



# Morphology of the mouthparts of the spittlebug *Philagra albinotata* Uhler (Hemiptera: Cercopoidea: Aphrophoridae)



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## ABSTRACT

Mouthparts associated with feeding behavior and feeding habits are important sensory and feeding structures in insects. To obtain a better understanding of feeding in Cercopoidea, the morphology of mouthparts of the spittlebug, *Philagra albinotata* Uhler was examined using scanning electron microscopy. The mouthparts of *P. albinotata* are of the typical piercing–sucking type found in Hemiptera, comprising a cone-shaped labrum, a tube-like, three-segmented labium with a deep groove on the anterior side, and a stylet fascicle consisting of two mandibular and two maxillary stylets. The mandibles consist of a dorsal smooth region and a ventral serrate region near the apical half of the external convex region, and bear five nodules or teeth on the dorsal external convex region on the distal extremity; these are regarded as unique features that distinguish spittlebugs from other groups of Hemiptera. The externally smooth maxillary stylets, interlocked to form a larger food canal and a smaller salivary canal, are asymmetrical only in the internal position of longitudinal carinae and grooves. One dendritic canal is found in each maxilla and one in each mandible. Two types of sensilla trichodea, three types of sensilla basiconica and groups of multi-peg structures occur in different locations on the labium, specifically the labial tip with two lateral lobes divided into anterior sensory fields with ten small peg sensilla arranged in a 5 + 4 + 1 pattern and one big peg sensillum, and posterior sensory fields with four sensilla trichodea. Compared with those of previously studied Auchenorrhyncha, the mouthparts of *P. albinotata* may be distinguished by the shape of the mandibles, the multi-peg structures and a tooth between the salivary canal and the food canal on the extreme end of the stylets. The mouthpart morphology is illustrated using scanning electron micrographs, and the taxonomic and putative functional significance of the different structures is briefly discussed.

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## 1. Introduction

Cercopoidea, the second most species-rich superfamily in the Cicadomorpha, comprises approximately 3000 described species. The superfamily has been classified into five families: Cercopidae, Aphrophoridae, Clastopteridae, Machaerotidae and Epipygidae (Cryan and Svenson, 2010). Adults feed on the leaves and stems of a variety of plants by inserting their maxillary stylets into the xylem elements and suck the sap (Leopold et al., 2003). The saliva of some species, such as the sugarcane spittlebug, *Mahanarva fimbriolata*, contains toxic enzymes and feeding by these insects on plants may

result in the blockage of conducting channels causing desiccation of the leaves (Nunes and Camargo-Mathias, 2006).

Mouthparts are important sensory and feeding structures in insects and their structure is closely associated with feeding behavior and feeding habits (Ma et al., 2013). Understanding the structure of mouthparts and accompanying sensilla is relevant, not only in host plant selection studies, but also in the study of feeding behavior and sensory physiology (Rani and Madhavendra, 2005). In addition, because mouthpart structures may be characteristic of all members of a genus, family or order of insects, knowledge of mouthpart morphology is useful for classification and identification (Gullan and Cranston, 2005). Previous work on the ultrastructural morphology of Hemiptera mouthparts, using light and scanning electron microscopy, has mainly focused on Heteroptera (Anderson et al., 2006; Boyd, 2003; Cobben, 1978), Aphidoidea (Forbes, 1969;

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Pollard, 1973; Razaq et al., 2000), Psyllidae (Garzo et al., 2012; Liang et al., 2013) and Aleyrodidae (Rosell et al., 1995; Walker and Gordh, 1989) of Sternorrhyncha, and Cicadellidae (Tavella and Arzone, 1993; Leopold et al., 2003; Wiesenborn, 2004; Zhao et al., 2010; Ammar and Hall, 2012) and Fulgoroidea (Brentassi and de Remes Lenicov, 2007; Dai et al., 2014; Liang, 2005) of Auchenorrhyncha. Apart from some brief notes on the stylets of *Philaenus spumarius* (L.) (Pollard, 1971) and *Notozulia entreriana* (Berg) (Paladini et al., 2008), and the interlocking pattern of maxillae and mandibles of *Aphrophora alni* (Fallén) (Cobben, 1978), fine structure of the mouthparts in spittlebugs has received little attention. Information on the distribution of the sensilla on the mouthparts, the relationship between mouthpart structure and function in feeding and the utility of this information for classification of spittlebugs is not available.

In the present work, the mouthpart morphology of *Philagra albinotata* Uhler is described to provide comparative data elucidating variation within the family, contribute to knowledge of feeding behavior, and also to reveal characters that may be useful for future studies in taxonomy and phylogeny of Auchenorrhyncha, especially within Cercopoidea.

## 2. Material and methods

### 2.1. The insect

Adult *P. albinotata* were collected at Huoditang in Ningshan, Shaanxi Province, China in July 2011, using a sweep net and preserved in 75% ethanol. Voucher specimens were deposited in the Entomological Museum of Northwest A&F University, Yangling, Shaanxi Province, China.

