Sensory structures on maxillary and labial palps of *Tenebrio molitor*

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**Abstract**

The yellow mealworm beetle *Tenebrio molitor* L. (Coleoptera Tenebrionidae) is an important stored products pest, although its larvae are also one of the most promising for food and feed. Although *T. molitor* has been widely investigated, there remains a lack of information about the sensory structures on maxillary and labial palps. Here, it was investigated the external morphology and distribution pattern of the sensilla on mouthpart palps of *T. molitor* larvae using scanning electron microscopy. In *T. molitor*, the maxillary palp has three segments, whereas the labial palp has two segments. Based on the morphology of the features of the sensilla on the apical articles of both the maxillary and labial palps, we classified them into nine types: sensilla trichoidea types 1 and 2, sensilla digitiformia, sensilla chaetica, sensilla coeloconica, sensilla basiconica types 1 and 2, sensilla styloconica types 1 and 2. A total of 23 sensilla on maxillary palps and 13 sensilla on labial palps were found. In particular, the sensilla basiconica form the sensory complex located on the tip of each palp. In *T. molitor*, the numbers of sensilla present on the sensory complexes do not differ between maxillary and labial palps, as 13 units.

**Key words:** mealworm, mouthpart palps, sensilla, scanning electron microscope.

**Introduction**

Insect mouthparts are adapted for ingestion of many types of food, and the most distinguishing characteristic of the behavior of an insect can be defined by the functionality of their mouthparts (Moon et al., 2008). Insects are covered with sensory structures known as sensilla, where sensory neurons, responsible for perception of smell, taste, sound, touch, vision, proprioception, and geo-, thermo-, and hygroreception are found (Shields, 2011). Traditionally, sensillum types have been classified on the basis of the morphology of their cuticular parts, as well as location on the insect (Zacharuk, 1985; Zacharuk and Shields, 1991). Most of these structures are generally distributed on the labio-maxillary complex, and they can show a variety of forms and characteristics in relation to their various functions, in terms of contact chemoreception, mechanoreception, thermo- and hygro-reception (Aleksiev et al., 2005; Giglio et al., 2003; 2013). In particular, the number and distribution of the mouthpart sensilla have an important role in the detection of feeding resources and the evaluation of their quality prior to ingestion (Zacharuk and Shields, 1991; Liu et al., 2011; Song et al., 2014). Moreover, the variety of sensilla provides the most important cues to help in the location of their specific microhabitat (Shorey, 1973), and for chemical communication (Regnier and Law, 1968). The morphology and distribution pattern of sensory structures on mouthpart palps have been studied in Coleoptera larvae such as *Oryzaephilus surinamensis* (L.) (Silvanidae) (Roppel et al., 1972), *Entomoscelis americana* Brown (Mitchell et al., 1979) and *Psylliodes chrysocephala* L. (Chrysomelidae) (Bartlett et al., 1999), *Speophyes lucidus* (Delar) (Corbière-Tichané, 1973), and *Cteniceria destructor* (Brown) (Elateridae) (Zacharuk, 1971), *Tribolium confusum* du Val (Ryan and Behan, 1973) and *Tribolium castaneum* (Herbst) (Tenebrionidae) (Seada and Hamza, 2018), *Carabus lefebvrei* Dejean (Carabidae) (Giglio et al., 2013) and *Ips typographus* L. (Scolytidae) (Hallberg, 1982). However, much less attention has been directed toward the sensory structures associated with mouthpart of *Tenebrio molitor* L. (Coleoptera Tenebrionidae). Currently, on *T. molitor* larvae only one type of sensillum (digitiformium) was identified on maxillary palps (Honomichi and Guse, 1981). The yellow mealworm beetle, *T. molitor*, is well-known in stored food products worldwide, as larval stages and adult beetles cause damages and high economic losses, due to their feeding activities, in stored grains, seeds, and grain products in mills (Aribi et al., 2006). These beetles have chewing mouthparts, their growth is optimum in flour and other processed cereal products (Aitken, 1975). Moreover, its larvae are considered one of the most common edible insects for food and feed (van Huis et al., 2013). Because of the lack of data in the literature regarding sensory structure on mouthpart of *T. molitor* larvae, the goal of this study was to investigate the external morphology and the distribution pattern of sensilla on maxillary and labial palps. Moreover, a morphological comparison among first instar, third instar and last instar larvae was carried out.

**Materials and methods**

**Insects**

*T. molitor* larvae were obtained from cultures maintained in a climate-controlled chamber at the Entomology Laboratory of the Polytechnic University of Marche, Italy. The rearing conditions were set at 28 ± 0.5 °C and 60 ± 10% relative humidity, with a photoperiod of 0:24 (light:dark).

