

RESEARCH ARTICLE

Fine structure of the epicuticular secretion coat and associated glands of Pedipalpi and Palpigradi (Arachnida)

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Abstract

Pedipalpi Latreille, 1810 is a poorly studied clade of arachnids comprising the whip spiders (Amblypygi Thorell, 1883), short-tailed whip scorpions (Schizomida Petrunkevitch, 1945) and whip scorpions (Thelyphonida Cambridge, 1872). It has recently been shown that whip spiders coat their exoskeleton with a solid cement layer (cerotegument) that forms elaborate microstructures and turns the cuticle into a super-hydrophobic state. The amblypygid cerotegument provides taxonomic information due to its fine structural diversity, but its presence and variation in the sister groups was previously unknown. The present contribution reports the surface structure of the cuticle in species of Palpigradi, Thelyphonida, and Schizomida to determine if these taxa possess a solid epicuticular secretion coat. Scanning electron microscopy revealed that in addition to Amblypygi only species of Thelyphonida possess solid epicuticular secretion layers. Unlike in Amblypygi, in the Thelyphonida this layer does not usually form microstructures and is less rigidly attached to the underlying cuticle. A species of *Typopeltis* Pocock, 1894, which exhibited globular structures analogous to the amblypygid cerotegument, was an exception. Glandular structures associated with cement secretions in Amblypygi and Thelyphonida were considered homologous due to similar structure. Solid epicuticular secretion coats were absent from Schizomida, which is interpreted as a secondary loss despite the presence of slit-like glandular openings that appear to produce such epicuticular secretions. The micro-whip scorpion order Palpigradi Thorell, 1900 exhibited markedly different cuticular surface structures and lacked solid epicuticular secretions, consistent with the hypothesis that this order is not closely related to Pedipalpi. These results enhance the knowledge of the small, enigmatic orders of Arachnida.

KEYWORDS

Amblypygi, cerotegument, exoskeleton, Schizomida, Thelyphonida, Uropygi

1 | INTRODUCTION

The cuticle of arthropods is a multifunctional material. As the primary barrier to the environment, it plays a crucial role in defense, thermoregulation, locomotion, predation, and communication. It is a chitin-protein composite, forming several layers with different properties

and functions, that is, endo-, exo-, and epicuticle (Gallant & Hochberg, 2017; Vincent, 2002). Many physical properties and functions are accomplished by complex micro- and nano-structures and a vast diversity of secretions are exuded onto the epicuticular surface. In some arachnids (Alberti et al., 1981; Pugh et al., 1987; Wolff et al., 2017; Wolff, Schwaha, & Seiter, 2016) and myriapods (Adis

TABLE 1 Exemplar species of Palpigradi Thorell, 1900, Schizomida Petrunkevitch, 1945, and Thelyphonida Cambridge, 1872, with provenance data, and material examined for fine structure of epicuticle. Supplementary material is available here <https://phaidra.univie.ac.at/detail/o:1178645>

Thelyphonida Cambridge, 1872	Provenance	Ontogenetic stage	NHMW
Thelyphonida indet.	Vietnam	Early instar juvenile	
Thelyphonidae Lucas, 1835			
Hypoctoninae Pocock, 1899			
<i>Hypoctonus</i> sp.	Hoa Bin, Vietnam	Adult	29672
<i>Labochirus proboscideus</i> (Butler, 1872)	Kitulgala, Sri Lanka	Semi-subadult	29677
Mastigoproctinae Speijer, 1933			
<i>Mastigoproctus baracoensis</i> Franganillo, 1931	Baracoa, Cuba	Semi-adult	29633
<i>Mastigoproctus butleri</i> Pocock, 1894	Meta, Colombia	Adult	29674
<i>Mastigoproctus proscorpio</i> (Latreille, 1806)	San Pedro de Macoris, Dominican Republic	Adult	29631
<i>Mastigoproctus proscorpio</i> (Latreille, 1806)	Puerto Plata, Dominican Republic	Adult	29636
<i>Mastigoproctus tohono</i> Barrales-Alcalá et al., 2018	Arizona, United States	Semi-subadult	29680
<i>Ravilops kovariki</i> Teruel, 2017	Baoruco, Dominican Republic	Adult	29624
<i>Shelylongium pelegri</i> (Armas, 2000)	Pinar del Río, Cuba	Adult	29635
Thelyphoninae Lucas, 1973			
<i>Thelyphonus</i> cf. <i>doriae</i>	Sarawak, Malaysia	Subadult	29620
<i>Thelyphonus</i> cf. <i>hanseni</i>	Mindanao, Philippines	Adult	29621
<i>Thelyphonus manilanus</i> C.L. Koch, 1843	Luzon, Philippines	Adult	29622
<i>Thelyphonus</i> cf. <i>wayi</i>	Koh Kong, Cambodia	Adult	29670
<i>Thelyphonus</i> sp.	Tamil Nadu, India	Adult	29673
cf. <i>Thelyphonus</i> sp.	BaBe, Vietnam	Adult	29675
Typopeltinae Rowland & Cooke, 1973			
<i>Typopeltis crucifer</i> Pocock, 1894	Taiwan	Adult	29623
<i>Typopeltis dalyi</i> Pocock, 1900	N of Kuala Lumpur, Malaysia	Adult	29681
<i>Typopeltis guangxiensis</i> Haupt & Song, 1996	China?	Subadult	
<i>Typopeltis kasnakowi</i> Tarnani, 1900	Cat Ba, Vietnam	Adult	29676
<i>Typopeltis sinensis</i> (Butler, 1872)	Jiangsu, China	Subadult	29625
<i>Typopeltis</i> sp.	Malaysia	Subadult	29671
Schizomida Petrunkevitch, 1945			
Hubbardiidae Cook, 1899			
Hubbardiinae Cook, 1992			
<i>Rowlandius potiguar</i> Santos Ferreira & Buzatto, 2013	Brazil	Adult	
<i>Stenochrus portoricensis</i> Chamberlin, 1922	Cuba	Adult	
Palpigradi Thorell, 1900			
Eukoeneriidae Petrunkevitch, 1955			
<i>Eukoeneria ferratilis</i> Souza & Ferreira, 2011	Mato Grosso, Brazil	Adult	
<i>Eukoeneria florencae</i> (Rucker, 1903)	Mato Grosso, Brazil	Adult	