### 2.2. Sample preparation for SEM

The heads of randomly selected five female and male specimens respectively were freed from the body. The mouthparts were excised and dissected using fine dissecting needles under 40× magnification (Nikon SMZ 1500, stereomicroscope, Japan). Specimens were then fixed with 2.5% glutaraldehyde for 12 h at 4 °C, followed by washing in phosphate buffered saline (PBS, 0.1 M, pH7.2) for 15 min four times and cleaned in an ultrasonic cleaner for 15 s three times before dehydration in a graded series of 30%, 50%, 70%, 80%, 90% and 95% ethanol for 20 min each and 100% ethanol for 30 min twice. Specimens then underwent replacement in the mixture of 100% alcohol and 100% tertiary butyl alcohol mixed in sequential ratios (3:1, 1:1 and 1:3 by volume) for 15 min each time. Samples were then soaked in 100% tert-Butanol for 30 min before being placed into a freeze-drier with liquid CO<sub>2</sub> for 3 h. Thereafter, the samples were mounted on aluminum stubs with double-sided copper sticky tape and sputtered with gold/palladium (40/60) in a LADD SC-502 (Vermont, USA) high resolution sputter coater. The samples were subsequently examined with a Hitachi S-3400N scanning electron microscope operated at 15 kV (Zhao et al., 2010).

All measurements are given as mean ± S.E.

## 3. Results

### 3.1. Gross morphology of mouthparts

As in other Auchenorrhyncha, the mouthparts of *Philagra albinotata* arise from the posteroventral part of the head capsule (Fig. 1A) and are typical piercing–sucking form (Fig. 1A and B) composed of the labrum (Lm) (Figs. 1B and 2B), a tube-like labium (Lb) (Fig. 1) and a stylet fascicle (Sf) consisting of two mandibular

(Md) and two maxillary stylets (Mx). The three-segmented labium has a longitudinal groove in the middle of the venter, called the labial groove (Lg) (Fig. 1B), which envelops the stylet fascicle comprising two inner maxillary stylets partially surrounded by two shorter and serrate-edged mandibular stylets, and proximally by the small cone-shaped labrum. The surface of the labium has different types of sensilla symmetrically arranged on both sides of the labial groove or positioned on the labial apex (Fig. 1B).

### 3.2. Labrum

The cone-shaped labrum (Lm) is very short ( $411 \pm 6 \mu\text{m}$ ,  $n = 5$ ) (Fig. 2A and B), and is attached to the anterior margin of the anteclypeus and overlies the 1st and 2nd labial segments (Figs. 1B and 2A). The surface of the labrum is covered with a few scattered short triangular spines (Fig. 2C). Some clusters of pegs on the lateral edge and the terminus are more distinct and palmate (Fig. 2C).

### 3.3. Labium

The labium (Lb) (also known as the rostrum or proboscis) is tubular in shape and composed of three segments (Fig. 1C). The anterior surface of the labium is bisected by a deep labial groove (Lg) (Fig. 1B), extending its entire length, enclosing two mandibular and two maxillary stylets. Sensilla are mainly distributed symmetrically on each side of the labial groove (Lg) and distally, but fewer occur on the dorsal and lateral surface (Fig. 1B and C). The tip of the labial segment is flattened with an opening from which the apices of the stylets are extended (Figs. 1B, C, and 5B).

The total length of the labium is  $2109 \pm 20 \mu\text{m}$  ( $n = 5$ ). It is broad and of uniform width through most of its length with the distal segment widening near the tip. The first labial segment is the shortest ( $567.3 \pm 9.6 \mu\text{m}$ ,  $n = 5$ ) with most of it concealed by the overlapping anteclypeus (Fig. 1B). There are scarcely any sensilla trichodea I on this segment (Fig. 1C).

The second labial segment is the longest ( $781 \pm 17 \mu\text{m}$ ,  $n = 5$ ) of the three segments (Figs. 1C and 2A), with a small round tumid area close to the first segment (Fig. 3A). There are two types of sensilla, sensilla trichodea I and sensilla basiconica I. Most sensilla trichodea I (s.t.I) are arranged symmetrically on each side of the labial groove (Fig. 2A and D) and on the lateral surface, and a few are arranged on posterior surface near the junction of the middle and third segments (Fig. 3A). Sensilla trichodea I, ranging from  $75 \mu\text{m}$  to  $140 \mu\text{m}$  in length, are slender and slightly curved with pointed tips. The surface has longitudinal grooves that spiral slightly around the shaft, and gradually converge on the obverse side, gradually disappearing near the distal part (Fig. 2D and E). Sensilla basiconica I (< $30 \mu\text{m}$  in length) are short, stout basiconic pegs with a blunt tip, with a minute longitudinal groove in the shaft, arise from sunken pits (Fig. 2D), and are randomly distributed on the ventrum and dorsum of the labium. Numerous clusters of short denticles are scattered over the anterior surface of second segment (Figs. 2A and 3A).

The third labial segment is  $761 \pm 5 \mu\text{m}$  ( $n = 5$ ) long, tubular, of uniform width from base to apical 1/4 then widens to the apex (Figs. 1C, 3B and D). There are some sensilla trichodea I and several sensilla basiconica I arranged on each side of the labial groove and randomly distributed on the dorsum of the labium (Fig. 3B, D, F and G). Two sensilla basiconica II are present on each side of the junction of the second and third segments (Fig. 3D and E).

The labial tip forms two lateral lobes separated by the anterior stylet groove and posterior groove. Each lateral lobe has an anterior sensory field located laterad of the stylet groove and a posterior sensory field located behind the stylet groove (Fig. 4C). Each

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