Sociobiology 72(4): e11823 (December, 2025) DOI: 10.13102/sociobiology.v72i4.11823

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Antennal Sensilla of Workers of *Melipona quadrifasciata anthidioides* Lepeletier (Apidae, Meliponini) Removing and non-Removing Dead Brood: Morphology, Morphometry and Quantity

Jossimara N. de Jesus[1,2], Johnnatan D. de Freitas[3], Paulo R. R. Mesquita[1], Carlos Alfredo L. de Carvalho[2]
Frederico M. Rodrigues[1], Cândida Maria L. Aguiar[4]

1 - Agricultural Technological Center of the State of Bahia, Salvador, Brazil
2 - Federal University of Recôncavo of Bahia, Cruz das Almas, Brazil
3 - Federal Institute of Alagoas, Maceió, Brazil
4 - State University of Feira de Santana, Feira de Santana, Brazil

Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 12 June 2025
Initial acceptance 18 October 2025
Final acceptance 04 November 2025
Publication date 23 November 2025

Keywords
Hygienic bee, olfactory system, stingless bee, unhygienic bee.

Corresponding author
Jossimara N. de Jesus
Universidade Federal do Recôncavo da Bahia (UFRB)
Rua Rui Barbosa s/nº - CEP: 44380-000
Cruz das Almas, Bahia, Brasil.
E-Mail: apismara@yahoo.com.br

Abstract

The sophisticated chemical communication of bees allows the natural control of pathogens and diseases in the colony, through hygienic behavior (HB), in which dead or sick brood are removed, improving colony health. In this study, we investigated the morphology, morphometry, and quantity of sensilla in workers of *Melipona quadrifasciata anthidioides* Lepeletier, removing and non-removing dead brood in the HB test. From the micrographs obtained through scanning electron microscopy, the sensilla trichodea (STr), basiconic (SBa), placodea (SPl), coeloconic (SCo), campaniform (SCa), ampullacea (SAm), chaetica (SCha), and their subtypes were morphologically characterized. We highlight the identification of sensilla STr 7, setae (SSe), placodea 2 (SPl 2), Bohm's bristles (BhB), and branched bristles (BB), not previously reported in this species. Morphometry indicates that removing workers have longer average antenna length and segment lengths than non-removing bees, as well as longer lengths of the SCo, SPl 1, SSe, STr 1, STr 2, STr 3, and STr 4 sensilla. Regarding the quantification of sensilla, no significant difference was found between the groups. Given that chemical stimuli elicit bee HB, sensilla may play a fundamental role in hygienic bee lineages, and there is a need to further explore this relationship to understand bee HB better.

Introduction

Insects can interact with conspecifics, the environment, and other organisms through the antennal olfactory system, a communication mechanism essential to their survival and reproduction (Elgar et al., 2018; Kannan et al., 2022). Efficiency in communication is challenging for social insects due to the division into castes, which requires synchrony to perform activities (Grüter & Keller, 2016). Additionally, the overlapping of generations in the colony makes it more susceptible to diseases, parasites, and various contaminants due to high intergenerational contact rates (Swanson et al., 2009).

Throughout evolutionary history, eusocial bees have developed a type of biological control against pathogenic organisms and contaminants within colonies, known as hygienic behavior. This is a genetically based behavior (Bigio et al., 2013), in which capable worker bees identify volatile compounds emanating from dead or sick brood and remove them, eliminating the focus of infection in the colony (Wilson-Rich et al., 2009; Bigio et al., 2013; Evans & Shutler, 2023).

Hygienic behavior has been associated with the olfactory system, as workers from colonies with hygienic behavior exhibit greater olfactory sensitivity than those from unhygienic colonies (Masterman et al., 2000; Spivak et al., 2003; Gramacho &

ISSN: 0361-6525

Spivak, 2003; Mondet et al., 2015). The recognition of odors emanating from dead or sick brood occurs through olfactory sensilla, cells specialized in sensory perception, which are found mainly in the antennae of insects (Cruz-Landim, 2009). These cells contain OBPs (Odorant Binding Proteins) that bind to chemical compounds that trigger removal behavior in bees (Gramacho et al., 2003; Wagoner et al., 2020; Gebremedhn et al., 2023). Sensilla are linked to receptor neurons, which process information based on the type of signal received and trigger a behavioral response (Chen et al., 2003; Yan et al., 2020).

There are several types of sensilla, which are classified based on their size, shape, and function, among which chemoreception (basiconic porosa, trichodea, and placodea), hygroreception and thermoreception (ampullacea, campaniform and coeloconic), contact chemoreception and mechanoreception (chaetica), mechanoreception (setae, Bohm's Bristles and Branched Bristles) are described (Kaissling & Renner, 1968; Van Baaren et al., 1999; Fialho et al., 2014; Ravaiano et al., 2014). There are several studies aimed at understanding the distribution pattern of sensilla in *Apis mellifera* L. (Stort & Moraes-Alves, 1999; Frasnelli et al., 2010; Fialho et al., 2014). However, the sensilla of stingless bee species have been little investigated. There are some studies on the sensilla of *Melipona scutellaris* Latreille (Nascimento et al., 2013; Carvalho et al., 2017), *Tetragonisca angustula* (Latreille) (Dohanik et al., 2017) and *Melipona quadrifasciata* Lepeletier (Moraes & Cruz-Landim, 1972; Azevedo et al., 2008; Ravaiano et al., 2014). In studies using *Melipona quadrifasciata*, especially in Ravaiano et al. (2014), 13 types of sensilla were identified. On the other hand, until now, only Gramacho et al. (2003) investigated the relationship between colony hygiene and the pattern of olfactory sensilla in the honeybee.

In this study, we investigated the hypothesis that there are differences in the morphology, size, and quantity of olfactory sensilla between *Melipona quadrifasciata anthidioides* workers from hygienic (removing) and non-hygienic (non-removing) colonies. To test our hypothesis, we characterized morphologically and morphometrically the antennal segments and antennal sensilla of worker bees, both those that removed dead brood and those that did not.

Materials and Methods

Selection of colonies

We used 25 colonies of *Melipona quadrifasciata anthidioides* installed in the meliponary of the Federal University of Recôncavo da Bahia (UFRB), in the municipality of Cruz das Almas, state of Bahia, Brazil. Colonies were categorized as hygienic or unhygienic based on freezing brood with liquid nitrogen. A colony was considered hygienic when workers removed more than 90% of dead brood within 48 hours (Spivak & Reuter, 1998; Jesus et al., 2017). After screening, four colonies that presented 100% removal of the dead brood in three consecutive tests were selected to act as donors of brood discs of the hygienic lineage. On the other hand, the colonies that presented the lowest percentages of removal (0% to 57%) were chosen as donors of brood discs of the non-hygienic lineage (n=4). The discs containing brood in the pupal stage were removed from the hygienic and non-hygienic colonies and stored separately, based on hygiene level, in plastic cages lined with voile fabric. The bees were kept in a BOD (Biological Oxygen Demand) incubator at 27 ± 5 ºC and 70 ± 5% humidity, with a 24-h scotophase, until worker emergence. The newly emerged bee workers were removed from the BOD incubator daily and marked with numbered tags affixed to the dorsal thorax surface for individual identification and age control. After marking, the bees were transferred to two observation colonies, one containing workers of hygienic ancestry and the other with workers of unhygienic ancestry.

