

## ***Morphology of sensilla on the ovipositor of the palm borer *Paysandisia archon* (Burmeister, 1880) (Lepidoptera: Castniidae): new data***

### **Morphologie des sensilles sur l'ovipositeur de *Paysandisia archon* (Burmeister, 1880) (Lepidoptera: Castniidae): nouvelles données**

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**Abstract.** Sensilla on the ovipositor of the palm borer, *Paysandisia archon* Burmeister (Lepidoptera: Castniidae) have been investigated by scanning electron microscopy to understand their involvement in oviposition and to provide a morphological base for future ultrastructural and electrophysiological studies. The egg-laying organ is a developed telescopic ovipositor, ending in two sensory lobes provided with three types of sensilla: sensilla chaetica of different lengths and diameters (4 subtypes), sensilla trichodea, and sensilla basiconica (3 subtypes). Tactile aporous sensilla chaetica C1, C2, and C3 are distributed over the entire surface of the lobes and constitute 51% of the total sensilla. Presumed taste sensilla chaetica C4, occur in small quantities, 8% of the total sensilla, on the distal part of the lobes. Multiporous sensilla trichodea and multiporous sensilla basiconica B1, representing 19% of the total sensilla are candidates for olfactory chemoreception. An olfactory or hygroreceptive function, or carbon dioxide reception, may be attributed to sensilla basiconica B2 and B3. *P. archon* is a new example of a moth possessed of an ovipositor with multiporous sensilla. It is the first time that several morphological types of multiporous sensilla are described in the same species. Behavioural and electrophysiological studies will be required to verify the role of the different sensilla during the choice of an oviposition site.

**Keywords:** Lepidoptera, *Paysandisia archon*, female, ovipositor, sensilla, aporous, multiporous.

**Résumé.** Les sensilles sur l'ovipositeur du papillon du palmier, *Paysandisia archon* Burmeister (Lepidoptera: Castniidae) ont été identifiées en microscopie électronique à balayage pour connaître leur implication lors de l'oviposition et fournir une base morphologique pour des études ultrastructurales et électrophysiologiques ultérieures. L'organe de ponte est un ovipositeur développé et télescopique terminé par deux lobes sensoriels pourvus de trois types de sensilles : sensilles chétiformes de différentes longueurs et diamètres, avec 4 sous-types, sensilles trichoïdes, et sensilles basiconiques avec 3 sous-types. Les sensilles chétiformes sans pore C1, C2 et C3, à fonction tactile, sont réparties sur l'ensemble des lobes et constituent 51% du total des sensilles. Les sensilles chétiformes C4, présumées gustatives, sont en petite quantité, 8% du total des sensilles, sur la partie distale des lobes. Les sensilles trichoïdes multipores et les sensilles basiconiques multipores B1, représentant 19% du total des sensilles, sont des candidates pour la fonction olfactive. Une fonction olfactive ou hygro-réceptrice, ou une sensibilité au dioxyde de carbone peut être attribuée aux sensilles basiconiques B2 et B3. *P. archon* est un nouvel exemple de lépidoptère dont l'ovipositeur a des sensilles multipores. C'est la première fois que plusieurs types morphologiques de sensilles multipores sont décrits chez une même espèce. Des études comportementales et électrophysiologiques seront nécessaires pour vérifier le rôle des différentes sensilles durant le choix du site de ponte.

**Mots-clés :** Lepidoptera, *Paysandisia archon*, femelle, ovipositeur, sensilles, sans pore, multipores.

## **INTRODUCTION**

In the 1990s, the palm borer *Paysandisia archon* (Burmeister 1880) (Lepidoptera: Castniidae) (Fig. 1) was accidentally introduced into Southern Europe with new palm species originating from Argentina and Uruguay. It damages endemic and ornamental palms and could become a major economical threat in the Maghreb, for example for the date palm, *Phoenix dactylifera*, an important food crop, which is also a potential host (Dresher & Jaubert 2003). Therefore, developing methods for monitoring *P. archon* populations is important (Delle-Vedove *et al.* 2012). This pest also attacks the doum palm *Chamaerops humilis* which is the only species of palm native to the Mediterranean Basin. Following the arrival of *P. archon*, this palm which is a characteristic component of biodiversity at all Mediterranean levels (infra, thermo or meso) is threatened with eradication (Castellana *et al.* 2018).

The biology of the palm borer is not very well known; its larvae, boring into the palm crown at the base of the fronds, are to be found throughout the year in all larval instars. The oviposition step is particularly crucial in the Lepidoptera, because the hatching larvae are often relatively immobile and thus depend on the judicious choice of food plant by the female adult (Feeny *et al.* 1983, Renwick, 1989). After mating, the laying of the first eggs occurs rapidly and sometimes even the

same day on which the adults mated; females continue to lay eggs throughout their life (Delle-Vedove *et al.* 2012).

Thanks to morphological and ultrastructural studies on the antennae of *P. archon*, Sarto i Monteys *et al.* (2012) and Verdolini (2013) showed that the olfactory equipment (multiporous sensilla trichodea, basiconica and auriculica) is compatible with the perception of host-plant volatiles. The role of antenna in the location of the palms hosts was evaluated by electroantennograms to monitor responses to ester and terpene compounds previously identified as volatiles of damage/fermenting palm tissue. The two sexes showed responses to all of the chemicals tested, with greater responses from the females, providing a significant sex dose effect (Ruschioni *et al.* 2015). To assess the presence of a pheromone gland in the ovipositor, morphological, electrophysiological and chemical investigations were carried out. Riolo *et al.* (2014) were already reporting the absence of female pheromone. In *P. archon*, ovipositor extrusions are not related to calling behaviour, but might be involved in the female physiological state, for instance egg load, or in thermoregulation activity. Furthermore, the midlegs of males are modified; the last tarsomere bears distally a pair of brush-like structures, the paronychialia, which are assumed to release male sex pheromone into the air (Frérot *et al.* 2013, Hamidi & Frérot 2020).

