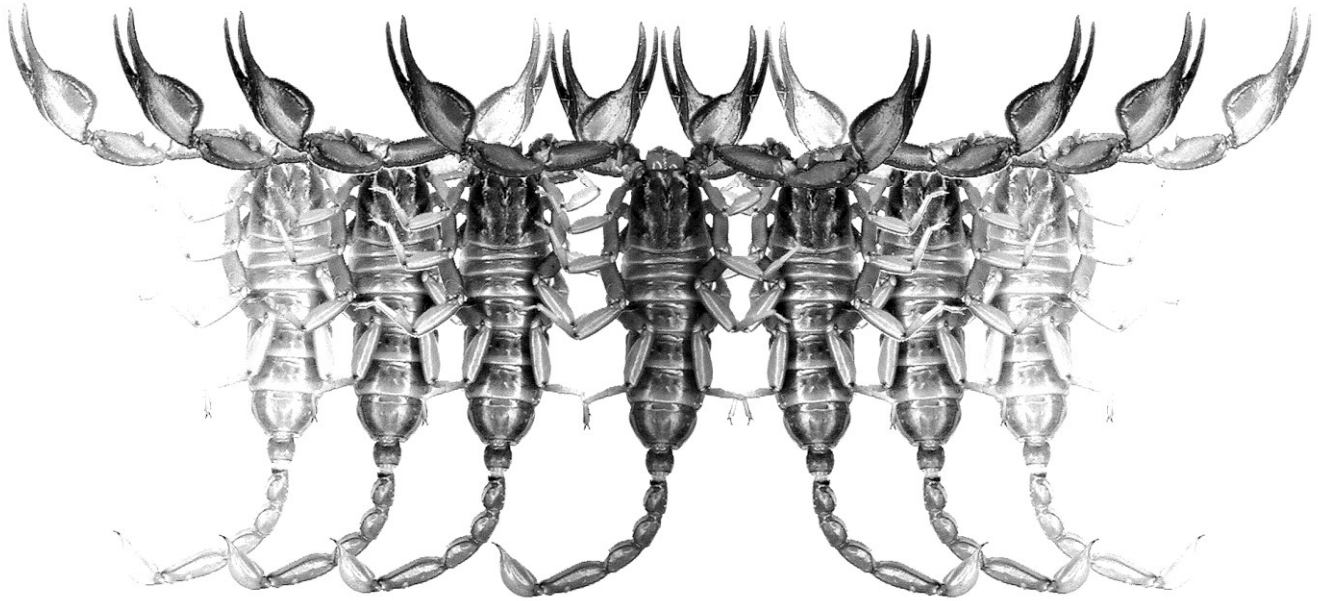


Euscorpius

Occasional Publications in Scorpiology



**Etudes on Iurids, VII. An SEM Study of External
Morphology of *Calchas birulai* Fet et al., 2009
(Scorpiones: Iuridae)**

Mehlika Benli, Ersen Aydın Yağmur & Victor Fet

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Etudes on iurids, VII. An SEM study of external morphology of *Calchas birulai* Fet et al., 2009 (Scorpiones: Iuridae)

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<http://zoobank.org/urn:lsid:zoobank.org:pub:C464B014-AB5F-4C28-88DC-2379A910CE8F>

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Summary

External morphology of a recently described scorpion species, *Calchas birulai* Fet et al., 2009 (Iuridae), was investigated in detail using Scanning Electron Microscopy (SEM). The samples were collected from Halfeti District, Şanlıurfa Province, Turkey. We investigate and depict, in particular, the cuticle, chelicerae, genital operculum, sternum, stigmata, wax pores, and sensory organs (trichobothria, setae, pectines, slit sensilla, constellation array).

Introduction

The relict scorpion family Iuridae, found in Aegean-Anatolian area, includes four genera: *Iurus* Thorell, 1876, *Calchas* Birula, 1899, *Protoiurus* Soleglad et al., 2012, and *Neocalchas* Yağmur et al., 2013. The genus *Calchas* has been of great interest among scorpion taxonomists due to its basal phylogenetic position in superfamily Iuroidea, and a number of unique features (Fet et al., 2009). In the most recent revision, Yağmur et al. (2013) described two new species and transferred *C. gruberi* Fet et al., 2009 to new genus *Neocalchas*. The genus *Calchas* currently includes four allopatric species: *C. nordmanni* Birula, 1899 (type species), *C. birulai* Fet et al., 2009, *C. anlasi* Yağmur et al., 2013, and *C. koss-wigi* Yağmur et al., 2013. The genus is subendemic to Turkey (with only one locality confirmed outside of Turkey, from northern Iraq).

Calchas birulai is the most widespread species of this genus in southeastern Turkey (Yağmur et al., 2013). It lives under stones in limestone or basaltic areas covered by steppe or scrub vegetation; very little is known about the habitat or biology of this species. Here, we offer the first detailed SEM study of external morphology of *Calchas birulai*, in addition to the features illustrated by Fet et al. (2009).

Material and Methods

Specimens were collected by E.A.Y. in Halfeti District (Şanlıurfa Province, Turkey), 2 km south of Savaşan Village, under stones, on 30 March 2008. The

area is covered by scrub and steppe vegetation, with limestone rocky areas. Samples were prepared according to standard SEM methods and deposited in the collection of the Museum of Turkish Arachnology Society in 70% ethanol. The SEM study was done at University of Ankara. The specimens were rehydrated with ethanol series (from 70%, 50%, 30% ethanol to deionized water). To clean the surface, specimens were rinsed in 0.3% Tween 20 (Merck) for 20 min and washed by water. They were then dehydrated with ethanol series (30% - 100% ethanol), dried at 30 °C, and coated with a thin layer of gold in Polaron SC 502 sputter coater. The specimens were examined at an accelerating voltage of 15 kV under JEOL JSM 6060 LV Scanning Electron Microscope, and the electron micrographs were recorded.

Results and Discussion

A number of scorpion morphological studies have employed SEM technique (e.g. Ivanov & Balashov, 1979; Graham & Fet, 2006; Fet et al., 2006, 2009; Lourenço, 2007; Kovařík et al., 2010; Lourenço & Leguin, 2010). Most of these works, however, were restricted to specific sensory organs or other specialized structures. The only scorpion species for which comprehensive SEM micrographs are available, both for external and internal organs, is probably *Smeringurus mesaensis* Stahnke (Vaejovidae) (Farley, 1999, 2001). In the present study, external morphology of *Calchas birulai* is examined in detail and presented on SEM

micrographs. We base our description, in part, on that of Fet et al. (2009).

Cuticle. As in most arthropods, scorpion body is covered by chitinous cuticle. Its surface granulation and other ornamentation varies with species. In *C. birulai*, granules with smooth apical area are present on carinae of dorsal portions (tergites) of mesosomal segments (Fig. 1). Intercarinal areas of tergites are completely covered by coarse or fine granules (Figs. 1–4). Surface of these granules as well as between granules have irregular, non-linear cuticular ornamentation (Figs. 4–5). Such ornamentation is present in *C. birulai* throughout the body except sternites (ventral portions of mesosomal segments). Sternites lack any granules or carinae; however, some white homogeneous spots are present on their surface. Under higher magnification, it becomes apparent that each white spot is comprised of local cuticular ornamentation, less expressed in the middle of a spot, where also slit-shaped wax pores are detected (Fig. 6). Granulation was not observed on leg surface, which instead is covered only with cuticular ornamentation (Fig. 5).

Sensory setae. Scorpion body is covered with sensory setae of cuticular origin (Figs. 3–4, 10, 12, 18–20, 24–30, 32–34, 36b), which are known to have chemosensory and mechanoreceptive function (Farley, 1999, 2001). See also below for specialized setae such as *trichobothria* (on pedipalps) and *peg sensilla* (on pectinal organs).

Carapace. At the anterior margin, carapace has a small emargination, five irregularly distributed, distinct setae as well as many small setae and scattered coarse granules (Fig. 7). Area between the median eyes is relatively smooth (Fig. 8). Posterolateral margins of carapace are covered by small and moderate sized granules (Fig. 9). Small granules present on lateral margins of carapace. Mediolateral ocular carinae are present and granular, reaching to median eyes (Fig. 7). Median eyes and eye tubercle are rather small and located anteriorly of the middle of carapace. A couple of setae is present behind the median eyes (Fig. 8). Two lateral eyes exist; posterior eye is slightly larger than the other. Posteromedian furrow begins from median eyes and extends toward posterior margin of carapace. Two less expressed, S-shaped posterolateral furrows begin from posterior margin and extend toward anterolateral margin (Fig. 9).

Chelicerae. Each chelicera consists of a basal segment, a manus (Fig. 10h) with fixed finger (Fig. 10f), and movable finger (Fig. 10g). The movable finger has four denticles on its dorsal edge: basal (Fig. 10a), median (Fig. 10b), subdistal (Fig. 10c), distal (Fig. 10d);

and one ventral distal denticle (Fig. 10e). The fixed finger (Fig. 11a) has four denticles on its dorsal edge: basal (Fig. 11b) and median (Fig. 11c) conjoined on common trunk, subdistal (Fig. 11d), and distal (Fig. 11e); and one basal denticle on ventral edge.

Chelicerae are bordered by coxae of pedipalps and legs I–II. A cluster of setae is located on the dorsal surface of the fixed finger (Fig. 10k). Manus is covered by small granules. Coarse, scarce setae are present on the surface of the fixed finger and manus (Fig. 10j).

Serrula. This comb-shaped structure is located ventrally on the fixed finger of chelicera (Fig. 12a). Scorpion serrula was discussed in detail by Graham & Fet (2006) and identified for the taxa of Iuridae. Fet et al. (2009) reported serrula in *C. birulai*. Over 20 lined-up, sharp tines were present on the serrula of specimens examined by us (Figs. 13a, 14a). While the tines were mostly broken in the specimen examined by Fet et al. (2009), they were generally intact in our specimen. The function of the serrula is yet unknown but it could assist in feeding, or in cleaning cheliceral setae cluster or pedipalp fingers. Dorsally of serrula, we observed specialized setae that are shaped differently from other scorpion setae. At higher magnification, one can see that these setae are covered by numerous barbs (Figs. 13b, 14b), which appear to help in feeding by serving as filtration devices (Farley, 1999). Modified setae of a similar nature, but with fewer barbs, are visible inside the mouth (Figs. 12, 15, 16).

Genital operculum. Genital operculum (a lid covering genital opening) is located posteriorly from the sternum. It is shaped differently in male and female. In *C. birulai*, operculum is completely divided longitudinally. As a whole, it is slightly protruded outward. Sclerites are large, subtriangular, and approximately as wide as long. In males, posterior side is oval-shaped and curving outward; anterior side is extended forward and sharply pointed (Fig. 17). In females, in contrast to the male, the anterior side of the operculum is curved outward (Fig. 18). The posterior side of the female genital operculum curves inward and is flanked posteriorly by a very unique structure of subfamily Calchinae, a so-called prepectinal plate in females, absent on males (Fig. 18) (see Fet et al., 2009 for more details).

Sternum. The scorpion sternum is located ventrally between the coxae of legs III and IV. The shape of the sternum has been used in the classification of scorpion families. In *C. birulai*, sternum is pentagonal, Type 2 according to Soleglad & Fet (2003a). There is a sharp apical point anteriorly, with a depression behind it. The anterior and lateral edges are nearly linear. An emargination is present at the posterior edge (Fig. 19). The

sternum is wider than its length; its lateral lobes are convex.

Mesosoma. Intercarinal areas of the segments are shagreened and covered with scattered coarse granules. Posterior margins of tergites I–VI are granulated, tergites III–VI with scattered coarse granules (Fig. 20). Tergite VII with two pairs of granulate carinae (Fig. 21). Sternites III–VI are smooth and lustrous; sternite VII is rough. Laterally, sternites bear sparse setae (Fig. 22).

Stigmata. Scorpions have four pairs of book lungs, located on mesosomal segments III to VI. Their paired openings, stigmata (or spiracles), are located close to the edge of sternites. Stigmata in *C. birulai* have elliptical shape (Fig. 23). Scorpions are capable of close the spiracle, protecting themselves and surviving in adverse environments.

Metasoma. Dorsal and dorsolateral carinae crenulate on segments I–IV. Dorsal carinae on segments I–IV and dorsolateral on segments I–III carinae terminate with a spinoid granule. Lateral carinae complete and crenulate on I, present on one-half of II, on 30 % of III, and absent on IV. Ventrolateral and ventromedian carinae crenulate. Dorsolateral carinae of segment IV terminate at articulation condyle (Fig. 24). Segment V has dorsolateral, lateral, ventrolateral, and single ventromedian carinae. Dorsolateral carinae, lateral carinae, ventrolateral and ventromedian carinae crenulate. Lateral carinae present on two-thirds of posterior side. Ventromedian carina terminates in straight line (Fig. 25). Intercarinal areas of segments I–IV are smooth ventrally, with scattered granulation laterally; segment V with heavy granulation on ventral surface. Metasoma has sparse setation.

Telson. Telson includes a pair of venom glands. In *C. birulai*, vesicle is elongated; aculeus (sting) is short and abruptly curved. Ventral surface of vesicle is covered with medium sized granules, posterior ventral granules are larger. Scattered setae are located on ventral surface of vesicle (Fig. 26a). Subaculear tubercle is reduced (Fig. 26b). Subaculear setal pair (SSP) is located at base of aculeus, on subaculear tubercle (Fig. 26c). Paired venom gland openings are present at the end of the aculeus (Fig. 27).

Pedipalps. Pedipalps, the chelate second pair of prosomal appendages, are the largest scorpion appendages, used for hunting and defense. Pedipalps consist of coxa, trochanter, femur, patella, and chela. Chela has two segments: chela manus with fixed finger (a modified tibia) and movable finger (modified tarsus). Specialized mechanosensory setae (trichobothria) on pedipalps detect air vibrations.

Femur. Dorsointernal and ventrointernal carinae are serrate, dorsoexternal carina crenulate, ventroexternal

one-fifth which is granulate; internal (*I*) weak, rounded, not continuous, with small granules (Fig. 30).

Constellation array. This sensory organ (a field of specialized sensilla) of unknown function, located subterminally on the external aspect of fixed finger, was recently discovered by Fet et al. (2006). Number of constellation array sensilla varies with scorpion species. Fet et al. (2006) reported that *Neocalchas gruberi* (referred to as *Calchas nordmanni*) had 15 sensilla, the largest number so far recorded in scorpions (see also Fet et al., 2009, fig. 14). For *Calchas birulai* from Nemrut Dağı, Fet et al. (2009, fig. 15) reported 8 sensilla. We also observed 8 sensilla in examined Halfeti specimens (Figs. 29, 31).

