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Research paper

Morphology and sensilla of the enigmatic Bhutan pine aphid *Pseudessigella brachychaeta* Hille Ris Lambers (Hemiptera: Aphididae) – A SEM study

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ABSTRACT

The aphid tribe Eulachnini is unusual in being both diverse in species number and specializing on conifer hosts. The Bhutan pine aphid *Pseudessigella brachychaeta* Hille Ris Lambers, 1966; the only known species of its genus and native of the Himalayan region, displays characteristics of both the Palearctic genus *Eulachnus* and the Nearctic *Essigella*. Species of all three genera, as well as eulachnine *Cinara* subgenus *Schizolachnus*, share the same ecological niche, feeding almost exclusively on the needles of true pines. To obtain a better understanding of this enigmatic aphid and its biological context within the Eulachnini, we conducted a scanning electron microscopy study of the fine morphology and sensilla of the live-bearing and egg-laying forms of the species. The general morphology most resembles that of species of the genus *Eulachnus* but in detail *Pseudessigella* also displays unique features. Many types of sensilla, including coeloconic, campaniform, placoid and trichoid sensilla occur at different locations on the antennae, mouthparts, and legs. The rhinariolum on the second antennal segment is described and illustrated for the first time in Lachninae and a second rhinariolum-like sensillum, hitherto unknown in aphids, is also described on the second antennal segment. We discuss the morphological features of *Pseudessigella* and the distribution of antennal and labial sensilla with respect of their possible taxonomic and functional significance.

1. Introduction

Hemipterans, characterized by their piercing-sucking mouthparts, constitute one of the most diverse and economically-important orders of insects (Weintraub and Beanland, 2006). Within this group, the Sternorrhyncha (aphids, psyllids, scale insects and white flies) play an extremely significant ecological and economic role as plant pests and virus vectors (Eastop, 1977; Ng and Perry, 2004; Hühnlein et al., 2016). Although Aphididae represent one of the best known and investigated hemipteran families, morphological analyses using SEM methods are still fragmentary and based on studies of the most characteristic features (Bromley et al., 1979; Harrington, 1985; Wieczorek et al., 2011; Ammar et al., 2013). Still unavailable for most aphid groups are descriptions of the distribution and grouping of the different types of sensilla on various body parts, including the appendages, the texture of the dorsal cuticle, the patterns of wax secretions, and other anatomical details useful for their classification.

At 403 species, the aphid subfamily Lachninae is second in size only to the Aphidinae (Favret, 2016). Its phylogeny is one of the most studied and well-resolved among aphids (Normark, 2000; Chen et al., 2016), yet several important questions remain, including regarding the high rate of speciation and the host- and geography-related relationships among the conifer-feeding tribe Eulachnini. Several eulachnine species are regarded as pests of pines, spruces, firs and other trees of forests and urban greenery (Carter and Maslen, 1982; Alford, 2012). Four aphid genera comprise the Eulachnini: *Cinara* Curtis, 1835; *Eulachnus* Del Guercio, 1909; *Essigella* Del Guercio, 1909 and *Pseudessigella* Hille Ris Lambers, 1966. These last three genera and including the *Cinara* subgenus *Schizolachnus* Mordvilko, 1909, feed almost exclusively along the needles of species of true pines (*Pinus* L., 1753). *Essigella*, *Eulachnus*, and *Pseudessigella* comprise a characteristic group of

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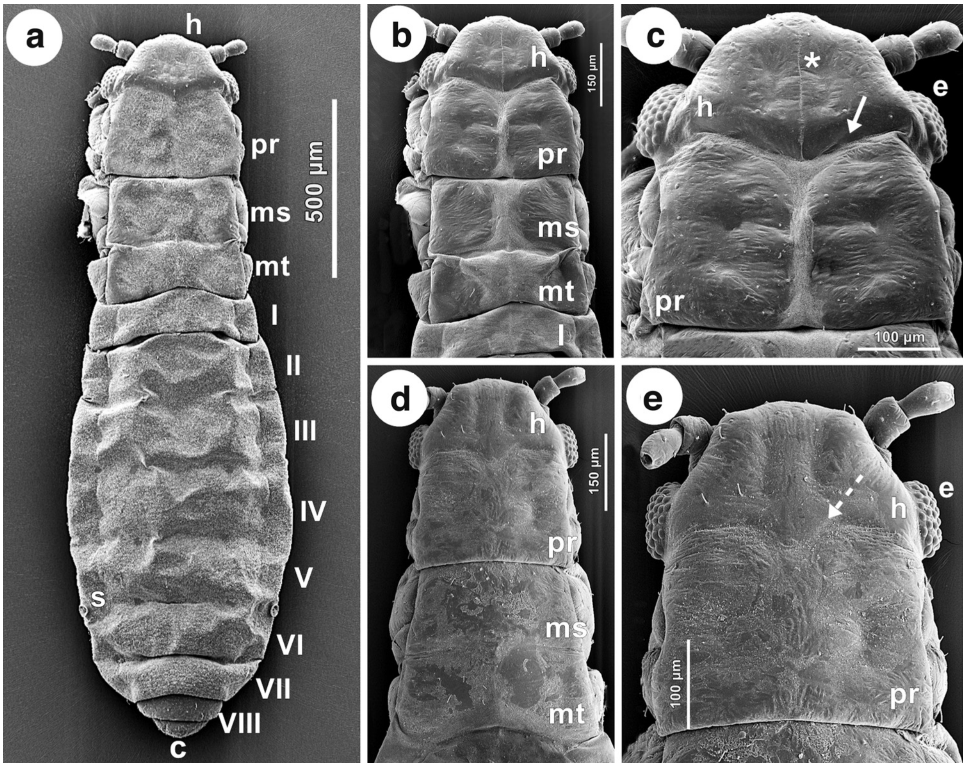

Fig. 1. Scanning electron micrographs showing the general view, head and thoracic characters of *P. brachychaeta*: (a) general morphology of apterous vivipara, h – head, pr – pronotum, ms – mesonotum, mt – metanotum, I–VIII – abdominal segments I–VIII, s – siphunculi, c – cauda; (b) head and thorax of the larva with well-developed sclerites on both sides of thoracic segments and still separated tagmata and segments; (c) sclerotized and separated head and pronotum of larva with membranous area between head and pronotum and pronotal sclerites (arrow) and visible epicranial suture (asterisk); (d) head and thorax of adult apterous vivipara with head fused with pronotum, separated from mesonotum which is itself fused with metanotum; (e) fused head and pronotum of apterous vivipara female with small suture between the tagmata (dotted arrow).

narrow-bodied aphids, sometimes collectively treated as the subtribe Eulachnina (Sorensen, 1990). Although *Eulachnus* and *Essigella* are relatively well-known and well-studied, *Pseudessigella* has been a missing element of every morphological, taxonomic, and phylogenetic study on Lachninae (e.g. Normark, 2000; Chen et al., 2016).

Pseudessigella brachychaeta Hille Ris Lambers, 1966; the only species of its genus, was described from Pakistan as having the antennae of *Essigella* but the claws and other body characteristics of *Eulachnus* (Hille Ris Lambers, 1966). Whereas Hille Ris Lambers (1966) described *Pseudessigella* as being closely related to *Eulachnus*, Sorensen (1987, 1991) emphasized its phylogenetic proximity to *Essigella*. The matter is in no way resolved. In order to better understand the evolutionary diversification of one of the most important aphid groups and in particular to compare three genera of aphids all found in the same ecological niche, we undertook a thorough scanning electron microscopy morphological study of *P. brachychaeta*. In addition to its general morphology, we paid special attention to the structure of the antennae and mouthparts and the sensilla found thereon.

2. Material and methods

2.1. Insect collecting

Adult apterous viviparae, oviparae, and larvae were collected in the Yousmarg region, the state of Jammu and Kashmir in India from *Pinus wallichiana* Jackson, 1938 in November 2015; Ali Akbar leg., Kanturski & Favret det. Voucher specimens are deposited at the Ouellet-Robert entomological collection, University of Montreal, Canada.

2.2. Scanning electron microscopy

Field collected individuals of *P. brachychaeta* were preserved in 70% ethanol for several days and prepared following the method described by Kanturski et al. (2015). The samples were transferred into a 6% phosphotungstic acid (PTA) solution in 70% ethanol for 24 h. Dehydration proceeded using serial baths of 80%, 90%, and 96% ethanol for 20 min each, and two baths of absolute ethanol for 30 min each. Dehydrated specimens were subsequently dried in a series of baths of a ratio of 1:3, 1:2; 2:3 hexamethyldisilazane (HMDS) and absolute alcohol for 30 min each, followed by two 30-min baths in undiluted HMDS. Samples were mounted on aluminum stubs with double-sided adhesive carbon tape and sputter-coated with gold in a Pelco SC-6 sputter coater (Ted Pella Inc., Redding, CA, USA) to obtain a layer approximately 25 nanometers thick. The samples were imaged with a Hitachi SU8010 field emission scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) at 5.0, 7.0 and 10.0 kV accelerating voltage with a secondary electron detector.