Hamidi & Frérot (2016) studied the different steps of oviposition behaviour. "Gravid females were significantly more attracted by the palm crown than virgin females. The landing site is always the upper part of the palm crown. Subsequent to alighting on the crown, pre-oviposition behaviour was characterized by the following steps. As a first step, the female in motion tapped the substrate with its antennae in order to probe it. As a second step, the female halted and probed the substrate with both its antennae and ovipositor that was introduced quickly into the interstices of the palm fibers. Subsequently, the ovipositor was deeply introduced into the fibrous part of the upper part of the crown and eggs were laid. About ten eggs can be laid in the same place. Finally, the female starts to move again and recommences the same behavioural sequences. Approximately half of the ovipositing females studied scratched the substrate with their intermediate legs". Sarto i Monteys & Aguilar (2005) previously emphasized that most eggs were found within the fibre webs closer to or within the palm crowns and were not glued to the fibres, remaining loose within their thick layers (1-2 cm inside). The young larvae, after feeding on the young palm, bore into the apex of the palm stem. According to the results of Hamidi & Frérot, it is possible that contact receptors on the ovipositor are involved in the assessment process. Similarly, mechano- and chemosensory sensilla on the ovipositor of the potato moth, *Phthorimaea operculella* are involved in evaluating potential sites for oviposition (Fenemore 1988). Both tarsal and ovipositor contact chemoreceptors have also been described for *Ostrinia nubilalis* and their role in the recognition of host-plant surfaces has been suggested (Marion-Poll *et al.* 1992).

Based on a rapid study of 3 ovipositors, *P. archon* seems to possess multiporous sensilla with presumably an olfactory function (Faucheux *et al.* 2015). The aim of the present study is to confirm this initial observation by examining 5 other ovipositors, whereas for many authors, an olfactory function is unusual with moth ovipositors (Faucheux 2017).

## MATERIAL AND METHODS

*P. archon* specimens were obtained from infested plants of *Phoenix dactylifera* in the municipality of Narbonne, 11100-France by Daniel Fousse. For scanning electron microscopy (SEM) study, 5 ovipositors were cut, cleaned in acetone, dehydrated in pure alcohol, and mounted on specimen holders. After coating with gold and palladium, preparations were examined in a Jeol J.S.M. 6400F SEM at 10 kV. Counts of the sensilla were made by using the SEM at different magnifications. The total number of pores on the surface of sensillum was measured directly from SEM micrographs. Sensillum terminology follows Zacharuk (1980), Altner & Prillinger (1980), and Faucheux (1999).

Abbreviations used: s, sensilla; SEM, scanning electron microscopy.

## RESULTS

The *P. archon* ovipositor consists of the 8<sup>th</sup> uromere which forms the ovipositor base, and the 9<sup>th</sup> and 10<sup>th</sup> uromeres are fused together and connected to the base by an intersegmental membrane (Riolo *et al.* 2014). The last uromeres are visible on Fig. 3a. The ovipositor is telescopic (Sarto i Monteys *et al.* 2012) and at rest, the terminal lobes, formerly named "anal papillae", are visible (Fig. 2). It is approximately 1.5-2.0 cm when fully exerted (Sarto i Monteys & Aguilar 2005). In cross section, its circumference is round except in the pointed portion that is laterally flattened and provided with a ventral furrow which separates the two sensory lobes. The

sensory lobes are oval-shaped (length: 840  $\mu\text{m}$ , width: 335  $\mu\text{m}$ ) (Fig. 3b). Their cuticle without microtrichia is slightly mameloned and ribbed in places (Fig. 5a).

Three morphological types of sensilla were identified on the ovipositor lobes of *P. archon*: sensilla chaetica (4 subtypes C1, C2, C3, and C4), sensilla trichodea (T), and sensilla basiconica (3 subtypes B1, B2, B3). The morphological characteristics of different types and subtypes are summarized in Table 1. Numbers, percentages, and presumed function of types and subtypes are listed in Table 2.

Sensilla chaetica of subtypes 1, 2, 3 (C1-C3)

Sensilla chaetica C1-C3 are for the most part long, prominent and gently tapering hairs whose distal part forms a pointed tip (Figs. 3c, 4). The hair-shaft has 20-30 thin longitudinal striae, only visible at high magnifications. Its base is not tightly inserted into the socket itself but is separated by 5-6  $\mu\text{m}$  and connected with it, thanks to a membrane (e.g. C1, Fig. 5a). On broken hairs, can be seen the lumen of the hair which is filled with a spongy extracellular material which harbours a very narrow lumen whose diameter is one fifteenth of that of a hair in certain sensilla (Fig. 5a, d). Sensilla chaetica C1-C3 have no pores and are thus typical aporous sensilla chaetica (ASC).



Figure 1. Female *Paysandisia archon* (photograph Gérard Beaulieu).

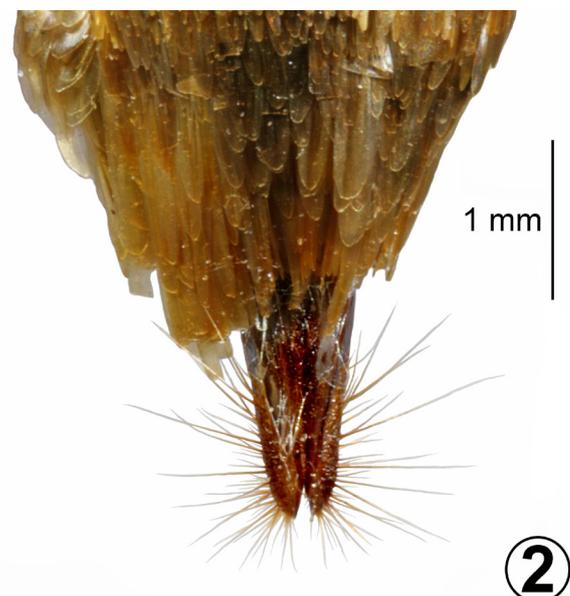


Figure 2. *Paysandisia archon*: ovipositor (photograph Gérard Beaulieu).

