

Research article

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## The pretarsus of the honeybee

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**Abstract.** Although the honeybee (*Apis mellifera* L.) is a well-studied species, the functional morphology of its pretarsal structure is still not fully understood. We conducted an in-depth scanning electron microscopic study on these complex structures to contribute to the comprehension of the pretarsal structure-function relationships. As a result, this study has provided valuable information on the ultrastructure of the pretarsus, and in particular on the spines of the unguitactor surface and the small spines and scalloped surface of the claws with longitudinal grooves. Special attention was given to the adhesive contact zone of the arolium with its highly specialized fibrillary cuticle texture. Remarkably, several of the observed pretarsal structures, such as the pyramidal structures on the unguitactor and the thin hairs on both the grooved claws, and the hairs of the manubrium have not been previously described. All observed structures in this study were characterized with respect to their possible physiological and mechanical roles.

**Keywords.** Honeybee, pretarsus, arolium, arcus, manubrium, planta, unguitactor.

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## Introduction

All insects including the honeybee (*Apis mellifera* L.) possess three pairs of segmented legs attached to the thorax. The honeybee's segmented leg (Fig. 1) consists of the coxa, the trochanter, the femur, the tibia and five tarsomeres, comprising the basitarsus, three small tarsomeres and the pretarsus (ASPERGES 2011).



Figure 1 – The hind-most leg of a honeybee with pollen-loaded corbiculum attached to the tibia.

Although the honeybee is a well-studied species, the functional morphology of its pretarsal structure is still not completely elucidated. Using light microscopy SNODGRASS (1956) described all parts of the pretarsus accurately, but did not propose any structure-function relationship. It was later suggested that the pores on the pretarsus could possibly secrete footprint pheromones (LENSKY *et al.* 1985; BILLEN 1986). In 2003, as part of an extensive electron microscopic study, GOODMAN (2003) was the first to provide a general description of the pretarsal functional morphology. In addition, JARAU *et al.* (2004) and WILMS & ELTZ (2008) proposed the occurrence of epithelial glands around the tendon to mark food sources. In order to contribute to the elucidation of the functional morphology of the pretarsal structures, we conducted an in-depth scanning electron microscopic study on these complex structures. In this way, we aimed to provide more detailed information on the ultrastructure allowing better comprehension of the pretarsal functional morphology.

## Material and methods

### Light microscopy

Amputated whole legs from fresh honeybees were immersed in 10% KOH in water (25°C). After a few days the solution turned brown. The solution was refreshed (every two days) until it remained colorless and the legs had become transparent. This clearing operation could take up to two weeks and more. Following the clearing operation the legs were rinsed in water followed by dehydration in a graded ethanol series (30%, 50%, 70%, 95%). Whole legs or parts of legs were mounted on glass slides within a drop of water for further observation using an Olympus CH2 equipped with a Nikon Coolpix 950 camera, and a Zeiss “Primo Star” microscope.

### Scanning electron microscopy

Fresh amputated legs were fixed for 24 hours in 2% glutaraldehyde buffered in 0.05 M sodium cacodylate (pH 7.3) and 0.15 M saccharose. The fixed legs were rinsed for  $2 \times 10$  minutes in 0.05 M sodium cacodylate and 0.15 M saccharose (pH 7.3). Subsequently, the legs were dehydrated in a graded ethanol series of 30%, 50%, 70% and 95% for 30 minutes each step. The legs were critical-point dried with a Polaron critical-point dryer. To enable observation by SEM the samples were Au/Pt sputter coated using a Leica E:ACE600 Sputter coater. The SEM images were first recorded using a FEI Quanta 200FEG-SEM scanning electron microscope.

The images presented in this report are only SE (secondary electron) images. These images were mostly used to reveal the surface morphology of the specimen. SE image contrast is mostly generated by differences in SE emission efficiency with topography.

## Results

### Tarsus

Each leg has five tarsomeres (Fig. 2) of which the first or most proximal one is the basitarsus. The basitarsus of a worker bee has specialized functionalities, such as collecting pollen. The next three more distal tarsomeres are rather small. The foot ends in the pretarsus. There are no muscles in the segments behind the tibia, however a long unguitractor tendon runs through all the segments from the femur down to the pretarsus.

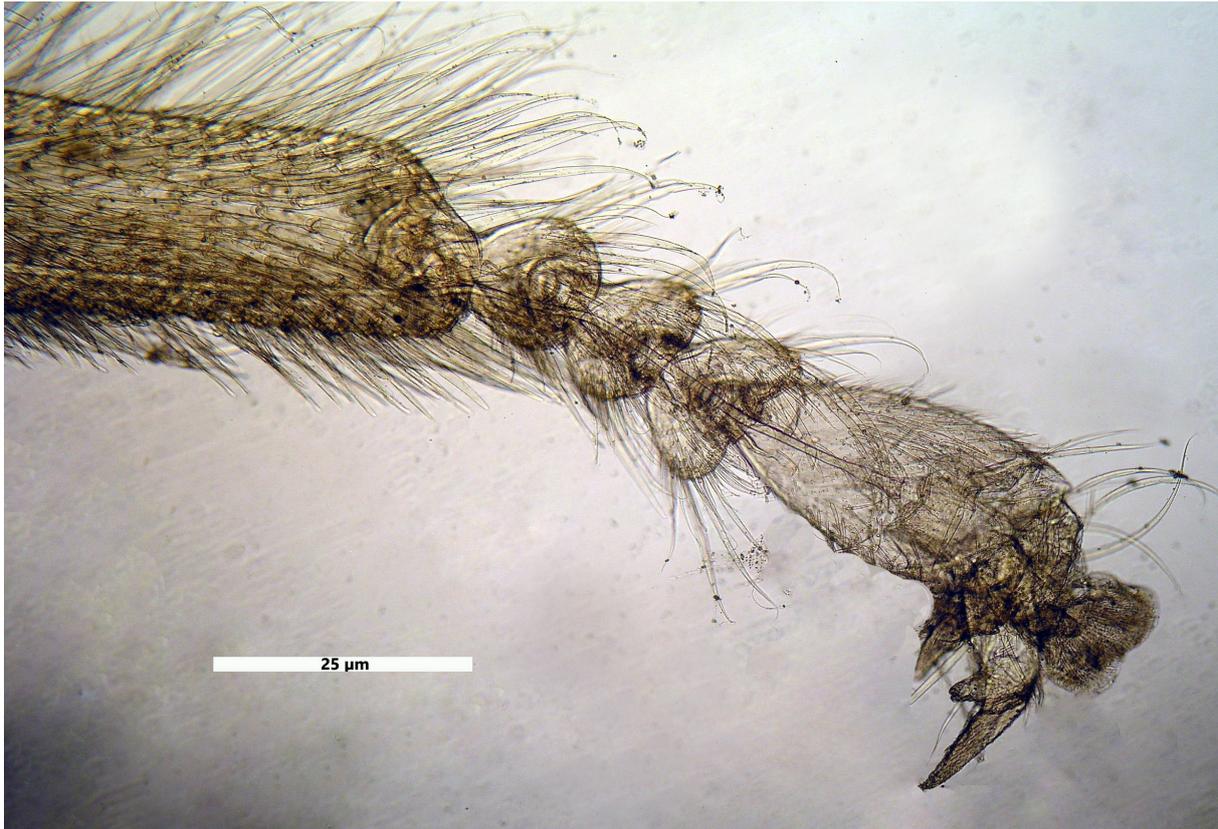


Figure 2 – Detailed image of the tarsomeres. The basitarsus in the left upper corner is more distally followed by three tarsomeres and the pretarsus. The long unguitractor tendon runs through all the leg segments.