Chelal finger dentition. Median denticle (MD) rows are oblique and slightly imbricating, numbering 6/6 and 7/7 on fixed and movable fingers; 5/5 and 6/6 internal denticles (ID) and 5/5 and 6/6 outer denticles (OD) are present on fixed and movable fingers, respectively. No accessory denticles present. Total number of MD denticles on movable finger is 55 (Fig. 32). Many small setae are present around rounded. Dorsal and internal surfaces sparsely granulate, ventral smooth, and external surface with a line of serrate granules (Fig. 21).

Patella. Dorsointernal and ventrointernal carinae are serrate, dorsoexternal carina granulate, ventroexternal roughly rounded, and exteromedian carina granulate. Intercarinal area is shagreened.

Chela. In *C. birulai*, the chelae are strong and heavily carinated. Chela fingers are short, without scalloping. Intercarinal areas on chela manus and fingers are shagreened, but distal part of fingers is smooth. **Chelal carinae** comply with the “8-carinae configuration” (Soleglad & Fet, 2003b). Digital (*D1*) carina strong, smooth to granulate; dorsosecondary carina (*D3*) present on basal half only, smooth; dorsomarginal (*D4*) rounded, continuous, with granules; dorsointernal (*D5*) weak, sparsely granulated (Fig. 29); ventroexternal (*V1*) strong and granulated proximally, terminating at external condyle of movable finger; ventrointernal (*V3*) strong and smooth, continuous to internal condyle; external (*E*) strong, continuous, essentially smooth except for proximal fingerblade denticles; setae located near inner denticles (ID) are larger and more conspicuous. Denticles are curved inwards (Figs. 32–33), which could provide more grip for hunting.

Trichobothria (Figs. 29, 34, 35a) are specialized mechanoreceptory setae detecting air currents (Farley, 1999, 2001). In scorpions, trichobothria are located only on femur, patella, and chela (but not on its movable finger) of pedipalps along with regular setae; however, they are easily discernible due to a longer and thinner seta (shaft, hair) emanating from a cup-shaped areola (base). Trichobothrial patterns of subfamily Calchinae

are depicted and discussed in detail by Fet et al. (2009) and Yağmur et al. (2013).

Wax Pores. Scorpion body surface is covered with a layer of hydrophobic wax, which protects the body from water loss as well as from physical impacts and bacterial or fungal infections. Wax is produced by body and excreted through lidless wax pores. Wax pores are observed abundantly throughout *C. birulai* body (Figs. 37–41). Some wax pores are surrounded by cuticular folds (Fig. 37) while others are slit-shaped (Fig. 38).

Legs. Tibial spurs are present on legs III and IV; two pedal (basitarsal) spurs are present on all four legs (Figs. 42–43). Tibial spur (Fig. 44) is an especially unique feature of subfamily Calchinae (genera *Calchas* and *Neocalchas*), the only group of scorpions that has it outside of “basal” families Buthidae and Pseudochactidae. See Fet et al. (2009: 20) for a more detailed discussion.

Tarsus and basitarsus are heavily covered with large socketed setae, especially on ventral surface (Figs. 43, 45, and 46).

In addition to setae, non-socketed spinules are present on leg tarsi (Fig. 46b), which likely improve traction assisting in walking and climbing. Fet et al. (2009, fig. 17) indicate that, in *Neocalchas gruberi*, non-socketed spinules occur in clusters on ventral surface of juveniles but their number is reduced in adults. These spinules are an important trait in systematics of Iuridae.

A peculiar rosette-shaped cuticular structure is present symmetrically on both sides at the distal end of basitarsus, next to basitarsal-tarsal joints; it has variable number of protrusions (Fig. 43d). This structure was previously reported for *Neocalchas gruberi* (Fet et al., 2009, fig. 19).

On basal portions of basitarsus and tarsus, there are chemosensory slit sensilla (Figs. 43c, 45a, 48). Mechanoreceptory setae are present around ungues (Fig. 47), which perceive vibrations from the ground.

Pectinal organs, or pectines, unique for scorpions, are found in all scorpion species. They are located posteriorly from genital operculum. Pectines are composed of anterior lamellae, median lamellae, fulcra, and teeth (plates). Number of pectinal teeth varies with species, and is usually sexually dimorphic. Fet et al. (2009) reported for *C. birulai* pectinal teeth number 5 in females, and 6 in males. Our examined female specimen of *C. birulai* has 4/5 pectinal teeth; male specimen had 6/6 teeth (Figs. 49–50). The surface of pectinal teeth is covered with non-linear ornamentation (Fig. 51). Sensory setae are found throughout pectines.

Pectinal teeth bear sensory fields with specialized, multiple *peg sensilla* (Figs. 51–53) shaped in *Calchas* as short, stocky extensions emanating from a cavity (Fig.

53); their shape varies considerably with species. Carthy (1966, 1968) was first to study these structures. Ivanov & Balashov (1979) and Foelix & Müller-Vorholt (1983) showed that pectines act as mechanoreceptors and chemoreceptors. Later, it was demonstrated that peg sensilla perceive pheromones; see Gaffin & Brownell (2001) for a detailed review of scorpion chemoreception.

Acknowledgments

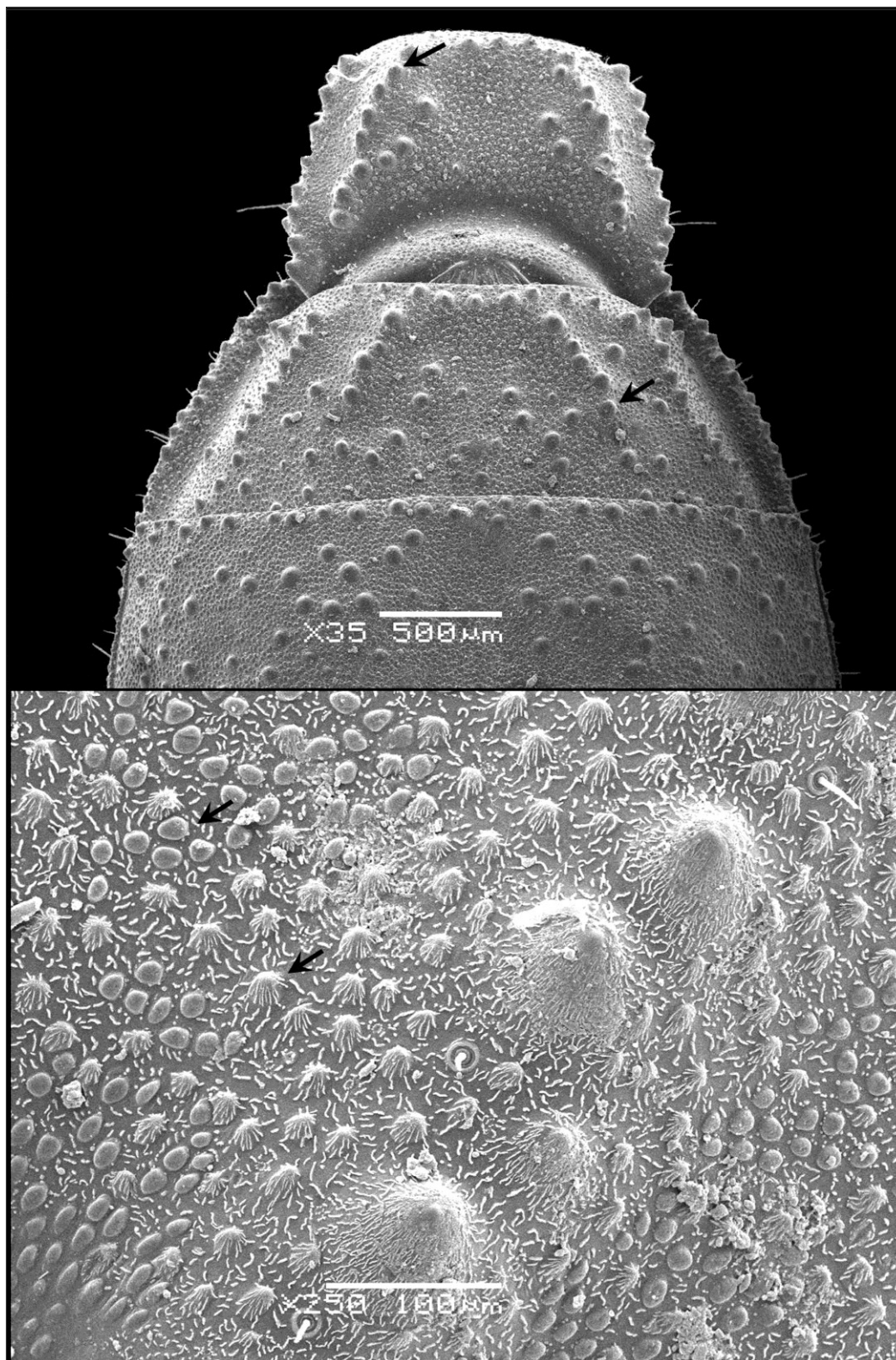
We thank Michael E. Soleglad for his help with processing this manuscript.

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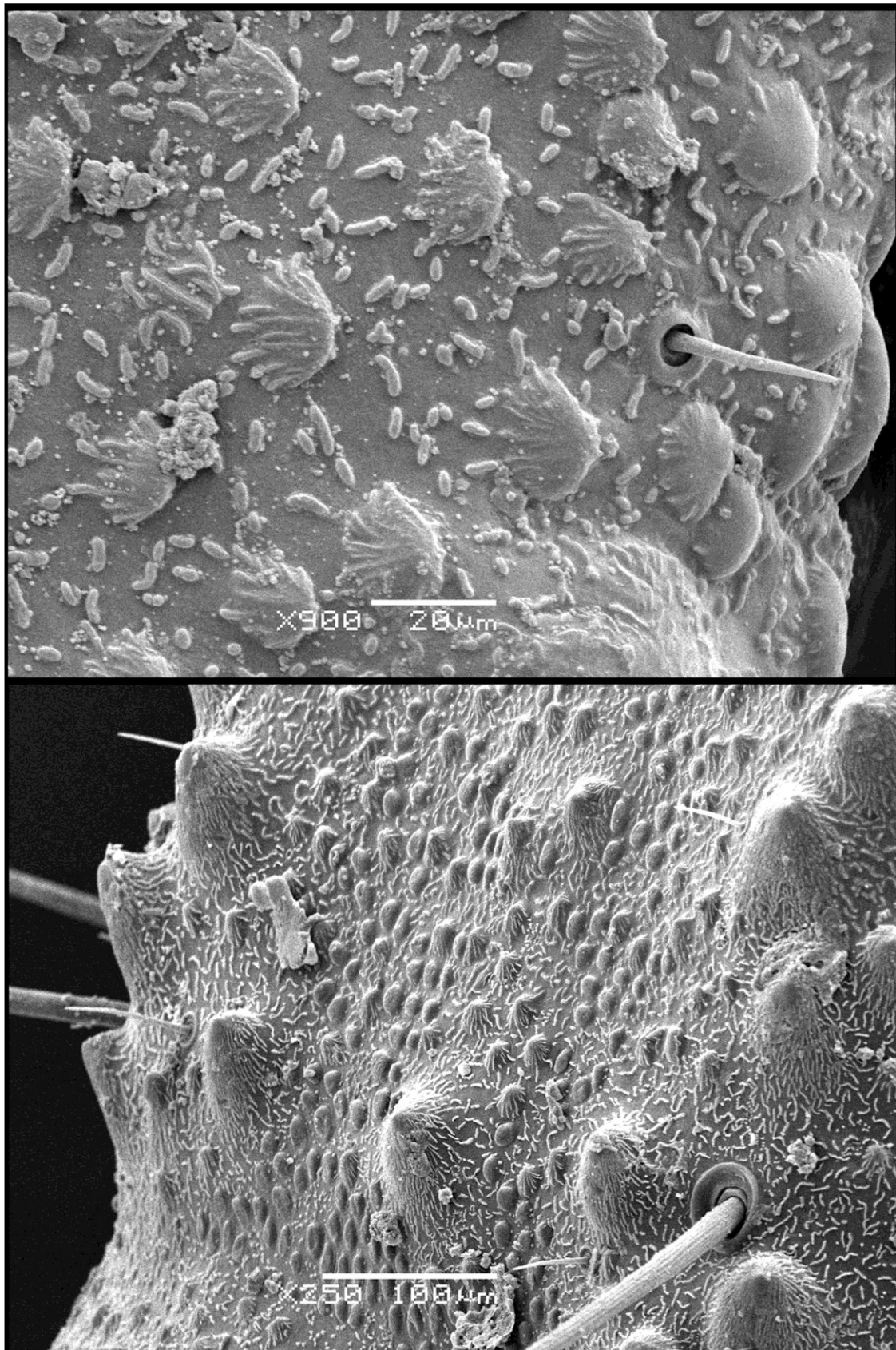
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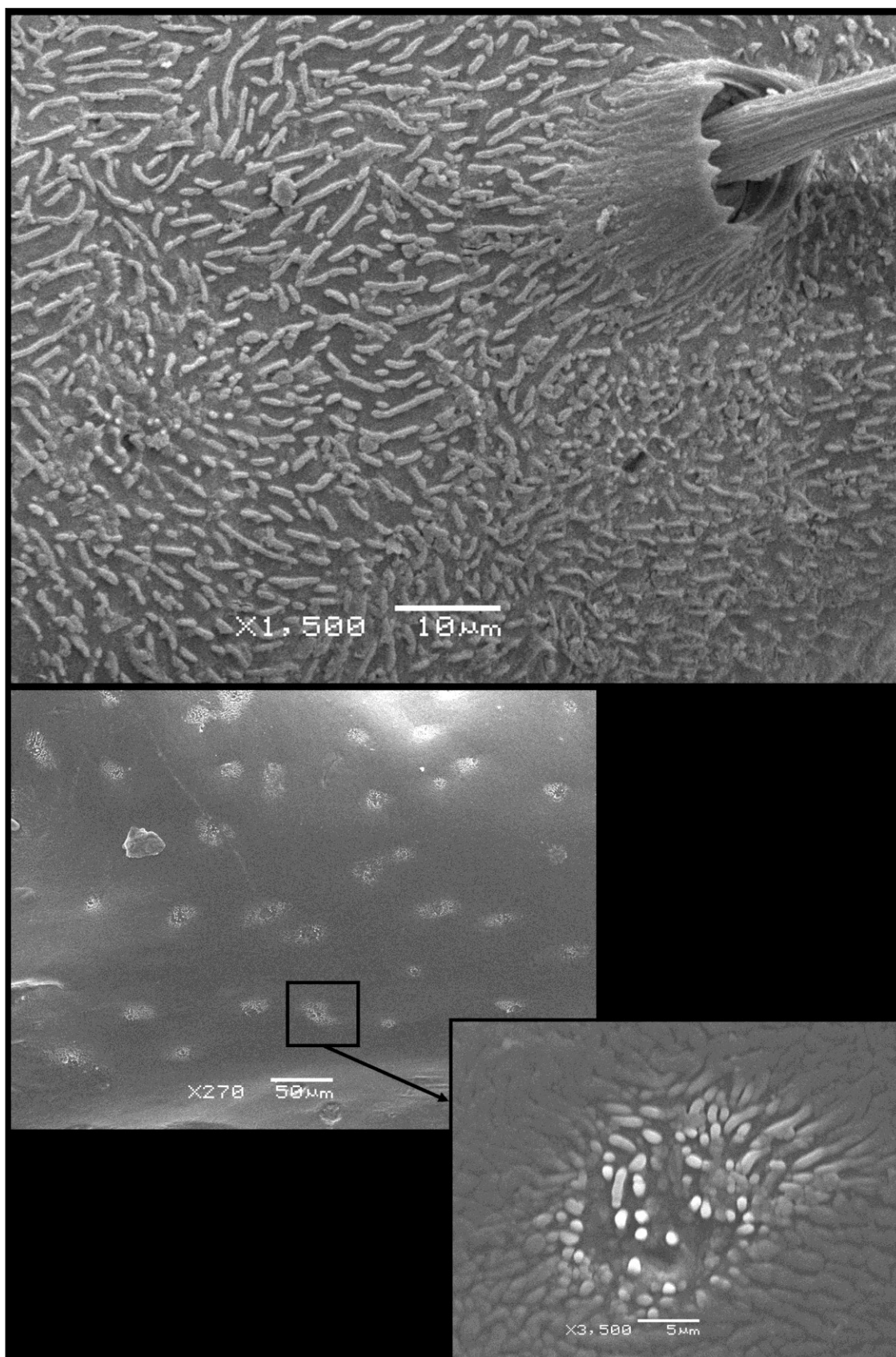
APPENDIX
Scanning Electronic Images (SEMs) of *Calchas birulai*