2.3. Terminology and abbreviations

ANT – antennae; ANT I–V – antennal segments I–V; BASE – basal part of the last antennal segment, PT – processus terminalis of the last antennal segment; ABD I–VIII – abdominal segments I–VIII; HT I – first segment of hind tarsus; HT II – second segment of hind tarsus. Sensilla terminology follows Bromley et al. (1979, 1980).

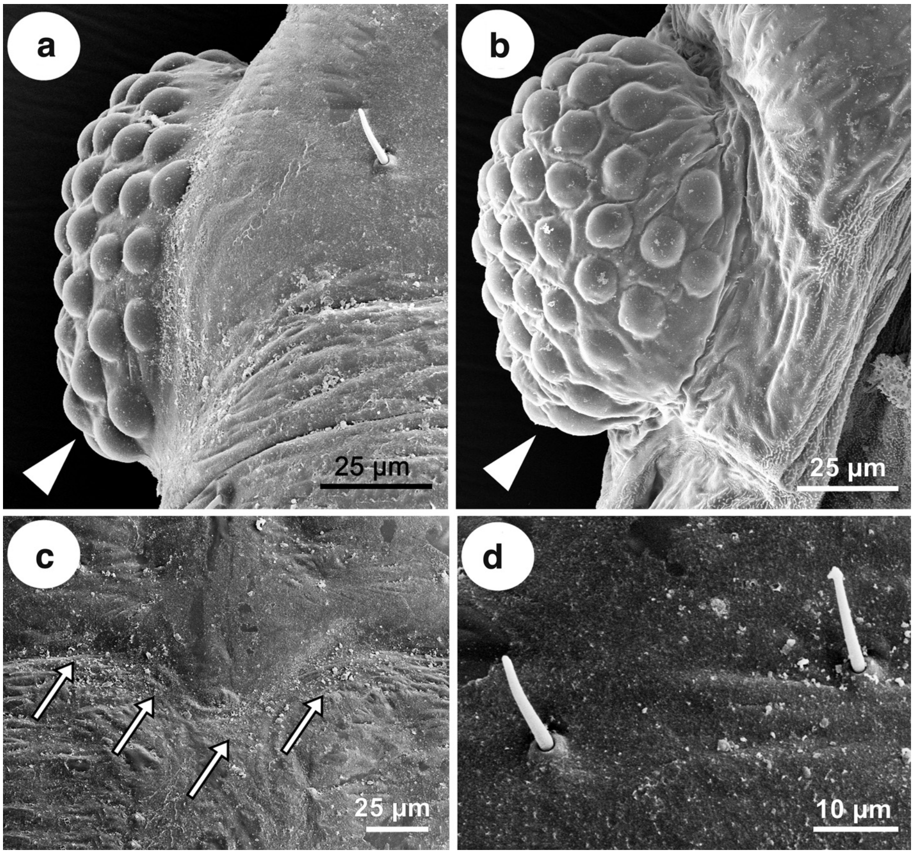

Fig. 2. SEM of the compound eye and head surface of *P. brachychaeta*: (a) dorsal, (b) ventral side of the compound eye without ocular tubercle; three ommatidia lying closer together indicate the vestigal triommatidium (white arrowheads); (c) surface and border between head and pronotum with suture between the tagmata (white arrows); (d) surface of cuticle and short and rigid mechanosensilla of head with blunt or with expanded apices.

3. Results

3.1. General morphology, head and thorax

Immature and adult forms of the genus *Pseudessigella* are characterized by a narrow body with certain fused segments. The head of adults is fused with the pronotum (Fig. 1a, d, e) with a visible suture in form of a shallow groove (Figs. 1 e, 2 c). The head is well-sclerotized with a rounded frons. The compound eyes are well developed but consist of a relatively small number (45–50) of clearly-separated ommatidia (size of one ommatidium10–12 µm). There are no ocular tubercles. The triommatidia are significantly reduced, visible only as a group of three ommatidia slightly larger than the others (each approximately 16 µm in diameter) (Fig. 2a, b). The head and thorax have a small number of setae, apparently functioning as mechanosensilla (Fig. 1d, e). The mechanosensilla on the head are short (18–20 µm) and rigid, either blunt or with expanded apices. The sockets are low with wider bases (Fig. 2d). The pronotum is one of the largest segments and well-separated from the mesonotum. The meso- and metanotum are fused, although a suture visibly separates them (Fig. 1a, d, e). The tergites of abdominal segments I and VIII are the only ones to be fully separated, unfused with neighboring segments. The other abdominal tergites (i.e., ABD II through ABD VII) are fully fused albeit visibly distinguishable (Fig. 1a). The juvenile stages are more heavily sclerotized, a large tergite covering the head and prothorax and another the meso- and metathorax. These four segments are more distinct in the larva than in the adult (Fig. 1b, c). A well-developed epicranial suture is visible in the larva, whereas the head capsule is completely smooth in the adult (Fig. 1c, e). The sclerotization of thoracic segments is faded in the adult morphs.

3.2. Antennae

The five antennal segments are rather short and carry only a few short and rigid, type I trichoid sensilla (4–9 µm) on ANT III–IV and V BASE. The scape and pedicel (ANT I and II) are more or less smooth and the flagellum is imbricated with many grooves and recesses (Fig. 3a, b). ANT IV is clearly narrower at the base than at the apex, with one medium-sized and more or less rounded multiporous placoid sensillum (primary rhinarium) protruding from the segment surface (Fig. 3c). ANT V is widest in the middle with a narrow base and an extremely short PT. This segment also bears a large, rounded primary sensorium in the form of a flat plate, as well as accessory rhinaria, in two groups, under and next to the primary sensorium (Fig. 3d). The PT bears five very short (5–7 µm), robust, rigid, and pointed type II trichoid sensilla, with well-developed hemispherical sockets: three apically and two subapically (Fig. 3e).

The antennal sensilla are developed into two general types: the aforementioned rhinaria on ANT IV and V, and a poorly-investigated sensillum present only on the pedicel (ANT II), known as the rhinariolum (Fig. 4a, b). The rhinariolum is situated on the ventral side of the segment and in *Pseudessigella* is characterized by a relatively big aperture (2.50-3.00 µm) with a well-developed, ring-like edge (Fig. 4a). From the aperture protrudes a sunken, tubular, coeloconic sensillum (0.80-1.00 µm) with 10–12 circular projections (Fig. 4b). A second type of sensillum, situated on the dorsal side of the segment near the campaniform sensillum, differs from the rhinariolum by its extremely small size (Fig. 4c, d). The

