

Ultrastructural Morphology of Tarsus of *Aspidomorpha dorsata* (F.) (Coleoptera: Hispididae)¹

Xin Bian,^{2,3} Zheng Liu^{2,3} and Ai-Ping Liang^{2,4}

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Abstract The ultrastructural morphology of the tarsus of the adult beetle, *Aspidomorpha dorsata* (F.) (Coleoptera: Hispididae), is described for the first time, using scanning electron microscopy observations. The tarsus of *A. dorsata* consists of 5 tarsomeres and the distal pretarsus consists of a pair of ungues. The foretarsus and midtarsus are similar to each other whereas the hindtarsus exhibits specialization. The ungues, which articulate dorsally at the distal end of the tarsus, are broad basally (about 169 - 178 µm long and 126 - 133 µm wide), gradually taper into an acute apex over apical half, with the surface of the ungues being rough dorsally and longitudinally grooved innerly and ventrally. There are three small, parallel, accessory ungues (about 35 - 80 µm long) on the ungues. The surface of the accessory ungue is smooth. The ventral surface of the first, second and third tarsomeres is covered with dense adhesive setae. Each seta consists of two parts: a setal shaft and a modified apex (terminal plate). Four types of adhesive setae, viz. the pointed setae, the spatulate setae, the discoidal setae and the drop-shaped setae, are recognized based on the shape of the setal tip. The pointed setae are about 29 - 39 µm long, have acute, pronged and curved tips and are found on tarsomeres I and II of the hind legs and on the edge of tarsomeres I and II of the fore and middle legs. The spatulate setae are about 34 - 55 µm long, have spatulate, pronged and curved terminal plates and are present on tarsomere III of three pairs of legs. The discoidal setae are about 23 - 24 µm long, have discoidal tips and are found on tarsomeres I and II of fore and middle legs. A few drop-shaped setae are found on tarsomere II of three pairs of legs. The distribution of the 4 types of setae is different on the 3 pairs of legs. This suggests that the fore, middle and hind legs exhibit different adhesive functions during the climbing.

Key Words *Aspidomorpha dorsata*, Hispididae, pretarsus, ultrastructure, scanning electron microscopy

The Insecta include the most prospering species in the earth, and they are able to move and climb on various natural surfaces with foot pads which have particular adhesive ultrastructures (Gladun and Gorb 2007). The tibia, tarsus and pretarsus are important appendages for locomotion in insects. The high efficiency attachment system on their legs allows the insects to adhere to the different natural surfaces and has attracted many scientists for several centuries since the understanding of the structure of the insect legs. Their attachment mechanism will be useful to the research of robots and the development of adhesive materials (Chen and Gao 2007).

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²Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, P.R. China.

³Graduate University of Chinese Academy of Sciences, Beijing 10049, P.R. China.

⁴Corresponding author. E-mail: liangap@ioz.ac.cn.

Among insects, the structure and morphology of the adhesive appendages have evolved into different types (Arzt et al. 2003). Previous studies have revealed 2 general kinds of attachment systems in insects: the hairy attachment system, such as beetle pads (Stork 1983), and the smooth attachment system, such as the arolia and euplantulae of grasshoppers (Slifer 1950, Kendall 1970).

The Coleoptera is the largest group of insects, and beetles live almost everywhere. The beetles are good at grasping their host plants with the ventral soles of the tarsus. Yet, the tarsal ultrastructure has been studied on only very limited beetle species (Stork 1980a, Betz 2003, Liu and Liang 2013).

Aspidomorpha dorsata (F.) (Coleoptera: Hispididae) is a common beetle species widely distributed in southwest China (Yunnan, Hainan and Guangxi Provinces) and in some other countries in southeastern Asia (Chen 1986). The species is harmful to many economic crops and ornamental plants such as rice (*Oryza sativa* L.), maize (*Zea mays* L.), sugarcane (*Saccharum* sp.) and *Ipomoea* sp. (Gressitt 1952, Medvedev and Dan 1982, Reid 1998). Both males and females of this species are good at grasping the leaves and moving on the surface of leaves.

