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Diversity of olfactory structures: A comparative study of antennal sensilla in Trichoptera and Lepidoptera

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ABSTRACT

The antenna is the main sensory organ of insects, housing different types of sensilla dedicated to detect chemical cues, motion, humidity and temperature. Sensilla are divided into different types based on their wall structure and morphology. Among the olfactory sensilla, there is an enormous variation in the numbers and morphological types present in different insect taxa. The reasons for this variation remain obscure, though there may be a correlation between sensillum morphology and the characteristics of the stimulus that the olfactory sensory neurons inside the sensillum detect. Here, we report the first comparative analysis of the morphology and ultrastructure of sensilla from *Rhyacophila nubila* (Rhyacophilidae: Trichoptera) and three species of Lepidoptera, *Eriocrania semipurpurella* (Eriocraniidae), *Lampronia capitella* (Prodoxidae), and *Bicyclus anynana* (Nymphalidae), which use different chemical types of pheromones. Our results, together with a thorough literature review, suggest a shift in major types of olfactory sensilla, from a high proportion of sensilla placodea or auricillica in Trichoptera and the most basal moth lineages (including Eriocraniidae), respectively, to sensilla trichodea in the more derived Lepidoptera (including Prodoxidae and the Ditrysia clade), which parallels the change in the types of sex pheromones used.

1. Introduction

Olfaction plays a critical role in insects, underlying behaviors such as host-seeking, mate-finding and enemy-avoidance. The olfactory system of an adult insect consists of two pairs of main olfactory appendages on the head, the antennae and the palps. The antennae are usually covered with scales and other structures, often hair-like, called sensilla, involved in the detection of chemical, mechanical and thermal stimuli (Hansson and Stensmyr, 2011; Schneider, 1964). Based on their wall structure (i.e., pores and wall properties) and external appearance, sensilla are classified into different morphological types, e.g. trichodea, basiconica, chaetica, coeloconica, ascoidea, vesiculoclada, auricillica, placodea, styloconica, ampullacea, squamiforma, campaniforma, and Böhm's bristles (Hallberg and Hansson, 2003; Keil, 1999). Different insect species possess various subsets of these types, and in different relative abundances. Also, the different types of sensilla are used for distinctive functions, for instance sensilla trichodea, basiconica, coeloconica, vesiculoclada, auricillica, placodea and ascoidea are used for olfaction, sensilla chaetica for taste, sensilla styloconica and ampullacea for thermo- or hygrosensation, and sensilla squamiforma, campaniforma, and Böhm's bristles for mechanoreception (Hallberg and Hansson, 2003). The olfactory sensilla of insects generally contain

specialized structures, including multiple cuticular pores, facilitating detection of odor molecules (Schneider, 1964). The pores in the sensillum wall are connected to pore-tubules suspended in the sensillum lymph, which might possibly serve as a route for the odor molecules to reach the odorant receptors (ORs) in the dendrites of the olfactory sensory neurons (OSNs) (Carlson, 1996; Larter et al., 2016; Steinbrecht, 1996). The OSNs are responsible for the detection of different sets of odor molecules. Insect olfactory sensilla typically contain 2–3 OSNs (Andersson et al., 2009; Hallberg and Hansson, 1999; Ljungberg et al., 1993; Yuvaraj et al., 2013) but sometimes more depending on taxon (reviewed in Keil, 1999).

The number of sensilla on the antenna varies widely between species, for instance, psyllids (Sternorrhyncha: Psyllidae) may only have four olfactory sensilla (Kristoffersen et al., 2006; Yuvaraj et al., 2013), whereas moths and butterflies (Lepidoptera) may have tens of thousands sensilla, belonging to several morphological types (Steinbrecht, 1970; Ansebo et al., 2005; Hallberg and Hansson, 2003; Wee et al., 2016). The difference in antennal architecture may be the result of adaptation to different ecological niches (Hallberg and Hansson, 1999; Hansson and Stensmyr, 2011), although evidence supporting this suggestion remains elusive. The different morphology of the sensilla may also be advantageous for specific tasks. For instance, long sensilla

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trichodea can be organized to create basket-like sieves to capture sex pheromone molecules in male moths (Steinbrecht, 1996). Indeed, sensilla trichodea house the OSNs tuned to pheromones in *Drosophila* and many moth species (Ansebo et al., 2005; Clyne et al., 1997; Hallberg et al., 1994; Ljungberg et al., 1993; Pophof et al., 2005). On the other hand, the ORs that detect sex pheromones (Yuvaraj et al., 2017) in the basal moth *Eriocrania semipurpurella* (Lepidoptera: Eriocraniidae) are located in sensilla auricillica, as suggested by single sensillum recordings (Larsson et al., 2002).

In most species of moths, females produce long-range sex pheromones to attract males for mating (Ando et al., 2004; Löfstedt et al., 1991). Moth pheromone communication has been well characterized in terms of pheromone biosynthesis, and to some extent olfactory reception (Ando et al., 2004; Jurenka, 2004; Löfstedt et al., 2016; Zhang and Löfstedt, 2013). Moth pheromones are divided into different types based on their site of production, chemical structure and biosynthetic origin (Löfstedt et al., 2016). Type 0 pheromones are short-chain secondary alcohols or ketones, which are similar to general plant volatiles (Kozlov et al., 1996; Löfstedt et al., 2016). This type is used by a few old lineages of Lepidoptera, as well as the sister group Trichoptera (caddisflies; Fig. 1). Type I pheromones are C₁₀–C₁₈ acetates, alcohols, and aldehydes, which are used by approximately 75% of the moth species (Ando et al., 2004; Löfstedt et al., 2016).

Because olfactory sensilla are key elements of olfaction by allowing the odor molecules to enter the internal environment, it is possible that major changes in pheromone signalling, such as the transition from structurally dissimilar Type 0 to Type I pheromones in Lepidoptera (Löfstedt et al., 2016), have been associated with modifications of sensillum morphology or alterations in the relative abundance of sensillum types. The antennal morphology and sensillar ultrastructure have been investigated in many families within the Lepidoptera (Supplementary information), and most of the species from these families use Type I sex pheromones (Löfstedt et al., 2016). To our knowledge, the antennal morphology of moth species using Type 0 pheromones has so far only been studied in a single species, namely *E. semipurpurella* (Larsson et al., 2002). No studies have compared the antennal architecture or diversity of olfactory sensilla between species using Type 0 or Type I sex pheromones.

