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Research paper

Morphological comparison of proboscis sensilla between Plusiinae and Noctuinae (Lepidoptera: Noctuidae)

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ABSTRACT

In higher Lepidoptera the proboscis and its sensilla vary from species to species in different feeding guilds. However, whether such morphological differences have a phylogenetic significance remains unclear. Here we compared the morphology of proboscis sensilla in 19 representative species of Plusiinae and four of Noctuinae using scanning electron microscopy. In Plusiinae four types of sensilla (sensilla chaetica, sensilla basiconica, sensilla styloconica, and sensilla coeloconica) were present on the proboscis, with cuticular processes only in the proximal region. In Noctuinae three types of sensilla (sensilla chaetica, sensilla basiconica, and sensilla styloconica) were found on the proboscis, with cuticular processes on its whole length. Morphological differences of proboscis sensilla between Plusiinae and Noctuinae mainly involve the shape, number, and length of sensilla styloconica and sensilla chaetica. The sensilla styloconica of Plusiinae are significantly shorter and fewer than those of Noctuinae. The styli of sensilla styloconica are glabrous in Plusiinae and are longitudinally ribbed in Noctuinae. These significant morphological differences are useful in distinguishing the two subfamilies, suggesting that proboscis sensilla may provide useful characters in the systematic and phylogenetic analyses at the subfamily level within Noctuidae.

1. Introduction

Higher Lepidoptera typically have siphoning mouthparts, with their proboscis consisting of a complex fluidic system derived from two extremely elongated galeae. The proboscis functions like a drinking straw through which floral nectar and other liquid substances are imbibed (Scoble, 1992; Kingsolver and Daniel, 1995; Krenn, 2010; Monaenkova et al., 2012). Each galea bears various types of sensilla, which play important roles in feeding activities of Lepidoptera (Salama et al., 1984; Krenn et al., 2005; Krenn, 2010; Faucheux, 2013; Lehnert et al., 2013; Zaspel et al., 2013). Proboscis sensilla exhibit remarkable differences with regard to their type, morphology, location and number, and are associated mainly with the feeding habits of their owners (Krenn et al., 2001; Krenn, 2010; Krenn and Aspöck, 2012; Zenker et al., 2011; Faucheux, 2013; Lehnert et al., 2016). Previous investigations of Lepidoptera proboscis sensilla are mainly concentrated on Nymphalidae, Riodinidae, Arctiidae, Erebidae, Pyralidae, Geometridae, Saturniidae, and Noctuidae (Faucheux, 1991; Büttiker et al., 1996; Paulus and Krenn, 1996; Krenn, 1998; Krenn and Penz, 1998; Walters et al., 1998; Krenn et al., 2001; Zaspel et al., 2011; Bauder et al., 2013). However, whether these morphological differences of sensilla have a phylogenetic significance remains unclear.

Noctuidae is the largest family in Lepidoptera with numerous agricultural pests of great economic significance (Zahiri et al., 2010). The classification of Noctuidae has recently undergone major shifts and rearrangements, especially at the subfamily level (Speidel et al., 1996; Lafontaine and Fibiger 2006; Mitchell et al., 2006). Plusiinae, a small subfamily of Noctuidae, is regarded as monophyletic based on considerable morphological, bionomical and biogeographical evidence (Kitching, 1987; Ronkay et al., 2008), and may eventually be raised to a family status (Weller et al., 1994).

In this paper, we compared the morphology of the proboscis and its sensilla in 19 representative species of Plusiinae and four of Noctuinae using scanning electron microscopy in order to prove the suitability of characters regarding proboscis sensilla for phylogenetic differentiation of Plusiinae and Noctuinae.

2. Materials and methods

2.1. Insect collection and preparation

Male and female individuals of 23 species from two subfamilies of Noctuidae (19 species in 17 genera of Plusiinae and four species in four genera of Noctuinae) were examined in this study (Table 1). The moths were obtained from collections in the Entomological Museum, Northwest A&F University, China. Dried proboscises were removed at the base of the head from pinned specimens using fine-tip forceps.

2.2. Scanning electron microscopy

For scanning electron microscopy (SEM), proboscises were cleaned with an ultrasonic cleaner in 75% ethanol for 30 s. After dehydration in a graded ethanol series, the samples were freeze-dried in

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Table 1
Information for specimens examined.

Subfamilies	Species	Localities
Plusiinae	*Anadevidia peponis* (Fabricius, 1775)	Henan
	Antoculeora locuples (Obethür, 1880)	Shaanxi
	Autographa excelsa (Kretschmar, 1862)	Shaanxi
	Cornutiplusia circumflexa (Linnaeus, 1767)	Xinjiang
	Diachrysia chrysitis (Linnaeus, 1758)	Xinjiang
	Diachrysia leonine (Obethür, 1884)	Jilin
	Diachrysia stenochrysis (Warren, 1913)	Hebei
	Diachrysia oberthueri (Ronkay, Ronkay and Behounek, 2008)	Gansu
	Erythroplusia rutilifrons (Walker, 1858)	Sichuan
	Euchalcia inconspicua (Graeser,1892)	Xinjiang
	Macdunnoughia confusa (Stephens, 1850)	Xinjiang
	Panchrysia ornate (Bremer, 1864)	Gansu
	Panchrysia tibetensis (Chou and Lu, 1982)	Yunnan
	Plusidia cheiranthi abrostoloides (Butler, 1879)	Liaoning
	Plusia festucae (Linnaeus, 1758)	Jiangsu
	Plusia putnami festata (Graeser, 1890)	Heilongjiang
	Polychrysia esmeralda (Oberthür, 1880)	Qinghai
	Polychrysia imperatrix (Draudt, 1950)	Yunnan
	Stigmoctenoplusia aeneofusa (Hampson, 1894)	Yunnan
	Syngrapha ain persibirica (Ronkay, Ronkay and Behounek, 2008)	Jilin
	Thysanoplusia intermixta (Warren, 1913)	Shaanxi
	Thysanoplusia orichalcea (Fabricius, 1775)	Shaanxi
	Trichoplusia ni (Hübner, 1803)	Shaanxi
	Zonoplusia ochreata (Walker, 1865)	Yunnan
Noctuinae	*Anaplectoides virens* (Butler, 1878)	Shaanxi
	Axylia putris (Linnaens, 1761)	Shaanxi
	Euxoa sibirica (Boisduval, 1837)	Shaanxi
	Xestia agalma (Pungeler, 1899)	Shaanxi

tertiary butanol. They were then mounted onto SEM stubs using a double graphite adhesive tape, coated with gold in a sputter coater, and viewed in a Hitachi S-3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 15 kV.

