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First-instar nymphal morphology and antennal sensilla in the *Kerria lacca* (Kerr, 1782) and *Paratachardina mahdihassani* (Kondo and Gullan, 2007) (Hemiptera: Tachardiidae)

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ABSTRACT

The external morphology and antennal sensilla, in the first-instar nymphs of both *Kerria lacca* (Kerr) and *Paratachardina mahdihassani* (Kondo and Gullan) is analyzed using the scanning electron microscope. The studies reveal that first-instar nymphs could be differentiated based on characteristics of head, antennae, labium, brachial plate, anal region and associated structures. Among these the most significant ones of descriptive significance include dorsal setae on head, number and arrangement of antennal sensilla, number of labial sensilla, quinquelocular spiracular pores and pseudospines on brachial plate and anal fringe nature, *i.e.*, sharp, acute setae or broad plates. Six morphologically distinct types of sensilla *viz.*, sensilla trichodea, basiconica, chaetica, coeloconica, campaniformia and flagellate are identified and described. The arrangement of these and their probable functions are discussed relating to morphology and location. The types and arrangement of antennal sensilla are very similar in both the species studied, but the sensilla basiconica subtypes, *i.e.*, sensilla basiconica type 2 (SB2) and sensilla basiconica type 3 (SB3) were restricted only to *P. mahdihassani*.

1. Introduction

The family Tachardiidae (=Kerriidae, Ben-Dov and Lit, 1998) constitutes morphologically distinct scale insects producing a resinous secretion forming a hard covering over the insect. The family includes nine genera and 90 species (Varshney, 2009). These are phloem feeding phytophagous insects with a wide range of hosts. Of these, the species of *Kerria* are known for their good quality, soft, commercially important resinous secretion called shellac, while the resin from those of *Paratachardina* is hard, horny and unsuitable for commercial purposes. Some species of the latter are also serious pests on woody plants, *e.g.*, *P. pseudolobata* (Kondo and Gullan, 2007).

The taxonomy of these scale insects is based on the morphological characteristics of adult females due to their longevity and conspicuousness as compared to their males and immature stages. The immature stages are less studied despite the presence of conserved morphological features and potential to resolve evolutionary relationships (Cook et al., 2000). The first-instar nymphs exhibit features absent in adult females, and little is known on their external morphology (Chamberlin, 1923; Imms and Chatterjee, 1915; Mahdihassan, 1964; Misra, 1931; Kapur, 1962; Kondo and Gullan, 2007).

In the phytophagous insects plant volatiles play an important role in locating the host plant, their acceptance and feeding. The detection of chemical stimuli is accompanied by an array of sensory capabilities, of the olfactory and gustatory sensilla present over the antennae and other insect body parts (Ananthakrishnan, 1988). The exploratory behaviour of hemipteran insects includes walking on plant surfaces allowing differentiation between plants with their antennae pointing forward and labium tapping the surface rapidly (Backus, 1988; Le Ru et al., 1995; Rani and Madhavendra, 2005). The antennal sensilla play an essential role being involved in detecting plant volatiles on the surface during exploration. There are several studies on antennal sensilla and their importance in hemipteran insects (Catala, 1997; Chinta et al., 1997; Le Ru et al., 1995; Onagbola et al., 2008; Rani and Madhavendra, 1995, 2005; Silva et al., 2010); however for lac insects there are only a few, that too light microscopic studies such as for *Kerria lacca* (Imms and Chatterjee, 1915; Kapur, 1962) and *P. pseudolobata* (Kondo and Gullan, 2007).

The present study focuses on the first-instar nymphs of *K. lacca* (Kerr) and *Paratachardina mahdihassani* (Kondo and Gullan), with a view to bring out their external morphology along with details of their antennal sensilla.

