

Candan & Özyurt Koçakoğlu, (2022) *Comm. J. Biol.* 6(2), 184-189. DOI: <u>10.31594/commagene.1187494</u>

Research Article/Araştırma Makalesi

e-ISSN 2602-456X

The Morphology of the Sensilla on the Proboscis of *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae)

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Received: 11.10.2022	Accepted: 05.12.2022	Published online: 09.12.2022	Issue published: 31.12.2022

Abstract: Proboscis structure and sensilla types are important morphological characters for the systematic analysis of Lepidoptera families. There is no study on proboscis structure and sensilla types of *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) despite the fact that it is an important pest. For this purpose, the sensilla types and proboscis structure of *A. crataegi* were investigated by using stereomicroscope and scanning electron microscope in detail. The results show that the proboscis of *A. crataegi* has three sensillum types (sensilla basiconica, sensilla trichodea, and sensilla styloconica). Sensilla basiconica consists of a sensory cone with a single terminal pore surrounded by a shallow socket and has a flat surface. Sensilla trichodea (chaetica) is bristle-shaped. The bristles of sensilla trichodea are poreless and smooth. Sensilla styloconicum has a smooth stylus, blunt tip, and long peg. In this study, the proboscis structure and sensilla types of *A. crataegi* were discussed with morphological similarities and differences of the other lepidopteran species' proboscis structure and sensilla types. Thus, they contribute to the understanding of proboscis structure and sensilla types in Lepidoptera including Pieridae.

Keywords: Sensilla basiconica, Sensilla trichodea, Sensilla styloconica, scanning electron microscope.

Aporia crataegi (Linnaeus, 1758) (Lepidoptera: Pieridae) Emme Hortumundaki Sensilla Morfolojisi

Öz: Lepidoptera familyalarının sistematik analizinde emme hortumu yapısı ve sensilla tipleri önemli morfolojik karakterlerdir. Zararlı bir tür olmasına rağmen *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae)'nin emme hortumu yapısı ve sensilla tipleri üzerine bir çalışma bulunmamaktadır. Bu amaçla *A. crataegi*'nin sensilla tipleri ve hortum yapısı stereomikroskop ve taramalı elektron mikroskobu kullanılarak detaylı bir şekilde incelenmiştir. Sonuçlar, *A. crataegi*'nin emme hortumunun üç sensilla tipleri (sensilla basiconica, sensilla trichodea ve sensilla styloconica) sahip olduğunu göstermektedir. Sensilla basiconica, sığ bir yuva ile çevrili tek bir terminal gözenek ve düz bir yüzeye sahip duyusal bir koniden oluşur. Sensilla trichodea (chaetica) kıl şeklindedir. Sensilla trichodea'nın kılları gözeneksiz ve pürüzsüzdür. Sensilla styloconicum'un kör uçlu, düz bir stilusu ve uzun bir peg kısmı vardır. Bu çalışmada A. crataegi'nin emme hortumu yapısı ve sensilla tipleri ile diğer lepidoptera türlerinin emme hortumu yapısı ve sensilla tiplerinin morfolojik benzerlikleri ve farklılıkları tartışılmıştır. Böylece, Pieridae dahil Lepidoptera'daki emme hortumu yapısı ve sensilla tiplerinin anlaşılmasına katkıda bulunulmuştur.

Anahtar kelimeler: Sensilla basiconica, Sensilla trichodea, Sensilla styloconica, taramalı elektron mikroskobu.

1. Introduction

Lepidoptera consists of approximately 150,000 species and constitutes the second largest class of insects (Bibi et al., 2022). The Black-veined White *Aporia crataegi* L. (Lepidoptera: Pieridae) is a trans-Palearctic species with high migratory activity, a pest of various fruit and berry crops, with population outbreaks causing complete deforestation (Ilyinskiy & Tropin, 1965; Tolman & Lewington, 2008; Maximov & Marushchak, 2012).

