

The Bhutan Pine Aphid *Pseudessigella brachychaeta* Hille Ris Lambers (Hemiptera: Aphididae: Lachninae) From India Reveals the Hitherto Unknown Oviparous Female and Dwarfish Male

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Mariusz Kanturski, Shahid Ali Akbar, and Colin Favret (2017) Here we describe the presence of the monotypic and poorly known aphid genus *Pseudessigella* Hille Ris Lambers (Hemiptera: Aphididae: Lachninae) in India. So far, the genus has only been known from Punjab, Pakistan. Representatives of *P. brachychaeta* Hille Ris Lambers were collected from *Pinus wallichiana* A.B. Jacks. in the Yousmarg region of the state of Jammu and Kashmir in India. Hitherto unknown oviparous females and dwarfish males, the latter reported in Eulachnini for the first time, are described and illustrated. The male's antennal sensilla and genitalic morphology are additionally studied and presented using Scanning Electron Microscopy. Notes on the biology, distribution, and previously overlooked generic features are given. We provide morphological identification keys to the genera of the tribe Eulachnini and to the species of aphid living on *P. wallichiana*.

Key words: Aphid, Dwarfism, Eulachnini, Genitalia, Pest.

BACKGROUND

Over 5000 species of aphid (Hemiptera: Aphididae) are known globally (Favret 2017), of which approximately 653 are recorded from India (Agarwala and Ghosh 1984). Fifty-one aphid species feed on more than 34 species of forest trees in the Kashmir Himalaya (Bhagat 2011). Most conifer-feeding aphid species are found in the four genera of the tribe Eulachnini of the subfamily Lachninae (Aphididae) (Blackman and Eastop 1994, Chen et al. 2016, Favret 2017), the Indian fauna being represented by 36 species (Ghosh 1982), of which 23 from Northwest India (Ghosh and Singh 2000).

The eulachnine genus *Pseudessigella* Hille Ris Lambers includes a single species, *P.*

brachychaeta Hille Ris Lambers, that lives on *Pinus wallichiana* A.B. Jacks. in Murree in Punjab, Pakistan (Hille Ris Lambers 1966). *Pseudessigella* is the most poorly known genus of the tribe Eulachnini, although it resembles species of the genera *Eulachnus* Del Guercio and *Essigella* Del Guercio (Kanturski et al. 2017). These three genera comprise a group of narrow-bodied Eulachnini aphids, sometimes referred to collectively as the subtribe Eulachnina (Sorensen 1990), that live on the leaves of Pinaceae (in contrast with the broader-bodied species of the genus *Cinara* Curtis, subtribe Cinarina) (Ghosh 1982, Kanturski et al. 2015). *Pseudessigella brachychaeta* differs from *Eulachnus* species by having a 5-segmented antenna, and from *Essigella* species by having a membranous dorsum and tarsi with typically-

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shaped claw apices. Until recently, *Pseudessigella* was known only from the type series of the type species from Pakistan, represented only by the apterous viviparous morph (Hille Ris Lambers 1966) and India where during field studies in 2015 and 2016, in the area of Yousmarg in the Budgam District of Jammu and Kashmir in India, many representatives of *P. brachychaeta* were found (together with specimens of *Cinara maculipes* Hille Ris Lambers and *C. lachnirostris* Hille Ris Lambers) on *Pinus wallachiana* for the first time (Kanturski et al. 2017).

Moreover, some specimens of *P. brachychaeta* represented hitherto unknown oviparous females and dwarfish apterous males, representing the species's sexual morphs and for which we give detailed descriptions below.

MATERIALS AND METHODS

Specimens examined: Specimens are deposited in the following institutions: Natural History Museum, London (BMNH); Muséum national d'Histoire naturelle, Paris (MNHN); University of Silesia, Katowice (UŚ). The material was preserved in 70% ethanol and mounted using the Kanturski and Wieczorek (2012) protocol. The specimens were examined using a Nikon Eclipse E600 microscope equipped with a DS-Fi2 camera.

PARATYPES, 4 slides (all with the same collection data): PAKISTAN, Punjab, Murree, 33.9°N × 73.4°E, on needles of *Pinus wallichiana*, Robert van den Bosch leg., 4.vii.1964, BMNH no. 1984-340 and MNHN no. P-VII-4d, 9 and 1 apterous viviparous females, respectively. INDIA (all same locality, host, and collector), Jammu & Kashmir, Yousmarg, 33.83°N × 75.30°E, on needles of *Pinus wallichiana*, Shahid Ali Akbar leg., 17.xi.2015, UŚ no. 11/15/01Ind - 5 apterous viviparous females, 3 larvae; 29.xi.2015, UŚ no. 11/15/02Ind - 12 oviparous females; 10.x.2016, UŚ no. 10/16/05Ind - 20 oviparous females, 20 males.

Morphological description: The following abbreviations are used (partly after Blackman and Eastop 1994): BL - body length (from the anterior border of the head to the end of cauda); BW - greatest body width across the middle of abdomen; H - head, width measured across the compound eyes; ANT - antennae or their length; ANT I-V - antennal segments I-V or their lengths (ratios of antennal segment lengths are given as, e.g., 'V:III'); LS III - length of the longest seta of ANT III; BD III - basal articular diameter of ANT III;

BASE - basal part of the last antennal segment or its length; PT - processus terminalis of the last antennal segment or its length; URS - ultimate rostral segment (4+5) or its length; FEMUR III - hind femur length; TIBIA III - hind tibia length; HT - first segment of the hind tarsus or its length; HT II - second segment of the hind tarsus or its length; ABD I-VIII - abdominal tergites I-VIII. Naming of male genitalic characters is after Wieczorek et al. (2011), sensilla terminology follows Bromley et al. (1979, 1980) and Kanturski et al. (2017).

Occurrence data are based on a detailed review of specimens that had been studied in museum collections and scientific literature. All localities were retrospectively georeferenced using Google Earth 7.1.2.2041 (Google 2014) – the coordinates were determined by the description of the locality (geographical projection, decimal degrees and datum: WGS84). Host plant localities are from the Global Biodiversity Information Facility (GBIF; <http://www.gbif.org/>). Only those with precisely formulated coordinates were selected from among the points of the occurrence of the host plant. Repetitions and imprecise data were deleted.

Scanning electron microscopy: Specimens for SEM analysis were preserved in 70% ethanol for several days. For preparation, a method modified from that of Kanturski et al. (2015) was used. From ethanol, the specimens were transferred into 6% phosphotungstic acid (PTA) solution in 70% ethanol for 24 hours. Dehydration followed a series of ethanol baths of 80%, 90%, 96% and two changes of 100% ethanol for 30 minutes each. Dehydrated specimens were dried using hexamethyldisilazane (HMDS) solution with absolute ethanol in proportions of 1:3, 1:2; 2:3 for 30 minutes each, followed by two changes of undiluted HMDS. Samples were mounted on aluminum stubs with double-sided adhesive carbon tape and sputter-coated in a Pelco SC-6 sputter coater (Ted Pella Inc., Redding, CA, USA). The specimens were imaged with a Hitachi SU8010 field emission scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) at 5, 10 and 15 kV accelerating voltage with a secondary electron detector (ESD, Faculty of Biology and Environmental protection in Katowice).

RESULTS

Description of sexual morphs (Figs 1-11; Table 1)

Shared characters of the sexual morphs of *Pseudessigella*

The sexual generation shares most features with the vivipara. Body narrow, slender, spindle-shaped (Figs. 1, 5). Head with large compound eyes but without ocular tubercles or visible triommatidia. ANT 5-segmented. ANT IV with one rounded primary rhinarium at the apex. ANT V with short PT, one rounded primary rhinarium, and with accessory rhinaria in two groups: one situated close to the major rhinarium and 3-4 tightly joined to each other under the major rhinarium. ANT V with 2 basal, 2 subapical, and 3 apical setae. ANT setae short and pointed, never longer than

the diameter of the antennal segments. URS short and blunt, segments 4 and 5 fused, without accessory setae. Dorsal thoracic setae short and blunt or apically expanded. TIBIA III setose, with blunt or slightly capitate setae dorsally and long and pointed setae ventrally. HT I with 1 dorsal and 5 ventral setae (Fig. 6e, f). First segment of prothoracic and mesothoracic tarsi without dorsal seta. HT II long and slender. Abdominal dorsum membranous, with numerous intersegmental muscle attachment plates and very small and rounded scleroites at setal bases. Each dorsal segment with 2 spinal, 2-4 pleural, and 2 marginal sclerites (Figs. 2a, 3i). Dorsal abdominal setae short and blunt or with expanded apices (Fig. 3j). Cauda broadly rounded with many long, fine and pointed setae. SIPH low, almost poriform, with very narrow siphuncular sclerites, always without setae (Fig. 6d).