Each observation colony consisted of approximately 200 marked bees aged 1-20 days (following Arathi et al., 2000), with an average of 10 per age. The observation colonies were set up in INPA model boxes (13 cm x 13 cm x 6 cm) with a 2 mm thick anti-reflective glass lid, installed in the Insect Behavior Laboratory of the Insecta Research Group at UFRB. The colonies were connected to the outside via plastic tubes running through the building wall, so the bees could carry out their external activities normally.

A section of brood disc containing seven larvae killed by freezing in liquid nitrogen was inserted into each observation colony, and filming of worker behavior immediately began. In the hygienic descent colony, we identified and captured the workers that removed the dead larvae within 24 h. The same procedure was carried out in the observation colony containing workers of unhygienic descent. In this case, bees were captured that were not involved in removing dead larvae or uncapping brood cells containing dead larvae. In this way, we obtained two groups: dead brood removing workers (RB), all from the observation colony categorized as hygienic, and non-removing dead-brood workers (NRB), all from the observation colony categorized as unhygienic.

Preparation of the antennas

Eight bees from each group (RB and NRB) underwent antennal excision, performed after anesthesia at low temperature (-20 °C). Subsequently, the collected individuals had their antennae (n = 16 per group, RB and NRB) separated into right and left pairs. The antennae were immediately immersed in a fixative solution (composed of 25% glutaraldehyde, 16% paraformaldehyde, and 0.2 M sodium cacodylate buffer). Then, the antennae underwent dehydration in a graduated series of ethanol (70%, 80%, and 95%) for 5 minutes each, and were placed on filter paper for 1 minute to evaporate the solvent.

Scanning electron microscopy - SEM

The antennae of the RB and NRB groups were mounted on double-sided carbon tape (SPI Supplies, PA, USA) and fixed to a Stub (sample holder), which was placed on the Quorum Q150R ES metalizer and sprayed with gold at 45 mA for 200 seconds. The antennae were examined, and images were captured using a scanning electron microscope (Tescan VEGA-3 LMU, Brno, Kohoutovice, Czech Republic) (10 kV~ 20 kV) at the Instrumental Analysis Laboratory of the Federal Institute of Alagoas (IFAL).

Terminology and data analysis

The images obtained from the left and right antennae and sensilla of workers from the RB and NRB groups were treated and classified according to the morphological criteria previously described for bees (Frasnelli et al., 2010; Ravaiano et al., 2014; Huang et al., 2023). We considered the region of the antenna close to the scape and pedicel as "basal", the region close to the tip of the antenna as "apical", and the portion between the basal and apical region was called "medial".

Measurement of antennal segments and sensilla was performed using ImageJ software (v. 1.50i). The length and width of the antennal segments were obtained from 10 antennae (five right and five left) from each group (RB x NRB). The length of sensilla was measured in 8 antennae of each group (RB x NRB), with 40 sensilla being measured per type and subtype. Coeloconic sensilla (SCo) measurement was obtained from a total of 30 sensilla units per group (RB x NRB). Sensilla quantification was carried out in an area of 130 µm2 in flagellomere 10, with eight antennae evaluated per group. SCo sensilla were not considered in this analysis due to insufficient numbers in flagellomere 10.

To facilitate understanding of the different types of sensilla, they were grouped by basic shape: Thread-like, Plate-like, and Hole-shaped. The results were expressed as mean ± standard deviation, and statistical analysis was performed using XLStat version 7.8 (Addinsoft, 2019). Data normality was verified using the Shapiro-Wilk test. Differences between the RB and NRB groups were analyzed by one-way ANOVA, and when significant effects were detected ($p < 0.05$), means were compared using Tukey's post hoc test.

Results

Using SEM micrographs of the antennae of worker bees of *M. q. anthidioides* that remove and do not remove dead broods, it was possible to identify and characterize the morphology and morphometry of 19 types of antennal sensilla, according to morphological criteria of species of the same order, family, and/or genus previously described. Among these, the sensilla setae, Bohm's bristles and branched bristles, as well as the trichodea seven and placodea two subtypes, had not been reported in the literature for *M. q. anthidioides*.

Morphological analysis of sensilla

The antenna of worker bees of *M. q. anthidioides* contains sensilla in both the ventral and dorsal parts (Fig 1A); The flagellum of workers is composed of 10 subunits called flagellomeres (Fig 1B). Morphological characterization indicated the presence of trichodea sensilla represented by seven subtypes (STr 1, STr 2, STr 3, STr 4, STr 5, STr 6, and STr 7); basiconic sensilla and two subtypes (SBa 1 and SBa 2); setae (SSe); chaetica and two subtypes (SCha 1 and SCha 2), placodea and two subtypes (SPl 1 and SPl 2); ampullacea (SAm); coeloconic (SCo); campaniform (SCa); Bohm's bristles (BhB) and branched bristles (BB).

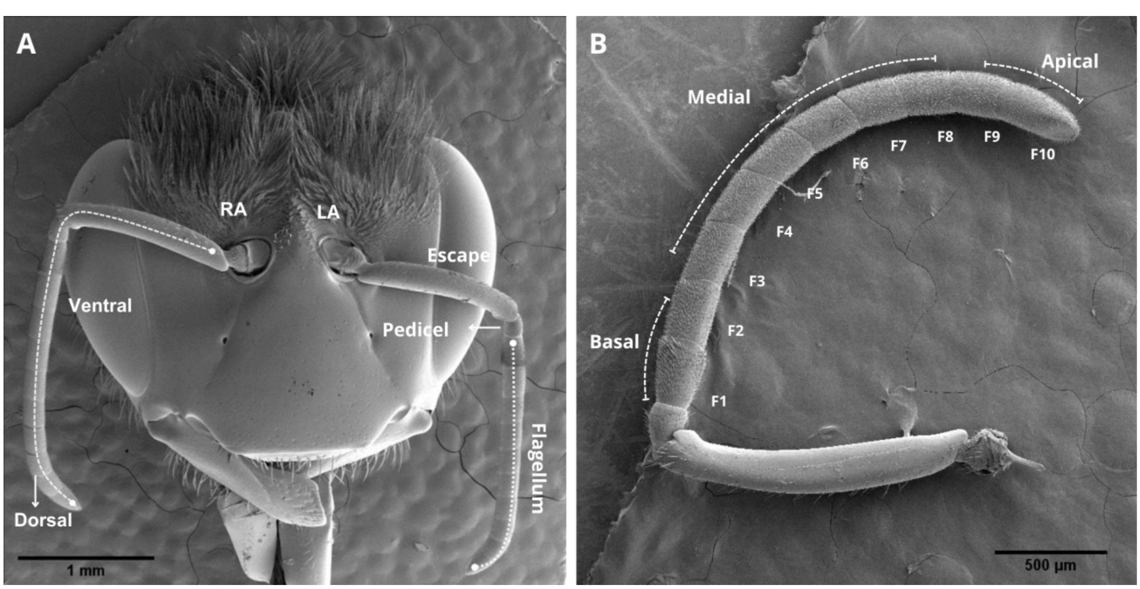

Fig 1. Morphology of the antenna of *Melipona quadrifasciata anthidioides*: (A) SEM micrograph of the head showing the right (RA) and left (LA) antennae, escape, pedicel, and antennal flagellum; (B) Antenna with emphasis on the basal, medial, apical portions and the flagellomeres (F1 – F10) that make up the antennal flagellum.