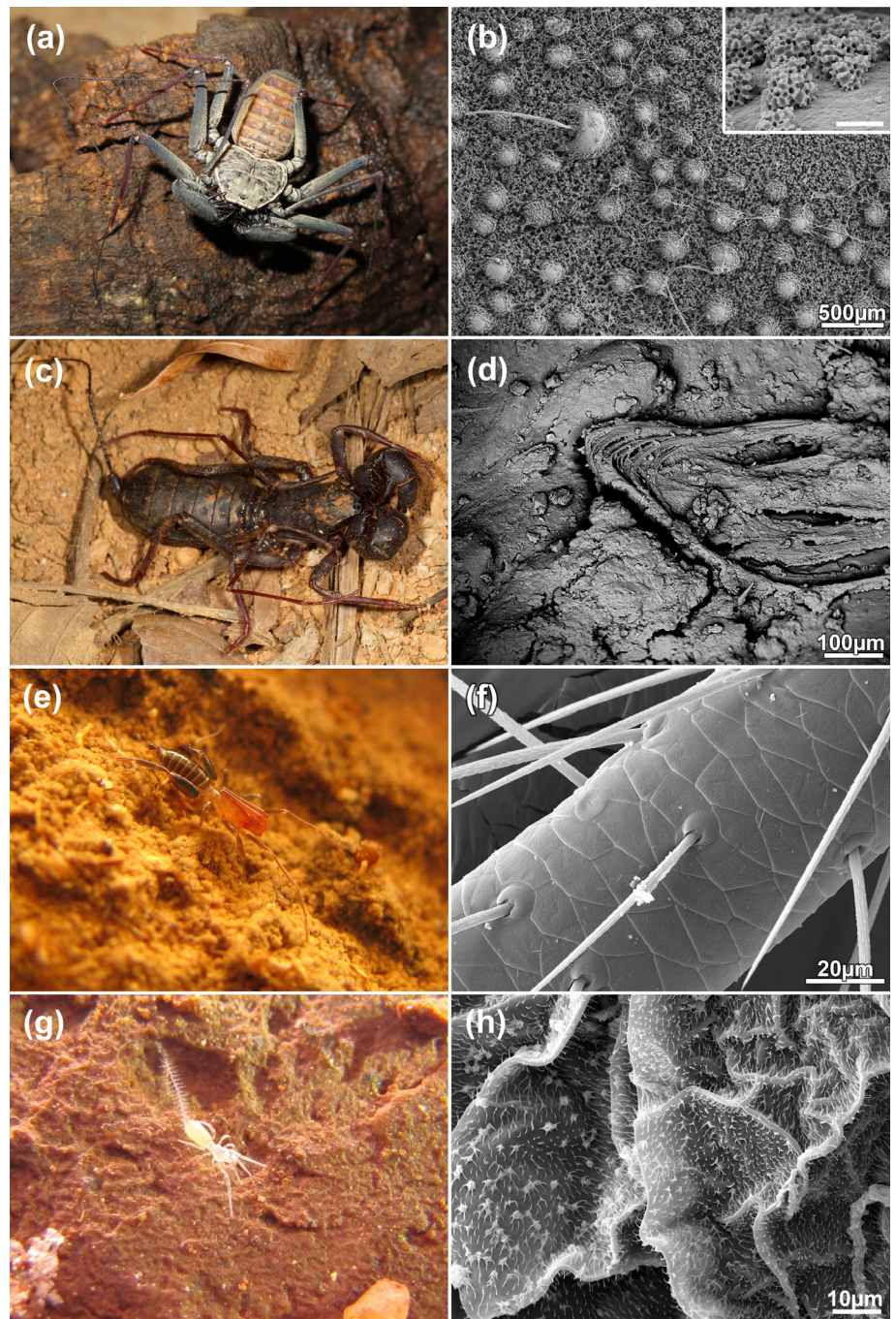
Note: Generally, one sample per species was analyzed. Specimens were preserved in 80% ethanol, except *Typopeltis sinensis* (Butler, 1872), for which a freshly air dried exuvium was used. Specimens were captive bred from wild caught individuals by the first author. Vouchers are deposited in the Naturhistorisches Museum Wien (NHMW), Austria. Specimens without NHMW numbers were destroyed in the study after identification.

et al., 1998), such secretions form a hard crust consisting of elaborate microstructures that strongly modify the surface properties of the cuticle – such rigid, micro-structured cement layers are called cerotegument. In contrast to the more widespread surface modification by epicuticular wax secretions that form crystals, the cerotegument is usually more inert, with a more complex structure, and assumed to be based on structural proteins (Wolff, Schwaha, & Seiter, 2016). Where

known, its function has been linked to plastron respiration to enhance oxygen uptake during submergence in water (Adis et al., 1998; Pugh et al., 1987; Wolff, Schwaha, & Seiter, 2016).

Cerotegument structures recently attracted the attention of different disciplines. For example, a comparative study of the cerotegument structure of the arachnid order Amblypygi Thorell, 1883 (whip spiders) revealed enormous variation across the families and

FIGURE 1 Live habitus (a), (c), (e), (g) and scanning electron micrographs of carapace (b), (d), (f), (h) of Pedipalpi Latreille, 1810 and Palpigradi Thorell, 1900: (a)–(b) amblypygid, *Phrynus decoratus* Teruel & Armas, 2005; (c) and (d) thelyphonid, *Mastigoproctus butleri* Pocock, 1894; (e) and (f) schizomid, *Rowlandius potiguar* Santos, Ferreira & Buzatto, 2013; (g) and (h) palpigrade, *Eukoenenia florenciae* (Rucker, 1903). Inset (b) illustrates hierarchical microstructure of cement layer in *Amblypygi* Thorell, 1883, scale bar = 2 μm



even within genera, offering a new, informative character system for their systematics (Wolff et al., 2017). Furthermore, it has been shown that the amblypygid cerotegument turns the initially hydrophilic cuticle into a super-hydrophobic state (Wolff, Schwaha, & Seiter, 2016). This is the result of a self-assembling process of colloid secretion, produced by two types of secretory cells (Filippov et al., 2017; Wolff, Schwaha, & Seiter, 2016). As it is accomplished by a single fluid, this system is of interest for biomimetics to inspire new technologies of surface modifications. The evolution of a complex cerotegument in amblypygids also plays a role in the microbial ecology of these arachnids. The secretions have been found to promote the growth of an

epicuticular mycobiome, with as yet unknown ecological functions (Gibbons et al., 2019).

Amblypygi are placed within Pedipalpi Latreille, 1810, a larger clade of predatory tropical and subtropical arachnids characterized by raptorial pedipalps, an elongated, antenniform first pair of legs providing sensory functions, and maternal care behavior, in which the first instar (prenymph) is defenseless and carried by the mother until the first molt (Wolff et al., 2015). Pedipalpi are the sister group of spiders (Araneae Clerck, 1757) and comprise the orders Amblypygi, Schizomida Petrunkevitch, 1945 (short-tailed whip scorpions) and Thelyphonida Cambridge, 1872 (whip scorpions or vinegaroons), the