In this study, we describe and compare the antennal morphology and sensillar structures of two moth species representing two of the most basal lepidopteran lineages (*E. semipurpurella*: Eriocraniidae; *Lampronia capitella*: Prodoxidae), one species of butterfly (*Bicyclus anynana*; Lepidoptera: Nymphalidae), and one species of Trichoptera (*Rhyacophila nubila*; Rhyacophilidae), using scanning and transmission electron microscopy. We hypothesize that the use of different pheromone types among these species may be associated with differences in the types of sensilla present, or sensilla frequencies. The caddisfly, *R. nubila* belongs to the sister group of Lepidoptera, and uses similar Type 0 pheromone compounds as *E. semipurpurella* (Löfstedt et al., 1994). The currant shoot borer moth, *L. capitella*, belongs to the first lepidopteran lineage using female-produced Type I sex pheromone compounds (Fig. 1; Löfstedt et al., 2016). Butterflies form a relatively derived monophyletic clade within Lepidoptera, but unlike moths, they do not use female-produced sex pheromones. In contrast, butterflies use male-produced pheromones for short-range courtship behaviour, which makes them interesting for the present comparison. Some of the male-produced pheromone compounds of the tropical butterfly *B. anynana* and several of its congeners, are structurally similar to typical pheromone compounds in moths (Nieberding et al., 2008; Bacquet et al., 2015). We also present an overview of antennal morphological studies from Lepidoptera and Trichoptera, and summarize previously reported electrophysiological data recorded from the various morphological sensillum types. We observe variation in the occurrence of different types of sensilla among the four species studied and throughout the lepidopteran phylogeny.

2. Material and methods

2.1. Insects

Pupae of *R. nubila* were collected from fresh water streams near Sjöbo, Sweden (55°41'13.2"N, 13°21'24.6"E), and adults were allowed to emerge in the laboratory. Adult males of *E. semipurpurella* were collected, using pheromone traps, from a birch forest in Skrylle, close to Lund, Sweden (55°38'51.0"N, 13°41'28.1"E). Male and female *L. capitella* adults were collected by hand from black currant fields near Roskilde, Denmark (55°36'26.8"N, 11°58'35.2"E). The squinting bush browns, *Bicyclus anynana*, were derived from a lab-reared population maintained at the University of Cambridge, UK, originally founded from 80 gravid females collected in Malawi in 1988 (courtesy of Dr. Oskar Brattström).

2.2. Scanning electron microscopy (SEM)

The antennae from live insects were dissected and immersed in a freshly prepared fixative solution, containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M l⁻¹ cacodylic buffer (pH 7.4) for 24 h at 4 °C. The antennae were then dehydrated in a graded ethanol series followed by critical-point drying (BAL-TEC CPD 030). The dried specimens were carefully glued onto SEM stubs, and sputter-coated with gold (Cesington 108 auto, 45 s, 20 mA). The preparations were viewed using a scanning electron microscope (SEM; Hitachi SU3500) at 5 kV.

2.3. Transmission electron microscopy (TEM)

The fixated antennae (see above) were post-fixed in 1% osmium tetroxide in 0.1 M l⁻¹ cacodylic buffer for 2 h at 4 °C. The specimens were dehydrated in a graded ethanol series and embedded in epoxy resin (Agar 100) via acetone. Semi-thin sections (1.5 µm) were made using a Leica EM UC7 ultratome with a glass knife and stained with Richardson's solution (Richardson et al., 1960) to examine the orientation of the tissue in the trimmed block. Ultra-thin sections (50 nm) were made using a Leica EM UC7 ultratome with a diamond knife. The sections were mounted on copper grids and stained with 2% uranyl acetate (30 min) and lead citrate (4 min), and then examined using a JEOL JEM 1400 Plus transmission electron microscope. The images captured from both SEM and TEM were edited using Adobe Photoshop and Illustrator software (www.adobe.com/).

2.4. Data analysis

The different types of sensilla were discriminated based on their morphological features defined in the literature (Hallberg and Hansson, 1999; Ivanov and Melnitsky, 2016). The abundance of different types of sensilla along the antennae of *R. nubila* varied and thus we counted the sensilla on segments from the proximal, middle and distal parts of the antenna, i.e., segment number 7, 20 and 30, respectively. For *E. semipurpurella* and *L. capitella*, the numbers of different types of sensilla were counted on a middle segment of the antennae, as the different types show a homogenous distribution along the antennae. In *B. anynana*, the sensilla were only present on the club-shaped distal segments and therefore the sensilla on the middle segment of the club were counted. The absolute number and relative abundance (percent of total sensillum count) of the different sensillum types are presented as means of three replicates, four in the case of *L. capitella*. There was no difference in sensillum numbers between male and female *B. anynana*, hence counts from male and female antennae were pooled.

2.5. Antennal morphology of other lepidopteran species

We compiled data on previously reported antennal morphology, sex pheromone compounds, and electrophysiological recordings from species

