



King Saud University

Saudi Journal of Biological Sciences

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SAUDI BIOLOGICAL SOCIETY

ORIGINAL ARTICLE

Pretarsus structure in relation to climbing ability in the ants *Brachyponera sennaarensis* and *Daceton armigerum*



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Received 24 February 2016; revised 7 June 2016; accepted 18 June 2016

Available online 24 June 2016

KEYWORDS

Climbing;
Pretarsus;
Arolium gland;
Claws;
Formicidae

Abstract We studied the external and internal pretarsus structure of the ants *Brachyponera sennaarensis* and *Daceton armigerum* in relation to their very different climbing ability. *B. sennaarensis* is a ground-dwelling species that is not able to climb vertical smooth walls. They have a pair of straight pretarsal claws with an average claw tip angle of 56 degrees, while the ventral tarsal surface lacks fine hairs that touch the substrate. They have no adhesive pad on the vestigial arolium, while the arolium gland is very small. *D. armigerum*, on the other hand, is an arboreal and thus well-climbing species with a very strong grip on the substrate. Their pretarsal claws are very hooked, with a claw tip angle around 75 degrees. They have dense arrays of fine hairs on the ventral tarsal surface, a well-developed arolium and arolium gland. These clearly different morphological characteristics are in line with the opposite climbing performance of both species.

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1. Introduction

Among the social Hymenoptera, ant workers are distinctly different from bees and wasps by their wingless nature, which inevitably forces them to a substrate-bound life. This lifestyle nevertheless goes along with considerable differences in mobility, ranging from subterranean species not exposed to open air and sun- or moonlight, hypogaecic species that live and forage in the leaf litter, ground-dwelling species which forage in or on the surface of the soil or in the leaf litter, to arboreal species that nest within living or dead tissue of trees. Their terrestrial habitat faces them with various kinds of substrates on which they walk or climb. It is obvious that the distal tarsal segments,

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Peer review under responsibility of King Saud University.



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that are the ants' direct contact region with the substrate, are of crucial importance in their movements. Several elegant biomechanical studies have studied how ants walk on smooth versus rough surfaces (Endlein and Federle, 2008, 2015; Federle et al., 2001). These studies illustrate how pretarsal adhesive pads and dense arrays of tarsal hairs play an essential role besides the claws. For the claws, both their shape (straight versus hooked) and angle plays a role in the ant's climbing ability, with a larger angle allowing better climbing (Orivel et al., 2001).

Besides these structural mechanisms, also exocrine gland secretions can play a role in leg movements of ants. Ant legs can contain over 20 glands (Billen, 2009; Billen et al., 2013), although the function of most of these remains as yet unknown. Several glands are located near the articulations between leg segments, which makes it likely that they have a lubricant role. One of the most common leg glands, found in the 6 legs of all Hymenoptera, is the arolium gland in the pretarsus. Pretarsus movements can cause hydraulic compression of the gland reservoir sac, liquid being pumped into the arolium with extension of the arolium as the result (Federle et al., 2001). By this direct effect on the arolium shape by its extension and deflation, the arolium gland is of crucial importance in the insect's climbing properties.

The impetus to examine external and internal tarsal structure in the present study were two very opposite observations during recent field trips by one of us (JB). When studying the samsum ant *Brachyponera sennaarensis* in Saudi Arabia (Al-Khalifa et al., 2015) to look at their general exocrine system (Billen and Al-Khalifa, 2015, 2016), we were struck by the obvious inability of workers to escape from an open Petri dish (Fig. 1A). On the other hand, when collecting the arboreal *Daceton armigerum* in northern Brazil a few months later to look at their abdominal sternal glands (Hölldobler et al., 1990), we were amazed by the extremely strong grip that workers of this ant had on the surface, as attempts to catch them with soft entomological forceps mostly failed: the forceps just slipped off the ants, as the ants seemed to be almost glued to the substrate.