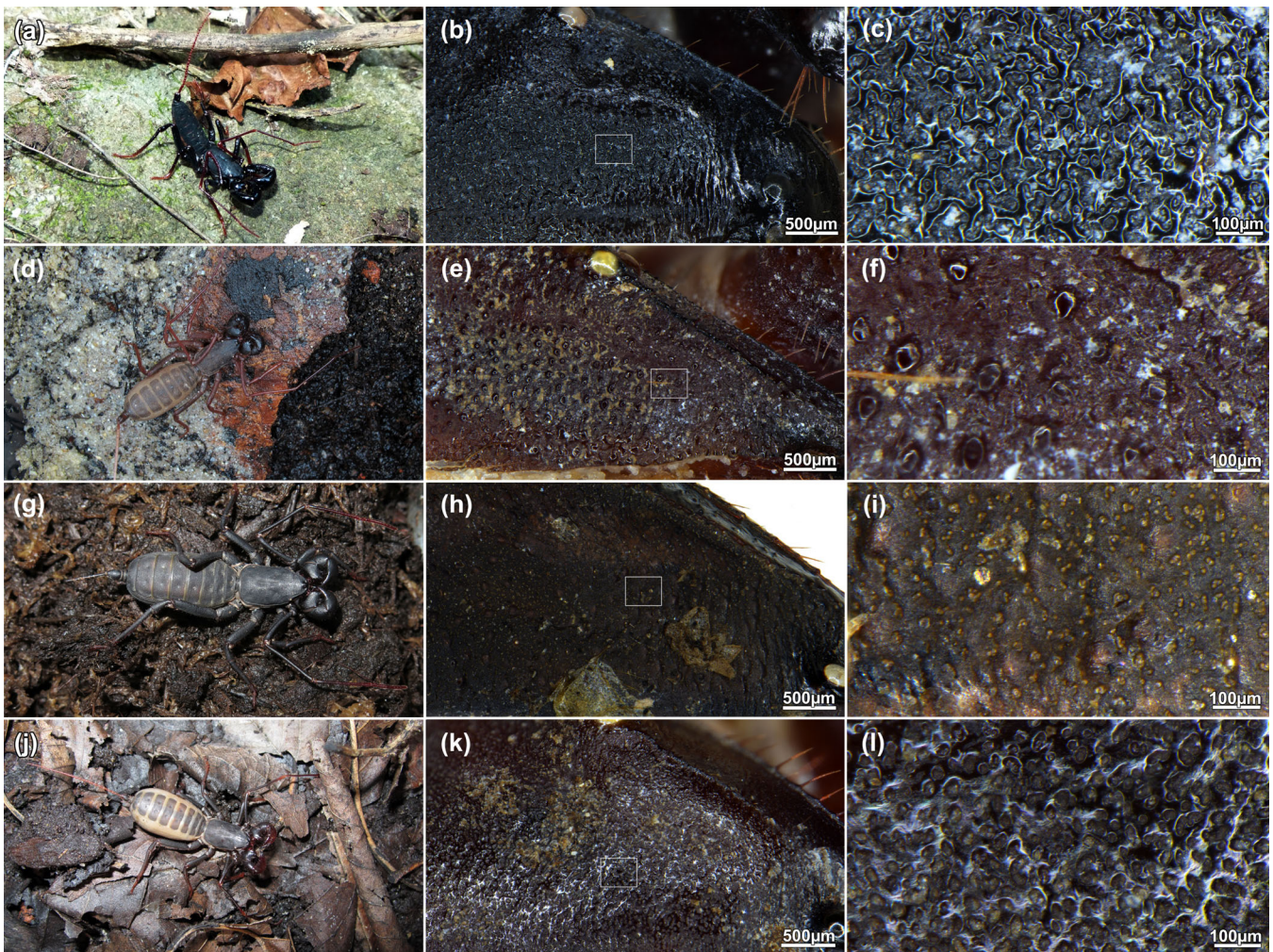


FIGURE 2 Live habitus (a), (d), (g), (j) and light microscopy (reflected light, true color) of carapace (b), (c), (e), (f), (h), (i), (k), (l) of Thelyphonida Lucas, 1835, dorsal aspect: (a)–(c) hypoctonine, *Labochirus proboscideus* (Butler, 1872); (d)–(f) mastigoproctine, *Mastigoproctus baracoensis* Franganillo, 1931; (g)–(i) typopeltine, *Typopeltis sinensis* (Butler, 1872); (j)–(l) thelyphonine, *Thelyphonus* cf. *doriae*

latter two orders forming the clade known as Uropygi Latreille, 1804 (Clouse et al., 2017; Garwood et al., 2016; Giribet, 2018; Shultz, 2007; Weygoldt & Paulus, 1979). Another order, Palpigradi Thorell, 1900 (micro-whip scorpions), shares some characters with Pedipalpi, but its phylogenetic affinities remain unclear (Giribet, 2018).

Thelyphonida is a small order of large-bodied arachnids originating about 333 million years ago in tropical Pangea (Clouse et al., 2017). The 125 extant species are placed into a single family, Thelyphonidae Lucas, 1835, containing four subfamilies, Hypoctoninae Pocock, 1899, Mastigoproctinae Speijer, 1933, Thelyphoninae Lucas, 1973, and Typopeltinae Rowland & Cooke, 1973 (Harvey, 2003; Prendini, 2011; Seraphim et al., 2019). No comprehensive phylogenetic study exists for Thelyphonida, the systematics of which are in severe need of re-evaluation. New character systems, in combination with classical morphological characters and molecular data, may assist in that effort.

In their comparative study of cerotegument structures in Amblypygi, Wolff et al. (2017) included a thelyphonid, *Typopeltis crucifer* Pocock, 1894 as outgroup. The whip scorpion also exhibited a cerotegument layer, but with an amorphous structure, lacking the regular micro-patterns present in the cerotegument of whip spiders, suggesting that the cerotegument may not be apomorphic in Amblypygi, but evolved earlier. However, no other studies of epicuticular secretions in Thelyphonida have since been presented to test this hypothesis. Several glandular openings were illustrated for various genera of Schizomida (e.g., Giupponi et al., 2016; Pinto-da-Rocha et al., 2016; Santos & Pinto-da-Rocha, 2009), without any interpretation of their function. This may be because scanning electron microscopy (SEM) is rarely conducted on arachnids outside the orders Araneae Clerck, 1757 and Acari Leach, 1817, and usually involve cleaning protocols which may remove secretion layers from the epicuticle.

The present study aimed to clarify the distribution of the cerotegument among the three orders of Pedipalpi and describe in more

FIGURE 3 Scanning electron micrographs of carapace of hypotoonine Thelyphonida Cambridge, 1872, dorsal aspect: (a) and (b) *Hypotoonus* sp.: (a) amorphous layer concentrated on left side, illustrating several cracks; (b) regular amorphous layer covering cuticle, with few patches uncovered and illustrating smooth cuticular surface. (c) and (d) *Labochirus proboscideus* (Butler, 1872): (c) flake-like appearance of amorphous layer in upper half; lower half illustrating uncovered, indented, and punctate cuticle; (d) almost uncovered smooth cuticle illustrating minute slit-like gland opening (arrow) and amorphous patches presumably covering gland openings