Thread-like sensilla

The following types of sensilla were identified in this group: trichodea, chaetica, basiconic, setae, branched bristles, and Bohm's bristles. Trichodea sensilla (STr) are distributed from the pedicel to the flagellum of the antennae and comprise seven subtypes (Fig 2). Subtype 1 (STr 1) consists of a structure whose morphology is slightly conical and inclined towards the apical end of the antenna; subtype 2 (STr 2) presents a curvature directed to the surface of the flagellomere, also towards the antennal apex. Both subtypes were observed from the second flagellomere of the basal region of the antenna to the apical portion. STr 3 has a cuticular wall with longitudinal striations, a broad base, and a subtly thin sickle-shaped apical end and is inclined towards the surface of the antennal cuticle; STr 4 has a slightly broad base that narrows along its length, and the cuticular surface of STr 4 also has longitudinal striations. Trichodea sensilla subtypes 3 (STr 3) and 4 (STr 4) were mainly localized to the most basal flagellomere. Subtype 5 (STr 5) has a slightly broad base, and, along the length, as it approaches the tip, the structure curves towards the antennal apex; subtype 6 (STr 6) is characterized by a thin structure with a rounded apical tip. Both STr 5 and STr 6 subtypes are localized on the apical flagellomere. Subtype 7 (STr 7) was found in the basal flagellomere, and a structure with a slightly broad base and pointed end, with a surface showing longitudinal striations. These sensilla are inclined parallel to the surface of the flagellum.

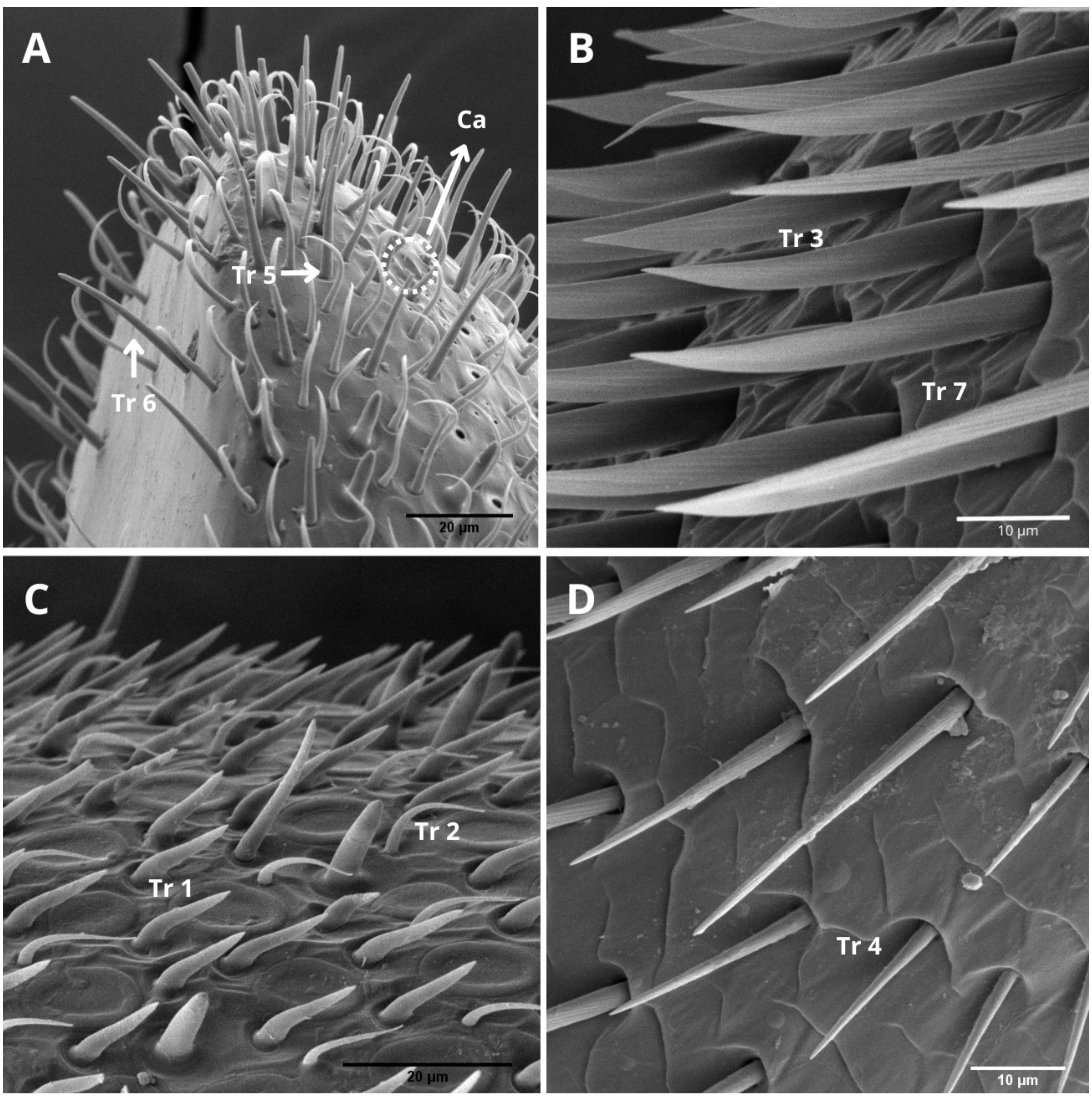

Fig 2. SEM micrographs of sensilla on the antennal flagellum of *Melipona quadrifasciata anthidioides*: (A) apical portion of the antenna sensilla trichodea (Tr 5 and Tr 6), campaniform (Ca); (B) basal portion of sensilla trichodea (Tr 3 and Tr 7); (C) medial portion of trichodea (Tr 1 and Tr 2); (D) basal portion of sensilla trichodea (Tr 4).

Sensilla chaetica (SCha) were classified into two subtypes, SCh 1 and SCh 2 (Figs 3A and 3B). SCh 1 are long with a broad base and a tapered apical end, presenting longitudinal striations on the surface; they are located on the pedicel. SCh 2, on the other hand, appear as short and uniformly broad structures with longitudinal striations and a rounded apical end; they are located mainly in the basal flagellomeres. The sensilla have a structure with a broad base that subtly tapers along the length and has a rounded and curved tip towards the basal portion of the antenna (Fig 3D).