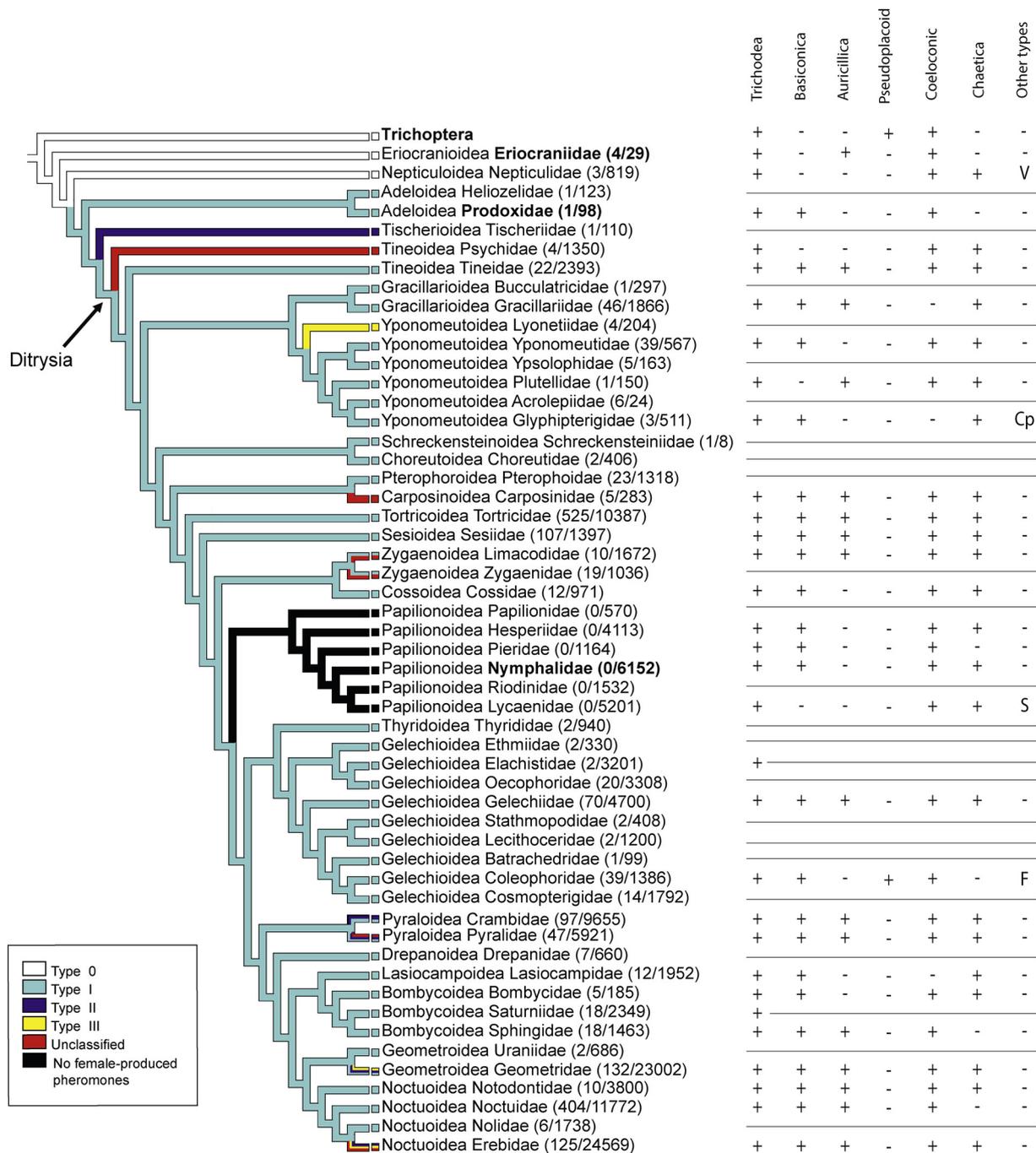


Fig. 1. Phylogenetic tree of major lepidopteran lineages and the sister group Trichoptera using the parsimony criterion. Numbers in parentheses after each taxon indicate approximate number of pheromones and attractants reported followed by the number of species in each taxon. The tree is mapped with sex pheromone types (for detailed description, see Löfstedt et al., 2016), and the occurrence of common antennal sensillum types is indicated next to the family: present (+), absent (-) and no data (line). Other types of sensilla: V, vesiculocladium; Cp, chemoreceptor pegs; S, stellate; F, surcatea. The tree is adapted from Löfstedt et al. (2016) with the data on morphological sensillum types added. Note: the families and order that contain the four species analyzed here are highlighted in bold.

across the lepidopteran phylogeny (Fig. 1; Supplementary information). Only families in which female sex pheromones have been identified were included in the phylogeny and the compilation (exceptions are the Papilionoidea families). A maximum of two species per family were included with preference given to species with reported morphological and electrophysiological data, and identified pheromones. The phylogenetic tree with pheromone types mapped was adapted from Löfstedt et al. (2016), and the antennal morphological data of different families were added (Fig. 1). The families in the supplementary information summarizing electrophysiological data from various sensillum types follow the order of the phylogenetic tree in Fig. 1.

3. Results

3.1. General antennal morphology

The antennae of all four species in this study are filiform and consist of three parts: scape, pedicel, and a long flagellum divided into segments. The flagellar segments are divided into dorsal and ventral surfaces with sensilla distributed most abundantly on the ventral surface. Fine hair-like cuticular structures called microtrichia are found on the antennal surface except in *L. capitella* (Fig. 2, 3 and 5). The dorsal surface and most of the proximal flagellar segments of *E. semipurpurella*