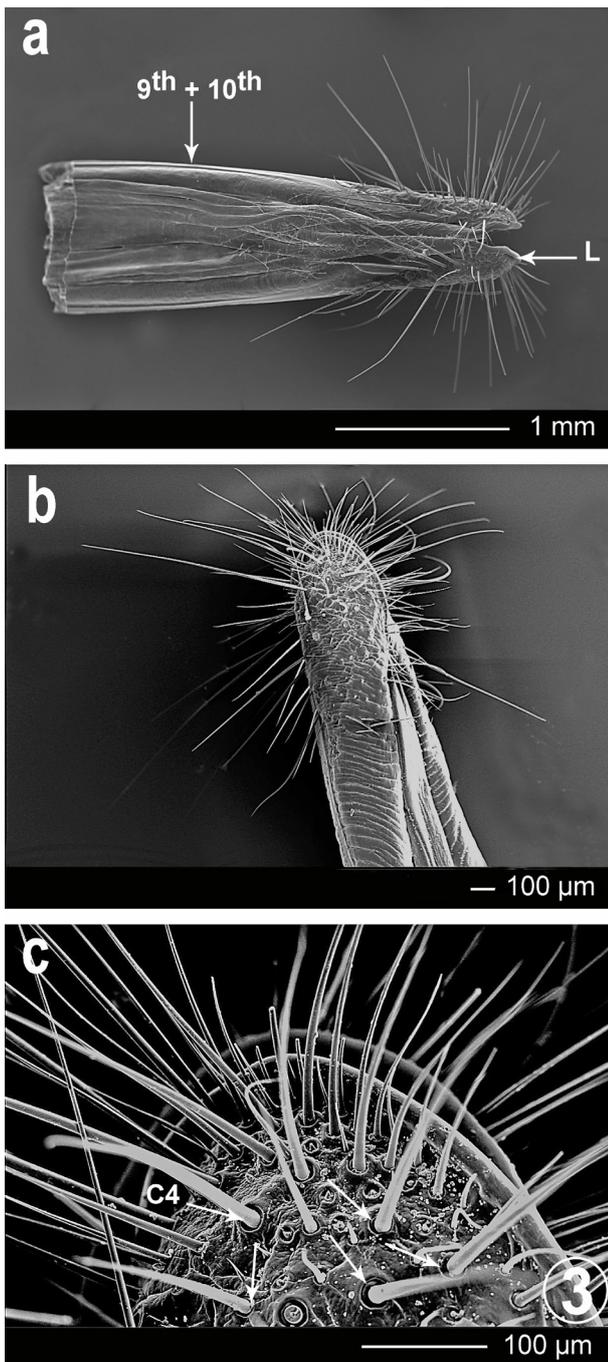


Figure 3. Ovipositor of *Paysandisia archon*, **a**, ventral view, terminal part of female last abdominal segments 9 and 10, 9<sup>th</sup>+10<sup>th</sup>, and two sensory lobes, L; **b**, lateral view showing a lobe; **c**, distal view of lobe showing sensilla chaetica subtype 4, C4 and arrows (SEM).

According to their length and basal diameter, these sensilla have been classified under 3 subtypes (Tab. 1). They are all spread out over the surface of the lobes. The longest ones (C1), measuring 900  $\mu\text{m}$ , 700  $\mu\text{m}$ , and 500  $\mu\text{m}$ , are erected perpendicularly to the surface of the lobes and contribute to the doubling of their width (Fig. 4). Those of average length (C2) (400  $\mu\text{m}$ ) are also more or less placed at right angles. The shortest sensilla (C3) (200  $\mu\text{m}$ , 110  $\mu\text{m}$ ) are oriented towards the rear of the lobes. All of these sensilla are delicately and longitudinally striated at a rate of 20-30 striae according to the subtype. Among these long sensilla, can be found scattered a few rare and much shorter sensilla, measuring 16  $\mu\text{m}$  or 13

$\mu\text{m}$ . The total number of s. chaetica C1-C3 on the two lobes is 107 (Tab. 2) which thus constitutes 51.4 % of the total sensilla.

Sensilla chaetica of subtype 4 (C4) can be distinguished from previous s. chaetica by the shape of the hair that is comparatively stouter than aporous s. chaetica, ASC (Fig. 3c). The hair diameter is constant from the base up to at least a third of the length whereas that of ASC regularly diminishes from the base to the tip. The variation in length of C4 is also less than in the ASC (Tab. 1). The walls of C4 are thick but an internal lumen is present (Fig. 5b, c) unlike the ASC (i.e. C2, Fig. 5d). They are blunt-tipped and the alleged terminal pore is not visible in the micrographs. They are all situated on the distal part of the lobes (Fig. 3ca). Their number is always reduced compared to that of the ASC. Their average number (16) represents 7.7% of the total (Tab. 2). We have classified them with the uniporous sensilla chaetica (USC).

Sensilla trichodea are hairs of shorter length than that of sensilla C3; they are slender, blunt-tipped, and curved in their distal half (Fig. 5a,e,g). The distal sensilla (Fig. 5a) are shorter than the proximal ones (Fig. 5e). The hair has some twenty longitudinal striae corresponding to alignments of wall pores (Fig. 5f). The pore density is about 4-5 pores/ $\mu\text{m}^2$ . According to Zacharuk (1980), they are multiporous s. trichodea (MST). Most of the sensilla are situated in the distal region of the lobes where they are often found in groups of two (Fig. 5a). Their average number on the ovipositor is 24 and corresponds to 11.5% of the total sensilla (Tab. 2).

#### Sensilla basiconica B1, B2, B3

Sensilla basiconica of subtype 1, B1, are short, thorn-shaped, more or less slender pegs, inserted into a depression of the ovipositor cuticle, without a visible socket. They are often grouped at the apex of the lobes (Figs. 5a, 6a). The wall is perforated with more or less linear pores which are distributed according to about fifteen longitudinal ridges (Fig. 6b). These are multiporous s. basiconica, MSB1, whose pore density is 5-6 pores/ $\mu\text{m}^2$ . Approximately 16 MSB1 are present on both lobes and amount to 7.7% of the total sensilla (Tab. 2).