The Basiconic sensilla (SBa) have a broad base, this shape extends uniformly along its length and has a rounded apical end. These sensilla were characterized by subtypes SBa 1 (short structure) and SBa 2 (long structure) (Fig 3C). Its distribution occurs from the first flagellomere to the apex of the antenna.

Already, sensilla setae (SSe) have a broad base inserted in a superficial depression, a slightly tapered end and subtly curved towards the antennal apex. They present longitudinal striations along the cuticular surface (Fig 4A). These sensilla were observed along the flagellum. The Branched bristles (BB) sensilla comprise long, branched structures that have longitudinal striations on the surface and are present on the scape (Fig 4B), while Bohm's bristles (BhB) sensilla are short, pointed structures, whose cuticle surface is smooth; they were observed on the antenna pedicel forming clusters (Fig 4C).

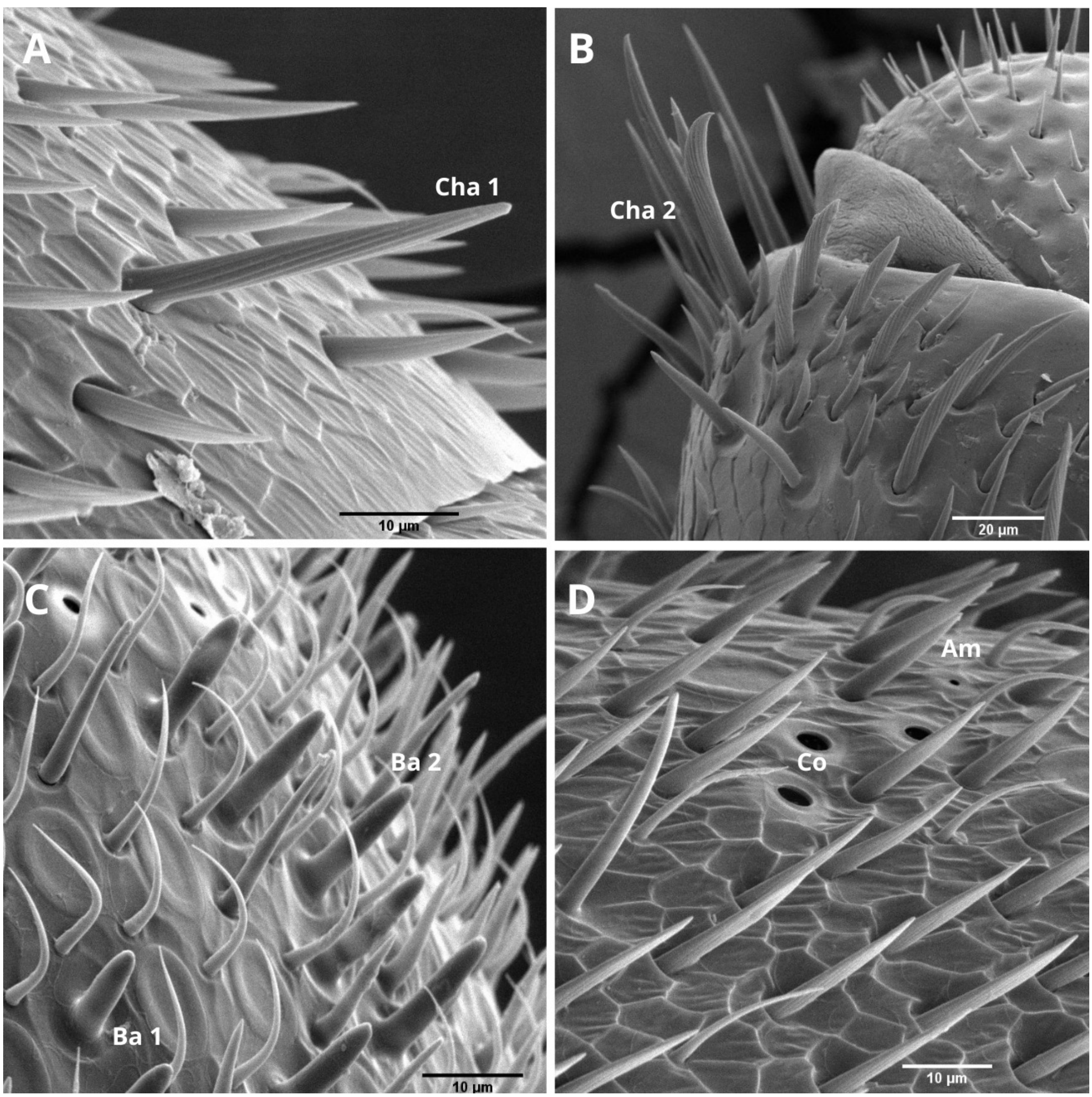

Fig 3. SEM micrographs of sensilla in the antennal segments of *Melipona quadrifasciata anthidioides*: (A) flagellum basal portion of sensilla chaetica (Cha 1); (B) scape of sensilla chaetica (Cha 2); (C) flagellum medial portion basiconic sensilla (Ba 1 and Ba 2); (D) flagellum medial portion coeloconic sensilla (Co) and ampullacea (Am).