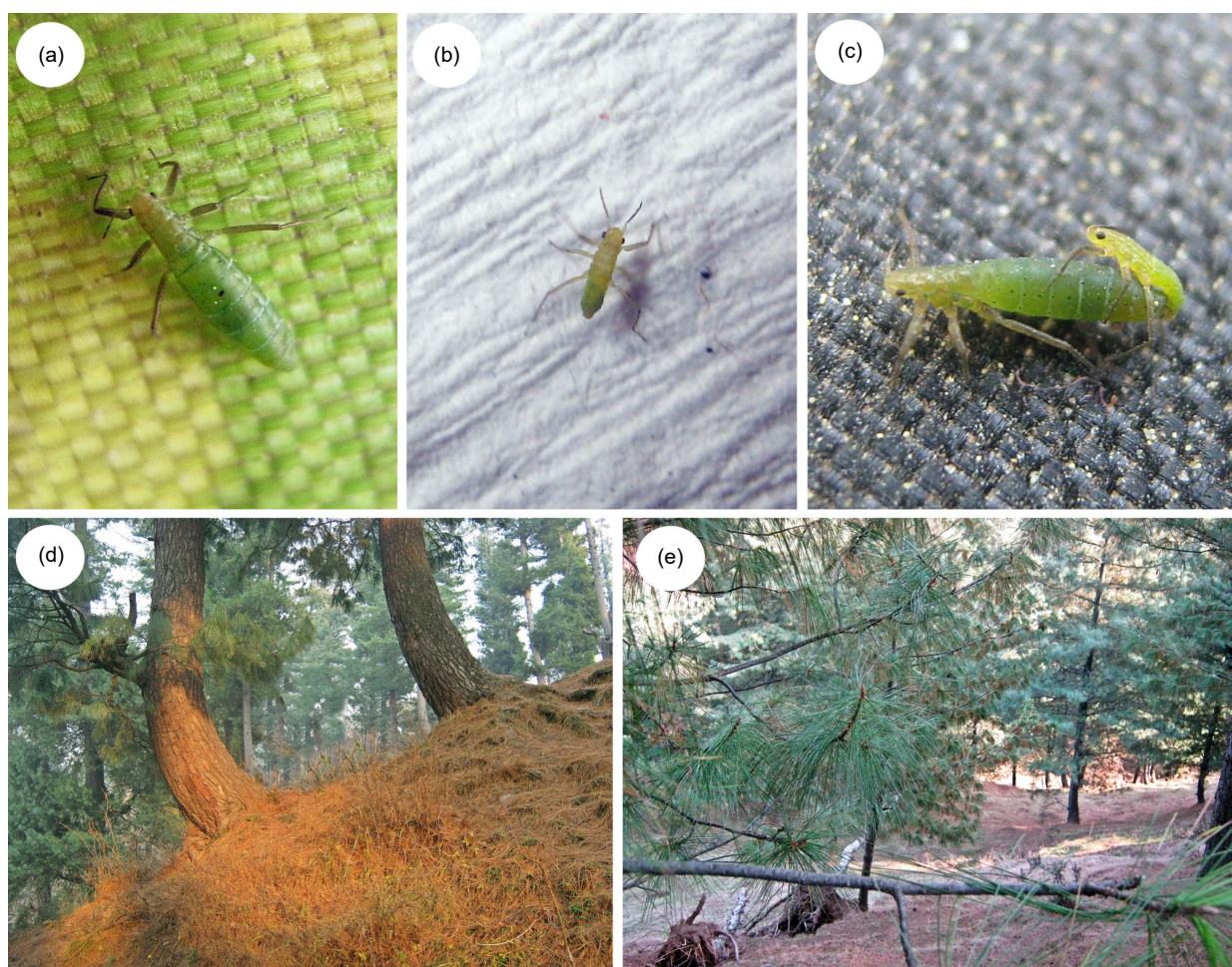


Fig. 1. *Pseudessigella brachychaeta* in life: (a) ovipara; (b) male; (c) sexuales in copula, (d, e) *P. wallichiana* and habitat in the mountain area of Yousmarg (2396 m [7861 ft]) in India.

Oviparous female – description based on 10 specimens

(Figs. 1-3, 5; Table 1)

In life: Body narrow, membranous, with distinct segments and well-developed segmental sutures; these sutures with light wax and therefore paler than the segments.

Colour: Head and prothorax yellow. ANT I-III yellow, ANT IV and V brown. Meso- and metathorax yellowish-green. Legs brown to dark brown. Abdominal segments green, SIPH with poorly-visible (Figs. 1a, c).

Slide-mounted: Colour: Head and thorax sclerotized, light brown. ANT entirely light brown, or with ANT III pale at the base and middle and light-brown at the apex. Legs uniformly light brown. Abdomen membranous with light brown intersegmental muscle attachment plates and very small light brown sclerites at setal bases. Siphuncular sclerites light brown. Genital plate, ABD VIII and cauda sclerotized, light brown (Fig. 2a).

Other morphological characters: H width $0.73\text{--}0.81 \times \text{ANT}$. H chaetotaxy: dorsal side with

10-12 short setae, blunt or with slightly expanded apices; ventral side with 10 long, fine and pointed setae, $17\text{--}27 \mu\text{m}$. ANT (Fig. 3a) $0.18\text{--}0.19 \times \text{BL}$. ANT III similar in length to ANT IV + V. ANT IV shorter than ANT V (Fig. 3a). ANT V slightly swollen in the middle (Fig. 3b), PT/BASE $0.12\text{--}0.15$. Other antennal ratios: V:III $0.48\text{--}0.60$, IV:III $0.39\text{--}0.45$. Antennal chaetotaxy: ANT I with 4-6, ANT II with 4-5, ANT III with 6-8, and ANT IV with 1-3 setae, LS III $0.70\text{--}0.88 \times \text{BD III}$. Rostrum short, reaching behind the middle coxae. URS (Fig. 3c) approximately $0.30 \times \text{ANT III}$, $0.60\text{--}0.62 \times \text{ANT V}$, $0.28 \times \text{HT II}$ (Fig. 3d). Thoracic setae $20\text{--}22 \mu\text{m}$ on pronotum, $15\text{--}25 \mu\text{m}$ on mesonotum, $25\text{--}27 \mu\text{m}$ on metanotum. TIBIA III only very slightly swollen, with $18\text{--}34$ pseudosensoria (Fig. 3e) located only ventrally, occupying the middle $3/5$ of the length of the tibia. Pseudosensoria irregular in size and shape, from small, rounded, and 8-shaped, to large ones formed from the merging of smaller ones (Figs. 3f, g). TIBIA III setae $20\text{--}42 \mu\text{m}$. HT II $1.08\text{--}1.15 \times \text{ANT III}$, $1.99\text{--}2.22 \times \text{ANT V}$. Dorsal abdominal setae $30\text{--}32 \mu\text{m}$ on ABD I-VI and $25\text{--}30 \mu\text{m}$ on ABD VII-VIII. Subgenital plate slightly indented in the middle (Fig. 3i), with $20\text{--}25$ setae.

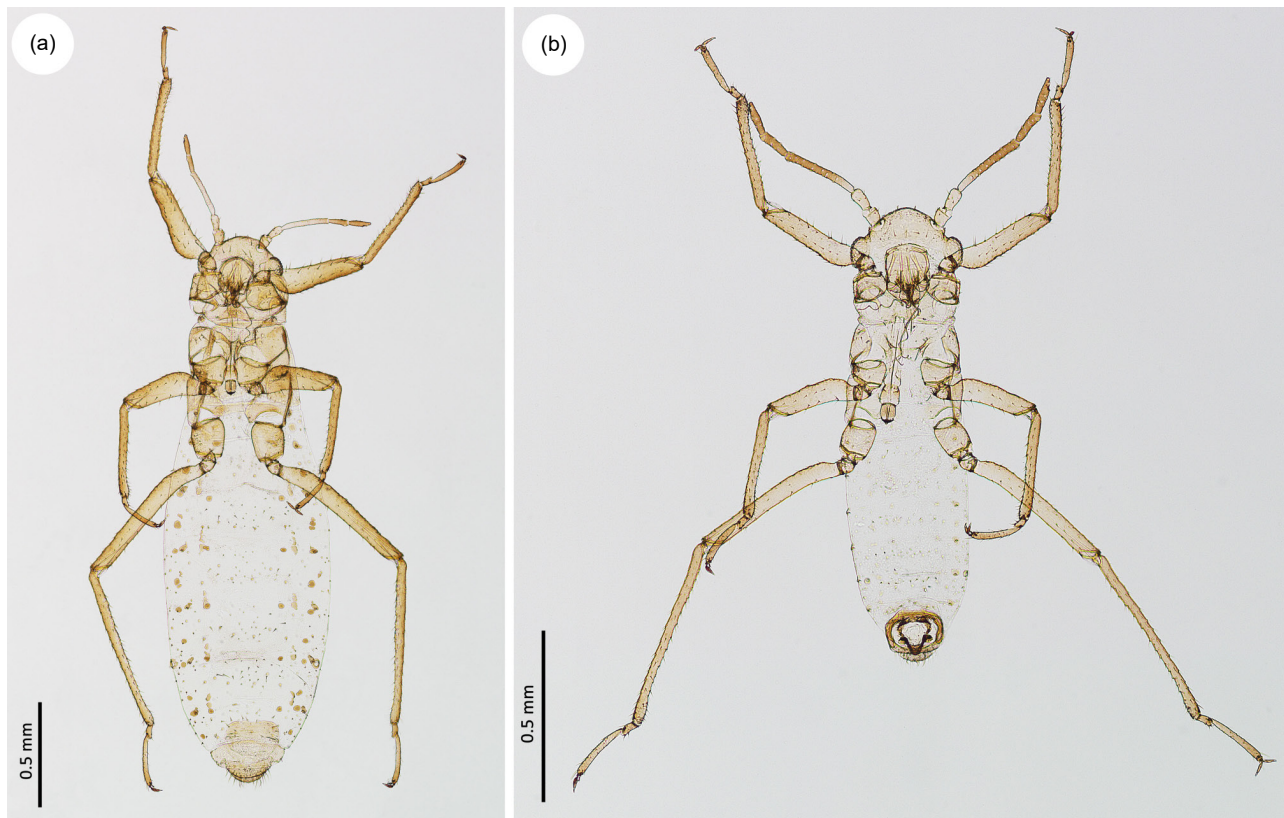


Fig. 2. Mounted specimens of *P. brachychaeta*: (a) ovipara; (b) male.

