges of the developing sternite on segment 7. Scale, 500 μm.

Figure 32: Ventral view of embryo mesosoma of *Pandinus imperator* (supine) at a later stage of development than Figure 31. SEM. Sternites (Ste) are becoming evident on the ventral surface of opisthosomal segments 4−6 (O4−O6), and some spiracles (Sp) can be identified. The flaps of the genital operculum (GO) are evident anterior to the pectines (P). The pectines are attached only at their proximal ends, and they have distinct anterior and posterior regions with a row of teeth at the posterior margin. Some setae (bristles, B) are present on the ventral surface of the mesosoma, but they are not prominent and may be deteriorating as compared with their distribution and size in Figure 31. Scale, 1 mm.