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Ultrastructure of antennal and posterior abdominal sensilla in *Chlorophorus caragana* females

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ABSTRACT

Chlorophorus caragana Xie & Wang is a destructive wood-boring beetle that damages *Caragana* spp. bushes and is distributed in desert areas in north-west China. Using scanning electron microscopy and transmission electron microscopy, we observed the morphology and ultrastructure of antennal and posterior abdominal sensilla in *C. caragana* females, to discuss the putative functions of these sensilla in host location and oviposition behaviors.

In total, seven types (24 subtypes) of sensilla were located on the antenna and posterior abdomen. On the antenna, there were Böhm's bristles (BB.); four subtypes of sensilla chaetica (Ch.1–Ch.4) characterized by non-porous surfaces and sensillum-lymph cavities without dendrites; two subtypes of sensilla trichodea (Tr.1 and Tr.2) with a tip pore and dendrites surrounded by dendritic sheaths; dome-shaped sensilla (Dom.) emerging from a deep cavity with one tip pore; four subtypes of sensilla basiconica (Ba.1–Ba.4) and one type of sensilla auricillica (Au.) with a porous cuticular surface and dendrites in the sensillum-lymph cavity; and one type of sensilla styloconica (Sty.) with grooves on the cuticular wall. On the posterior abdomen, there were four subtypes of sensilla chaetica (Ch.5–Ch.8); three subtypes of sensilla trichodea (Tr.3–Tr.5); and three subtypes of sensilla basiconica (Ba.5–Ba.7; Ba.5 had no groove in the cuticular wall, Ba.6 had one tip pore, and Ba.7 was located in a cuticular cavity). The antennal sensilla were believed to be mechanosensitive, chemosensitive, and sensitive to humidity and temperature, and to play roles in mating, host location and oviposition. The abdominal sensilla are believed to be related to oviposition behaviors.

1. Introduction

Chlorophorus caragana Xie & Wang (Coleoptera: Cerambycidae) is a newly discovered beetle species occurring in the arid and semiarid regions of north-west China that mainly damage *Caragana davazamcii* Sancz. (Fabales: Papilionaceae) and *Caragana microphylla* Lam. (Fabales: Papilionaceae). Female individuals of *C. caragana* lay eggs in bark crevices on the plants without making oviposition scars. After hatching, the larvae bore into trunks and branches. Previous research on the species has focused mainly on its ecology and on plant-derived attractants (Zong et al., 2012, 2013).

The choice of oviposition site directly affects the development of the next generation of insects (Bovill et al., 2013; Fatouros et al., 2012; Liu et al., 2012; Masaki, 1986; Van Loon, 1996; Woodcock et al., 2013; Zhang et al., 2012). Female insects locate hosts by means of complicated behaviors in which sensilla play important sensing roles. For many insects, the antenna are important for olfaction, and sensilla distributed on the ovipositors are often used in oviposition behavior (Rice et al., 1973; Smallegange et al., 2008; Spänhoff et al., 2003a; Spiegel et al., 2005; Sukontason et al., 2007).

Research on morphology and ultrastructure of sensilla has mainly been focused on the insect orders Diptera, Lepidoptera, Hymenoptera, Coleoptera, etc. (De Kramer, 1985; Hunger and Steinbrecht, 1998; Klein, 1987; Merivee et al., 2002; Ozaki et al., 2005; Phillips and Vande Berg, 1976; Ren et al., 2012; Shanbhag and Singh, 1992; Shigekazu and Kuwabara, 1965; Yokohari, 1983; Zhang et al., 2013a). Studies of sensillar morphology and ultrastructure in Coleoptera have involved insects in the families Curculionidae, Chrysomelidae, Buprestidae, and Scarabaeidae. Little research has been focused on the family Cerambycidae (Bartlet et al., 1999; Chamorro et al., 2012; Crook et al., 2008; Dai and Honda, 1990; Dyer and Seabrook, 1975; Farazmand and Chaika, 2012; Jourdan et al., 1995; Kim and Yamasaki, 1996; Lopes et al., 2002; Liu et al., 2012; Merivee et al., 1999, 2000, 2010; Romero-López et al., 2013; Saïd et al., 2003; Srivastava, 2003). Only a few authors have studied sensilla on insect ovipositors.

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In this study, we used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to observe the morphology and ultrastructure of antennal and posterior abdominal sensilla of *C. caragana* females. We discuss the putative functions of these sensilla in host and oviposition site location behaviors to provide theoretical evidence for oviposition mechanisms, and to enhance knowledge of the regulation of chemical ecology in this long-horned beetle.

2. Materials and methods

2.1. Insects

Adults of *C. caragana* were lured into field traps using plant attractants in Ling-wu County (38.05′ N, 106.30′ E), Ningxia Hui Autonomous Region, P. R. China, and collected during July and August 2012. Only complete and clean adult *C. caragana* bodies were used.

2.2. Scanning electron microscopy

Healthy *C. caragana* females were fixed in 2.5% glutaraldehyde (Agar Scientific Ltd., Plano GmbH, Wetzlar, Germany) in phosphate-buffered saline (PBS) (0.1 M, pH 7.2) with 0.06% Tween 20 (Sigma Chemical Company) (PBST) for 20–24 h. Carbon tetrachloride was then used to clean the specimens. The antennae and ovipositors of female beetles were dissected at the basal region and cleaned sequentially using PBST and then ethanol (40%) in an ultrasonic wave cleaner, each for 380 s. The samples were dehydrated with a graded series of ascending ethanol concentrations. After air-drying, the samples were fixed onto a stub with double-sided carbon adhesive tape (SPI Supplies Division of Structure probe, Inc.) and sputter-coated with gold three times during a 45 s period while rotating the stub (E-1010, Hitachi, Tokyo, Japan). A Hitachi S-3400N SEM was used to observe the samples at 10–25 kV.

