THE DISTRIBUTION OF CAMPANIFORM SENSILLA ON THE APPENDAGES OF *MINDARUS* SPECIES (HEMIPTERA: APHIDIDAE)[1]

Laurent Montagano[2] and Colin Favret[2,3]

ABSTRACT: The proprioceptive function of a campaniform sensillum is to perceive stresses and changing forces on the cuticle, facilitating insect locomotion including walking and flight. In the aphid genus *Mindarus* (Hemiptera: Aphididae), campaniform sensilla are often clustered in groups and are found at specific locations on the insect's appendages. Ring-shaped, approximately 5µm in diameter, these sensilla are found in predictable numbers on the pedicel (1), the trochanter (4), the femur (2-4), and the distitarsus (1). Those situated on the wings are variable in number and size. The wing sensilla form five identifiable groups: four on the forewing and one on the hindwing, with an average of 4 or 6 sensilla per group. Light and electron scanning microscopy was conducted to reveal their external anatomical detail. The pattern and distribution of campaniform sensilla does not appear to inform *Mindarus* taxonomy.

KEY WORDS: *Acyrthosiphon pisum*, anatomy, Mindarinae, morphology, proprioception, Sternorrhyncha, wing

INTRODUCTION

Campaniform sensilla are proprioceptive sense organs which convert forces into electrical discharge by detecting strains or deformations in the insect cuticle (Pringle, 1937). They are usually under 25µm in diameter and possess a dome-like cap set within a ring-like canal in the cuticle where the nerve endings are located (Keil, 1997). Campaniform sensilla are usually found in clusters at various locations on the insect body, including the legs, wings and wing bases, halteres, head, thorax, abdomen, antennae, mouth parts, cerci, and ovipositor (Snodgrass, 1993). Ridget et al. (2003) and Hofmann and Bässler (1982) showed that campaniform sensilla are used to collect information regarding the forces applied to cockroach and stick insect legs, respectively. More specifically, they can detect substrate engagement, a function essential in locomotion (Zill et al., 2010, 2014; Ichikawa, 2014). It has also been suggested that campaniform sensilla carry out an important role in feedback for controlling movements or for adjusting to changing external forces or body weight (Noah et al., 2004; Zill et al., 2011, 2014; Ichikawa, 2014). Pringle (1948) and Dickerson et al. (2014) highlight the fact that campaniform sensilla are present on the wings and halteres of certain insects. These allow insects to detect strains caused by the deformation and movement of the wings, thereby gathering critical information concerning flight dynamics.

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[2] University of Montreal, Department of Biological Sciences, Biodiversity Centre, 4101 rue Sherbrooke est, Montreal, Quebec, H1X 2B2 Canada. E-mail: laurentmontagano@hotmail.com

[3] Corresponding author E-mail: ColinFavret@AphidNet.org

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Although campaniform sensilla have been identified in all principal insect orders (Snodgrass, 1993), they have not been studied in smaller insects. In aphids (Hemiptera: Aphididae), the campaniform sensillum on the pedicel is the only one to have attracted any attention. Dunn (1978) published images obtained through electron scanning microscopy of what he called coeloconic and campaniform sensilla. It was also reported that Johnston's organ is contained in the distal end of the aphid pedicel and that a single campaniform sensillum is co-located on the exterior of the pedicel of certain species (Bromley et al*.,* 1980; Yang et al*.,* 2009). Although Gimpel and Miller (1996) drew a sensillum on the pedicel and McKenzie (1967) on the trochanter of mealy bugs (Hemiptera: Coccoidea), to our knowledge, Stroyan (1984) is the only aphid taxonomist to include campaniform sensilla in his drawings, although he did not label them.

Mindarus (Hemiptera: Aphididae) is a poorly studied aphid genus exhibiting a large number of plesiomorphic character states. A number of cryptic species await description (Favret, 2009). In our search for taxonomically informative morphological characters, we studied the external anatomy and the distribution of campaniform sensilla on the appendages of *Mindarus* species.

