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DIPLOMARBEIT

Titel der Diplomarbeit

„Comparative morphology of proboscides in Nymphalidae
(Lepidoptera) – Phylogenetic and functional conclusions“

verfasst von

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angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2013

Studienkennzahl lt. Studienblatt:

A 439

Studienrichtung lt. Studienblatt:

Diplomstudium Zoologie

Betreut von:

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1. Abstract

The Nymphalidae are not only the most species-rich butterfly family, they also have been controversial concerning their intrafamilial phylogenetic relationship for a long time. The aim of the present study was to identify new morphological characters of the lepidopteran proboscis and associated structures that could expand the existing data set and may be useful for subsequent phylogenetic analysis.

The proboscis of the Lepidoptera consists of the two elongated galeae and is a specialized adaptation for sucking up fluids. It evolved once within the Glossata and therefore bears a lot of structures that are related to this kind of food intake. The proboscis morphology of 53 species, representing all widely recognized lineages of the Nymphalidae was investigated using light microscopy. The composition of the outer galeal wall, cuticular spines, sensilla trichodea, sensilla styloconica and the food canal were examined and described for all investigated nymphalid species. The plesiomorphic conditions were determined by outgroup comparison, using ten species of closely related families, including Lycaenidae, Riodinidae, Pieridae, Papilionidae and HesperIIDae. Twelve characters and 27 character states were used to generate a morphological data matrix. Preliminary results indicate that the composition of the outer galeal wall is potentially informative at subfamilial level. Most of the characters show a high intrafamilial variability, which may be a result of convergent development.

Butterflies are often associated with nectar uptake from tubular flowers. Statistical analyses indicate that some proboscis characters developed in connection with different feeding habits. A longer tip region, longer sensilla trichodea in the mid region of the proboscis, more densely arranged and longer sensilla styloconica and a higher density of sensilla basiconica seem to be adaptations to the exploitation of alternative food sources like rotting fruits, dung or tree sap.

The results are discussed in a systematical, ecological and functional context.

2. Introduction

2.1. Phylogeny of Nymphalidae

The Nymphalidae (Lepidoptera) are the most species-rich butterfly family within the Papilionoidea, containing about 6000 species and showing a worldwide distribution, except for the Arctic and Antarctica (Ehrlich 1958, Ackery 1984, Ackery et al. 1999). Since many years, members of this diverse family have been a subject of interest in several fields of biology, i.e. genetics, behavioral and ecological research. Although the Nymphalidae are a well-studied group, the members of the Nymphalidae and their relationship among each other have long been a matter of contention. The first attempt to clarify the systematic relationship of this family was the work of Müller (1886), who used larval characters for a classification. From that time on the attention of various works was focused on nymphalid phylogeny. In the last decades nearly every new publication presented a different systematic concept. Members of this butterfly family are easy to recognize because of two distinct characters, namely reduced forelegs and antennae with two ventral grooves, so-called tricarinate antennae (Ackery 1984). Although a few taxa are quite well-supported by some characters and have been recognized as monophyletic groups in several studies (e.g. Libytheinae, Heliconiinae, Charaxinae) others have been mixed and rearranged, because of weak support or lack of synapomorphies for a stable definition (e.g. Limenitidinae, Nymphalinae, Morphinae). The position of Libytheinae as the sister taxon to all other Nymphalidae was first recognized by Ehrlich (1958) due to the presence of fully functional forelegs in females and was confirmed by many other authors (De Jong et al. 1996, Wahlberg et al. 2003, Freitas and Brown 2004, Wahlberg et al. 2009). Danaini, Ithomiini and Tellervini, often treated as subfamilies in earlier works, are considered as a monophyletic group (Danainae) by most authors (Brower 2000, Wahlberg et al. 2003, Freitas and Brown 2004, Wahlberg et al. 2009). Ehrlich (1958) and Ackery (1984) used characters from early stages as well as adult characters for their analysis. The work of Freitas and Brown (2004) covered many nymphalid taxa by using many morphological characters from adult butterflies as well as characters from immature stages, which can be crucial for phylogenetic analysis. Molecular studies (Brower 2000, Wahlberg et al. 2003) reinforced some traditional views, and provided new approaches for intrafamilial classification. The latest work on nymphalid phylogeny (Wahlberg et al. 2009), based on morphological and molecular data revealed 12 subfamilies with a strong support on monophyly and relationship among subfamilies.

2.2. Proboscis morphology

The proboscis of adult Glossata is composed of the two extremely elongated galeae (Figure 1). All other major components of the mouthparts, except the labial palps, are reduced (Eastham and Eassa 1955, Krenn et al. 2005, Stekolnikov and Korzeev 2007, Krenn 2010). Each galea forms a functional unit containing a trachea, muscles, nerves, one or two septa and different types of sensilla (Eastham and Eassa 1955, Krenn and Mühlberger 2002). After eclosion the galeae interlock and are held together by linking structures on the dorsal and ventral side along the entire length of the proboscis (Krenn 1997). These linking structures are composed of cuticular processes. On the ventral side a tight-closing connection between the galeae is enabled by a row of interlocking hooks, whereas on the dorsal side series of overlapping plates (legulae) ensure the formation of a central food canal (Eastham and Eassa 1955, Hepburn 1971, Krenn and Kristensen 2000). At the apical region of the proboscis the dorsal legulae are elongated and with slits between them. These drinking slits enable the uptake of fluid into the food canal in this particular region, called tip region (Paulus and Krenn 1996, Krenn and Kristensen 2000). The food canal is bounded by the inner, concave walls of the galeae lined with cuticular plates (Eastham and Eassa 1955, Hepburn 1971, Krenn and Kristensen 2000, Paulus and Krenn 1996). The surface of the external galeal wall has an annulated appearance because of alternating dark- and light-coloured cuticle. The dark cuticle is considered as hard exocuticle, while the light cuticle seems to be flexible meso- and endocuticle. The alternation of these different kinds of cuticle is responsible for the flexibility of the proboscis (Hepburn 1971).

Cuticular spines originate on the outer galeal wall and differ in shape as well as in their distribution along the galea. These spines are believed to hold the proboscis spiral in a fixed resting position because they hook into the ridges of the outer galeal wall of the underlying proboscis coil (Krenn 1990).

Three types of sensilla occur along the lepidopteran proboscis. Sensilla trichodea are bristle-shaped mechanoreceptors which originate from a collared socket and are distributed along the external surface of the galeae (Paulus and Krenn 1996, Krenn 1998). These mechanosensitive sensilla may give information about the width and depth of a corolla during flower-visiting and the correct resting position of the proboscis (Krenn 1990, Krenn and Kristensen 2000). Sensilla basiconica are chemoreceptive sensilla. They are arranged in several irregular rows on the outside of the galea and in one row on the median sides of the galea which form the food canal. These sensilla consist of a blunt sensory cone and a hemispheric socket (Altner and Altner 1986, Krenn 1998, Paulus and Krenn 1996, Walters et al. 1998). Inoue et al. (2009) showed that sensilla basiconica in the food canal respond to sucrose. The most prominent sensilla on the

lepidopteran proboscis are the sensilla styloconica. They are restricted to the tip region and have been considered as combined chemo/mechanosensilla. It is assumed that they help to find the opening of corolla tubes and respond to sucrose solution (Altner and Altner 1986, Krenn 1998, Walters et al. 1998). They consist of a distinct stylus and an apical sensory cone. Especially within the Nymphalidae the sensilla styloconica show a tremendous variety concerning the shape and size of the stylus (Paulus and Krenn 1996, Petr and Stewart 2004).

2.3. Proboscis morphology in connection with phylogeny

The first attempt to interpret morphological proboscis characters in context with phylogeny was done by Börner (1932), who suggested that the sensilla styloconica can be used as informative characters in phylogeny. Paulus and Krenn (1996) investigated the proboscis morphology of 71 European species of all recognized families within the Papilionoidea, focusing on the composition of the outer galeal wall, cuticular spines and the shape and distribution of sensilla styloconica. Petr and Stewart (2004) compared seven morphological characters of sensilla styloconica in 76 species of North American Nymphalidae. Zaspel et al. (2011) investigated the proboscis morphology of 36 noctuid species, belonging to the family of Calpinae, with regards to taxonomic relevance and association with feeding behaviors.