2.3. Transmission electron microscopy

The antennae and ovipositors of female beetles were dissected at the basal region and fixed in 2.5% glutaraldehyde in PBST (0.1 M, pH 7.2) for 20–24 h. After washing in PBST for 3 h, the samples were post-fixed in 1.0% osmium tetroxide (Simec, Zofingen, Switzerland) in PBS at 25 °C and then washed carefully with PBST. The samples were dehydrated using a graded series of ethanol solutions and embedded in Spurr's resin. Different types of sensillum were pre-located under an MD2500 luminescence microscope. Ultra-thin sections measuring 60–100 nm were cut using a Leica UC6 ultra-microtome and mounted on Formvar-coated 100 mesh copper grids. The samples were double-stained with 1% uranyl acetate for 20 min, and lead citrate for 10 min, then observed using a TEM (Hitachi model H-7500) at 80 kV.

2.4. Data analysis

Sensilla were identified based on their appearance and size, and the naming systems of Schneider (1964) and Zacharuk (1985) were applied. The ultrastructure of sensilla was described based on the research of Tarumingkeng et al. (1976), Zacharuk (1980) and Shanbhag et al. (1999).

The length, the base diameter and the size of each sensillum were measured by using Image J (U.S. National Institute of Health). At least 30 sensilla of each type were measured. For all the data, anterior view SEM pictures were used to avoid the impact of shooting angle on data. Pictures were processed with Photoshop CS5 (Adobe).

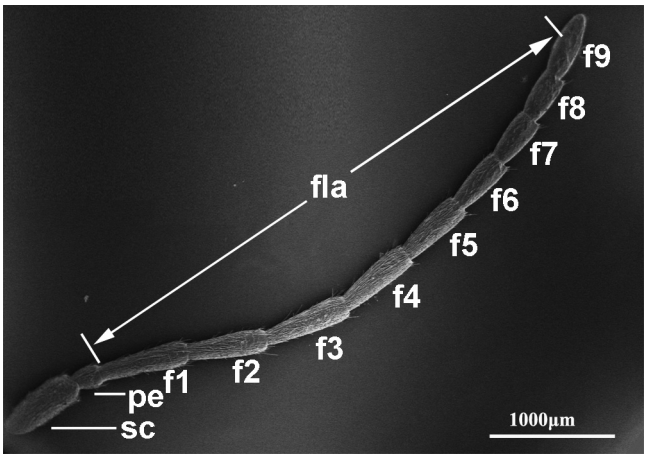

Fig. 1. Female antenna of *C. caragana* under SEM. Showing antenna, consisting by scape, pedicel and 9 flagellomeres. Fla, flagellum; f1–f9, 1st till 9th flagellomeres; pe, pedicel; sc, scape.

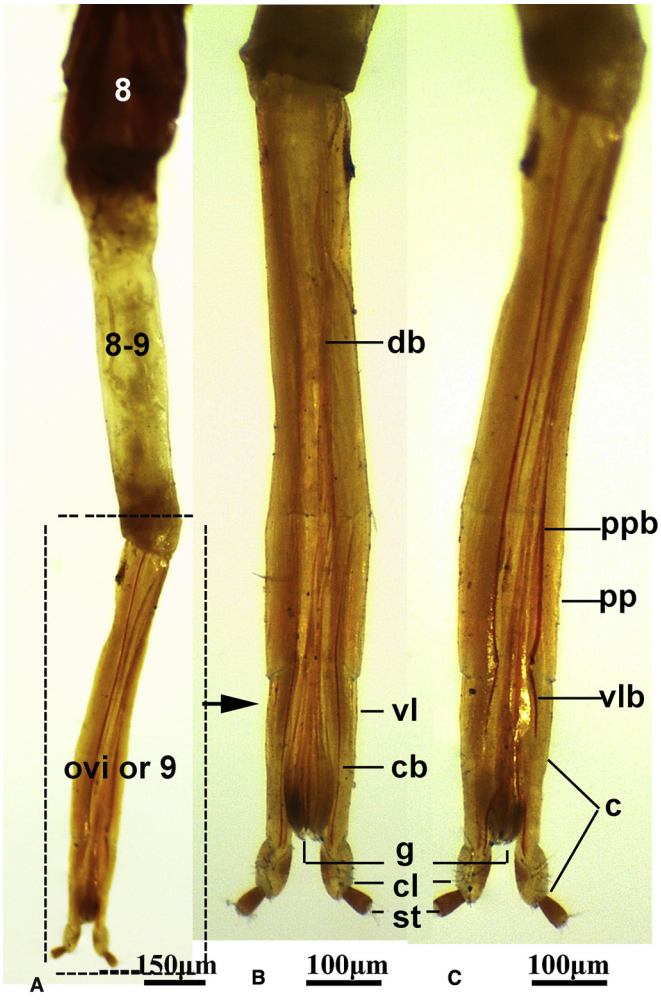

Fig. 2. Posterior segments of the female abdomen under light microscopy (elongated status). (A) The 8th and 9th abdomen, the inter-segmental membrane between 8th and 9th abdomen, and the ovipositor. (B) Dorsal view of the ovipositor. (C) Ventral view of the ovipositor. 8, 8th segment; 8–9, inter-segmental membrane of 8th and 9th segments; 9, 9th segment; c, coxite; cb, coxite baculum; cl, coxite lobe; db, dorsal baculum; g, genopore; ovi, ovipositor; pp, paraproct; ppb, paraproct baculum; st, stylus; vl, valvifer; vlb, valvifer baculum.