**Scanning electron microscopy**

Scanning electron microscopy was performed on the maxillary and labial palps of the last instar *T. molitor*...
larvae (n = 20). The larvae were anesthetized by exposure to cold temperature (−18 °C) for 60 seconds, and then dipped into 60% alcohol. The labio-maxillary complex was carefully excised using fine forceps under a stereomicroscope. These samples were dehydrated through a series of graded ethanol concentrations (60%, 70%, 80%, 90%, 95%, 99%) with 15 minutes in each.

After dehydration, the 99% ethanol was substituted with pure hexamethyldisilazane (Fluka, Sigma-Aldrich, Buchs, Switzerland), and the specimens were left to dry under a hood under room conditions; this final step was repeated twice. The samples were then mounted on stubs using conductive double-sided black adhesive tape. They were coated with gold using a sputter unit (SCD 040; Balzers Union, Vaduz, Liechtenstein), taking care to position them in different orientations to provide clear views of the ventral, dorsal, and lateral sides. The gold-coated samples were examined using scanning electron microscopy (FE-SEM Supra 40; Carl Zeiss NTS GmbH, Oberkochen, Germany/ XL 30; Philips, Eindhoven, The Netherlands), at an acceleration voltage of 7 kV to 10 kV and a window width of 9 mm to 10 mm. The data were processed and analyzed using the Smart-SEM software, version 05.06 (Carl Zeiss Microscopy, Jena, Germany).

Statistical analysis

The data for the measurements from the digitized images were processed as means ± standard deviation (Zar, 1999).

Results

The head of T. molitor larvae (first instar, third instar and last instar larvae) is prognathous and has well-developed chewing mouthparts that consist of the clearly visible labrum, which forms the roof of the upper part of the mouth, a pair of strongly sclerified mandibles that are triangular in shape and a large maxillo-labial complex that is ventrally located, to close the lower part of the mouth (figure 1A). This maxillo-labial complex has maxillary (figure 1B) and labial palps (figure 1C) that are inserted on a large galea-lacinia complex and on a developed prementum, respectively. The maxillary palp has three segments (figure 1B) and the labial palp has two segments (figure 1C). Based on the morphology of its external features, the sensilla observed along the apical articles and on the tip of both maxillary and labial palps of all instars of T. molitor larvae were classified into nine types: trichoidea types 1 (figure 2A) and 2 (figure 2A-B), digitiformia (figure 2A,C), chaetica (figure 2A,E), coeloconica (figure 2D), basiconica types 1 (figure 3A-F,I,L) and 2 (figure 3A-F,L), and styloconica types 1 (figure 3A-G,I,L) and 2 (figure 3A,C,E,H,I).

No differences are present on the sensory structures organization among the first instar, third instar and last instar larvae of T. molitor (figure 3A-F).

On maxillary palps of all instars it was observed a total of 23 sensilla belonging to sensilla trichoidea types 1 and 2, sensilla digitiformia, sensilla chaetica, sensilla coeloconica, sensilla basiconica types 1 and 2, and sensilla trichoidea types 1 and 2, sensilla digitiformia, sensilla chaetica, sensilla coeloconica, sensilla basiconica types 1 and 2, and sensilla...
Representative scanning electron microscopy images of the maxillary palps of *T. molitor*. (A) Segment III of the maxillary palp. (B) Sensillum trichoideum type 2 (T2). (C) Sensillum digitiformium (Dg). (D) Sensillum coeloconicum. (E) Sensillum chaeticum (Ch). SC, sensory complex. Scale bars: 20 µm (A, B); 5 µm (C); 1 µm (D, E).

Meanwhile on the labial palps of all instars it was observed a total of 13 sensilla belonging to basiconica types 1 and 2, and styloconica type 1.

In all instars, on the tip of both maxillary and labial palps, a sensory complex, composed by the 13 sensilla, is present (figure 3A-F). This structure differs between maxillary and labial palps based on the typology and distribution of the sensilla carried on it. On the maxillary palp, the sensory complex is composed by sensilla basiconica types 1 and 2 and styloconica types 1 and 2 (figure 3A,C,E). A sensilla basiconica type 2 is located in the center of the sensory complex, randomly surrounded by two sensilla styloconica type 1 and ten sensilla basiconica type 1.

**Sensilla trichoidea**

The sensilla trichoidea (figure 2A,B) are long and they end in a fine, sharp tip. There are two types of sensilla trichoidea.