The aim of the present study is to identify additional morphological characters of the proboscis and to create a character matrix that expands the existing data set and may be important for subsequent phylogenetic analysis.

3 Material and Methods

3.1. Taxon sampling

Fifty-three species representing all major lineages of the Nymphalidae were selected as ingroup taxa. The character states were determined by outgroup comparison, using ten species of closely related families, including Lycaenidae, Riodinidae, Pieridae, Papilionidae and Hesperidae. Additional information for outgroup comparison was gathered from several studies (Paulus and Krenn 1996, Petr and Stewart 2004). In order to find out if derived character states developed convergently or not, character states were mapped onto a cladogram based on the latest nymphalid phylogeny (Wahlberg et al. 2009) (Figure 7). The ingroup and outgroup species are listed in Table 1. The specimens were obtained from the Lepidoptera Collection of the Natural History Museum Vienna.

Table 1. Ingroup and outgroup species used in the study. The classification of Nymphalidae follows Wahlberg et al. 2009. Classification of outgroup species after <http://tolweb.org/Ditrysia/11868> (13.09.2013).

Nymphalidae		
Subfamily	Tribe	Species
Libytheinae		<i>Libythea celtis</i> (LAICHARTING, [1782])
		<i>Libytheana carinenta</i> (CRAMER, [1777])
Danainae	Danaini	<i>Amauris niavius</i> (LINNAEUS, 1758) <i>Lycorea cleobaea</i> (GODART, 1819)
	Tellervini	<i>Tellervo zoilus</i> (FABRICIUS, 1775)
	Ithomiini	<i>Mechanitis lysimnia</i> (FABRICIUS, 1793) <i>Ithomia drymo</i> (HÜBNER, 1816)
Charaxinae	Charaxini	<i>Charaxes varanes</i> (CRAMER, 1764)
	Preponini	<i>Agrias sardanapalus</i> (BATES, 1860) <i>Archaeoprepona chalciope</i> (HÜBNER, [1823])
Apaturinae		<i>Apaturopsis cleochares</i> (HEWITSON, 1873) <i>Doxocopa agathina</i> (CRAMER, [1777])
Satyrinae	Morphini	<i>Morpho achilles</i> (LINNAEUS, 1758)
	Amathusiini	<i>Taenaris onolaus</i> (KIRSCH, 1877) <i>Zeuxidia aurelius</i> (CRAMER, [1777])
	Brassolini	<i>Caligo oileus</i> (C. & R. FELDER, 1861) <i>Dasyophthalma creusa</i> (HÜBNER, [1821]) <i>Narope cyllastros</i> (DOUBLEDAY, [1849]) <i>Bia actorion</i> (LINNAEUS, 1763)
	Haeterini	<i>Pierella lamia</i> (SULZER, 1776)
	Melanitini	<i>Melanitis leda</i> (LINNAEUS, 1758) <i>Gnophodes chelys</i> (FABRICIUS, 1793)
	Satyrini	<i>Bicyclus mandanes</i> (HEWITSON, 1873)

Material and Methods

		<i>Magneptychia libye</i> (LINNAEUS, 1767)
		<i>Godartiana muscosa</i> (BUTLER, 1870)
		<i>Maniola jurtina</i> (LINNAEUS, 1758)
		<i>Melanargia galathea</i> (LINNAEUS, 1758)
		<i>Eteona tisiphone</i> (BOISDUVAL, [1836])
Calinaginae		<i>Calinaga buddha</i> (MOORE, 1857)
Heliconiinae	Acraeini	<i>Altinote dicaeus</i> (LATREILLE, [1817])
	Heliconiini	<i>Dione junio</i> (CRAMER, [1779])
		<i>Dryadula phaetusa</i> (LINNAEUS, 1758)
		<i>Heliconius erato</i> (LINNAEUS, 1764)
	Argynnini	<i>Euptoieta hegesia</i> (CRAMER, [1779])
Limenitidinae	Limenitidini	<i>Limenitis populi</i> (LINNAEUS, 1758)
	Neptini	<i>Cymothoe herminia</i> (GROSE-SMITH, 1887)
		<i>Neptis rivularis</i> (SCOPOLI, 1763)
	Adoliadini	<i>Aterica galene</i> (BROWN, 1776)
Cyrestinae		<i>Cyrestis thyodamas</i> (BOISDUVAL, 1846)
Pseudergolinae		<i>Dichorragia nesimachus</i> (DOYÈRE, [1840])
Biblidinae	Biblidini	<i>Biblis hyperia</i> (CRAMER, [1779])
		<i>Eurytela dryope</i> (CRAMER, [1775])
	Epicaliini	<i>Catonephele numilia</i> (CRAMER, [1775])
		<i>Myscelia orsis</i> (DRURY, [1782])
	Epiphilini	<i>Epiphile iblis plusios</i> (GODMAN & SALVIN, 1883)
	Eubagiini	<i>Dynamine mylitta</i> (CRAMER, 1782)
	Callicorini	<i>Callicore texa titania</i> (SALVIN, 1869)
Nymphalinae	Nymphalini	<i>Araschnia burejana</i> (BREMER, 1861)
		<i>Vanessa myrinna</i> (DOUBLEDAY, 1849)
		<i>Vanessa atalanta</i> (LINNAEUS, 1758)
	Melitaeini	<i>Chlosyne lacinia</i> (GEYER, 1837)
		<i>Eresia lansdorfi</i> (GODART, 1819)
	incerte sedis	<i>Kallimoides rumia</i> (WESTWOOD, 1850)
Outgroup		
Family	Subfamily	Species
Lycaenidae	Lycaeninae	<i>Lycaena phlaeas</i> (LINNAEUS, 1761)
	Theclinae	<i>Laothus phydela</i> (HEWITSON, 1867)
Riodinidae	Riodininae	<i>Riodina refracta</i> (STICHEL, 1910)
		<i>Calydna calamisa</i> (HEWITSON, 1854)
Pieridae	Pierinae	<i>Delias belisama</i> (CRAMER, [1780])
		<i>Pieris bryoniae</i> (HÜBNER, 1805)
Papilionidae	Papilioninae	<i>Graphium sarpedon</i> (LINNAEUS, 1758)
		<i>Papilio machaon</i> (LINNAEUS, 1758)
Hesperiidae	Pyrginae	<i>Erynnis montanus</i> (BREMER, 1861)
	Euschemoninae	<i>Euschemon rafflesia</i> (MACLEAY, [1826])

3.2. Morphological studies

All proboscis features were examined using a light microscope (Nikon Labophot-2). Proboscides were taken from pinned museum specimens and soaked in diluted lactic acid for five to seven days to bring back flexibility. Afterwards, the galeae were rinsed in distilled water and 30 % ethanol. After this treatment the galeae could be separated easily from each other. Subsequently the galeae were embedded in polyvinyl-lactophenol on microscope slides and enclosed with a coverslip. Sketches of proboscides and selected characters were done using a Nikon drawing tube. Measurements were taken using a flexible measuring tape. To obtain the real length of the structures, measurements were divided by a coefficient, depending on the magnification of the microscope. Proboscides were photographed on an Olympus CX41 microscope equipped with an Olympus E330 digital camera. Photos were edited and compiled with Adobe Photoshop CS6. Illustrations were drawn using Adobe Illustrator CS6.