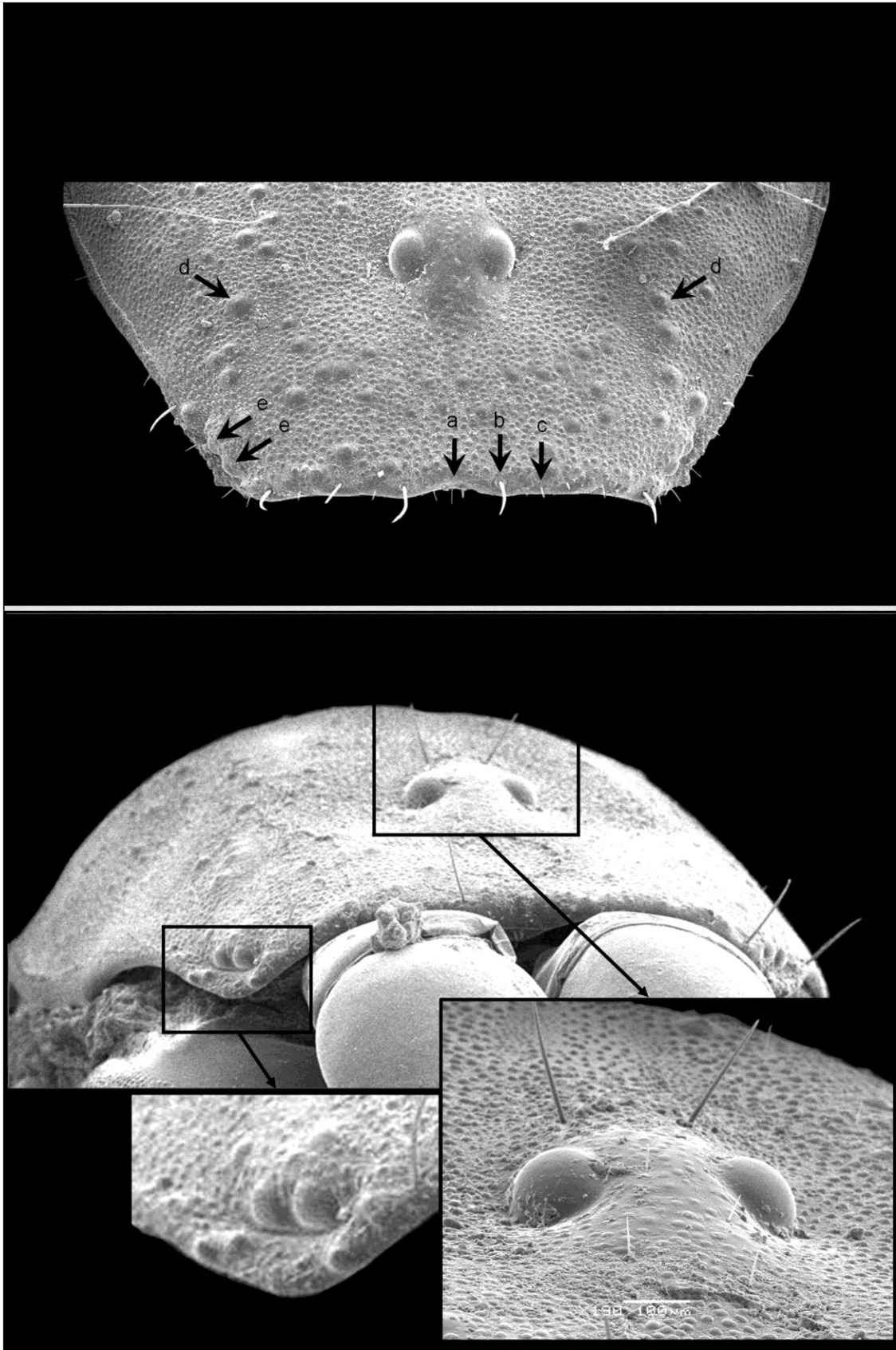
Figures 1–2: Granulation and ornamentation on cuticle. **1 (top).** Coarse granules on carinae of mesosomal tergites. **2 (bottom).** Fine granules in intercarinal area.



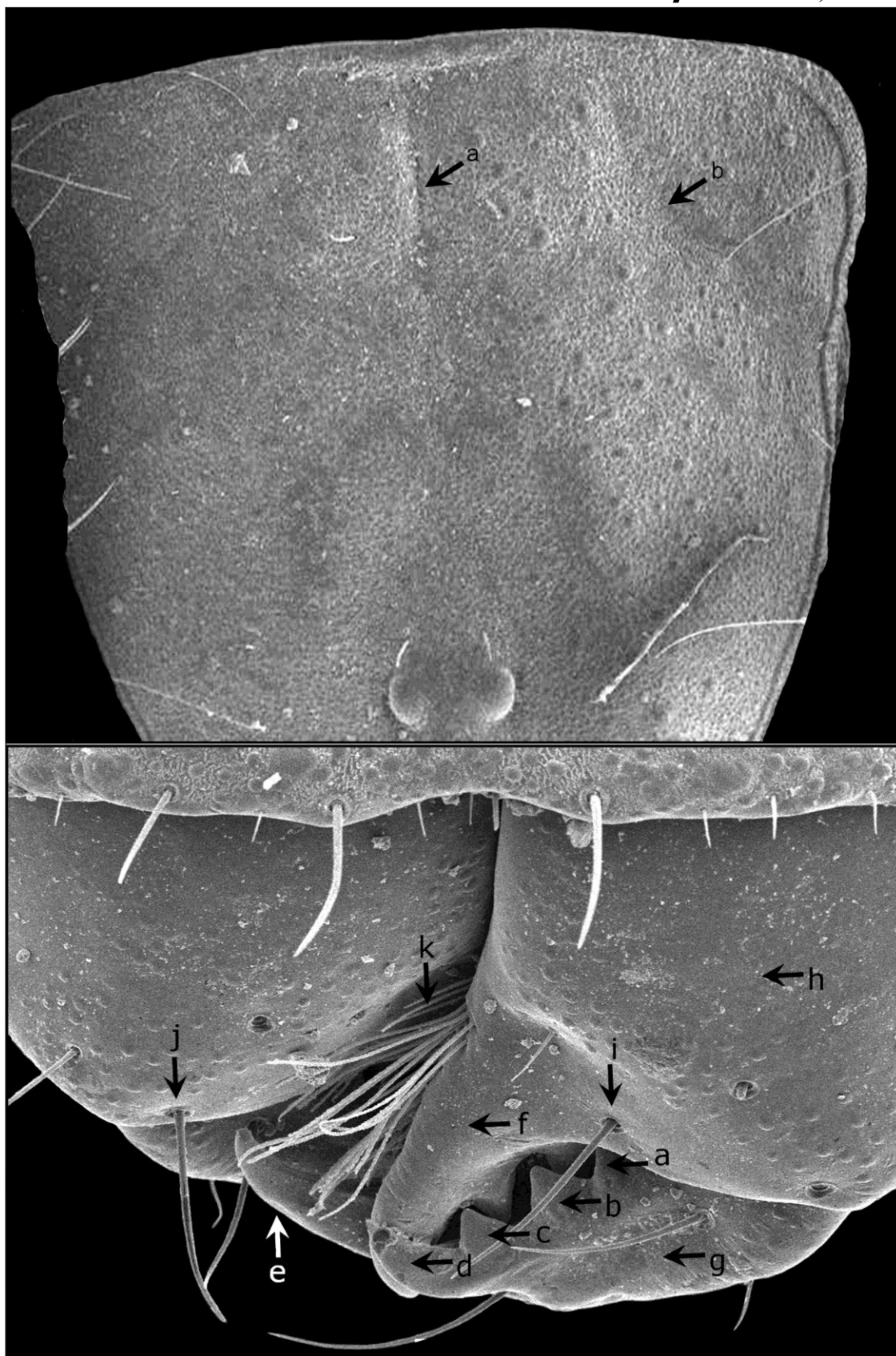
Figures 3–4: Granulation and ornamentation on cuticle. **3 (top).** Fine granules and cuticular ornamentation on mesosomal tergites. **4 (bottom).** Coarse granules, fine granules, and cuticular ornamentation of metasomal segment V.



Figures 5–6: Granulation and ornamentation on cuticle. **5 (top).** Cuticular ornamentation on legs. **6 (bottom).** Cuticular ornamentation on sternites and the same under high magnification.



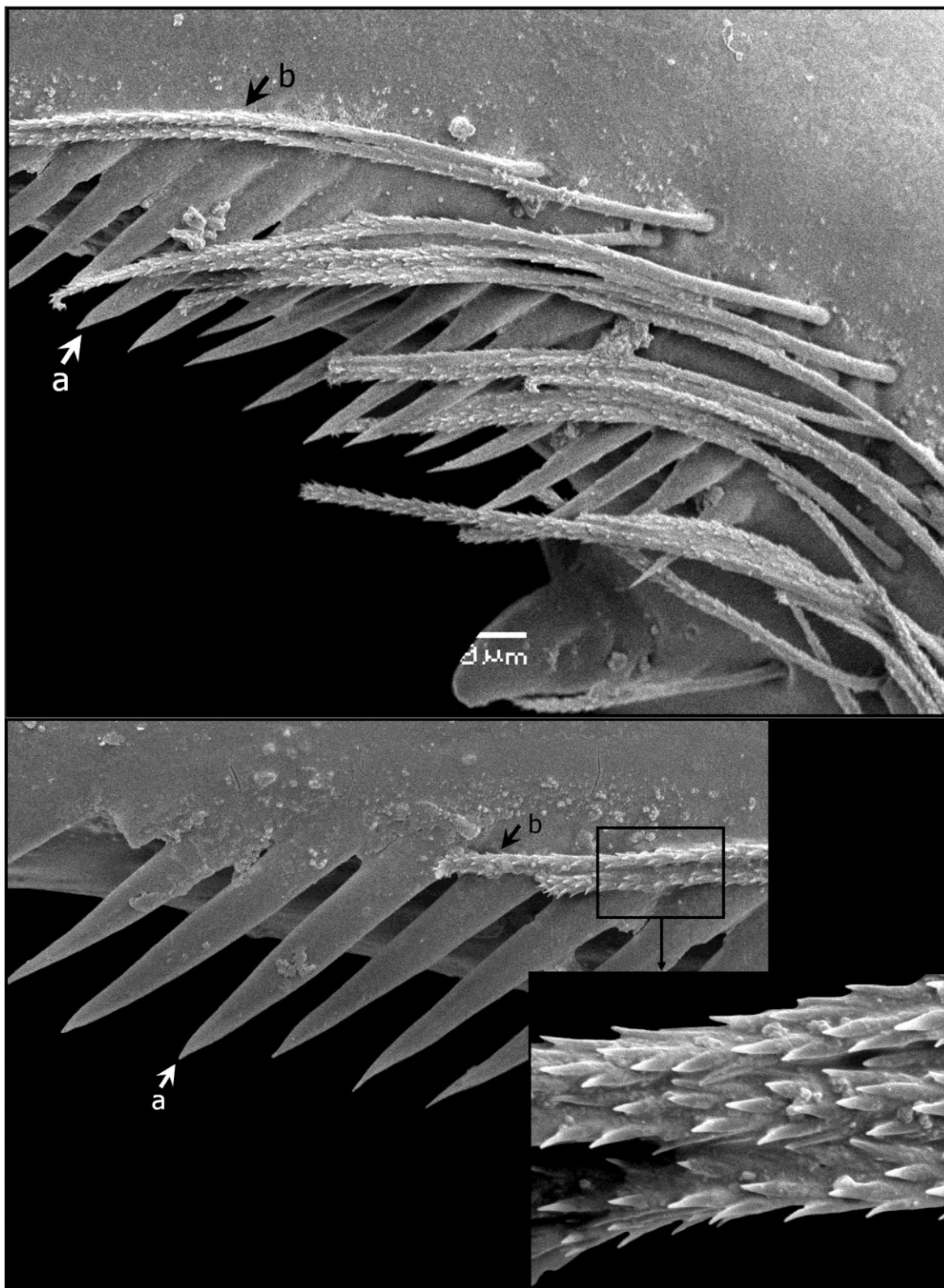
Figures 7–8: Carapace. 7. Anterior part of carapace: a, anterior depression; b, large seta; c, small seta; d, mediolateral ocular carinae; e, lateral eyes. 8. Median and lateral eyes.