2.3. Terminology

Terminology for proboscis morphology follows Shields (2008) and Faucheux (2013). In order to avoid ambiguity, we explain here the following less-widely used terms as we use in this paper:

Tip region: the hydrophilic region of the proboscis (Lehnert et al., 2013), beginning by rows of intake slits leading into the food canal (Krenn, 1998; Lehnert et al., 2013).

Stylus shoulders: the distal part of the stylus near the sensory peg (Petr and Stewart, 2004).

2.4. Measurements and statistical analyses

Proboscises and sensilla were measured and counted for 30 individuals of each species (15 males and 15 females). The lengths of proboscis, sensilla chaetica, external sensilla basiconica, internal sensilla basiconica, and sensilla styloconica were measured using a spreading sheet and the Imaris 7.2.3 software. The mean number and standard deviation were analyzed using the Predictive Analytics Software Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test ($\alpha = 0.05$) was used to compare measurements between Plusiinae and Noctuinae with software SPSS 20.0.

3. Results

3.1. General morphology of the noctuid proboscis

The mouthparts of adult Plusiinae and Noctuinae are of the typical siphoning type, consisting of two extremely elongated galeae, which are interlocked to form a sucking tube. In the resting position, the proboscis is coiled in a tight spiral beneath the head between the setose three-segmented labial palps. The outer surface of proboscis is covered with cuticular processes and various types of sensilla. Sexual dimorphism was not detected in the proboscis of any species.

3.2. Proboscis sensilla of Plusiinae

The sensilla equipment of the 19 species of Plusiinae examined is the same in all species, all of which bear four morphological types of sensilla on the galeae: bristle-shaped sensilla chaetica, uniporous sensilla basiconica, glabrous sensilla styloconica, and particular sensilla coeloconica (Fig. 1). Shapes, lengths, numbers as well as distributions of these sensilla do not differ significantly among species of Plusiinae (Figs. 5 and 6), therefore we use *Anadevidia peponis* as the representative of this subfamily.

The proboscis length of *A. peponis* is 11.87 ± 2.25 mm (10.73–12.84 mm, $n = 30$). The proboscis can be divided into the proximal, bend, distal, and tip regions. When uncoiled, the proximal region makes up 35% of the total length; the bend region comprises 12% of the total length; the distal region constitutes 41%; and the tip region, where fluids are taken up into the food canal, comprises 12% of the total length.

The proximal region of proboscis is covered with hair-like cuticular processes (Fig. 1A and B). The bend, distal, and tip regions are devoid of cuticular processes. All the four types of sensilla are present on the tip region, of which the sensilla chaetica and sensilla basiconica are rare and scattered, and the sensilla chaetica are the shortest next to sensilla basiconica.

3.2.1. Sensilla chaetica

Sensilla chaetica are more frequent and longer on the proximal region than on the bend, distal, and tip regions of the proboscis (Fig. 1A), with a mean number of 65.90 ± 2.71 ($n = 30$) per galea. Sensilla chaetica each possess an aporous sensory bristle with a length of 91.47 ± 3.24 µm ($n = 30$), extending from a collared socket (Fig. 1B). Sensilla chaetica are rare on the tip region.

3.2.2. Sensilla basiconica

External sensilla basiconica each have a uniporous sensory cone in a length of 3.97 ± 0.16 µm ($n = 30$) and protrude from a circular and sometimes slightly elevated area of the wall on the external surface of proboscis (Fig. 1C). The mean number of sensilla is 23.97 ± 1.36 ($n = 30$) per galea.

The shape of internal sensilla basiconica is similar to that of the external sensilla basiconica (Fig. 1D). The mean length of the sensilla was 4.21 ± 0.28 µm ($n = 30$). With a mean number of 10.83 ± 0.16 ($n = 30$) per galea, sensilla basiconica are rare in the food canal.

3.2.3. Sensilla styloconica

Sensilla styloconica are confined to the tip region of proboscis and are generally composed of an elongated stylus and a short sensory cone that protrudes from the apex of stylus. The stylus is cylindrical, glabrous, and is devoid of longitudinal ribs. The shoulders are completely smooth, without apical shoulder spines. The sensory cone, lo-