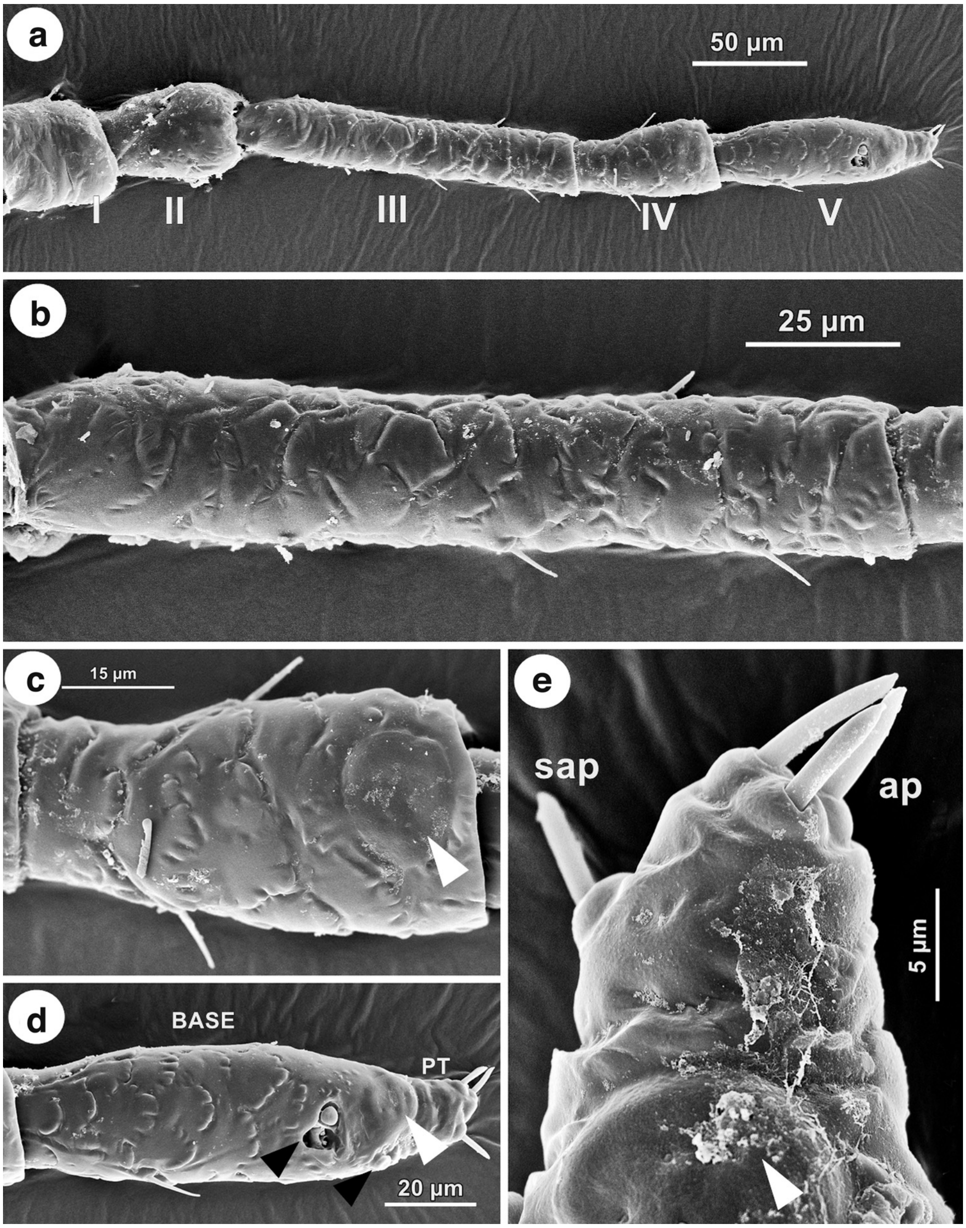

Fig. 3. SEM of antenna of *P. brachychaeta*: (a) antenna general view, I–V – antennal segments I–V; (b) imbricated ANT III with few, short, rigid, type I trichoid sensilla; (c) ANT IV with rounded and flat multiporous placoid sensillum (primary rhinarium) (white arrowhead); (d) ANT V with two kinds of primary rhinaria: one big and rounded multiporous placoid sensillum (major rhinarium) (white arrowhead) and accessory rhinaria (black arrowheads); (e) distal end of BASE with the major rhinarium (white arrowhead) and PT of ANT V, ap – three type II trichoid sensilla (apical setae), sap – one of the two type II trichoid sensilla (subapical setae).

aperture is much smaller (0.90-1.100 µm) than in the rhinariolum, surrounded by a flat border. This new sensillum seems to contain 5–6 flat lobate outgrowths (Fig. 4d).

The primary rhinaria form three morphologically different groups: 1) big, multiporous placoid sensilla – single, rounded, flat plates on ANT IV and V (on ANT V it is called the major rhinarium), diameter approximately 30–40 µm (Fig. 3c, d; 4 g); 2) two small multiporous placoid sensilla (accessory rhinaria) – mushroom-shaped and with a diameter approximately 4–5 µm (Fig. 4g, i, j); 3) four sunken coeloconic sensilla (accessory rhinaria) – diameter approximately 0.75-1.00 µm (Fig. 4g, j, k). The surface of the placoid sensillum is flat, grained and densely covered by small pores, about 10–15 per 1 μm^2. The surface of the big multiporous placoid sensillum is flat with 4–6 small but distinct cavities with more or less globular structures (Fig. 4e, g, h). The globular structure is sunken into the plate surface, distinct, grainy, and not porous (Fig. 4f). The accessory rhinaria are divided into two different types of sensilla: a single mushroom-shaped, small, multiporous placoid sensillum near the major rhinarium, and a group of sensilla under the major rhinarium – the second mushroom-shaped small multiporous placoid sensillum with a group of sunken coeloconic sensilla (Fig. 4g). Both small placoid sensilla are characterized by a porous surface with a central shallow recess. One of the small placoid sensilla (near the major rhinarium) is situated on a flat and exposed surface (Fig. 4i). The second small placoid sensillum together with its associated coeloconic sensilla, lies in a deep cavity with well-developed septa – one between the placoid sensillum and the group of coleoconic sensilla, the second dividing the four coeloconic sensilla into two groups (Fig. 4j). The coeloconic sensilla are sunken pegs with 8–10 finger-like projections (microtrichia) (Fig. 4k).

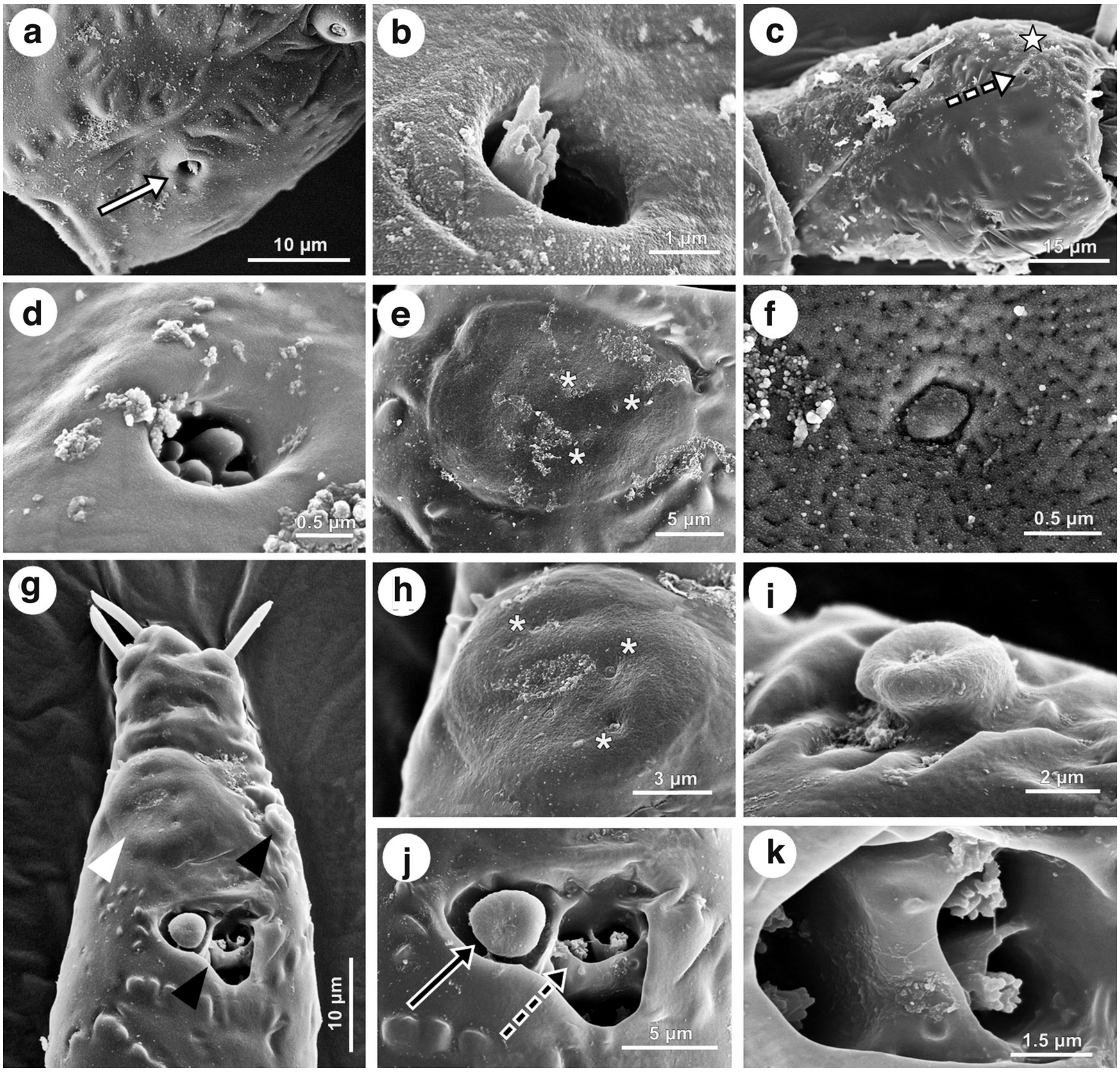

Fig. 4. SEM structure of antennal sensilla of *P. brachychaeta*: (a) ventral side of ANT II with the single rhinariolum; (b) structure of the rhinariolum; (c) dorsal side of ANT II with very small sensillum near the campaniform sensillum (white star) resembling the rhinariolum; (d) morphology of the sensillum resembling the rhinariolum; (e) general structure and surface of the placoid sensillum (primary rhinarium) on ANT IV with flat, porous plate with small recesses (white asterisks); (f) detailed structure of the surface of the placoid sensillum plate with the recess and globular structure; (g) location of the placoid sensillum (major rhinarium) (white arrowhead), small placoid and sunken coeloconic sensilla (accessory rhinaria) (black arrowheads) on ANT V; (h) surface of the placoid sensillum (major rhinarium) on ANT V with porous plate and small recesses with globular structures (white asterisks); (i) morphology of the one exposed small placoid sensillum (accessory rhinarium) lying near the major rhinarium with porous surface; (j) deep cavity under the major rhinarium with the second mushroom-shaped and small placoid sensillum (arrow) separated from four sunken coeloconic sensilla (dotted arrow); (k) structure of the four sunken coeloconic sensilla divided into two groups.