MATERIALS AND METHODS

Light microscopy was conducted on specimens of several species of *Mindarus* Koch, *M. kinseyi* Voegtlin, *M. pinicola* (Thomas), and *M. victoria* Essig, borrowed from the United States National Aphid Collection (Beltsville, MD) and the Illinois Natural History Survey (Champaign, IL). We used a Zeiss Imager.M2 microscope, an AxioCam HR camera, and ZEN Pro software (Oberkochen, Germany) to observe and photograph the aphids. Specimens were observed and photographed under brightfield, phase contrast, and differential interference contrast.

We compared the general distribution, measured the size, and counted the number of sensilla in 27, 30, and 21 adult, alate, female, viviparous specimens of *Mindarus kinseyi*, *M. pinicola*, and *M. victoria*, respectively. With the Zen Pro imaging software, we measured the circular diameter and the two-dimensional surface area of the sensilla. The sensilla are imperfectly circular, so we thus measured the largest possible diameter and used the outer edge as a border for the area. We compared the sizes of the different sensilla with analyses of variance with R software, version 3.1.1.

Eight adult, alate, unidentified, ethanol-preserved *Mindarus* specimens, dehydrated progressively to 100% ethanol, were observed using an environmental scanning electron microscope (Hitachi TM 1000 tabletop SEM, Tokyo, Japan). Ethanol-preserved specimens are more difficult than fresh specimens to fully dehydrate and prepare for sputter-coating. Light microscopy revealed that the forms of the sensilla of *Acyrthosiphon pisum* (Harris) (Aphidinae) and *Mindarus* were substantially similar. Because fresh specimens of *Mindarus* were unavail-