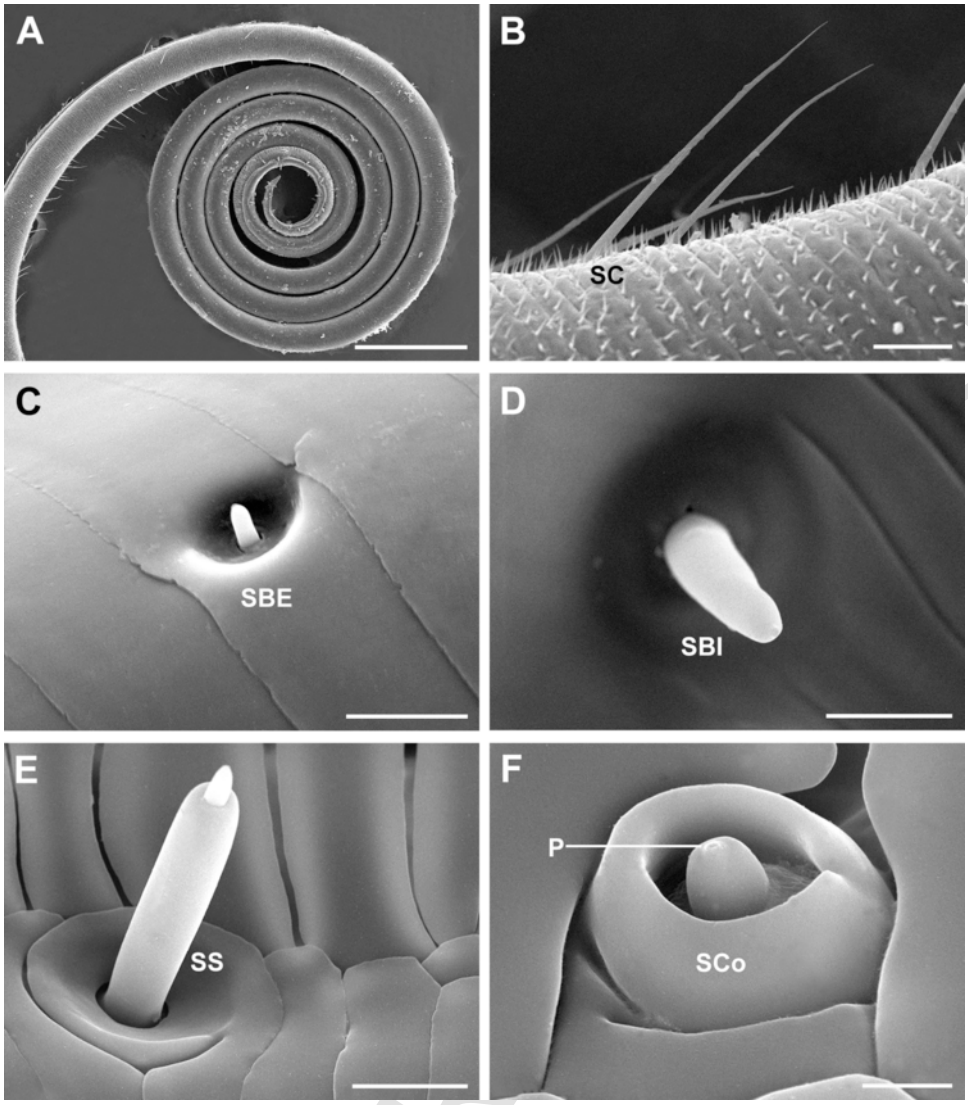

Fig 1. Proboscis sensilla of *A. peponis* (Plusiinae). (A) The proboscis. (B) Sensilla chaetica on the proximal region of the proboscis. (C) Magnification of an external sensillum basiconicum (SBE). (D) An internal sensillum basiconicum (SBI) in the food canal. (E) A glabrous sensillum styloconicum (SS) on the tip region. (F) A sensillum coeloconicum (SCo) on the tip region and the pore (P) on the sensillum. Scale bars: (A) = 500 µm; (B) = 30 µm; (C) and (E) = 10 µm; (D) = 5 µm; (F) = 2 µm.

cated centrally at the apex of stylus, is not tilted away from the longitudinal axis of stylus (Fig. 1E).

Each galea possesses 14.07 ± 1.05 (13–17, $n = 30$) sensilla styloconica. The total length of the sensilla is 19.60 ± 2.18 µm (16.32–23.98 µm, $n = 30$). The length of the sensory cone is 3.01 ± 0.02 µm (2.97–3.04 µm, $n = 30$).

3.2.4. Sensilla coeloconica

The sensillum coeloconicum has a glabrous sensory cone, 1.0–1.9 µm in diameter, located in a deep cavity of the galeal cuticle. The cavity is ring-shaped with a small nick. The external diameter of the cavity varies from 6 to 8 µm. The sensory cone hardly reaches the top level of the cavity. A terminal pore is visible on the sensory cone (Fig. 1F).

Sensilla coeloconica are confined to the tip region of proboscis, next to sensilla styloconica. Each galea bears one longitudinal row of sensilla coeloconica, 11.83 ± 1.28 ($n = 30$) in number.

3.2.5. Pollen grains on the proboscis

Various kinds of pollen grains were found on the tip region next to sensilla styloconica of the proboscises of *Antoculeora locuples*, *Autographa excels*, *Erythroplusia rutilifrons*, and *Diachrysia stenochrysitis* (Fig. 2), indicating that these moths are flower-visiting species.

3.3. Proboscis sensilla of Noctuinae

The sensilla equipment is very similar among *Anaplectoides virens*, *Axylia putris*, *Euxoa sibirica*, and *Xestia agalma*, with three morphological types of sensilla on the galea: bristle-shaped sensilla chaetica, uniporous sensilla basiconica, and prominent sensilla styloconica (Figs. 3 and 4). Shapes, lengths, numbers and distributions of sensilla differ insignificantly among the species of Noctuinae (Figs. 5 and 6), therefore we use *A. virens* as the representative of this subfamily.