**Sensilla trichoidea type 1**

These sensilla (figure 2A) are characterized by an elongated cuticular shaft that is $28.27 \pm 0.05$ µm long and that decreases in diameter from the base, which is $1.24 \pm 0.01$ µm width ($n = 10$), toward the apex; it is inserted in a specialized flexible socket. There are two ventrally located sensilla of this type on each maxillary palp, more specifically on the distal part of the second article.
Figure 3. Representative scanning electron microscopy images of the sensory complex of *T. molitor* of the first, the third and the last instar larvae. (A-B) Last instar larvae: sensory complexes of the maxillary (A) and labial (B) palps. (C-D) Third instar larvae: sensory complexes of the maxillary (C) and labial (D) palps. (E-F) First instar larvae: sensory complexes of the maxillary (E) and labial (F) palps. (G) Sensillum styloconicum type 1 (S1). (H) Sensillum styloconicum type 2 (S2). (I) Sensillum basiconicum type 1 (B1). (L) Sensillum basiconicum type 2 (B2). Scale bars: 5 µm (A-D,); 2 µm (E, F, I ); 1 µm (H, L); 400 nm (G).

Sensilla trichoidea type 2
These sensilla (figure 2A,B) are characterized by an elongated cuticular shaft that is 11.86 ± 0.02 µm long, and thus shorter than for sensilla trichoidea type 1, which decreases in diameter from the base, at 0.55 ± 0.02 µm wide (n = 10), toward the apex; it is inserted in a specialized flexible socket. There is one sensillum trichoideum type 2 on each maxillary palp, which is located on the internal part of the third article, just above the sensilla chaetica.

Sensilla digitiformia
The sensilla digitiformia (figure 2A,C) can be seen as elongated finger-like ‘pegs’ that lie in individual grooves that are 39.53 ± 0.16 µm long and 2.48 ± 1.18 µm wide (n = 10). For each maxillary palp, one sensillum digitiformium is proximally located on the dorsal side.

Sensilla chaetica
The sensilla chaetica (figure 2A,E) are a cuticular apparatus that consists of an outstanding hair shaft. This sensilla is 2.58 ± 0.24 µm long and gradually tapers from the base, which is 0.81 ± 0.07 µm in diameter (n = 10), to the blunt tip with smooth walls, and it is inserted in a specialized, flexible socket. There are up to three sensilla chaetica on each maxillary palp, which are located on the apical article, proximal to the internal side, and opposite to the digitiformium sensillum.

Sensilla coeloconica
The sensilla coeloconica (figure 2D) can be seen as small and rounded apertures (0.57 µm²) of 0.85 ± 0.02 µm in diameter (n = 10). Up to three sensilla coeloconica are externally and basally located on the apical segment of each maxillary palp.
Sensilla basiconica
There are two types of sensilla basiconica (figure 3).

**Sensilla basiconica type 1**
These sensilla (figure 3A-F,LL) appear as cone-shaped structures with a rough surface, which are $5.58 \pm 0.14 \mu m$ long and $2.50 \pm 0.03 \mu m$ wide (n = 10). There are 8 and 10 sensilla basiconica type 1 on the sensory complex of maxillary and labial palps.

**Sensilla basiconica type 2**
These sensilla (figure 3A-F,LL) appear as globe-like structures that are $2.61 \pm 0.05 \mu m$ long and $4.70 \pm 0.03 \mu m$ wide (n = 10). There is one sensilla basiconica type 2 on the sensory complex of maxillary and labial palps.

Sensilla styloconica
There are two types of sensilla styloconica (figure 3).

**Sensilla styloconica type 1**
These sensilla (figure 3A-G,LL) have the shape of a cylinder, at $1.10 \pm 0.17 \mu m$ long and $0.97 \pm 0.13 \mu m$ wide (n = 10). They are inserted into larger cylinders that are $2.55 \pm 0.18 \mu m$ long and $2.55 \pm 0.18 \mu m$ wide (n = 10), which arise from a rigid socket. There are two sensilla styloconica type 1 on the sensory complex of maxillary and labial palps.

**Sensilla styloconica type 2**
These sensilla (figure 3A-C,E,H,II) appear as cone-shaped structures with apical finger-like cuticular projections. They are $4.34 \pm 0.06 \mu m$ long and $3.79 \pm 0.08 \mu m$ wide (n = 10), and are inserted in cylinders that are $2.51 \pm 0.17 \mu m$ long and $3.79 \pm 0.08 \mu m$ wide that arise from a rigid socket (n = 10). There are two sensilla styloconica type 2 on the sensory complex of maxillary palps.