2. Material and methods

Workers of *B. sennaarensis* were collected from a natural colony located between the root system of a date palm tree at Naa'm, Huata bani Tamim region in the south of Riyadh, Saudi Arabia. Workers of *D. armigerum* were obtained from a *Tapirira guianensis* (Anacardiaceae) tree on the grounds of Museu Paraense Emílio Goeldi in Belém, Brazil. For claw angle measurement (Fig. 2), the pretarsal claws of 10 forelegs, 10 midlegs and 10 hindlegs of each species were photographed at high magnification in frontal view with an Olympus BX-51 microscope, and trigonometric measurements made using the Olympus DP-soft version 3.2 programme. Tarsi of the three leg pairs of both species (10 samples per leg type) were mounted on stubs, coated with gold and examined in a JEOL JSM-6360 scanning microscope. Another set of 3 tarsi of each leg pair of both species were fixed in cold 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, the tarsi were embedded in Araldite and longitudinally

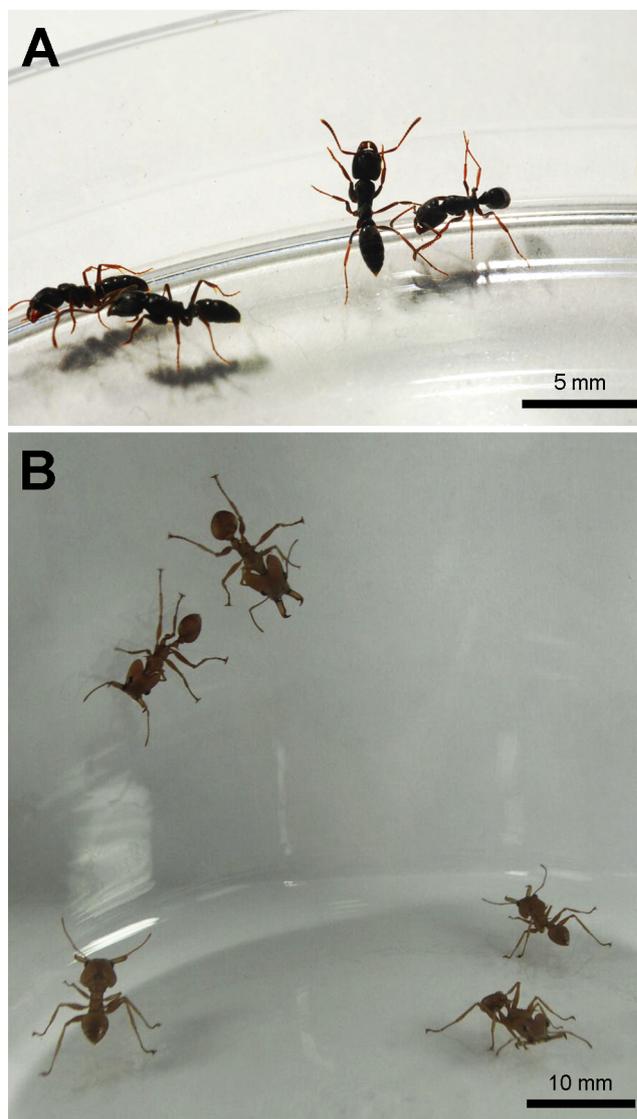


Figure 1 A. Workers of *Brachyponera sennaarensis* walking along the inner wall of an open Petri dish, from which they cannot escape (photograph Ricardo Oliveira). B. Workers of *Daceton armigerum*, that easily climb vertical glass walls (photograph Rony Almeida).

sectioned with a Leica EM UC6 ultramicrotome. Serial semi-thin sections with a thickness of 1 μm were stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope. All microscopy images in this article are shown with the distal part to the right.