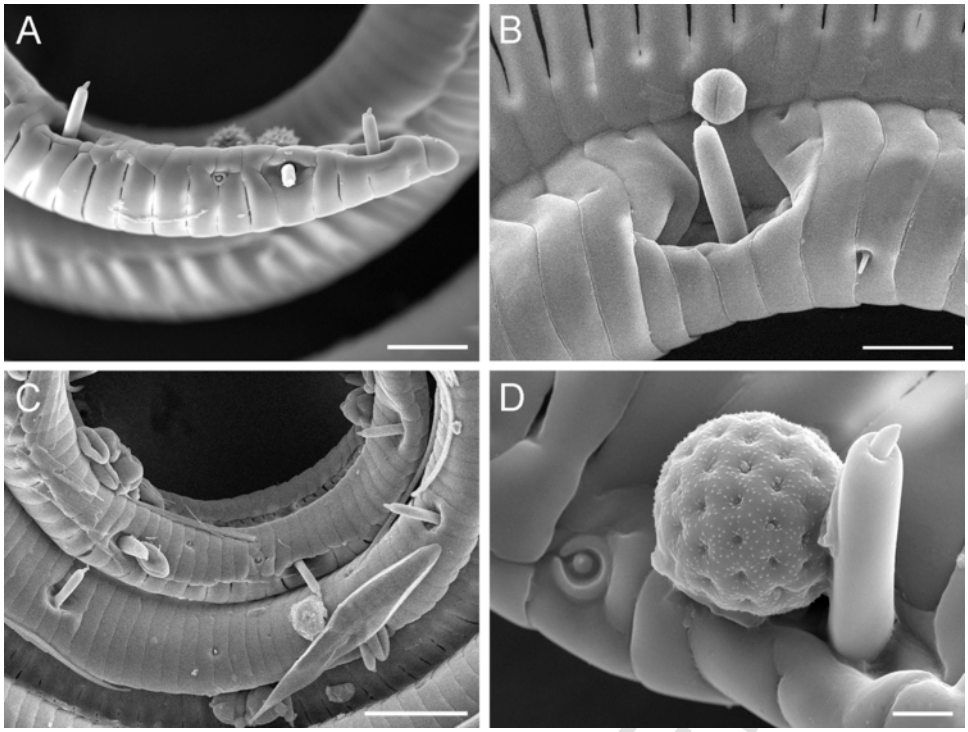

Fig. 2. Proboscises of Plusiinae that have pollen grains on the tip region. (A) *Antoculeora locuples*. (B) *Autographa excels*. (C) *Erythroplusia rutilifrons*. (D) *Diachrysia stenochrysitis*. Scale bars: (A) = 30 μm; (B) = 20 μm; (C) = 50 μm; (D) = 5 μm.

The proboscis length of *A. virens* is 12.65 ± 3.28 mm (10.35–14.96 mm, $n = 30$). The proboscis can be divided into the proximal, bend, distal, and tip regions. When uncoiled, the proximal region makes up 38% of the total length; the bend region comprises 10% of the total length; the distal region constitutes 39%; and the tip region comprises 13% of the total length.

The outer surface of proboscis is fluted with cuticular processes, whose shape varies in different regions of the proboscis. The cuticular processes are hair-like in the proximal and bend regions (Fig. 3A), but shorter and more spine-like toward the distal and tip regions (Fig. 3B and C).

Different types of sensilla are located on different regions of the proboscis: sensilla chaetica are present on the external surface; external sensilla basiconica occur on the external surface; internal sensilla basiconica exist on the inner wall of the food canal; and sensilla styloconica are confined to the tip region.

3.3.1. Sensilla chaetica

Sensilla chaetica are the most numerous type of sensilla on the proboscis of *A. virens*, with a mean number of 205.50 ± 9.86 ($n = 30$) per galea. Each sensillum chaeticum has an aporous sensory bristle extending from a collared socket and possesses a grooved surface (Fig. 3B). Each bristle is inserted into a distinct sunken socket, protruding at an angle of 30°–45° from the surface on the proximal and bend regions of the proboscis. They tend to be upright toward the tip region (Fig. 3B and C). Bristles vary greatly in length (2.88–139.64 μm) and are longer on the ventral side than on the dorsal side of the proboscis (Fig. 3D). They are the longest on the ventral side of proximal region (128.46 ± 3.40 μm), becoming gradually shorter and being the shortest in the tip region (3.40 ± 0.17 μm).

3.3.2. Sensilla basiconica

Sensilla basiconica occur on both the external and internal surfaces of the galea (Fig. 4A and B) and have a short blunt-tipped sensory cone, which bears a single terminal pore and a short dome-shaped socket. External sensilla basiconica are arranged in irregular rows throughout the dorsal and lateral sides of the proboscis, while internal sensilla basiconica form a single row in the food canal on each galea.

3.3.3. Sensilla styloconica

Sensilla styloconica are concentrated on the tip region of the proboscis, with each sensillum consisting of a stout basal stylus and a short apical sensory cone. Their styli possess six or seven smooth-edged longitudinal ribs separated from each other by furrows or flutes (Fig. 4C and D). The sensilla styloconica with six longitudinal ribs are more abundant than those with seven. The gaps between two adjacent cuticular processes are directly in the face of the ribs of the stylus (Fig. 4C). From the basal to middle part of the tip region, sensilla styloconica are arranged in three rows and then become scattered toward the apex. On the dorsolateral side of the tip region, sensilla styloconica are widely spaced.

3.4. Comparisons of proboscises and their sensilla between Plusiinae and Noctuinae

Considerable morphological differences of proboscises and their sensilla between Plusiinae and Noctuinae mainly concern distributions and shapes of cuticular processes, types of sensilla, forms, numbers, and lengths of sensilla styloconica, numbers and lengths of sensilla chaetica, and numbers of external and internal sensilla basiconica (Figs. 5 and 6; Table 2). No distinct differences were found between species within each of the two subfamilies (Figs. 5 and 6).