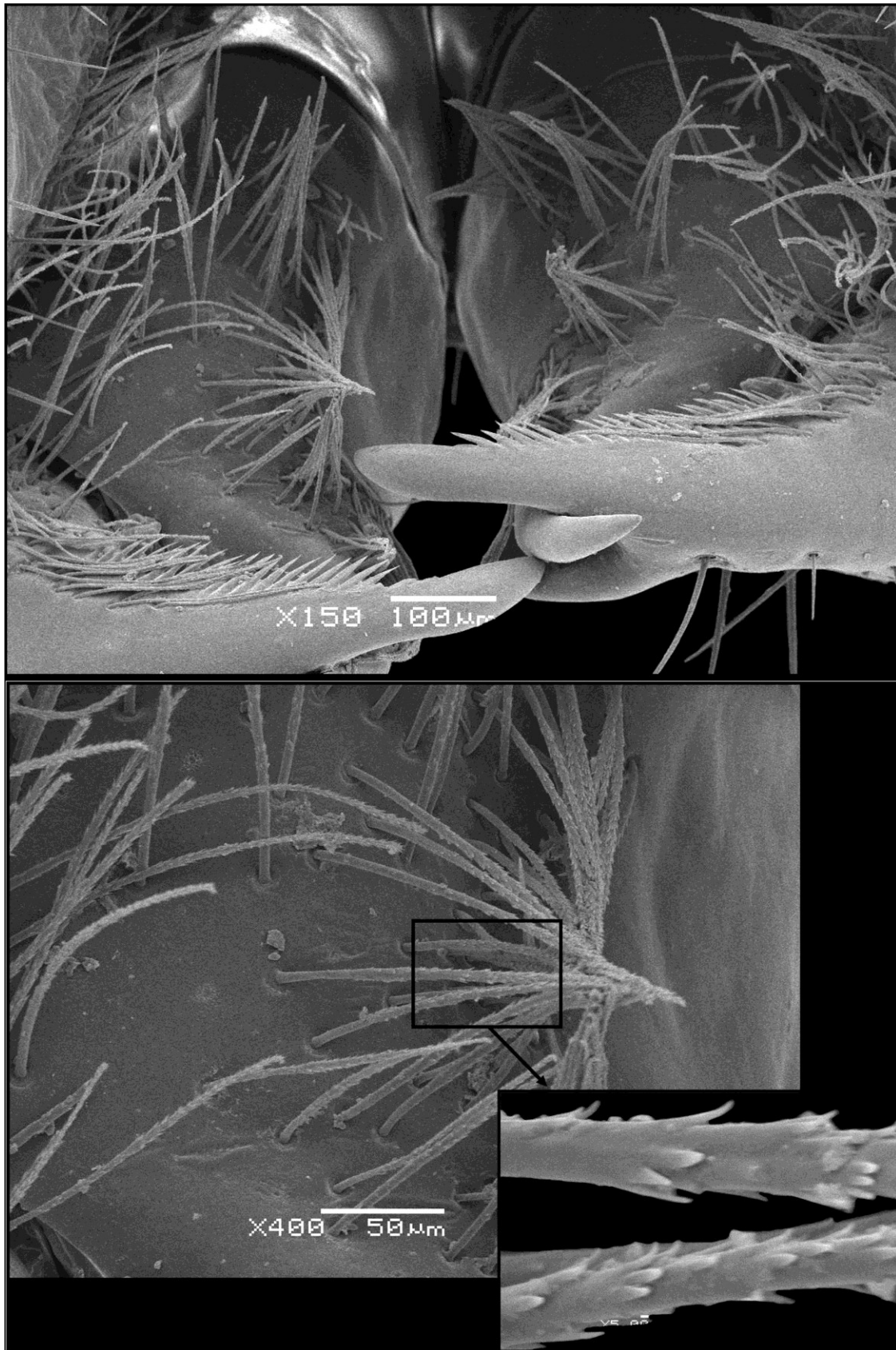
Figures 9–10: Carapace. **9 (top).** Posterior part of carapace: a, posteromedian furrow; b, posterolateral furrow. Chelicerae. **10 (bottom).** Chelicerae from dorsal aspect: a, basal denticle; b, median denticle; c, subdistal denticle; d, distal denticle; e, ventral distal denticle; f, fixed finger; g, movable finger; h, manus; k, seta cluster; j, long seta.



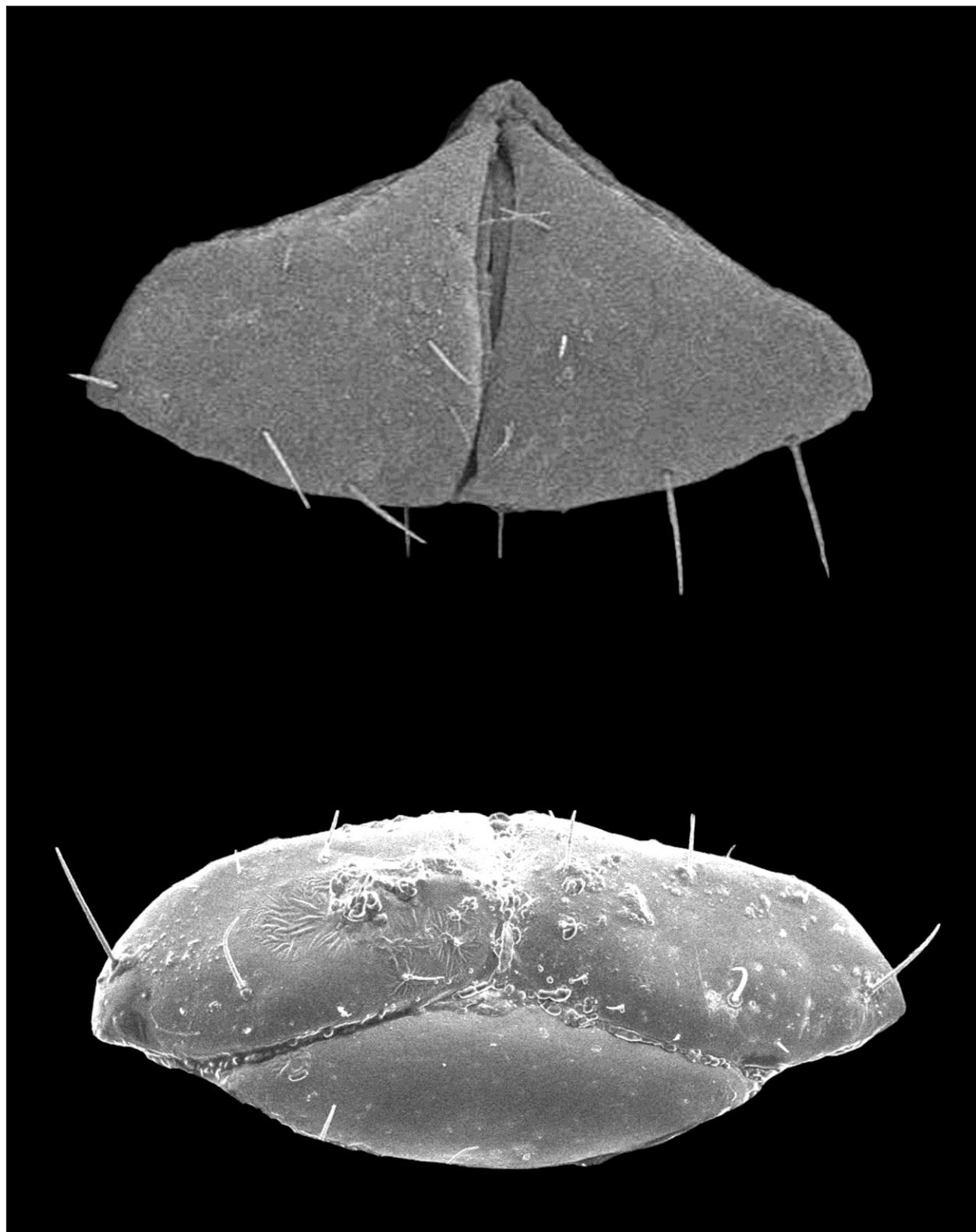
Figures 11–12: Chelicerae. **11 (top).** Chelicerae from anterodorsal aspect: a, fixed finger; b, basal denticle; c, median denticle; d, subdistal denticle; e, distal denticle. **12 (bottom).** Chelicerae and pedipalp coxa, ventral aspect: a, serrula; b, specialized setae; c, pedipalp coxa.



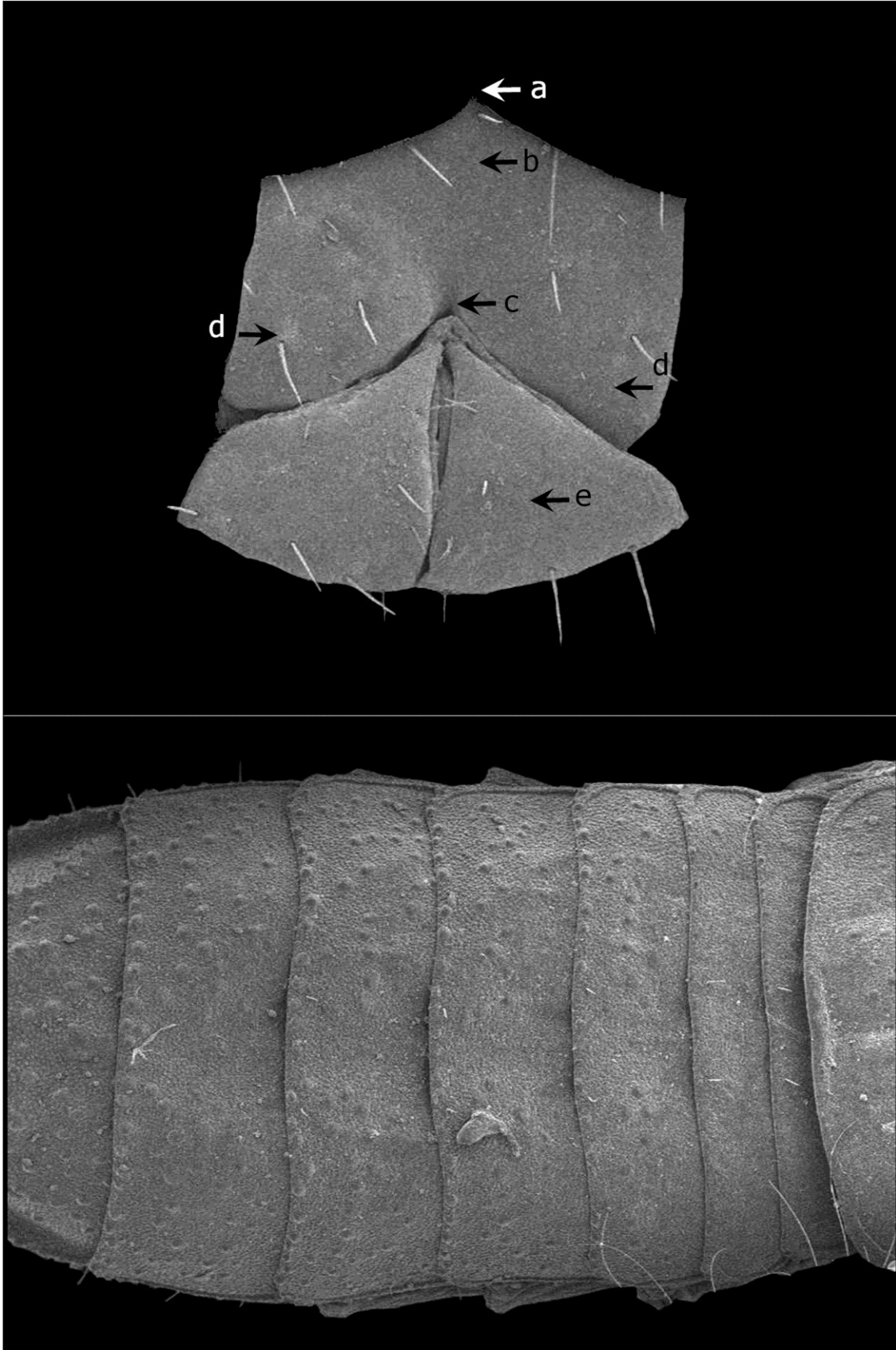
Figures 13–14: Chelicerae: a, serrula; b, specialized setae.



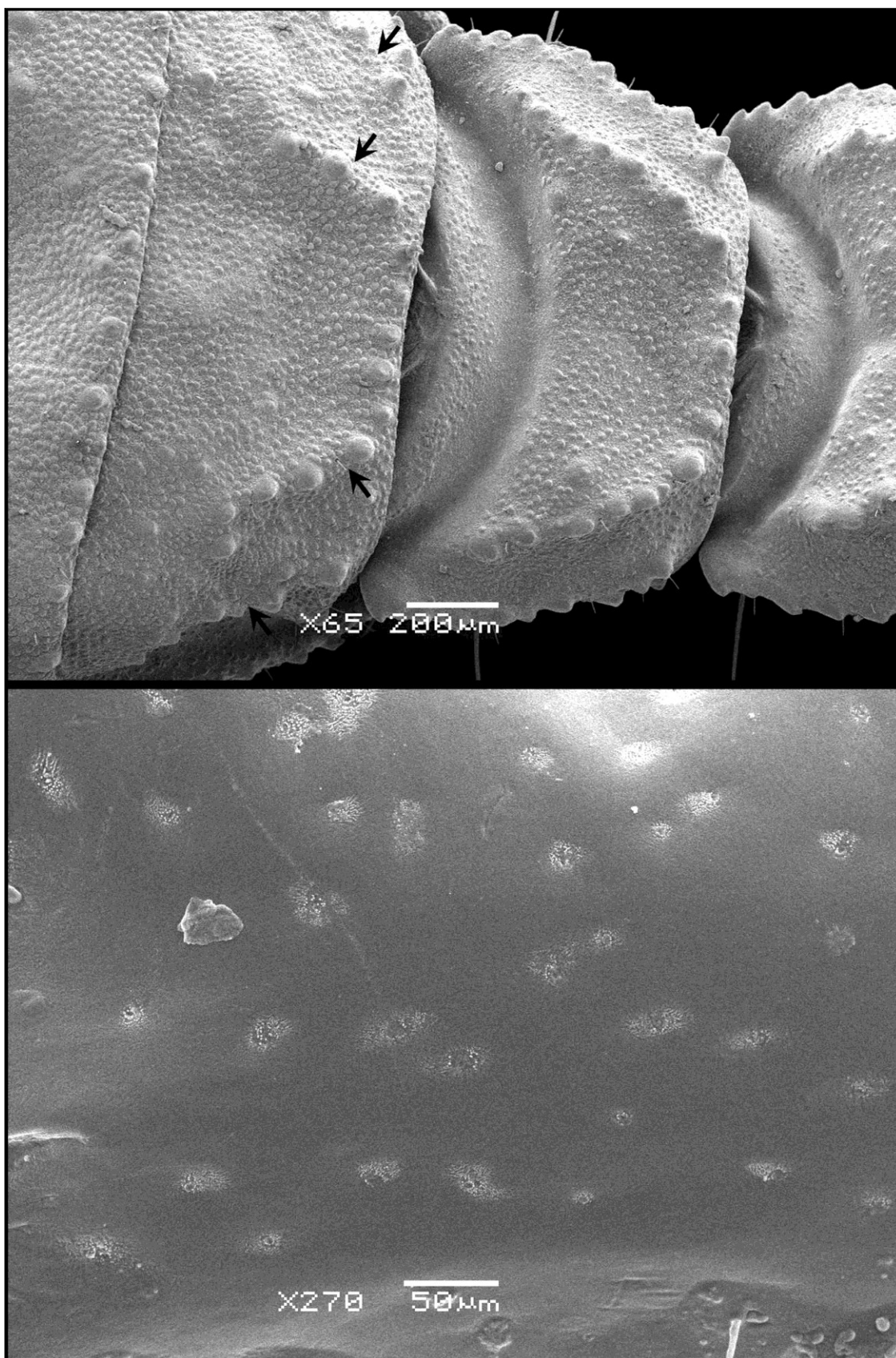
Figures 15–16: Specialized setae, inner side of the mouth.