3. Results

3.1. Climbing ability

B. sennaarensis workers cannot climb smooth vertical walls, as became apparent when they were put in an open Petri dish (Fig. 1A). They walk along the inner vertical walls, attempting to climb, but cannot get any grip, and therefore are not able to escape. *D. armigerum* workers are excellent climbers in nature,

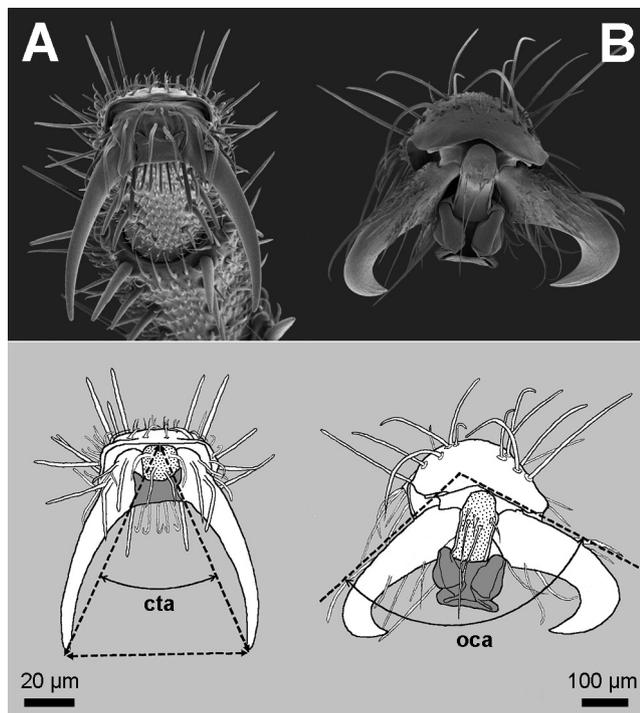


Figure 2 Frontal view scanning micrographs of the midleg pretarsus claws of *Brachyponera sennaarensis* (A) and *Daceton armigerum* (B). The corresponding schematical drawings indicate the way of measuring claw angles: claw tip angle (cta) according to Orivel et al. (2001) is calculated using the triangle formed by the two claw tips and the upper median tip of the manubrium, overall claw angle (oca) is calculated using the main claw direction in its proximal third. The arolium is shown in darker grey, the manubrium appears stippled.

where they show an extremely strong grip on the substrate. When put in a glass container, they can very easily walk the smooth vertical walls (Fig. 1B).

3.2. External structure

The pretarsal claws differ considerably in both species, both in their general shape and in their claw angle. *B. sennaarensis* has almost straight claws with a claw tip angle around 56 degrees and an overall claw angle around 43 degrees in all legs (Fig. 2A; Table 1). *D. armigerum*, in contrast, has conspicuously hooked claws in all legs with a claw tip angle of over 73 degrees; the overall claw angle is even almost twice as large (Fig. 2B; Table 1).

The ventral surface of the distal tarsomeres shows relatively few and rather short hairs in the three leg pairs of *B. sennaarensis*, besides two parallel rows of long stiff bristles, that even prevent that the shorter hairs touch the substrate (Fig. 3A–C). In contrast, the tarsomeres of the three leg pairs of *D. armigerum* ventrally show dense patches of long hairs, that are especially conspicuous in tarsomeres 3 and 4 (Fig. 3D–F).

In between the basis of the claws, the distal tip of the pretarsus (= tarsomere 5) contains the arolium (Fig. 2). As in most ants, the arolium is extendable in *D. armigerum* (Fig. 4A–E), where in *B. sennaarensis* it is reduced to a small and smooth cushion-like structure (Fig. 4F). During the extension process in *D. armigerum*, the inner arolium portion that contains hundreds of microscopic short hairs of hardly 5 µm is turned outward (Fig. 4B and D), bringing these hairs in contact with the substrate.