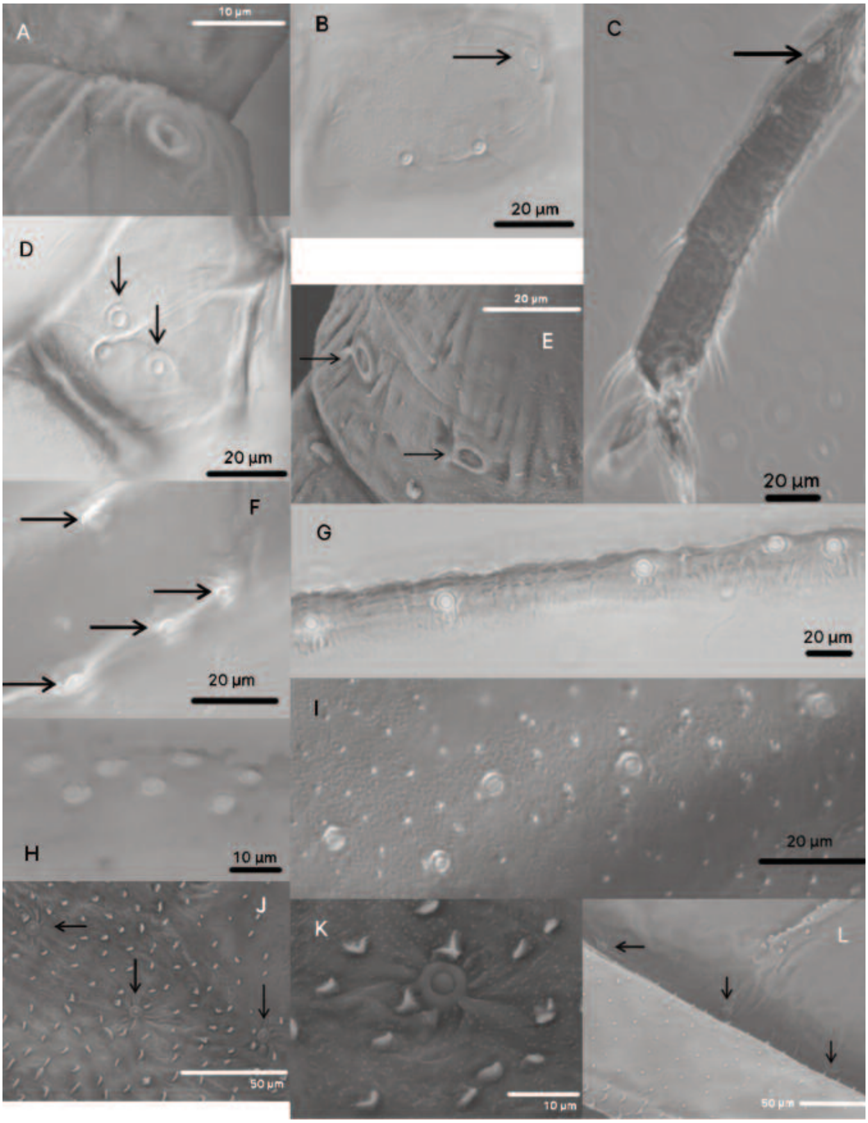

Fig. 1. Light and scanning electron micrographs of individual or clusters of campaniform sensilla on appendices of *Mindarus* species or, where indicated, *Acyrthosiphon pisum*. (A), (B) pedicel; (C) distitarsus; (D) trochanter; (E) trochanter of *A. pisum*; (F) femur; (G) forewing subcosta; (H) base of forewing; (I) base of hindwing; (J) (K) pterostigma of *A. pisum*; (L) at junction of subcosta and cubital vein of *A. pisum*.

able during the study, to capture the best possible images of campaniform sensilla, lab-reared alate *A. pisum* specimens were dissected to obtain anterior wings and legs. The body parts were coated with 10 nm of a mixture of gold and palladium through cathodic pulverization using a low vacuum coater Leica EM ACE200 (Wetzlar, Germany). Imagery was captured with the same Hitachi SEM.

RESULTS

Campaniform sensilla were found at nine different locations on the aphid body: the pedicel, trochanter, femur, distitarsus, and in five distinct groups on the wings (Table 1). There were no differences in sensillum distribution or size among the *Mindarus* species, although sensilla from different body parts were often of different sizes. In order to increase our sample size and compare the sensilla at different locations, data were pooled (Table 1). The overall morphology of specific *Mindarus* and *Acyrthosiphon* sensilla was identical, therefore the *A. pisum* SEM images are presented to show details (Figs. 1E, 1K, 1L).

Table 1: Sensilla size at nine locations on the body of 81 *Mindarus* specimens

Location	Number		Diameter (µm)		Area (μm^2)	
	Mean	Range	Mean	SD	Mean	SD
Pedicel	1		5.34	0.86	18.29	4.17
Trochanter	4		4.65	0.58	17.92	5.31
Femur	3	2-4	5.05	0.84	20.08	6.72
Distitarsus	1		4.97	0.84	18.37	5.41
Fw basal	4.347	2-8	6.45	0.79	26.49	7.81
Fw subcosta	6.077	2-12	7.26	1.36	41.79	16.62
Fw junctions	4.316	2-8	6.57	1.02	30.54	9.76
Fw pterostigma	4.078	1-8	6.87	1.23	39.07	14.06
Hw basal	6.082	2-10	4.18	1.15	16.10	7.31

Two sensilla are found on each of the anterior and posterior faces of the trochanter, located at approximately three-quarters of the length along the trochanter (Figs. 1D, 1E). Each sensillum may be flush with the surrounding cuticle or may protrude slightly. The ventral face of the femur has 2-4 sensilla. In most cases, they are close to the base of the femur, near its joint with the trochanter; rarely, the most distal sensillum may be located as far as a third of the length of the femur. They are usually aligned along the femur, with the second sensillum often slightly closer to the first than to the third (Fig. 1F). When not aligned, the second sensillum is shifted dorsally in relation to the other two. Unlike the sensilla from the antenna and other leg segments, the femoral sensilla vary in number: three are seen most often, but only two are present on the femora of some immature individuals. A fourth sensillum, dorsal to the other three, appears on some adults (Fig. 1F). Of the antenna and leg sensilla, those of the femur are generally the largest in area (Table 1). A single campaniform sensillum is located on the tarsus, positioned dorsally at the base of the distitarsus (Fig. 1C). The tarsal sensillum has a typical diameter and surface area and though always round, its protruding inner ring is similar to that of the pedicel; it is never upraised as it sometimes is on the trochanter or femur.