The basitarsus and the small subsegments of the tarsus move freely with respect to one another by monocondylic articulations. The articulations shown in Fig. 3A–B consist of flexible membranes arising from a large ‘elbow’- or ‘knee’-like joint. The same type of connection exists between the basitarsus and the tibia. The tendon of the musculus retractor unguis of the femur and tibia, called “retrotractor of the claws”, ends in the pretarsus on the unguitractor and arcus.

### Pretarsus

The pretarsus is a complex structure (Fig. 4A–B) consisting of two segments: the complex foot and the fifth most distal tarsomere. On the foot two pairs of claws can be distinguished. Between the claws a soft arolium can be seen, including the arcus, a dark ‘U-shaped’ band on the upper part of the arolium.

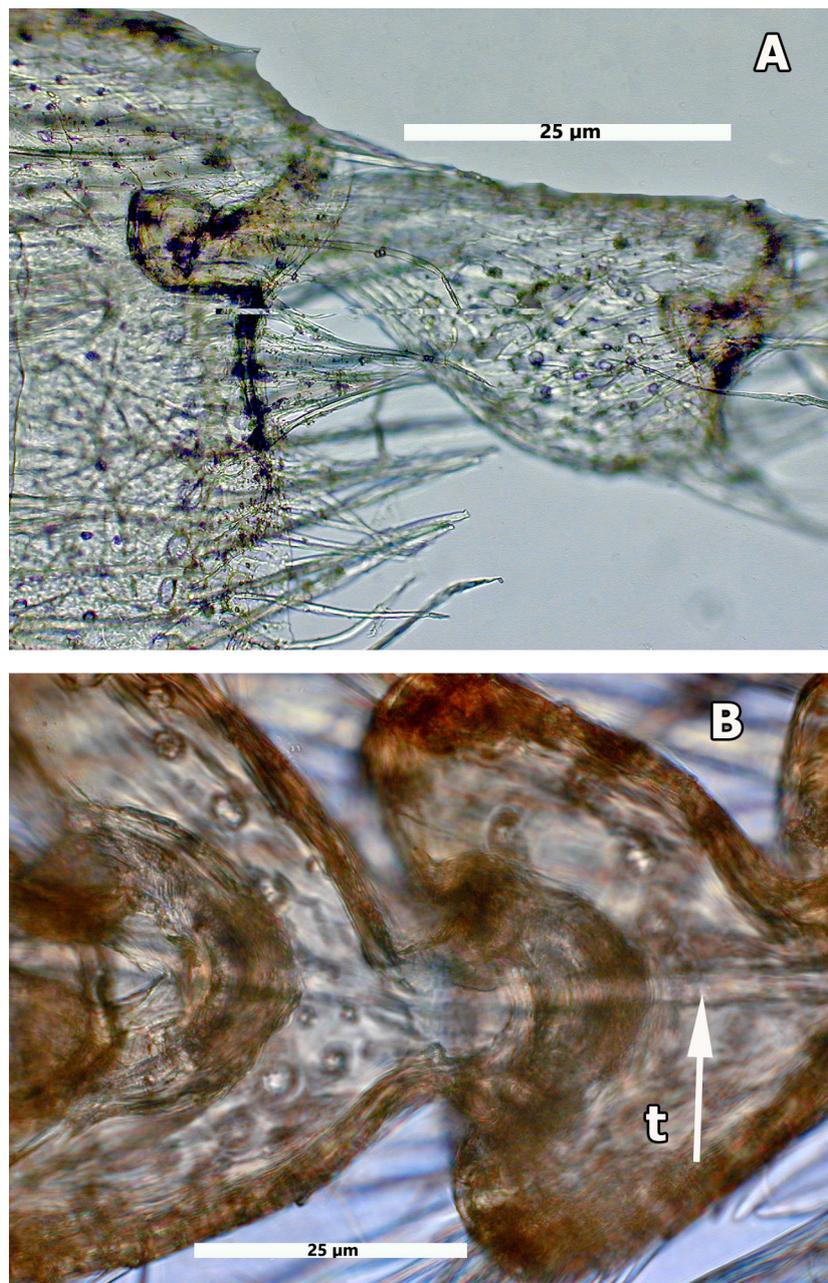


Figure 3 – A. Micrograph of the leg joints between the basitarsus and the second tarsomere. B. Second and third tarsomere with ‘knee-like’ joint and in the middle the unguitactor tendon (t) (arrow) clearly visible.

The arolium, the ‘sole’ of the foot, is connected to the planta, a small sclerotized plate, attached to the unguitactor. The manubrium is located on top of the planta. The unguitactor is connected to the long unguitactor tendon (Figs 5–7) coming from the tibia and femur musculus retractor unguis through the different tarsomeres.

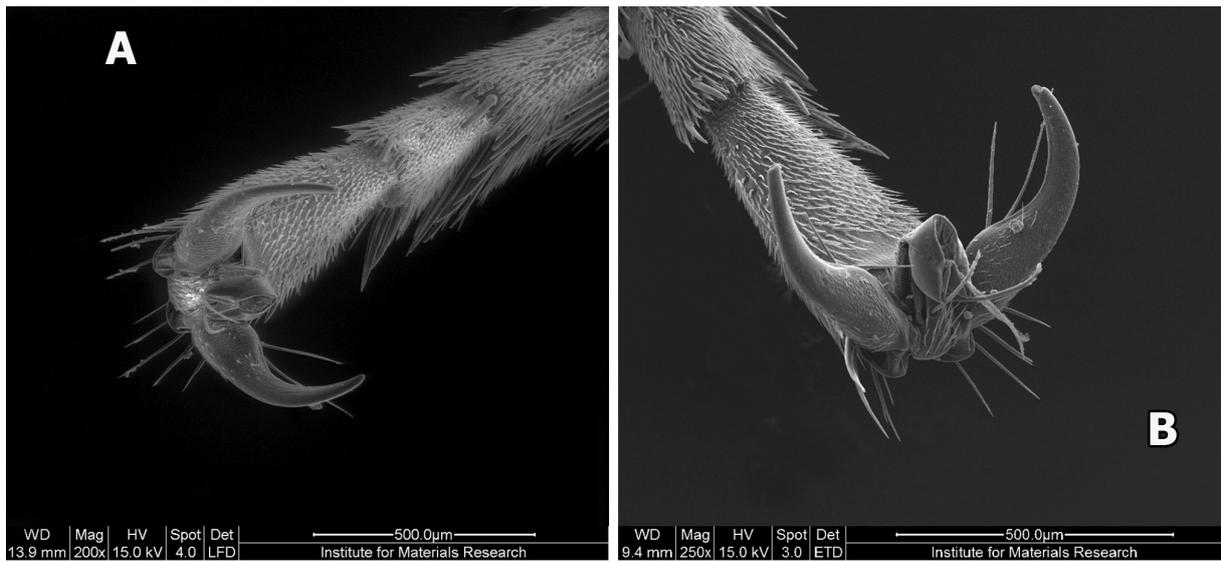


Figure 4 – A–B. SEM of the pretarsus.