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2. Materials and methods

The first-instar nymphs of *K. lacca* and *P. mahdihassani* were collected form Namkum, Ranchi, on *Flemingia macrophylla* and Jarakabande State Forest, Bangalore, Karnataka, on *Pongamia pinnata*, respectively along with the mature adult females. The identification of the first-instar nymphs was established from adult females as given in Varshney (1976) and Kondo and Gullan (2007). The specimens were placed overnight in 10% KOH, to clear the internal body contents, followed by cleaning in distilled water under Leica EZ4 stereozoom microscope at 35×. The specimens were then stained for 40 min using polychromatic stain prepared of phosphomolybdic acid, orange G, aniline blue (WS) with acid fuchsin in distilled water followed by dehydrating in a graded series of ethyl alcohol 50, 70, 90 and 100%, followed by clearing in grades of xylene 30, 50 and 100% and mounting in DPX mountant. Measurements for first-instar nymphs are based on slide mounted specimens using Leica DM1000 compound research microscope with an ocular micrometre. The measurements given are based on a sample ($n = 10$) and are given as range in microns.

For scanning electron microscopic (SEM) studies the first-instar nymphs were fixed in 2.5% glutaraldehyde for 12 h at 4 °C, followed by rinsing in phosphate buffer saline (PBS – 0.1 M, pH 7.2) for 10 min and cleaned in ultrasonic mini cleaner for 30 s. The specimens were then dehydrated in graded series of 70, 80, 90 and 100% ethyl alcohol, and then dried with chemical dryer 1,1,1,3,3,3-hexamethyldisilazane (HMDS). The specimens were then mounted on aluminium stubs with double sided silver tape and sputter coated with palladium (18 nm) in a SC7620 mini sputter coater. Studies were done with Zeiss EVOMA10 scanning electron microscope at 20 kV and 10 Pa between 246× and 105.58 k×.

The terms used to describe the first-instar nymphs are those of Kondo and Gullan (2007) and for antennal sensilla those of Snodgrass (1935), McIver (1975), Altner and Prillinger (1980), Koteja (1980) and Zacharuk (1980). Most of the antennal sensilla of insects are mechanoreceptors, but chemoreceptors also common. The cuticular mechanoreceptors fall mainly into two main categories as – the hair sensilla (sensilla chaetica and sensilla trichodea) and the campaniform or dome-like sensilla (McIver, 1975) whereas chemosensilla includes the uniporous and multiporous sensilla (Zacharuk, 1980). Sensilla often occur singly, but may be grouped together forming functional units (Chapman, 1998). Sensilla chaetica have thick, smooth walls and blunt-tip without a socket, whereas trichodea are smooth walled, curved and pointed with a socket (McIver, 1975; Altner and Prillinger, 1980). Sensilla campaniform are dome or cupola-shaped structures with central dome usually slightly convex (Koteja, 1980). Sensilla basiconica are thick walled, conical and with a sharp-apex whereas coeloconic are thin walled discernible pegs with its bottom inserted into an invagination (Koteja, 1980). Sensilla flagellate are similar to hair sensilla, smooth walled, slender and set into a flexible socket. The subtypes in sensilla trichodea and basiconica are based on length of sensilla.

3. Results and discussion

3.1. External morphology – first-instar nymph

3.1.1. K. lacca: mounted material (Fig. 1A–N)

Body elongate oval, narrowing towards posterior end, 510–640 μm long and 220–280 μm wide (Fig. 1 A and B).

Dorsum. Derm membranous, dorsal segmentation clear, with rugose sculpturing. Dorsal setae present (Fig. 1C), each 32.5–47.5 μm long, supra-anal plate rectangular with a median cleft and anal fringes of short type, continuous and 30–65 μm long surrounding anal ring, each plate 40–55 μm long, 60–70 μm wide; dorsal surface of anal region with a short subapical seta and a very long apical tactile seta 340–525 μm long almost equal to body length (Fig. 1J). Anal ring 6 sectored with 6 anal setae and perisetal pore (Fig. 1K).