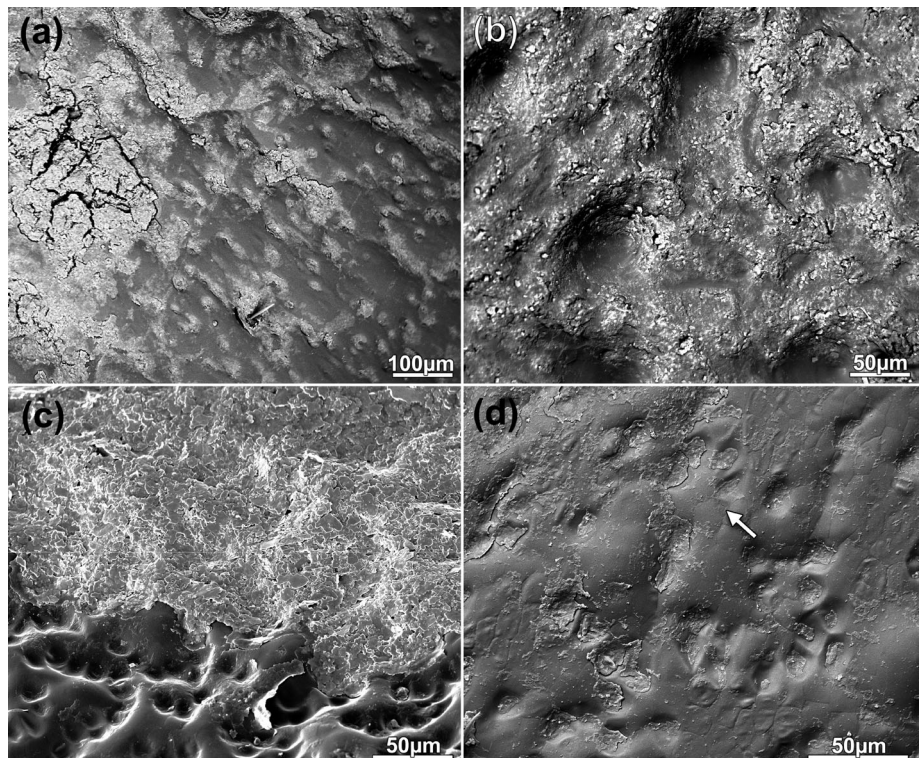
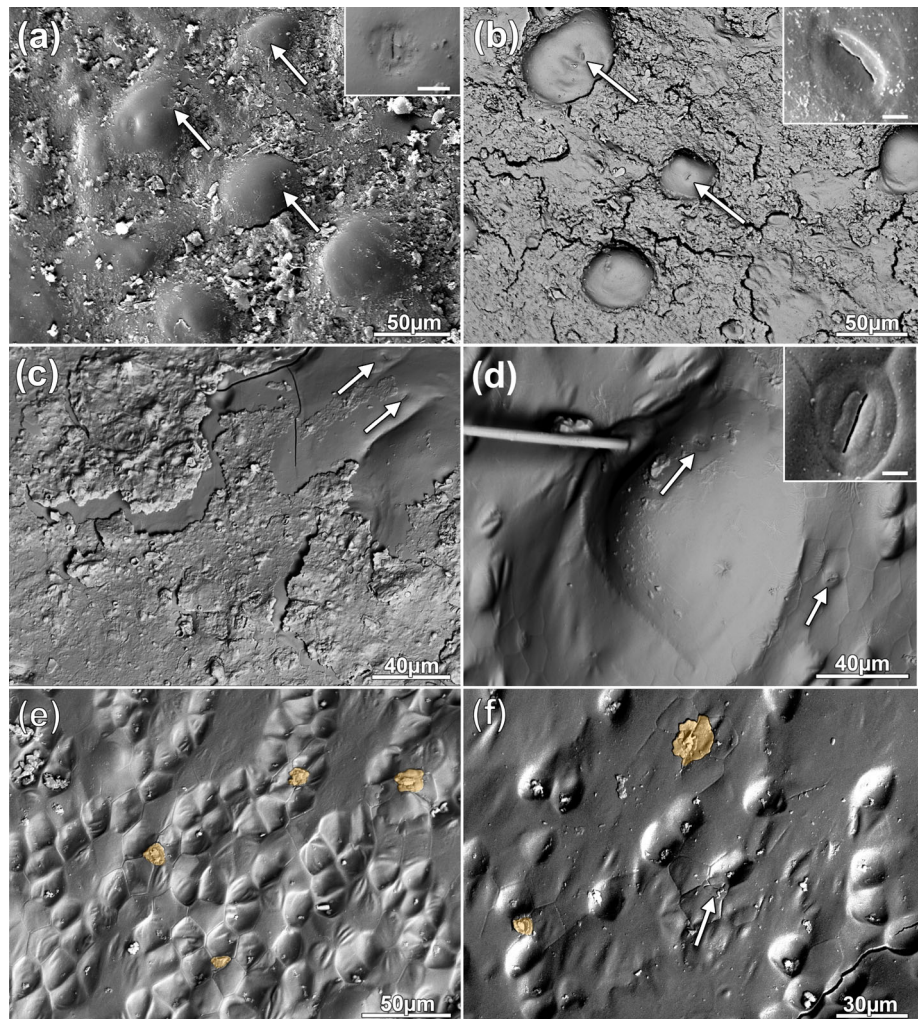


FIGURE 4 Scanning electron micrographs of carapace of mastigoproctine Thelyphonida Cambridge, 1872, dorsal aspect: (a) *Mastigoproctus proscorpio* (Latreille, 1806): note tubercles which commonly have slit-like gland openings (arrows), without amorphous layer covering cuticle; inset illustrates slit-like gland opening, scale bar = 5 µm. (b) *Mastigoproctus baracoensis* Franganillo, 1931: tubercles free of amorphous layer and slit-like gland openings (arrows); inset illustrates slit-like gland opening, scale bar = 2 µm. (c) and (d) *Sheylayongium pelegri* (Armas, 2000), backscatter images: (c) part of amorphous layer disrupted showing smooth areas of cuticle with slit-like gland openings (arrows); (d) area of cuticle with amorphous layer removed, illustrating small tubercle with slit-like gland opening cuticle beneath (arrows); inset illustrates detail of slit-like gland opening, scale bar = 2 µm. (e) and (f) *Ravilops kovariki* Teruel, 2017: (e) mostly smooth cuticle uncovered by amorphous layer except at several patches bordered by three or four cuticular tiles, presumably area of gland openings, marked in orange; (f) patches of amorphous layer with slit-like gland opening in area lacking amorphous layer (arrow), presumably area of gland openings with epicuticular secretion coat marked in orange