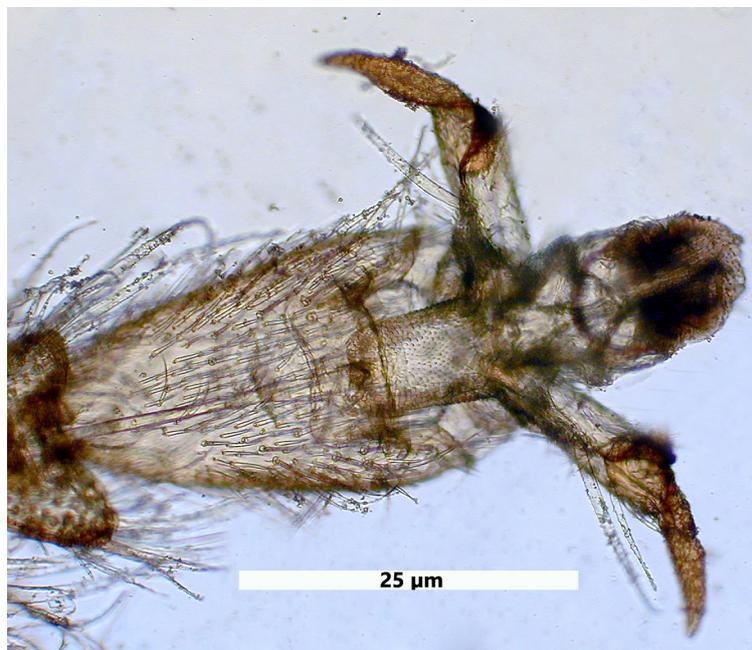


Figure 5 – Pretarsus with the fifth tarsomere and the foot.

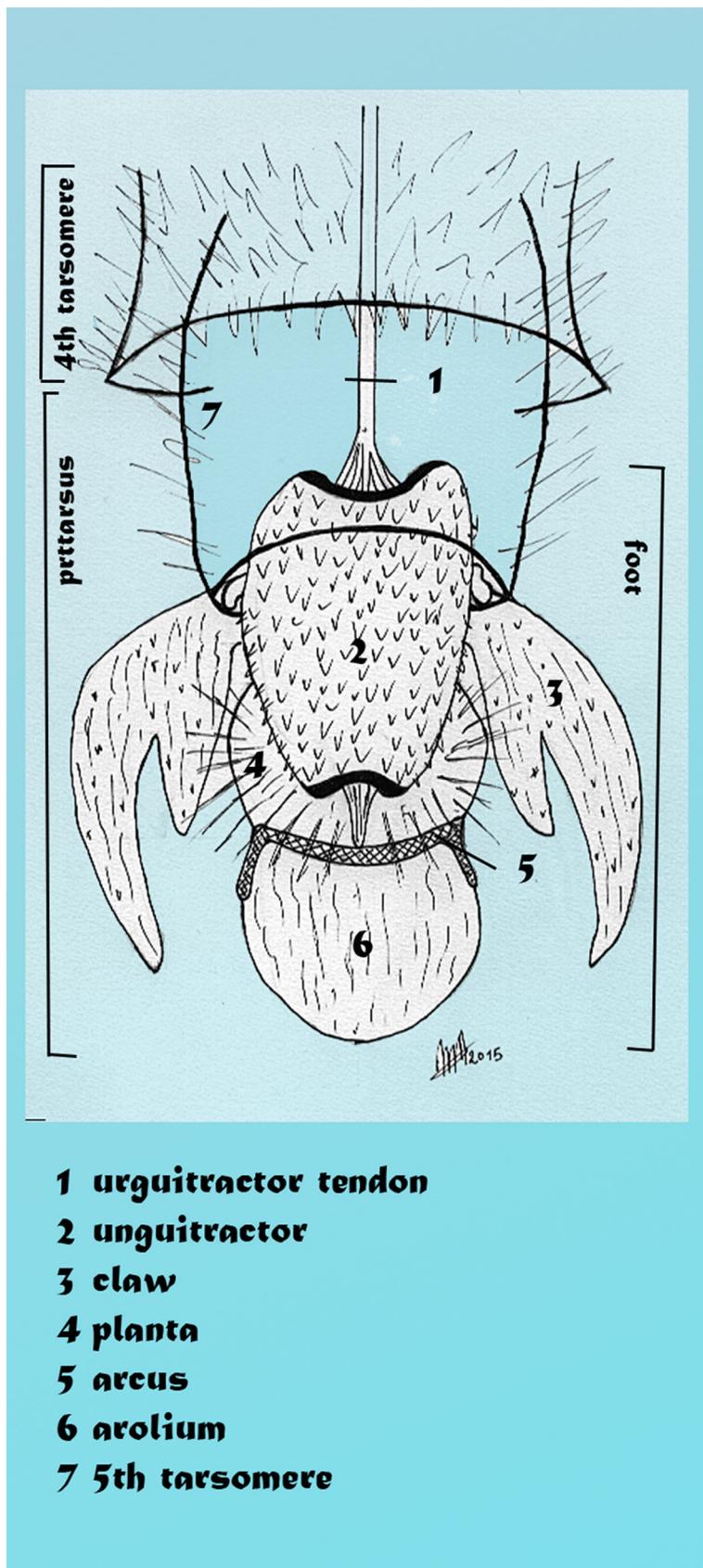


Figure 6 – Scheme of the pretarsus.

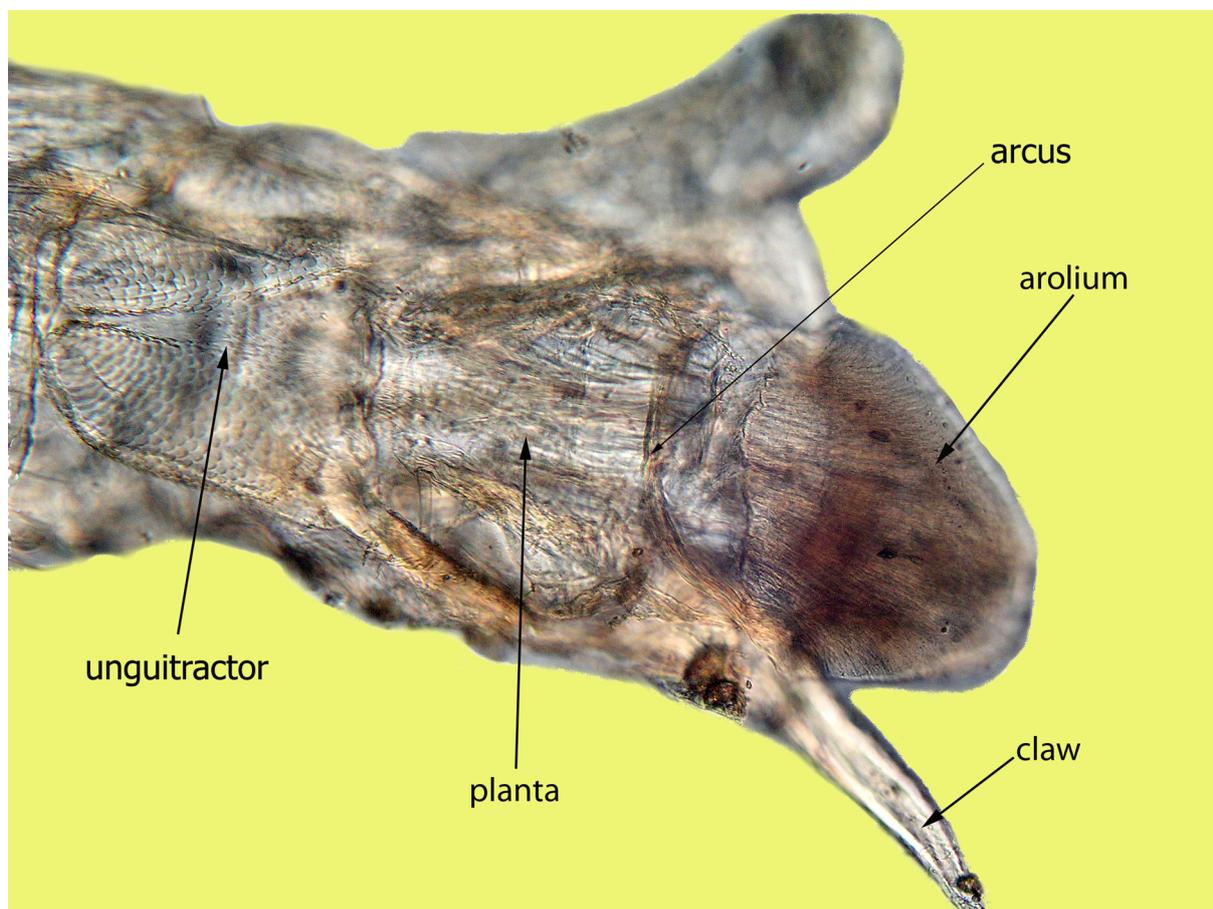


Figure 7 – Foot of the honeybee.