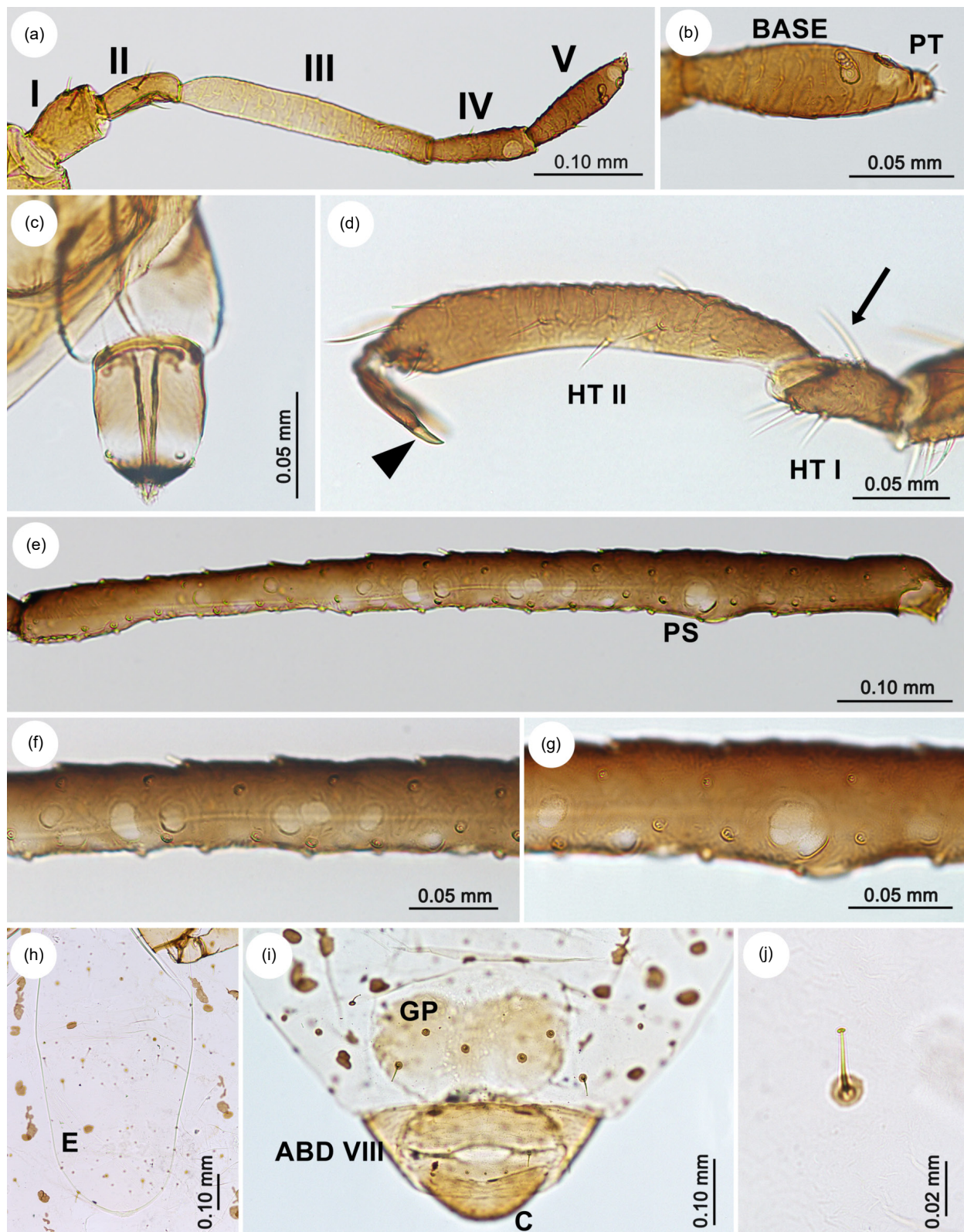


Fig. 3. Morphological details of *P. brachychata* ovipara: (a) ANT I-V; (b) ANT V with base and processus terminalis (PT); (c) URS; (d) hind tarsus with three of five ventral and single dorsal seta (arrow) visible on the first segment (HT I), the second segment with claws with pointed apices (arrowhead); (e, f, g) TIBIA III with pseudosensoria of different shapes; (h) egg (E) inside abdomen; (i) posterior abdomen with cauda (C), ABD VIII, and genital plate (GP); (j) dorsal abdominal seta.

Dwarfish apterous male – description based on 10 specimens

(Figs. 1, 2, 4, 5, 6-8; Table 1)

In life: Body small, dwarfish, membranous.

Colour: Head and thorax yellow to light green. ANT brown. Legs pale yellow to light brown. Abdominal segments light green to green, SIPH poorly-visible. (Figs. 1b, c).

Slide-mounted: Colour: Head and thorax lightly sclerotized, yellow to pale brown. ANT light brown, except ANT I, ANT II, and basal part of ANT III pale to yellowish. Legs yellow with femora pale. SIPH light brown. Genitalia sclerotized, light brown (Fig. 2b).

Other morphological characters: H with convex frons. H width $0.59-0.66 \times \text{ANT}$. Head setae short, with blunt or slightly expanded apices, $10-15 \mu\text{m}$. ANT (Fig. 4a) $0.38-0.42 \times \text{BL}$. ANT III almost as long as ANT IV + V, with 18-25 secondary rhinaria situated from about $1/3$ of its length to its apex. ANT IV (Fig. 4b) only slightly shorter than ANT V, with 8-12 secondary rhinaria. PT/BASE $0.09-0.14$, with 7-11 secondary rhinaria (Fig. 4c). Secondary rhinaria on all ANT segments small, rounded and slightly protuberant. Other antennal ratios: V:III $0.47-0.54$, IV:III $0.45-0.47$. Antennal chaetotaxy: ANT I with 5, ANT II with 5, ANT III with 5-6, and ANT IV with 2-3 setae, LS III $0.75-0.90 \times \text{BD III}$. Rostrum reaching behind hind coxae. URS $0.24-0.28 \times \text{ANT III}$, $0.45-0.57 \times$

ANT V, $0.27-0.31 \times \text{HT II}$. Thoracic setae $7-13 \mu\text{m}$ on pronotum, $10-13 \mu\text{m}$ on mesonotum, and $10-16 \mu\text{m}$ on metanotum. TIBIA III setae $19-40 \mu\text{m}$. HT II $0.84-0.90 \times \text{ANT III}$, $1.54-1.85 \times \text{ANT V}$. Dorsal abdominal setae very short, blunt, or with expanded apices, $11-20 \mu\text{m}$ on ABD I-VI, $15-20 \mu\text{m}$ on ABD VII-VIII. The genital area strongly sclerotized and easily visible. Parameres fused basally, basally connected to a sclerotized ring surrounding the whole genital area (Fig. 4d). The separate projections of parameres short and stocky, located above the basal part of phallus. Phallus short and flattened, with rounded apex. Parameres and basal part of phallus both bear short pointed setae (Figs. 4e, f).

