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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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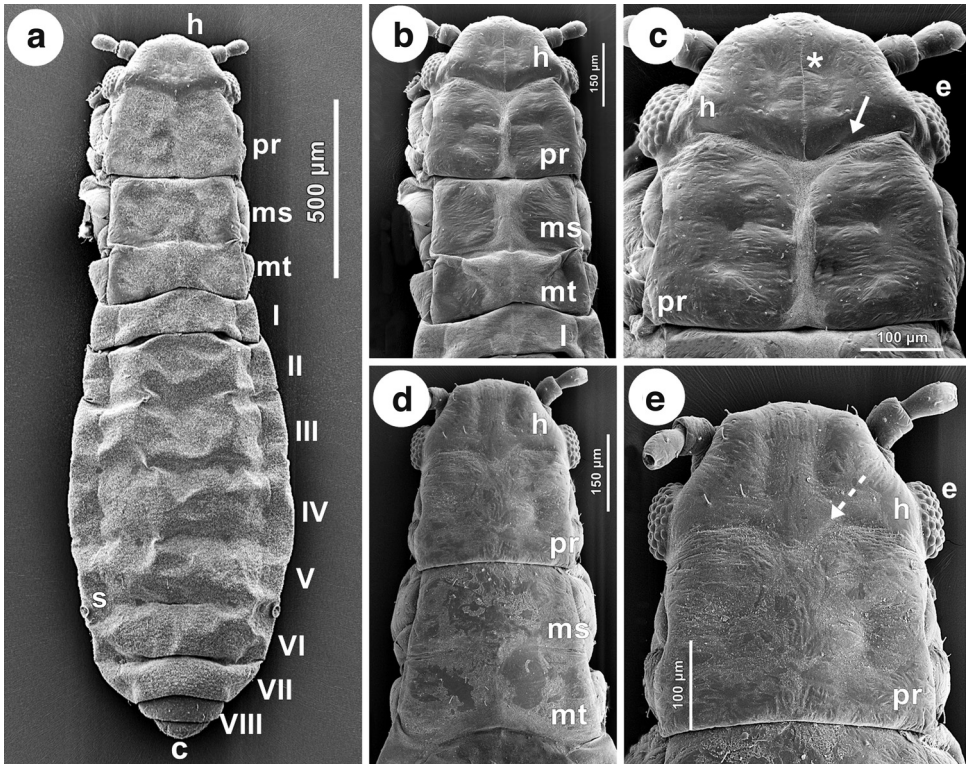

Fig. 1. Scanning electron micrographs showing the general view, head and thoracic characters of *P. brachychaeta*: (a) general morphology of apterous vivipara, h – head, pr – pronotum, ms – mesonotum, mt – metanotum, I–VIII – abdominal segments I–VIII, s – siphunculi, c – cauda; (b) head and thorax of 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 brachychaet*a 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).