3.3. Selected proboscis characters

The following proboscis characters were examined: (1) the composition of the outer galeal wall; (2) the shape and extension of cuticular spines on the outside of the proboscis; (3) the average length of ten sensilla trichodea at 25 %, 50 % and 75 % proboscis length; (4) the sensilla basiconica on the outer galeal wall; (5) the length of the tip region, measured from the apex of the proboscis to the ending of the modified dorsal legulae (Figure 3a); (6) the number of rows of sensilla styloconica per galea; (7) the coverage of sensilla styloconica along the tip region; (8) the number of sensilla styloconica on both galeae; (9) the density of sensilla styloconica on both galeae expressed as number of sensilla per 100 μm ; (10) the average length of the stylus of ten sensilla styloconica measured from the base of the stylus to the origin of the sensory cone; (11) the average length of ten sensory cones; (12) the shape of the stylus; (13) the structuring of the stylus surface; (14) the shape and arrangement of appendages on the apical part of the stylus; (15) the position of the sensory cone on the apical part of the stylus; (16) the number of sensilla basiconica inside the food canal on one galea; (17) the density of sensilla basiconica inside the food canal on one galea expressed as sensilla per 1 mm galeal length; (18) the surface condition of the food canal.

All measurements were done with one specimen per species. Nomenclature for sensilla styloconica follows Petr and Stewart (2004).

3.4. Statistical analysis

Statistical analyses were done using Microsoft Excel 2010 and SPSS 21 software for Windows. Differences between the two feeding guilds (flower-visiting and non-flower-visiting) were tested using the Mann-Whitney *U* test. The significant level was set at $p < 0.05$.

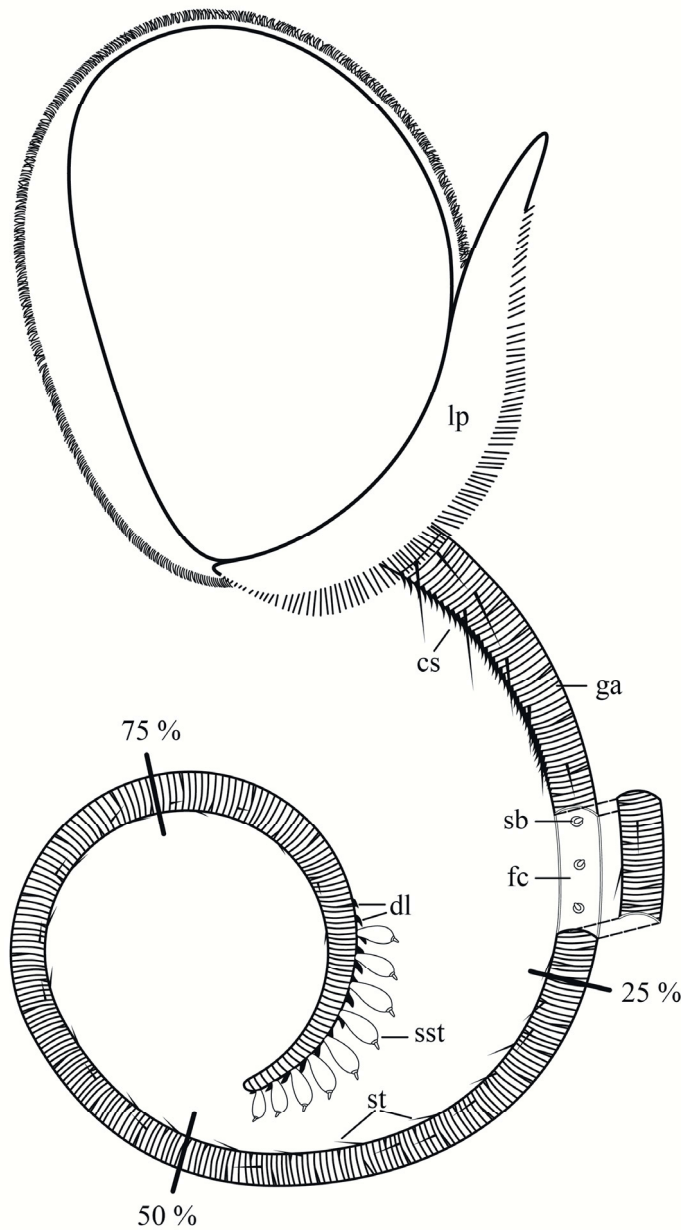


Figure 1. Schematic illustration of a nymphalid butterfly's head in a lateral view. The galeae (ga) originate between the labial palps (lp). Cuticular spines (cs) are restricted to the ventral side of the proboscis. Bristle-shaped sensilla trichodea (st) are distributed over the entire surface of the galeae. Sensilla styloconica (sst) are restricted to the tip region. The tip region is characterized by modified dorsal legulae (dl). Intake slits of the tip region allow the uptake of fluids into the food canal (fc), which is equipped with sensilla basiconica (sb). Length of sensilla trichodea (st) was measured at 25 %, 50 % and 75 % proboscis length.

4. Results

4.1. Overview of proboscis morphology in Nymphalidae

4.1.1. Composition of the outer galeal wall

The outer galeal wall consists of light cuticle, in which dark cuticle is embedded in most species. The surface texture of the galea differs with respect to the presence and formation of the dark cuticle. Regarding the composition of the outer galeal wall three major types can be distinguished: (1) proboscides with elements of dark cuticle along the entire length (Figure 2a - 2h), (2) proboscides where the dark cuticle is restricted to the proximal region (Figure 2i, 2j) and (3) proboscides without dark cuticle (Figure 2k, 2l).

In species where areas of dark cuticle occur along the entire proboscis they appear as vertical and partly branched stripes. These stripes can be either straight (Figure 2a, 2b, 2d, 2h), corrugated (Figure 2f) or shaped like strings of pearls (Figure 2c, 2e). In the proximal region the stripes of dark cuticle can change into individual, round shaped elements at the dorsal and ventral margin of the galea. Individual elements of dark cuticle can also occur between the stripes (Figure 2g). In the distal region the stripes are either proceeding straight from the dorsal to the ventral side (Figure 2b, 2d, 2f) or they show a characteristic bend on the lateral side of the galea (Figure 2h).

In species where dark cuticle occurs only in the proximal region of the proboscis, it forms thin stripes which are surrounded by some small individual and irregularly arranged components of dark cuticle. Individual components are also clustered on the dorsal side of the proboscides (Figure 2i). The distal region consists of light cuticle only (Figure 2j).

Proboscides without dark cuticle have a uniform structure since they are exclusively composed of light cuticle along the entire galea (Figure 2k, 2l).

4.1.2. Extension and shape of cuticular spines

Cuticular spines are present on the proboscides of most investigated species. They are found on the ventral side of the galeae (Figure 1). If present, these spines always originate on the most basal part of the proboscis and cover between 1.8 % and 100 % of the total proboscis length (Figure 4, Appendix Table 4). Two different types of cuticular spines can be distinguished, broad and triangular spines, where the length corresponds approximately to the width (Figure 3d), and slender and acute spines, with the length not less than one and a half times longer than the width (Figure 3c).