Margin. Outline smooth except for indentation near anterior spiracle and on each side of abdominal segments. Eyes located on margins area lateral to each antennal scape (Fig. 1C). Brachial cleft surrounded by 5 bordering setae (Fig. 1G), each 12.5 μm long. Pseudocerarius present on margins ventral to supra-anal plate, each 25–35 μm long, composed of pseudospines with a sclerotized base, each pseudospine 15.0–22.5 μm long (Fig. 1J).

Venter. Derm membranous with rugose sculpturing. Antennae 195–220 μm long (Fig. 1E and F), scape 15–25 μm and pedicel 20–25 μm long with 4 flagellomeres, each measuring, 55–70 μm, 20–30 μm, 20–30 μm and 50–60 μm respectively, 3rd segment being longest. Mouthparts normal; clypeolabral shield 180–205 μm long, 85–100 μm wide; labium 2 segmented, 55–70 μm long, 65–75 μm wide with 6 pairs of sensilla (Fig. 1H). Legs well developed; foreleg: trochanter 30–40 μm, femur 85–95 μm, tibia 65–75 μm and tarsus 60–75 μm long. Midleg: trochanter 25–35 μm, femur 80–100 μm, tibia 70–90 μm and tarsus 70–85 μm long. Hindleg: trochanter 30–35 μm, femur 75–90 μm, tibia 75–95 μm and tarsus 70–80 μm long (Fig. 1I). Tarsal digitules, capitate each 25.0–32.5 μm long. Claws each 25–30 μm long, with a denticle and having a pair of capitate digitule, apex of each digitule slightly surpassing apex of claw (Fig. 1L). Anterior and posterior spiracles similar, 17.5–20.0 μm and 17.5–22.5 μm long, respectively, each with a well developed muscular plate. Spiracular disc-pores present, each pore 2.5–5.0 μm wide with 5 loculi (quinquelocular); pores totalling 6–10 between anterior spiracle and body margin in brachial furrow surrounded by 5 thin bordering setae (Fig. 1G), and 1 spiracular disc-pore anterior to each posterior spiracle. Pairs of long median abdominal setae present on each abdominal segments (Fig. 1M) and a seta on each side on area between fore coxa and mid coxa, and between mid coxa and hind coxa, each seta 32.5–45.0 μm long; with 3 pairs of setae in a longitudinal line extending from interantennal area towards mouthparts (Fig. 1D). Ventral microducts present along margins (Fig. 1N). Submarginal setae slender, of 2 categories, distributed as follows: (i) short setae, each 7.5 μm long, 6 in number anteriorly placed on each side between eyes (Fig. 1C) and, 1 on mid area between each eye and brachial cleft, 1 present of each side of brachial cleft, and (ii) very short setae, present on segmental pairs of abdomen (Fig. 1N).

3.1.2. P. mahdihassani: mounted material (Fig. 2A–N)

Body elongate, oval, narrowing towards posterior end, 460–510 μm long and 200–220 μm wide (Fig. 2 A–B).

Dorsum. Derm membranous, dorsal segmentation clear with rugose sculpturing. Dorsal setae absent (Fig. 2C), supra-anal plate irregularly rectangular with a median cleft and anal fringes short type, *i.e.*, fringes shorter than anal setae, occurring as thick broad plates, continuous, surrounding anal ring, each fringe 20 μm long, anal plate 20 μm long and 40–45 μm wide; dorsal surface of anal region with a pair of short subapical seta and a long apical tactile seta 110–195 μm. Anal ring 4 sectored with 6 anal setae and perisetal pore (Fig. 2J and K).

Margin. Outline smooth except for indentation near anterior spiracle and lower abdominal segments. Eyes located on margins area lateral to each antennal scape (Fig. 2C). Brachial cleft with 3 thick stout bordering setae, each 10.0–12.5 μm long (Fig. 2G). Pseudocerarius present on margins ventral to supra-anal plate 25–40 μm long, composed of pseudospines with a sclerotized base, each pseudospine 17.5–25.0 μm long (Fig. 2K).

Venter. Derm membranous with rugose sculpturing. Antennae 115–140 μm long (Fig. 2E and F), scape 10–15 μm and