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Abstract

Lepidoptera is the most suitable insect order to comprehend the connections between nutrition and mouthparts. The proboscis, which is typically tightly coiled between the labial palps, is a flexible organ used by most adult butterflies for extracting flower nectar and other liquids. We examined the proboscis structure and sensilla of *Maniola jurtina* Linn. by scanning electron microscope (SEM) in order to contribute to the relationship between food selection and proboscis structure in Satyrinae Sensilla chaetica (s. trichodea) of various lengths is the most common species along the proboscis. Sensilla styloconica is of pluricarinate type. It is densely located only in the distal region and is in rows. It has a long quill with 6 ribs (flat protrusions) and a relatively short nail. In the study, sensilla lengths were compared with the sensilla lengths of some other lepidopteran species.

1. Introduction

Lepidopteran proboscis is one of the most remarkable body parts among insect groups in terms of nutrition. The diversity and structure of lepidopteran proboscis are intricately linked to their feeding behaviors and their capacity to ingest fluids [1-5]. While many species are known to be anthophilous, some also exhibit a varied diet, including mud puddles, sugary substances, sweat, tears, and wound exudates from mammals [6-13]. The proboscis, a flexible tube specialized for liquid food intake, typically adopts a coiled ring shape at rest. However, during feeding, it unfurls, revealing distinct regions. The proximal region is closest to the head, while the bend region emerges at approximately one-third of the proboscis's length [14]. The distal region, which is relatively lengthy, features a flexible apex containing several slits responsible for food intake [15, 16]. In this region, approximately 10–20% features dorsal legulae modifications characterized by wider interlegular spaces and reduced galeal surface area. These adaptations aid in fluid passage into the alimentary canal, contributing to an overall hydrophilic profile [3-4]. The proboscis consists of a pair of c-shaped galeae. Each galea is equipped with cuticular processes and several types of sensilla that are essential for nutritional activities [1, 17]. These sensilla vary depending on feeding habits [5, 18-20]. The form and function of the lepidopteran proboscis have been studied in various taxa encompassing numerous families [10, 15, 21-24].

There are six sensilla types in butterflies [1, 25]. Three of the most commonly seen sensilla types are: s. chaetica (=trichodea), s. basiconica and s. styloconica. Sensilla chaetica is located on the entire galea surface. Acting as a mechanoreceptor, this sensilla provides information about the width and depth of the tubular flower during flower scanning [26]. Sensilla basiconica are composed of a sensory cone, either domed or pointed, and a shallow, flexible socket [1, 27]. Present across various insect groups and distributed throughout the body, they serve as essential structures for chemosensory functions [28]. Sensilla basiconica exist in two forms: external and inner sensilla basiconica. Inner sensilla basiconica are notably fewer in number compared to those on the outer surface and are typically confined to the distal part of the proboscis [29]. The most characteristic sensilla type in lepidopterans is sensilla stylochonica.

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They are outstanding chemo-mechanic receptors limited to the apex region [27]. It is the sensilla type in which the most differentiation is observed [25]. The potential systematic importance of these senses in butterflies have been acknowledged by some researchers (example Borner, [30]) yet this issue has not been evaluated enough in systematic aspects. In their examination of the proboscis, Paulus and Krenn [16] documented 19 European satyrid species, a number of which are also present in Turkey. In a study about proboscises of butterflies conducted in North America, proboscis of 17 Satryinae species was examined [26]. In Turkey, there are only 3 publications regarding Lepidoptera proboscis [24, 31-32].

Given their biology, diversity, and distribution, Satyrinae constitutes a prominent group within butterfly communities [33-40]. The extensive diversity of Satyrinae, both in terms of species richness and morphology, has resulted in considerable uncertainty and taxonomic challenges in their classification. Comparative morphological studies on sensilla located in different places in insects may contribute to classification.

This research involves a detailed SEM examination of the proboscis morphology and sensilla types, and mouthparts in specimens of *Maniola jurtina* from Turkey. Sensilla have been compared with other lepidopter species and discussed later. The results contribute to the proboscis morphology of Satyrinae. This will be beneficial for studies that are intended to be conducted later related to the nutritional behaviors of adult lepidopters.