3.3. Mouthparts

The mouthparts (630–650 μm from the proximal part of clypeus to the distal part of R V) are like those of other aphids, consisting of the postclypeus, the anteclypeus, a short labrum, and the well-developed labium (rostrum). The labium of Lachninae consists of five segments (Fig. 5). The postclypeus bears three fine and pointed type I trichoid sensilla, 26–33 μm long. There is also one long, fine and pointed type I trichoid sensillum on the anteclypeus. The labium is tubular in shape. The first segment is short, 66–76 μm, membranous, largely covered by the anteclypeus and labrum and is without any sensilla. The second segment is the longest (220–246 μm), with a visible labial groove (Fig. 5a, b) and approximately 15–16 type II trichoid sensilla. The sensilla are short (6–8 μm), fine or slightly rigid and pointed, with well-developed hemispherical sockets (Fig. 6a, b). They generally are distributed symmetrically on each side of the labial groove (Fig. 5b). The third segment is similar in length as the fourth (62–65 μm). Two pairs of short, rather rigid and pointed type II trichoid sensilla occur on this segment: one pair (3–4 μm) is positioned on each side of the labial groove, the other two (6–8 μm) are placed marginally (Fig. 5c). The distal end of the rostrum is short and blunt, divided into an almost square-shaped fourth segment (40–45 μm) and a very short (15–19 μm) and broadly conical fifth segment. The fourth segment bears four pairs of two kinds of sensilla: one pair of short and delicate type II basiconic sensilla (6–7 μm) on the proximal part (called basal setae) and three pairs of type I trichoid sensilla (known as primary setae) on the distal part of the segment. The sensilla of this segment have slightly flattened sockets and are longer (12–17 μm) than those on segment II (Fig. 6c, d). On the fourth segment there are no trichoid sensilla adjacent to the labial groove (Fig. 5d). The fifth segment is the shortest and bears type III basiconic sensilla on the tip (Fig. 7a). This segment forms an opening for the protrusion of the stylet bundle; the sides of the opening bear seven pairs of type III basiconic sensilla, arranged symmetrically. These sensilla are short (3–8 μm) stout pegs, inserted in a conical socket (Fig. 7b).

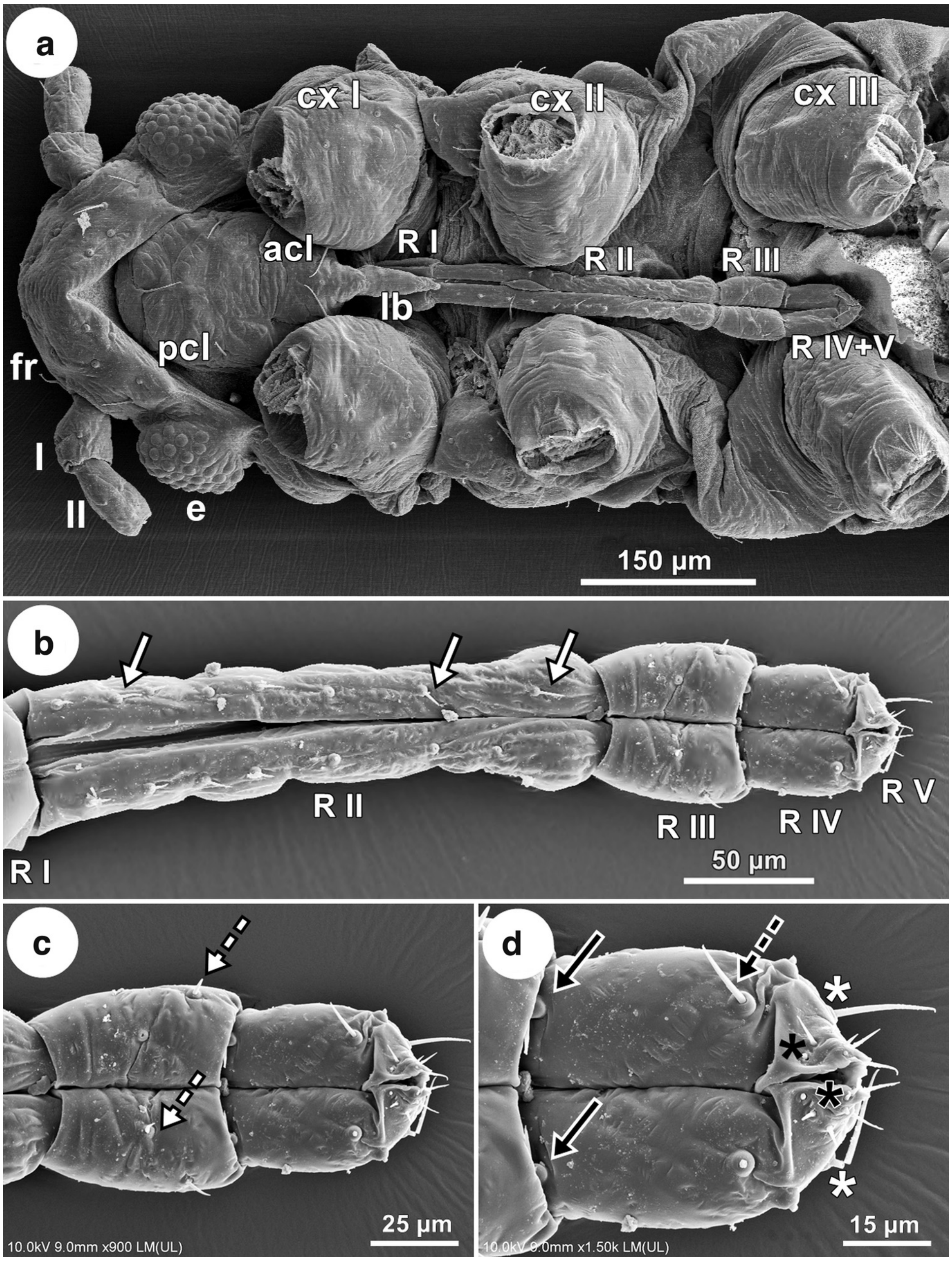

Fig. 5. SEM of mouthparts of *P. brachychaeta*: (a) ventral side of head and thorax, fr – frons, I, II – ANT I and II, e – eye, cx I–III coxae I–III, pcl – postclypeus, acl – anteclypeus, lb – labrum, RI-V – rostral segments I-V; (b) general structure of labium and distribution of the trichoid sensilla on the second segment (white arrows), RI-V – rostral segments I-V; (c) location of the trichoid sensilla (dotted arrows) on third rostral segment; (d) ultimate rostral segments (R IV + V) with the type II basiconic sensilla (black arrows) hidden by third segment, type II trichoid sensilla (white asterisks) and type III basiconic sensilla on the tip (black asterisks).

3.4. Legs

The legs of *Pseudessigella* are characterized by thick and short fore femora (Fig. 8a) and typical lachnine, relatively slender, mid and hind femora. The type I trichoid sensilla on the legs are relatively few, rather short (10–32 μm), rigid, with capitate apices on the dorsal side and pointed apices on the ventral side (Fig. 8b, 9 g, h, i). Small groups (2–4) of campaniform sensilla are present on the ventral aspect of the trochanters and proximal areas of the femora (Fig. 9a, d). These campaniform sensilla are oval or rounded (8–12 μm), rather flat with gentle edges (Fig. 9b, e). The central plate of the sensillum is flat and bears a minute pore near one edge (Fig. 9c). The tibiae, especially the hind tibiae, are imbricated. In oviparae, the hind tibiae also bear many pseudosensoria, covering almost the entire length, mostly from the ventral side (Fig. 8c). The pseudosensoria can be classified in three general types: (1) big (26–28 μm) and protuberant, (2) medium (15–17 μm) and 8-shaped, and (3) small (7–8 μm), sunken, and rounded or mushroom-shaped (Fig. 8f, g). The surface of the pseudosensorium is densely covered by small pores (30–37 per μm^2) (Fig. 8e) but no secretion was observed. The tarsi, as in all Aphididae, are composed of two tarsomeres and, as in most Lachninae, they are elongated (Fig. 8h). HT I (basitarsus) bears five setae ventrally (Fig. 8i). HT II (distitarsus) bears one small and rounded campaniform sensillum basally on the dorsal side (Fig. 9f). The pretarsus is normal, bearing two claws with single, curved and pointed apices, and very short (7–8 μm) and pointed parempodia (empodial setae) (Fig. 8j).