In our study of the leg morphology of the beetle species, the ultrastructure of the tarsus of *A. dorsata* was examined with the scanning electron microscope. This paper presents descriptions of the tarsal ultrastructures of male *A. dorsata* for the first time. Information on the external morphology, number, density, measurements, and distribution of the adhesive setae found on the tarsus of the species are provided.

Materials and Methods

Specimens. Two dried, pinned museum specimens of adult males, collected at Mengla County, Yunnan, China on 6 September 2004 were observed with scanning electron microscopy. The specimens were obtained from the Zoological Museum, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Scanning electron microscope (SEM). The dried specimens were intenerated and then cleared in the 2% PBS (phosphate buffer saline). The tarsi were removed from the body under the stereomicroscope, dehydrated in an ascending series of ethanol, critical-point-dried, glued on the sample stage with carbon adhesive tape, sputter-coated, and examined with the Quanta 200 (FEI Co. Ltd., Oregon, USA) scanning electron microscope, operated at an accelerating voltage of 10 kV at room temperature. The size and the number of the unguis and setae were measured and analyzed with the Image-Pro Plus 6.0. The morphological terminology used in this paper follows those of Beutel and Gorb (2001) and Gorb and Beutel (2001).

Results

Gross morphology of the tarsus. As in other species of Coleoptera, *A. dorsata* has adhesive pads with dense tenent setae which are distributed on the ventral surface of the tarsus (Stork and Evans 1976, Eisner and Aneshansley 2000). The tarsus of *A. dorsata* consists of 5 tarsomeres and 2 unguis (Figs. 1A–C). Only 4 tarsomeres are visible, and the ventral surfaces of the basal 3 tarsomeres are covered with adhesive setae.

The proximal tarsomere I is the smallest and approximate spherical with discoidal and pointed setae. Tarsomere II is bigger and looks like a trapezoid and is covered with several kinds of setae. The distal tarsomere III is covered with the spatulate setae