Sensilla basiconica of subtype 2, B2, are the shortest sensilla whose length varies from 7.0  $\mu\text{m}$  to 9.5  $\mu\text{m}$ . They are shell-shaped or more pointed (Fig. 6e, d). The base of the peg is sunk into a very large socket from which it is separated by 3-4  $\mu\text{m}$  and connected via a membrane (Fig. 6c, e). The wall is slightly rough (Fig. 6d, f); the presence of wall pores is hypothesized. Their number for the two ovipositor lobes is the largest of all the s. basiconica (see Fig. 5a) and represents 18% of the total sensilla.

Sensilla basiconica of subtype 3, B3, partly resemble sensilla B1 (length, shape, absence of socket) but differ because of the appearance of the wall (Fig. 7a, b): this has a slightly transversal striation with presumed wall pores. They are rare and constitute about 3% of the total sensilla.

## DISCUSSION

Three types of sensilla are present on the ovipositor lobes of *P. archon*: aporous s. chaetica (4 subtypes C1, C2, C3, and C4), multiporous s. trichodea (T), and s. basiconica (3 subtypes: B1 with wall pores, B2 and B3 with suspected wall pores).

The aporous sensilla chaetica are the longest and more numerous on the ovipositor of *P. archon*. Most of these sensilla are pointed either towards the apical extremity of lobes or transversally as regards the latter. A contact mechanoreceptive (tactile) function has been found for

Table 1. Morphological characteristics (in  $\mu\text{m}$ ) of different sensillum types or subtypes on the ovipositor of *Paysandisia archon*. ?, pores hypothesized but not clearly visible in the studied material ; n = 20.

Sensillum type	Length	Basal width	Socket width	Pores
chaeticum C1	$870.4 \pm 23.7$	$12.3 \pm 1.2$	$29.3 \pm 2.0$	no pore
chaeticum C2	$345.8 \pm 14.2$	$10.4 \pm 0.8$	$22.8 \pm 1.7$	no pore
chaeticum C3	$152.8 \pm 14.2$	$7.3 \pm 0.5$	$13.7 \pm 0.9$	no pore
chaeticum C4	$160.9 \pm 10.3$	$13.6 \pm 1.9$	$25.2 \pm 1.4$	terminal pore?
trichodeum T	$59.4 \pm 6.8$	$5.2 \pm 1.3$	$13.1 \pm 2.2$	wall pores
basiconicum B1	$16.9 \pm 7.3$	$3.8 \pm 0.7$	----	wall pores
basiconicum B2	$8.5 \pm 1.5$	$4.3 \pm 0.5$	$14.4 \pm 1.3$	wall pores?
basiconicum B3	$18.1 \pm 1.8$	$4.0 \pm 0.2$	----	wall pores?

Table 2. Mean numbers ( $\pm$  SE), percentages and presumed function of sensilla on two ovipositor lobes of *Paysandisia archon* (5 females)

Sensillum type	mean number	%	presumed function
chaeticum C1	$30.5 \pm 5.7$	14.47	tactile
chaeticum C2	$55.8 \pm 6.4$	26.52	tactile
chaeticum C3	$22.1 \pm 3.9$	10.47	tactile
chaeticum C4	$16.2 \pm 1.8$	7.66	gustative?
trichodeum T	$24.3 \pm 4.2$	11.52	olfactive
basiconicum B1	$16.4 \pm 2.7$	7.76	olfactive
basiconicum B2	$38.5 \pm 4.5$	18.28	hygroreceptive?
basiconicum B3	$7.2 \pm 0.6$	3.32	olfactive? CO <sub>2</sub> reception?
All sensillum types	210	100	