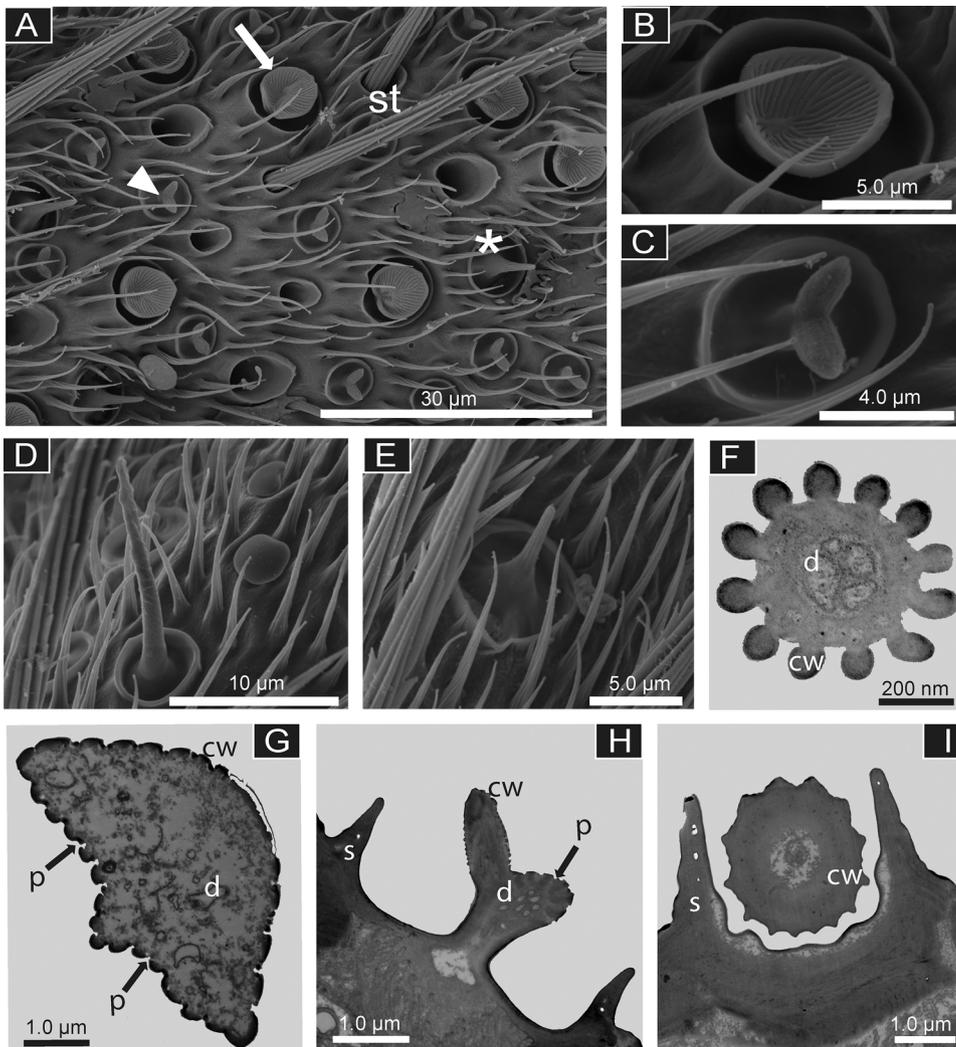


Fig. 2. Scanning and transmission electron micrographs of female *Rhyacophila nubila* antennae. (A) The external morphology and occurrence of chemosensory sensilla: mushroom-like pseudoplacoid (arrow), forked pseudoplacoid (arrowhead), coeloconic (*) and serrated trichoid (st). Close-up views of (B) mushroom-like pseudoplacoid, (C) forked pseudoplacoid, and (D) short curved smooth trichoid sensilla. (E) Short peg-like sensillum coeloconicum with a terminal pore, no microtrichia or scales were found contrary to what is found in higher Lepidoptera. (F–J) TEM cross-sections of different sensilla; cw, cuticular wall; s, socket; p, pores; d, dendrites. (F) Sensillum coeloconicum, (G) mushroom-like pseudoplacoid, (H) forked pseudoplacoid, and (I) serrated trichodeum. In B–E all sensilla are surrounded by circular ridges.

and *B. anynana* are covered with scales. By contrast, no scales are found on the antennae of *L. capitella*. The *R. nubila* antennae are covered with numerous long serrated and ridge-bearing trichoid sensilla. The general appearance of the long serrated and ridge-bearing trichoid sensilla resembles the scales present in *E. semipurpurella* and *B. anynana*.

3.2. *Rhyacophila nubila*

The nomenclature for the *R. nubila* sensillum types follows that of Ivanov and Melnitsky (2016). The filiform antennae of *R. nubila* consist of 45 segments, and both sexes have five types of sensilla: long serrated and ridge-bearing trichoid, short curved smooth trichoid, mushroom-like pseudoplacoid, forked pseudoplacoid, and sensilla coeloconica (Fig. 2A). In males, the forked pseudoplacoids are the most abundant type of sensillum followed by mushroom-like pseudoplacoids, whereas sensillum trichodeum and mushroom-like pseudoplacoids are the most abundant types in females, but depending on antennal segment (Table 1). The cuticular surface of both mushroom-like and forked pseudoplacoid sensilla is penetrated by numerous pores (Fig. 2B, C, G and H). We found a few peg-shaped sensilla coeloconica on the ventral side of the antenna (Fig. 2E). There are also short curved smooth trichoid sensilla (~15 µm) clustered in groups on the basal segments of the antennae (Fig. 2D). No obvious sexual dimorphism could be observed in terms of the presence of sensillum types, but there is a

difference in absolute and relative abundance of different sensilla (Table 1). Transmission electron micrographs (TEM) revealed that the cuticle wall of the mushroom-like pseudoplacoid sensilla is very thin and the sensilla are innervated by > 25 dendrites in the lymph (Fig. 2G). The forked pseudoplacoid sensilla are morphologically similar to sensilla basiconica, thin-walled, multiporous and innervated by several (> 20) dendritic projections (Fig. 2C and H). The sensilla coeloconica (~5 µm) are double-walled and have about four dendritic projections (Fig. 2F). The long serrated and ridge-bearing trichoid sensilla are thick-walled without any dendritic projections similar to that of scales found in derived moths suggesting these are not olfactory sensilla (Fig. 2I).

3.3. *Eriocrania semipurpurella*

The antenna of male *E. semipurpurella* has 40 segments with four types of putative olfactory sensilla (females could not be collected in the field), including sensilla auricillica, trichodea, and coeloconica (Fig. 3A), and occasional antennal segments also housed very few sensilla styloconica (Fig. 3C). The rabbit ear-shaped sensillum auricillicum (~10 µm long) is the major type distributed across the antennal surface (Fig. 3A and C, Table 1). Sensilla auricillica show fine longitudinal punctuated grooves with depressions and ridges where pores were observed. Around 10 multiporous trichoid sensilla