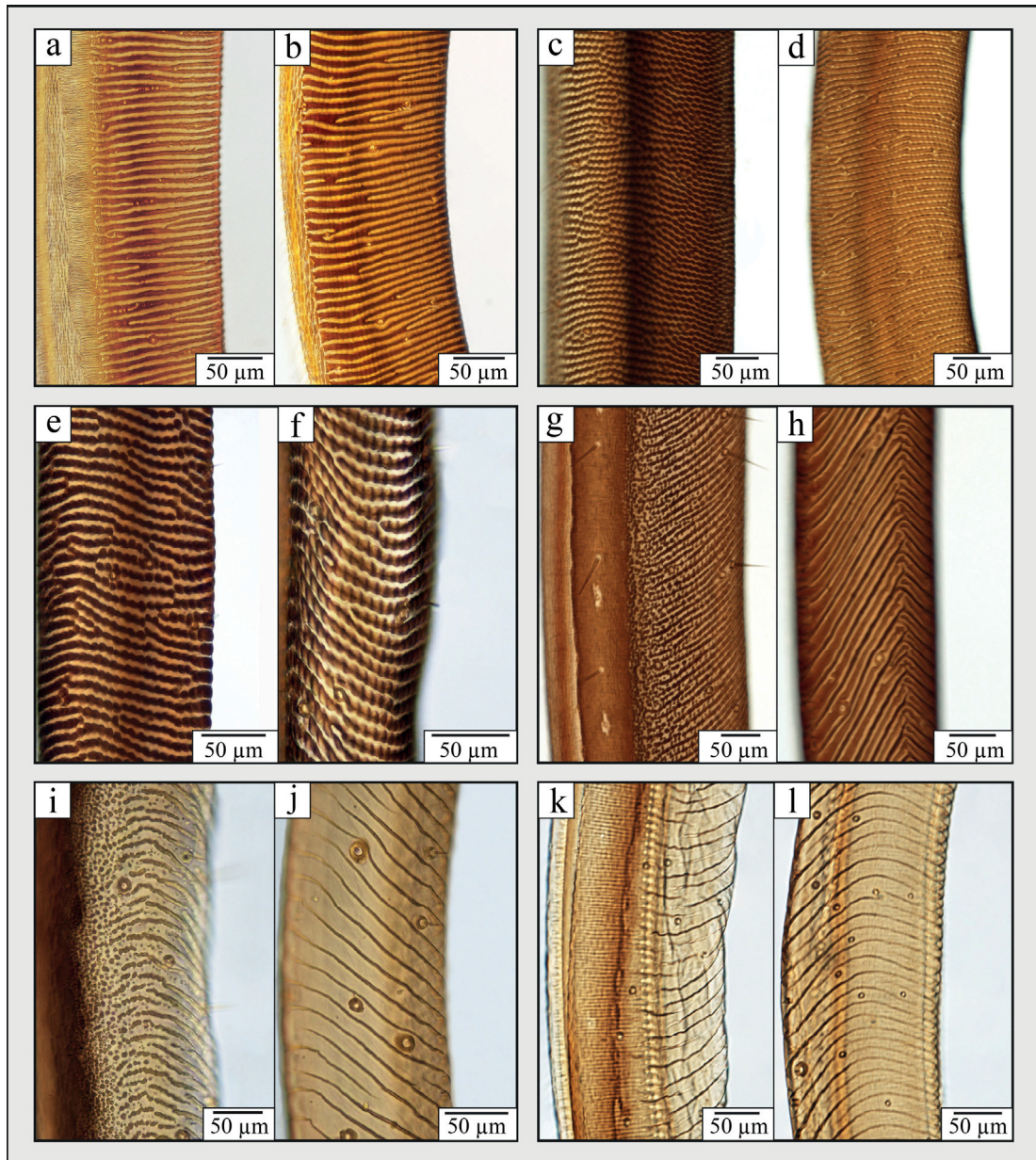


Figure 2. Composition of the outer galeal wall. Proximal (**a**) and distal region (**b**) of *Libytheana carinenta* (Libytheinae); proximal (**c**) and distal region (**d**) of *Biblis hyperia* (Biblidinae); proximal (**e**) and distal region (**f**) of *Chlosyne lacinia* (Nymphalinae); proximal (**g**) and distal region (**h**) of *Dione juno* (Heliconiinae); proximal (**i**) and distal region (**j**) of *Cymothoe herminia* (Limenitidinae); proximal (**k**) and distal region (**l**) of *Melanitis leda* (Satyrinae).

4.1.3. Sensilla trichodea

The total length of the sensilla trichodea ranges between 2.8 μm and 279.2 μm (Figure 4, Appendix Table 6). Sensilla trichodea are divided into three categories: (1) short sensilla trichodea (average length of ten sensilla shorter than 50 μm), (2) sensilla trichodea of intermediate length (average length of ten sensilla shorter than 100 μm) and (3) long sensilla trichodea (average length of ten sensilla longer than 100 μm).

Apart from considerable differences concerning the sensilla length, also the distribution of the differently sized sensilla trichodea along the proboscis varies between species. Five different categories can be distinguished: (1) the average sensilla length can decrease from the proximal to the distal region, (2) or vice-versa. (3) The average sensilla length can be longest in the mid region and shortest in the distal region or (4) longest in the mid region and shortest in the proximal region of the proboscis. (5) The average length can be longest at the proximal region and shortest in the mid region.

In most cases the average length of the sensilla trichodea does not change much between the three proboscis regions. Only in a few species (*Dione juno*, *Dryadula phaetusa*, *Catonephele numilia*, *Myscelia orsis*, *Vanessa myrinna*) the sensilla in the proximal region are on average at least three times longer than in the mid region of the proboscis.

4.1.4. Sensilla basiconica on the outer galeal wall

This type of sensilla is rather inconspicuous, due to their small size. They are numerous and arranged in several irregular rows along the entire proboscis length. Because they are hardly recognizable using light microscopy, they are not studied in detail in the various taxa.

4.1.5. Length of the tip region

The length of the tip region varies conspicuously among species (Figure 5, Appendix Table 4). The total length varies between 0.6 mm and 3.8 mm. The relative tip region length ranges between 5.5 % and 30.6 % of the total proboscis length. Species are divided into two groups: (1) species with a short tip region (relative tip region length shorter than 15 % of the total proboscis length) and (2) species with a long tip region (relative tip region length higher than 15 % of the total proboscis length).