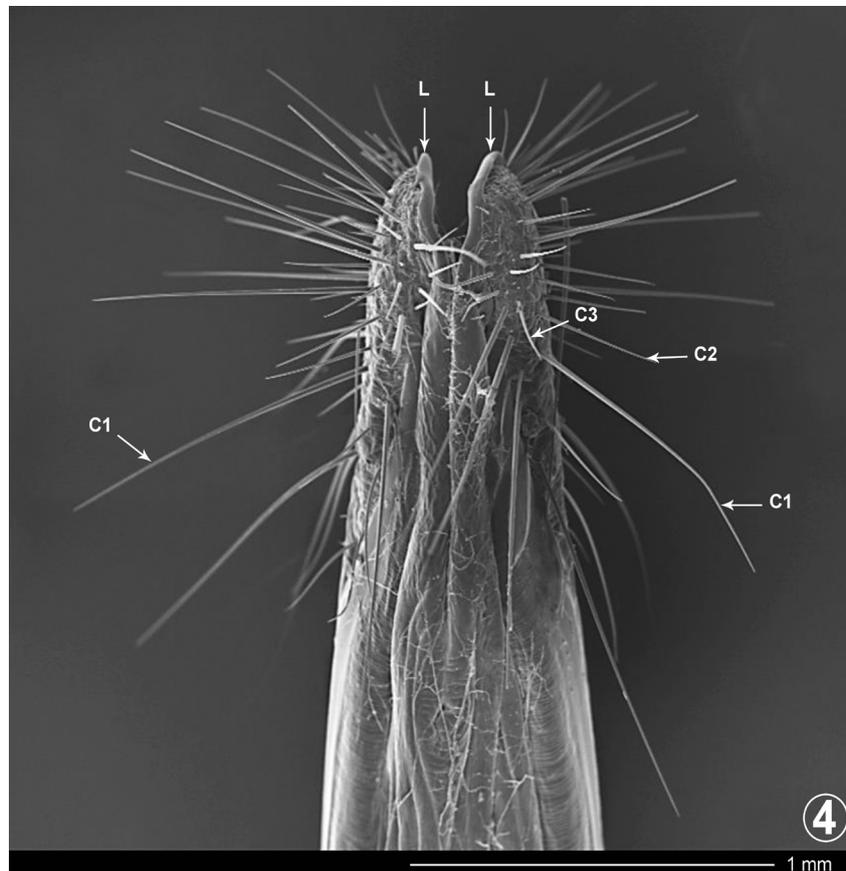
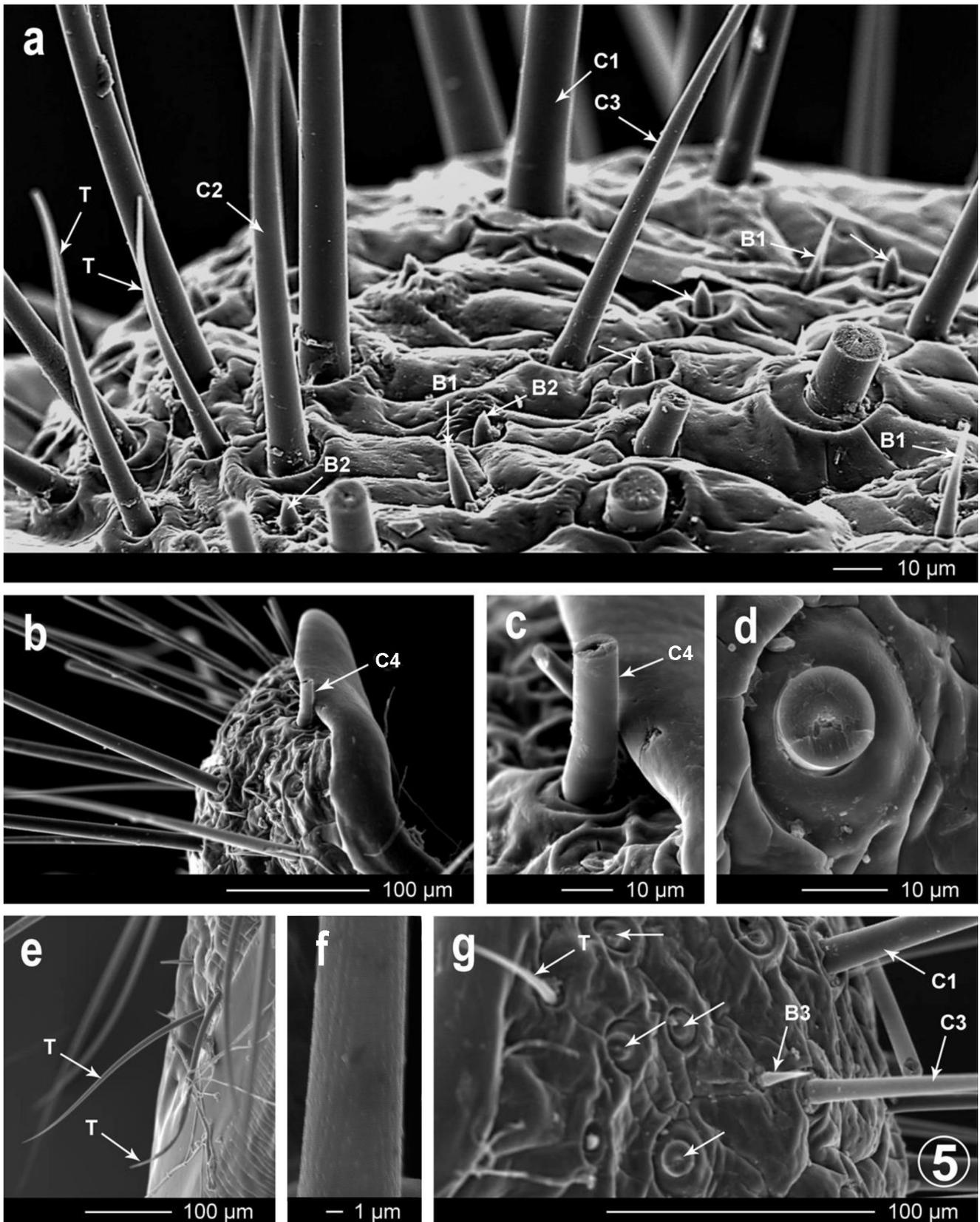


Figure 4. Ovipositor of *Paysandisia archon*, ventral view of sensory lobes, L, showing three subtypes of sensory chaetia C1, C2, and C3 (SEM).



**Figure 5.** Ovipositor of *Paysandisia archon*, **a.** distal part of lobe showing sensilla chaetica subtypes 1, C1; 2, C2; 3, C3; sensilla trichodea, T; sensilla basiconica subtype 1, B1; subtype 2, B2 and arrows; **b.** distal part of lobe showing broken sensillum C4; **c.** detail of C4 inserted into basal socket; **d.** broken base of sensillum C2 with thick wall and absence of internal lumen; **e.** base of lobe with long sensilla trichodea, T; **f.** detail of sensillum trichodeum with longitudinal lines of pores; **g.** distal part of lobe with sensilla C1 and C3, broken bases of sensilla chaetica C2 (arrows), short sensillum trichodeum, T, and sensillum basiconicum subtype 3, B3 (SEM).