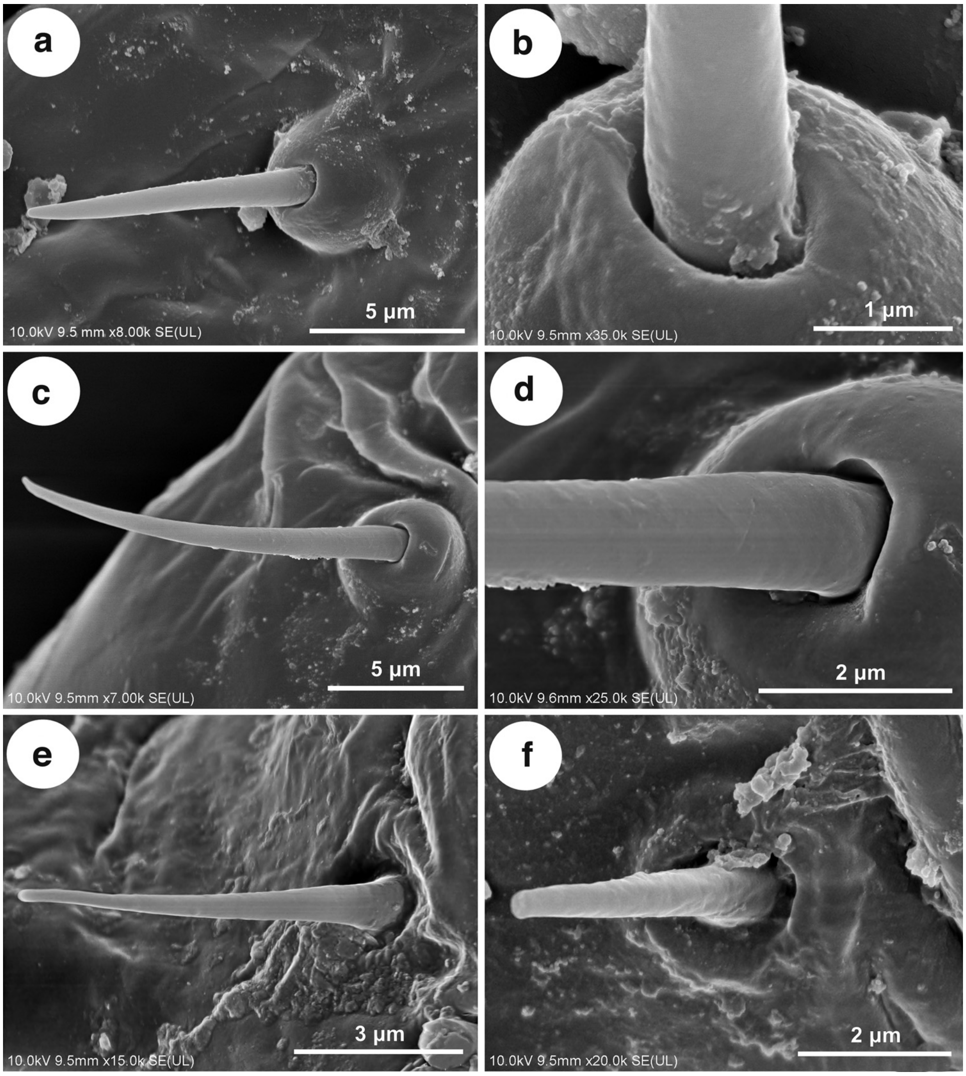

Fig. 6. SEM of mouthpart sensilla of *P. brachychaeta*: (a) structure of the type I trichoid sensillum of the second segment; (b) socket of the type I trichoid sensillum of the second segment; (c) structure of the type I trichoid sensillum of the third segment; (d) socket of the type I trichoid sensillum of the second segment; (e, f) structure and sockets on the type III basiconic sensilla on the tip of the last segment.

3.5. *Abdomen*

The abdominal cuticle is relatively delicate on most segments but strongly sclerotized on ABD VIII. The cauda is very short and semicircular, similar in size to the anal plate (Fig. 10a). Both ABD VIII and the cauda are covered by minute spinules (Fig. 10d). The short siphunculi are situated between ABD V and VI, devoid of reticulation, and bear a well-developed, smooth flange (Fig. 10b). The siphuncular sclerite is very small. The genital plate is also more or less sclerotized, smaller and less sclerotized in the apterous vivipara (Fig. 10c), bigger and heavily sclerotized in the ovipara (Fig. 10e). The dorsal abdominal surface is covered in reticulation composed of polygonal cells separated by electron-darker lines (Fig. 11a, b). At higher magnification, the polygons are seen to be filled with sclerotic, ridge-like reinforcing (Fig. 11c), whereas the lines demarcating the polygons are where the sclerotization is reduced (Fig. 11b). The dorsal surface of ABD VI and VII additionally bear minute spines on the lines separating the polygons (Fig. 11d, e). Dorsal type I trichoid sensilla are few, short (9–23 μm), rigid, and capitate or capito-flabellate. The setae are characterized by a smooth surface and small sclerotized, wart-like bases (Fig. 11f, g). Each abdominal segment bears from one to three intersegmental muscle attachment plates (17–30 μm), especially on the pleural and marginal surface (Fig. 11h). Every one of these plates consists of five to eight subunits (9–14 μm) (Fig. 11i).

4. Discussion

Hemiptera SEM studies have focused on true bugs (Brożek and Zettel, 2014), plant hoppers (Brożek and Bourgoin, 2013) and moss bugs (Spangenberg et al., 2013). Due to their economic importance, Sternorrhyncha have also been the focus of attention, for example with psyllids (Drohojowska et al., 2013), whiteflies (Dubey and Ramamurthy, 2013; Franielczyk-Pietyra and Wegierek, 2016) and scale insects (Sirisena et al., 2015). Detailed morphological SEM studies of aphids have been few, with most work focusing on antennal sensilla (Shambaugh et al., 1978; Bromley et al., 1979, 1980) and mouthparts (Forbes, 1969; Adams and Fyfe, 1970; Brożek et al., 2015; Ge et al., 2016). Recently, authors expanded the field with SEM analyses to complement research on aphid reproduction (Wieczorek et al., 2011, 2012), systematics (Wieczorek et al., 2015) and evolution (Depa et al., 2015). Aphid morphology has also been elucidated more generally (Yang et al., 2009; Brożek et al., 2015). Here, we sought to broaden the general knowledge of the morphol-

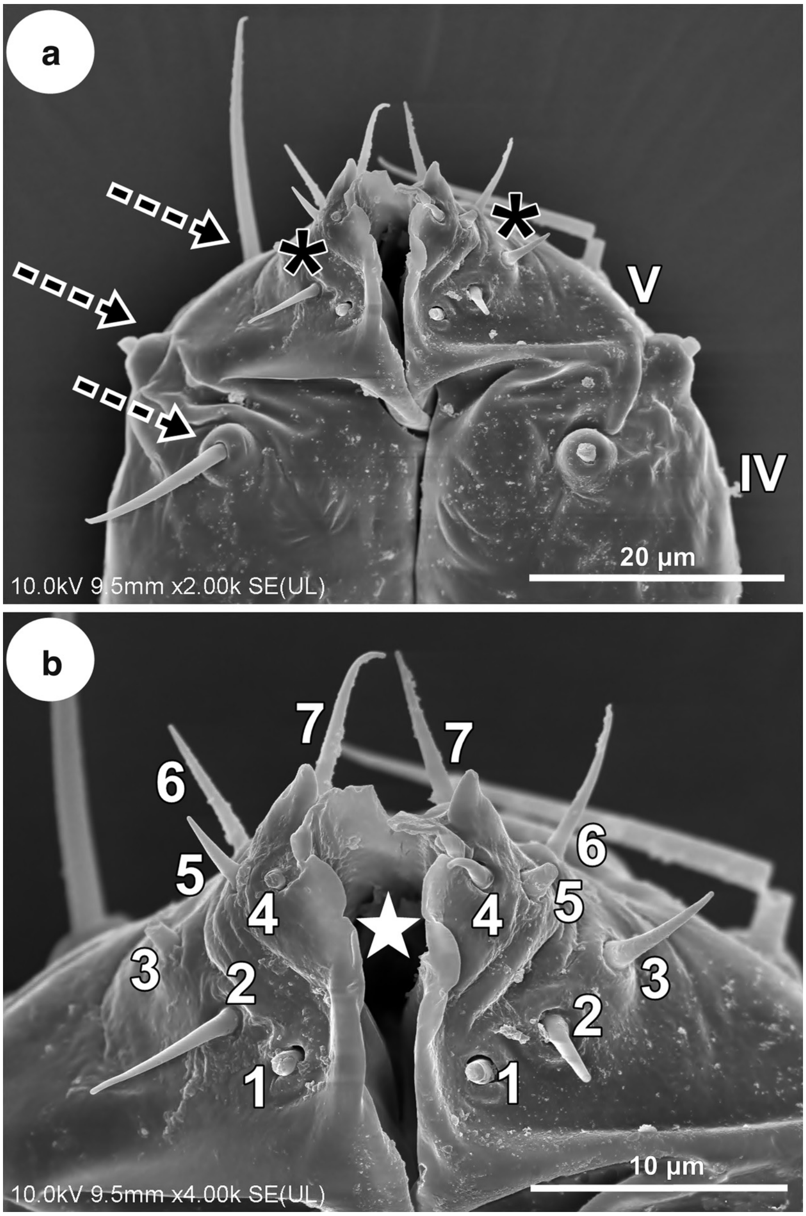

Fig. 7. SEM of the last rostral segments of *P. brachychaeta*: (a) three pairs of type I trichoid sensilla (primary setae) (dotted arrows) and type III basiconic sensilla on the tip (black asterisks), IV, V – fourth and fifth rostral segments; (b) anterior view of the fifth rostral segment showing seven pairs of type III basiconic sensilla around the stylet opening (white star).

ogy and sensory systems of aphids, in particular of an enigmatic conifer-feeding aphid, and possible missing link between Palearctic and Neartic groups.