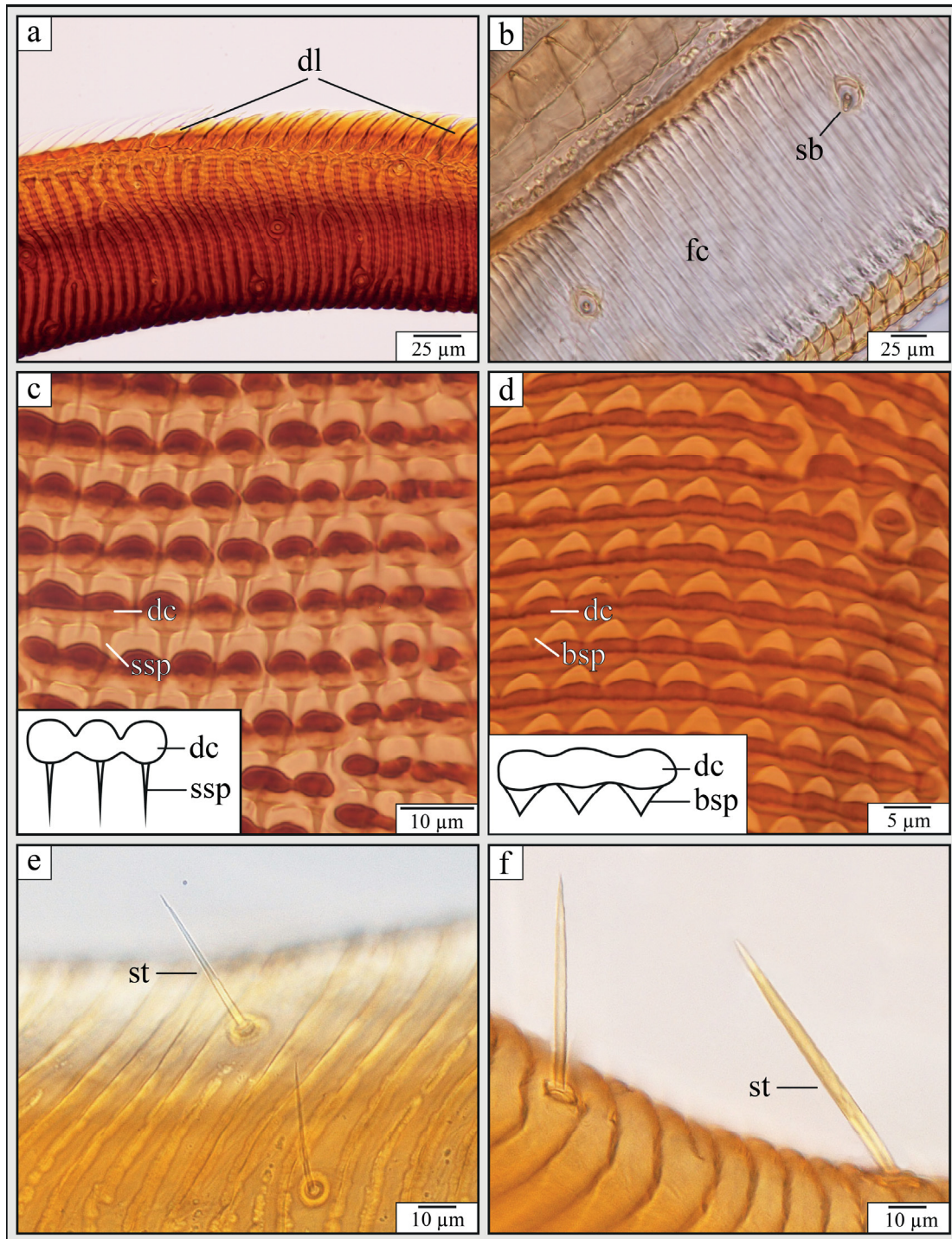


Figure 3. (a) End of the tip region characterized by modified dorsal legulae (dl), which become gradually shorter (*Amauris niavius*). (b) Sensilla basiconica (sb) in the food canal (fc) of *Cymothoe herminia*. (c) Slender and acute spines (ssp) originating from areas of dark cuticle (dc) (*Amauris niavius*). (d) Broad and triangular spines (bsp) originating from areas of dark cuticle (dc) (*Biblis hyperia*). (e) Short sensilla trichodea at 25 % proboscis length in *Aterica galene*. (f) Sensilla trichodea of intermediate length at 50 % proboscis length in *Aterica galene*.

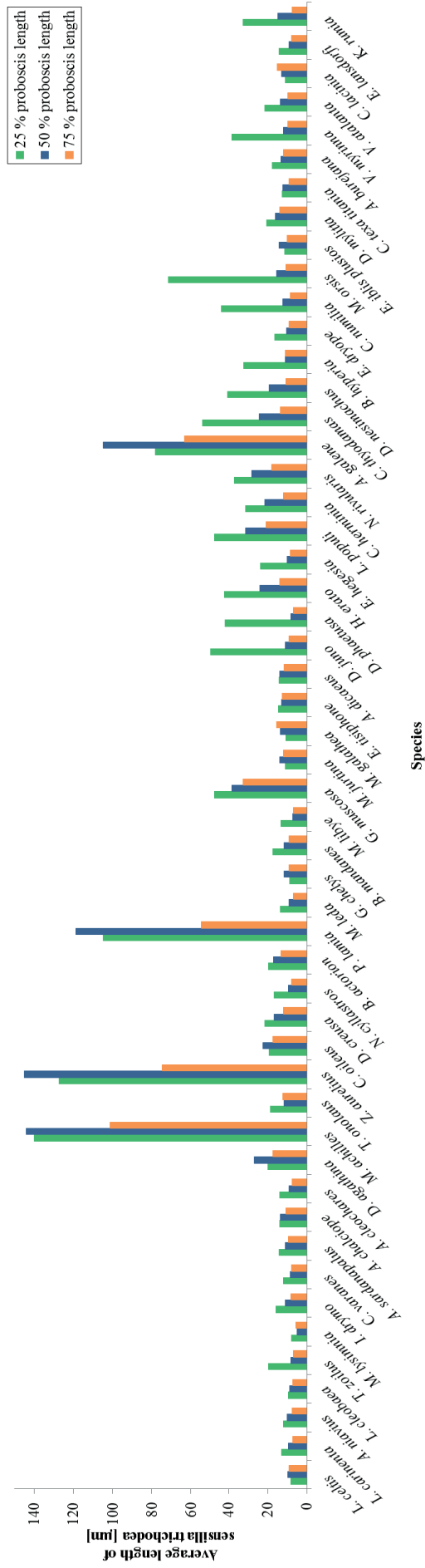


Figure 4. Average length of sensilla trichodea at 25 %, 50 % and 75 % proboscis length of the investigated nymphalid species.

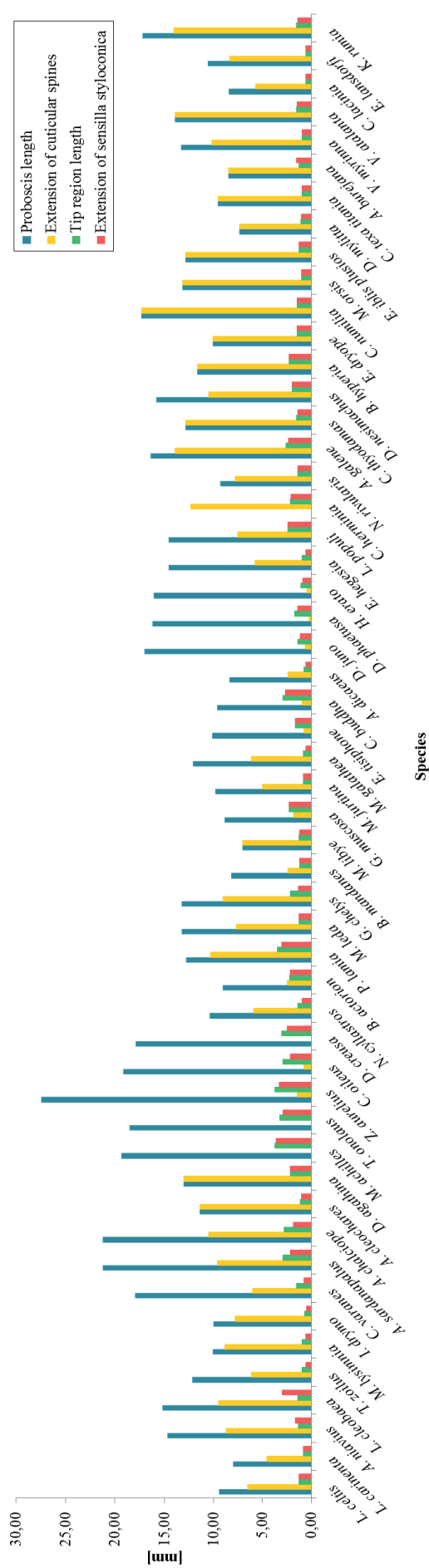


Figure 5. Proboscis length, extension of cuticular spines, tip region length and extension of sensilla styloconica of the investigated nymphalid species.

4.1.6. Sensilla styloconica

The extension of sensilla styloconica on the tip region varies greatly and ranges between 0.5 mm and 3.6 mm (Figure 5, Appendix Table 4). The relative extension of sensilla styloconica ranges between 4.1 % and 27.6 % of the total proboscis length. In most of the species the extension of sensilla styloconica corresponds approximately to the length of the tip region.

Along the tip region sensilla styloconica are either arranged in one row (Figure 6b, 6h, 6i), two rows (Figure 6c, 6d, 6f, 6g, 6j) or three rows (Figure 6e) on one galea (Appendix Table 5). If the sensilla are arranged in two or three rows, they are either arranged on the dorsal side of the tip region (Figure 6f, 6g, 6j) or distributed across the whole proboscis tip (Figure 6a, 6c, 6d, 6e).

The number of sensilla styloconica on both galeae ranges between 15 sensilla (*Mechanitis lysimnia*) and 375 sensilla (*Biblis hyperia*). Density values range from 2.5 sensilla per 100 μm (*Lycorea cleobaea*) to 16.7 sensilla per 100 μm (*Neptis rivularis*). Sensilla styloconica can be arranged very tightly, forming a brush-like structure (Figure 6f, 6g, 6j) or they are more widely spaced, not touching each other (Figure 6a - 6e, 6h, 6i).

The average stylus length ranges between 3.5 μm (S.D. = 0.5, N = 10) in *Mechanitis lysimnia* and 141.9 μm (S.D. = 10.8, N = 10) in *Neptis rivularis*. According to the average stylus length, sensilla styloconica are divided into three categories: (1) short sensilla styloconica (average stylus length of ten sensilla shorter than 50 μm), (2) sensilla styloconica of intermediate length (average stylus length of ten sensilla shorter than 100 μm) and (3) long sensilla styloconica (average stylus length of ten sensilla longer than 100 μm).