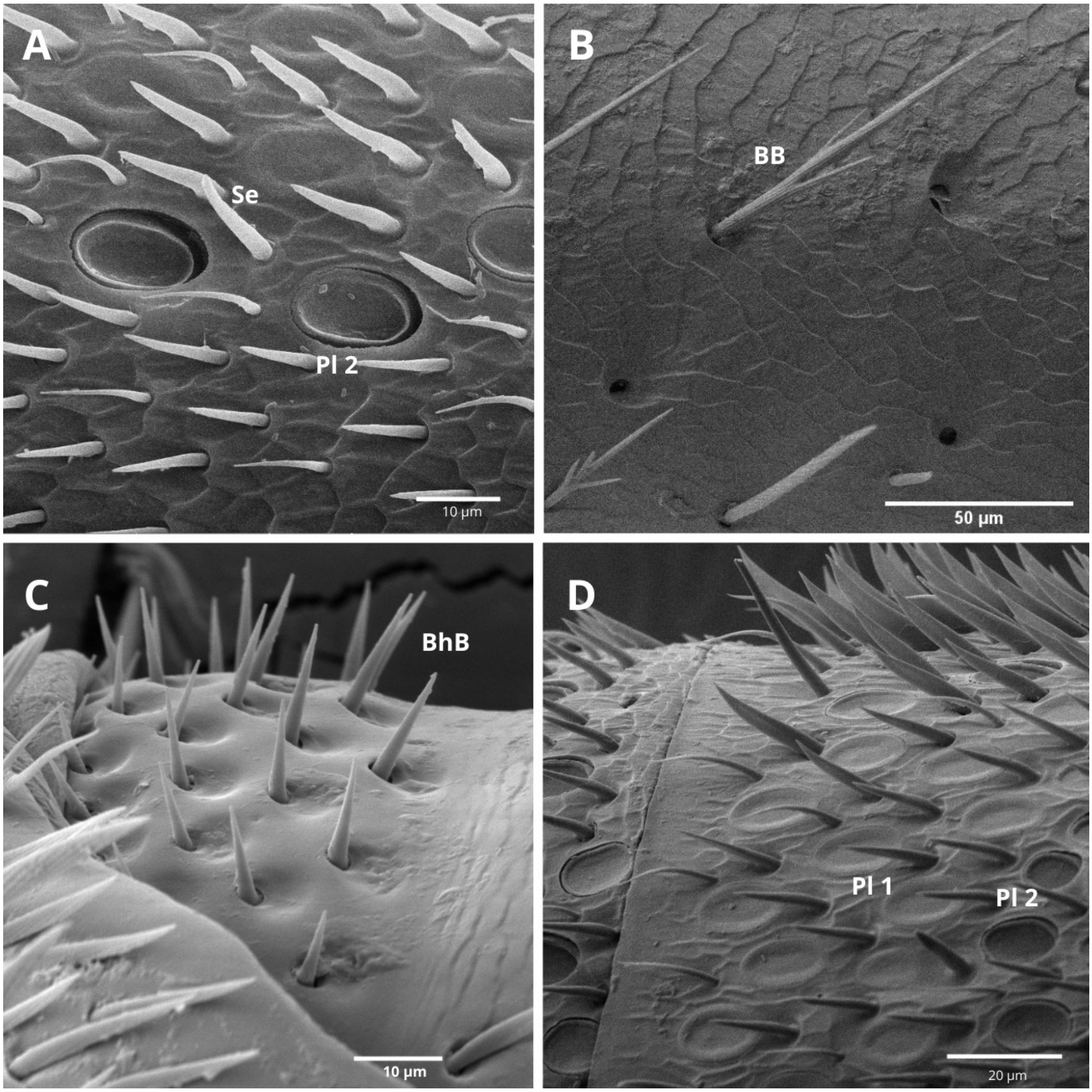

Fig 4. SEM micrographs of sensilla in the antennal segments of *Melipona quadrifasciata anthidioides*: (A) flagellum medial portion sensilla setae (Se) and placodea (Pl 2); (B) scape sensilla branched bristles (BB); (C) pedicel sensilla Bohm's bristles (BhB); (D) placode flagellum (Pl 1 and Pl 2).

Plate-like sensilla

Two types of sensilla were identified in this group: placodea and campaniform. Sensilla placodea (SPl) have a rounded plate shape with variations in their morphology that led to their classification into two morphotypes, SPl 1 and SPl 2 (Figs 4A, 4D). SPl 1 exhibits a projected rough edge, and the center of the plate presents a slight depression in relation to the surface of the flagellum. In contrast, SPl 2, despite presenting similar characteristics to SPl 1, has a deeper central region of the plate and has a contour with a prominent stria and a narrower border compared to SPl 1. In some structures, it was possible to verify that SPl 2 appears to detach from the cuticle, forming a space between the edge of the sensilla and the cuticle. Both subtypes are distributed along the flagellum to the apex of the antenna. Campaniform sensilla (SCa) have a shallow cavity with a small central circular projection on the cuticular surface of the flagellomere (Fig 2A). These sensilla were observed in the apical portion of the flagellum.

Hole-shaped sensilla

Similar to the previous group, only two types of sensilla were identified: ampullacea and coeloconic. Ampullacea sensilla (SAm) are characterized as a hole inserted at the level of the phlegelomere cuticle (Fig 3D). The coeloconic sensilla (SCo) are also characterized by a hole, but much smaller than in the ampullacea sensilla (Fig 3D). From the analysis of the occurrence of sensilla along the antennal segments, it was evident that in the scape and pedicel, there is a lower diversity of sensilla in relation to the flagellum, with only the branched bristle sensilla being identified in the scape and only Bohm's bristles and branched sensilla in the pedicel. bristles, chaetica 1 and 2, setae, and trichodea 4 and 7.

Morphometric and Quantitative Analysis

From the micrographs of the antennae of the RB and NRB groups, a statistical difference was verified between the groups in relation to the morphometry of the antennal segments (scape, pedicel, and flagellum) (Table 1) and flagellomeres (Table 2). In removing bees (RB), the average total length of the antenna and the segments that compose was higher than that observed in non-removing bees (NRB). From the morphometric data on the length of each flagellomere, a statistical difference was observed in flagellomeres F1, F2, and F3 between removing and non-removing bees (Table 2). Regarding width, all flagellomeres of removing needles (RB) presented mean values higher than those of non-removing bees (NRB) (Table 2).

Table 1. Morphometry of the antennal segments of *Melipona quadrifasciata anthidioides*.

Groups	Antenna length (mm)	Scape		Pedicel	
		length (mm)	width (mm)	length (mm)	width (mm)
RB	4.39 ± 0.26a	1.32 ± 0.10 a	0.21 ± 0.02 a	0.18 ± 0.04 a	0.17 ± 0.01 a
NRB	3.86 ± 0.48b	1.20 ± 0.14 b	0.19 ± 0.03 b	0.14 ± 0.03 b	0.15 ± 0.01 b

Mean values ± SE of the length and width of the antennal segments analyzed from 10 antennas (5 right and 5 left) from each group (Removing Bees = RB x Non-removing Bees = NRB). Measurements were obtained from a total of 10 replicates for each group. Values (mean ± SE) followed by different lowercase letters in the same column indicate statistical difference (Tukey test, p < 0.05).

Table 2. The mean length of each flagellomere of *Melipona quadrifasciata anthidioides*.

Flagellomere	length (µm)			width (µm)		
	Removing Bees	Non-removing Bees	p	Removing Bees	Non-removing Bees	p
F1	285.58 ± 15.70 a	247.61 ± 31.96 b	p = 0.003	216.96 ± 8.57 a	180.52 ± 14.50 b	p < 0.000
F2	281.97 ± 15.16 a	255.98 ± 28.81 b	p = 0.021	210.93 ± 5.45 a	184.85 ± 15.50 b	p < 0.000
F3	270.08 ± 17.45 a	249.60 ± 22.31 b	p = 0.035	201.26 ± 6.67 a	181.63 ± 16.98 b	p = 0.003
F4	256.72 ± 13.40 a	246.40 ± 19.94 a	p = 0.191	197.58 ± 9.40 a	178.21 ± 17.01 b	p = 0.006
F5	243.03 ± 5.36 a	228.12 ± 24.97 a	p = 0.081	199.11 ± 11.42 a	178.91 ± 18.22 b	p = 0.008
F6	242.59 ± 7.56 a	229.14 ± 25.03 a	p = 0.121	201.10 ± 12.91 a	180.26 ± 20.47 b	p = 0.014
F7	231.03 ± 7.86 a	220.69 ± 24.50 a	p = 0.220	204.21 ± 14.92 a	178.72 ± 15.25 b	p = 0.001
F8	229.66 ± 15.89 a	212.82 ± 33.73 a	p = 0.170	205.87 ± 8.08 a	184.66 ± 15.45 b	p = 0.001
F9	231.70 ± 13.17 a	211.84 ± 33.51 a	p = 0.098	203.05 ± 7.91 a	184.30 ± 15.98 b	p = 0.004
F10	342.64 ± 19.30 a	320.07 ± 37.57 a	p = 0.108	191.27 ± 9.50 a	161.63 ± 12.86 b	p < 0.000

Mean values ± SE of the length and width of flagellomeres analyzed from 10 antennae (5 right and 5 left) from each group (RB x NRB). Measurements were obtained from a total of 10 replicates for each group. values (mean ± SE) followed by different lowercase letters on the same line indicate statistical difference (Tukey test, P < 0.05).

