Morphological observation and characterization of the *Pseudoregma bambucicola* with the scanning electron microscope

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**A B S T R A C T**

Both leica microscopic camera system and scanning electron microscopy was used to observe and characterize the feet, back, abdomen, antennae and mouthparts of the *Pseudoregma bambucicola* from the bamboo, *Bambusa multiplex*. The possible functions of all the external morphological characteristics of the *P. bambucicola* were described and discussed in detail, which offers a basis for further enriching the biology, phylogeny and ecological niche of the *P. bambucicola*. Moreover, the morphological results should contribute to morphological identification and differentiation of the *P. bambucicola* from other aphids in the same family.

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**1. Introduction**

*Pseudoregma bambucicola* (Homoptera: Hormaphididae) is an elliptical polyplide, with 3 mm in length and 2 mm in width of adult. The body is dark brown and covered with white wax powder. *P. bambucicola* reproduction is apomixes, thus it has strong reproductive capacity and presents a fixed damage. In addition, the aphid is a typical phytophagous insect which can cause the physiological lesions of plants by blocking and affecting normal photosynthesis and respirations. Often, the *P. bambucicola* mainly infects the twigs and stems of the bamboo and carries numerous important plant viruses. Its infection not only affects normal leaves crimpling but also induces stoody molds or causes bamboo dead hardest. Currently the researches of this aphid mainly limit in the ecology, behavior and chemical control. No literature was reported about morphological and structural characteristics of the *P. bambucicola*. Therefore, this study described and preliminary identified the possible functions of the external structures of the *P. bambucicola* by scanning electron microscopic observation. These results may lay the foundation for further studies of the phylogeny and morphologies of the *P. bambucicola*, and provide evidence for the morphological differentiation of the *P. bambucicola* from other aphids in the same family by some organs.

**2. Materials and methods**

**2.1. Ethics statement**

No specific permissions were required for the activities conducted in this study. The location is neither privately-owned nor protected. The experiments did not involve endangered or protected species.

**2.2. Pseudoregma bambucicola**

The *P. bambucicola* were collected from the *Bambusa multiplex*, which plants in Leshan city, Sichuan province, China.

**2.3. Preparation and observation of specimens**

About (number) of *P. bambucicola* were immersed in 70% ethanol for 12 h, and then in the carbon tetrachloride solution for 52 h. All immersed aphids were transferred in 100%, 90%, 80% and 70% ethanol, respectively, for 30 min per each change; 70% ethanol was used as medium to clean for ultrasonic purpose. Afterwards, 70%, 80%, 90% and 100% ethanol were employed to dehydrate the
aphid specimens successively, 30 min per each change, and then sticking table and spraying gold were carried after the critical point drying. Finally, the specimens were subjected to observation of morphological ultra-structures under a scanning electron microscope and the Leica microscopic camera system was used for observing the whole aphid, and taking photographs of all the parts of polypides.

3. Results

3.1. Mouthparts

The *P. bambucicola* has typical sucking mouthparts which grow in the ventral back of head, and beak is thick and short. The lower lip extends and forms a beak, which covers the scalpella, and the inside of beak caves and forms the oral groove (Fig. 1). Both sides of the oral groove which connect with the base at the top have same punctiform protuberances in size, and extend to the bottom of the groove. The *oral groove* used to protect stylets which places in the oral groove without feeding. In addition, the beak is cylindric with 109 μm length, and divided into two palpable elbows which have obvious distinction in texture. The section nearby the base/C24 37 μm, and the section close to the tip/C24 72 μm. The diameter becomes small from base to tip gradually and reaches to oval groove appeared constriction, and its back become smaller (see Figs. 2 and 3).

The receptors on the mouthparts of *P. bambucicola* mainly distribute on the surface of beak, with three types: Sensilla basiconic, Sensilla chaetica and Sensilla trichodea, particularly, the distribution of axial symmetry on the surface of the beak. According to Schnieder (1964) nomenclature (Xuqing et al., 2016; Zhao et al., 2012). There are two (one per side) Sensilla trichodea close to the root and six (three per side) nearby the top. Moreover, the surface of sensilla trichodea is smooth, and it turns to fine near top gradually with curved growth. The basal part of the Sensilla trichodea vertically grows in the collar protrusion pit of the high about 1 μm, inconsistent with the feature description of Sensilla trichodea in Schnieder. The reason may be involved with the study object of Schnieder is Lepidoptera insect antennae (Zhao et al., 2012). The Sensilla basiconic is distributed in the top of beak. Moreover, it is symmetrical distribution with the oral groove and scalpella as the axis. Two flanks are sixteen (eight each side) with rough surface and grow in the shallow depression with a diameter of 1 μm deep. Specially, it becomes thin from base to top inch by inch. In addition, there are two Sensilla chaetica (one each side) at the oral groove with the base and top intersection. The surface of Sensilla chaetica is smooth with vertical growth and no winding.

However, compared with the mouthparts of *Myzus persicae* with Winged parthenogenetic, the beak of *P. bambucicola* is palpable shorter than Winged parthenogenetic *M. persicae*. The beak of Winged parthenogenetic *M. persicae* is ligulate and whose long about 280 μm. Besides, there is an obvious difference in the texture and the part near the base that is ~140 μm length with a large of irregular punctiform bulge. The surface of beak is symmetrical distributed with twelve Sensilla trichodea, and the section near the top is eight, the base section is four (Zhao et al., 2012).

3.2. Antenna and frontal elevation

There are numerous sensillum in the antenna of aphids, including primary rhinarium, secondary rhinarium, Johnston’s organ, Campaniform sensillum, trichoid sensillum, joint receptors, and sixth segment.