The average sensory cone length ranges between 2.5 μm (S.D. = 0.8, N = 10) in *Ithomia drymo* and 36.6 μm (S.D. = 12.1, N = 10) in *Pierella lamia*. Most species have short sensory cones (average length of ten sensory cones shorter than 19.5 μm), long sensory cones (average length of ten sensory cones longer than 19.5 μm) occur only in a few species. In most of the species the stylus is prominent, except for the subfamily of Danainae, where the stylus is extremely reduced, hardly protruding the surface of the outer galea (Figure 6a).

Sensilla styloconica show a remarkable variety concerning their morphology. Classification and nomenclature for sensilla styloconica follows Petr and Stewart (2004). These differences concern the shape of the stylus, the structuring of the stylus surface and the position of the sensory cone as well as the shape, number, and arrangement of appendages on the apical part of the stylus. The shape of the stylus can be cylindrical referred to as cylindriform (Figure 6a - 6d, 6f), flattened referred to as platyform (Figure 6g - 6j) or with an asymmetrical cross-section referred to as asymmetriform (Figure 6e). The surface of the stylus can have a smooth

appearance, the stylus can be equipped with smooth longitudinal ridges termed a pluricarinate stylus (Figure 6d, 6e) or the stylus can have serrated longitudinal ridges termed a pluridentate stylus (Figure 6c). An aspinate stylus has no apical appendages (Figure 6a, 6b, 6i); whereas a paucispinate stylus is equipped with one or two oppositely positioned lobe-shaped appendages (Figure 6j). The apical region of the stylus can be equipped with a circular crown of spines, a so-called equicoronate stylus (Figure 6g) or with an elliptical crown of spines, a so-called inequicoronate stylus (Figure 6h). The sensory cone is either situated centrally (Figure 6a-6h, 6j) or eccentrically on the apical part of the stylus (Figure 6i).

4.1.7. Food canal

In contrast to the sensilla basiconica on the outer galeal wall, sensilla basiconica inside the food canal are arranged in a single row (Figure 3b). In all species the sensilla basiconica are more densely arranged at the basal and the tip region of the proboscis.

The number of sensilla basiconica on one galea ranges between 24 sensilla (*Altinote dicaeus*, *Chlosyne lacinia*) and 70 sensilla (*Taenaris onolaus*). Density values per galea range between 1.9 sensilla per 1 mm (*Dione juno*, *Euptoieta hegesia*) and 5.2 sensilla per 1 mm (*Narope cyllastros*) (Appendix Table 4). Densities up to 3.5 sensilla per 1 mm galeal length are considered as low; densities over 3.5 sensilla per mm galeal length are considered as high.

In most of the investigated species the surface of the food canal is smooth, except for the protruding sensilla basiconica. However, two members of the subfamily Biblidinae (*Eurytela dryope*, *Callicore texa titania*) have small spines on the food canal surface across the entire length.

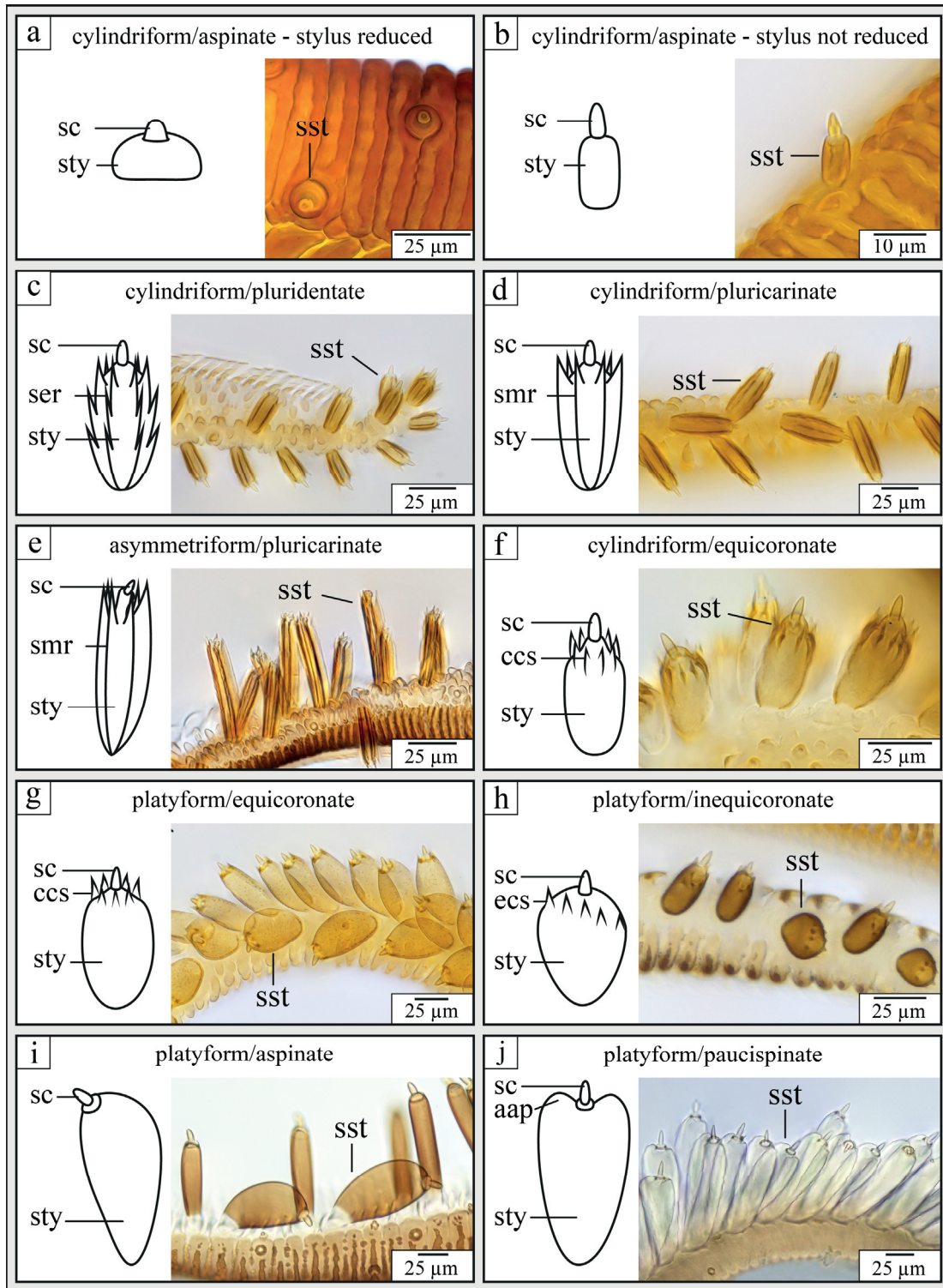


Figure 6. Different types of sensilla styloconica. Every sensillum consists of a stylus (sty) and a sensory cone (sc). The stylus can be equipped with smooth ridges (smr) or serrated ridges (ser). The apical region of the stylus can bear a circular crown of spines (ccs), an elliptical crown of spines (ecs) or lobe-shaped appendages (aap). (a) *Amauris niavius*, (b) *Tellervo zoilus*, (c) *Melanargia galathea*, (d) *Maniola jurtina*, (e) *Libytheana carinenta*, (f) *Araschnia burejana*, (g) *Vanessa atalanta*, (h) *Vanessa myrinna*, (i) *Dryadula phaetusa*, (j) *Aterica galene*.

4.2. Proboscis characters of the investigated nymphalid taxa

Libytheinae

Both species have areas of dark cuticle along the entire proboscis. The stripes of dark cuticle are straight and continuous from the dorsal to the ventral side of the galea (Figure 2a, 2b). Slender and acute spines reach from the basal region to the mid region of the proboscis in both species. The proboscides of both species are equipped with very short sensilla trichodea. On the proboscis of *Libytheana carinenta* the average sensilla length decreases from the proximal to the distal region. The average sensilla length in *Libythea celtis* is longest in the mid region and shortest in the proximal region of the proboscis.