Figures 17–18: Genital operculum. 17 (top). Male. 18 (bottom). Female (with prepectinal plate).



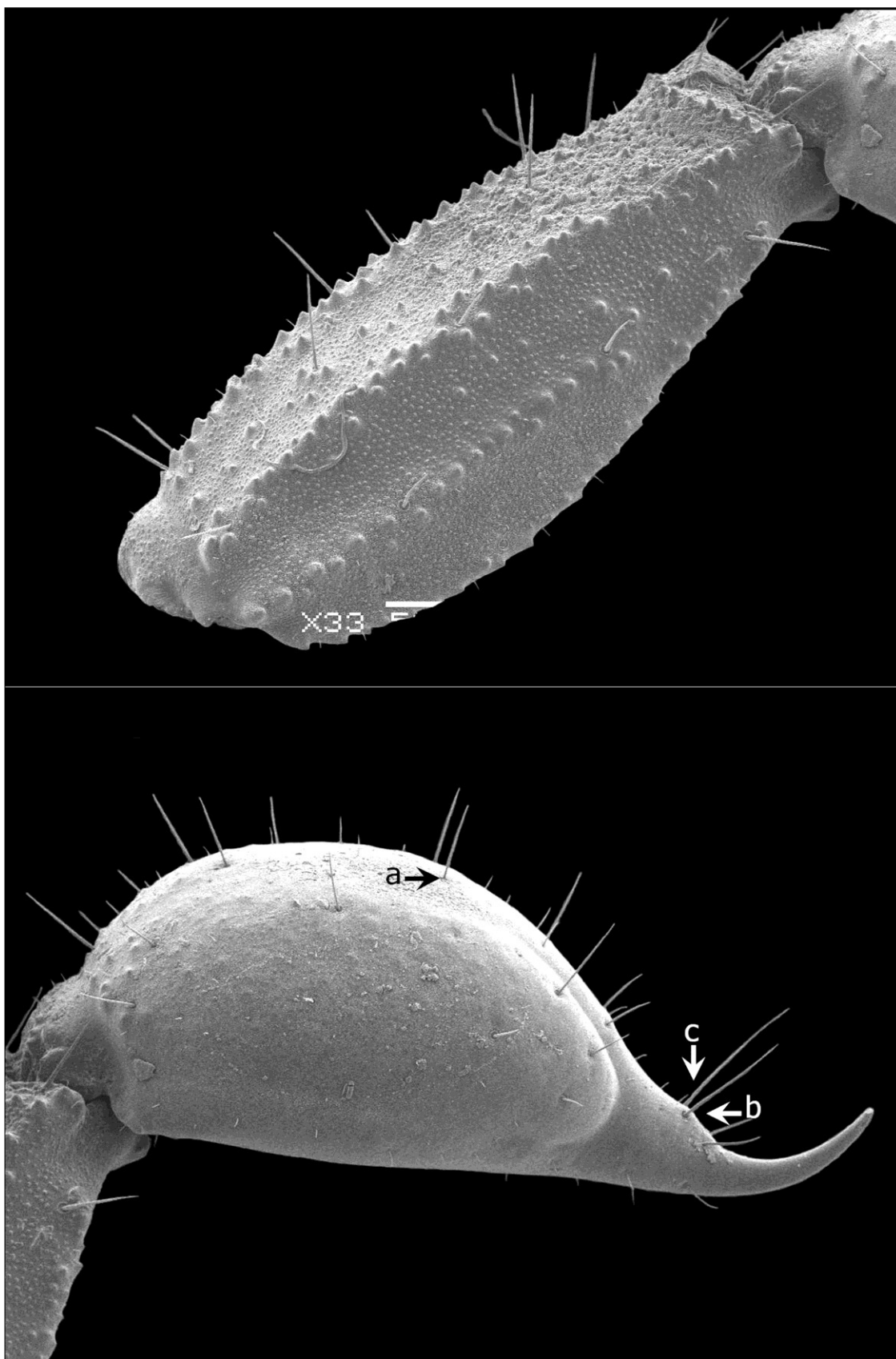
Figures 19–20: Sternum and genital operculum. **19 (top).** Sternum: a, apex; b, apical depression; c, posterior emargination; d, lateral lobes; e, genital operculum. **20 (bottom).** Mesosoma.



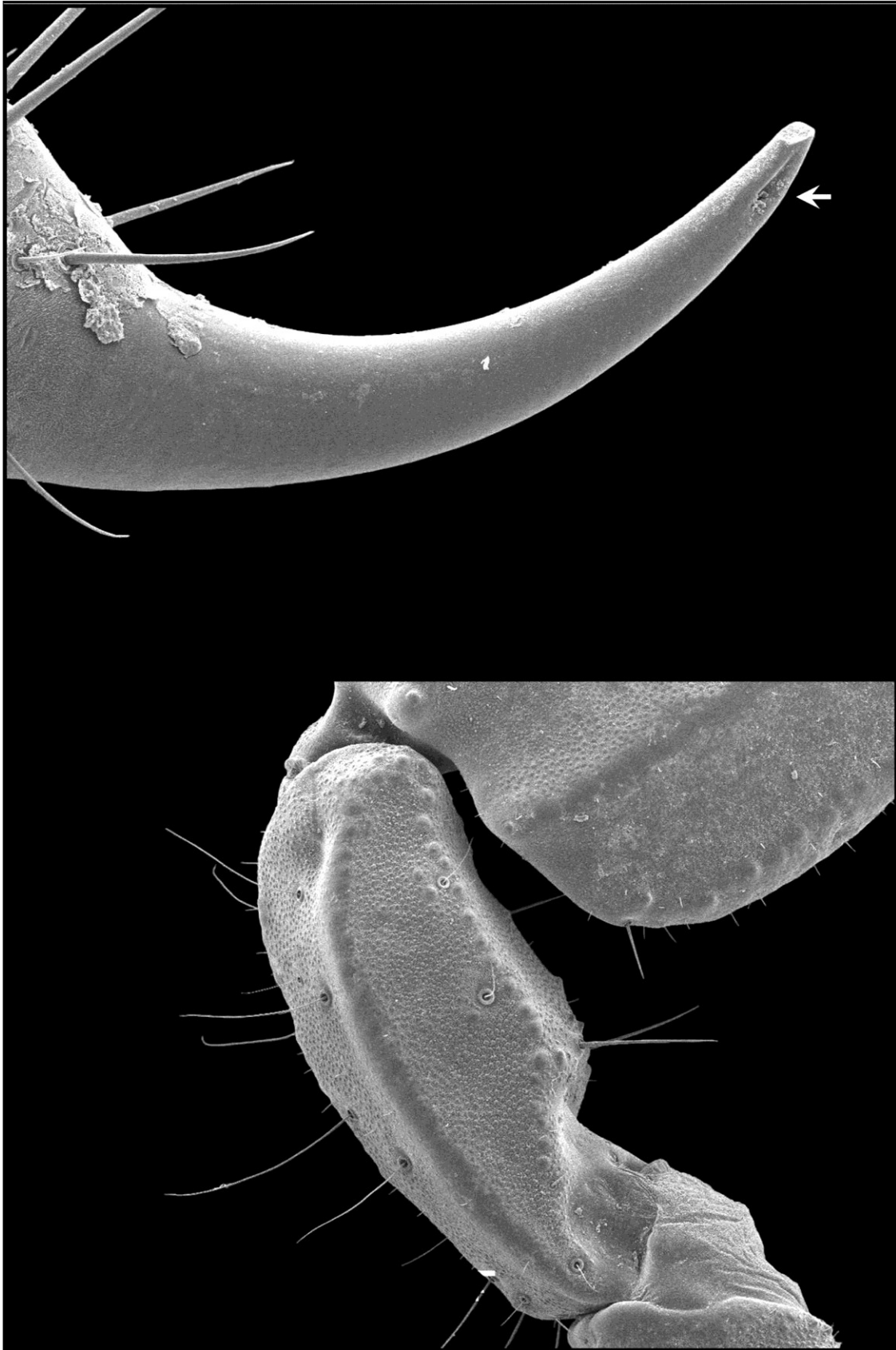
Figures 21–22: 21 (top). Mesosomal segment VII and metasomal segment I. 22 (bottom). Surface of a sternite.



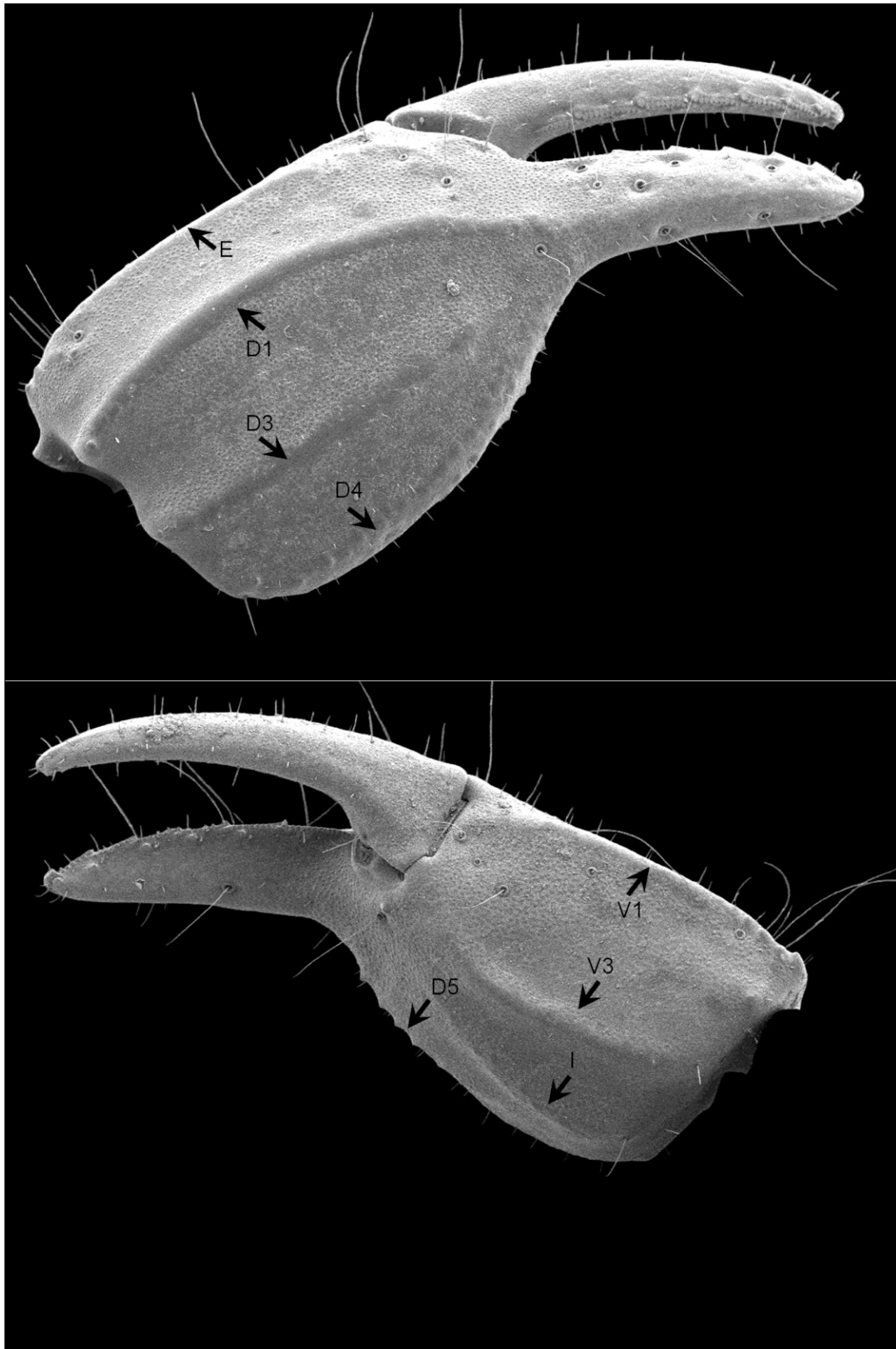
Figures 23–24: 23 (top). General view of a stigma (spiracle) and its inner lamellae. 24 (bottom). Metasomal segments I–IV.