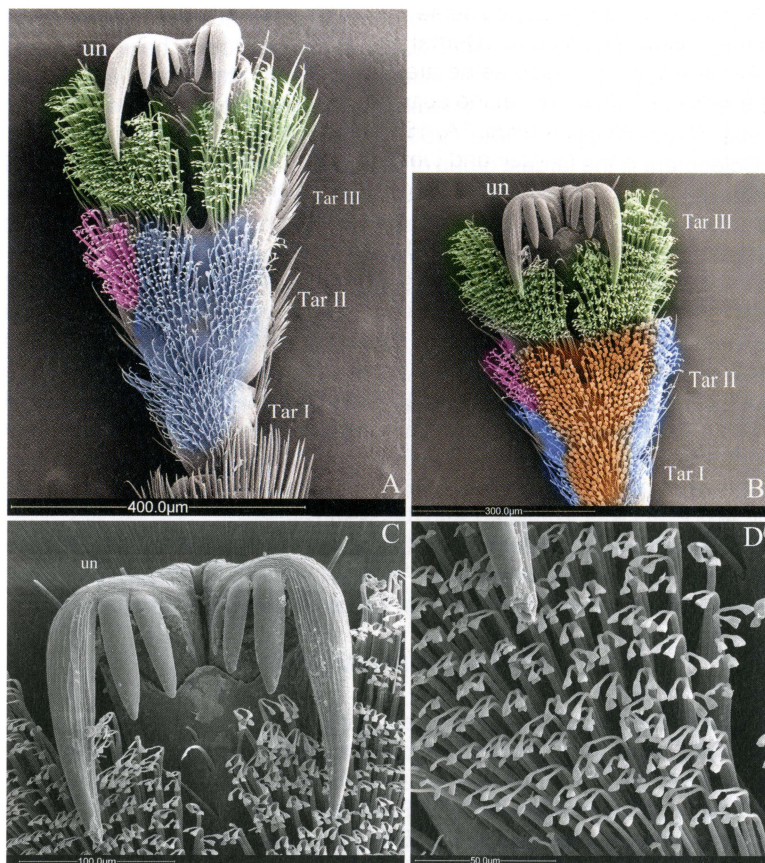


Fig. 1. Scanning electron micrographs of tarsus of *A. dorsata*. A, B. Tarsi of hind and fore legs, ventral view, showing the external morphology of three attachment pads and unguis. The color of orange indicates the discoidal setae, blue indicates the pointed setae, green indicates the spatula-tipped setae and pink indicate the drop-shaped setae C. Ventral view of unguis. D. Array of spatula-tipped and pronged setae on the Tar III of fore and hind legs, ventral view, showing the morphology and structure of this type of setae. Abbreviations: un, unguis; tar I, the first tarsomere; tar II, the second tarsomere; tar III, the third tarsomere.

(Figs. 1A, 1B). The unguis are broad basally and gradually taper into an acute apex. There are 3 pairs of accessory unguis located on the inner, basal edge of the unguis (Fig. 1C).

Tenant setae types and distribution. Four types of tenant setae - pointed setae, spatulate setae, discoidal setae, and the drop-shaped setae - were observed on the tarsal tarsomeres of *A. dorsata* (Figs. 1 - 3). The former 2 kinds of setae are bent downward and are pronged (Figs. 1D, 2C-F). The discoidal setae are not pronged

(Figs. 3A, 3B). A few drop-shaped setae were also observed (Figs. 3C, 3D). The tips of these setae exhibit a different external morphology.

Pointed setae. The pointed setae are mainly found on tarsomeres I and II of the hind leg (more than 89%) and on the edge of the tarsomeres I and II of male fore and middle legs (less than 27%) (Figs. 1A, 1B, blue color). These setae, which are about 30 - 39 μm in length, are slender and tubular with a smooth surface and are pronged over apical half (Figs. 2C, 2D). Their tips gradually taper into an acute apex and bend