Having only been collected once before in 1964 (Hille Ris Lambers, 1966), ours is the first SEM description of the general morphology and sensilla of *Pseudessigella brachychaeta*, a representative of the tribe Eulachnini and the subtribe Eulachnina (Sorensen, 1990). The morphology of *Pseudessigella* generally resembles that of the genus *Eulachnus* (Kanturski et al., 2015). Its body is relatively small and spindle-shaped as in all Eulachnina species. In contrast with many *Eulachnus* species, however, *Pseudessigella* is characterized by having short, five-segmented antennae and very short trichoid sensilla (setae on antennae, dorsal side of body and legs). Of the trichoid sensilla of Aphididae, the type I are the most variable between species, depending on the aphids systematic position, ecological niche and behavior. Sensilla in *P. brachychaeta* have a different size and the shape of their apices vary according to their location on the body. The type II trichoid sensilla are as in other Aphididae, rather short, robust and more or less pointed (Shambaugh et al., 1978), but lacking pores, as in *Myzus persicae* (Sulzer, 1776) (Ban et al., 2015). The other sensilla on the antennal flagellum are similar to those of other aphids. The multiporous placoid sensilla (primary rhinaria) are the largest and are present on ANT IV and V. In contrast with most aphid species, in *Pseudessigella* these sensilla are not embedded in a cavity but instead are exposed and without flange. A similar situation was observed on ANT VI of another Lachninae species, *Tuberolachnus salignus* (Gmelin, 1790) (Shambaugh et al., 1978). In that species, another type of long, fine and pointed type I trichoid sensillum was observed, as well as another arrangement of the accessory rhinaria in relation to the major rhinarium. Notably, in *P. brachychaeta*, the accessory rhinaria of the last ANT segment form two discrete groups. One of the small placoid sensilla is separated from the second small placoid sensillum and the coeloconic sensilla, these

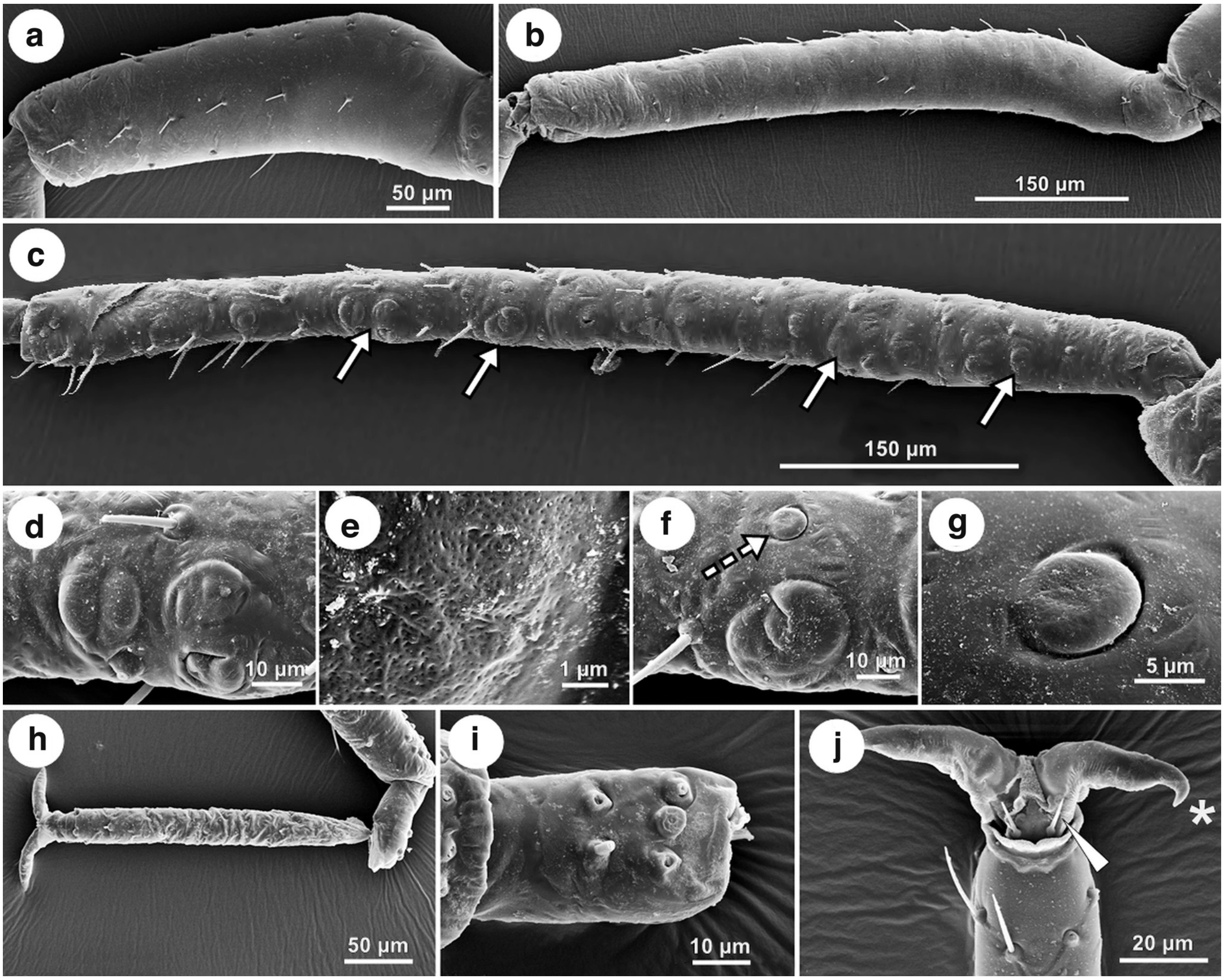

Fig. 8. SEM of legs and pseudosensoria of *P. brachychaeta*: (a) strongly swollen fore femur; (b) normal shaped, slightly elongated hind femur; (c) hind tibiae of ovipara with pseudosensoria (white arrows); (d) different shapes of pseudosensoria; (e) porous surface of the pseudosensoria; (f) two different pseudosensoria with small, mushroom-shaped pseudosensorium (dotted arrow); (g) slightly porous structure of the mushroom-shaped pseudosensorium; (h) end of tibia and tarsus of hind leg (setae broken); (i) ventral side of HT I with bases of five broken ventral setae; (**j**) distal part of HT II with short and pointed parempodia (arrowhead) and claws with single, pointed apices (white asterisk).

Table 1
Morphometric data for various sensilla of representatives of *P. brachychaeta*.

General function	Sensilla type	Distribution	Diameter (rhinaria) or length (setae)
Chemoreceptors and higroreceptors	Big placoid sensilla	Antennal segment IV and V	30–40 µm
	Small placoid sensilla	Antennal segment V	4–5 µm
	Coleoconic sensilla	Antennal segment V	0.75-1.00 µm
	Rhinariolum	Antennal segment II (ventral side)	2.50-3.00 µm
	Rhinariolum-like sensillum	Antennal segment II (dorsal side)	0.90-1.100 µm
Mechanoreceptors	Trichoid sensilla – type I	Antennal segments I–V BASE	4–9 µm
		Legs	10–32 µm
		Dorsal side of body	9–23 µm
	Trichoid sensilla – type II	Antennal segment V PT	5–7 µm
		Second segment of labium	6–8 µm
		Third segment of labium	3–8 µm
	Basiconic sensilla – type II	Fourth segment of labium	6–7 µm
	Basiconic sensilla – type III	Fifth segment of labium	3–8 µm
	Campaniform sensilla	Trochantera, femora and HT II	8–12 µm

latter being located under the primary rhinarium. This same small placoid sensillum, like the big placoid sensillum, is exposed, lacking any cavity or flange. This distribution of accessory rhinaria is unlike that of other aphids thus-far studied, where all the sensilla are often aggregated with the major rhinarium (Shambaugh et al., 1978; Bromley et al., 1979; Sun et al., 2013). In the related genus, *Eulachnus* (i.e., *E. rileyi* (Williams, 1911)), the accessory rhinaria lie across the segment under a more or less exposed major rhinarium. Also many coeloconic sensilla are found between the placoid sensilla in *Eulachnus* (Kanturski, unpublished data). The rhinariola are the least studied of the aphid antennal sensilla. Krzywiec (1968) reported for the first time the presence of these structures