### Claws

There are two pairs of hollow, strongly sclerotized claws (Fig. 8A–B), each claw tapering to a point. Each claw exists of two lobes of unequal length with long stout spines on the outer surface. The lobes of the claws are outgrowths of the membranous lateral walls of the base of the tarsus. They arise between the articular condyle above and the auxiliary sclerite below, just at the top of the unguitractor plate. They are freely flexible but not muscled. The claws are unable to grip on hard or smooth surfaces. When the claw touches a substrate, it flexes as the points of the claws spread sideways. This causes a chain reaction on the unguitractor, the planta plate and further on the arcus, pulling down the arolium, which spreads out on the smooth surface to be gripped.

SEM micrographs (Fig. 9A–B) show that the claw surface is not smooth but scalloped with longitudinal grooves on both outer and inner surfaces. The surface is covered (Fig. 10) with very small, short spines. These seem to have a tactile or sensory role.

### Arolium

The arolium (Fig. 11A) is a soft cuticular sac located between the two pairs of claws at the front of the foot. The upper surface of the arolium is covered with very short hairs while the undersurface is almost

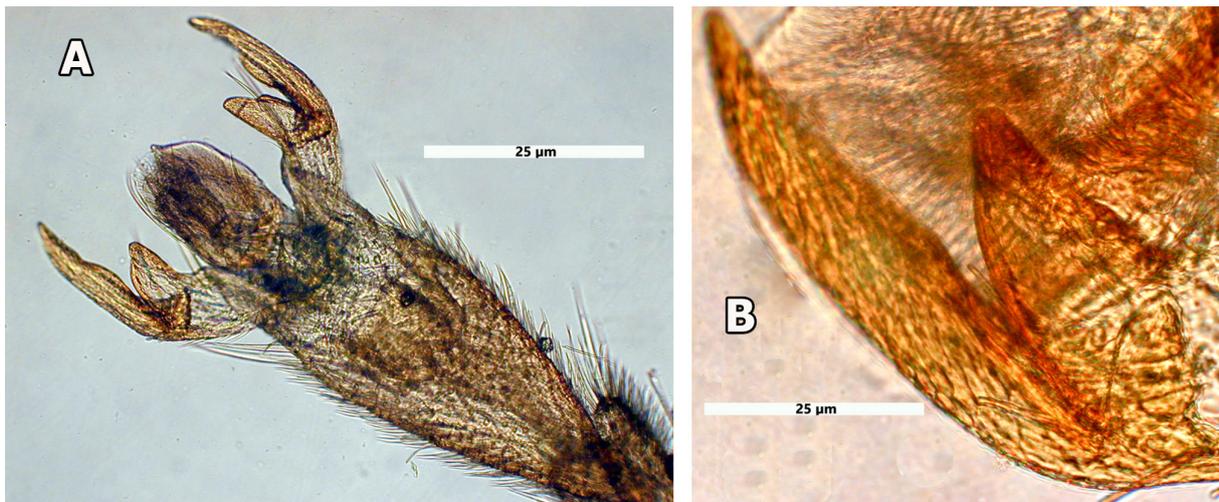


Figure 8– A. Claws, each longer lobe ending in a point. B. Each claw consists of two lobes of unequal length.

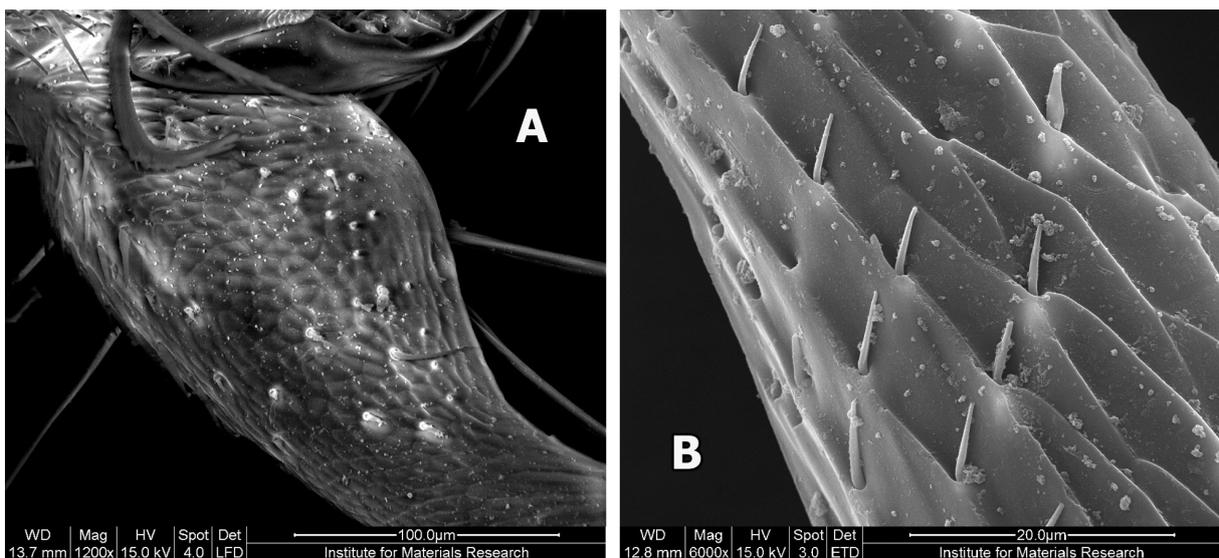


Figure 9 – A. The claw surface possesses very small, short spines. B. The surface of the claws is scalloped with longitudinal grooves.

entirely bare as observed by SNODGRASS (1956). Its adhesive contact zone has a highly specialized fibrillary, textured cuticle (FEDERLE *et al.* 2001). Observing the distended arolium by light microscopy or by SEM it is clear that the arolium is covered with parallel grooves (Fig. 11B) that facilitate a grip on smooth surfaces. It can be hypothesized that pheromones are secreted from the arolium.

The arolium is shaped by two hard pretarsal sclerites, the arcus and the manubrium. The arolium can be observed in a retracted or distended state. In general, the claws touch the surface before the arolium. When the foot is placed on a smooth surface the claws retract. The arolium unfolds and extends on the surface. According to FEDERLE *et al.* (2001) the adhesion of the arolium to smooth surfaces is enabled by a thin liquid film between the surface and the arolium. Note that the arolium never extends without a retraction of the claws. When the arolium unfolds, the unguitactor plate is drawn back completely into



Figure 10 – The claw surface is covered with very small, short spines that seem to have a tactile or sensory role.