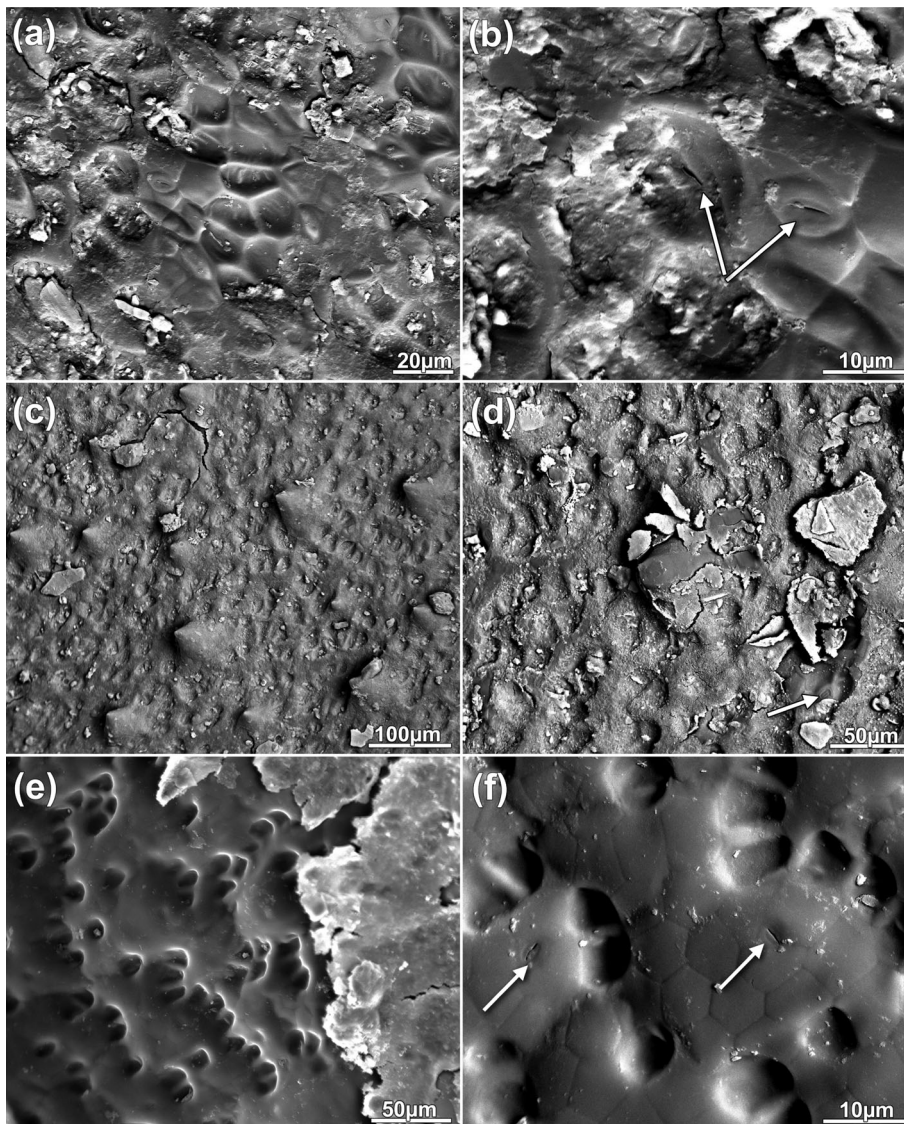


FIGURE 5 Scanning electron micrographs of carapace of thelyphonine Thelyphonida Cambridge, 1872, dorsal aspect: (a) and (b) *Thelyphonus* cf. *henseni*: (a) cuticular area mostly free of amorphous covering, illustrating smooth surface with slit-like gland openings; (b) slit-like gland openings (arrows). (c)–(d) *Thelyphonus* cf. *wayi*: (c) regular amorphous layer covering cuticle; layer less coarse than in some other species; distinct spines evident on cuticle; note crack in amorphous layer at top; (d) exfoliating amorphous layer with spine uncovered (center), illustrating smooth cuticle; note slit-like gland opening at lower right (arrow). (e)–(f) *Thelyphonus* *manilanus* C.L. Koch, 1843: (e) cuticular area with amorphous layer (at right), broken and illustrating smooth cuticle; (f) slit-like gland openings (arrow)

detail its variation within Thelyphonida, to assess its potential as a new character system for systematics. A fine structural analysis of the cuticle was conducted on 21 exemplar species representing all four subfamilies of Thelyphonidae, two exemplar species of the schizomid family Hubbardiidae Cook, 1899, and two exemplar species of the palpigrade family Eukoeneniidae Petrunkevitch, 1955.

2 | MATERIAL AND METHODS

2.1 | Taxon sample

Twenty-one species of Thelyphonida, two species of Schizomida, and two species of Palpigradi were studied (Table 1). Thelyphonid identifications were based on Rowland and Cooke (1973), Haupt (1996, 2004a, 2004b, 2009), and Haupt and Song (1996). Voucher specimens of thelyphonids are deposited in the Naturhistorisches Museum, Wien (Vienna, Austria; NHMW).

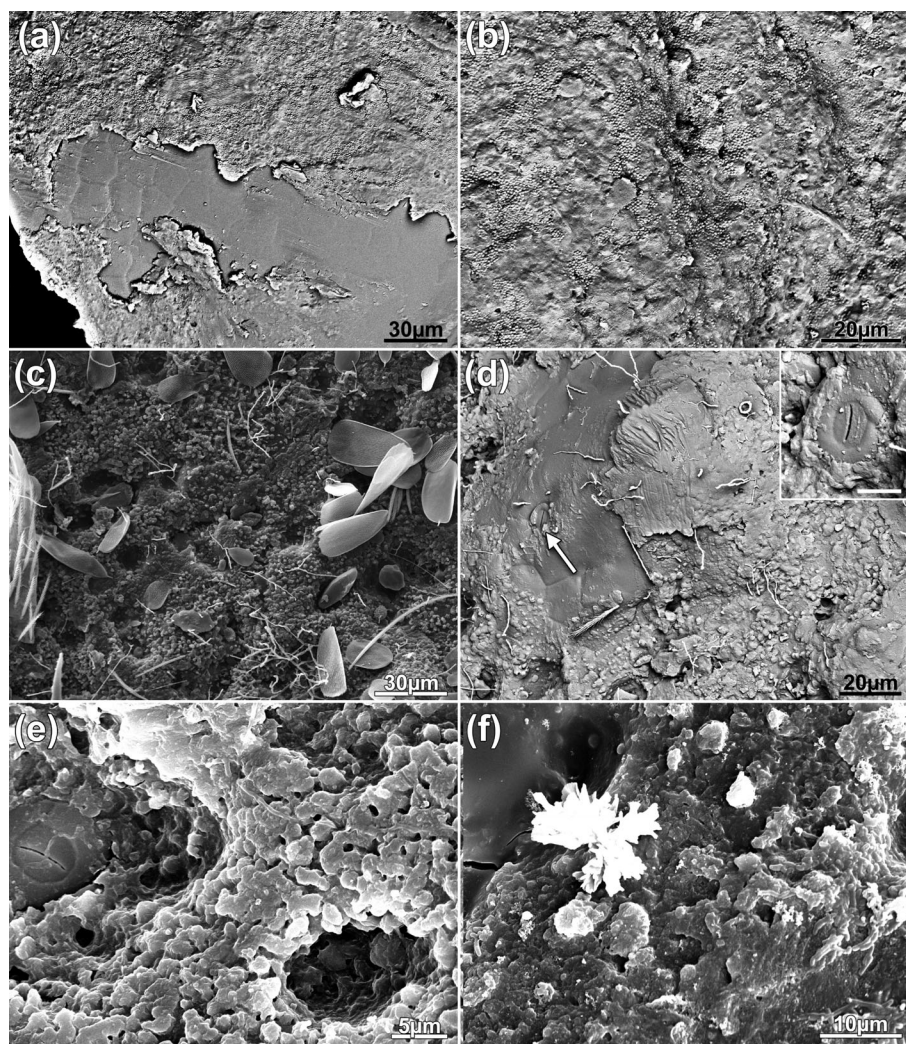
2.2 | Scanning electron microscopy

Half the carapace (Figures 1 and 2) or, for smaller taxa, the entire specimen, was air-dried or dehydrated in an ethanol series of increasing concentration, followed by chemical drying with hexamethyldisilazane (HMDS) or critical point drying in a Leica CPD300 (Leica Microsystems, Wetzlar, Germany). Dried specimens were mounted on standard aluminum stubs, sputter-coated with gold (approximately 20–40 nm) on a JEOL JFC-2300HR sputter coater (Akishima, Japan) and examined in a JEOL IT300 SEM.