The wings feature the largest number of campaniform sensilla. They are found in distinct locations on the forewing in four identifiable groups, here referred to as the "basal", "subcosta", "junctions", and "pterostigma" groups (Table 1). The hindwing also carries a group of sensilla near its base. Those on the forewing are much larger in diameter and area than those on the antenna and leg. In contrast, the hindwing's sensilla are the smallest (Table 1). The forewing basal group is located near the base of the forewing on the posterior half of the subcostal vein (Figs. 1H, 2). It can be distinguished from all other groups because these sensilla are organized in one or several rows. The average number of sensilla in the basal group is four, with 79% of groups having between 3 and 5. As with the campaniform sensilla on the rest of the body, those on the wings are shaped like a swollen ring. The forewing subcosta group is composed of bulky sensilla arranged in a row along a large portion of the dorsal subcosta (Figs. 1G, 2). The row of sensilla begins close to the intersections of the subcosta with the cubital veins (cu-1a and cu-1b) and ends at the pterostigma. Most of the time (89%), 4 to 8 of these sensilla can be found on a single wing. The forewing junctions group is found on the ventral face of the forewing, on the posterior face of the subcosta or on the membranous part of the wing immediately posterior to the subcosta (Fig. 1L). Like the subcosta group, these sensilla are arranged linearly, but centered near the intersections of the subcosta and the cubital veins (Fig. 2). The sensilla in the forewing junctions group create minute folds on the wing along their circumference (Fig. 1L). The forewing pterostigma group is found on the ventral face of the pterostigma towards the distal end of the wing (Fig. 2). The pterostigma sensilla are harder to see and are not clustered like those of the basal or junctions groups. Like the junctions sensilla, the pterostigma sensilla are located ventrally and also create folds on the wing all along their circumference (Figs. 1J, K). Three-quarters of specimens (74%) had between 3 and 5 sensilla in the forewing pterostigma group. Much resembling and probably serially homologous to the forewing basal group, the hindwing group is apparently haphazardly distributed at the base of the hindwing (Figs. 1I, 2). The hindwing sensilla are very small, even when compared to those found on the tarsus (Table 1), and vary in number: 4 to 7 in 73% of cases.

Fig. 2. Fore and hind wing of *Mindarus* indicating the distribution of campaniform sensilla. X-markers indicate sensilla located dorsally (Fw basal, Fw subcosta, and Hw basal), O-markers are ventrally located sensilla (Fw junctions, Fw pterostigma).

DISCUSSION

The function of the campaniform sensillum on the distal end of the pedicel of vegetable aphids is to detect stresses from the movement of the flagellum (Dunn, 1978). Bromley et al. (1980) suggested that Johnston's organ, the pedicel campaniform sensillum, and antennal joint receptors all work as a single, coordinated multifunctional unit whose main task is antennal proprioception. It seems likely that the several campaniform sensilla, whether on the leg or the wing, also act as a unit. Meresman et al. (2014) studied the stimuli with which an aphid rights itself during a fall and concluded that the righting behavior was caused by a reflex of the tarsus. Perhaps it is the tarsal campaniform sensilla that detect the aphid's position during its fall.

Ridgel et al. (2003) found that campaniform sensilla increase in size and number as cockroaches grow in size and suggested that it is the need to detect more force. Therefore, larger and more numerous sensilla located on the forewings of aphids compared to the hindwing, pedicel or legs may simply be because the forewings detect a larger range of forces. Aphid wings certainly flex more in flight than do their legs while walking. This may also explain why aphids possess few campaniform sensilla compared to larger insects such as tenebrionid beetles whose claws alone can harbor 25 to 45 sensilla each (Ichikawa et al., 2014).

The present study was born of the idea that the size, number, or distribution of campaniform sensilla may inform species-level aphid taxonomy. On the contrary, however, we found that they were very regular across the diversity of the genus *Mindarus*. Preliminary studies of the distribution of the more distantly related genus *Uroleucon* suggest that there may be little variation even between genera and subfamilies. There is yet much to learn about how campaniform and other proprioceptive organs function, especially in coordination, but it does not seem that their external morphology contains much taxonomic/phylogenetic signal for aphids.

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