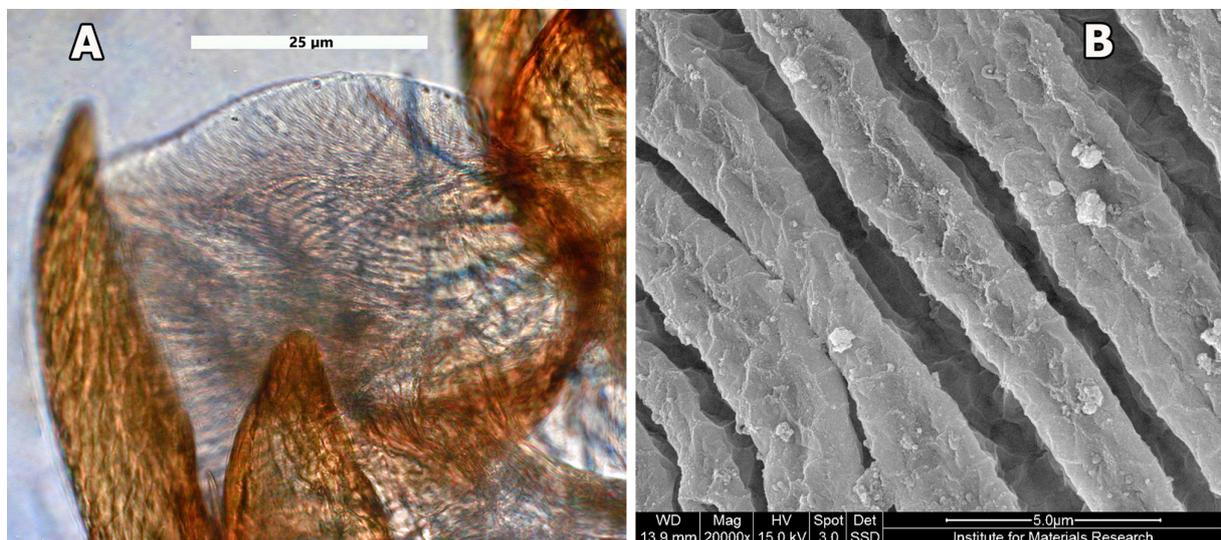


Figure 11 – A. Distended arolium with parallel grooves. B. The adhesive contact zone has a highly specialized fibrillary cuticle texture.

the fifth tarsomere. When the leg is lifted from the surface, the claws extend and the arolium deflates (Fig. 12A–B) until it detaches from the surface.

The arolium (Fig. 13) in the passive position, half retracted, presents dorsally a deep cavity between the upturned lateral walls.



Figure 12 – A. Retracted arolium. B. Distended arolium.

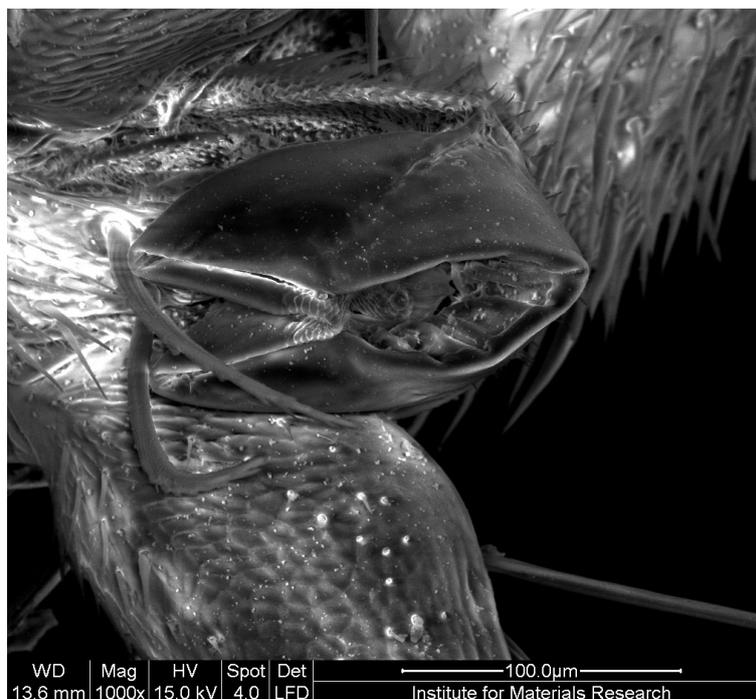


Figure 13 – In passive position (half retracted) the arolium presents dorsally a deep cavity between the upturned lateral walls.

### Arcus

The arcus (Fig. 14) is the thick dark brown, “U”-shaped structure at the upper part of the arolium. A contraction by the unguitractor tendon causes the arolium to flatten thereby retracting or distending the arcus.

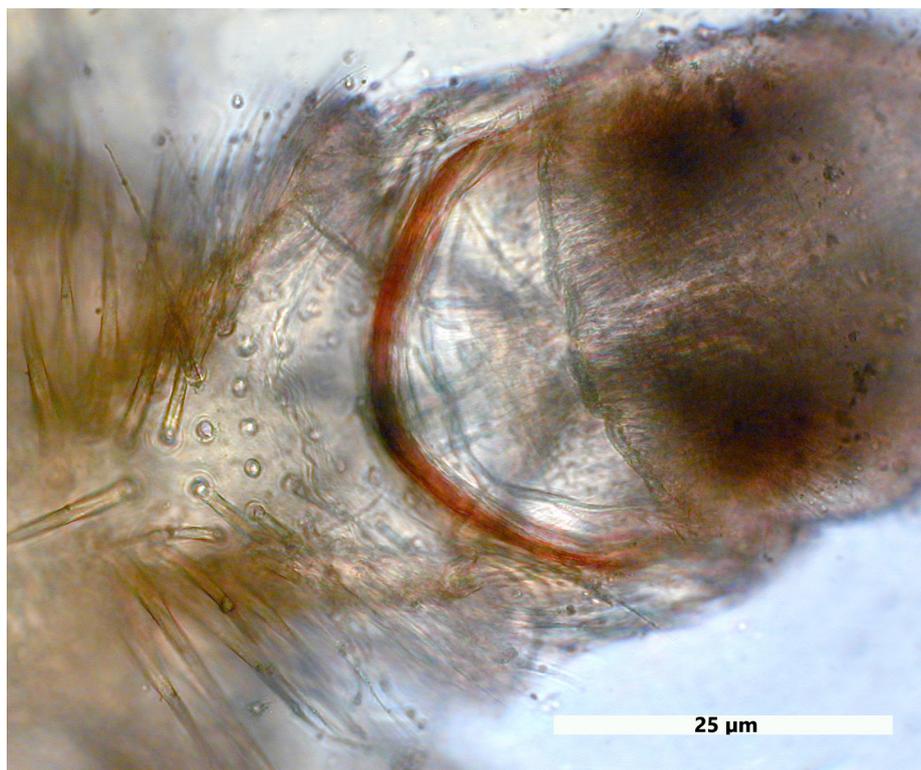


Figure 14 – The arcus, a dark brown “U”- shaped structure.

### Planta

The planta connects the unguitactor with the arolium. As described by GOODMAN (2003) the surface (Figs 15A–B, 16) of this planta plate is densely covered with strong distally-diverging spines. As mentioned by STELL (2012) the traction force on the unguitactor plate is transferred to the planta plate, which, by itself, acts on the arcus. The last one pulls down the arolium and causes it to spread out thereby enabling grip on smooth surfaces such as flower petals or window glass.