SEM morphology of the male of *P. brachychaeta*

General morphology: The male is, like the other morphs, characterized by a spindle-shaped body (Fig. 5b). Almost all dorsal segments are fused. The head is fused with the pronotum, but a distinct suture between them is visible (Fig. 6a). The meso- and metanotum are fused with the abdominal segments, the ensemble of these segments being separated from the pronotum. ABD I is less distinctly separated from other segments (Fig. 5b). The head is characterized by a convex frons with very short setae (trichoid sensilla), and large compound eyes without visible triommatidia (Fig. 6b). The URS is as in other morphs of the

Table 1. Morphological measurements (in mm) of oviparae ($n = 10$) and males ($n = 10$) of *P. brachychaeta*

Character	Oviparous female	Male
BL	2.500-3.100	1.320-1.410
BW	0.900-0.980	0.350-0.380
HW	0.420-0.460	0.330-0.360
ANT	0.550-0.570	0.545-0.565
ANT III	0.200-0.230	0.210-0.225
ANT IV	0.900-0.950	0.100-0.105
ANT V	0.110-0.120	0.105-0.120
BASE	0.100-0.105	0.095-0.105
PT	0.012-0.015	0.010-0.015
URS	0.065-0.070	0.055-0.060
III FEMORA	0.650-0.750	0.440-0.480
III TIBIAE	0.730-0.850	0.550-0.600
HT I	0.070-0.080	0.050-0.055
HT II	0.230-0.250	0.185-0.200
SIPH sclerite	0.050-0.062	0.017
Genital plate length	0.160-0.180	-
Genital plate width	0.250-0.300	-

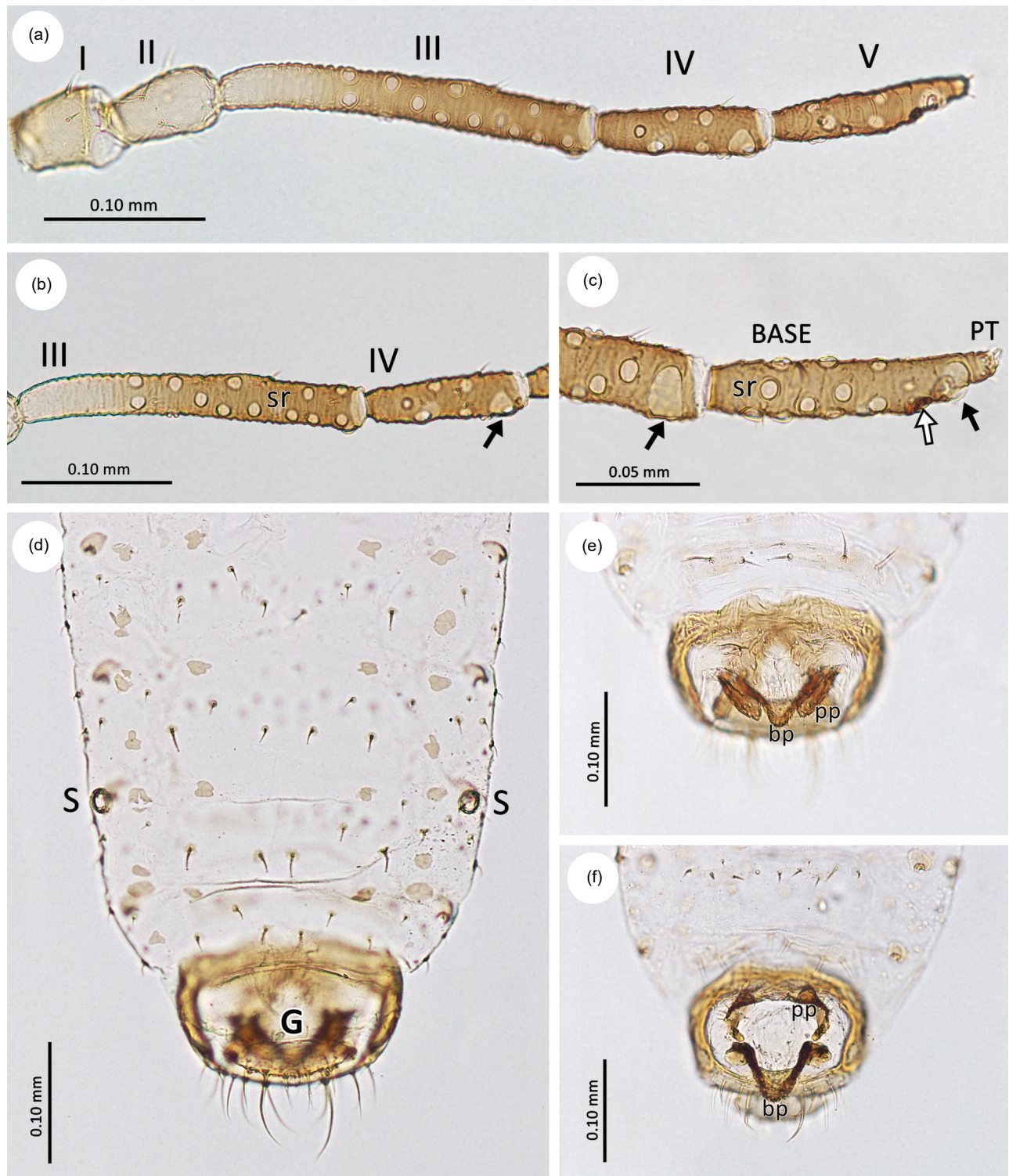


Fig. 4. Morphological details *P. brachychaeta* of male: (a) ANT I-V; (b) ANT III with secondary rhinaria (sr) and ANT IV with secondary rhinaria and primary rhinarium on the apex (black arrow); (c) end of ANT IV and ANT V with secondary rhinaria (sr) and primary rhinaria (black arrows). Primary rhinaria on ANT V present as large major rhinarium (black arrow) and small accessory rhinaria (white arrow); (d) dorsum of abdomen with siphunculi (s) and ventrally located genitalia (G) out of focus; (e) ventral view of the genitalia with paramere projections (pp) fused basally and connected to the basal part of phallus (bp) and the broad, sclerotic, genitalic ring; (f) posterior ventral view of the genitalia showing the size and shape of paramere projections (pp) and the basal part of phallus (bp).

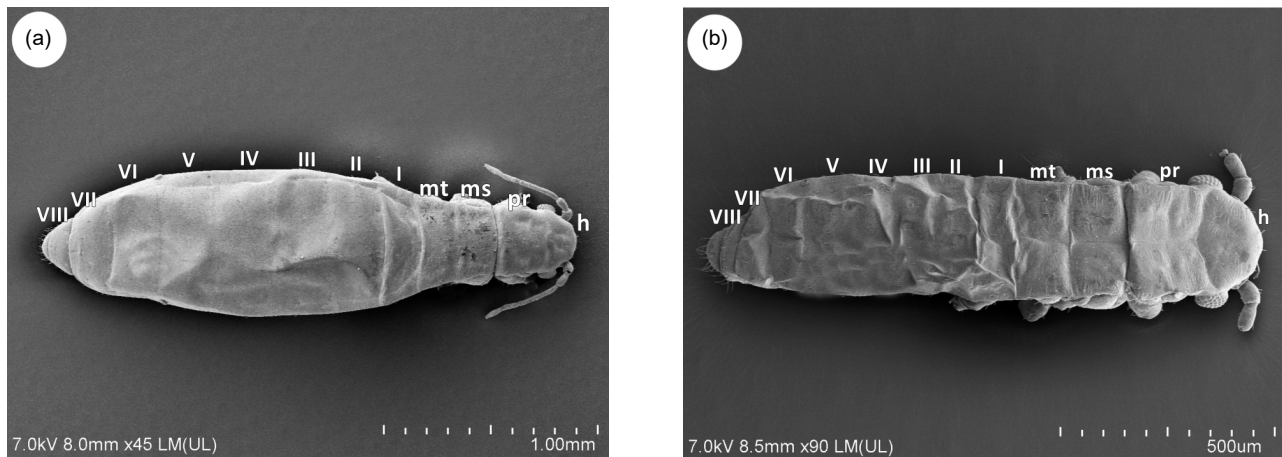


Fig. 5. SEM micrographs of *P. brachychaeta*: (a) ovipara and (b) male: head fused with pronotum, mesonotum fused with metanotum, distinct ABD I (less so in male), fused ABD II-VII (but with visible sutures), and distinct ABD VIII; h-head, pr-pronotum, ms-mesonotum, mt-metanotum, I-VIII-abdominal terga.