2. Material and Method

In this study, the proboscis of two male specimens of *M. jurtina* were examined. The specimens taken from Prof. Dr. Çalışkan's collection are kept in Gazi University Zoology Museum. The ultrastructure of the sensilla of the proboscis in adults of *M. jurtina* is elucidated using SEM imaging. For the terminology used here in proboscis and sensilla morphology, see Bänzigerl [9, 41], Büttiker et al. [8], Speidel et al. [23], Altner and Altner [27], Faucheux [42, 43], Hallberg [44] remove. For the scanning electron microscope imaging (SEM), mouthparts and proboscises of Maniola jurtina individuals have been cleaned with a brush and dried by air before being positioned with a double-sided tape on SEM tabs. They were later coated with a layer of gold using a Polaron SC 502 Sputter Coater and examined with a 10 kV Jeol JSM 6060 LV SEM. While stretching the butterflies, the proboscis of the softened samples were lengthened and fixed with the help of a needle.

Sensilla lengths were measured with references taken from the beginning and end points of sensilla in SEM images. The example examined in the study is from the second author's master's thesis.

3. Results

The components of the mouthparts of Maniola jurtina are shown in Figure 1. The head of the individual shown in Figure 1a is damaged. The proboscis is four spirals (Fig. 1a). Galeae are hinged by dorsal and ventral legulae. Long bristle-shaped scales project from the pilifer and contact the nearby dorsal galeal surface. The maxillary palpus is fist-shaped next to the proboscis base (Fig. 1b). The proboscis is roughly threefold compared to the labialy palpus. The galeal surface has a rough texture, lacks spines, and has a spiny web-like structure near the dorsal ligulae. There are three types of sensilla in Maniola jurtina: Sensilla basiconica, sensilla styloconica, and sensilla chaetica (Figs. 2, 3, 4). Sensilla chaetica can be observed in various lengths and scattered on lateral and ventral sides in the proximal and distal regions of the galea (Figs. 2a, 2b, 3a). Sensilla basiconica can be observed in irregular rows on the external surface of the proboscis (Figs. 2a, 2b, 3a). Sensilla styloconica are in distal region of the proboscis. There has a long stylus with 6 ribs (Smooth Ridges) and a relatively short peg. Tips of the ribs bear six (Shoulder Spines) sharp spines. Length of the shoulder spines are shorter than the peg (Fig. 3b). Sensilla basiconica consist of a conical sensory structure with a flat surface surrounded by a shallow socket. Inner sensilla basiconica form a single row on the food canal (Fig. 4a). They consist of a long stylus and a short sensory nail placed in a slightly dome-shaped socket (Fig. 4b). The sensilla were not seen in the maxillary palps in the samples examined. However, pits on the palps attract attention. These pits are likely to be sensilla pits. The sensilla on the pits may have been severed because the head of the specimens was damaged.

4. Discussion

Most Satyrinae species are diurnal adults with limited dispersal capabilities, typically flying close to the ground, particularly in shaded forest areas (understory) [45-48]. Host plants of Satyrinae are mostly monocots. They are also used with certain such eudicot families as Fabaceae and Menispermaceae, and some species have been recorded feeding on Lycopodiophyta (Selaginellaceae), Bryophyta (Neckeraceae) and gymnosperms (Cycadaceae) [49-52]. Adults of most species are polyphagous and feed on the nectar of different species.



Figure 1a-b. Head and mouthparts of *Maniola jurtina* in oblique frontal view, **a.** proboscis (pr) in recoiled resting position 1: complex eye (e), pilifer (pf), maxillar palpus (mp), labial palpus (lp), **b.** Basal proboscis each proboscis (pr), dorsal legulae (dl), pollen (pl)



Figure 2a-b. Galea surface in *M. jurtina*, and the distribution of sensilla positioned on the galea surface, **a.** sensilla chaetica (sc), sensilla basiconica (sb), sensilla styloconica (st), **b.** Enlarged image of s. chaetica (sc), s. basiconica (sb)



Figure 3a-b. Distal region of proboscis of *M. jurtina*, a. sensilla chaetica (sc), sensilla basiconica (sb), sensilla styloconica (st), b. Enlarged image of sensilla styloconica



Figure 4a, b. Food canal of the proboscis of *M. jurtina*, a. intrasensilla basiconica (sb), b. Enlarged image of sensilla basiconica.