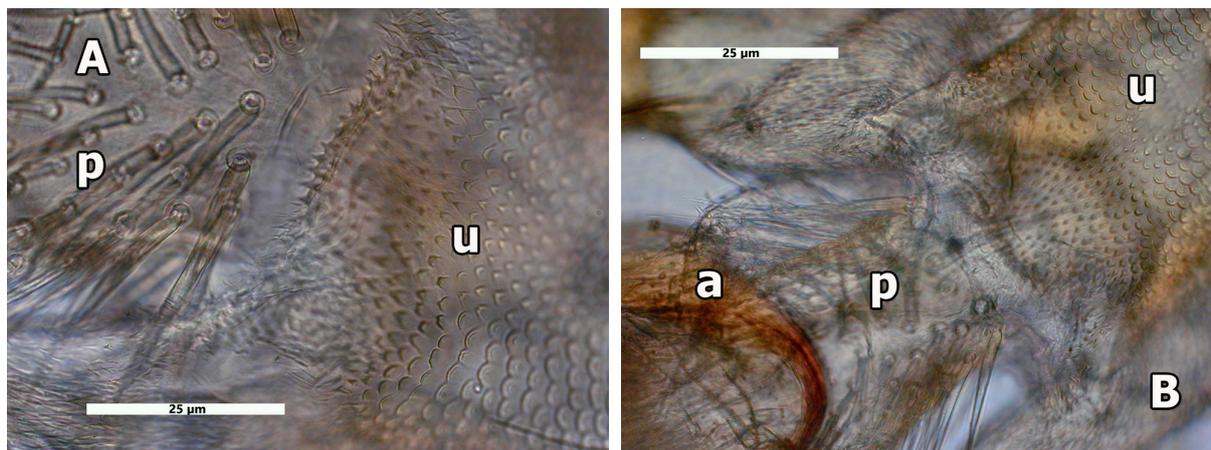


Figure 15 – A. Micrograph of planta (p) and unguitactor (u) with spikes. B. Micrograph showing the connection between arcus (a), planta (p) and unguitactor (u).

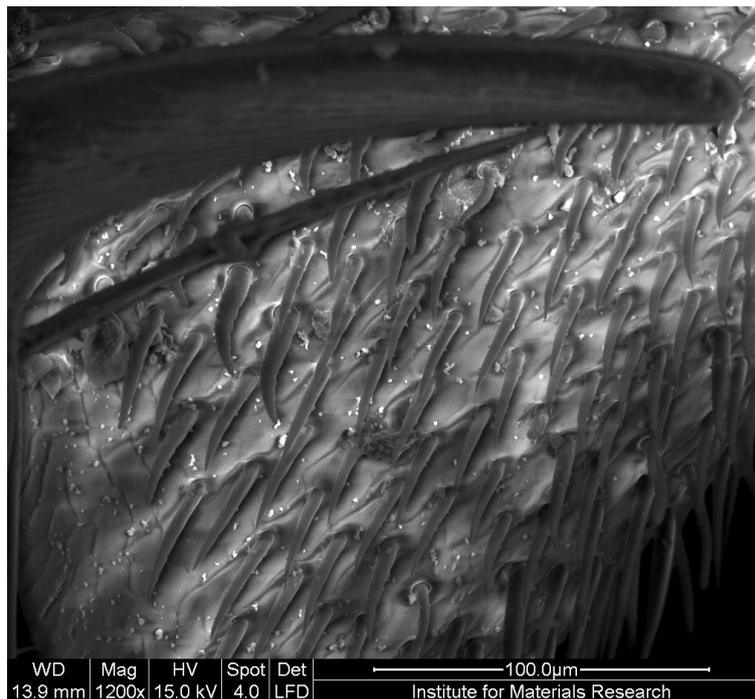


Figure 16 – The planta with spines.

GOODMAN (2003) suggested that the tarsal glands, located in the fifth tarsomere of each leg, produce ‘the footprint pheromones’, oily colorless secretion(s) with low volatility. The secretion product(s) originating from a reservoir near the unguitactor in the fifth tarsus spread over the surface of the planta. In agreement with GOODMAN (2003) and STELL (2012), we found no connection between the secretory reservoir of the Arnhart gland and the foot parts. Therefore, the secretory products most likely flow straight to the arolium.

### Unguitractor

The proximal side or front of the unguitactor plate extends into the pretarsus along a membranous fold, and the outer or distal end attaches to the planta (Fig. 17) with a thin membrane. It is attached to the base of the claws with a tendon-like elastic structure. On its distal side, the unguitactor plate is attached to the weakly-sclerotized hairy planta plate, which connects to the arolium by the arcus.

Based on light microscopic observations, GOODMAN (2003) stated that “the unguitactor plate is covered with a scaly surface”. However, the light microscopic micrographs presented here (Fig. 15A–B) do not confirm this statement. When viewing under a slightly inclined angle, lots of pyramidal spines can be seen in the transition region between the unguitactor and the planta (Fig. 15A–B). The SEM micrographs (Figs 18, 19A–B) provide detailed images of the surface, densely covered with pyramidal-shaped spines. When the unguitactor touches a rough surface the Van der Waal forces are strong, which, in combination with the grip of the claws, results in a firm grip. On a smooth surface the Van der Waal forces are too weak for a sufficient grip of the claws. In that case, the unfolded adhesive arolium clings to the substrate thereby providing sufficient grip.

From the femur and tibia muscles a long unguitactor tendon passes (Figs 17, 20A, 21) through all tarsomeres and connects to the unguitactor plate. In agreement with their observations in the bumblebee (*Bombus terrestris* L.), JARAU *et al.* (2005, 2012) also reported the presence of footprint secretions for the stingless bee (*Melipona seminigra* Friese). The secretion produced by the glands around the unguitactor tendon is released at the base of the unguitactor plate. WILMS & ELTZ (2008) also proposed

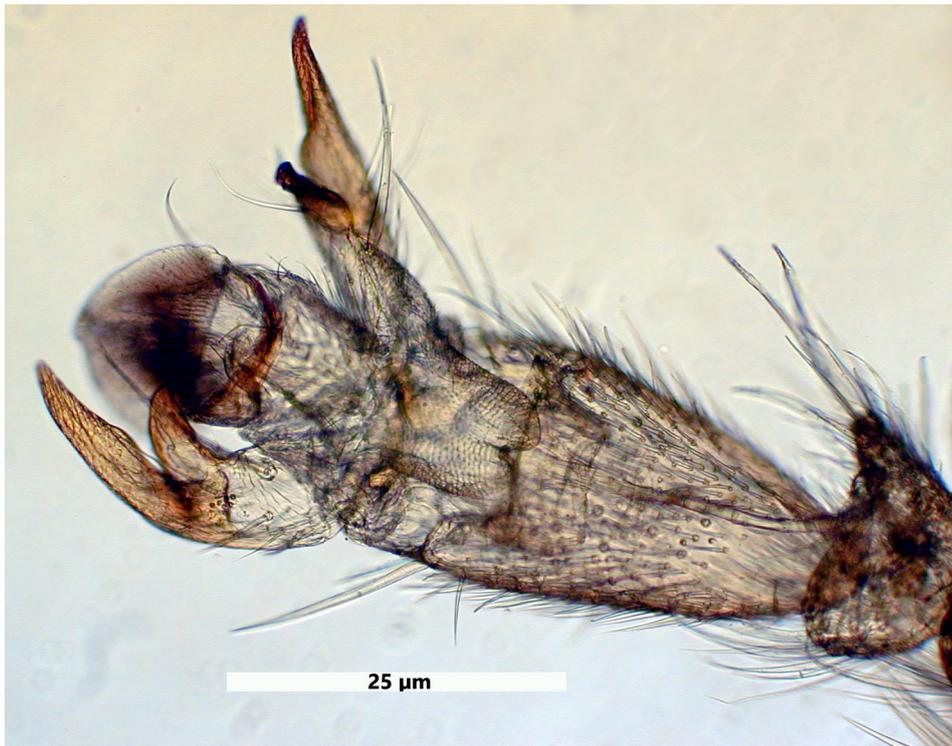


Figure 17 – Overview of the pretarsus, the foot and tarsus The unguittractor is covered at one side by the tarsus.