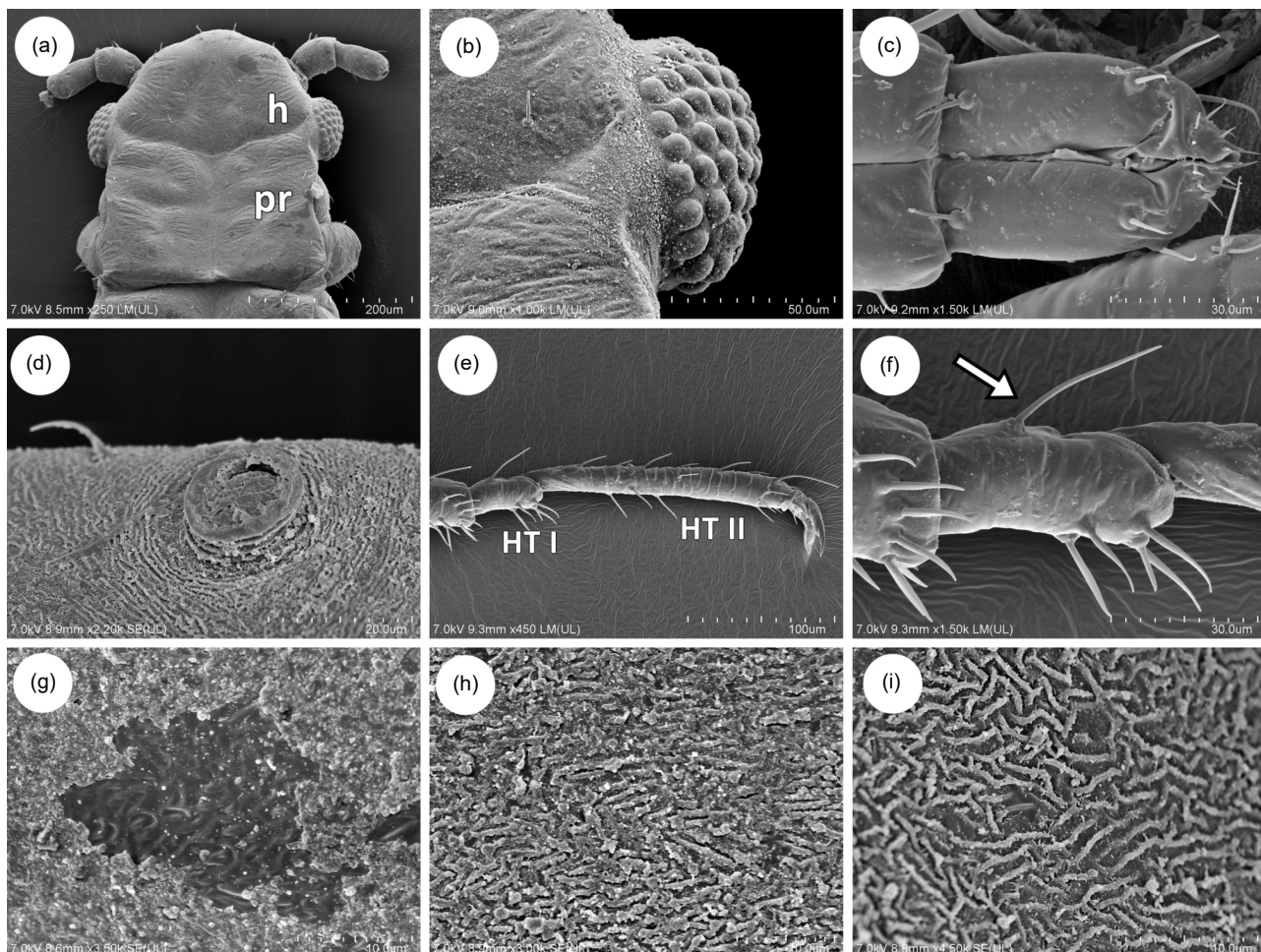


Fig. 6. SEM of morphological features of *P. brachychaeta* male: (a) head fused with pronotum; (b) compound eye without ocular tubercle; (c) short and blunt URS lacking accessory setae; (d) small, almost poriform siphunculus with visible flange; (e) hind tarsus with long HT II; (f) HT I with one dorsal (white arrow) and five ventral setae (four setae + one sense peg); (g) wrinkled cuticle of thorax with globular wax secretions; (h) regular and tightly fitting wax secretions on ABD I-VI; (i) wrinkled cuticle covered by wax secretion on ABD VII-VIII.

species, with one pair of type-II basiconic sensilla, three pairs of type-II trichoid sensilla, and seven pairs of type-III basiconic sensilla (Fig. 6c).

Although no secretions are observed in life, the SEM study revealed that both sexuales are covered by thin layer of wax. The cuticle is slightly ruffled and evenly covered by regular-shaped secretions. On the head and thorax, the wax is rather granular (Fig. 6g), whereas on the abdomen it is in the form of small, thin strips or rolls (Figs. 6h, i).

Sensilla morphology: The antennal sensilla of the male *P. brachychaeta* are distributed on the pedicel and the flagellum. The pedicel bears one rhinariolum, located near the segment's apex (Fig. 7a). It has a relatively small aperture (diameter, 1.8-2.0 μm) and a robust, protruding coeloconic sensillum, 0.8-1.0 μm wide, this latter with 5-7 very short projections (Fig. 7b). The flagellar segments are characterized by the presence of differently-sized and developed placoid and coeloconic sensilla (rhinaria) (Fig. 7c). ANT III bears, ventrally, rounded secondary rhinaria (small placoid sensilla) of different sizes (diameter, 4-11 μm) (Fig. 7d). The rhinaria are developed as more or less protuberant plates with delicate rings at the base or as narrow cavities without rings (Fig. 7e). Secondary rhinaria (small placoid sensilla) on ANT IV and V are very similar as those on ANT III but less protuberant. Primary rhinaria on ANT IV and V are rounded and rather flat (diameter, 14-21 μm), without a visible ring (Figs. 7f, g). Accessory rhinaria on ANT V are developed into two morphological types of sensillum: (1) mushroom-shaped placoid and (2) sunken coeloconic. A single, mushroom-shaped, small, placoid sensillum, located next to and under the major rhinarium (big placoid sensillum), lies in a broad cavity with a well-developed flange with transverse struts. A second group of rhinaria is located under the major rhinarium and consists of a second mushroom-shaped placoid sensillum, and 2-3 sunken coeloconic sensilla, all of which are located in a deep cavity with many thickened struts (Fig. 7h). The diameter of the mushroom-shaped, small, placoid sensillum is approximately 5-6 μm . The antennae also bear, on all segments, type-I trichoid sensilla in the form of very short, rigid and pointed setae (Figs. 7a, i). The PT bears 3 apical and 2 subapical type-II trichoid sensilla (Fig. 7j). The rest of the body bears a few type-I trichoid sensilla consisting of short and rigid, straight or curved setae with blunt or expanded apices (Figs. 7k, l). Type-I trichoid sensilla on the legs are short- to medium- sized setae with slightly

capitate (especially on the dorsal side of the tibia) or pointed apices (on the ventral side of the tibia) (Fig. 7m).

Genitalic morphology: Compared to the males of other aphid species the genitalia of *P. brachychaeta* are large relative to body size. They consist of a pair of parameres and the phallus (sclerotized basal part + large aedeagus) (Fig. 8a). The aedeagus is membranous, 65-72 μm long, and 65-75 μm wide in the middle. The length of the aedeagus is about 12-13% of the length of the insect's body. The genitalia are located between the cauda and the anal plate, these latter both covered by long, fine, and pointed setae. The genitalia connect smoothly with a broad, strongly sclerotized ring, comprising the end of the abdomen on the ventral side (Fig. 8b). The sclerotic ring connects to a pair of parameres that are fused basally and have broadly-spread projections (Fig. 8c). Each paramere projection is triangle-shaped, 18-20 μm long, 28-33 μm wide at the base, slightly wrinkled, with a rounded, smooth-cuticle apex, and with 18-19 fine and pointed setae (trichoid sensilla). These setae are longer (15-23 μm) on the outer side of the paramere and shorter (7-12 μm) on the inner side and on the apex. The fused basal parts of the parameres bear 8-10 short (4-10 μm) and pointed setae (Figs. 8c, d). The phallus is shaped as a broad triangle basally, and has a rounded apex. It is 48-50 μm long, 52-55 μm wide basally, and bears 16-20 setae 7-10 μm long on its outer sides. It is vertically wrinkled, with 6-7 sensilla-like structures, approximately 2-3 μm long and 2 μm wide, in the form of membranous pegs each on a rounded sclerotic base (Fig. 8e). Male larvae bear sclerotized genitalic buds in form of two small and smooth projections, each with 4-6 setae (Fig. 8f).

Egg morphology: The egg of *P. brachychaeta* is approximately 1.25-1.35 mm long and 0.30-0.35 mm wide, spindle-shaped, with narrow rounded ends (Figs. 9a, b). One pole is characterized by a small, rounded protuberance (Figs. 9c, d), also visible inside the abdomen in slide-mounted oviparae (Fig. 3h). Two membranes are visible on the chorion: the external one is slightly wrinkled (Fig. 9e), whereas the inner one is smooth, globular, and porous, or covered in wax (Fig. 9f).

Copulation and oviposition of the sexual morphs

In the first half of October, adult males and oviparae were observed copulating on the pine needles. The male contacts the dorsal side of female's abdomen with the ventral side of ABD I-V