3.3. Internal structure

Longitudinal sections through the pretarsus show in the three leg pairs of both species the presence of the arolium gland. The gland consists of a sac-like and thickened back-folded continuation of the tegumental epithelium. A thin cuticle forms the lining of the arolium gland reservoir, that extends into the arolium. In its proximal part, the reservoir sac is pierced by the heavily sclerotized unguitraction tendon, that will make contact with the unguitraction plate to regulate the flexion of the pretarsal claws (Fig. 5). The appearance of the arolium gland, however, differs considerably in both species. In *B. sennaarensis*, the gland is small and occupies only the most distal part of the pretarsus, the epithelium thickness is around 10 µm, and the reservoir volume is limited (Fig. 5A–C). In *D. armigerum*, the gland occupies a considerable part of the pretarsus, the epithelial thickness is around 40 µm, and the reservoir lumen is large (Fig. 5D–F).

4. Discussion

Myrmecologists who keep ants in artificial nests are very familiar with the use of fluon to coat the vertical walls of the foraging arenas to prevent the ants from escaping. In an earlier study on climbing ability in ants, Orivel et al. (2001) examined the relationship between climbing and pretarsal claw structure in 15 *Pachycondyla* species. They found that all arboreal species had horn-shaped claws with a claw tip angle of more than 90 degrees, an arolium with a well-developed adhesive pad, and were able to walk upside down on glass; most ground-dwelling species had straight claws with a claw tip angle around 50–60 degrees, lacked an adhesive pad, and were not able to

Table 1 Pretarsal claw angle measurements in the fore-, mid- and hindlegs ($n = 10$ of each) of *Brachyponera sennaarensis* and *Daceton armigerum* (measurements done as illustrated in Fig. 2).

	<i>Brachyponera sennaarensis</i>		<i>Daceton armigerum</i>	
	Claw tip angle	Overall claw angle	Claw tip angle	Overall claw angle
Foreleg	56.05 ± 5.68	42.52 ± 3.16	72.93 ± 5.81	130.58 ± 10.40
Midleg	56.27 ± 5.11	43.10 ± 11.81	77.82 ± 10.70	145.05 ± 16.67
Hindleg	56.34 ± 5.45	43.98 ± 14.23	73.59 ± 9.78	143.11 ± 12.61