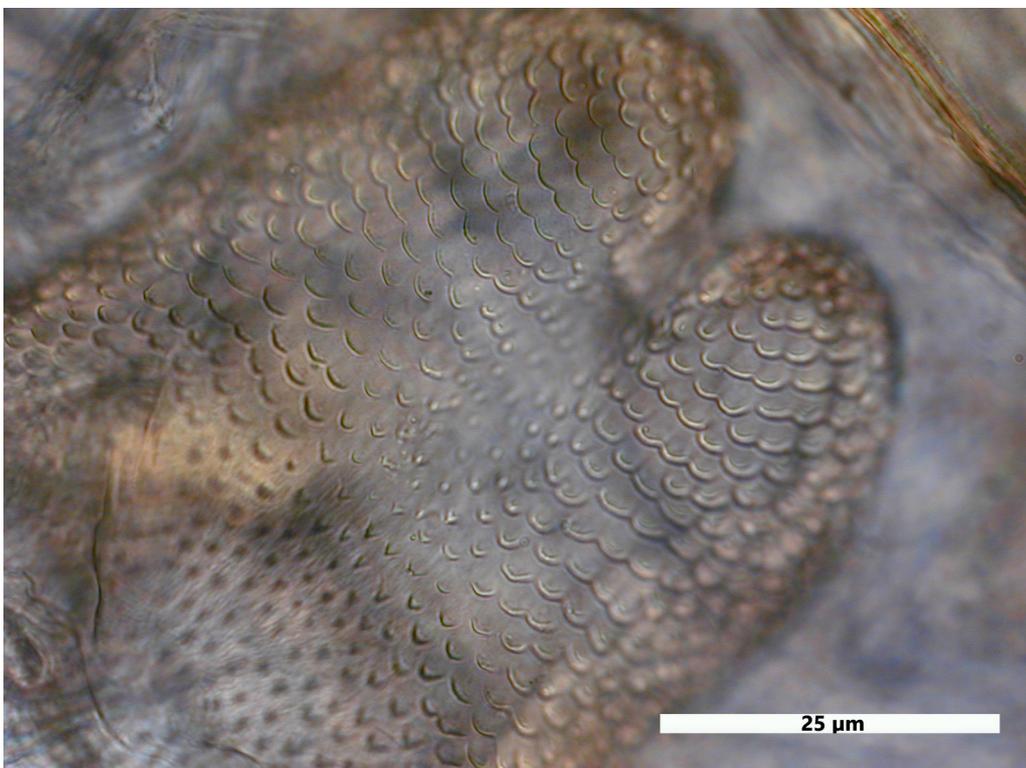


Figure 18 – The “scaly” appearance of the unguittractor, which is not strictly scaly, but possesses pyramidal spines.

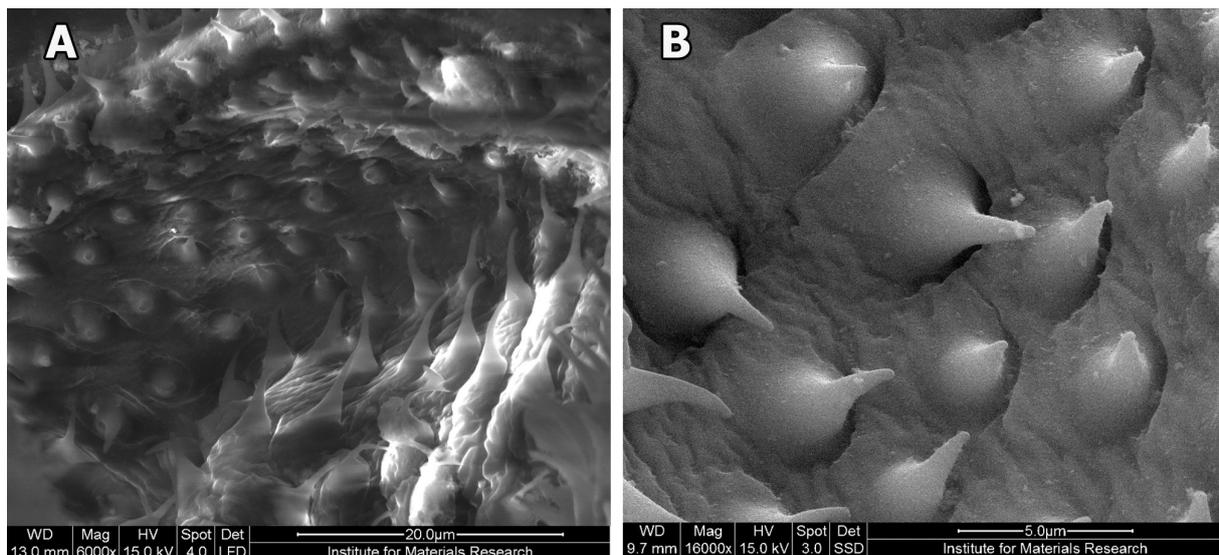


Figure 19 – A–B. The unguitactor is covered with pyramidal-shaped spines.

the occurrence of an epithelial gland around the tendon in the honeybee. However we could not find any indication for the existence of one or more pores from which such secretion could be released. Studies on honeybees (LENSKY *et al.* 1985) and wasps (BILLEN 1986) using transmission electron microscopic techniques (TEM) have confirmed the absence of pores, which could not be located either in the cuticle of the arolium, or in the cuticle of the fifth tarsomere. So the glandular secretion mechanism remains unsolved. Most likely, the tendon glands rather than the tarsal glands (Arnhart's gland) discharge footprint secretions in honeybees, bumblebees and stingless bees. BILLEN (1984) found that this is, however, not the case for ants.

There is a junction (Fig. 20A–B) of the unguitactor tendon to the arolium by the arcus. When the muscle contracts, the tendon draws on the membranes of the foot and the two auxiliary sclerites on either side of the unguitactor plate. The leverage causes the claws to flex down to provide attachment to the surface. The arolium between the claws usually turns upwards.

Foot rupture from the fifth tarsomere (Fig 21) making the long unguitactor tendon visible. This tendon is emerging from the fifth tarsomere making the long unguitactor tendon visible in the other tarsomeres.

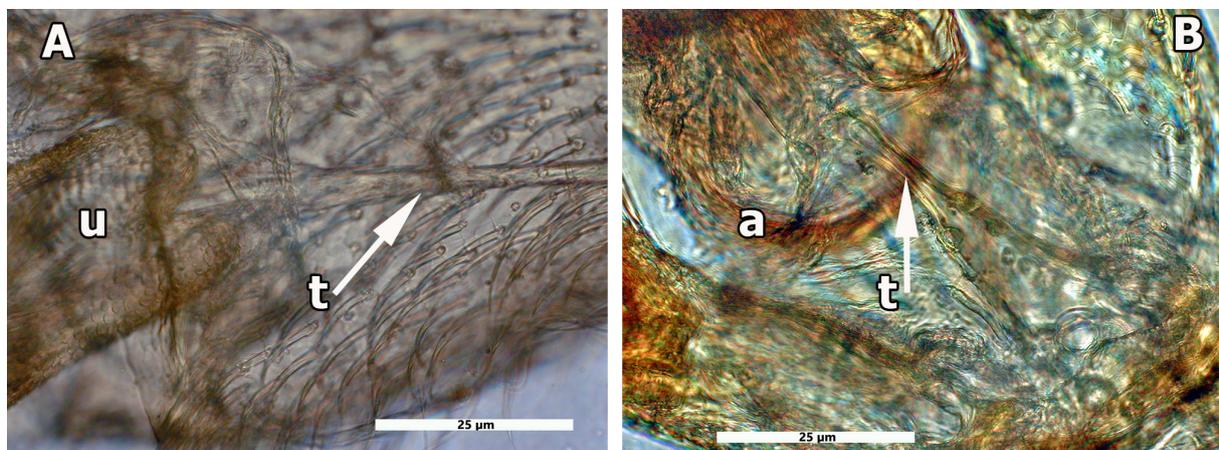


Figure 20 – A. Part of the unguitactor (u) with the attached tendon (arrow, t). B. The junction of the tendon (arrow, t) from the unguitactor to the arolium by the arcus (a).