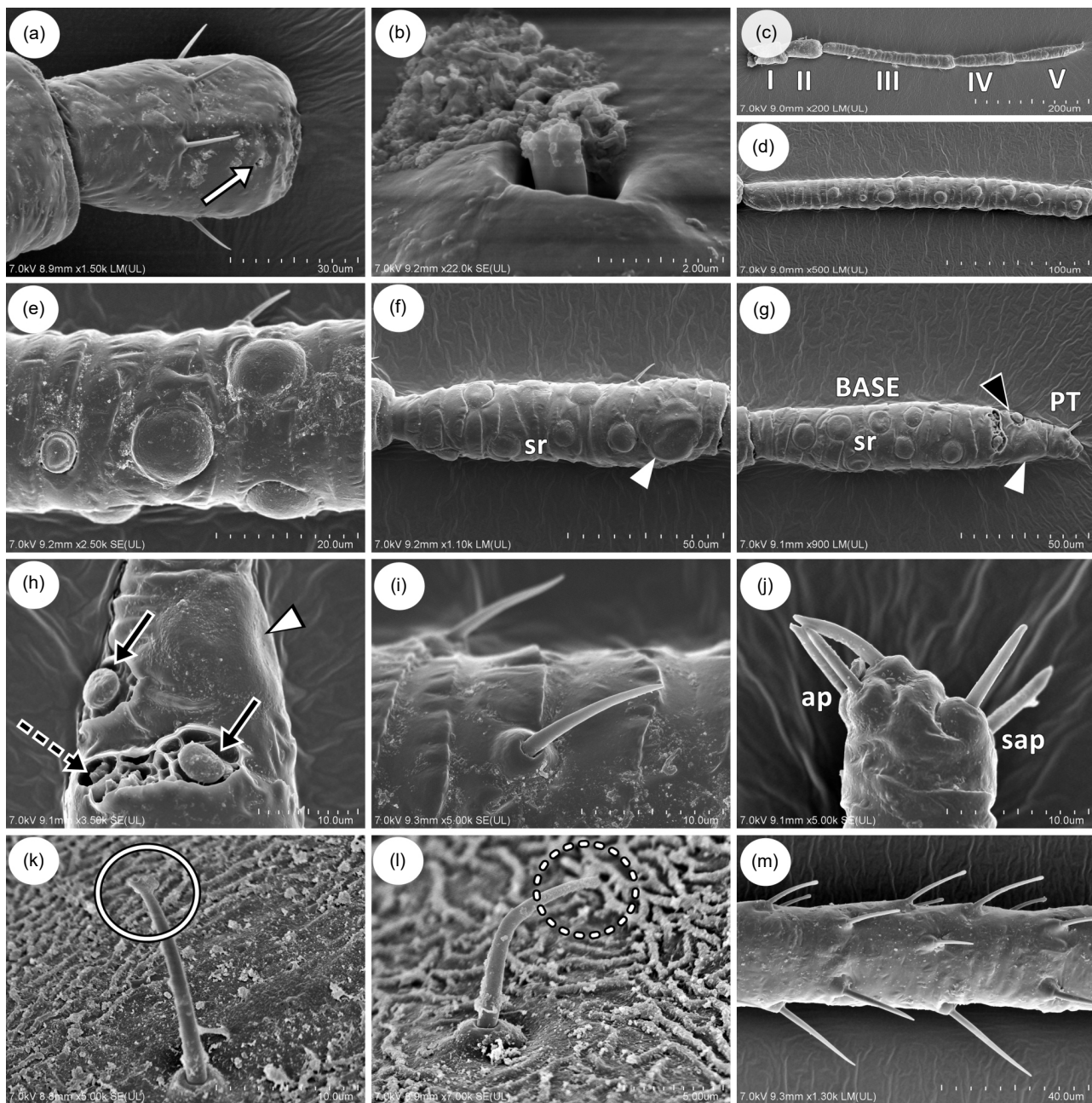


Fig. 7. SEM of sensilla of *P. brachychaeta* male: (a) ventral side of pedicel with short, rigid and pointed type-1 trichoid sensilla and single rhinariolum near the distal end of the segment (white arrow); (b) structure of the rhinariolum showing small aperture and coeloconic-like sensillum with poorly-developed projections; (c) antenna segments I-V; (d) secondary rhinaria (small placoid sensilla) on ANT III; (e) different shapes and sizes of the secondary rhinaria (small placoid sensilla) on ANT III; (f) secondary rhinaria (small placoid sensilla) (sr) and primary rhinarium (large placoid sensillum) (white arrowhead) on ANT IV; (g) sensilla on ANT V: primary sensilla in form of major rhinarium (large placoid sensillum) (white arrowhead) and accessory rhinaria (black arrowhead) on the apex of BASE and secondary rhinaria (small placoid sensilla) on the whole length of BASE of ANT V; (h) structure of primary rhinaria on ANT V: large and rounded major rhinarium (white arrowhead), and two kinds of accessory rhinaria – mushroom-shaped small placoid sensilla (solid arrows) and sunken coeloconic sensilla (dotted arrow); (i) type-I trichoid sensillum as found on antennal segments I-IV and ANT V BASE; (j) type-II trichoid sensilla on ANT V PT, arranged into three apical (ap) and two subapical (sap) setae; (k) straight trichoid sensillum with expanded apex as distributed mostly on the spinal and pleural regions of the body; (l) slightly curved and blunt trichoid sensillum as distributed mostly on the marginal regions of the body; (m) trichoid sensilla on tibia: with capitate or blunt apices dorsally and with pointed apices ventrally.

(VI). The abdomen is strongly curved and the male genitalia adhere tightly with the genital area of the ovipara. The male clasps the female between ABD III and IV with his fore legs and between ABD V and VI with his middle legs. His hind legs hold him on the needle or other surface (Fig. 1c). The males die at the end of October: by mid-November, none could be found. Egg laying starts by the middle of October, but oviparae persist as late as the end

of November and into December. Eggs are laid singly, more or less near the middle of the length of the needle. The freshly-laid egg is light green, becoming darker then completely black and shiny (Figs. 10 a, b). The eggs of *Pseudessigella* are easy to distinguish from those of *Cinara*, the latter being larger, more oval in shape (with both ends rounded), brown, and laid on the needle in groups of 6-10 (Fig. 10 c).

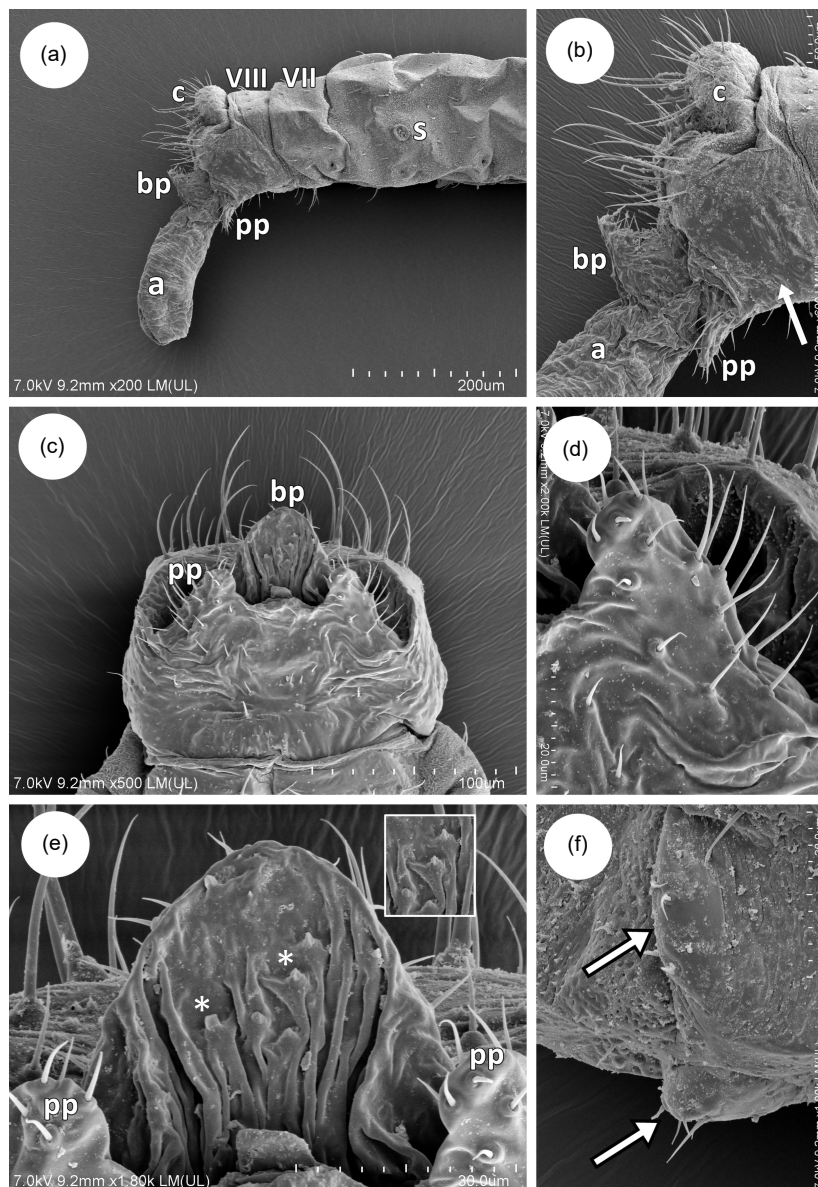


Fig. 8. SEM of genitalia *P. brachychaeta* male: (a) side view of abdomen showing the structure of the genitalia consisting of sclerotized parameres (pp) and everted phallus composed of a sclerotized basal part (bp) and membranous aedeagus (a), with ABD VII- VIII, siphunculus (s), cauda (c); (b) magnified side view of the genitalia area with sclerotized band (white arrow), cauda (c), paramere projection (pp), basal part of phallus (bp), aedeagus (s); (c) ventral view of genitalia showing the parameres fused basally but with separate projections (pp) and the basal part of the phallus (bp); (d) paramere; (e) basal part of phallus (bp), between the paramere projections (pp), bearing sensilla-like structures (asterisks); (f) genitalic buds in last instar.