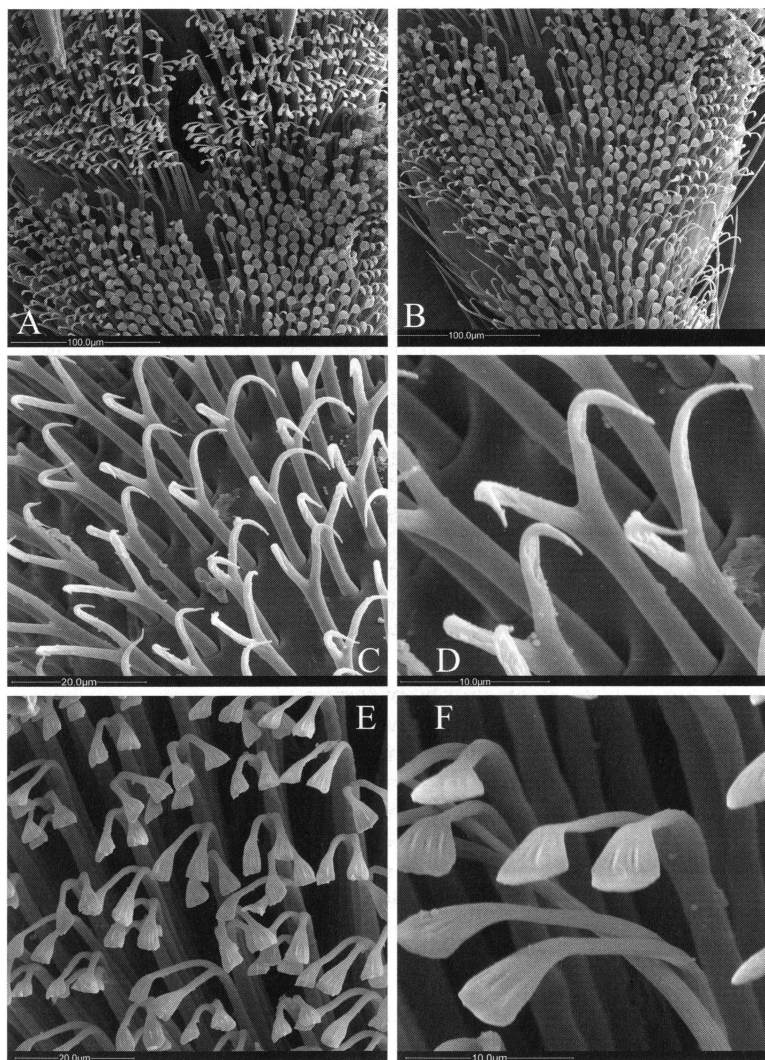


Fig. 2. Scanning electron micrographs of two types of setae. A, B. Array of all types of setae, ventral view. C. Array of pointed and pronged setae. D. Pointed setae. E. Array of spatula-tipped and pronged setae. F. Spatula-tipped setae.

downward. The surface of the pointed seta is smooth. There are approximately 3 pointed setae in each $100 \mu\text{m}^2$ on the average.

Spatulate setae. The spatulate setae mainly distribute on tarsomere III of 3 pairs of legs (more than 98%), whereas a small quantity of them is located on tarsomeres I and II (less than 10%) (Figs. 1A, 1B, green color). This type of setae is straight at the base, and gradually becomes pronged and deflexed at the tip. The seta is about $34 - 50 \mu\text{m}$ in length. Each seta has 2 spatulate, broad and flat tips with 2 - 3 grooves on the dorsal surfaces (Figs. 2E, 2F). The area is about $13.75 \pm 1.626 \mu\text{m}^2$. There are approximately 4 or 5 spatulate setae in each $100 \mu\text{m}^2$ on average. This type of setae was found in many beetle species, and their morphology is diverse.

Discoidal setae. The discoidal setae are specific for male beetles and are absent in females. They are distributed only on tarsomeres I and II of the male fore and middle legs (about 65 - 70%) (Fig. 1B, orange color). This type of tenent setae is straight at the base and not pronged at the tip. The seta is about $24 - 29 \mu\text{m}$ in length. Each seta has a discoidal and smooth tip with the central part lower than the border (Figs. 3A, 3B). The diameter of the discoidal tip is about $6.69 \pm 0.268 \mu\text{m}$. There are approximately 4 discoidal setae in each $100 \mu\text{m}^2$ on average. This type of setae may play an important role in the mating behavior and is found in males of various beetles. There are some differences in the size and shape of discoidal setae among different beetle species (Stork 1980, Pelletier and Smilowitz 1987, Bullock and Federle 2011).

Drop-shaped setae. The drop-shaped setae are mainly distributed on tarsomere II of the 3 pairs of legs (Figs. 1A, 1B, pink color). This type of seta is also straight at the base, and becomes pronged and bent downward at the tip. The seta is about $32 - 53 \mu\text{m}$ in length. There are approximately 4 drop-shaped setae in each $100 \mu\text{m}^2$ on average. The drop-shaped tip is relatively flat and board (Figs. 3C, 3D).

The size and the distribution of these kinds of tenent setae are different among the 3 pairs of legs (Tables 1, 2). In the tables we describe the size with mean $\pm$ SD, and the unit is μm .

Discussion

There are a variety of attachment systems employing different principles of adhesion. Our study demonstrates that the setae on 3 pairs of legs of *A. dorsata* exhibit different external morphology, diverse numbers, and distribution ratios. It is likely that these differences reflect varying biological functions for each leg and each seta type. We found that the total numbers of all these types of setae are similar. The number of setae on different tarsomeres is related with the area of each pad, and the tip of setae is of vital importance for the function.