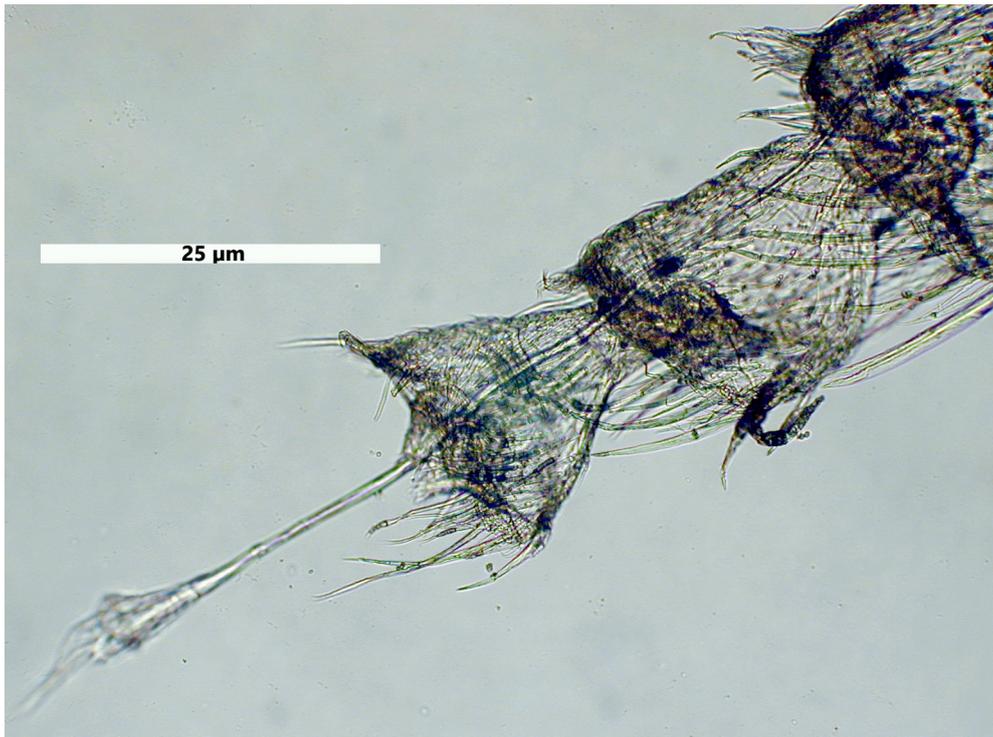


Figure 21 – Foot ruptured from the fifth tarsomere making the long unguitactor tendon visible emerging from the fifth tarsomere, and inside in the fourth and third tarsomeres.

### Manubrium

The manubrium (Fig. 22A–B) is hinged between the claws. It is a sclerotic plate (Fig. 23A–B) bearing five or six long bristles. These bristles are covered with small tactile or sensory hairs. The plate is attached at one side to the arcus of the arolium.

When the manubrium is put down on the arcus, after being stimulated by the long bristles, the arcus will stimulate the arolium to unfold.



Figure 22 – A–B. On the top of the foot the manubrium, bearing five or six long bristles is visible.

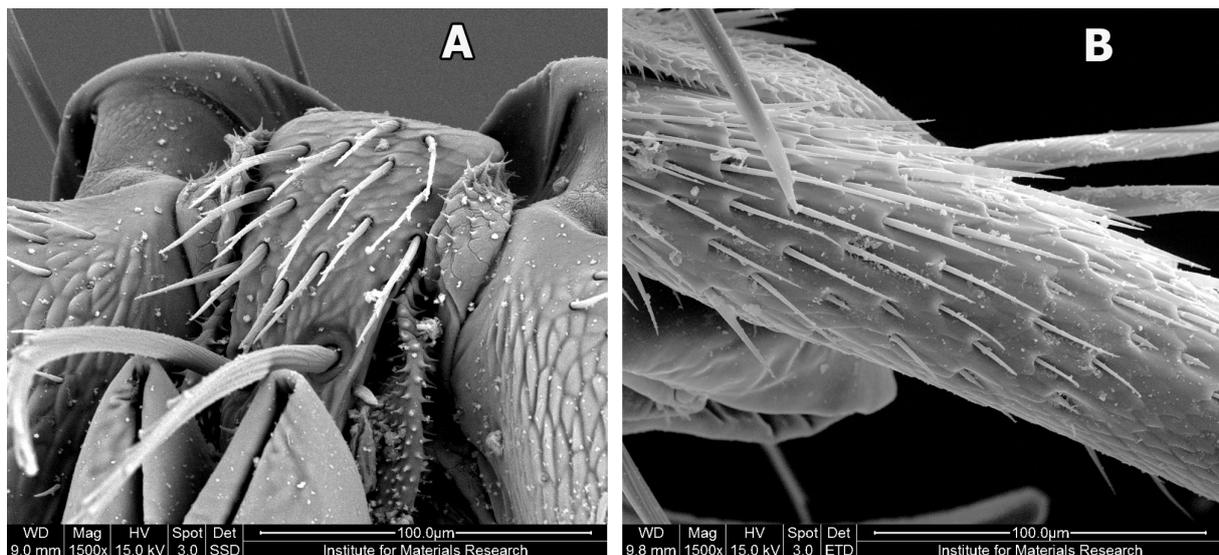


Figure 23 – A–B. Front view of the manubrium with detail of one bristle.

### Discussion and conclusion

The pretarsus is a complex structure, divided into two segments: the complex foot and the tarsus. This study confirms and elaborates on earlier studies regarding the complex honeybee foot organization. It is clear that our in-depth study using SEM to visualize surface details has allowed us to bring forward new information. The spiny appearance of the planta, the scaly surface at the end and the pyramidal-shaped spines in the more proximal part of the unguitactor plate, the details of the bristles on the surface of the manubrium, as well as the identification of the longitudinal grooves and very small sensory spines on the surface of the claws, and finally the parallel grooves on the surface of the arolium, are clear examples of innovative documentation. We also elucidated the junction of the tendon from the unguitactor to the arolium by the arcus.

Based on our morphological observations, and the findings from previous studies, we tried to comprehend the attachment mechanism of the honeybee foot to both rough and smooth surfaces. Also important was the fact that we did not find any evidence for the Arnhart's tarsal gland to be involved in the release of the predicted footprint pheromones as suggested for the bumblebee (*Bombus terrestris*) (WILMS & ELTZ 2008). The fact that we did not observe secretory pores either in the cuticle of the arolium, or in the cuticle of the fifth tarsomere is in agreement with the absence of such pores in honeybees (LENSKY *et al.* 1985) and wasps (BILLEN 1986), although JARAU *et al.* (2005) did report the presence of footprint secretions in the stingless bee (*Melipona seminigra* Friese). In the honeybee, the secretion produced by the glands around the unguitactor tendon is released at the base of the unguitactor plate. Most likely, the tendon glands rather than the tarsal glands (Arnhart's gland) discharge footprint secretions in honeybees, bumblebees and stingless bees although this is, however, not the case for ants (BILLEN 1984).

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