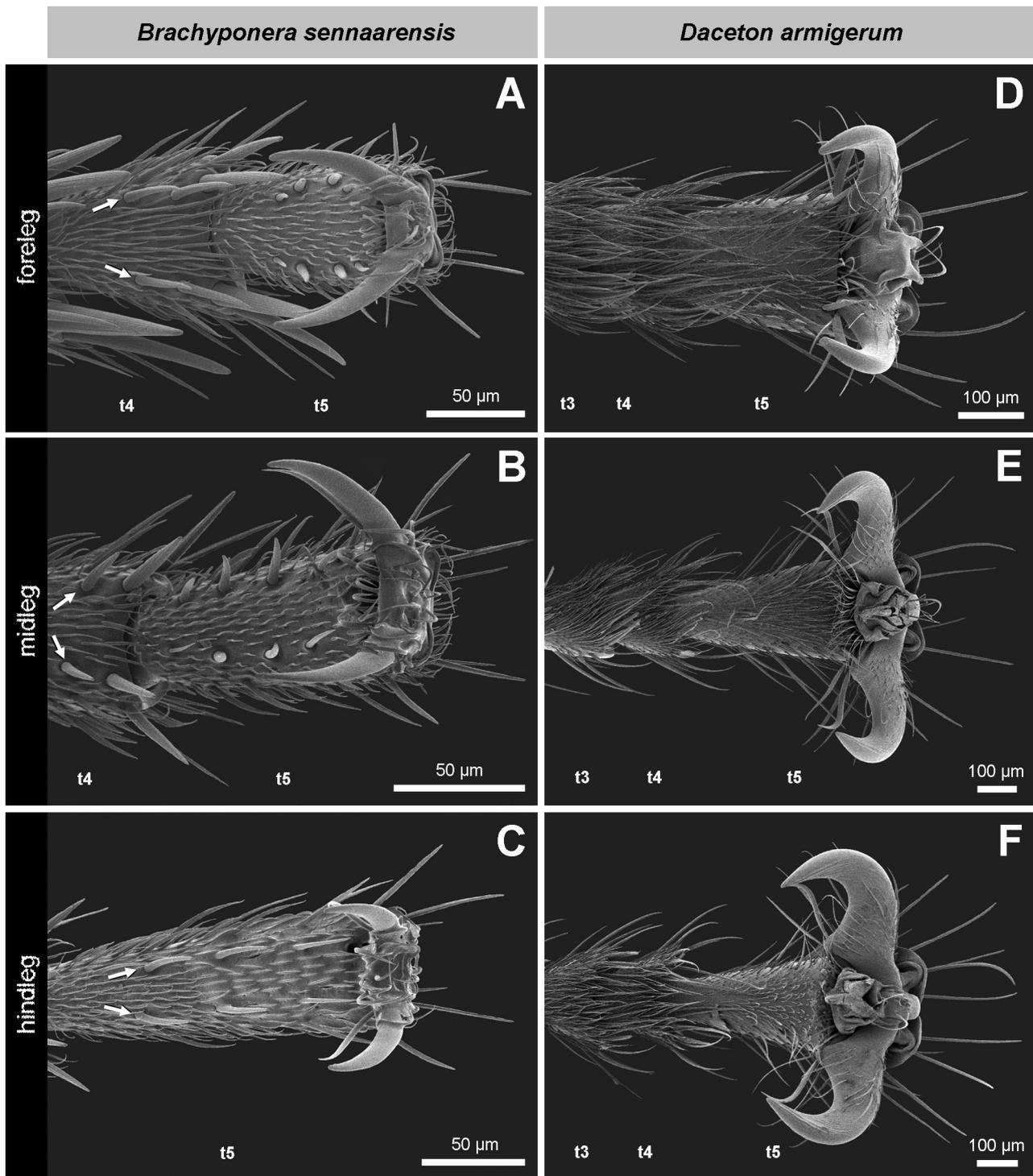


Figure 3 Scanning micrographs of the ventral surface of the distal tarsi of fore-, mid- and hindlegs of the two species, showing the very different shape of the pretarsal claws. Note the two rows of stiff ventral bristles (only the most proximal pair indicated by white arrows) and sparse hairs in *Brachyponera sennaarensis*, opposed to the very hairy appearance of the tarsi in *Daceton armigerum* (t3, 4, 5: tarsomeres 3, 4, 5).

walk upside down (Orivel et al., 2001). Workers of our ground-dwelling study species *B. sennaarensis* (that lives from small prey as well as plant seeds) fit well in the latter group with their straight claws, a claw tip angle of 56 degrees and vestigial arolium (note *B. sennaarensis* until recently was also classified in

the genus *Pachycondyla* – Schmidt and Shattuck, 2014). *B. sennaarensis* workers moreover are extremely poor in climbing, as they cannot even escape from an open Petri dish.

A very opposite situation occurs in workers of the arboreal *D. armigerum* (a general predator on medium-sized insects),