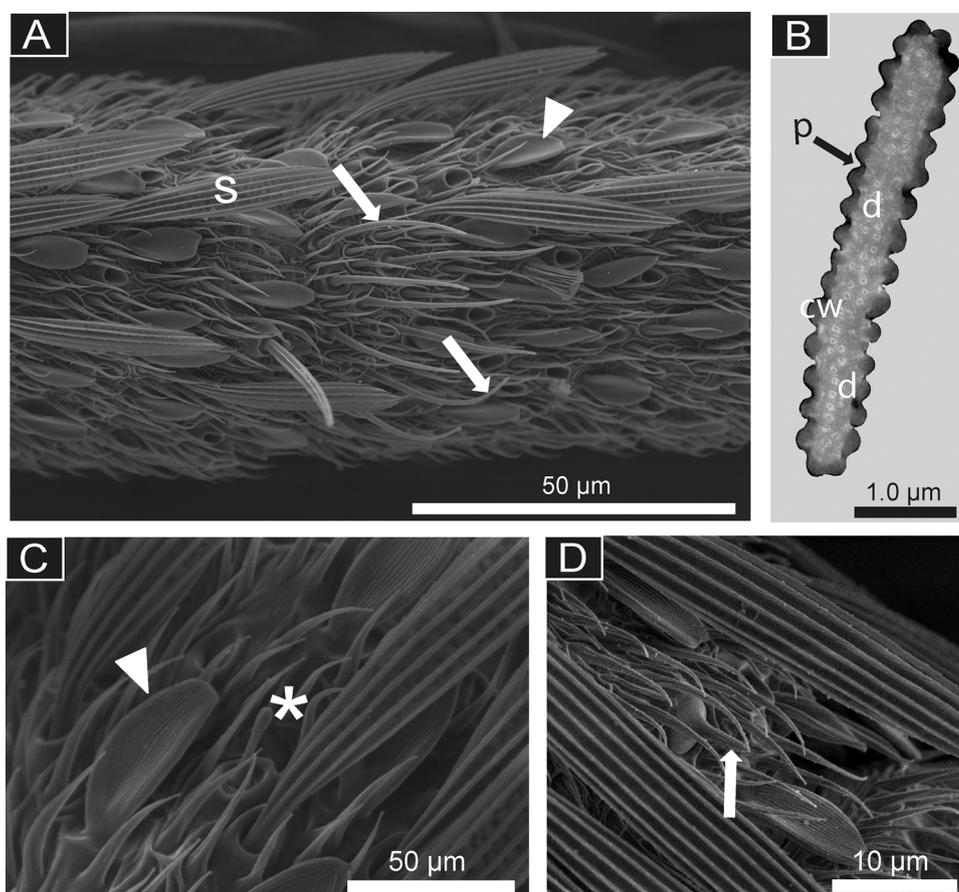


Fig. 3. Scanning and transmission electron micrographs of male *Eriocrania semipurpurella* antennae. (A) General view of major sensillum types and scales: sensillum auriculicicum (arrowhead), sensillum trichodeum (arrows), S (scale). (B) Cross section of sensillum auriculicicum; cw, cuticular wall; p, pores; d, dendrites. (C) Sensillum auriculicicum (arrowhead) and styloconicum (*). (D) Peg shaped sensillum coeloconicum (arrow) without any surrounding ridges or microtrichia.

(~20–25 μm long) were found on the base of the proximal segments on the ventral side of the antenna, similar to earlier findings by Larsson et al. (2002). The abundance of both sensilla coeloconica (~4–5 μm long) and sensilla styloconica (~6 μm long) was low (Table 1; Fig. 3A, C and D). Our TEM micrographs revealed numerous pores distributed on the surface of sensilla auriculica, and the cuticle was thin-walled. The sensilla auriculica are innervated by three sensory cells, with approximately 100 dendritic branches per sensillum (Fig. 3B; Larsson et al., 2002).

3.4. *Lampronia capitella*

The antennae of male and female *L. capitella* consist of 27 segments and house four types of putative olfactory sensilla: long trichodea, short trichodea, basiconica and coeloconica (Fig. 4A–C). The grid- or net-like antennal surface without scales is unique to *L. capitella* among the four species studied (Fig. 4A–C). The sensilla trichodea are found across the antenna and constitute the most abundant type (Table 1). Based on their surface substructure and length, sensilla trichodea are subdivided into two subtypes, long (~65–70 μm) and short trichodea (~35–40 μm). Long sensilla trichodea are more abundant in males than in females (Table 1). On the other hand, short sensilla trichodea and sensilla basiconica are more abundant in females than in males (Table 1). The surface of the trichoid sensilla is smooth at the base, but forms a typical spiral ridge towards the top (Fig. 4C). Sensilla basiconica are short (~10–15 μm) and multiporous (Fig. 4B). Sensilla coeloconica are short (~3–5 μm) and located in deep depressions fenced by a ring of scales (Fig. 4A). TEM micrographs revealed that sensilla trichodea are thick-walled with few pores and innervated by 2–5 dendritic projections (Fig. 4D). Sensilla basiconica are thin-walled and innervated by numerous (> 50) dendritic branches (Fig. 4E). Sensilla

coeloconica are double-walled and contain 3–4 dendritic projections, which are immersed in the central lumen (Fig. 4F).

3.5. *Bicyclus anynana*

The antennae of *B. anynana* consist of 34 segments (flagellomeres). The antennae are divided into compartments by three longitudinal ridges separating two grooves running from the base to the tip, a common feature found among butterfly antennae (Carlsson et al., 2013; Odendaal et al., 1985). The 10–12 distal segments form the bulb and host the highest density of sensilla. We found four types of sensilla, i.e. sensilla trichodea, basiconica, coeloconica, and chaetica (Fig. 5A–C). So far, only a few morphological studies have been performed on the antennae of butterflies, hence, our naming of sensillum types is based on morphological and ultrastructural similarities to moth sensilla, and suggestions from previous studies of other nymphalid species (Carlsson et al., 2013). No sexual dimorphism was found between the antennae of male and female *B. anynana*. Sensilla trichodea are ~20–25 μm long, and the most abundant sensillum type (Table 1; Fig. 5C and D). Sensilla basiconica are short (~7–8 μm long), multiporous, and hidden in pits fenced by a ring of scales. Sensillum basiconicum is the second most abundant sensillum type, distributed on both the dorsal and ventral side of the club (Table 1; Fig. 5C and E), whereas sensilla trichodea are not found on the dorsal side of the club. Sensilla coeloconica are (partly) covered by the scales, preventing us from obtaining clear SEM micrographs for this sensillum type, but we could still count them and obtain TEM micrographs of cross sections (Fig. 5I). Sensilla chaetica are triangular straight hairs (~25 μm long) showing radial ridges, with their base inserted into a cuticular socket. No pores were found along the bristle, though a typical pore was present on the tip, suggesting a role in contact chemoreception (Fig. 5F). TEM micrographs revealed that

Table 1

Absolute (#) and relative (%) abundances of antennal sensillum types with putative olfactory function in *Rhyacophila nubila* (Rnub), *Eriocrania semipurpurella* (Esem), *Lampronia capitella* (Lcap) and *Bicyclus anynana* (Bany). Values are presented as means and range of three to four replicates. Note: for *R. nubila*, sensilla abundances varied depending on antennal segment, with the analyzed segment (S) numbers indicated in bold.