We found a number of pronged and deflexed setae. These pointed-setae provide very little adhesive force in a simple pull-off situation (Bullock and Federal 2011). But, when the substratum makes contact with the tips of this type of setae, the pointed setae can exhibit pad force when the pad is either pressed against the substratum or sheared in proximal direction (Majidi et al. 2005). Thus, the apparent function of the pointed setae is to produce friction rather than adhesion (Bullock and Federal 2011). Based on our observation, the pointed setae are mainly found on tarsomeres I and II of the hind leg. So, the likely function of this beetle's hind legs, which are covered with the pointed setae, is to generate more friction than adhesion. On the remaining 2 pairs of legs, we found only this type of setae around the edge of the tarsi. These setae might contribute to friction force when the bugs move. Moreover, the pointed setae are

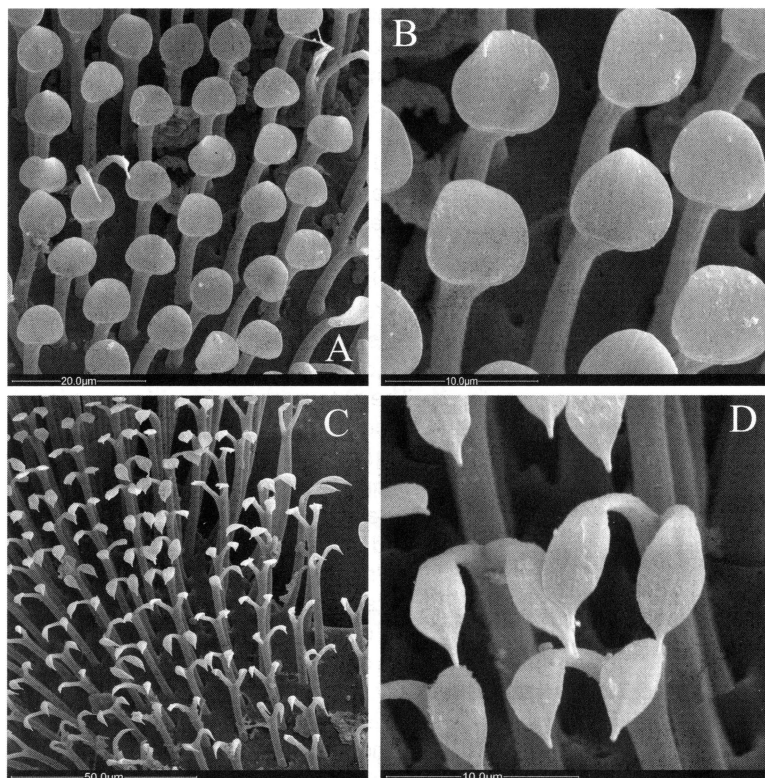


Fig. 3. Scanning electron micrographs of two types of setae. **A.** Array of discoidal setae. **B.** Discoidal setae. **C.** Array of drop-shaped and pronged setae. **D.** Drop-shaped setae.

pronged over apical half. The specialized morphology increases the contact area, thereby enhancing the friction force.

The spatulate setae (bifid setae) each divide proximal to their tips into 2 branches. The function of this external morphology is to reduce the intersetae distance and to increase the efficient contact area available for adhesion (Stork 1980b, 1983). Similar to the pointed setae, the tips of this kind of seta appears to be angled to the surface when not in contact (Bullock and Federal 2011). Grooves are also found longitudinally on the dorsal surface of the tips of the spatulate setae. The legs may acquire more friction from this specialized structure. These setae are found on tarsomere III of 3 pairs of tarsi, and their observed distribution pattern is similar.

The discoidal setae are observed only on the fore and middle legs of male beetles. Based on our observation, the terminal plates of these setae are not bent and the surface of discoidal tips is smooth. This seta is the shortest but has the largest adhesive force among the 3 types of setae. The specialized tip may help to increase the adhesive force by allowing the load to be distributed across the entire tip (Bullock and Federal 2011). The geometry can reduce the stress concentration and increase the adhesion (del Campo et al. 2007, Gorb and Varenberg 2007, Spuskanyuk et al. 2008).