Morphological key to Eulachnini genera

1. Body long and narrow, at least three times longer than wide; SIPH low, siphuncular sclerite without setae; triommatidion absent or vestigial, no ocular tubercle; 4th and 5th rostral segment fused (URS) or not readily distinguishable, blunt, lacking accessory setae; on needles of Pinaceae; with or without wax 2 (Eulachnina)
- Body oval, less than three times as long as wide; SIPH raised as sclerotic cone, siphuncular sclerite usually with numerous setae; triommatidion present, located on ocular tubercle; 4th and 5th rostral segment usually distinct, pointed apically, usually bearing accessory setae; usually on ligneous parts of Cupressaceae and Pinaceae; if on needles of *Pinus*, covered in fluffy wax (subgenus *Schizolachnus*) *Cinara* Curtis (Cinarina)
2. Antenna 6-segmented; very lightly to moderately covered in wax; HT I with 2 dorsal setae *Eulachnus* Del Guercio
- Antenna 5-segmented; usually without or with very little wax; HT I with 1-4 dorsal setae 3
3. Pretarsal claw with bifurcate apex; HT I with 2-4 dorsal

- setae *Essigella* Del Guercio
- Pretarsal claw simple; HT I with 1 dorsal seta
..... *Pseudessigella* Hille Ris Lambers

Key to Aphididae on *Pinus wallichiana* (characters of *Cinara* species partly after Blackman and Eastop 2017)

1. Compound eye present only as triommatidion. On roots
..... *Prociphilus himalayensis* Chakrabarti
- Compound eye normal, formed from numerous ommatidia. On aerial parts of plant 2
2. Antenna with 5 segments 3
- Antenna with 6 segments 4
3. Claw of HT II with bifurcate apex; HT I with 2-4 dorsal setae *Essigella californica* (Essig)
- Claw of HT II with simple apex; HT I with 1 dorsal seta
..... *Pseudessigella brachychaeta* Hille Ris Lambers
4. URS short and blunt, without accessory setae. Body long and narrow, spindle-shaped. SIPH without setae
..... *Eulachnus rileyi* (Williams)

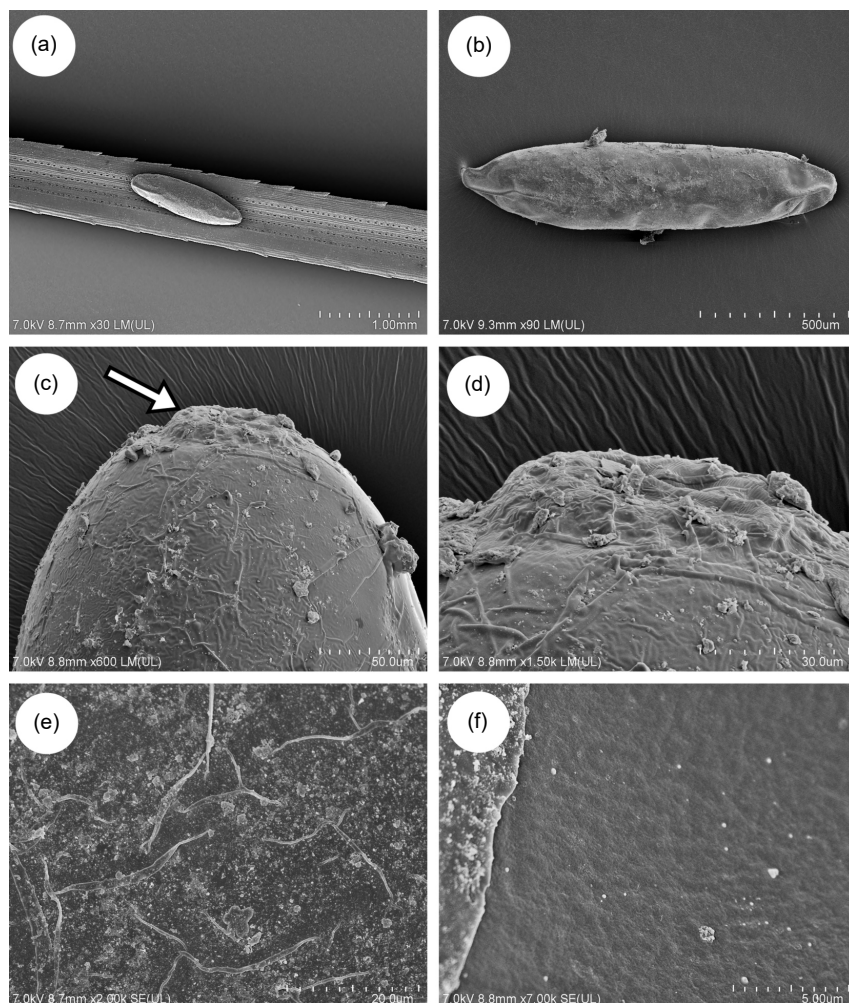


Fig. 9. SEM of *P. brachychaeta* eggs: (a) egg located on *P. wallichiana* needle; (b) close-up of egg; (c, d) rounded ring on one of the poles of the chorion (white arrow); (e) slightly wrinkled external layer of chorion; (f) grainy and porous internal layer of chorion.

- URS long and pointed, with at least two accessory setae. Body oval or pear-shaped. SIPH on setose sclerite 5
- 5. Dorsal abdominal setae short and peg-like, 0.04 mm or shorter. SIPH on small sclerite having a maximum diameter

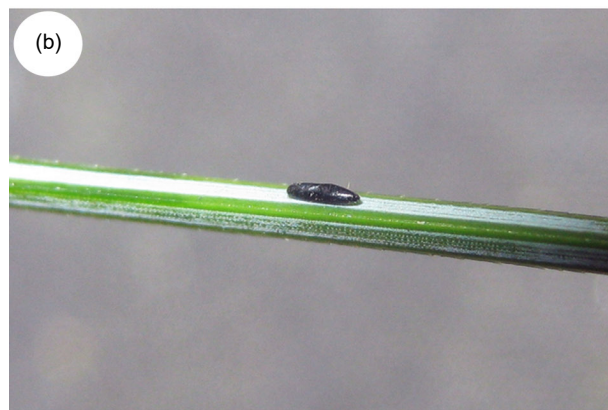
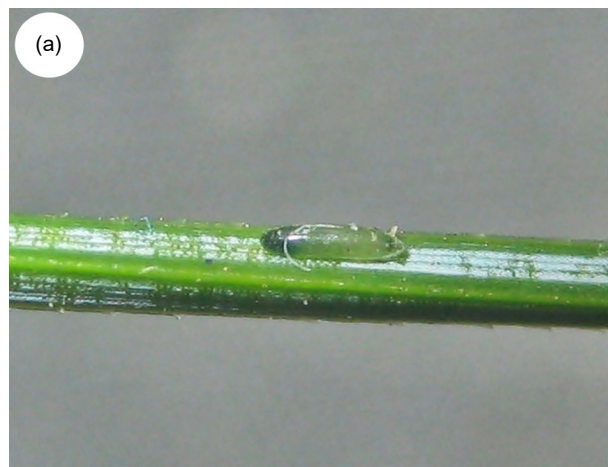


Fig. 10. Eggs of *P. brachychaeta* and *Cinara maculipes* on *P. wallichiana* needle: (a) freshly-laid single egg of *P. brachychaeta*; (b) *P. brachychaeta* egg several days old; (c) comparison of single eggs of *P. brachychaeta* (upper two needles) and a group of *Cinara maculipes* eggs laid in row (lower needle).

- equal to or less than the length of the fourth rostral segment 6
- Dorsal abdominal setae much longer than 0.06 mm. SIPH on well-developed sclerite with a maximum diameter usually much greater than length of the fourth rostral segment 7
- 6. Femur and tibia conspicuously maculate. ANT VI always shorter than ANT V. BL more than 3.70 mm *Cinara maculipes* Hille Ris Lambers
- Femur and tibia not maculate. ANT VI longer than ANT V. BL less than 2.9 mm *Cinara lachnirostris* Hille Ris Lambers
- 7. Fourth rostral segment with more than 7 accessory setae. Dorsum with paired sclerotized patches or groups of sclerites at least on ABD I and VIII *Cinara eastopi* Pinter
- Fourth rostral segment with fewer than 7 accessory setae. Dorsum dotted with small sclerites and scleroites on ABD I-VI and long cross-bars on ABD VII-VIII 8
- 8. Dorsal area between SIPH with only a few small and rounded scleroites at setal bases. HT I 0.16-0.22 mm, without dorsal setae *Cinara pinimaritimae* (Dufour)
- Dorsal area between SIPH with many irregularly-shaped scleroites at setal bases. HT I 0.22-0.33 mm, with dorsal setae *Cinara pinea* (Mordvilko)

DISCUSSION

Morphology of the sexual morphs

The importance of description of unknown morphs or life stages has been reported in many groups of insects as a first step to better understanding their biology and evolution (Kalandyk-Kołodziejczyk 2016; Szpila et al. 2017). The various apterous female morphs in the subfamily Lachninae are known to be similar in their general and even in their detailed morphology. Fundatrices (stem mothers) in the genus *Stomaphis* Walker are often very similar to other viviparous generations without any significant morphological differences (Depa and Kanturski 2014). On the other hand, some fundatrices in the genus *Eulachnus* Del Guercio can be easily distinguished from other viviparae as they have pseudosensoria on the hind tibiae. Contrary to the norm, oviparae may lack pseudosensoria (e.g., *Stomaphis* or *Pterochloroides* Mordvilko) requiring examination of much less obvious morphological characters such as modest differences in the size or pilosity of the genital plate (Wieczorek et al. 2013; Depa et al. 2015).