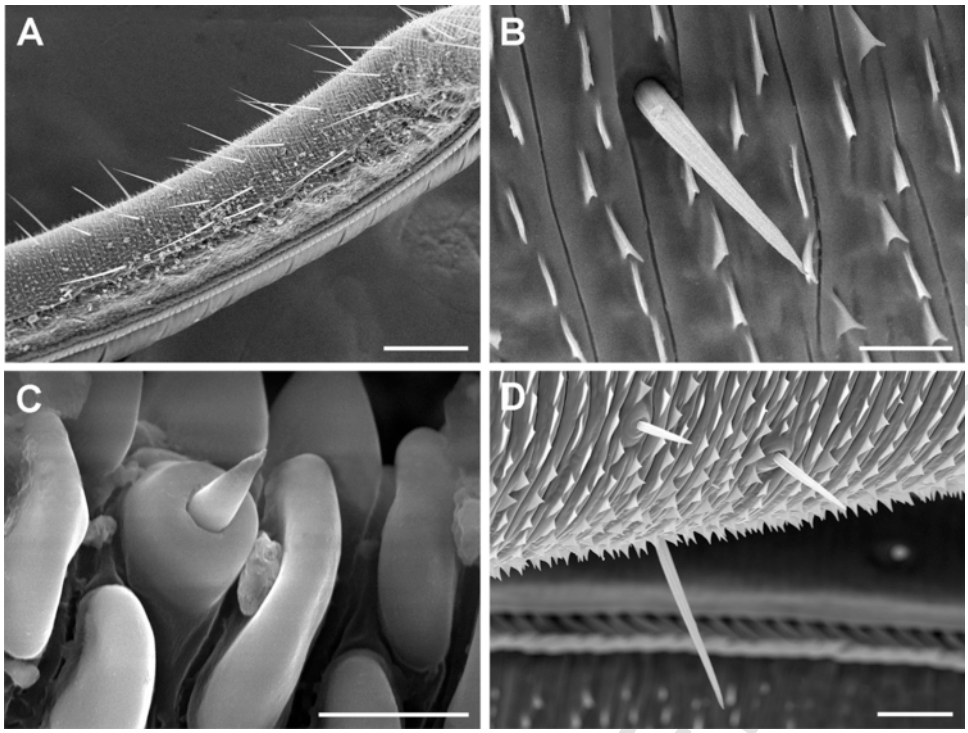

Fig. 3. Sensilla chaetica of *A. virens* (Noctuinae). (A)–(C) Sensilla chaetica on the proximal, bend, and distal region of the proboscis. (D) Sensilla chaetica (SC) on the bend region of the proboscis, showing that the sensilla chaetica on the ventral side are longer than those on the dorsal side. Scale bars: (A) = 100 µm; (B) and (D) = 20 µm; (C) = 3 µm.

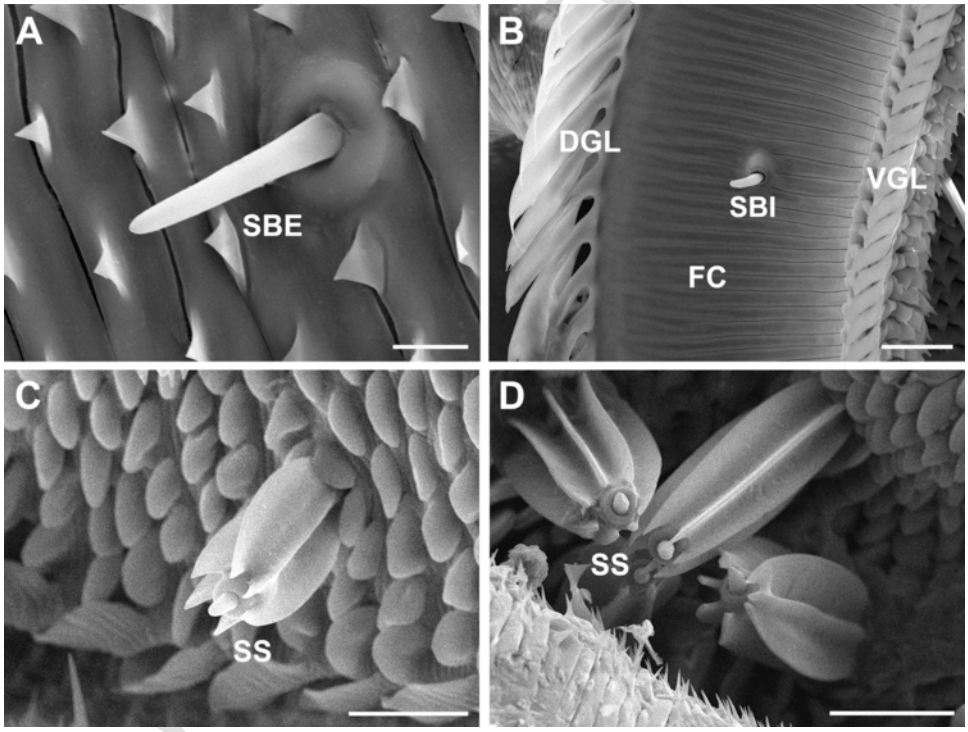

Fig. 4. Sensilla basiconica and sensilla styloconica of *A. virens* (Noctuinae). (A) Magnification of an external sensillum basiconicum (SBE). (B) Inner view of proboscis, showing that the food canal (FC) is interlocked by dorsal (DGL) and ventral galeal linkages (VGL) and featuring an internal sensillum basiconicum (SBI) in it. (C) A sensillum styloconicum with six longitudinal ribs on the tip region. (D) Sensilla styloconica with seven longitudinal ribs on the tip region. Scale bars: (A) = 5 µm; (B) and (D) = 20 µm; (C) = 15 µm.

Sensilla chaetica on the proximal region of proboscis are significantly shorter in Plusiinae than in Noctuinae (Fig. 5A; Table 2). External sensilla basiconica, internal sensilla basiconica and sensilla sty-