Table 1. Measurements (μm , mean $\pm$ SD) of attachment structure of the legs of *Aspidomorpha dorsata*.

Attachment structure	Fore leg	Middle leg	Hind leg
Ungues ($n = 5$)	126.53 $\pm$ 7.328	127.87 $\pm$ 10.011	133.22 $\pm$ 3.669
Base width	171.30 $\pm$ 4.572	169.91 $\pm$ 7.529	178.59 $\pm$ 3.777
Ungues length	80.47 $\pm$ 2.641	66.00 $\pm$ 2.773	72.40 $\pm$ 3.768
Accessory unguis I length	65.65 $\pm$ 6.423	60.34 $\pm$ 4.087	67.50 $\pm$ 1.637
Accessory unguis II length	35.13 $\pm$ 1.762	41.55 $\pm$ 1.225	42.02 $\pm$ 1.877
Accessory unguis III length			
Tar I ($n = 7$)	31.81 $\pm$ 5.637	39.27 $\pm$ 10.354	38.87 $\pm$ 8.370
Pointed seta length	absent	absent	absent
Spatulate seta length	24.59 $\pm$ 2.285	24.26 $\pm$ 2.538	absent
Discoidal seta length			
Tar II ($n = 7$)	31.33 $\pm$ 3.179	33.29 $\pm$ 7.745	29.95 $\pm$ 4.156
Pointed seta length	42.80 $\pm$ 10.083	55.40 $\pm$ 6.391	34.12 $\pm$ 4.196
Spatulate seta length	23.80 $\pm$ 4.650	28.63 $\pm$ 7.230	absent
Discoidal seta length			
Tar III ($n = 7$)	absent	absent	absent
Pointed seta length	48.53 $\pm$ 6.096	50.85 $\pm$ 10.909	49.58 $\pm$ 6.333
Spatulate seta length	absent	absent	absent
Discoidal seta length			

The function of the special drop-like setae on the tarsomere II is not known at present. Further study of their function is needed.

The pretarsus of *A. dorsata* contains a pairs of unguis. The tarsal setae are of greater importance on smooth surfaces, whereas the unguis seem to be more important on rough substrata (Betz 2002). Our examination showed that the morphology of the unguis is unique. The unguis are broad basally and gradually become tapered. Three small accessory unguis are observed on each unguis. There are several longitudinal grooves on the inner and ventral surface of the unguis, but the surface of the accessory unguis is smooth. With these special unguis, the beetle can grab the tiny unevenness of the substratum firmly. Moreover, the longitudinal grooves on the surface of the unguis can produce additional friction.

In general, all types of tenent setae of *A. dorsata* mentioned above contribute to the attachment and mobility/movement on diverse surfaces in natural environments. The structure guarantees survival and reproduction. The study of the ultrastructure of the legs is of significance in engineering. However, some critical problems remain unresolved. For instance, how forces in natural fibrillar adhesive systems scale single setae to the whole-animal level (Bullock and Federal 2011). In the near future, scientists can design the bio-inspired synthetic adhesive devices.

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Table 2. The number of the 3 types of setae observed on tarsomeres I, II and III and the total number of setae of 3 pairs of legs of *Aspidomorpha dorsata*.

Seta type	Fore leg	Middle leg	Hind leg
Tar I	22	31	54
Pointed seta	absent	absent	absent
Spatulate seta	49	58	absent
Discoidal seta	71	89	54
SUM			
Tar II	58	42	167
Pointed seta	10	5	20
Spatulate seta	128	108	absent
Discoidal seta	196	155	187
SUM			
Tar III	absent	absent	absent
Pointed seta	533	520	581
Spatulate seta	absent	absent	absent
Discoidal seta	533	520	581
SUM			
SUM	800	764	822

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