The flagellum corresponds to the largest antennal segment. The sensilla observed in this segment were: trichodea 1 to 7; basiconic 1 and 2; chaetica 1 and 2; placodea 1 and 2; coeloconic; campaniform; setae and ampullacea, whose length and quantity measurements of each type of sensilla between the RB and NRB groups are presented in Table 3. The morphometric results demonstrated that there was a statistical difference between seven types/subtypes of sensilla from removing and non-removing bees, such as: SCo (p = 0.010), SSe (p = 0.000), SPl 1 (p = 0.044), STr 1 (p = 0.005), STr 2 (p = 0.002), STr 3 (p = 0.018), STr 4 (p = 0.000) (Table 3), with the greatest length found in the removal bees (RB). On the other hand, there was no statistical difference for the measurements of sensilla BhB (p = 0.435), SCha 1 (p = 0.246), SBa 1 (p = 0.108), SBa 2 (p = 0.099), STr 5 (p = 0.157), STr 6 (p = 0.348) and STr 7 (p = 0.619) (Table 3).

Specifically, for the basiconic sensilla (SBa 1 and SBa 2), as described in the morphological analysis, differences in size were observed, confirming the classification of the subtypes (Table 3). This difference was observed within the RB group (p < 0.0001) and within the NRB group (p < 0.0001). No difference in subtypes was observed between the RB and NRB groups. Regarding the morphometry of the placodea (SPl 1 and SPl 2) and chaetica (SCha 1 and SCha 2) sensilla, it was not possible to verify whether there is a statistical difference between their respective subtypes, as not enough of these structures were found to allow measurement.

The subtypes of trichodea sensilla (STr 1 and STr 2) showed no statistical difference, as well as STr 3 and STr 4, and STr 5 and STr 7. About the quantification of sensilla, there was no significant difference in the number of sensilla between the groups of removing and non-removing bees.

Table 3. Morphometry and quantification of sensilla in *Melipona quadrifasciata anthidioides.*

Sensilla	Removing Bees	Non-removing Bees	Removing Bees	Non-removing Bees
	length (µm)		N° of sensilla 300µm²	
BhB	12.23 ± 1.51 a	11.86 ± 2.54 a	*	*
BB	-	-	*	*
SCo	3.60 ± 0.48 a	3.08 ± 0.48 b	-	-
SSe	19.59 ± 3.49 a	16.74 ± 3.11 b	14.1 ± 7.1 a	9.9 ± 8.7 a
SPl 1	15.55 ± 1.13 a	14.93 ± 1.57 b	32.8 ± 9.9 a	40.1 ± 11.3 a
SPl 2	-	-	*	*
SCha 1	67.99 ± 10.87 a	64.84 ± 13.14 a	*	*
SCha 2	-	-	*	*
SBa 1	8.83 ± 1.91 aA	8.14 ± 1.88 aA	7.4 ± 6.4 a	4.6 ± 2.8 a
SBa 2	14.49 ± 1.09 aB	14.05 ± 1.24 aB	6.0 ± 3.1 a	6.4 ± 2.9 a
STr 1	15.65 ± 1.98 aA	14.40 ± 1.88 bA	55.0 ± 17.1 a	68.0 ± 30.6 a
STr 2	15.78 ± 2.83 aA	13.67 ± 2.92 bA	24.1 ± 10.6 a	17.0 ± 10.6 a
STr 3	36.81 ± 7.50 aB	32.59 ± 8.06 bB	*	*
STr 4	39.43 ± 7.73 aB	33.42 ± 6.29 bB	4.1 ± 2.1 a	4.1 ± 3.9 a
STr 5	21.63 ± 2.12 aC	20.81 ± 2.95 aC	17.9 ± 9.7 a	10.4 ± 13.0 a
STr 6	26.30 ± 4.02 aD	25.48 ± 3.64 aD	4.0 ± 2.1 a	2.3 ± 1.3 a
STr 7	22.63 ± 2.88 aC	22.22 ± 4.25 aC	*	*
SAm	-	-	*	*
SCa	-	-	*	*

Mean values ± SE of the length of sensilla analyzed from 8 antennae of each group (RB brood removing workers x NRB non- removing dead brood). Measurements were obtained from a total of 40 sensilla per type and subtype. SCo measurement was obtained from a total of 30 sensilla units per RB and NRB group. To quantify sensilla, 8 antennae per group were evaluated. Values (mean ± SE) followed by different lowercase letters in the same row and uppercase letters in the same column indicate statistical difference (Tukey test, $p < 0.05$). The presence (-) indicates that there were not enough numbers for analysis and (*) they were not found.

The sensilla BB, SPl 2, SCha 1 and 2, STr 3 and 7, and SCa were not present in flagellomere 10 (Table 3).

Discussion

The sensilla identified in worker bees of *M. q. anthidioides*, removing and non-removing dead brood, agree with those previously identified in the same species (Ravaiano et al., 2014), in addition to being similar to those indicated in other species of bees included in the tribes Meliponini, Bombini, and Euglossini, as well as the honeybee *A. mellifera* (Fialho et al., 2014; Huang et al., 2023). Additionally, in our study, the sensilla setae, Bohm's bristles, and branched bristles were identified in both RB and NRB, as well as the subtypes trichodea 7 and placodea 2, which had not been previously reported in *M. q. anthidioides*. Such sensilla have also been observed in other bee species, such as *M. scutellaris* (Carvalho et al., 2017), *Bombus atiapes* Smith, *Bombus breviceps* Smith, *Bombus flavescens* Smith, and *Bombus terrestris* (L.) (Ren et al., 2023).

Based on the morphological characterization of sensilla and the presence of all types of sensilla in both groups, we concluded that there is no difference regarding the typology of sensilla between worker bees that remove (RB) and those that do not remove dead brood (NRB), refuting the hypothesis on the existence of typological differences between the sensilla of the RB and NRB groups. In contrast, the morphometric analysis of sensilla revealed differences between groups in the length of seven types/subtypes of sensilla (SCo, SPl 1, SSe, STr 1, STr 2, STr 3, and STr 4), corroborating the hypothesis that there are differences of sensilla size between dead brood removing (RB) and non-removing dead-brood (NRB) workers, an unprecedented result in the literature on hygienic behavior in stingless bees.