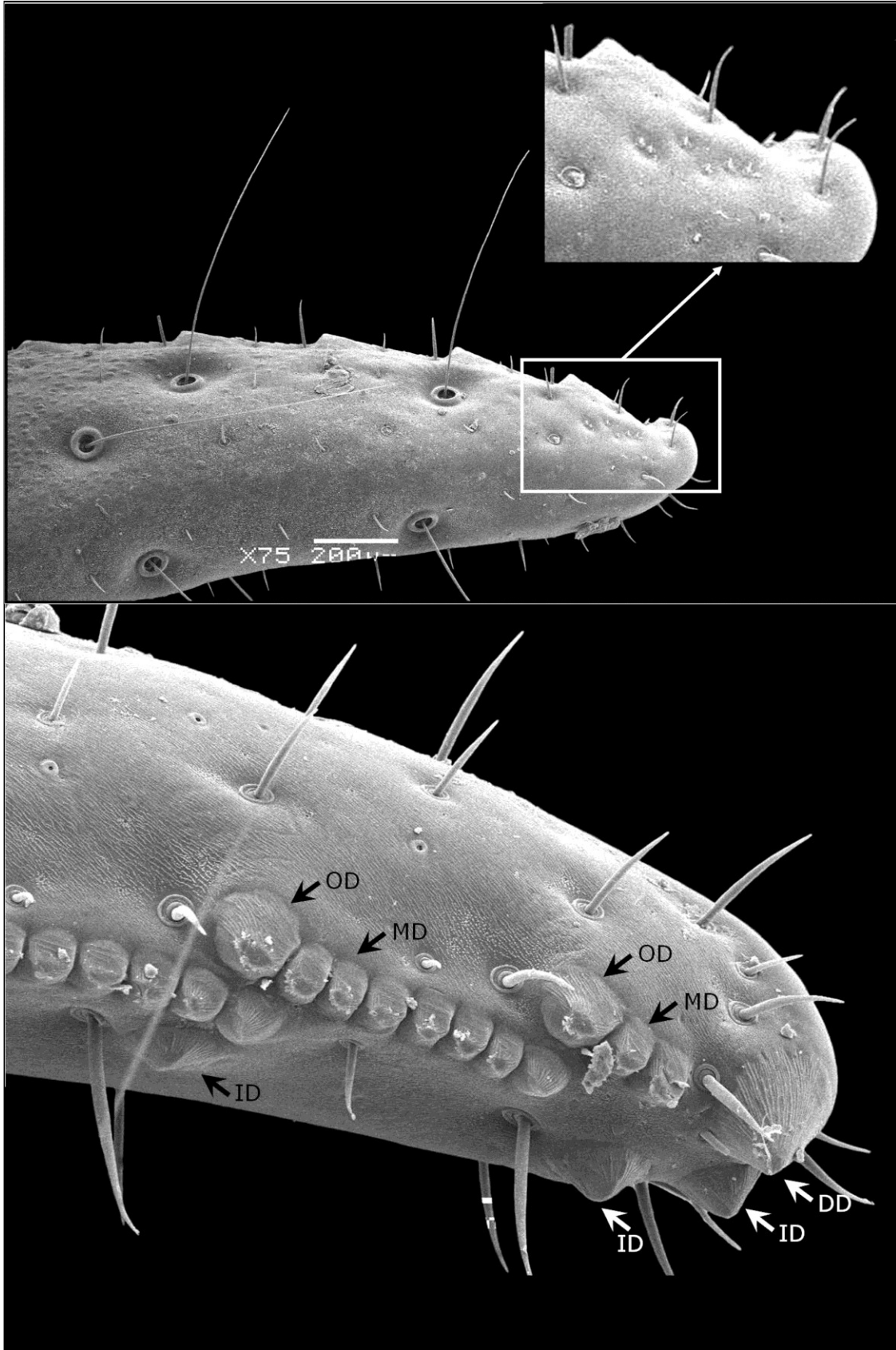
Figures 25–26: 25 (top). Metasomal segment V. 26 (bottom). Telson: a, setae; b, reduced subaculear tubercle; c, subaculear setal pair (SSP).



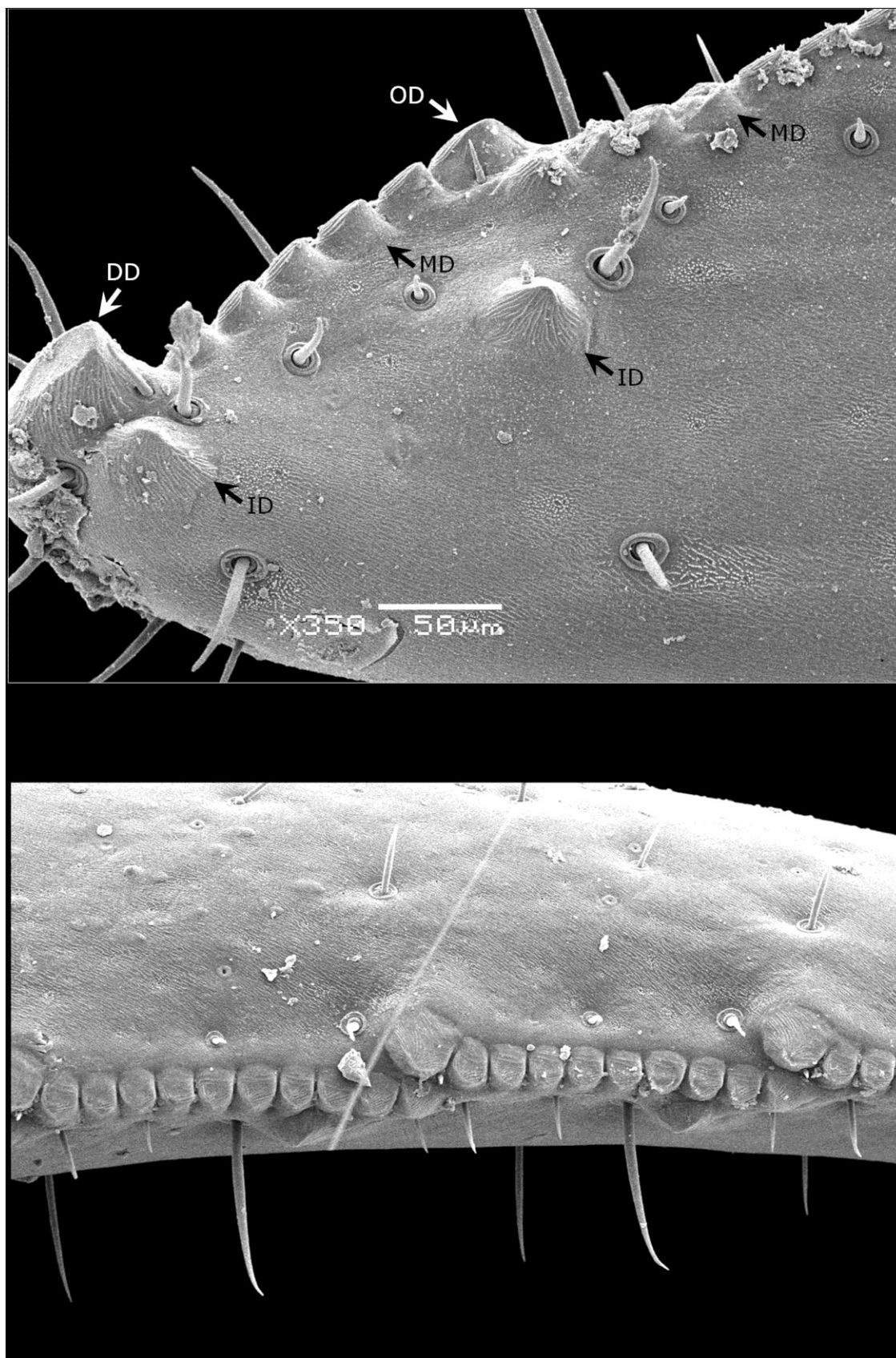
Figures 27–28: 27 (top). Venom gland opening. 28 (bottom). Pedipalp patella, dorsal aspect.



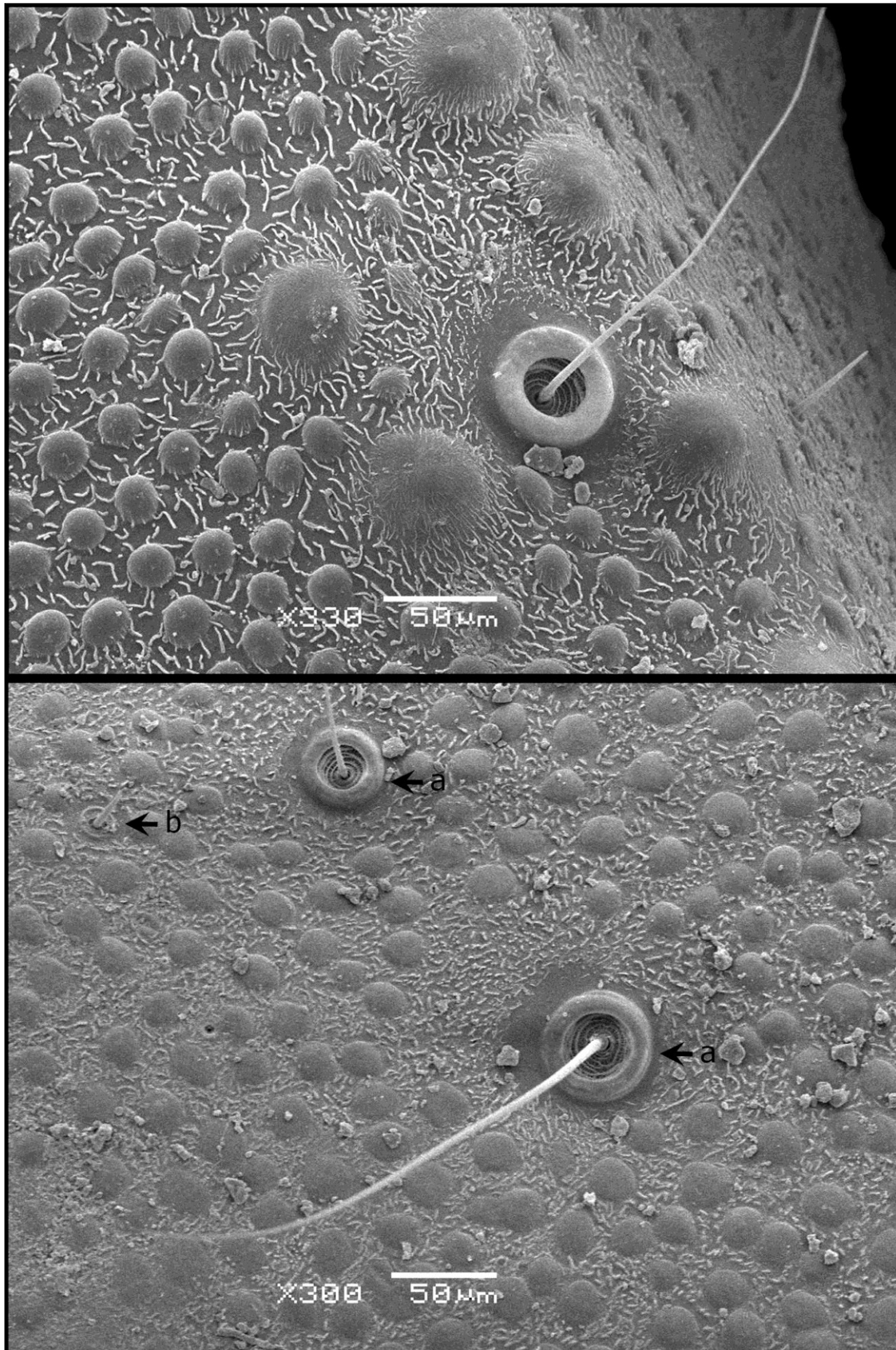
Figures 29–30: Pedipalps. **29 (top).** Pedipalp chela, dorsal aspect. **30 (bottom).** Pedipalp chela, ventral aspect.



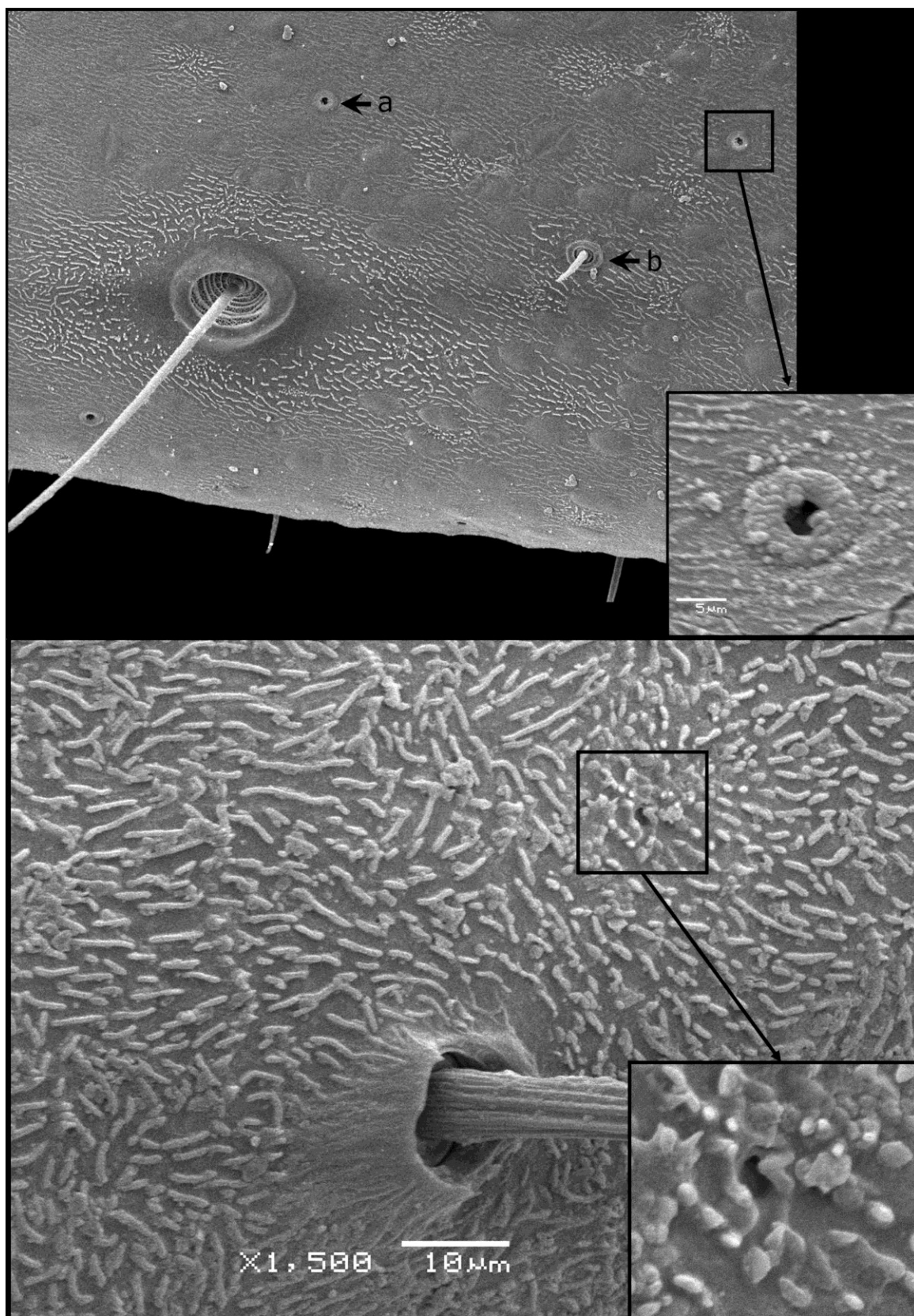
Figures 31–32: Pedipalps. **31 (top).** Constellation array. **32 (bottom).** Pedipalp chela movable finger, distal tip: MD, median denticle groups; DD, distal denticle; OD, outer denticles; ID, inner denticles.