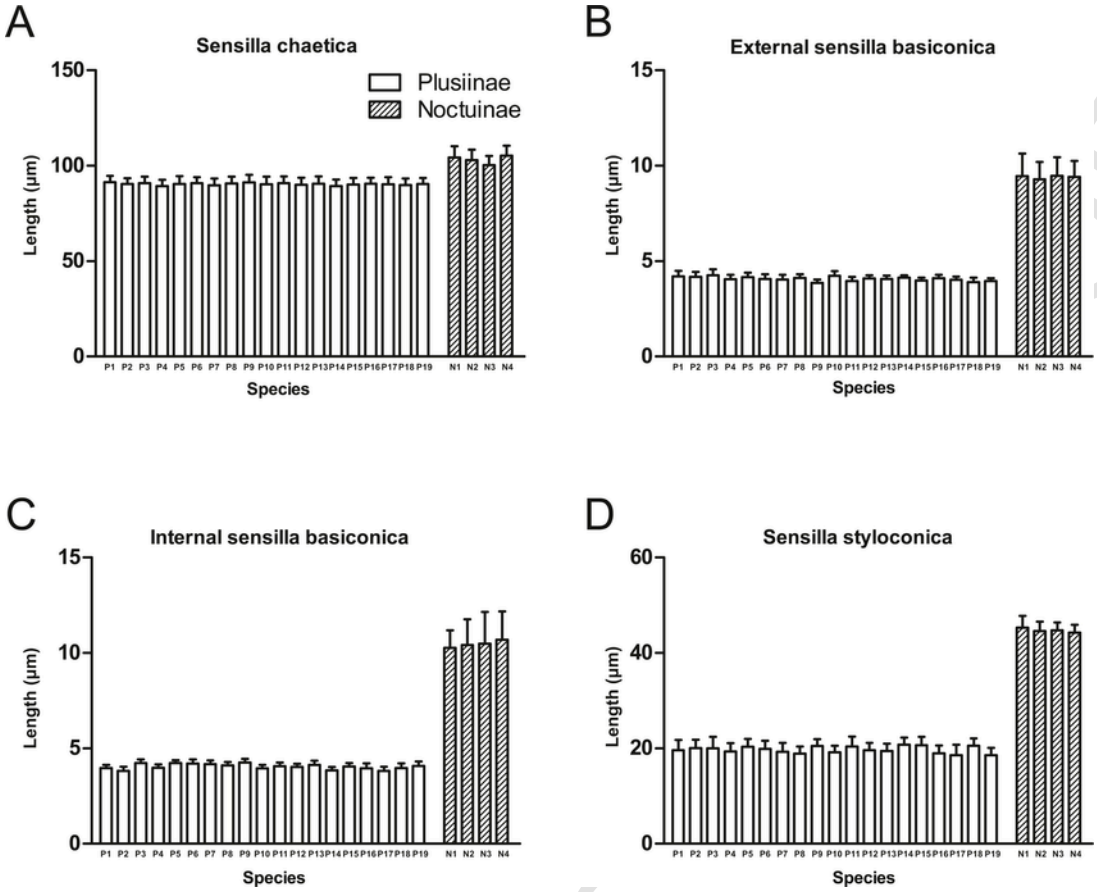

Fig. 5. Mean lengths of proboscis sensilla of Plusiinae and Noctuinae. $n = 30$ for each species investigated. (A)–(D) Mean lengths of sensilla chaetica on the proximal region, external sensilla basiconica, internal sensilla basiconica and sensilla styloconica of species in Plusiinae and Noctuinae. P1: *A. peponis*; P2: *A. locuples*; P3: *A. excels*; P4: *C. circumflexa*; P5: *D. chrysitis*; P6: *D. stenochrysis*; P7: *E. rutilifrons*; P8: *E. inconspicua*; P9: *M. confusa*; P10: *P. ornate*; P11: *P. cheiranthi*; P12: *P. festucae*; P13: *P. esmeralda*; P14: *S. aeneofusa*; P15: *S. ain persibirica*; P16: *T. intermixta*; P17: *T. orichalcea*; P18: *T. ni*; P19: *Z. ochreata*; N1: *A. virens*; N2: *A. putris*; N3: *E. sibirica*; N4: *X. agalma*.

loconica on the proboscis of Plusiinae are significantly shorter than those of Noctuinae (Fig. 5B–D; Table 2).

The number of sensilla chaetica, external sensilla basiconica, internal sensilla basiconica, and sensilla styloconica on the proboscis of Plusiinae are significantly fewer than those of Noctuinae (Fig. 6; Table 2). Sensilla coeloconica are present only on the proboscis of Plusiinae.

4. Discussion

4.1. Morphological diversity of the proboscis and its sensilla

The morphology of proboscis and its sensilla differs significantly between Plusiinae and Noctuinae, but not among genera and species within the same subfamily. This implies that the sensilla may provide useful characters in the phylogenetic analysis of Noctuidae at the subfamily level, but less valuable at the generic and species levels.

The proboscis of higher Lepidoptera may bear six morphological types of sensilla: chaeticum, basiconicum, styloconicum, coeloconicum, filiformium, and campaniformium, with the first three types most common (Krenn, 1998, 2010; Faucheux, 2013). Based on our present study, Plusiinae bear four types of sensilla on their proboscis, while Noctuinae bear only three types of sensilla. Both subfamilies bear sensilla chaetica, sensilla basiconica, and sensilla styloconica on their proboscises, showing that they are the common types of sensilla. The tip region of Noctuinae proboscis is characterized by numerous sensilla styloconica with six or seven longitudinal ribs, suggesting that Noctuinae are typical nectar-feeding species, as in the nectar-feeding *Noctua pronuba* (Büttiker et al., 1996). Plusiinae are also nectar-feeders because pollen grains are found on their proboscises (Fig. 2).

Based on our study, all the 19 species in 17 genera of Plusiinae have sensilla coeloconica on their proboscises. Sensilla coeloconica were also discovered on the proboscis of the sphingids *Acherontia atropos*, *Macroglossum stellatarum*, *Sphinx ligustri*, *Hemaris fuciformis*, *Hyles livornica*, and *Proserpinus proserpina* (Faucheux, 2013). However, our study was focused on the external morphology, and does not give an unambiguous clue to the biological role and function of the sensillum.