The morphometry of the antennae also revealed differences between the RB and NRB groups for the average length of the antennae, the length of the scape, the pedicel, and the F1, F2, and F3 flagellomeres, as well as the width of the scape, the pedicel, and the 10 flagellomeres, with the values found for the RB group being significantly higher.

Regarding the number of sensilla in flagellomere 10, there was no statistical difference between the RB and NRB groups of *M. q. anthidioides*, as also observed for *A. mellifera* (Gramacho et al., 2003). However, in this study, the authors did not indicate whether the individuals used to obtain the micrographs actively participated in the removal of brood in a preliminary test, as carried out in our study.

Although the density of sensilla (i.e., the number of sensilla counted in an area of 130µm^2) was similar between the two groups, the workers in the RB group had larger and wider antennae than the NRB workers. Therefore, they have a larger total surface antennal area and probably harbor a greater total number of sensilla than NRB workers. These results support our hypothesis that the total number of olfactory sensilla in workers that remove dead broods (RB) is greater than in those that do not remove dead broods (NRB).

Our findings suggest that the degree of hygiene of a colony is related both to the total number of olfactory sensilla on the antennae and to the size of some types of sensilla, which would confer greater olfactory sensitivity to bees of hygienic lineage. Based on the literature, olfactory sensitivity may be associated with the binding of odor molecules to Odorant Binding Proteins present in chemoreceptor olfactory sensilla (Guarna et al., 2015; McAfee et al., 2018; Wagoner et al., 2020; Gebremedhn et al., 2023). In *M. q. anthidioides*, among the chemoreceptor sensilla STr 1, STr 2, STr 3, STr 4, and SPl 1 were significantly larger in removing bees (RB) than in non-removing bees (RNB).

The antennae of worker bees *M. q. anthidioides* presented a diversity of types and subtypes of sensilla, which probably play a crucial role in the initiation of hygienic behavior. To our knowledge, this is the first study to investigate morphometric, morphological, and the quantity of sensilla traits in stingless bees and their relationship with hygienic behavior. The results showed that, morphologically, the sensilla of hygienic and non-hygienic bees do not differ from each other. However, morphometrically, there are differences in some types and subtypes of sensilla, as well as in the size of the antenna, although quantitatively, there were no significant differences. This information can be useful to improve the understanding of its association with hygienic behavior and further explore this mechanism, considering its importance for behavioral and electrophysiological studies that can help promote practices that improve the health of *M. q. anthidioides* colonies.

Acknowledgements

We would like to thank the Federal Institute of Alagoas – IFAL for providing the structure of the Microscopy Laboratory to obtain the micrographs. This work was carried out with the support of the Coordination for the Improvement of Personnel Higher Education-Brazil (CAPES) – Financing Code 001, linked to the Postgraduate Program in Agricultural Sciences at the Federal University of Recôncavo of Bahia (Scholarship for JNJ) and from the National Council for Scientific and Technological Development (CNPq), Process 305950/2021-5 (Scholarship for CALC).

Authors' Contributions

JNJ: Conceptualization, investigation, methodology, writing-original draft, writing-review & editing.
JDF: Investigation and methodology.
PRRM: Formal analysis, methodology and writing-original draft.
CALC: Conceptualization, methodology and writing-original draft.
FMR: Conceptualization and writing-original draft.
CMLA: Conceptualization, writing-original draft and writing-review e editing.

All authors read and approved the final manuscript.

References

Addinsoft. (2019). XLSTAT statistical and data analysis solution. Long Island, NY.

Arathi, H.S., Burns, I. & Spivak, M. (2000). Ethology of hygienic behaviour in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioural repertoire of hygienic bees. Ethology, 106: 365-379. https://doi.org/10.1046/j.1439-0310.2000.00556.x

Azevedo, D.O., Matiello-Guss, C.P., Rönnau, M., Zanuncio, J.C. & Serrão, J.E. (2008). Post-embryonic development of the antennal sensilla in *Melipona quadrifasciata anthidioides* (Hymenoptera: Meliponini). Microscopy Research and Technique, 71: 196-200. https://doi.org/10.1002/jemt.20539

Bigio, G., Schürch, R. & Ratnieks, F.L.W. (2013). Hygienic behaviour in honey bees (Hymenoptera: Apidae): effects of brood, food, and time of the year. Journal of Economic Entomology, 106: 2280-2285. https://doi.org/10.1603/EC13076

Carvalho, W.J., Fujimura, P.T., Bonetti, A.M., Goulart, L.R., Cloonan, K., Silva, N.M. & Leal, W.S. (2017). Characterization of antennal sensilla, larvae morphology and olfactory genes of *Melipona scutellaris* stingless bee. PloS One, 12: e0174857. https://doi.org/10.1371/journal.pone.0174857

Chen, H.H., Zhao, Y.X. & Kang, L. (2003). Antennal sensilla of grasshoppers (Orthoptera: Acrididae) in relation to food preferences and habits. Journal of Biosciences, 28: 743-752.

Cruz-Landim, C. (2009). Abelhas: morfologia e função de sistemas. São Paulo: Editora UNESP, 408p

Dohanik, V.T., Souza, E.A., Lisboa, L.C.O., Zanuncio, J.C. & Serrão, J.E. (2017). Development of antennal sensilla of *Tetragonisca angustula* Latreille, 1811 (Hymenoptera: Meliponini) during pupation. Brazilian Journal of Biology, 77: 284-288. https://doi.org/10.1590/1519-6984.12515

Elgar, M.A., Zhang, D., Wang, Q., Wittwer, B., Thi Pham, H., Johnson, T.L., Freelance, C.B. & Coquilleau, M. (2018). Focus: ecology and evolution: insect antennal morphology:

the evolution of diverse solutions to odorant perception. The Yale Journal of Biology and Medicine, 91: 457-469

Evans, J.V. & Shutler, D. (2023). The importance of *Apis mellifera* olfaction to social immunity. Journal of Apicultural Research, 64: 150-158. https://doi.org/10.1080/00218839.2023.2246233

Fialho, M.D.C.Q., Guss-Matiello, C.P., Zanuncio, J.C., Campos, L.A.O. & Serrão, J.E. (2014). A comparative study of the antennal sensilla in corbiculate bees. Journal of Apicultural Research, 53: 392-403. https://doi.org/10.3896/IBRA.1.53.3.07

Frasnelli, E., Anfora, G., Trona, F., Tessarolo, F. & Vallortigara, G. (2010). Morpho-functional asymmetry of the olfactory receptors of the honeybee (*Apis mellifera*). Behavioural Brain Research, 209: 221-225. https://doi.org/10.1016/j.bbr.2010.01.046