2.3 | Light microscopy

Specimens were preserved in 80% ethanol, studied and photographed with a Nikon SMZ-25 stereomicroscope (Nikon, Tokyo, Japan) equipped with a Nikon DS-Ri1 microscope camera, and measured using NIS-Elements BR software. Live habitus photographs were

FIGURE 6 Scanning electron micrographs of carapace of typopeltine Thelyphonida Cambridge, 1872, dorsal aspect: (a), (b) *Typopeltis crucifer* Pocock, 1894: (a) amorphous layer partially scraped off cuticle, illustrating smooth cuticular tiles; (b) amorphous layer illustrating regular minute globular microstructure. (c)–(e) *Typopeltis sinensis* (Butler, 1872): (c) cuticle illustrating various structures (e.g., insect scales) attached to amorphous layer; (d) uncovered slit-like gland opening on smooth cuticle (arrow); inset illustrates gland opening, scale bar = 5 μ m; (e) granular structure of secretions and gland openings. (f) *Typopeltis dalyi* Pocock, 1900: irregular granular secretions covering cuticle



taken with a Canon PowerShot G12. Digital images were processed using Adobe Photoshop® 8.0 to optimize contrast and brightness.

3 | RESULTS

3.1 | Epicuticular structure in Thelyphonida

The carapace of hypoctonine (Figure 3), mastigoproctine (Figure 4), thelyphonine (Figure 5) and typopeltine (Figures 6 and 7) thelyphonids was similar in most exemplar species. An epicuticular secretion coat superficially covers the cuticle of most species. This layer was thin and could be amorphous, appearing flake-like (Figure 3(c)), rugose or uneven (Figures 3(a),(b), 4(a)–(c), and 5(b)–(d)). The epicuticular secretion coat was usually irregularly granular in typopeltine species (Figure 6) but occasionally included foreign structures, such as pieces of collembolan cuticle, insect scales, crystalline structures or bristles (e.g., Figures 6(c),(d) and 7(a),(b)). Coiled wax-like secretions were regularly observed on top of the epicuticular secretion coat in one species of *Typopeltis* (Figure 7(a),(b)). Several globular granules were present in high densities on the carapace of *Typopeltis dalyi* Pocock,

1900 (Figure 7(c)–(f)). Each globule possessed a rough surface composed of multiple slender filaments, also located next to the globules (Figure 7(d),(f)). This species also exhibited an unusual abundance of microorganisms in and on the epicuticular secretion layer.

The epicuticular secretion coat was often cracked or broken, revealing the smooth, underlying cuticle (Figures 3(a),(c), 4(a),(c), 5(a)–(e), and 6(a),(d)). Such damage or even absence of the epicuticular secretion coat is artificial, probably caused by sample preparation, which suggests the epicuticular secretion coat is not firmly and permanently attached to the epicuticle.

The epicuticular secretion coat is likely secreted by epidermal glands with slit-like openings in the cuticle (Figures 3(d), 4(a)–(d),(f), 5(a),(b),(e),(f), and 6(d)), usually only 3–4 μ m in length, and surrounded by three or four cuticular tiles (e.g., Figure 4(d),(f)). In cases in which the epicuticular secretion coat was mostly detached, patches remained surrounding what were presumed to be gland openings (see Figure 4(e),(f)), supporting the notion that these glands are responsible for secreting the epicuticular secretion coat.

The cuticle below the epicuticular secretion coat was composed of smooth, polygonal cuticular tiles (Figures 4(d)–(f), 5, and 6(a),(d)). Some species possessed tubercles or small spines (Figures 3(b), 4(a)–

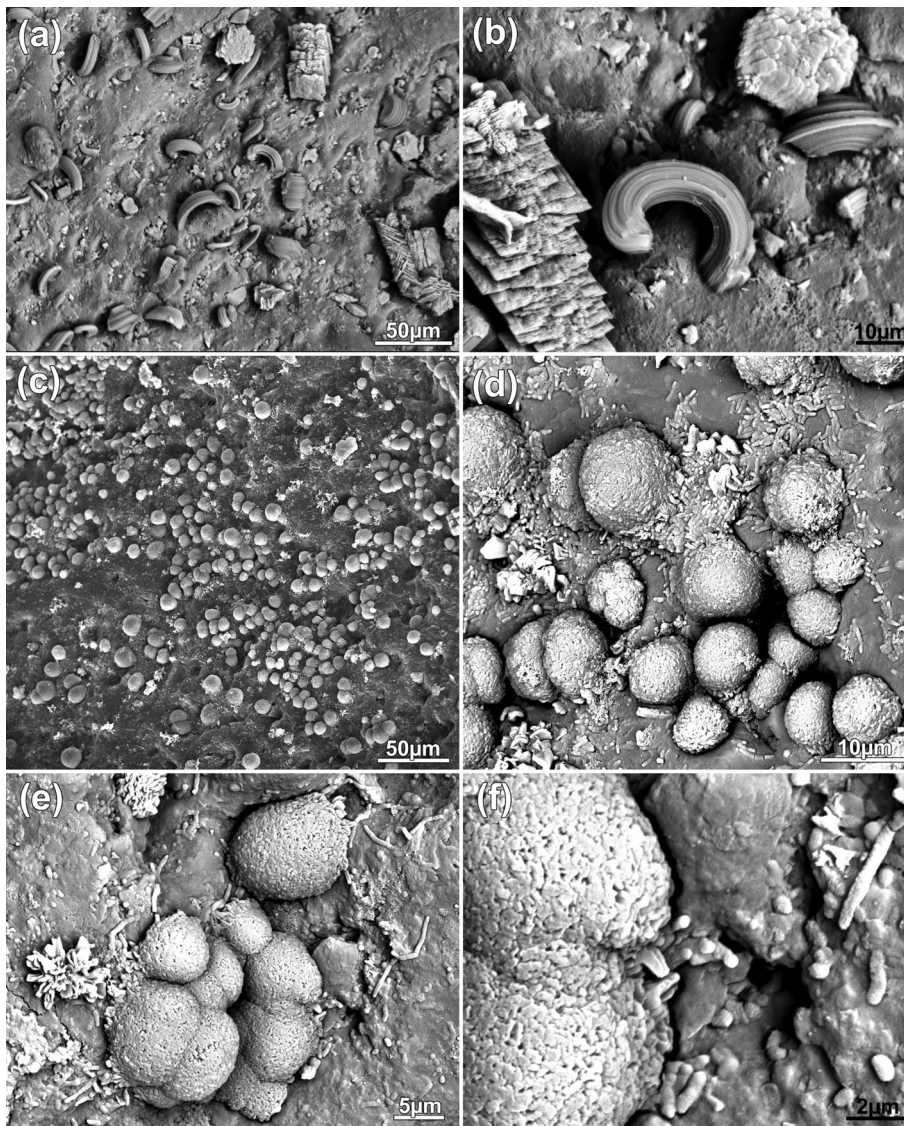


FIGURE 7 Scanning electron micrographs of carapace of typopeltine Thelyphonida Cambridge, 1872 with unusual surface structures, dorsal aspect: (a)–(b) *Typopeltis* sp.: (a) cuticle illustrating multiple coiled wax-like secretions; (b) single wax-like secreted rod illustrating longitudinal ridges. (c)–(f) *Typopeltis dalyi* Pocock, 1900: (c) several globular structures on amorphous layer; (d)–(f) globular structures

(d), and 5(c),(d)). Multiple depressions, reflecting the regular arrangement of cuticular tiles, were observed in many species (Figures 4(d)–(f) and 5(a),(e),(f)).