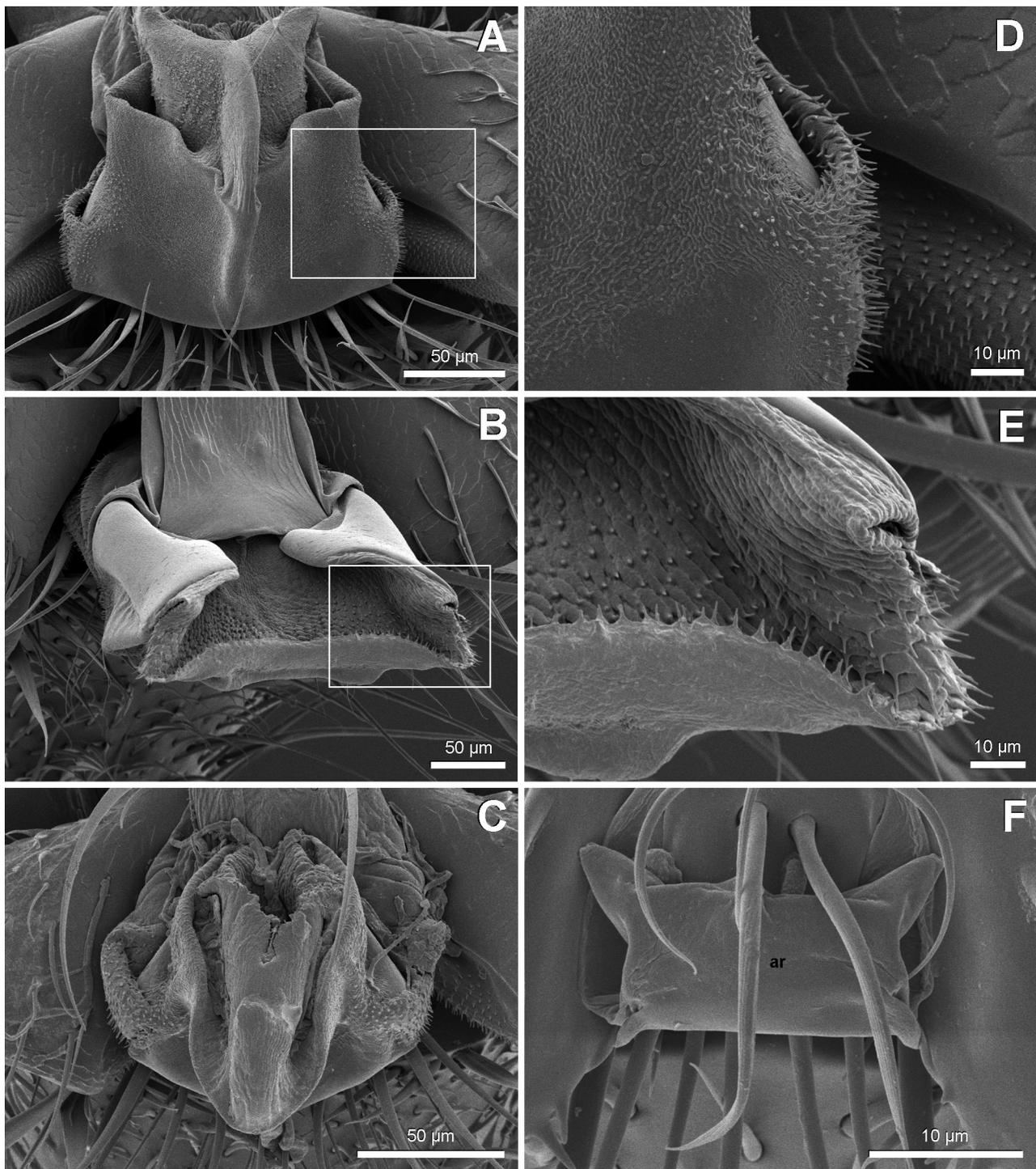


Figure 4 A–C. Scanning micrographs of the arolium in *Daceton armigerum* at various stages of its extension, white frame areas are enlarged in D and E, showing hundreds of microscopic short hairs (A–D midleg), F. The very much reduced and smooth cushion-shaped arolium (ar) of *Brachyponera sennaarensis* foreleg.

that show an extremely strong grip on the substrate in nature, and that very easily climb vertical glass walls. Their pretarsal structure can be compared with that of the arboreal *Pachycondyla* species studied by Orivel et al. (2001), with a well-developed adhesive pad on the arolium and horn-shaped pretarsal claws. Although their claw tip angle around 75

degrees is lower than the 90 degrees of the arboreal *Pachycondyla* species, *Daceton* has an overall claw angle around 140 degrees because of its claws being even more hooked than these of *Pachycondyla*. These strikingly hooked *Daceton* claws are very likely the reason why workers of this species display such extraordinary strong grip on the substrate.