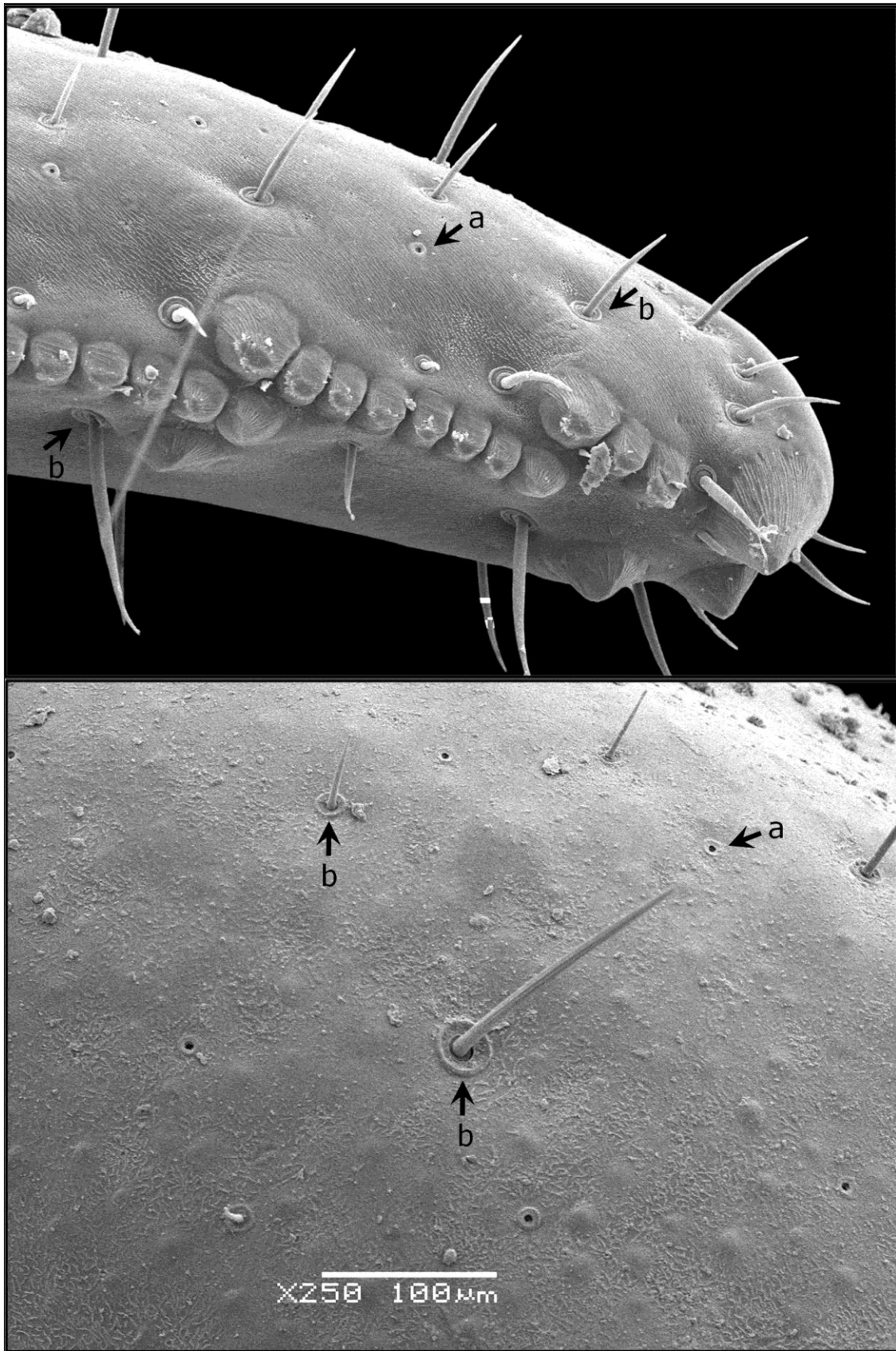
Figures 33–34: Pedipalps. **33 (top).** Pedipalp chela fixed finger, distal tip: MD, median denticle groups; DD, distal denticle; OD, outer denticles; ID, inner denticles. **34 (bottom).** Chelal movable finger.



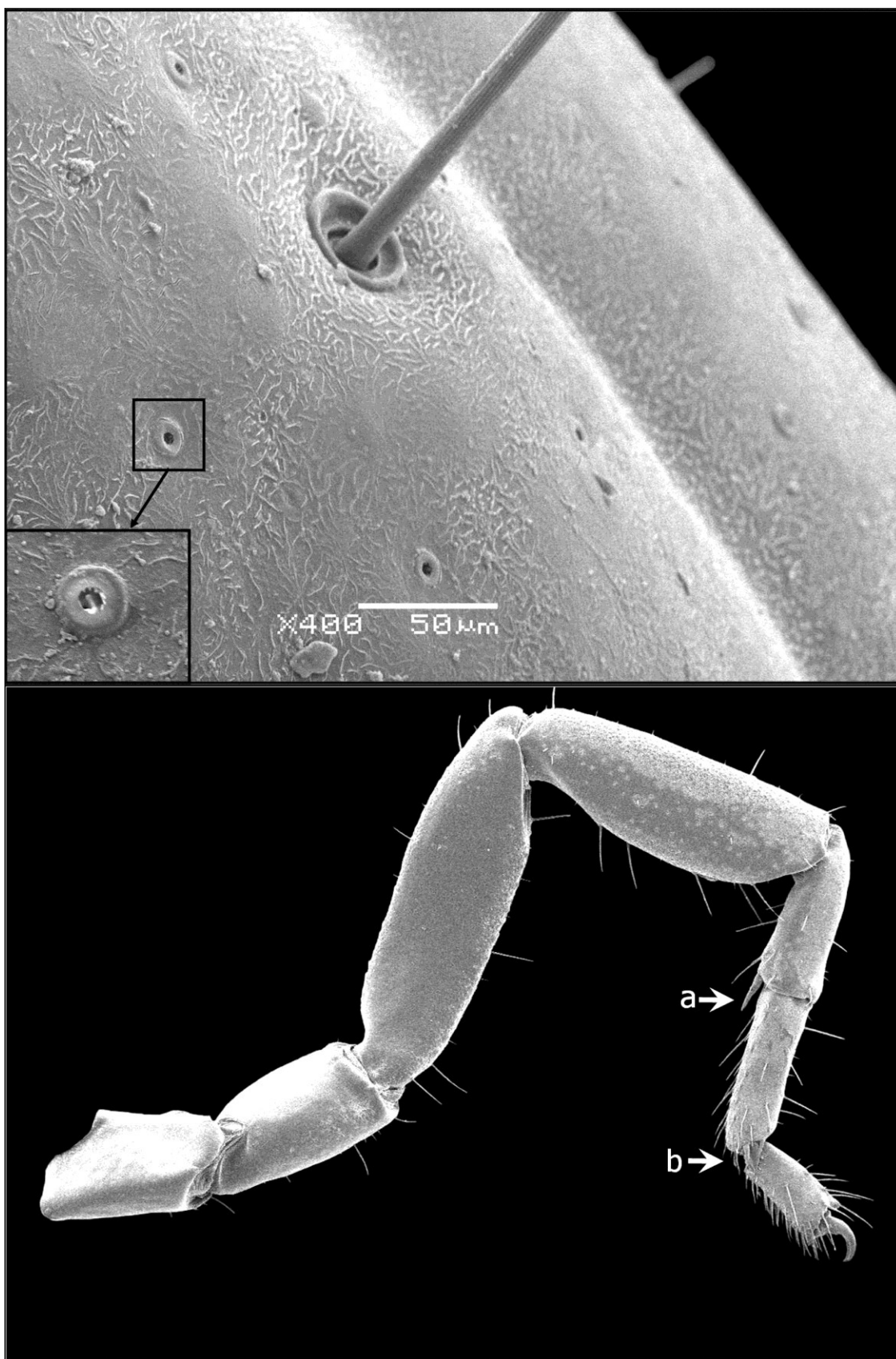
Figures 35–36: Pedipalps. 35 (top). Trichobothrium. 36 (bottom): a, trichobothria; b, seta.



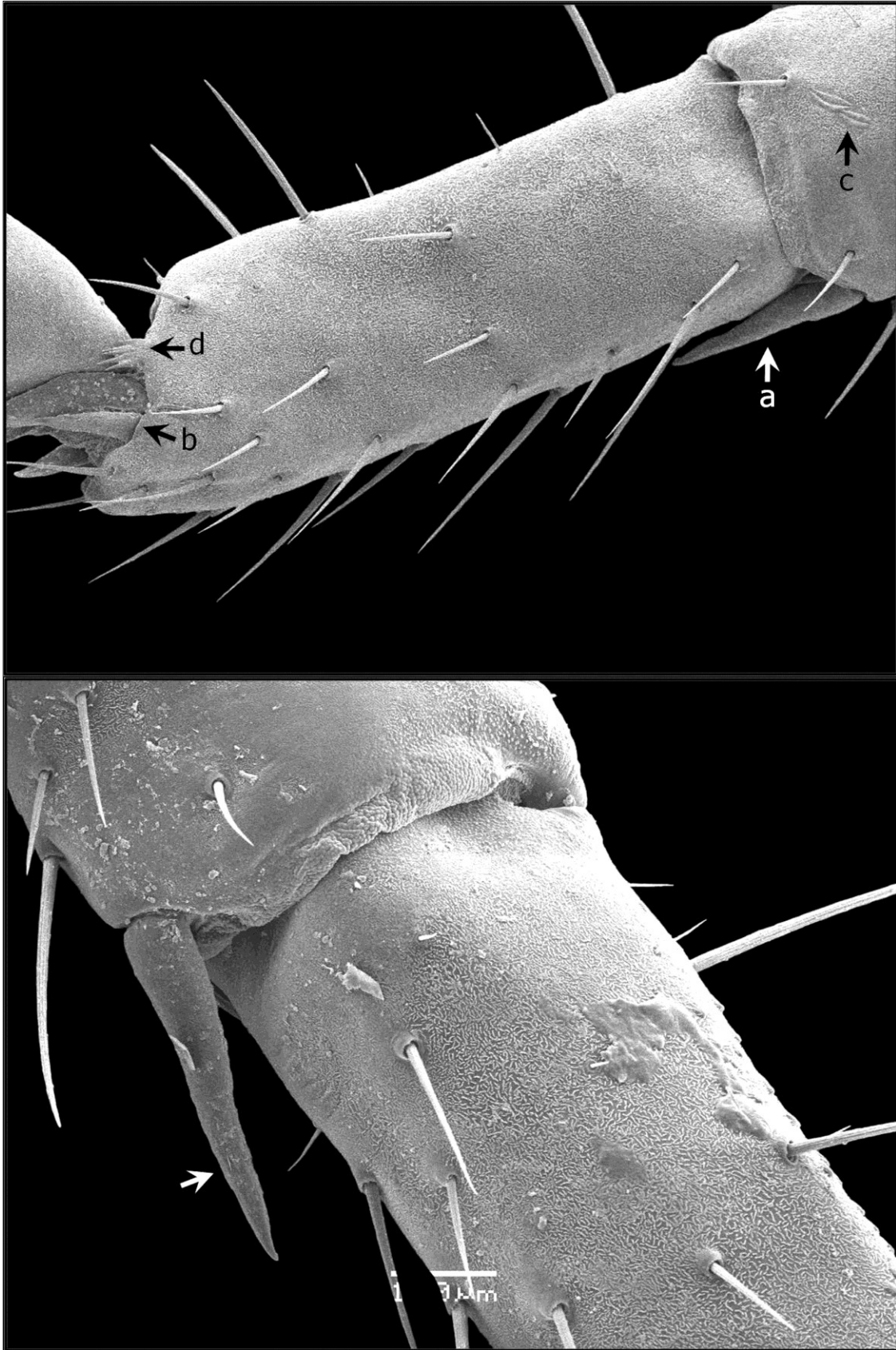
Figures 37–38: Wax pores. **37 (top).** Wax pores on pedipalp chela, next to a trichobothrium. **38 (bottom).** Wax pore on leg, next to a large seta.



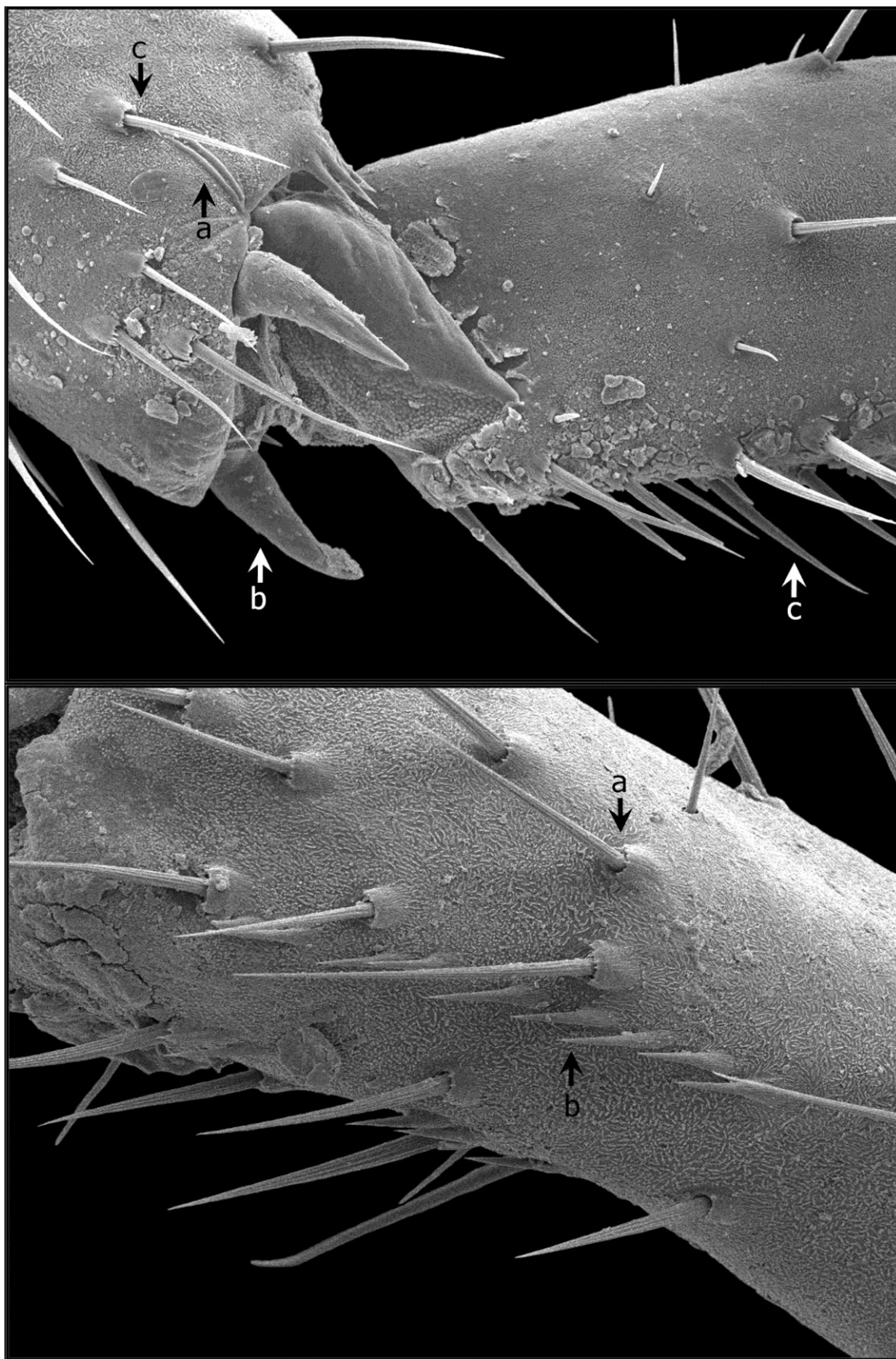
Figures 39–40: Wax pores. 39 (top): a, wax pore on chela movable finger; b, seta. 40 (bottom): a, wax pore on telson; b, seta.