Most adult Lepidoptera proboscis obtain their nutrients by sucking only liquid with their sucking mouthparts in the form of rostrum. Liquid food is drawn into the mouth by the sibarium or pharynx muscles. The proboscis of butterflies is highly elongated, composed of thin maxillary galea, and this proboscis can be elongated by an increase in hemolymph pressure (Gullan & Cranston, 2014). The proboscis is equipped with microtrichia, a row of lamellata, several porous sensilla, and a small muscle attached to the tubules along with the bristle-shaped sensilla (Krenn & Kristensen, 2004). Sensilla play important roles in feeding behavior and host site

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(Zaspel et al., 2013). The outer surface of the Lepidopteran proboscis can bear six morphological sensilla types: basiconicum, chaeticum, styloconicum, filiformium, coeloconicum, and campaniformium. The first three types are the most common. There are four types of pore systems: porous, single-pore, multi-porous, and singlepore-multi-porous (Faucheux, 2013). Sensillum styloconicum has a long stylus and a shorter peg (Paulus & Krenn, 1996). Sensillum basiconicum has a shorter stylus than peg. This sensilla is considered as a chemo or mechanical sensor (Krenn, 1998). Sensillum trichodeum is a hair-like sensory organ. It is considered mechanically sensitive (Krenn, 1998). The morphology and sensilla distribution of the proboscis differ depending on their feeding habits (Faucheux, 2013). Types, sizes, and shapes of sensilla on proboscis differ interspecifically.

This study contributed to the studies of all Lepidoptera species including Pideridae by describing the variation of sensilla types on proboscis composition in *Aporia crataegi* and comparing them with other lepidopteran species.

2. Material and Methods

Ten adult *Aporia crataegi* were collected from Ödek Village, Sivas province, Türkiye in June 2019. The proboscises from *A. crataegi* were cleaned and photographed under an Olympus SZX7 stereomicroscope (SM). Then, they were dehydrated in ethanol series (from 70 to 99%). Next, the proboscises were transferred to hexamethyldisilazane (HMDS) and air dried (Nation, 1983). After drying in air, samples were sprayed with gold (Polaron SC 502). Proboscises were placed in the stubs. Then, they were examined by scanning under electron microscope (SEM) (JEOL JSM 6060 LV) at 10 kV.

3. Results

The proboscis is a tubular structure which consists of the two elongated proboscis (galea) (Figs. 1-3). Proboscis of *Aporia crataegi* is long and is characterized by 3 different types of sensilla (basiconica, trichodea, and styloconica) according to their external morphology (Figs. 4-8). At rest, the proboscis of *A. crataegi* forms 5-6 spirals in a convoluted state (Figs. 2a, b). The proboscis was about 8

mm long. The surface of the proboscis showed a spiny appearance. The spines (microtrichia) extend from the proximal point to the tip of the proboscis in all areas of the galea (Figs. 3a, b). Dorsal legulae form overlapping double rows of finger-like projections (Fig. 3b). Various sensilla types are found along the entire proboscis. Three morphological types can be found on the exterior of the proboscis as sensilla basiconica, sensilla trichodea, and sensilla styloconica (Figs. 4-8). Sensilla basiconica consists of a cone-shaped sensory cone with a single terminal pore surrounded by a shallow socket and has a flat surface (Figs. 4a, b). Sensilla basiconica is $3 \mu m \log and 2\mu m$ wide. There are numerous bristle-shaped sensilla trichodea (chaetica) of varying lengths from proximal to distal to the galea. Sensilla trichodea are arranged in irregular rows on the surface of the galea. Sensilla trichodea of A. crataegi vary 2.77-22.5 µm long. The bristles of sensilla trichodea are poreless and smooth (Figs. 5-6). The galea is tuberculous toward its distal end (Fig. 7b). Sensilla styloconica is located distal to the galea (Figs. 7a, b). Sensilla styloconicum has a smooth stylus, blunt tip, and long peg (Figs. 6-8). Peg is 5 µm long 2 µm wide.