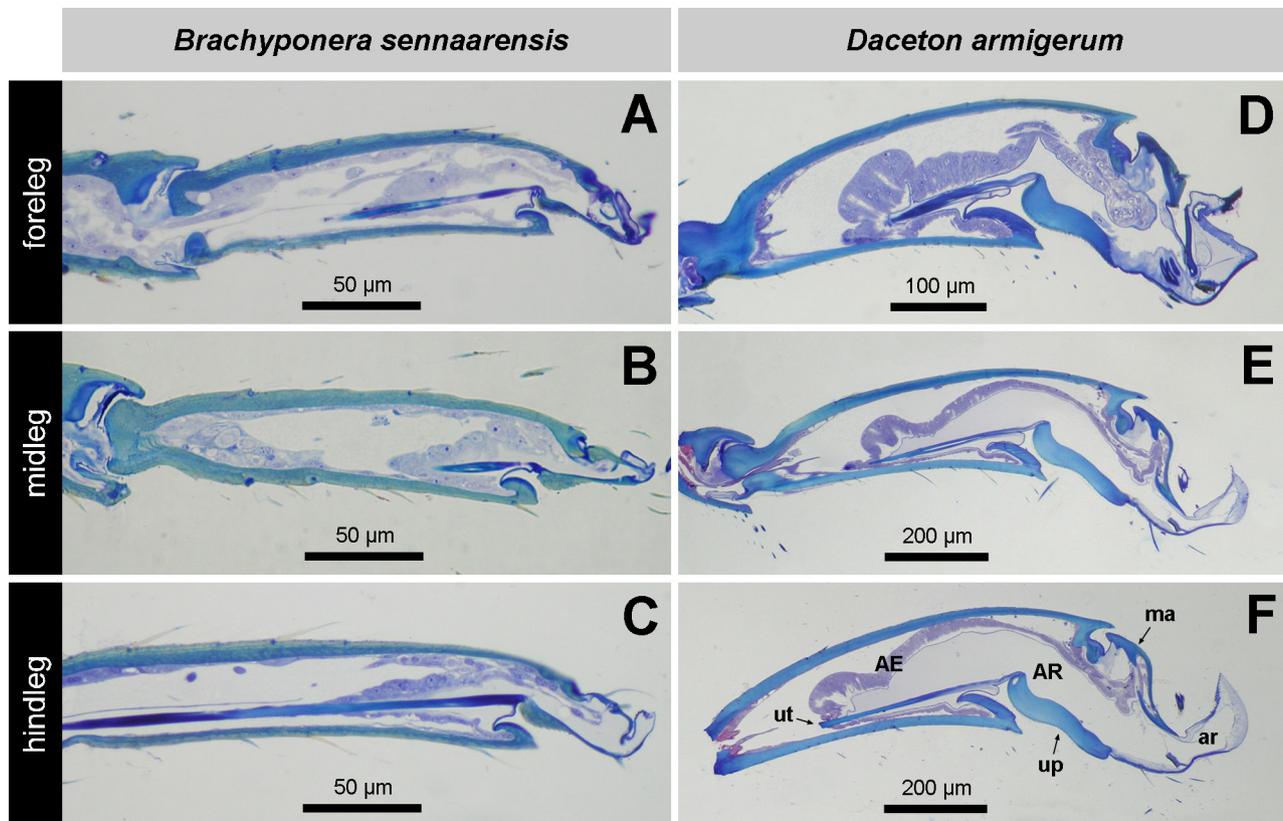


Figure 5 Longitudinal semithin sections through the central midline of the worker pretarsus of fore-, mid- and hindlegs of the two species. Note the much better developed arolium gland as well as the arolium itself in *Daceton* than in *Brachyponera*. For convenience, the various structures are only indicated in 5F, although they can be seen on all subfigures: AE: arolium gland epithelium, AR: arolium gland reservoir, ar: arolium, ma: manubrium, up: unguitractor plate, ut: unguitractor tendon.