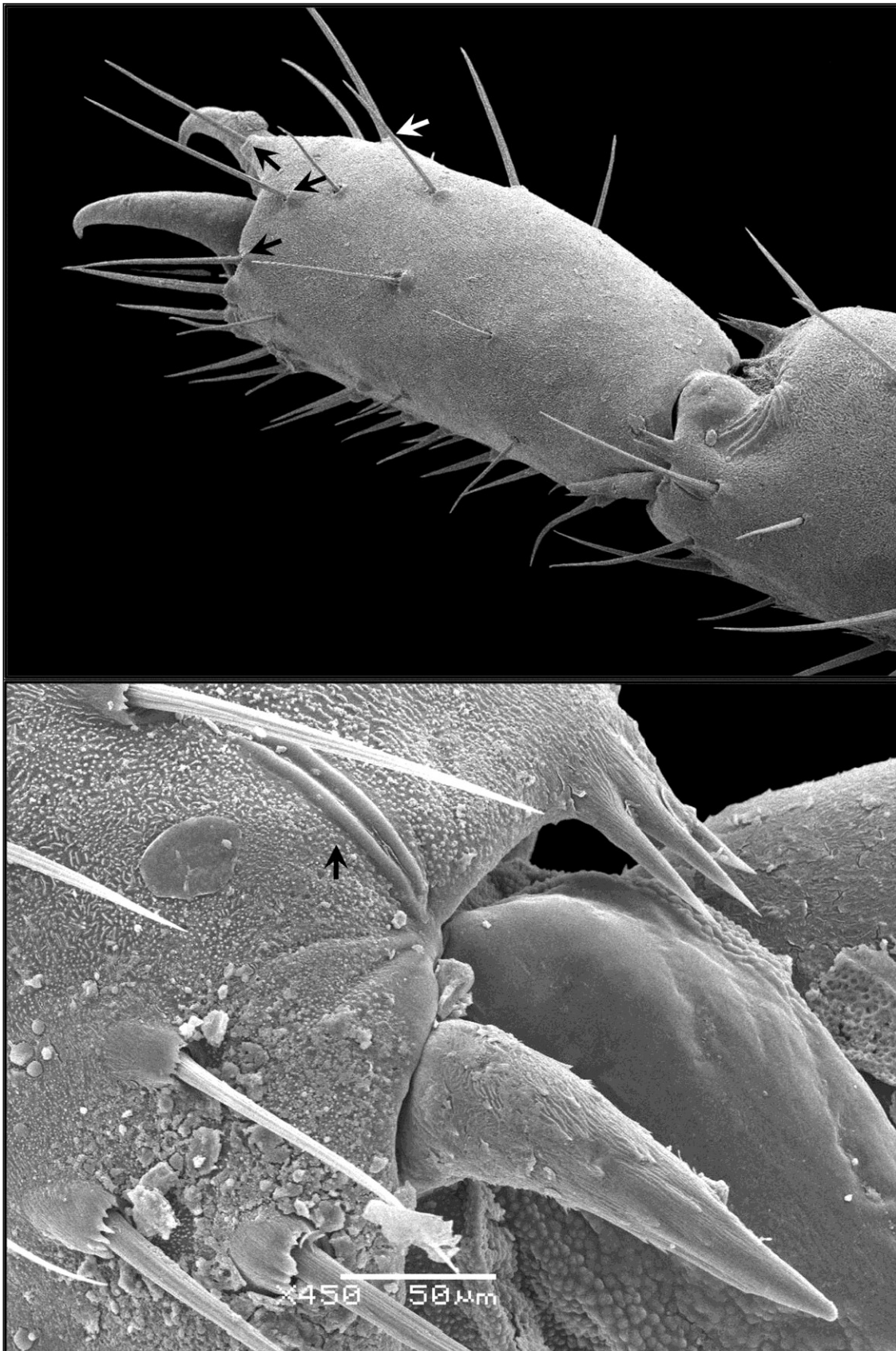
Figures 41–42: Wax pores. **41 (top).** Wax pore on telson, next to a large seta. Legs. **42 (bottom).** Leg: a, tibial spur; b, pedal spur.



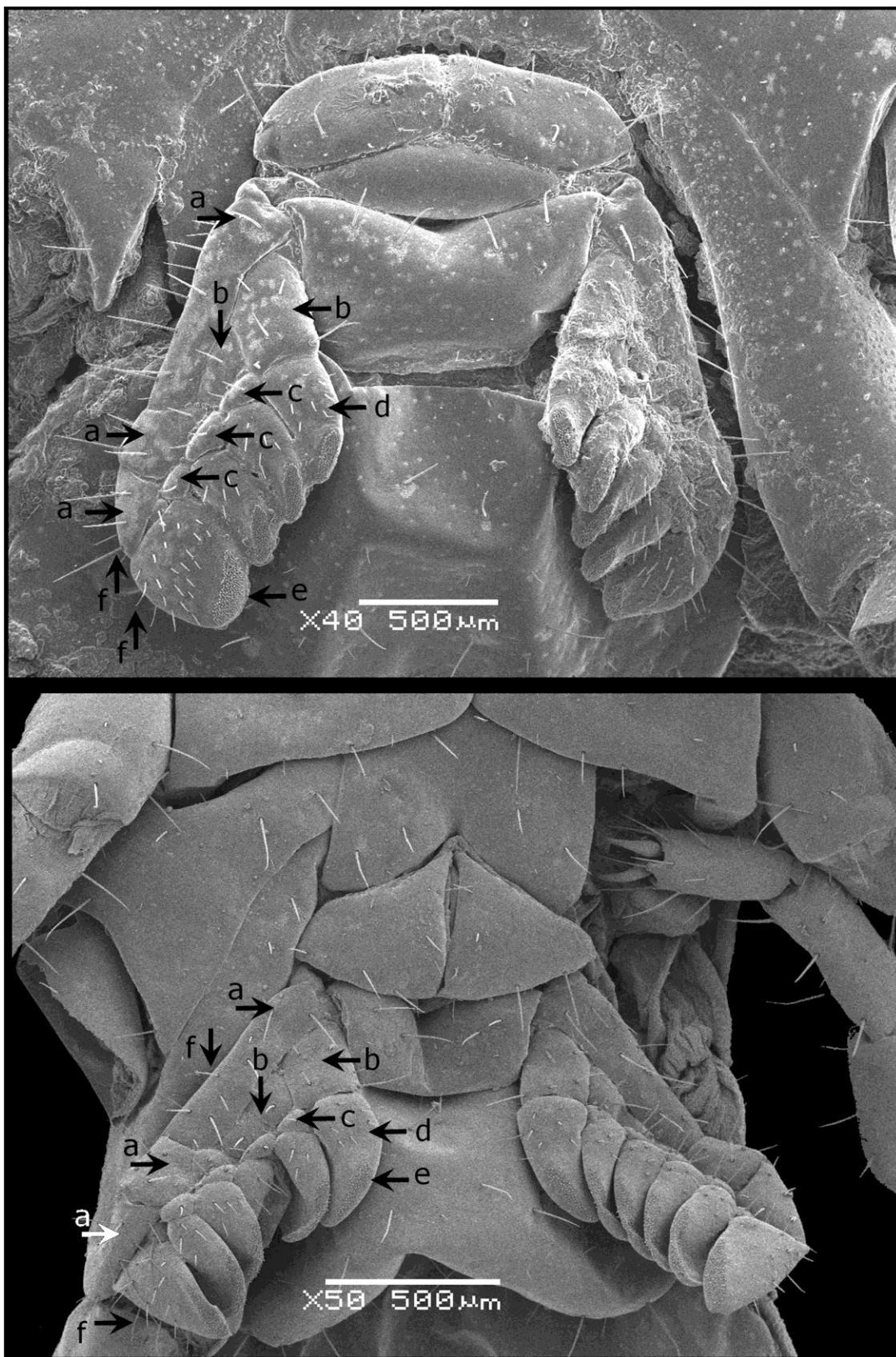
Figures 43–44: Legs. **43 (top).** Leg: a, tibial spur; b, basitarsal spur; c, basal slit sensilla on basitarsus; d, rosette-shaped structure. **44 (bottom).** Leg: tibial spur.



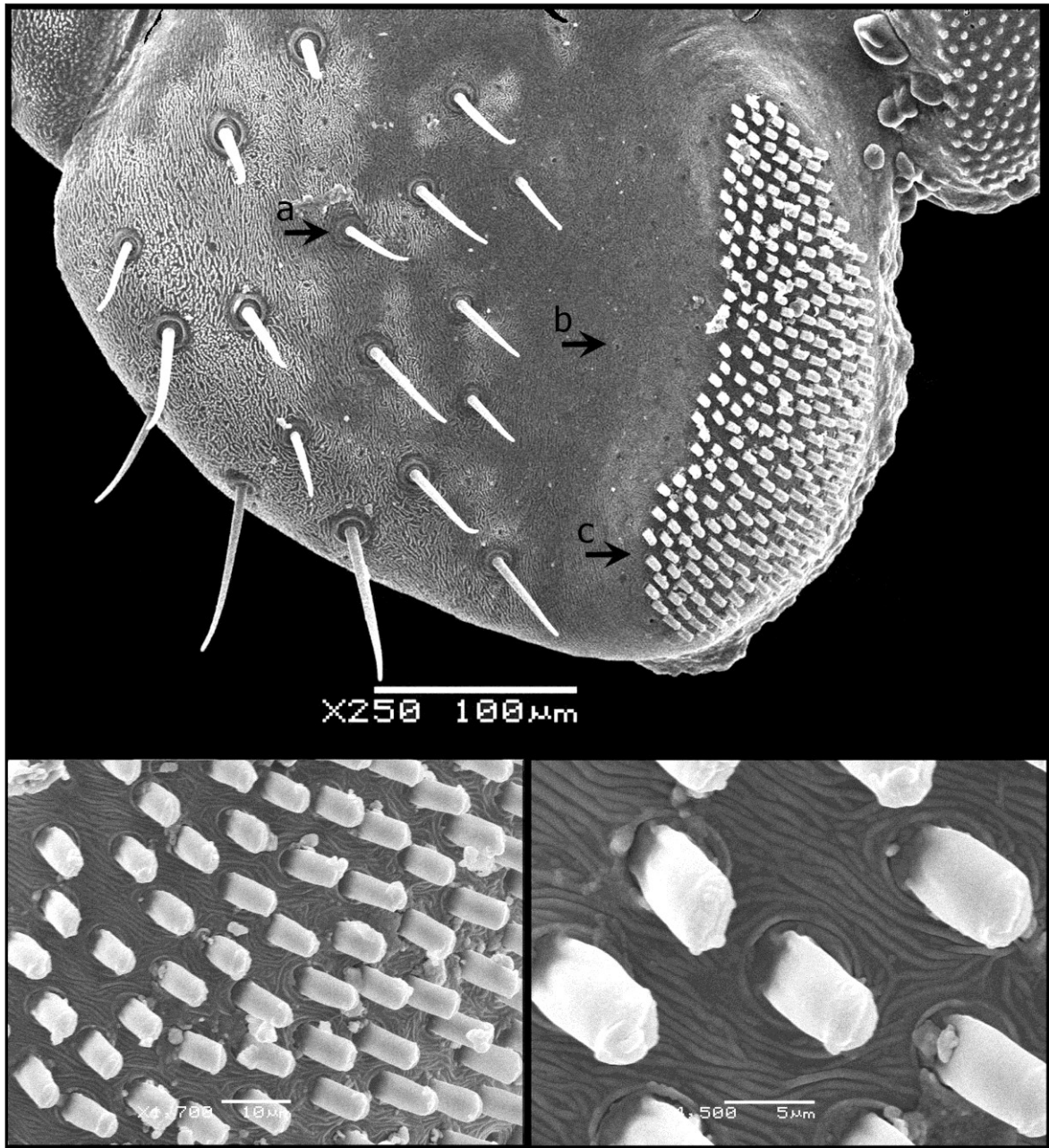
Figures 45–46: Legs. **45 (top).** Leg: a, slit sensilla; b, basitarsal spur; c, socketed setae on basitarsus and tibia. **46 (bottom).** Leg: a, socketed setae; b, non-socketed spinules.



Figures 47–48: Legs. 47 (top). Sensory setae. 48 (bottom). Slit sensilla on basitarsus under high magnification.



Figures 49–50: Pectinal organs. **49 (top).** Female pecten: a, marginal lamellae; b, median lamellae; c, fulcra; d, pectinal teeth; e, peg sensilla; f, setae. Note a prepectinal plate. **50 (bottom).** Male pecten: a, marginal lamellae; b, median lamellae; c, fulcra; d, pectinal teeth; e, peg sensilla; f, sensory setae.



Figures 51–53: Pectinal organs. **51 (top).** Pectinal teeth: a, sensory setae; b, wax pore; c, peg sensilla. **52–53 (bottom).** Peg sensilla.