Figure 1. a, b. The general view of proboscis (SM, SEM). Pr-proboscis, Lp-labial palp.



Figure 2. a, b. The lateral view of proboscis (SM, SEM).

4. Discussion

We identified three types of sensilla on the proboscis of adult *A. crataegi*. The morphology of these sensilla was compared with the other Lepidopteran species. Proboscis lengths vary according to the nutrient the butterfly is fed. There has been a process of evolution during which the length of the proboscis to receive pollen from flowers with long spurs has been increasing. It is seen that the helix number of proboscis of *A. crataegi* is 5–6. In *Pieris rapae* (Linnaeus, 1758) (Pieridae) and *Inachis nio* (Linnaeus, 1758) (Nymphalidae), the number of coils varies from 3.5 to 7

coils (Krenn, 1990). The proboscis coil number of *Helicoverpa armigera* (Hübner, 1805) (Noctuidae), *Mythimna separate* Walker, 1865 (Noctuidae), *Scotogramma trifolii* (Hufnagel, 1766) (Noctuidae) and *Protoschinia scutosa* (Denis & Schiffermüller, 1775) (Noctuidae) is 4-5 (Chen et al., 2019; Zhang et al., 2021).

The length of the proboscis varied. The proboscis of *A. crataegi* is about 8 mm long. The proboscis of *Tuta*

absoluta (Meyrick, 1917) (Gelechiidae) is 1.5 mm long (Abd El-Ghany & Faucheux, 2022). The proboscis lengths of *H.* armigera (Noctuidae) and *M. separate* (Noctuidae) are ~11.38 mm and ~10 mm (Chen et al., 2019). The proboscis length in *Pieris brassicae* (L. 1758) (Pieridae) is 16 mm, in *Macroglossum stellatarum* (L. 1758) (Sphingidae) has 25-28 mm, in *Sphinx ligustri* (L. 1758) (Sphingidae) has 37-42 mm (Amsel, 1938). The proboscis of *Vanessa cardui* (Linnaeus, 1758) (Nymphalidae) is 11.5–15.5 mm long (Krenn, 1998).



Figure 3. a, b. The spines (microtrichia) extend from the proboscis surface (SEM). Mt- microtrichia, Dl-Dorsal ligulae.



Figure 4. a, b. Sensilla basiconica (Sb) which is composed of a sensory cone with a smooth surface and a single terminal pore (Tp) (SEM).



Figure 5. a, b. Bristle-shaped sensilla trichodea (St) which varied in lengths (SEM).



Figure 6. a, b. Sensilla trichodea (St) and sensilla styloconica (Ss) (SEM).



Figure 7. a, b. Sensilla styloconica (Ss) which is located distal to the proboscis (SEM).



Figure 8. a, b. Sensilla styloconica (Ss)which have a smooth stylus, blunt tip, and long peg (SEM).

Sensilla are classified according to the presence or absence of pores and the morphology of the sensilla (basonica, chaetica, styloconica, filiformia, coeloconica, and campaniformia) (Faucheux, 2013). In *A. crataegi* proboscis, there are a total of three major types of sensilla as in *Parara guttata* (Bremer & Grey, 1852) (Hesperiidae), *Colias fieldii* Ménétriés, 1855 (Pieridae), *Celastrina oreas* (Leech, [1893]) (Lycaenidae), *Sasakia charonda* (Hewitson, 1863) (Nymphalidae), *Acraea issoria* (Hübner, 1819) (Nymphalidae), *Stichophthalma neumogeni* Leech, [1892]