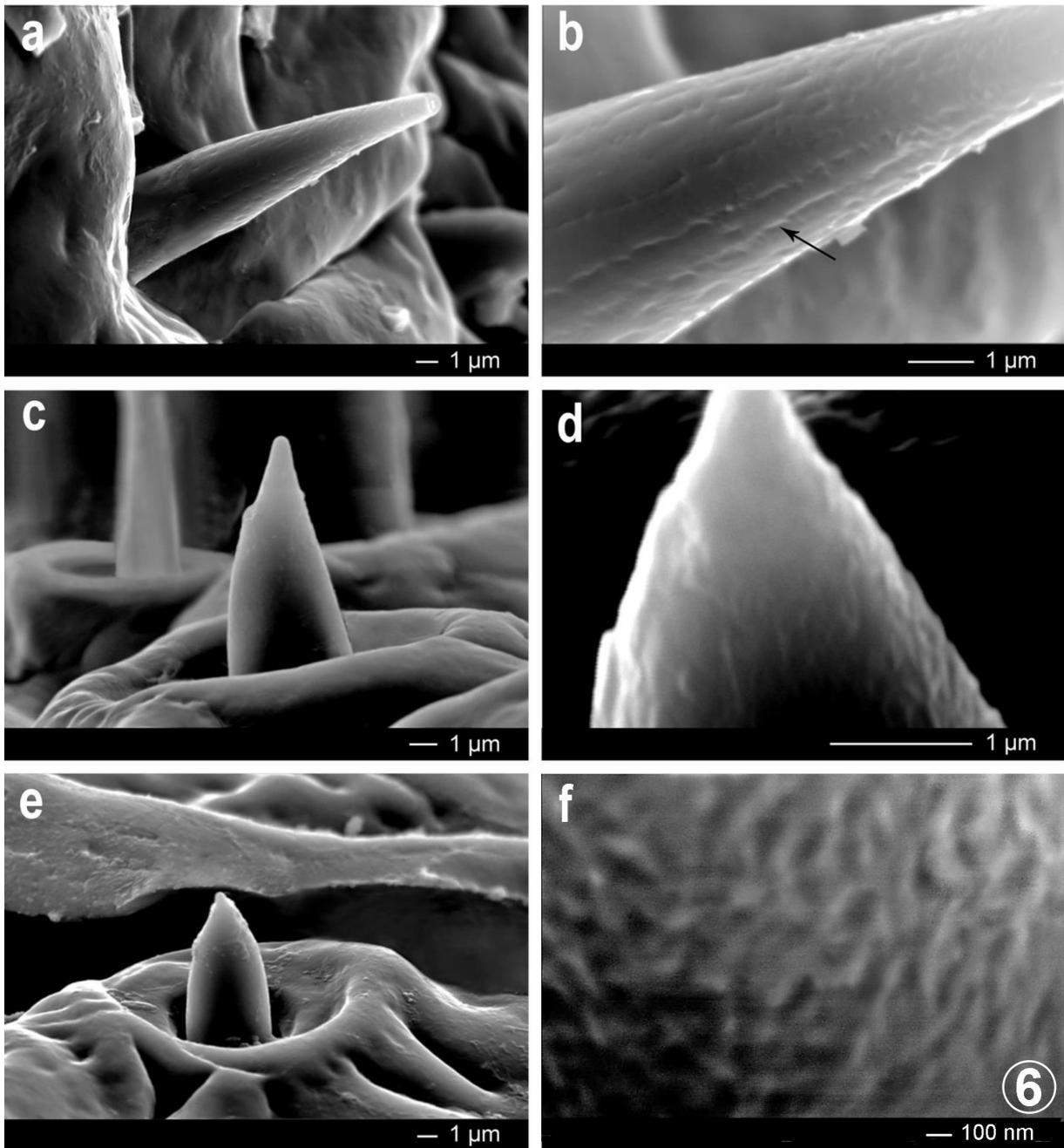


Figure 6. Ovipositor of *Paysandisia archon*, **a.** sensillum basiconicum subtype 1, B1; **b.** detail of B1 with lines of wall pores (arrow); **c. e.** Sensilla basiconica subtype 2, B2; **d.** tip of B2; **f.** wall of B2 (SEM).

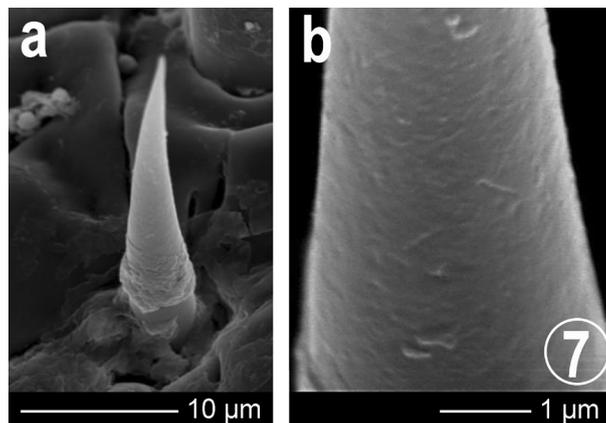


Figure 7. Ovipositor of *Paysandisia archon*, **a.** sensillum basiconicum subtype 3, B3; **b.** detail of wall with putative pores (SEM).

them (Zacharuk 1985). In the palm borer, they allow the ovipositor to mechanically explore, at some distance, the egg-laying substrate as well as the environment in all directions. According to their localization at the base of the ovipositor lobes, the proximal sensilla do not intervene during the tactile prospection of the ovipositor on the egg-laying substrate. They are stimulated during movements of protraction and retraction of the ovipositor. This explains why their direction (towards the front or the back, or transversally) is so diverse, and also their short length.

The tactile function of the ovipositor has been envisaged or demonstrated in most moth species. It is clear that the physical properties of an oviposition substrate are extremely important in determining where eggs are laid (Meisner *et al.* 1974, Traynier 1975, Fenemore 1978). Picard (1913) stated that the potato tuber moth *Phthorimaea operculella* oviposited on rugose rather than on smooth surfaces, and in cracks and depressions, and that oviposition was stimulated by the contact of the tip of the abdomen with rough surfaces. Under field conditions, a considerable proportion of eggs was laid in soil adjacent to host plants rather than on the plants themselves. Traynier (1975) concluded that preferred oviposition substrates provided irregularities that were large enough to accommodate eggs and considered the surface texture to predominate over light/shade, moisture, and the presence of host-plant juices. It appears that the stimulus for oviposition in the potato moth is primarily tactile (Fenemore 1988). Females of the Mediterranean flour moth, *Ephestia kuehniella* preferentially deposit their eggs in fissures or interstices (Ullyett 1945). This author noted an «indifference» of the females to the laying medium and showed that they can lay their eggs as easily in talc as in flour. Later, Daumal & Boinel (1994) concerning the same species, observed that the presence of an interstice or a fissure remains the most important stimulus inducing oviposition. Tactile stimuli rather than chemicals solicit the oviposition behaviour in *Tineola bisselliella*. The moth may recognize the depth or configuration of the substrates by tactile stimuli thanks to aporous sensilla chaetica (Kan & Waku 1985). These previous findings are corroborated by the fact that the ovipositors of lepidopterans bear a large number (e.g. about 100 in *P. operculella*, 108 in *P. archon*) of prominent mechanosensory hairs (Fenemore 1978, Valencia & Rice, 1982, Faucheux this paper).