Sensilla styloconica are composed of variously-shaped styli and relatively shorter terminal sensory cones, and are the most remarkable sensilla on the proboscis (Krenn, 1998, 2010; Petr and Stewart, 2004). The styli can be divided into two groups: either completely smooth and ellipsoid or sculptured with longitudinal ribs and stellate (Faucheux, 1991; Krenn, 1998; Krenn and Penz, 1998; Petr and Stewart, 2004; Zaspel et al., 2011). Based on our present study, the styli differ greatly in shape between Plusiinae and Noctuinae. The styli are completely smooth in Plusiinae species, as in the sphingid *Acherontia atropos* (Faucheux, 2013) and the pyralids *Hypolimnas misippus* and *Junonia coenia* (Petr and Stewart, 2004). The styli are flattened and paddle-like, with the sensory cone situated off centre in

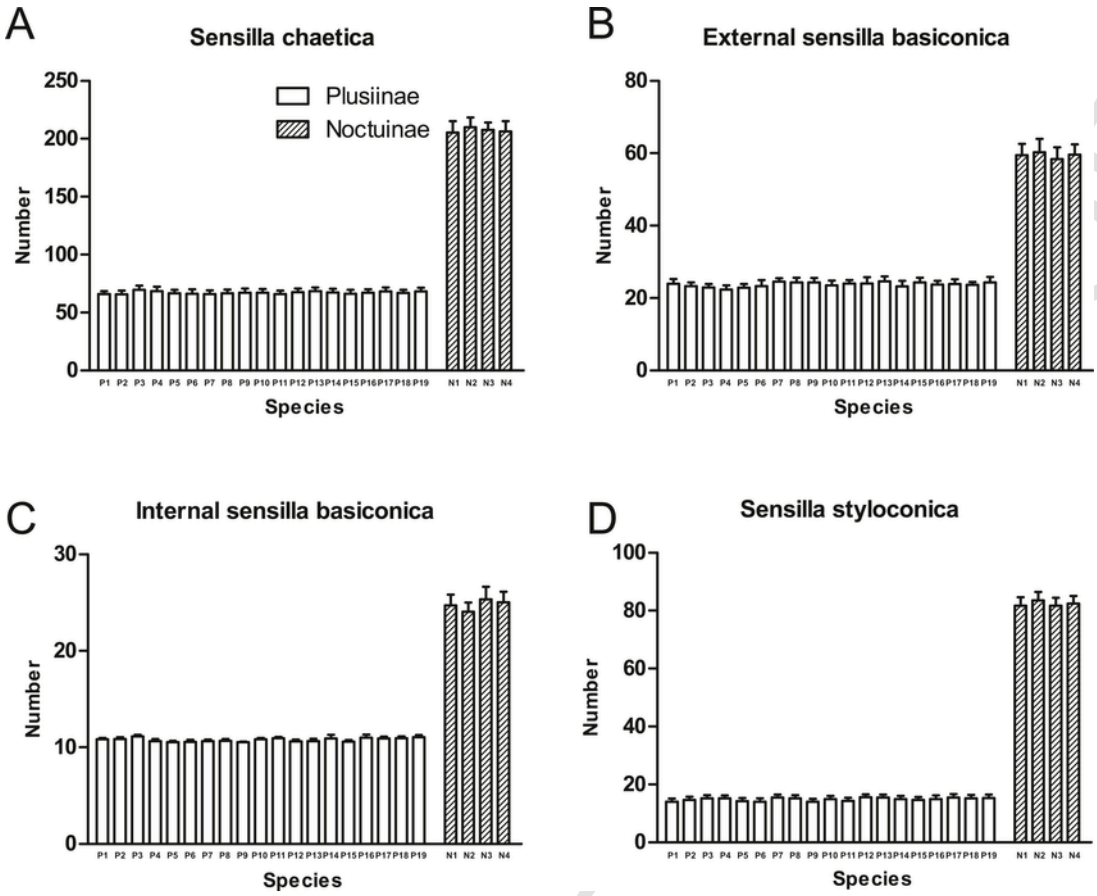

Fig. 6. Mean numbers of proboscis sensilla of Plusiinae and Noctuinae. $n = 30$ for each species investigated. (A)–(D) Mean numbers of sensilla chaetica, external sensilla basiconica, internal sensilla basiconica and sensilla styloconica on the proboscis of Plusiinae and Noctuinae. P1: *A. peponis*; P2: *A. locuples*; P3: *A. excels*; P4: *C. circumflexa*; P5: *D. chrysitis*; P6: *D. stenochrysis*; P7: *E. rutilifrons*; P8: *E. inconspicua*; P9: *M. confusa*; P10: *P. ornate*; P11: *P. cheiranthi*; P12: *P. festucae*; P13: *P. esmeralda*; P14: *S. aeneofusa*; P15: *S. ain persibirica*; P16: *T. intermixta*; P17: *T. orichalcea*; P18: *T. ni*; P19: *Z. ochreata*; N1: *A. virens*; N2: *A. putris*; N3: *E. sibirica*; N4: *X. agalma*.

Table 2
Differences of proboscis sensilla between Plusiinae and Noctuinae.

Sensilla		Plusiinae	Noctuinae	*t*-test	*t*-value
S. chaetica	Length (μm)	90.39 ± 3.45	103.21 ± 4.87	***	−27.414
	Number	67.21 ± 3.28	205.83 ± 8.26	***	−180.897
External s. basiconica	Length (μm)	4.05 ± 0.15	9.31 ± 0.44	***	−129.233
	Number	23.71 ± 1.84	59.45 ± 3.05	***	−123.886
Internal s. basiconica	Length (μm)	4.07 ± 0.12	10.71 ± 0.56	***	−129.954
	Number	10.79 ± 1.01	24.78 ± 1.47	***	−99.163
S. styloconica	Length (μm)	20.01 ± 2.78	44.46 ± 2.46	***	−89.312
	Number	14.99 ± 1.41	82.33 ± 3.01	***	−239.387
S. coeloconica	Number	12.17 ± 1.14	–	–	–

Data are presented as Mean ± SD. $n = 570$ in Plusiinae, $n = 120$ in Noctuinae.

*** $p < 0.001$ for student's *t*-test of significant difference of parameter value from 0.

H. misippus and *J. coenia* (Petr and Stewart, 2004). The sensory cone, however, is located centrally at the apex of stylus in Plusiinae.