Tytler, (Nymphalidae), Callerebia 1914 suroia (Laicharting, (Nymphalidae), Libythea celtis 1782) Rottemberg (Nymphalidae), Scotogramma trifolii Protoschinia (Denis & (Noctuidae), and scutosa Schiffermüller, 1775) (Noctuidae), (Ma et al., 2019; Zhang al.,2021). Helicoverpa armigera (Hübner, 1805) et (Noctuidae) has three major types of sensilla, including nine subtypes as sensilla basiconica (esb1, esb2, esb3, isb1, and isb2), sensilla chaetica (sch1 and sch2), and sensilla styloconica (ss1 and ss2) (Guo et al., 2018). The types of proboscis sensilla of *Iphiclides podalirius* (Linnaeus, 1758) (Papilionidae) and *Tirumala limniace* (Cramer, [1775]) (Nymphalidae) are sensilla chaetica, sensilla coeloconica, and sensilla basiconica (Ma et al., 2019). *T. absoluta* (Gelechiidae) has four sensillum types, that are aporous sensilla chaetica, uniporous sensilla chaetica, uniporous sensilla styloconica, and aporous sensilla squamiformia (Abd El-Ghany & Faucheux, 2022).

The length of the sensilla differs from species to species. Sensilla basiconica of *A. crataegi* is 3 μ m long. The cone measures 1.6 μ m in length in *Homoeosoma electellum* (Hulst, 1887) (Pyralidae), 4 μ m in *Monopis crocicapitella* (Clemens, 1859) (Tineidae), and 10 μ m in *Tineola bisselliella* (Hummel, 1823) (Tineidae) (Faucheux, 2013).

Sensilla trichodea of *A. crataegi* vary 2.77–22.5 μ m long. Sensilla trichodea lengths are ~4 μ m in *T. limniace* (Nymphalidae) and ~6 μ m in *I. podalirius* (Papilionidae) (Ma et al., 2019). Sensilla trichodea measures are 6–9 μ m in *Homoeosoma* sp. (Pyralidae), 8–15 μ m in *Adela reaumurella* (L. 1758) (Adelidae) (Faucheux 1991, 1995, 2005, 2009). In *V. cardui* (Nymphalidae), sensilla trichodea greatly varies in length (8–48 μ m) (Krenn, 1998).

Sensilla styloconica of Aporia crataegi (Linnaeus, 1758) (Pieridae) has one type. Sensilla styloconica length is $5 \mu m$. Sensilla styloconica in Avatha bubo (Geyer, 1832) (Erebidae) has two different types: the measure of first type 7.8-9.5 μ m in length and the second type is 5.9–7.4 μ m in length (Faucheux, 2013). Sensilla styloconica lengths are ~10 µm in P. guttata (Hesperiidae), ~11 µm in Co. fieldii (Pieridae), ~14 µm in Ce. oreas (Lycaenidae), ~15 µm in A. issoria (Nymphalidae), ~27 µm in Ca. suroia (Nymphalidae), ~44 µm in L. celtis (Nymphalidae) (Ma et al., 2019). Reinwald et al. (2022) defined sensilla styloconica length as 28.8 µm in Protambulyx strigilis (Linnaeus, 1771) (Sphingidae), 33.3 µm in Neococytius cluentius (Cramer, 1776) (Sphingidae), 25.4 µm in Sphinx pinastri Linnaeus, 1758 (Sphingidae), 28.3 in Euryglottis aper (Walker, 1856) (Sphingidae) and *Xylophanes pyrrhus* Rothschild & Jordan, 1906 (Sphingidae), and 31 µm in Manduca scutata (Rothschild & Jordan, 1903) (Sphingidae). Microtrichia observed on the surface of the galea are common on the proboscis and are likely to act as mechanical receptors (Molleman et al., 2005). They also help collect and fix the pollen (Gilbert, 1972).

Proboscis structure and sensilla types are important morphological characters in the systematic analysis of Lepidoptera families.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declared that there is no conflict of interest.

Author Contributions: Conception – S.C., N.Ö.K.; Design – S.C., N.Ö.K.; Supervision – S.C., N.Ö.K.; Materials – S.C., N.Ö.K.; Data Collection or Processing – S.C., N.Ö.K.; Analysis Interpretation – S.C., N.Ö.K.; Literature Review – S.C., N.Ö.K.; Writing – S.C., N.Ö.K.; Critical Review – S.C., N.Ö.K.

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