This was also noted by Wilson (1962) as ‘exceptionally long tarsal claws anchored the ant firmly to the bark surface and aided it in holding on to large or vigorous prey’.

While the pretarsal claws are important to get a good grip while walking or climbing on rough or natural substrates, they are not of much use during climbing on smooth surfaces. Endlein and Federle (2015) even show that the pretarsal segment of *Oecophylla smaragdina* workers during walking on a horizontal substrate do not even touch the substrate, and contacted the ground mainly with the 3rd and 4th tarsomeres. During vertical climbing or upside down walking, however, the adhesive pad of the arolium makes close contact with the substrate (Endlein and Federle, 2015). Besides the adhesive pad, also dense hair pads on the 3rd and 4th tarsomeres come in close contact with a smooth substrate during climbing, creating friction forces by pressing the long hairs sidewise onto the substrate. In a very elegant study, Endlein and Federle (2015) could even show that during vertical climbing the adhesive pads of the legs above the ant’s centre of mass created pulling forces in their contact with the substrate, while the legs below the centre of mass showed a pushing effect through contact with their hair pads on the 3rd and 4th tarsomeres. Dense hair pads on these tarsomeres do also exist in workers of *D. armigerum*, but are lacking in *B. senaarensis*. The latter, on the contrary, shows two rows of stiff bristles on the ventral tarsal segments, that even prevent the few existing hairs to come in touch with the substrate, which is yet another illustration of the clear inability to climb in this species. An interesting

observation in this regard is that termite workers cannot climb smooth vertical surfaces and do not have arolia, whereas most alate termites do possess arolia and are able to climb such surfaces (Crosland et al., 2005).

How do insects actually manage to hold on to vertical or inverse surfaces with the adhesive pads of their arolium and the hairy pads on the other tarsomeres? Older studies reported on the role of an adhesive oily secretion (Hasenfuss, 1977; Stork, 1983; Walker et al., 1985). Secretions from the arolium gland were supposed to reach the outside via the ventral groove (Arnhart, 1923; Lensky et al., 1985; Pouvreau, 1991), that forms the junction between the pretarsus and the unguitractor plate. Although such ventral groove does exist (see also Fig. 5), it does not provide any connection between the arolium gland and the exterior (Billen, 1986; Nijs and Billen, 2015 for wasps; Jarau et al., 2005 for stingless bees; Billen, 2009 for ants). Orivel et al. (2001) show suggestive scanning micrographs of balloon-like ‘arolium gland secretion’, which should be considered as artefacts, as real secretion of this kind would not be visible following SEM tissue preparation. The arolium gland, however, does seem to play a role in the adhesion process, not by releasing adhesive secretions as it has no structural contact with the exterior, but by acting as a hydraulic system that pumps liquids into the arolium (Federle et al., 2001). This results in an extension of the arolium, that goes along with the exposure of hundreds of microscopic hairs, that together act as an adhesive pad through capillary forces (Endlein and Federle, 2015). The excellent climbing performance of *D. armigerum* is

supported by the presence of a well-developed arolium gland, whereas the non-climbing ability of *B. sennaarensis* besides the external tarsal characteristics can also be linked with its much reduced arolium gland.

Acknowledgements

We are most grateful to An Vandoren and Alex Vrijdaghs for their assistance in section preparation and scanning microscopy, and to Ricardo Oliveira and Rony Almeida for their help with photography. This work was supported through research group project N° 340 from the Deanship of Scientific Research at King Saud University.

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