3.2 | Epicuticular structure in Schizomida

The cuticle of both exemplar species, *Rowlandius potiguar* Santos et al., 2013 and *Stenochrus portoricensis* Chamberlin, 1922, was generally smooth, lacking any epicuticular amorphous secretion coat, and arranged in overlapping tiles over most parts (Figure 8). Two different types of pores were observed. One type, with a slit-like opening, located within cuticular tiles of more roundish shape, was identical to the gland opening observed in Thelyphonida (Figure 8 (b)–(f)). The other type was triangular to rounded with a central pore and radially emerging cuticular ridges, creating a rosette-shaped appearance, and was usually located between cuticular tiles (Figure 8(c),(d)).

3.3 | Epicuticular structure in Palpigradi

The cuticle of both exemplar species of *Eukoenenia* Börner, 1901 was smooth and lacking distinct tiles (Figure 9). It was covered in short, regularly arranged microtrichia, sometimes with foreign particles clinging to the tips (Figure 9(c),(d)). The cuticular surface was regularly covered by nanopore openings, a few nanometers in length (Figure 9(a),(b)).

4 | DISCUSSION

The results presented here confirm the hypothesis of Wolff et al. (2017) that solid epicuticular secretions are present in Amblypygi and Thelyphonida. Such secretions were consistently found in all exemplar species of Thelyphonida. Unlike Amblypygi, however, these secretion layers were generally amorphous or irregular in Thelyphonida, with only one species exhibiting regular micro-patterns.

FIGURE 8 Scanning electron micrographs of cuticle of Schizomida Petrunkevitch, 1945: (a), (c), (e) *Stenochrus portoricensis* Chamberlin, 1922; (d)–(f) *Rowlandius potiguar* Santos, Ferreira & Buzatto, 2013: (a) anterior part of prosoma illustrating chelicerae and pedipalps, dorsal aspect; inset illustrates tiled structure of cuticle, scale bar = 10 μm ; (b) cuticular surface illustrating regular polygonal to hexagonal cuticular tiles; (c) rosette-shaped cuticular structure; (d) rosette-shaped gland opening; (e) cuticular surface of carapace illustrating slit-like gland openings at left and rosette-shaped gland opening at right. (f) slit-like gland opening (arrow) on round cuticular tile of leg

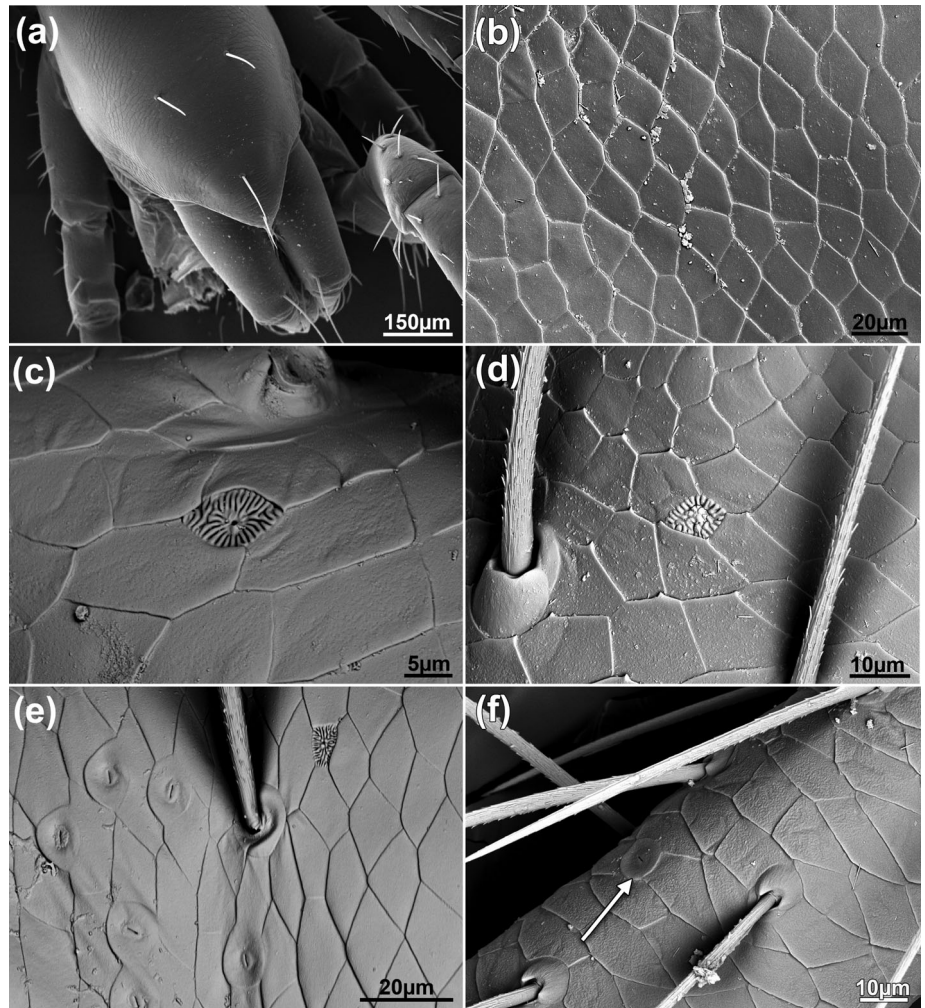
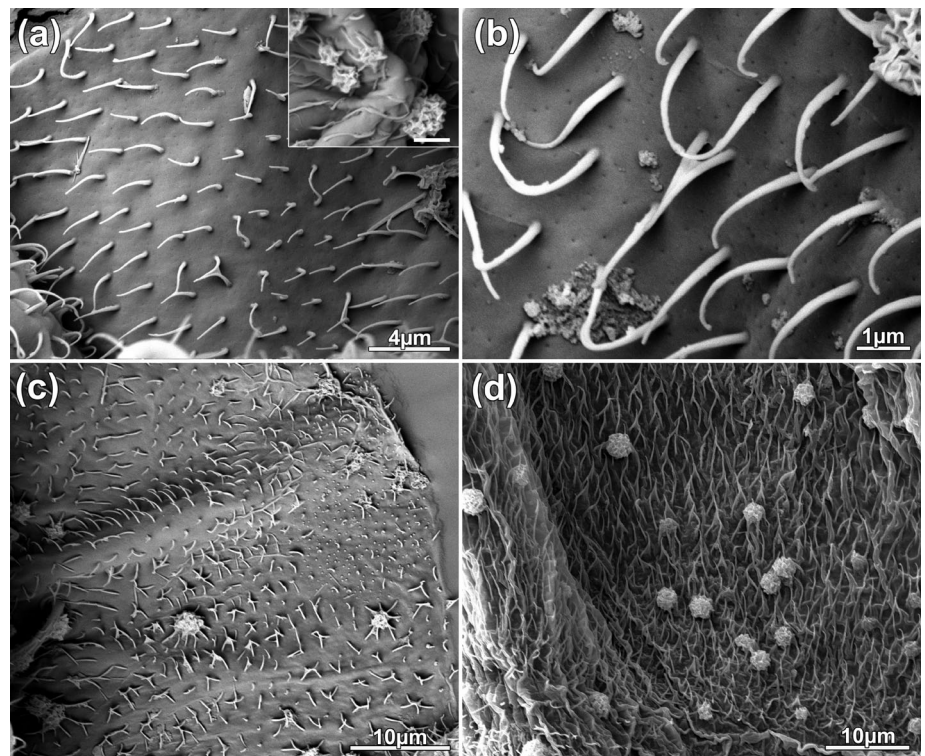