	Rnub		Esem	Lcap		Bany
	♂	♀	♂	♂	♀	♂ + ♀
Mp	S7:					
	% 34.6 (29–45)	% 25.2 (24.1–26.3)	–	–	–	–
	# 60.6 (59–63)	# 14.7 (14–15)				
	S20:					
% 22 (20.4–24.4)	% 21 (14.8–25)					
# 21.0 (20–22)	# 5.3 (4–6)					
S30:						
% 13.5 (11.5–16)	% 31.4 (30–33.3)					
# 7.6 (7–9)	# 3.3 (3–4)					
Fp	S7:					
	% 47.3 (42–50.7)	% 10.7 (7.1–14.5)	–	–	–	–
	# 88 (55–110)	# 6.3 (4–9)				
	S20:					
% 64.6 (63.4–66.3)	% 22 (12.5–33.3)					
# 62 (55–66)	# 4.6 (2–9)					
S30:						
% 77.1 (73.2–80.3)	% 28.6 (25–30)					
# 44 (41–49)	# 3.6 (3–4)					
Au	–	–	% 79.3 (73.1–82.8) # 22 (19–24)	–	–	–
T	S7:			Long	Long	
	% 16.1 (10.7–18.9)	% 60.1 (56.5–64.2)	% 18.2 (14.3–23.1)	% 82.6 (80.4–84.2)	% 59.6 (58–61.4)	% 57.7 (56.7–59.3)
	# 31 (14–41)	# 35 (34–36)	# 5 (4–6)	# 45 (42–48)	# 31 (29–35)	# 80 (76–86)
	S20:			Short	Short	
% 11.7 (10.5–12.5)	% 48.1 (46.1–50)		% 7.3 (5.5–10.7)	% 22.1 (21.2–24.6)		
# 11.3 (9–13)	# 11 (8–13)		# 4 (3–6)	# 11.5 (9–14)		
S30:						
% 8.8 (8.2–9.3)	% 31.1 (25–38.5)					
# 5 (5)	# 3.6 (3–5)					
B	–	–	–	% 6.9 (5.9–7.4) # 3.7 (3–4)	% 14.9 (12.2–18) # 7.7 (6–9)	% 29.3 (28.9–29.9) # 40.6 (39–42)
Co	S7:					
	% 1.9 (1.4–2.3)	% 4 (3.5–4.8)	% 2.5 (0–3.8)	% 3.2 (1.8–5.9)	% 3.4 (0–6)	% 12.9 (11.7–14.1)
	# 3.3 (3–4)	# 2.3 (2–3)	# 0.67 (0–1)	# 1.7 (1–3)	# 1.7 (0–3)	# 18 (17–19)
	S20:					
% 1.7 (1–2.9)	% 9.2 (3.7–12.5)					
# 1.6 (1–3)	# 2 (1–3)					
S30:						
% 0.6 (0–1.8)	% 8.9 (0–33.3)					
# 0.3 (0–1)	# 1 (0–2)					

Mp– Mushroom-like pseudoplocoid, Fp– Forked pseudoplocoid, Au– Auricillica, T– Trichodea, B– Basiconica, Co– Coeloconica.

sensilla trichodea are thick-walled and innervated by 2–5 dendritic projections (Fig. 5G). Sensilla basiconica are thin-walled and innervated by more than 100 dendritic branches (Fig. 5H). Double-walled sensilla coeloconica (Fig. 5I), and thick-walled sensilla chaetica (Fig. 5J) are found on the antennal surface. The number of dendrites for sensilla coeloconica and chaetica was not possible to determine from our TEM micrographs.

4. Discussion

We report the presence and abundance of sensillum types in four species, belonging to taxa that utilize different chemical types of sex pheromones, and in which studies of sensillar repertoires are scarce. We show that the caddisfly *R. nubila* has a different set of sensillum types compared to the lepidopteran species. However, we also observe, in *R. nubila*, some common sensillum types that are found throughout the Lepidoptera, such as sensilla trichodea and different forms of sensilla placodea.

Sensillum placodeum is the major type of sensillum on the antennae

of *R. nubila*, and it occurs in two different forms: mushroom-like pseudoplocoids and fork-shaped pseudoplocoids (Fig. 2). There are different forms of sensilla placodea reported, such as fungiform-, bi-blade-, dentate-, stellate-, bifurcated-, and corniculate placodea in Trichoptera (Ivanov and Melnitsky, 2016) and the placodeum-like sensilla auricillica in Lepidoptera (Anderson et al., 2000; Larsson et al., 2002). Considering the variation in structure, sensilla placodea appear to be heterogeneous across different insect taxa, and the different types may have evolved independently in different insect orders (Hallberg and Hansson, 1999). Previous studies suggest that the density of placoid or placoid-like sensillum types is high only in basal Lepidoptera and its sister group Trichoptera (Larsson et al., 2002; Melnitsky and Ivanov, 2016), although different forms of sensilla placodea (auricillica) occur throughout Lepidoptera at lower densities (Anderson et al., 2000; Ansebo et al., 2005; Ebbinghaus et al., 1998; Pophof et al., 2005). Furthermore, in lineages such as Sphingidae and Gelechiidae (both Lepidoptera), sensilla auricillica are sexually dimorphic (Supplementary information). In contrast, several lepidopteran lineages lack sensilla auricillica (Fig. 1). Two of the families that lack sensilla auricillica,