The antenna of the *P. bambucicola* is consisted of five parts: two sections near the root with the first wider than the second, and the fifth part is divided into base and whip that the whip is shorter than the base (Huang and Qiao, 2006). The antenna of all the aphids has primary rhinariums, and the whip and the fifth section have a primary rhinariums for each. A sensilla trichodea, a sensilla basiconic and others are on the antenna. Moreover, the frontal ele-

Fig. 1. The ultrastructure of mouthparts of the *P. bambucicola*.
vation is located on top of the head and between the antennae, and it is conical protuberance with 90.96 μm height. The diameter of the top of frontal elevation is 6.689 μm and the base diameter is about 47.39 μm. There are a large number of hairs on the frontal elevation 9.735 μm length.

The antenna of the *P. bambucicola* is four or five sections, both the Astegopteryx minuta and Cerataphis bambusifoliae Takahashi are five sections, but the Chaitoregma tattakana and Doraphis populi all are four sections. Howbeit, they are short and thick.

Compared the aphids in foushou-ensiform gallnut with the *P. bambucicola*, the sensillum of the *P. bambucicola* is less than the aphids in foushou-ensiform gallnut. Besides, the antenna sensillum of the aphids in foushou-ensiform gallnut are isolated circular or oblong rhinarias, and the size of the rhinarias is not uniform. Notably, the number of every section is different, and the third section is more than the fourth, fifth and sixth.

### 3.3. Abdomen

The abdomen is composed of 8 abdominal segments, and epidermal membranous has a tile pattern, the spacing of each 1.714–4.540 μm length. Scattered numerous feather with 34.44 μm on the abdomen. Furthermore, the cornicle is cylindrical, the caudae formed by the ninth abdominal segment specialization, and a long conic with a few of hairs on the edge (Figs. 4 and 5).

The cornicle of Takecallis arundinariae, Callaphididae, is short tube, and the Abdominal tergite I-VII each have a pair of longitudinal spots. Besides, the cornicle of Takecallis taiwanus also is short tube, but the Abdominal tergite I – V each have a pair of tumor. The cornicle of Cetatovacuna silvestrii is conical, the cornicle of Cerataphis bambusifoliae annular and the Abdominal tergite VIII is semilune, the cornicle of Chaitoregma tattakana truncation (Liu et al., 2016; Xie et al., 2016; Fang et al., 2006; Zhao and Ban, 2011). However, the cornicle of *P. bambucicola*, Hormaphididae, is shorter than others.

### 3.4. Foot

The aphids have three pairs of feet which is composed of the paturon, meropodium, throchanter, tibiae and digitus. Moreover, the foot is slightness, and the meropodium is about 584.1 μm length with a large number of hairs about 42.65 μm length. In
addition, the length of tibiae is about 878.9 μm, the meropodium is thicker than the tibiae and the digitus are two that about 200 μm.

The ratio of length scale of digitus II and I of foot is similar in 2.61–3.22 times (Fang et al., 2006) with other aphids in Hormaphididae, such as the Astegopteryx bambusifoliiæ, Astegopteryx minuta, Chaitoregma tattakana, Cerataphis bambusifoliiæ and Cetatovacuna silvestrii. The forms of digitus, claw and others are similarity (Fig. 6).

4. Discussion

In the long evolutionary process, the aphids are constantly adapting to the environment and host plants so that emerging all kinds of aphids in the same parts of the same plant, showing strong adaptability on the morphological characteristics of mouthparts, foot and other feeding and adhering. Therefore, the aphids have good adaptability to different host plants (Fang et al., 2011; Feng et al., 2016).

Phenotypic plasticity is the ability of an organism to exhibit different phenotypes when adapts to a biological or non biological environment, and has the genetic basis. The aphid is susceptible by its own genetic factors and the impact of external environmental factors to show phenotypic plasticity (Lei et al., 2000; Li et al., 2015). In the aphids, the end of beak is nearly triangular, nearly square, short blunt, wedge, Lance shaped and so on, and the end of beak of the *P. bambucicola* is short blunt. The mouthparts of the aphid are typical sucking mouthparts, and determine the feeding behavior by the sensilla on the mouthparts feel the information of feeding sites. Moreover, Bromley et al. described the presence of mechanoreceptors and chemoreceptors on the antennae of the Myzus persicae (Chen et al., 2006; Bromley and Dunn, 1980). Its function may be to feel and probe the characteristics of the host. The beak of the *P. bambucicola* is significantly shorter than the the alate viviparous female of *M. persicae*, and the number of Sensilla trichodea is significantly less than the alate viviparous female of the *M. persicae*. Besides, in the feeding process of the insects with sucking mouthparts, the twitching of the scalpellum is controlled by the deformation of the muscles and beak of scalpellum base. Therefore, the receptor of rostral surface may also be used to determine the degree of deformation of the beak so that control the depth of the mouthparts sucking (Wensler, 1977; Forbes, 1977; Liu and Liu, 2010). Importantly, this study describes the external morphological characteristics of the *P. bambucicola* in detail, and provides a basis for further researching the phylogeny and morphological characteristics of the *P. bambucicola*.

5. Authors’ contributions

XN and XMZ performed the majority of the study and analyzed the data, and contributed to drafting of the manuscript. ZL, LJL and
CBL contributed to partial study and discussion. MT contributed to partial analysis of the data. YJY conceived and designated the study plan, participated in all aspects of the study, provided funds, supervised the research. All authors read and approved the final manuscript.

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