Discussion and conclusions
Coleoptera larvae can inhabit different biotopes and have a wide range of trophic relationships, although they still show a uniform topography of their sensory organs (Alekseev et al., 2005). In this study, it was observed that *T. molitor* larvae have no morphological differences among instars. All of them have a large number of sensory structures located on maxillary and labial palps. Similar sensilla distribution pattern has been described on other larvae of Coleoptera families, such as Silvanidae (Roppel et al., 1972; Mitchell et al., 1979), Chrysomelidae (Mitchell et al., 1979; Bartlett et al., 1999), Elateridae (Zacharuk, 1985; Ruchty et al., 1998), Tenebrionidae (Ryan and Behan, 1973), and Scolytidae (Hallberg, 1982). Five morphological types of sensilla on the palps of *T. molitor* larvae were identified identically in all instars: trichoidea, digitiformia, chaetica, coeloconica and basiconica.

Two types of sensilla trichoidea on mouthpart palps of *T. molitor* larvae, which differ in length, were observed; in particular type 1 is longer than type 2. Trichoidea sensilla are located, on the lateral surface of the second and third segment of maxillary palps, respectively. Similar sensilla have been found in several coleopteran species such as *Leptinotarsa decemlineata* Say (Chrysomelidae) (Farazmand and Chaika, 2008), *Carabus lefebvrei* Dejean (Carabidae) (Giglio et al., 2013), *Triobium castaneum* (Herbst) (Tenebrionidae) (Ryan and Behan, 1973), *Anthrenus scrophulariae* (L.) (Dermentiidae) (Zaitseva and Sinitsina, 1982), *Oryzaephilus surinamensis* (L.) (Roppel et al., 1972) and *Entomoscelis americana* Brown (Silvanidae) (Mitchell et al., 1979). Sensilla trichoidea are mainly reported to function as mechanoreceptors (Schneider and Steinbrecht, 1968; Keil, 1997), although it has been suggested that they can act as both contact chemoreceptors and mechanoreceptors (Lewis, 1970). The shape and locations of sensilla trichoidea on *T. molitor* maxillary palps, suggest that their main functions are as mechanoreceptors for type 1 (Zacharuk, 1985; Farazmand and Chaika, 2008; Giglio et al., 2013), and both contact chemoreceptors and mechanoreceptors for type 2 (Steinbrecht, 1996; Giglio et al., 2003; Farazmand and Chaika, 2008).

One sensillum digitiformium was exclusively observed on the dorsal side of each maxillary palp of *T. molitor* larvae. This sensillum was previously described by Honomichi and Guse (1981) as aporous hair shafts and basally inserted into a narrow canal in the cuticle, with their longer distal parts positioned in a superficial groove on the maxillary palps. In other coleopteran species, such as *Carabus* spp. (Carabidae) (Giglio et al., 2003; 2013), *Orthosoma brunneum* Forster (Cerambycidae) (White et al., 1974), *Cantharis fusca* L. (Cantharidae), *Quiesus* sp. (Staphylinidae) and *Selatosomus nigricornis* Panzer (Elateridae) (Alekseev et al., 2005), sensilla digitiformia with a different shape and distribution pattern on larval mouthparts palps were observed. Sensilla digitiformia were reported as thermoreceptors, hygroreceptors (Honomichi and Guse 1981), CO$_2$ receptors (White et al., 1974) and mechanoreceptor (Zacharuk et al., 1977).

The sensilla chaetica observed on *T. molitor* larvae are located only on the lateral surface of maxillary palps, as observed also in *Ips typographus* L. (Scolytidae) (Mitchell et al., 1999) and *Psylliodes chrysocephala* L. (Chrysomelidae) (Isidoro et al., 1998). Sensilla chaetica are usually considered to have mechano- and gustatory functions (Isidoro et al., 1998; Mitchell et al., 1999). For the first time sensilla coeloconica have been described on palps. In *T. molitor* larvae, they appear as small and rounded apertures located externally and basally on the apical segment of each maxillary palp. These sensilla coeloconica have the cuticular peg completely embedded within the palp wall and can be ascribed to those described by Altner et al., 1985. Sensilla coeloconica are mainly reported as thermo- and hygro-receptors (Altner et al., 1981; Ruchty et al., 2009).

The sensilla basiconica observed on maxillary and labial palps of *T. molitor* larvae belong to type 1 and 2. Type 1 appears as a cone-shaped and type 2 as a globe-like structure, both can be ascribed to those described for coleopteran larvae of *Carabus* spp. (Carabidae) (Giglio et al., 2013).
The sensilla styloconica observed on maxillary and labial palps of *T. molitor* larvae belong to type 1 and 2. Type 1 appears as a shape of a cylinder, type 2 has a particular structure compared with the other sensilla basiconica types, as bears small apical finger-like cuticular projections as observed in *T. confusum* and *T. castaneum* (Ryan and Behan, 1973). Sensilla styloconica have been shown to be contact chemoreceptors (Jefferson et al., 1970; Albert and Seabrook, 1973), specialized to respond to water, salt, glucose and sucrose (Schoonhoven and Deither, 1966; Ishikawa et al., 1969), and thermos-/hygro-receptors (Schoonhoven, 1967; Hallberg et al., 1994).