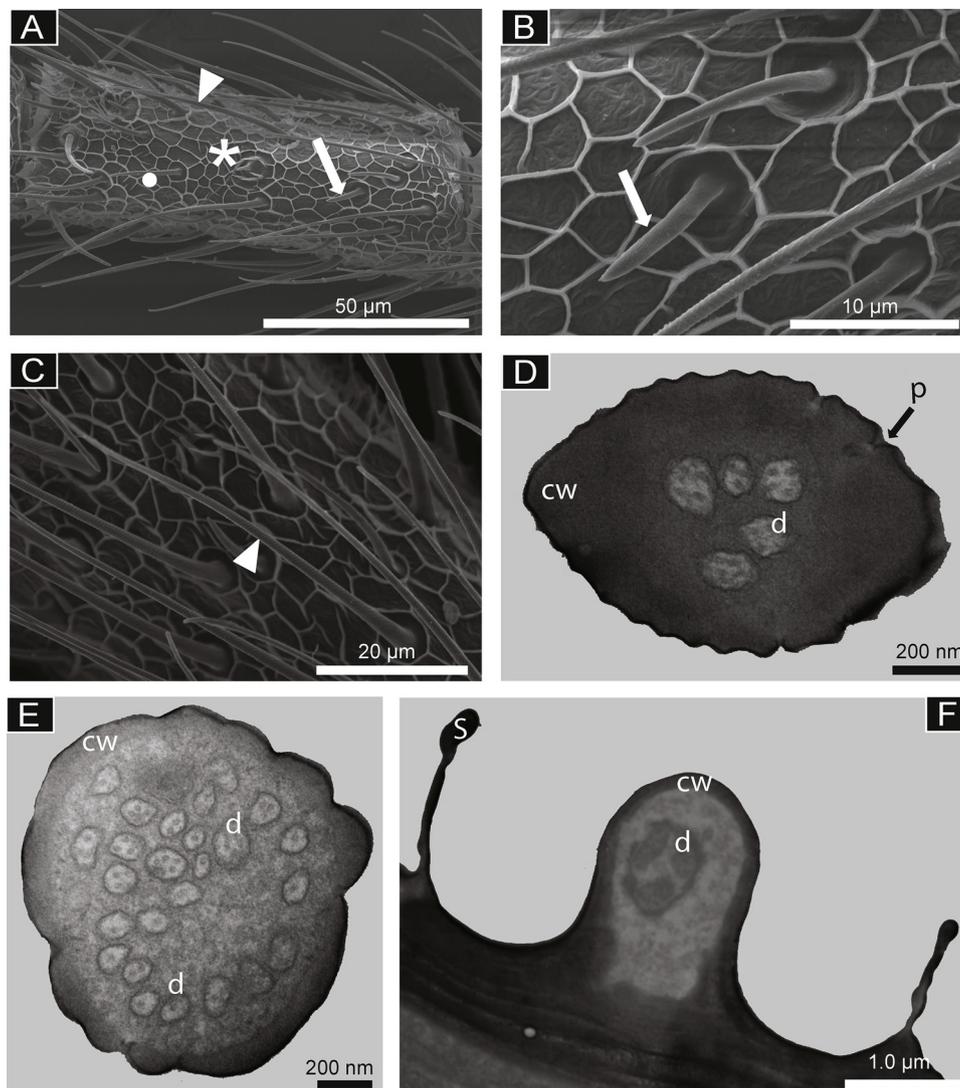


Fig. 4. Scanning and transmission electron micrographs of female *Lampronia capitella* antennae. (A) An antennal segment of *L. capitella* showing the presence of different morphological types of sensilla: basiconicum (arrow), long trichodeum (arrowhead), short trichodeum (*), and coeloconicum (*). Detailed surface morphology of (B) sensillum basiconicum and (C) trichodeum. Cross sections of different sensillum types: cw, cuticular wall; s, socket; p, pores; d, dendrites; (D) trichodeum, (E) basiconicum, and (F) coeloconicum.

viz. Glyphipterigidae and Coleophoridae, have additional olfactory sensillum types, i.e. chemoreceptor pegs and sensilla furcata, respectively (Fig. 1; Supplementary information).

R. nubila and *E. semipurpurella* use structurally similar Type 0 sex-pheromone compounds that also are similar to common plant volatile compounds (Kozlov et al., 1996; Löfstedt et al., 1994). These two species display high numbers of placoid and auricillic sensilla, respectively (Table 1; Figs. 2A and 3A). Electrophysiological recordings from sensilla auricillica of *E. semipurpurella* showed that these sensilla are involved in the detection of Type 0 pheromone compounds (Larsson et al., 2002). Considering the high number of sensilla placodea in *R. nubila*, it is possible that this sensillum type is responsible for pheromone detection. Also, the TEM micrographs showed similarities in the number of pores and dendritic projections between mushroom-like placoids and auricillic sensilla (Figs. 2G and 3B). Hence, it is possible that sensilla auricillica represent a modified form of mushroom-like placoids, and both types may be involved in the detection of structurally similar pheromone compounds (Type 0). However, in general, sensilla auricillica mostly respond to plant volatiles, including terpenes and green leaf volatiles (Supplementary information) (Ammagarahalli and

Gemeno, 2015; Anderson et al., 2000; Pophof et al., 2005). Apart from the pheromone-responsive auricillic sensilla of *E. semipurpurella*, an exception is also found in *Cydia pomonella* (Lepidoptera: Tortricidae) where two types of sensilla auricillica (called rabbit-eared shoehorn and regular shoehorn) house three OSNs each, with one of the OSNs responding to both plant odors, the major sex pheromone component, and a sex pheromone antagonist (Ansebo et al., 2005; Ebbinghaus et al., 1998). In *Spodoptera littoralis*, OR-OSN response correlations suggest that the herbivore-induced plant volatile DMNT activates SlitOR3 and 29, which are localized in sensilla auricillica (Binyameen et al., 2012; de Fouchier et al., 2017). DMNT shuts down upwind attraction of *S. littoralis* females when added to the host plant blend, and it also disrupts the attraction of *S. littoralis* males to the main pheromone compound (Hatano et al., 2015). In summary, the current physiological data suggest that sensilla auricillica have been adopted to serve important roles in both sexual communication and host finding, with the specific role being species- or sex-dependent. In addition to sensilla auricillica, OSNs located in sensilla basiconica respond to plant volatiles in a variety of species (Anderson et al., 1995, 2000; Andersson et al., 2009, 2012; Ansebo et al., 2005; Ndomo-Moualeu et al., 2014; Pophof et al., 2005)