Gebremedhn, H., Claeys Bouuaert, D., Asperges, M., Amssalu, B., De Smet, L. & de Graaf, D.C. (2023). Expression of molecular markers of resilience against *Varroa destructor* and bee viruses in Ethiopian honey bees (*Apis mellifera simensis*) focussing on olfactory sensing and the RNA Interference Machinery. Insects, 14: 436. https://doi.org/10.3390/insects14050436

Gramacho, K.P. & Spivak, M. (2003). Differences in olfactory sensitivity and behavioral responses among honey bees bred for hygienic behavior. Behavioral Ecology and Sociobiology, 54: 472-479. https://doi.org/10.1007/s00265-003-0643-y

Gramacho, K.P., Gonçalves, L.S., Stort, A.C. & Noronha, A.B. (2003). Is the number o antennal plate organs (sensilla placodea) greater in hygienic than in nonhygienic Africanized honey bees? Genetics and Molecular Research, 2: 309-316

Guarna, M.M., Melathopoulos, A.P., Huxter, E., Iovinella, I., Parker, R., Stoynov, I., Tam, A., Moon, K-M, Chan, Q.W.T., Pelosi, P., White, R., Pernal, S.F. & Foster, L.J. (2015). A search for protein biomarkers links olfactory signal transduction to social immunity. BMC Genomics, 16:1-16. https://doi.org/10.1186/s12864-014-1193-6

Grüter, C. & Keller, L. (2016). Inter-caste communication in social insects. Current Opinion in Neurobiology, 38: 6-11. https://doi.org/10.1016/j.conb.2016.01.002

Huang, X., Xu, X.L., Li, R.X., Wang, S. & Tian, L.X. (2023). Ultrastructure and distribution of antennal sensilla of *Bombus terrestris* (Hymenoptera: Apidae). Zoologischer Anzeiger, 302: 239-247. https://doi.org/10.1016/j.jcz.2022.12.006

Jesus, J.N., Chambó, E.D., Silva, G.S., Oliveira, N.T.E. & Carvalho, C.A.L. (2017). Hygienic behavior in *Melipona quadrifasciata anthidioides* (Apidae, Meliponini). Apidologie, 48: 504-512. https://doi.org/10.1007/s13592-017-0495-5

Kaissling, K.E. & Renner, M. (1968). Antennale rezeptoren für queen substance und sterzelduft bei der honigbiene. Zeitschrift fuer Vergleichende Physiologie, 59: 357-361. https://doi.org/10.1007/BF00365967

Kannan, K., Galizia, C.G. & Nouvian, M. (2022). Olfactory strategies in the defensive behaviour of insects. Insects, 13: 470. https://doi.org/10.3390/insects13050470

Masterman, R., Smith, B.H. & Spivak, M. (2000). Brood odor discrimination abilities in hygienic honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. Journal of Insect Behavior, 13: 87-101. https://doi.org/10.1023/A:1007767626594

McAfee, A., Chapman, A., Iovinella, I., Gallagher-Kurtzke, Y., Collins, T.F., Higo, H., Madilao, L.L., Pelosi, P. & Foster, L.J. (2018). A death pheromone, oleic acid, triggers hygienic behavior in honey bees (*Apis mellifera* L.) Scientific Reports, 8: 5719. https://doi.org/10.1038/s41598-018-24054-2

Mondet, F., Alaux, C., Severac, D., Rohmer, M., Mercer, A.R., Le Conte, Y. (2015) Antennae hold a key to *Varroa*-sensitive hygiene behaviour in honey bees. Scientific Reports, 5: 10454. https://doi.org/10.1038/srep10454

Moraes, R.L.M.S. & Cruz-Landim, C. (1972). Estudo comparativo de órgãos sensoriais em abelhas com diferentes tipos de comunicação. Revista Brasileira de Biologia, 32: 185-196.

Nascimento, M.A., Fernandes-Salomão, T.M. & Martins, G.F. (2013). Estudo comparativo das sensilas antenais de operárias de *Melipona scutellaris* (Hymenoptera: Apidae) de diferentes altitudes. EntomoBrasilis, 6: 64-67. https://doi.org/10.12741/ebrasilis.v6i1.252

Ravaiano, S.V., Ferreira, R.D.P., Campos, L.A.O. & Martins, G.F. (2014). The antennal sensilla of *Melipona quadrifasciata* (Hymenoptera: Apidae: Meliponini): a study of different sexes and castes. Naturwissenschaften, 101: 603-611. https://doi.org/10.1007/s00114-014-1184-0

Ren, C.S., Chang, Z.M., Zu, Z.Y., Han, L., Chen, X.S. & Long, J.K. (2023). Comparison of morphological characteristics of antennae and antennal sensilla among four species of bumblebees (Hymenoptera: Apidae). Insects, 14: 232. https://doi.org/10.3390/insects14030232

Spivak, M. & Reuter, G.S. (1998). Performance of hygienic honey bee colonies in a comercial apiary. Apidologie, 29: 291-302. https://doi.org/10.1051/apido:19980308

Spivak, M., Masterman, R., Ross, R., Mesce, K. A. (2003) Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. Journal of Neurobiology, 55: 341-354. https://doi.org/10.1002/neu.10219

Stort, A.C. & Moraes-Alves, M.M.B. (1999). Differences in the number of the antennal sensory structures of males of three honey bee types. Revista Brasileira de Biologia, 59: 161-166. https://doi.org/10.1590/S0034-71081999000100020

Swanson, J.A., Torto, B., Kells, S.A., Mesce, K.A., Tumlinson, J.H. & Spivak, M. (2009). Odorants that induce hygienic behavior in honeybees: identification of volatile compounds in chalkbrood-infected honeybee larvae. Journal of Chemical Ecology, 35: 1108-1116. https://doi.org/10.1007/s10886-009-9683-8

Van Baaren, J., Boivin, G., Le Lannic, J. & Nénon, J.P. (1999). Comparison of antennal sensilla of *Anaphes victus* and *Anaphes listronoti* (Hymenoptera: Mymaridae), egg-parasitoids of Curculionidae. Zoomorphology, 119: 1-8. https://doi.org/10.1007/s004350050076

Wagoner, K.M., Millar, J.G., Schal, C. & Rueppell, O. (2020). Cuticular pheromones stimulate hygienic behavior in the honey bee (*Apis mellifera*). Scientific Reports, 10: 7132. https://doi.org/10.1038/s41598-020-64144-8

Wilson-Rich, N., Spivak, M., Fefferman, N.H. & Starks, P.T. (2009). Genetic, Individual and Group Facilitation of Disease Resistance in Insect Societies. Annual Review of Entomology, 54: 405-423. https://doi.org/10.1146/annurev.ento.53.103106.093301

Yan, H., Jafari, S., Pask, G., Zhou, X., Reinber, G. D., Desplan, C. (2020) Evolution, developmental expression and function of odorant receptors in insects. Journal of Experimental Biology, 223: jeb208215. https://doi.org/10.1242/jeb.208215