Most sexual females of Lachninae, and especially Eulachnini, including *Pseudessigella*, exhibit the typical condition of a more-or-less swollen hind tibia bearing numerous pseudosensoria. The presence of pseudosensoria

on slightly swollen hind tibiae, as well as a longer and wider genital plate, clearly distinguishes the ovipara from the apterous vivipara. The oviparae are also larger, with a longer and wider abdomen (Fig. 5a), and possess longer setae on the dorsal side of body. The pseudosensoria of *P. brachychaeta* are located on the ventral side of the tibia and are easily visible, heterogeneous in shape and size. The pseudosensoria in *Eulachnus* are easily visible, usually homogeneous in shape and size, smaller and more numerous (30-100) than those in *Pseudessigella*, and located on the entire surface of the tibia. The oviparae in *Essigella* are unknown in many species; however, in those that have been described, the pseudosensoria tend to be heterogeneous, less numerous (5-27), and often difficult to distinguish (Sorensen 1994; Kanturski and Wieczorek 2014).

The males in Lachninae can be apterous or alate. The male of *P. brachychaeta* is small compared to the larger ovipara (Fig. 1), being 45-52% of her size. This male dwarfism is known in other aphid subfamilies such as Anoeciinae, Eriosomatinae, Hormaphidinae, Mindarinae, and Thelaxinae (Blackman and Eastop 1994, 2017; Wieczorek et al. 2011, 2014; Depa et al. 2015), but in these groups the oviparae are also smaller relative to the viviparous generations. *Stomaphis* is the only other lachnine genus to exhibit male dwarfism: the length of the male is 37-53% the length of the ovipara (Depa et al. 2015).

Morphologically, the *Pseudessigella* male resembles more the poorly-known male of *Essigella*, described in only two of 13 species (Sorensen 1994; Blackman and Eastop 2017). The *Essigella* male is only slightly smaller than the conspecific vivipara and ovipara, has slightly longer antennae and legs, and is characterized by a lack of siphunculi and the presence of secondary rhinaria only on ANT III (13-15) and ANT IV (8-10) (Sorensen 1994). The absence of siphunculi in male *Essigella* is probably an autapomorphy of the genus. The male of *Eulachnus* is the best known of the Eulachnina. With the exception of *E. isensis* Sorin from Japan, in which the male is apterous and smaller than the female, but not dwarfish (Sorin 2012), all *Eulachnus* males are alate. The *Pseudessigella* male shares with the male of *Eulachnus* the presence of siphunculi (although those in *Pseudessigella* are very small and poorly-developed) and secondary rhinaria on the last antennal segment (6-10 in *Pseudessigella* and 1-13 in *Eulachnus*). This latter character is also seen in some males of *Cinara* (Pintera 1968).

Also, the genitalic morphology of *Eulachnus* and *Pseudessigella* is very similar, especially the size, shape and development of parameres, which in both taxa are short projections, fused basally, and with a similar number of setae (trichoid sensilla) (Wieczorek et al. 2012). The basal part of the phallus is the most distinguishing part of the genital apparatus of these genera. In *Pseudessigella* this structure is evidently a flat and wide triangle with a broad, almost rounded apex, whereas in European *Eulachnus* it is either a more or less tubular, narrow triangle, with more pointed apex, or it is completely tubular and rounded (Kanturski et al. 2015). Sensilla-like structures reported on the basal ventral portion of the phallus have not been observed in any other aphid, albeit probably due to specimen positioning and preparation: variably positioned phallus or everted aedeagus. The membranous or faintly-sclerotized projections of these sensilla confirm their sensory function, probably during eversion and positioning of the aedeagus.

The genus-level features of *Pseudessigella*

The absence of dorsal setae on HT I was cited as a diagnostic character of *Pseudessigella* in both its original description (Hille Ris Lambers 1966) and its redescription (Sorensen 1991). However, we observed a single dorsal seta on HT I in apterous viviparae and sexuales from India. Re-examination of the type material of *P. brachychaeta* revealed that the viviparae all have one dorsal seta on HT I. The seta in question is simply broken off and missing in some prepared specimens, but remnants remain visible. Sorensen (1991) wrote that the head is unfused with the pronotum, although in all specimens, and indeed Sorensen's (1991) figure 1, the head and pronotum are clearly fused.

Biology, behavior, and distribution of *Pseudessigella brachychaeta*

The first records of oviparae and males provide new data on the biology of this species. *Pseudessigella brachychaeta* is a holocyclic species with a typical Lachnine life cycle of parthenogenetic, viviparous generations in the spring and summer, and a single sexual generation in the autumn (Depa 2013). Despite regular observations and sampling during the spring and summer, the alate viviparae remain unknown. This corroborates the hypothesis that

Pseudessigella is biologically more closely related to *Essigella* than to *Eulachnus*, as the alatae are also rare or unknown in many *Essigella* species (Sorensen 1991, 1994). In *Eulachnus*, alate viviparae are found frequently and are sometimes very numerous, especially in the spring (Carter and Maslen, 1982. The adult males of *Eulachnus* species occur in October (nymphs with genitalic buds were found in September) (Kanturski et al. (unpublished data)).

Until recently, *Pseudessigella* was the most poorly known genus in the tribe Eulachnini. Hille Ris Lambers (1966) provided a short description of the genus and only a text description of the species *P. brachychaeta*. Although Sorensen (1990) redescribed the genus and discussed its relationship with *Eulachnus* and *Essigella*, his studies were based only on the type series. Görür et al. (2011) reported the presence of *P. brachychaeta* in northeastern Turkey, well outside the natural range of *P. wallichiana*, and in the absence of additional information we consider this record suspect. Despite many investigations of the aphid fauna of northern India, with 310 recorded species one of the best investigated of the five biogeographical areas in India (Ghosh 1982; Agarwala and Ghosh 1984; Ghosh and Singh 2000), representatives of this genus were not recovered until now. Moreover, Naumann-Etienne and Remaudière (1995) reported that, despite a long-term (1987-1992) study of the aphid fauna of Pakistan, including in Murree, *P. brachychaeta* had not been seen since its original discovery.

The presence of *P. brachychaeta* in Yousmarg in India confirms that this species is montane and will likely be found in other places within the natural

range of *P. wallichiana*. Located at elevations between 1500 and 3600 m, the Himalayan white pine (Figs. 1d, e), also known as the Bhutan pine or the blue pine, has a native and restricted range in the mountainous areas of Afghanistan, Bhutan, China, Myanmar, Nepal, and Pakistan (Fig. 11) (Farjon and Page 1999). This pine species has been introduced in Europe, southern Africa, and North America (Orwa et al. 2009).

With this and another recent study (Kanturski et al. 2017), *Pseudessigella* has gone from being one of the least to one of the best well-known genera of Lachninae. Its phylogenetic placement close to *Essigella* and *Eulachnus* is not in doubt, but its various morphological, biological, and geographic similarities with one or both of these other genera suggest competing hypotheses of speciation that may be answered following a thorough cladistics analysis.

CONCLUSIONS

The present study confirms that the aphid genus *Pseudessigella* expands its range in the area of natural range of the host plant *P. wallichiana*. The presence of sexual morphs which are for the first time described in this paper confirm that the genus and species is characterized by a holocycle. Characters of the oviparous female and male clearly confirm that description of unknown morphs and life stages is crucial for the better understanding of evolution and phylogenetic relationships of many insect taxa, especially the studied tribe Eulachnini.

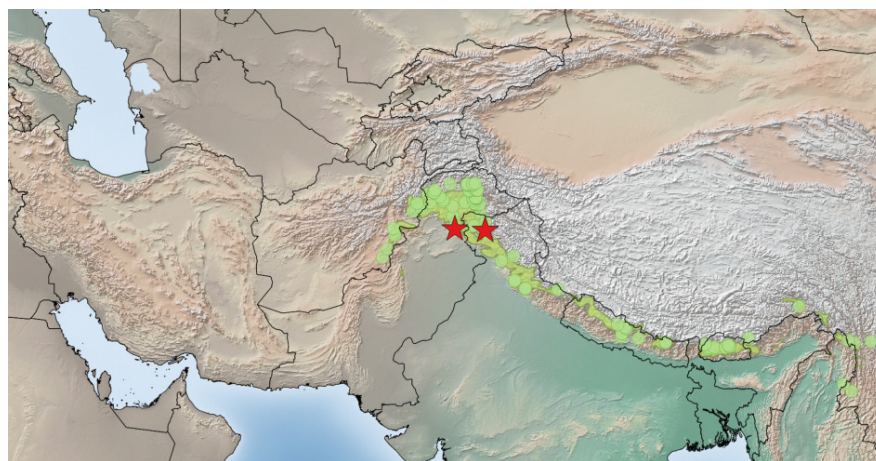


Fig. 11. Distribution of *Pinus wallichiana* as recorded by GBIF (green dots) and the two known localities of *P. brachychaeta* (red stars) in Pakistan and India.

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