All the aporous sensilla chaetica of ovipositors are innervated by a single neuron whose dendritic tip, or tubular body, is attached at the sensillum base (Banga *et al.* 2003, Klinner *et al.* 2016). In *M. sexta*, these sensilla were named s. trichodea by Eaton (1986). Based on their morphology, we suggest renaming them as s. chaetica according to the terminology by Altner & Prillinger (1980) (see also Klinner *et al.* 2016).

Contact mechanoreception may be the only function of the ovipositors (e.g. *Bombyx mori*). In detailed electrophysiological studies of the sensory hairs on the anal papillae of *B. mori*, Yamaoka & Hirao (1971, 1973) and Yamaoka *et al.* (1971) showed that these receptors actually received the tactile stimuli from the surface texture of the substrates and that the stimuli directly released the oviposition. Some ovipositors in moths only possess aporous sensilla chaetica, for instance: *Sitotroga cerealella* (Ma *et al.* 2017). However, it is also possible that uniporous sensilla morphologically similar to aporous sensilla chaetica have not been detected by the authors, given that the apical pore is often difficult to visualize in SEM.

### **Uniporous sensilla and gustatory function**

Physical properties of plant surfaces, unless of a specifically unique nature, cannot be solely responsible for selection of a particular plant species for oviposition. Such selectivity is much more likely to be conferred by chemical factors. However, it is difficult to see how external contact by the insect's ovipositor with the intact plant surface could detect juices within the plant tissue (Fenemore 1988). In *P. operculella*, Fenemore (1980) worked with a range of known host and non-host plants and found that the foliage of all species was rendered acceptable for oviposition when covered with a layer of muslin cloth suggesting that significant volatile chemical factors, stimulating or deterrent, were not involved. Plant juices extracted from the potato increased egg deposition. Such effects were negated by separating the oviposition substrate from the plant material by 1-2 mm, indicating that the chemical substances concerned were not appreciably volatile. The contact between the ovipositor and the substrate is therefore essential for egg-laying. This conclusion is supported by the presence of contact chemoreceptors (terminal-pore sensilla or uniporous sensilla) on the tip of the ovipositor.

Discovered for the first time in *Chilo partellus* and *Spodoptera littoralis* (Chadha & Roome 1980), uniporous sensilla were observed in *P. operculella* (Valencia & Rice 1982), *Eldana saccharina* (Waladde 1983), *Acrolepiopsis assectella* (Faucheux 1988b), *Prays citri*, *Choristoneura fumiferana* (Faucheux 1990), *Lobesia botrana* (Faucheux 1990, 2000; Maher & Thiery 2004, Maher *et al.* 2006), *Ephestia kuehniella* (Anderson & Hallberg 1990, Faucheux 1991). These sensilla always exist in limited numbers, about 10 in *P. operculella* (Valencia & Rice 1982), 8-10 in *Ephestia kuehniella* (Anderson & Hallberg 1990), 12 in *L. botrana* (Faucheux 2000), 12-15 in *Ostrinia nubilalis* (Faucheux 2017). Although the presumed terminal pore has not observed with SEM, sensilla chaetica subtype 4, C4, of *P. archon* offer many common traits with the “bimodal taste/tactile sensilla” of *E. kuehniella* (Anderson & Hallberg 1990) and the uniporous (UP) sensilla of *O. nubilalis* (Faucheux 1999). They could perform a gustatory function in the palm borer: “half of the ovipositing females of *P. archon* scratched the substrate, the palm crown, with their intermediate legs” thus releasing the juices of the plant (Hamidi & Frérot 2016).

### **Multiporous sensilla and olfactory function**

Multiporous sensilla were observed on ovipositors of two Tineidae, *Monopis crocicapitella* and *Monopis rusticella* (Faucheux 1987, 1988a, 1999), an Acrolepiidae, *Acrolepiopsis assectella* (Faucheux 1988b), a Cossidae, *Zeuzera pyrina* (Faucheux 1999), three Pyralidae, *Homoeosoma nebulella* (Faucheux 1991), *Homoeosoma electellum* (Faucheux 1995), *Homeosoma stypticellum* (Faucheux 2006), two Noctuidae, *Phlogophora meticulosa* (Faucheux 1996) and *Helicoverpa assulta* (Li *et al.* 2020), a Tortricidae, *Choristoneura fumiferana* (Banga *et al.* 2003), a Castniidae, *Paysandisia archon* (Faucheux *et al.* 2015), and a Sphingidae, *Manduca sexta* (Klinner *et al.* 2016). Like gustatory uniporous sensilla, multiporous sensilla are often in limited numbers, 2 in *M. crocicapitella* (Faucheux 1988a), 4-9 in *M. sexta* (Klinner *et al.* 2016), but are sometimes found in greater numbers, 33 in *H. electellum* (Faucheux 1995), 40 in *P. archon* (this paper).

Morphological types of sensilla basiconica on lepidopteran ovipositors can be classified as short sensilla basiconica (4 à 11 µm in *Homoeosoma* sp. and *Z. pyrina*, 8 µm for B2 in *P. archon*) and long sensilla basiconica (17 µm in *Monopis* sp., 17-18 µm for B1 and B3 in *P. archon*, 22 µm in *Acrolepiopsis*

sp., 100 µm in *P. meticulosa*). Although they do not possess the same morphology as the latter, the sensilla B2 of *P. archon* can be classified with the short sensilla basiconica. They are for the moment the only ones known to comprise a short cone surrounded by a large bulge. In most species possessing multiporous sensilla on their ovipositor, a single morphological type is known. The only exception is the spruce budworm *C. fumiferana*: Banga *et al.* (2003) described three multiporous types: types I and III sensilla were short and long multiporous hairs; type II sensilla were multiporous and also had a single terminal pore. The palm borer *P. archon*, provided with 2 or 3 types of multiporous sensilla, is a second example but none of these sensilla has a supplementary terminal pore. The uniporous-multiporous sensilla were mentioned only in three moths: *C. fumiferana*, *M. sexta*, and *H. assulta* (Banga *et al.* 2003, Klinner *et al.* 2016, and Li *et al.* 2020).