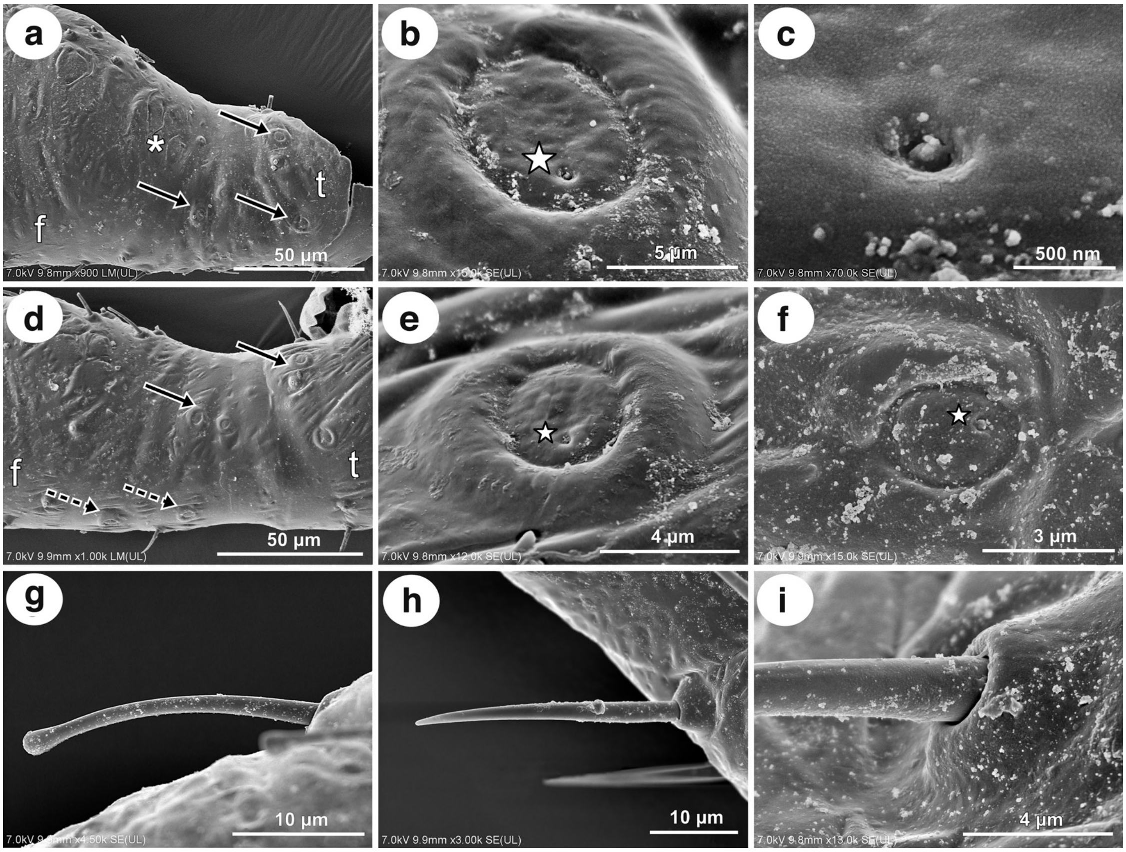

Fig. 9. SEM of campaniform and trichoid sensilla on the legs of *P. brachychaeta*: (a) location of campaniform sensilla on fused fore trochanter and femur with rufflings of the cuticle on femur (white asterisk), t – trochanter, f – femur; (b) structure of the campaniform sensillum on fore leg with small pore near the edge (white star); (c) grainy surface and pore of the campaniform sensillum; (d) oval (black arrows) and circular (dotted arrows) campaniform sensillum on fused hind trochanter and femur, t – trochanter, f – femur; (e) structure of the campaniform sensillum on hind leg with small pore near the edge (white star); (f) structure of the campaniform sensillum on second segment of hind leg with small pore near the edge (white star); (g) trichoid sensillum with capitate apex on the dorsal side of femur and tibia; (h) trichoid sensilla with pointed apices on the inner side of femur and tibia; (**i**) socket of trichoid sensillum of legs.

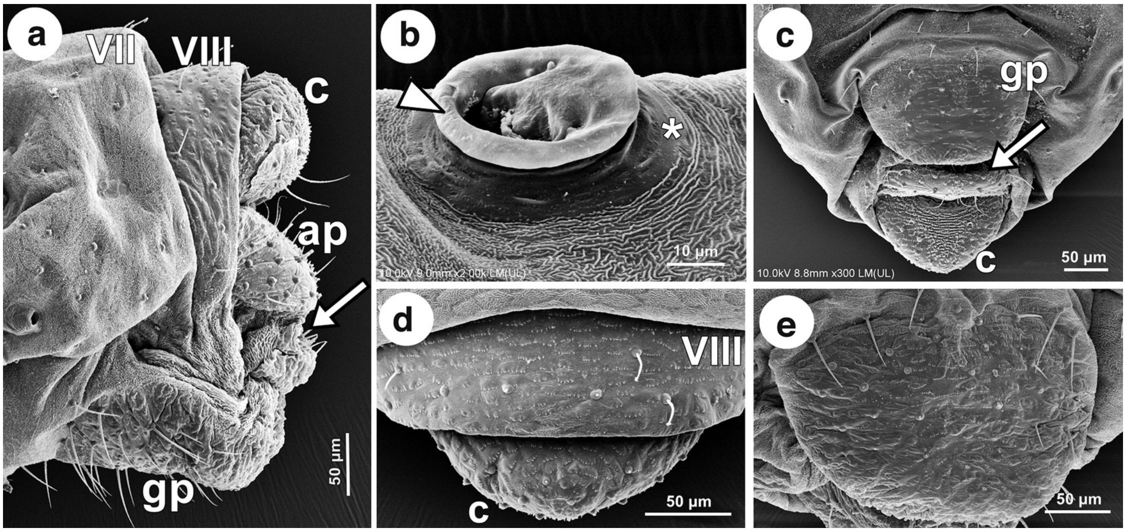

Fig. 10. SEM of abdominal structures of *P. brachychaeta*: (a) lateral view of abdomen showing the membranous seventh segment (VII), sclerotized eighth segment (VIII), short, semicircural cauda (c), anal plate (ap), rudimentary gonapophyses (white arrow), and genital plate (gp); (b) small, almost poriform siphunculus lying on very narrow siphuncular sclerite without setae (white asterisk) and with developed flange (white arrowhead); (c) ventral side of the end of abdomen of apterous vivipara showing the structure of genital plate and hidden rudimentary gonapophyses (white arrow); (d) dorsal side of the sclerotized eighth segment slightly covering the short, semicircular cauda; (e) structure of strongly sclerotized and more setose genital plate of the ovipara (some setae are broken but the sockets are visible).

in 24 species of Aphididae with simple, provisional drawings, and suggesting that they may have an olfactory function. Although Krzywiec (1968) named them rhinariellae (singular rhinariella) and other authors followed hers lead, the diminutive form of the Latin-derived rhinarium should be rhinariolum. Shambaugh et al. (1978) indicated "possible openings to rhinariellae" on ANT II of *Myzus per-*