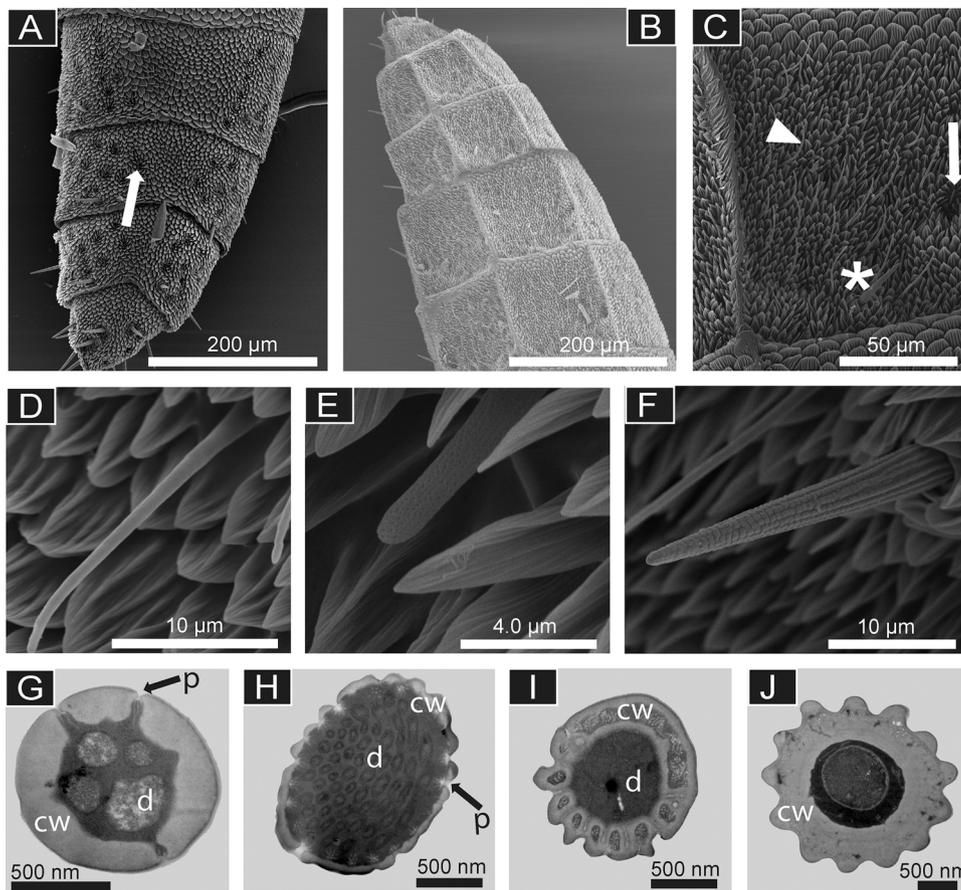


Fig. 5. Scanning and transmission electron micrographs of *Bicyclus anynana* antennae. (A) Dorsal side of the female antennal club showing hidden basiconic sensilla in scale-framed pits. (B) ventral side of the male antennal bulb showing the grooves and ridges forming segments. (C) An individual ventral segment with sensilla basiconicum (arrow), chaeticum (*) and trichodeum (arrowhead). Detailed surface structure of (D) sensillum trichodeum, (E) basiconicum, and (F) chaeticum. Cross sections of different sensillum types: cw, cuticular wall; s, socket; p, pores; d, dendrites. (G) Sensillum trichodeum, (H) basiconicum, (I) coeloconicum, and (J) chaeticum. Note: Pores connected to pore tubules in G and H.

and in *Drosophila*, OSNs in sensilla basiconica detect mainly fruit odors (de Bruyne et al., 2001).

Sensilla basiconica were found in both *B. anynana* and *L. capitella* representing the second most common sensillum type in these two species (after sensilla trichodea). However, this sensillum type was absent in *R. nubila* and *E. semipurpurella*, which is consistent with previous observations from Trichoptera and other basal Lepidoptera, although additional lepidopteran families also lack this sensillum type (Fig. 1; Supplementary information). The density of sensilla basiconica appears higher on the antennae of female *L. capitella* compared to the antennae of males, which is similar to previous findings in *Plodia interpunctella* and *Homoeosoma nebulella* (Faucheux, 1991; Ndomo-Moualeu et al., 2014). This possibly reflects the common role of basiconic sensilla to detect host plant cues, mostly relevant for females.

Sensilla trichodea are present in high numbers in the lepidopteran species that use sex pheromones other than Type 0, including *L. capitella* (using Type 1 pheromone) analyzed here (Table 1; Supplementary information). This is probably because this sensillum type is important for the detection of these more recent sex pheromone types (Ansebo et al., 2005; Hallberg et al., 1994; Jungberg et al., 1993; Pophof et al., 2005). Sensillum trichodeum was the most abundant type found in *L. capitella*, but also in *B. anynana*, in which potential pheromone detection and the function of trichoid sensilla are still unknown (Table 1). In many species, sensilla trichodea are differentiated into 2–3 subtypes according to size. For instance, in *L. capitella* we found two subtypes of sensilla trichodea. Several studies have shown that sex pheromone detecting OSNs are located in the long trichoids of males (Ebbinghaus et al., 1998; Hansson et al., 1995; Mochizuki et al., 1992). In some lepidopterans, e.g. *Synanthedon scitula* (Sesiidae) and *Plutella xylostella* (Plutellidae), females lack long trichoid sensilla, but have a larger number of medium sensilla trichodea than the males (Frank et al.,

2010; Wee et al., 2016). In *Bombyx mori*, the antennae of both sexes have abundant long sensilla trichodea, but only in males do these sensilla house OSNs that respond to the pheromone compounds bombykol or bombykal (Boeckh et al., 1965). In the females, one OSN in these trichoids is sensitive to benzoic acid, and the other to both 2,6-dimethyl-5-hepten-2-ol and linalool (Heinbockel and Kaissling, 1996; Priesner, 1979). Hence, OSNs located in the same type of sensilla may be tuned to different stimuli based on the needs of a particular sex or species. In *Heliothis subflexa* and *Heliothis virescens*, the shorter trichoids host OSNs tuned to minor pheromone components, whereas longer trichoids contain OSNs tuned to major pheromone components (Baker et al., 2004). At least in moths, the abundance of a particular type of sensillum may relate to its importance in detection of certain ecologically relevant odors. Baker et al. (2012) suggested that the relative abundances of differently tuned pheromone-responsive OSNs are not adaptations to maximize the sensitivity to an individual compound, but instead selected to accurately report the ranges of odor flux for each component in the pheromone plume. Pheromones are also detected by trichoid sensilla in *D. melanogaster* (Dweck et al., 2015; Kurtovic et al., 2007). On the other hand, in the swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae), sensilla circumfila are involved in the detection of sex pheromone, whereas no pheromone responses were recorded from its trichoid sensilla (Boddum et al., 2010). However, sex pheromone responses were recorded from trichoid sensilla, but not from sensilla circumfila, in another cecidomyiid (the Hessian fly, *Mayetiola destructor*) (Boddum et al., 2010), suggesting that the occurrence of pheromone responding OSNs might not necessarily be tightly linked to sensillum morphology.

In conclusion, *R. nubila* and *E. semipurpurella* (both using Type 0 pheromone) antennae house a large proportion of placoid sensilla of different types. The antennae of *L. capitella*, a species using a Type I sex

pheromone, resemble the antennae of the more derived ditrysian moths, with large numbers of sensilla trichodea. These results suggest a major shift in olfactory sensillum types between Trichoptera/the basal Lepidoptera using Type 0 pheromones and the more derived Lepidoptera using other pheromone types. Whether or not this pattern represents adaptations to newly evolved sex pheromone types remains to be further investigated, although a definite answer might be difficult to obtain due to the absence of Type 0 pheromones in derived Lepidoptera, and the absence of Type I pheromones among the most basal lineages. Future studies on additional species' sensillum morphology and their associated physiological responses are required to better understand these aspects of the insect olfactory system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.micron.2018.05.006>.

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