FIGURE 9 Scanning electron micrographs of cuticle of Palpigradi Thorell, 1900: (a), (b) *Eukoenenia ferratilis* Souza & Ferreira, 2011: (a) cuticle; insert illustrates structures attached to cuticular spines, scale bar = 2 μm ; (b) microtrichia and nanopores on cuticular surface. (c), (d) *Eukoenenia florenciae* (Rucker, 1903): (c) low density of microtrichia; (d) high density of microtrichia; note foreign particles ((c), (d))



Furthermore, the secretion coat was variable in thickness and appeared to be less rigidly connected to the cuticle in Thelyphonida than the cerotegument of Amblypygi. Therefore, the secretion crust of Thelyphonida is appropriately referred to as an epicuticular layer of an amorphous or granular structure, rather than a cerotegument.

In contrast to Amblypygi and Thelyphonida, solid epicuticular secretions were absent in the exemplar species of Schizomida and Palpigradi. However, Schizomida shared with the other two orders of Pedipalpi the slit-like openings, previously shown to be associated with a glandular apparatus that forms the colloidal particles in the whip spider cerotegument (Wolff, Schwaha, & Seiter, 2016). This may indicate that epicuticular secretions are also present in Schizomida, but do not form a rigid and durable crust, and were dissolved in the preservative or washed off in the preparation for SEM.

Ultrastructural analysis of the cuticle of another palpigrade, *Eukoenenina spelaea* (Peyerimhoff, 1902), was recently conducted by Franz-Guess and Starck (2020). As in the present contribution, no specific coating layer on the cuticle was observed. Short regular hairs, termed pubescence, covered most of the cuticle in all three palpigrade species investigated. Pore canals were observed in thin sections of *E. spelaea*, corresponding in size and arrangement to the pore openings observed in *Eukoenenina ferratilis* Souza & Ferreira, 2011 and *Eukoenenina florenciae* (Rucker, 1903). No specific glandular cells have been described in the epidermis of *E. spelaea*, which corroborates the lack of an epicuticular coating. A few attached particles of similar structure were detected in *E. spelaea* and the two exemplar species in the current study, but it is unclear whether these are products of the palpigrade or merely foreign particles.

Assuming that the sample in the present study is representative of the three orders of Pedipalpi, and that Pedipalpi and Uropygi are monophyletic, as widely accepted (e.g., Lozano-Fernandez et al., 2019; Noah et al., 2020), two alternative scenarios may be conceived for the evolution of the cerotegument in Pedipalpi. (1) The epicuticular secretion coat of Thelyphonida and the cerotegument of Amblypygi are not homologous, but rather independent derivations, perhaps from a common precursor, for example, a fluid secretion coat that evolved in a common ancestor of Pedipalpi. (2) Solid secretion crusts evolved in a common ancestor of Pedipalpi but were secondarily lost in Schizomida. If the primary function of the epicuticular secretion coat was camouflage, as suggested below, it would not be required by Schizomida, which are smaller, often humicolous and less exposed to visual predators. Alternatively, loss of the epicuticular secretion coat in Schizomida could be related to the small size of these animals (compared to Amblypygi and Thelyphonida) and have structural or physiological functions, such as a better gas exchange without epicuticular secretion coat.

The more regular structure of the cement coat of the thelyphonid, *T. dalyi*, is interpreted to be analogous, but not homologous, to the cerotegument of amblypygids. However, the globular structures observed in *T. dalyi* are approximately five times the size of those observed in Amblypygi (~10 vs. 2 μm in diameter, respectively). Whether this is also correlated with enhanced fluid repellence, as in Amblypygi, remains to be confirmed.

The slit-like gland openings are unique structures not yet observed in other arachnids. It can only be speculated as to whether their function is similar across Pedipalpi. In Amblypygi, these glands form a valve-like apparatus that opens after ecdysis to exude a colloidal secretion that provides the base material for the assembling microstructures (Wolff, Schwaha, & Seiter, 2016). Histological studies of the integument of Uropygi may clarify whether the obvious difference in secretion properties is mirrored by differences in the glandular apparatus. The rosette-like structures observed in the two exemplar Schizomida were previously described in other schizomid species (Pinto-da-Rocha et al., 2016; Santos & Pinto-da-Rocha, 2009) and resemble related structures in some Amblypygi interpreted as homologous to the slit-like openings due to the presence of intermediate forms (Wolff et al., 2017). Histological data are needed to test this hypothesis, however.

At present, nothing is known about the function of the epicuticular secretion coats of Uropygi. The amorphous crust observed in Thelyphonida may assist in camouflage, for example, by reducing reflectivity of the smooth cuticle and incorporating particles from the substratum, as in some harvestmen and mites, in which the epicuticular secretion coat is enriched with soil particles (Porto & Pérez-González, 2020; Schwangart, 1907; Wolff, García-Hernández, & Gorb, 2016; Wolff & Gorb, 2016). The attachment of foreign particles was observed in some thelyphonids, but not to the same extent as in the other arachnid taxa mentioned. The thelyphonid epicuticular secretion coat could also enhance the cuticular armor against predators, parasites and microorganisms. It is unlikely that the epicuticular secretion coat could transform the exoskeleton into a superhydrophobic state, as suggested for the cerotegument of Amblypygi (Wolff et al., 2017; Wolff, Schwaha, & Seiter, 2016), for this would require the presence of hierarchical micro- and nanostructures (Guo et al., 2011). Interestingly, unlike in Amblypygi, there was no evidence of an epicuticular mycobiome in Thelyphonida, suggesting that the secretion of Amblypygi exhibits distinct properties that promote, or at least do not suppress, fungal growth on the epicuticle.

The data on cuticular surface structure presented here are consistent with the placement of Palpigradi outside of Pedipalpi. Characters shared by all Pedipalpi studied in the present investigation and previous studies (Wolff et al., 2017) include tile-like patterning of the cuticle, slit-like gland openings, and near absence of setae or microtrichia. The cuticle of Palpigradi does not exhibit tile-like patterning or slit-like gland openings, but dense arrays of microtrichia are present instead.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and that no interests of any person or organization are affected by the information presented in the present manuscript.

AUTHOR CONTRIBUTIONS

Michael Seiter: Conceptualization; formal analysis; investigation; project administration; visualization; writing-original draft; writing-review & editing. **Thomas Schwaha:** Formal analysis; investigation; methodology; visualization; writing-original draft; writing-review & editing. **Rodrigo L. Ferreira:** Investigation; resources; visualization; writing-review & editing. **Lorenzo Prendini:** Resources; formal analysis; writing-review & editing. **Jonas O. Wolff:** Conceptualization; formal analysis; investigation; methodology; writing-original draft; writing-review & editing.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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