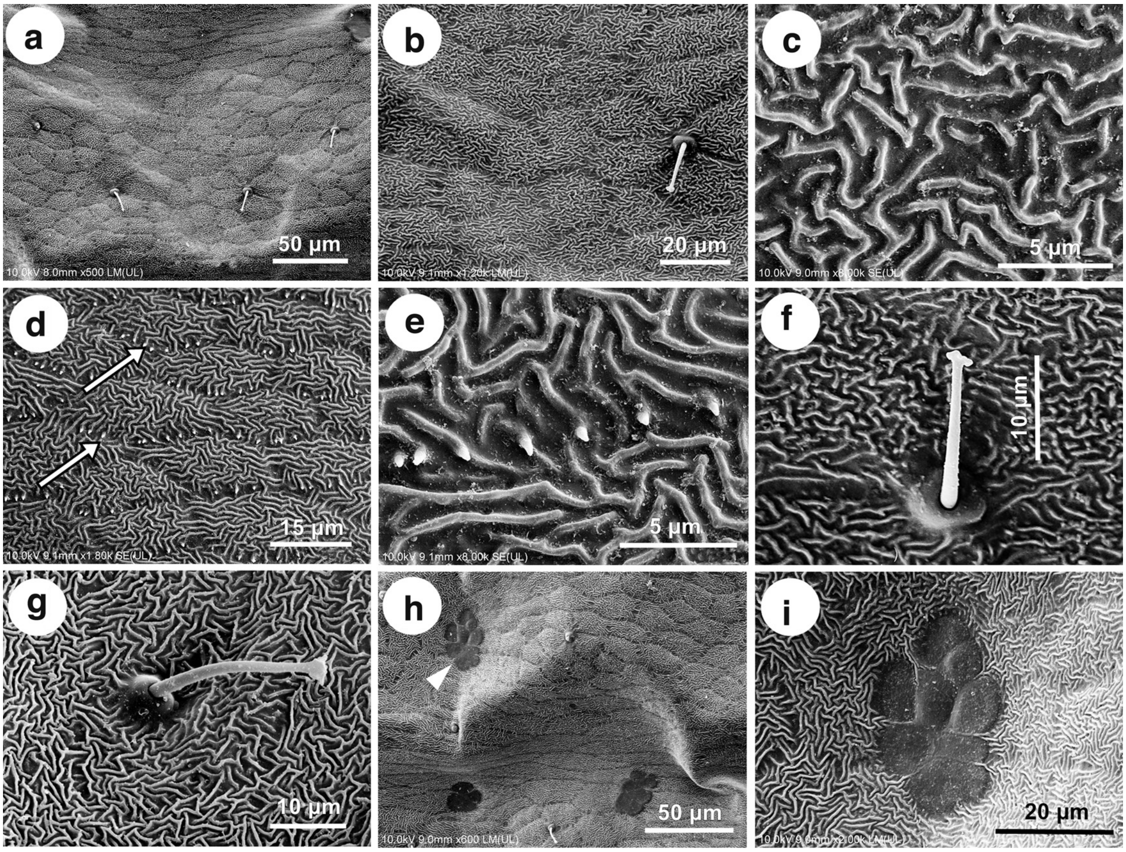

Fig. 11. SEM of the structure of the abdominal cuticle of *P. brachychaeta*: (a) dorsal side of abdominal tergites I–V cuticle showing reticulation with few trichoid sensilla; (b) structure of the reticulation forming folded polygons; (c) structure of the reinforcement of the cuticle in form of different sized ridges; (d) folded structure of polygons on abdominal tergites VI and VII with short spinules on the borders between the polygons; (e) structure of the spinules on abdominal tergites VI and VII; (f, g) trichoid sensilla on the dorsal side of abdominal segments with expanded apices; (h) dorsal side of abdominal segments with intersegmental muscle attachment plates (white arrowhead); (i) structure of the intersegmental muscle attachment plate.

sicae, *Chaitophorus populicola* Thomas, 1878 and *Therioaphis riehmi* (Börner, 1949). Bromley et al. (1979) showed that rhinariola are also present in *Nasonovia ribisnigri* (Mosley, 1841), although they called them coeloconic sensilla. Both of these studies showed only deep sunken pegs within cavities, whereas the rhinariolum of *P. brachychaeta* is characterized by a protruding peg, similar to *Pemphigus bursarius* (Linnaeus, 1758), where the structure is paired and with short pegs in a broad cavity (Dunn, 1978) (Table 1). We also found a previously unknown sensillum on ANT II. In contrast with the typical rhinariolum, this new "rhinariolium-like" sensillum is located on the dorsal (or outer) part of the pedicel, is much smaller, and has a differently-shaped stem. Because the morphology and the location of this sensillum are different from those of the rhinariolum, it likely has a different function. We will seek to better characterize this new sensillum in the future (Table 2).

The mouthparts of *P. brachychaeta* generally resemble those described in other Aphididae (Brożek et al., 2015; Ge et al., 2016) and Sternorrhyncha (Brożek, 2006; Garzo et al., 2012). The most distinctive structure is the labium, four-segmented in most Aphididae (Razaq et al., 2000; Ge et al., 2016) but five-segmented in Lachninae (Blackman and Eastop, 1994); the fourth and fifth segments often are collectively referred to as the ultimate rostral segment(s), or URS. Aphid mouthparts are often similar based on feeding behavior (Bradley, 1961; Heie, 1980) and the URS of *P. brachychaeta* is short and blunt with a very short apical tip, very similar to that of species in the genus *Eulachnus* (Kanturski et al., 2015), with which it shares a similar feeding site. The type and location of the labial trichoid and basiconic sensilla of *P. brachychaeta*, especially those on the second and third rostral segments, are similar to those of species from the genera *Eulachnus* (Kanturski, unpublished data), *Stomaphis* Walker, 1870 (Lachninae) (Brożek et al., 2015), *Eriosoma lanigerum* (Hausmann, 1802) (Eriosomatinae) (Ge at al., 2016) and *Myzus persicae* (Aphidinae) (Forbes, 1969). As seen in the European species of *Eulachnus*, but in contrast with other aphids, including other lachnines, the fourth labial segment of *P. brachychaeta* is devoid of trichoid sensilla (accessory setae) near the labial groove. There are also fewer basiconic sensilla on the tip of the last segment, a feature also seen *E. lanigerum* (Ge et al., 2016).

The legs of *P. brachychaeta* have a reduced number of trichoid sensilla (setae) on the femora and tibiae, these sensilla being short, rigid and capitate dorsally, and slightly longer and acuminate ventrally. The type, size and shape of the trichoid sensilla on the legs are similar across the aphids developmental stages and morphs (larva, vivipara and ovipara), with the hind tibiae of the sexual oviparae also bearing scent plaques (pseudosensoria). These structures are present in the oviparae of almost all aphids (Harrington, 1985). The species of some lachnine genera lack scent plaques, e.g., *Stomaphis* (Depa et al., 2015) and *Pterochloroides* Mordvilko, 1914 (Wieczorek et al., 2013). In comparison with the other species of Eulachnini, the hind tibiae of *P. brachychaeta* bear relatively few pseudosensoria. Species of the genus *Cinara* are known to have numerous (ca. 100–200) very small and rounded pseudosensoria (Harrington, 1985); less numerous but also abundant are the scent plaques in species of the genus *Eulachnus*. In *Pseudessigella*, the shape and size of these structures seems to be completely different than in *Eulachnus*, where the pseudosensoria are small and very flat, limited to small, rounded, porous fields with grainy secretions (Kanturski et al., 2015). The pseudosensoria of *P. bracychaeta* are

Table 2
Comparison of main features of antennal sensilla of *P. brachychaeta* and other species of Lachninae and Aphididae.

Species	Placoid sensilla in cavity	Small placoid sensilla in cavity	Sunken coleoconic sensilla	Edges of sensilla	Trichoid sensilla on ANT VI	Rhinariola	References
Pseudessigella brachychaeta	–	+/–	4	Smooth	A few short, rigid and blunt	Single with protruding peg	This study
Eulachnus rileyi	–	+	6–7	Smooth	Not numerous long fine and pointed	Not studied	Kanturski & Favret (unpublished data)
Tuberolachnus salignus	–	+	2–3	Smooth	Numerous, long fine and pointed	Not studied	Shambaugh et al. (1978)
Lachnus roboris	–	+	4	Smooth	Numerous, long, fine and pointed	Not studied	Kanturski & Favret (unpublished data)
Pemphigus bursarius	–	Not visible	1–2	Ciliated	Short, fine and pointed	Double with little protruding pegs	Bromley et al. (1979), Dunn (1978)
Aphis pomi	+	+	4	Ciliated	Short and blunt	Not studied	Bromley et al. (1979)
Acyrthosiphon pisum	+	+	3	Ciliated	Short, rigid and capitate	Not studied	Shambaugh et al. (1978)
Macrosiphum euphorbiae	+	+	4	Ciliated	Short and blunt	Not studied	Bromley et al. (1979), Dunn (1978)
Nasonovia ribisnigri	+	+	2–3	totally enclosed bycuticular fringe	Short and blunt	Single without protruding peg	Bromley et al. (1979)

among the largest in Lachninae and other Aphididae (Harrington, 1985). The legs, especially the trochantero-femoral areas also bear many campaniform sensilla with very small, single pores near their edges. Smaller, single campaniform sensilla are also present on the basal-dorsal side of the distitarsi.

The abdomen shows the typical morphology of Lachninae and Aphididae. Based on light microscopy studies, the abdominal cuticle of *P. brachychaeta* was known to be membranous, but other abdominal features were less discernable. The SEM work presented here has shown that the abdominal dorsum of *Pseudessigella* is densely pleated and wrinkled, bearing a reticulated pattern. Given its texture, it is probable that the dorsal cuticle of *P. brachychaeta* is strengthened, as in species of *Eulachnus* (Kanturski et al., 2015), yet without being covered by sclerites like some species of *Essigella* (Sorensen, 1994); abdominal sclerites occur in *P. brachychaeta* only on the eighth segment, cauda, and genital and anal plates. The siphunculi of *P. brachychaeta* are similar to those of *Eulachnus* and *Essigella*, being very short and located on very small sclerotic rings. Unlike all other conifer- or angiosperm-feeding Lachninae, however, the siphunculus-bearing rings of *Pseudessigella* are without setae.

The SEM morphological study of *P. brachychaeta* shows that although the species shares many features with other lachnines and especially other eulachnines, it also exhibits a number of unique characteristics. The cuticle structure, and the location, discrete morphology, and number of accessory rhinaria are promising character systems which may prove useful in the taxonomic and phylogenetic inference of these hemipterans, and in particular the several pine needle-feeding genera of eulachnines, *Cinara (Schizolachnus)*, *Essigella*, *Eulachnus*, and *Pseudessigella*. For lack of other SEM studies, ours mainly compared *Pseudessigella* with *Eulachnus*. It will be particularly interesting to compare in a phylogenetic context these two genera with the other two needle-feeding eulachnines.

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