Both species have a short tip region, equal to the extension of sensilla styloconica. Although both species have a high density of sensilla styloconica, they are loosely arranged and scattered over the entire tip region (Figure 6e). The styli of the sensilla styloconica are of intermediate length. The sensory cones are very short. The sensilla styloconica belong to the asymmetriform/pluricarinate type. The sensory cone is located centrally on the apical part of the stylus (Figure 6e). The food canal has a smooth surface. The density of the sensilla basiconica in the food canal is 3.7 sensilla per mm galeal length in *Libythea celtis* and 3.3 sensilla per mm galeal length in *Libytheana carinenta*.

Danainae

The species in this subfamily have dark cuticle along the entire proboscis. In the proximal region the stripes of dark cuticle have the shape of a strings of pearls. In the tribe Danaini (*Amauris niavius*, *Lycorea cleobaea*) the stripes change into individual fragments from the lateral to the ventral side. In the tribes Tellervini (*Tellervo zoilus*) and Ithomiini (*Ithomia drymo*, *Mechanitis lysimnia*) the stripes are continuous from the ventral to the dorsal side of the proboscis. The distal region of the proboscides is characterized by continuous stripes of dark cuticle with a corrugated surface. In all species slender and acute cuticular spines occur beyond the mid region of the proboscis (Figure 3c). All proboscides are equipped with short sensilla trichodea. Actually *Mechanitis lysimnia* has the shortest average sensilla length in all three proboscis regions of all investigated Nymphalidae. *Mechanitis lysimnia* has the longest sensilla trichodea in the proximal region and the shortest at the mid region of the proboscis. In the remaining species of the subfamily the average length of the sensilla trichodea decreases from the proximal to the distal region.

All species have a short tip region and the extension of sensilla styloconia is approximately about the same length. The sensilla are widely spaced in all species. Members of the Danaini

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have three rows, members of the Ithomiini two rows and *Tellervo zoilus* one row of sensilla styloconica. The sensilla of the Danaini and Ithomiini have extremely reduced styli which hardly protrude the galeal surface. They correspond to the cylindriform/aspinate type. The sensory cones are short and located centrally on the apical part of the stylus (Figure 6a). The sensilla styloconica of *Tellervo zoilus* also belong to the cylindriform/aspinate type but they are more prominent than the sensilla of the Danaini and Ithomiini (Figure 6b), even though the average length of the styli is also quite short (mean = 10.4 μm , S.D. = 1.4, N = 10).

All species have a smooth food canal surface. The density of sensilla basiconica ranges between 2.1 sensilla per mm galeal length in *Mechanitis lysimnia* and 3.8 sensilla per mm galeal length in *Ithomia drymo*.

Charaxinae

The surface of the proboscides is without dark cuticle. All species have slender and acute cuticular spines. In all species the spines end before the mid region of the proboscis. All species have short sensilla trichodea along the proboscis, decreasing in length from the proximal to the distal region.

The tip region is short. In all species of the subfamily the coverage of sensilla styloconica does not reach the ending on the intake slits of the tip region, in *Charaxes varanes* the coverage of sensilla styloconica even ends at the middle of the tip region. The sensilla styloconica are arranged in a single row on the dorsal margin of the proboscides. All species have widely spaced sensilla styloconica. The sensilla styloconica of the Charaxinae belong to the platyform/paucispinate type with styli of intermediate length in Preponini and short styli in *Charaxes varanes*. The sensory cones are short and situated centrally.

All species have a smooth food canal surface. The density of sensilla basiconica in the food canal is low and ranges between 2.8 sensilla per mm galeal length in *Agrias sardanapalus* and 3.4 sensilla per mm galeal length in *Charaxes varanes*.

Apaturinae

Both species have areas of dark cuticle across the whole proboscis. In the proximal region of the proboscis *Apatuopsis cleocharis* has straight stripes of dark cuticle that reach from the lateral to the ventral side of the galea. At the dorsal margin the dark cuticle is formed as individual round shaped elements which are arranged in rows. At the distal region the stripes of dark cuticle are straight and continuous from the dorsal to the ventral margin of the proboscis. *Doxocopa agathina* has straight and narrow stripes of dark cuticle in the proximal region. The stripes are surrounded by small and scattered individual elements which are clustered on the

dorsal margin. At the distal region the stripes of dark cuticle have the form of strings of pearls and are continuous from the dorsal to the ventral margin of the galea.

Apaturopsis cleochares has broad and triangular cuticular spines while *Doxocopa agathina* has slender and acute spines. In both species the spines occur along the entire proboscis length.

Both species have short sensilla trichodea in all three proboscis regions. In *Apaturopsis cleochares* the average length of the sensilla trichodea decreases from the proximal to the distal region. *Doxocopa agathina* has the highest average sensilla length in the mid region and the lowest in the proximal region of the proboscis.

The length of the tip region varies greatly between the two species. *Apaturopsis cleochares* has a short tip region, covering 9.9 % of the total proboscis length, while *Doxocopa agathina* has a long tip region, covering 16.6 % of the total proboscis length. The extension of sensilla styloconia is approximately the same length as the tip region. *Apaturopsis cleochares* has two rows and *Doxocopa agathina* three rows of sensilla styloconica. Both species have a brush-like arrangement of sensilla. The sensilla styloconica of *Apaturopsis cleochares* belong to the platyform/paucispinate type and have styli of intermediate length. The centric sensory cones are short. *Doxocopa agathina* has platyform/equicoronate sensilla styloconica with extremely long appendages forming the apical crown. The styli and the centric sensory cones are long.

The food canal has a smooth surface and a low density of sensilla basiconica in both species.

Satyrinae

All species of this subfamily have an outer galeal wall with a smooth and transparent surface without dark cuticle (Figure 2k, 2l).

Cuticular spines are slender and acute. The extension of spines is very diverse within the subfamily. *Morpho achilles*, *Taenaris onolaus* and *Dasyophthalma creusa* have no spines on their proboscides. On the proboscides of *Zeuxidia aurelius*, *Caligo oileus* and *Eteona tisiphone* the cuticular spines are restricted to a short area at the proximal region. In *Godartiana muscosa*, *Bia actorion* and *Bicyclus mandanes* the spines end before the mid region of the proboscis. In *Narope cyllastros*, *Pierella lamia*, *Melanitis leda*, *Gnophodes chelys*, *Maniola jurtina* and *Melanargia galathea* the cuticular spines reach beyond the mid region of the proboscides. *Magneptychia libye* has cuticular spines across the whole proboscis length.

Most of the species have short sensilla trichodea in all three proboscis regions. *Pierella lamia* and *Zeuxidia aurelius* have long sensilla trichodea at 25 % and 50 % proboscis length and sensilla of intermediate length at 75 % proboscis length. *Morpho achilles* has long sensilla trichodea in all three proboscis regions. The distribution of the differently sized sensilla trichodea along the proboscis varies greatly within the members of the subfamily. The average

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length decreases from the proximal to the distal region on the proboscides of *Dasyophthalma creusa*, *Narope cyllastros*, *Bia actorion*, *Melanitis leda*, *Bicyclus mandanes*, *Magneuptychia libye*, *Godartiana muscosa* and *Eteona tisiphone*. In *Melanargia galathea* the average length increases from the proximal to the distal region. *Morpho achilles*, *Zeuxidia aurelius*, *Caligo oileus* and *Pierella lamia* have the longest sensilla at 50 % and the shortest sensilla at 75 % proboscis length. *Gnophodes chelys* and *Maniola jurtina* have the longest sensilla at 50 % and the shortest at 25 % proboscis length. *Taenaris onolaus* has the longest sensilla at 25 % and the shortest at 50 % proboscis length.

The relative length of the tip region can be very short, as in *Melanargia galathea* with a tip region length corresponding to 6.8 % proboscis length, as well as very long like in *Pierella lamia* with a tip region length corresponding to 27.1 % proboscis length. The extension of sensilla styloconica is approximately the length of the tip region. Sensilla styloconica are arranged in two rows on the proboscides of *Taenaris onolaus*, *Caligo oileus*, *Dasyophthalma creusa*, *Maniola jurtina* and *Melanargia galathea*. The other species have one row of sensilla styloconica. In *Narope cyllastros*, *Melanitis leda*, *Maniola jurtina* and *Melanargia galathea* the sensilla styloconica are more widely spaced on the tip region. In the other species the sensilla are densely arranged giving the tip region a brush-like appearance. Within the subfamily sensilla styloconica with short, intermediate and long styli occur. Almost all species have short sensory cones except for *Pierella lamia* where the sensory cones are extremely long (mean = 36.6 μm , S.D. = 12.1, N = 10).