Krenn et al., [53] examined the sucking proboscis of six satyrid butterfly species, including Maniola jurtina, in their study of the proboscis structure of nymphalids. Their study revealed variations in the sensilla equipment, particularly in the length of sensilla chaetica and the shape and quantity of sensilla styloconica. Notably lengthy sensilla trichodea were observed extending beyond the midproboscis in numerous Heliconiini (as detailed in Krenn & Penz, [54]), certain Limenitidinae, some Brassolinae, all Morphinae, and Haetera piera (Satyrinae). In Morphinae and Haetera piera, stout sensilla trichodea were predominantly situated on the ventral sides of the galeae, spanning from the base of the proboscis to just short of the tip-region. In the samples we examined, needle-shaped extensions on the ventral sides of the galea are noteworthy. Sensilla caetica are remarkably long, varying in size from 11.7 to 25.1 µm. S. chaetica length of Aporia crataegi ranges from 2.77 to 22.5 µm [31], ~4 µm in Tirumala limniace and in Iphiclides podalirius, ~6 µm [55] in Vanessa cardui, 8-48 µm [56]. The size of chaetica varies even in the same individual.

The sensilla basiconica, consisting of a broad dome-shaped base and a short sensory cone, serves as a chemoreceptive [16, 25, 26, 28, 56]. Sensilla basiconica in the alimentary canal are sensitive to mechanical stimuli and likely function to control fluid selection within the digestive tract [29]. Fluid uptake is probably faster in individuals with short inner sensilla length. Sensilla basiconica of *M. jurtina* is 6 µm long. Sensilla basiconica of A. crataegi is 3 µm long [31], 1.6 µm in Homoeosoma electellum (Hulst, 1887), 4 µm in Monopis crocicapitella (Clemens, 1859) and 10 µm in Tineola bisselliella (Hummel, 1823) [25]. According to the data, sensilla basiconica length is not related to body height [29]. The fact that the length of the inner sensilla basiconica in M. jurtina is twice as long as that of A. crataegi supports this.

Sensilla styloconica can provide the insect with important information such as the location of nectar and the length of the pollen tube [55, 54]. Sensilla

styloconica increases the hydrophilicity and capillarity of the drinking area and helps fluid uptake from porous surfaces [3, 57]. Petr and Stewart [26] divided and named s. styloconica into groups according to their morphological structures. According to this study, sensilla styloconica of satyrids are divided into two groups: pluricarinate and pluridentate. Cylindriform/pluricarinate and cylindriform/pluridentate sensilla are unique of identifiers for some groups Satyrinae. Cylindrical/pluricarinate specific styli are to Coenonympha, Cercyonis, Neominois, and Oeneis; Cylindrical/pluricarinate styli are found only in Erebia, Gyrocheilus and Oeneis. The M. jurtina we examined also are the s.styloconica cylindriform/ pluricarinate type. The protrusions on the body of the cylindrical pencil are flat. They extend beyond the shoulders to the apical spines. Six apical shoulder spines form a symmetrical, rounded crown around the sensory nail.

Sensilla styloconica were observed to be longer and more abundant in non-flower-visiting nymphalids compared to flower-visiting species. Among species that visit flowers, there exists greater diversity in the shapes of sensilla styloconica. In Satyrinae, they feature longitudinally ribbed ends with apical spines, whereas in certain Nymphalinae members, they are either smooth and partially cylindrical (e.g., Ithomiinae) or smooth and flattened (e.g., Heliconiinae) [1]. The morphology of the sensilla styloconica in the examined sp. jurtina shows the characteristics of the satyrid group. The stylus has a cylindrical structure, unlike the either smooth and partially cylindrical sensilla styloconica seen in some members of the Nymphalinae group. The ribs on the body of the scion are smooth and have apical spines, 6 in number. These apical spines are arranged symmetrically to form a rounded crown around the sensory cone. The sensila styloconica of M. jurtina, studied by Krenn et al. [1], similarly has longitudinal projections ending in apical spines.

5. Conclusion and Suggestions

The results obtained show that Satyrinae are closer to Nymphalinae than Limenitiidae with their sensilla styloconica morphology. The usability of differences in the morphology of sensilla styloconica in the classification of higher categories in Satyrinae should be investigated.

Contributions of the Authors

S.S.Ç and Y.M. designed the study and wrote the article, S.C. contributed to Scanning Electron Microscope (SEM) images and writing the article.

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Conflicts of Interest Statements

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

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