Multiporous sensilla are considered as olfactory chemoreceptors (Zacharuk 1980), which justifies the hypothesis of an olfactory function in moth ovipositors and therefore a sensitivity to volatile organic compounds (VOCs) from the egg-laying substrate. They may also possess carbon dioxide receptors (Bogner 1990, Bogner *et al.* 1986). Until recently, the olfactory role of the moth ovipositors was unknown. Ovipositors have far fewer and smaller sensilla compared with the antenna; this may explain an almost complete absence of studies on the electrophysiology of ovipositors. Host discrimination is believed to be achieved by exploiting cues using mechanosensory and contact chemosensory sensilla present on the ovipositor (Van Lenteren *et al.* 2007). While olfaction has been largely investigated in the antennae, and the maxillary and labial palps of insects, only close-range chemical detection via gustation (contact chemoreceptors) has been found in egg-laying structures like the ovipositor (Yadav & Borges 2017). Several observations and experiments reveal the intervention of VOCs in oviposition behaviour. In addition to the physical effects of pubescence and the nature of the wax scales on the fruit and leaf surfaces of the apple, cherry, peach, and pear, codling moth (*Cydia pomonella*) oviposition was also strongly influenced by volatiles from the fruit of some host plants. Egg deposition on apple and pear would be favoured by the dissipation of  $\alpha$ -farnesene, an attractant from the fruit clusters of these hosts (Hagley *et al.* 1980).

The olfactory function of multiporous sensilla has been revealed in *Manduca sexta* thanks to electrophysiological techniques (Klinner *et al.* 2016). The authors determined whether the identified putative chemosensory sensilla served an olfactory function using single sensillum recordings. Their results demonstrated that a small number of sensory cells on the ovipositor function as olfactory sensory neurons (OSNs). Porous sensilla housed the dendrites of multiple neurons which responded to distinct sets of odorants. Furthermore, single sensillum recordings revealed neurons (named B-neurons) that did not respond to stimulation with a tested odorant. These neurons might be chemosensory but they may detect compounds not included in the panel of tested odorants. Klinner *et al.* (2016) suggested that they could also be involved in the detection of other modalities. The non-active neurons might be sensitive to CO<sub>2</sub>, gustatory cues, or possibly humidity. According to them, "The expression of the two putative CO<sub>2</sub>-receptor *MsexGR2* and *MsexGR3* (chemosensory receptor genes in *M. sexta* ovipositor tissue) further suggest that these sensory functions likely exist in the ovipositor." Mechano- and hygroreceptor functions in ovipositors have both been reported for many species of moths and suggested for *M. sexta* (Waladde 1983, Qiu *et*

*al.* 1998, Banga *et al.* 2003, Seada *et al.* 2016). Increased durations of ovipositor activities suggest that the females of *Lobesia botrana* and *Eupoecilia ambiguella* have the ability to recognize specific volatile organic compounds (VOCs) by the ovipositor, which could attract or repel females for egg deposition. The ovipositor activity of tasting, thanks to uniporous sensilla, is influenced by volatiles (Markheiser *et al.* 2020). This shows that there may be no essential difference as concerns function between a multiporous sensillum and an uniporous sensillum.

The two possibilities (detection of odors and sensitivity to CO<sub>2</sub>) may be envisaged concerning the sensilla trichodea and basiconica of the *P. archon* ovipositor. As has already been pointed out (Faucheux *et al.* 2015), the fact that, in rest, a large section of the terminal region of the telescopic ovipositor is exposed, contrary to most lepidopterans, may be of interest. Indeed, if the tactile sensilla only perform when in contact with the egg-laying substrate, the olfactory sensilla are stimulated at a distance by the odors from the host-tree, the palm, and to act, must be fully exposed to the air, which justifies their concentration at the apex of the lobes. Sensilla trichodea and sensilla basiconica B1, whose wall pores are visible on figures 5f and 6b, can be involved in the reception of volatile organic compounds from the palm.

The sensitivity to CO<sub>2</sub>, which could be the function of sensilla basiconica B2 and B3, seems likely in the case of the palm borer. Indeed, the cycle of development of the parasite takes place in a limited zone of the palm tree, between the base of the leaves and the last 40 cm underneath the final bud. The female lays her eggs near the crown of the palm tree, where most of the leaves gather at the summit of the stipe (Sarto i Monteys & Aguilar 2005). At this level, the petioles of the leaves are crowded together and carbon dioxide levels can be high. The oviposition would appear to be stimulated by these levels. As is the case of parasitic wasps (Yadav & Borges 2017), the *P. archon* ovipositor may act as a volatile sensor within a closed microcosm, as an olfactory organ that responded to volatiles and CO<sub>2</sub> in gaseous form. A hygroreceptive function for sensilla B2 or B3 cannot be excluded if one of these sensilla happens to possess a non-porous wall (Altner & Prillinger 1980).

## CONCLUSION

The present study corrects and completes a previous one (Faucheux *et al.* 2015); it offers a different interpretation of certain sensilla and has made possible the discovery of two new sensillum types. Apart from the tactile function usually attributed to the ovipositors of moths, it suggests a double chemoreceptive function, gustative or/and olfactory, for which 5 types or subtypes of sensilla (*s. chaetica* C4, *s. trichodea* T, *s. basiconica* B1, B2, and B3) are involved. Further ultrastructural studies will be necessary to specify the existence of pores and the number of sensory neurons for each sensillum type.

Our results show the need to observe several individuals in order to produce valid conclusions rather than accepting without question those of other researchers on the same subject. The first sensilla discovered on the ovipositor of lepidopterans were tactile aporous sensilla chaetica because they were the sensilla one would expect on an egg-laying organ. Subsequently, the uniporous sensilla basiconica with a gustative function were found; they have been searched for and reported on several moths. The multiporous sensilla with a presumed olfactory function were the last to be identified. They were discovered on several species. Each ovipositor

of most of the studied moths has only two sensillum types: aporous and uniporous or multiporous. *P. archon* is the only moth known at present to possess the three types with two morphological subtypes of multiporous sensilla. The sensory equipment of the ovipositor is important to know because this organ is the last to intervene before the egg-laying, and the three sensory functions (touch, taste, and smell) are thereafter likely to act.

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