Sensilla basiconica and styloconica are associated to olfactory and gustatory function (Zacharuk, 1985; Steinbrecht, 1996; Lopes et al., 2002).

Sensilla basiconica and styloconica, in all instars of *T. molitor* larvae, are exclusively present on the tip of both maxillary and labial palps forming a sensory complex. The sensory complex present on the maxillary palps bears all the four types of sensilla basiconica and styloconica, while on the labial palps sensillum styloconicum type 2 is missing. Sensory complexes similar to those described for *T. molitor* larvae, has been observed also in Carabidae (Giglio et al., 2003), Curculionidae, Scarabaeidae, Elateridae, Silvanidae (Alekseev et al., 2005) and Chrysomelidae (Farazmand and Chaika, 2008) families. Most studies have noted that the number of sensilla on the sensory complex in coleopteran larvae are either the same on the maxillary and labial palps, or differ by two to three sensilla (Alekseev et al., 2005). For example, there are 8 and 7 sensilla, respectively, in *Hydaticus* spp. (Dytiscidae), 12 and 14 in *Antherenura scrophulariae* L., 10 and 9 in *Attagenus smirnovi* Zhantiev (Dermestidae) (Zaitseva and Elizarov, 1980), 16 and 11 in *L. decemlineata* (Farazmand and Chaika, 2008), 12 and 9 in *Cassida* spp. (Chrysomelidae) (Rosciszewska, 1981), and 10 to 13 and 8 to 10 in species of the Curculionidae family (Tomkovich and Chaika, 2001). In *T. molitor*, the number of sensilla present on the sensory complexes do not differ between maxillary and labial palps, as 13 units. In *T. molitor* larvae, the shape and the distribution pattern of sensilla basiconica and styloconica suggests a role as contact chemoreceptors (Farazmand and Chaika, 2008; Alekseev et al., 2005; Giglio et al., 2013). In addition, the cuticle of the sensory complex is membranaceous, and so is likely to be flexible, which could allow the sensilla to move deeply into distal segments when the palp contacts with a feeding substrate (Farazmand and Chaika, 2008). Moreover, a reduction in the cuticular parts of the sensilla is related to the concept that active movements of the palps in the process of ingesting hard food would damage the exposed sensilla (Alekseev et al., 2005).

In all instars of *T. molitor* larvae we observed a total of 23 sensilla on the maxillary palps, and 13 on the labial palps. In general, the Coleoptera order is characterized by a mean of 10 to 16 sensilla on the mouthpart palps (Alekseev et al., 2005). However, in rare cases there can be more than 30 sensilla, or instead, sensilla can be totally absent (Alekseev et al., 2005). Higher numbers of sensilla should be considered as a progressive trend to facilitate the perception of information from the environment (Giglio et al., 2003). Sensilla on maxillary and labial palps are directly involved in the detection and in the evaluation of the quality of the feeding resources, through both tactile (Keil, 1997; Chapman, 1998) and chemical receptors (Steinbrecht, 1996; Liu et al., 2011). Indeed, the variety of sensilla, especially chemoreceptors, that has often been observed on the palps of larvae and adult stages of insects enable them to perceive several types of chemicals from both plants and feeding sources (Ma, 1972; Nagnan-Le Meillour et al., 2000; Tang et al., 2014). The differences in number and distribution of palp sensilla are related to the larval behavioural types and are an adaptive response to different lifestyles, feeding habits and use of space (Giglio et al., 2012). Infact, in *T. molitor* larvae there are no differences among instars, to support their tendency to have the same feeding habits and lifestyle.

This study provides new data on the typology and distribution of mouthpart palp sensilla. These results represent the starting point for future ultrastructural, electrophysiological and behavioural investigations.

**Acknowledgements**

This study was financially supported by the Università Politecnica delle Marche, Ancona, Italy, within the project “Edible insects: new frontiers in food-FOODIN”. Scanning electron microscopy data were obtained at the Laboratorio Microscopia Elettronica a Scansione (SIMAU; Università Politecnica delle Marche, Italy). The authors thank anonymous reviewers who helped in improving the manuscript.

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Received February 19, 2019. Accepted October 3, 2019.