On the contrary, the styli of Noctuinae bear longitudinal ribs, similar to those of *Choristoneura fumifernana* (Walters et al., 1998), *Helicoverpa armigera* and *H. assulta* (Wang et al., 2012), *Hypocala rostrata*, and *Eublemma pyrochroa* (Büttiker et al., 1996). The number of longitudinal ribs varies among different species. In *C. fumifernana* the sensillum styloconicum consists of a single sensory peg inserted at the top of the stylus, which is equipped with 6–9 ribs extending from the base to the sensory peg socket (Walters et al., 1998). The stylus bears six longitudinal ribs in *H. armigera* and *H. assulta* (Wang et al., 2012), and six to eight ribs in *H. rostrata* (Büttiker et al., 1996). In *E. pyrochroa* the sensilla styloconica are arranged in two rows with their styli compressed and bearing four or five longitudinal ribs (Büttiker et al., 1996). The styli of sensilla styloconica have six or seven longitudinal ribs in Noctuinae (Büttiker et al., 1996; Xue and Hua, 2014). The longitudinally-ribbed styli are likely an adaptation to holding up more pollen during visiting flowers.

The number of sensilla styloconica varies among different species, e.g. nine sensilla per galea in the pyralid *Homoeosoma electellum*, and 15 sensilla per galea in the oecophorid *Hofmannophila pseudospretella* (Faucheux, 1991, 2013). Their number even varies between the sexes of *A. bubo*: 76 sensilla per galea in the male, and 71 in the female (Altner and Altner, 1986). The number may also be associated with the feeding habits. Lacryphagous species have relatively fewer sensilla styloconica than the nectar-feeding species (Büttiker et al., 1996). *Heliothis virescens* and *Spodoptera littoralis* possess 60 and 130 sensilla styloconica, respectively, on the proboscis (Blaney and Simmonds, 1988). Based on our present study, Noctuinae possess more than 80 sensilla styloconica per galea, while Plusiinae possess only approximately 10 sensilla styloconica per galea.

The terms sensillum chaeticum and sensillum trichodeum are often used interchangeably in the literature (Krenn, 2010; Faucheux, 2013; Xue and Hua, 2014). Sensilla chaetica are generally regarded as mechanosensitive on the basis of ultrastructural studies (Krenn, 1998). Bristle-shaped sensilla on the proboscis may provide information on the diameters of the corolla and the depth of proboscis insertion during probing flowers (Krenn, 1998, 2010; Krenn and Penz, 1998; Krenn et al., 2005). Sensilla chaetica become shorter toward the tip so that they can assess the width and depth of the tube without blocking the entrance for the proboscis during feeding (Krenn, 1998; 2010).

Sensilla chaetica are different among species mainly in length and number. The longest sensillum chaeticum is likely 111.96 µm in flower-visiting and 214.00 µm in non-flower-visiting nymphalid species (Krenn et al., 2001). Sensilla chaetica are significantly longer and denser in Noctuinae than in Plusiinae (Figs. 5A and 6A). Sensilla chaetica on the tip region of proboscis are crucial in detecting the opening of corolla tubes (Faucheux, 2013). Sensilla chaetica on the tip region are rarer in Plusiinae than in Noctuinae, suggesting that the flower-visiting behaviors of Plusiinae are less vigorous than Noctuinae.

4.2. Systematic considerations

Prominent sensilla were found on proboscises in almost all the Lepidoptera (Krenn, 2010; Faucheux, 2013) and exhibit remarkable differences with regard to their type, morphology, distribution and number (Bänziger, 1975, 1982, 1988, 1992, 2007; Büttiker et al., 1996; Zaspel et al., 2007; Zenker et al., 2011; Bauder et al., 2013; Faucheux, 2013; Xue and Hua, 2014). Previous studies of the proboscis were mainly concerned with whether the morphology of proboscis sensilla was correlated with feeding habits (Büttiker et al., 1996; Krenn et al., 2001; Zenker et al., 2011; Bauder et al., 2013). Our present research may shed a light on their value in systematic and phylogenetic analyses in Noctuidae.

Sensilla styloconica show an amazing variety of shapes of their styli in Noctuidae. The styli are longitudinally ribbed in *Mamestra brassicae* (Nagnan-Le Meillour et al., 2000) and *Chabuata major* (Zenker et al., 2011) in Hadeninae, *Heliothis virescens* (Kvello et al., 2006) in Heliothinae, and *Agrotis ypsilon* (Xue and Hua, 2014) and *Noctua pronuba* (Büttiker et al., 1996) in Noctuinae. According to previous studies, Plusiinae is likely the only subfamily in Noctuidae that possesses sensilla styloconica with cylindrical glabrous stylus on the proboscis. The sensilla styloconica of Plusiinae are significantly different from those of other subfamilies in Noctuidae, providing further evidence to support the monophyly of Plusiinae.

According to Krenn and Kristensen (2000), a stylus equipped with 4–6 longitudinal ribs was found in some species of Prodoxidae, Adelidae and all Incurvarioidea, Palaephatoidea, and many Ditrysia (non-apoditrysians, Tortricidae, Arctiidae, Pyralidae, Noctuidae, Papilionoidea). Therefore, this type of stylus was regarded as the plesiomorphy for sensilla styloconica on the lepidopteran proboscis (Krenn and Kristensen, 2000). In this case, the styli devoid of longitudinal ribs likely represent an apomorphy state of sensilla styloconica in Plusiinae.

All the species of Plusiinae studied possess the same types of sensilla, especially the specific sensilla styloconica and sensilla coeloconica. They show similar distribution patterns on the proboscis and even similar lengths and numbers for all the proboscis sensilla. All these characters of Plusiinae are significantly different from those of Noctuinae, Hadeninae, and Heliothinae (Nagnan-Le Meillour et al., 2000; Kvello et al., 2006; Xue and Hua, 2014), providing additional evidence to support the monophyly of Plusiinae.

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