Maniola jurtina has cylindriform/pluricarinate sensilla styloconica (Figure 6d) and *Melanargia galathea* cylindriform/pluridentate sensilla styloconica (Figure 6c). The remaining species of the subfamily have platyform/paucispinate sensilla styloconica. The sensilla styloconica have centric sensory cones.

The food canal has always a smooth surface. The density of the sensilla basiconica ranges between 2.2 sensilla per mm galeal length in *Zeuxidia aurelius* and 5.2 sensilla per mm galeal length in *Narope cyllastros* which is the highest density within the investigated Nymphalidae.

Calinaginae

Calinaga buddha is the only species investigated from the subfamily. The surface of the galeal wall has no dark cuticle and a smooth and transparent appearance. Cuticular spines are slender and acute and are restricted to a short area at the proximal region, covering 9.8 % of the proboscis. Values for the average mean length of the sensilla trichodea are lacking, because most of the sensilla were broken off.

Calinaga buddha has the longest relative tip region length within the investigated Nymphalidae, corresponding to 30.6 % proboscis length. The extension of sensilla styloconica is approximately the length of the tip region. The sensilla styloconica are arranged densely in two rows forming a brush. The styli of the sensilla styloconica are long and correspond to the platyform/paucispinate type. The sensory cones are long and are situated centrally on the apical part of the stylus.

The surface of the food canal is smooth. The density of sensilla basiconica amounts 4.0 sensilla per mm galeal length.

Heliconiinae

In the proximal region of the proboscis the dark cuticle is forming straight and narrow stripes that are surrounded by small and unordered individual elements of dark cuticle. In the distal region there are broad stripes of dark cuticle with a sharp bend on the lateral side (Figure 2g, 2h).

All species have slender and acute cuticular spines. On the proboscides of *Dione juno*, *Dryadula phaetusa* and *Heliconius erato* the spines are restricted to a short area at the proximal region. On the proboscis of *Altinote dicaeus* the spines cover 29.4 % of the total proboscis length and on the proboscis of *Euptoietia hegesia* 39.6 % of the total proboscis length.

All species have short sensilla trichodea in all three proboscis regions, which decrease in length from the proximal to the distal region of the proboscis. In *Dione juno* and *Dryadula phaetusa* the average length of the sensilla trichodea at 25 % proboscis length is many times higher than at 50 % proboscis length. In the other species the length differences between the proboscis regions are not that distinct.

The relative tip region is short in all species of the subfamily and the extension of the sensilla styloconica is always a bit shorter than the tip region. The sensilla styloconica are arranged in one row and widely spaced. All species have short sensory cones but the styli of the sensilla styloconica can be short, of intermediate length or long. The sensilla styloconica belong to the platyform/aspinate type with an eccentrically situated sensory cone (Figure 6i).

The surface of the food canal is smooth. The density of sensilla basiconica is very low in all species.

Limnitiidinae

Areas of dark cuticle occur only in the proximal region of the proboscis. The dark cuticle is forming straight and narrow stripes that are surrounded by small and unordered individual elements of dark cuticle. The distal region is without dark cuticle (Figure 2k, 2l).

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All species have slender and acute cuticular spines and they extend over the mid region of the proboscis.

Most of the investigated species have short sensilla trichodea in all three proboscis regions which decrease in length from the proximal to the distal region. Only *Aterica galene* has sensilla trichodea of intermediate length at 25 % proboscis length and long sensilla trichodea at 50 % and 75 % proboscis length.

The relative tip region length is rather similar within the subfamily and ranges between 14.9 % total proboscis length in *Cymothoe herminia* and 16.8 % total proboscis length in *Limenitis populi*. The extension of sensilla styloconica corresponds approximately to the tip region length. The sensilla styloconica are arranged in one row in *Cymothoe herminia* and in two rows in the other species. The sensilla styloconica have long styli and are very densely arranged forming a brush-like structure. *Limenitis populi* has sensilla styloconica with long sensory cones; the other species have short sensory cones. The sensilla styloconica correspond to the platyform/paucispinate type with centrally situated sensory cones (Figure 6j).

The surface of the food canal is smooth. Density of sensilla basiconica ranges between 2.9 sensilla per mm galeal length in *Cymothoe herminia* and 4.8 sensilla per mm galeal length in *Neptis rivularis*.

Cyrestinae

Cyrestis thyodamas was the only species investigated from this subfamily. In the proximal and distal region of the proboscis the dark cuticle is shaped like strings of pearls. Cuticular spines are slender and acute and occur across the whole proboscis length.

At 25 % proboscis length the sensilla trichodea are of intermediate length and short at 50 % and 75 % proboscis length. The average mean length decreases from the proximal to the distal region.

The tip region length corresponds to 12.0 % of the total proboscis length. The extension of sensilla styloconica is a little bit shorter than the tip region, corresponding to 11.1 % of the total proboscis length. The sensilla styloconica are arranged in three rows on one galea and have a density of 11.4 sensilla per 100 μm . The sensilla are sparsely arranged and distributed across the whole tip region. The styli as well as the sensory cones of the sensilla are short. They correspond to the asymmetriform/pluricarinate type with a centrally situated sensory cone.

The surface of the food canal is smooth. The density of sensilla basiconica is low with 2.9 sensilla per mm galeal length.

Pseudergolinae

Dichorragia nesimachus is the only species investigated from this subfamily. In the proximal and distal region of the proboscis the dark cuticle is shaped like strings of pearls.

Slender and acute cuticular spines occur over the mid region of the proboscis.

The proboscis is equipped with short sensilla trichodea in all three proboscis regions which decrease in length from the proximal to the distal region.

The tip region is short, corresponding to 12.4 % of the total proboscis length. The extension of sensilla styloconica corresponds to the tip region length.

Sensilla styloconica are densely arranged in one row. The sensory cones are short and situated centrally on the apical part of the stylus. The sensilla belong to the platyform/paucispinate type.

The surface of the food canal is smooth. The density of the sensilla basiconica amounts 3.8 sensilla per mm galeal length.

Biblidinae

All species of the subfamily have dark cuticle on the entire proboscis. In the proximal region the dark cuticle is shaped like strings of pearls (Figure 2c). In the distal region the stripes of dark cuticle can be either straight (*Biblis hyperia*, *Eurytela dryope*, *Myscelia orsis* and *Callicore texa titania*) (Figure 2d) or shaped like strings of pearls (*Catonephele numilia*, *Epiphile iblis plusios* and *Dynamine mylitta*).

All species have broad and triangular spines that extend over the whole proboscis length (Figure 3d).

The sensilla trichodea are short in all three proboscis regions in all species except *Myscelia orsis* where the sensilla at 25 % proboscis length are of intermediate length. On the proboscides of *Myscelia orsis* and *Catonephele numilia* the sensilla trichodea at 25 % proboscis length are many times longer than the sensilla at 50 % proboscis length. In the remaining species the average sensilla length does not differ that much between the three proboscis regions. The average sensilla length decreases from the proximal to the distal region, except for *Epiphile iblis plusios*, where the average sensilla length is longest at 50 % proboscis length and shortest at 75 % proboscis length.

The relative tip region length varies greatly among the investigated species and ranges between 7.6 % of the total proboscis length in *Myscelia orsis* and 19.8 % of the total proboscis length in *Biblis hyperia*. The extension of sensilla styloconica corresponds to the length of the tip region in all species.

The sensilla styloconica are arranged in three rows in *Biblis hyperia*, in two rows in *Eurytela dryope*, *Catonephele numilia*, *Epiphile iblis plusios* and *Dynamine mylitta* and in one row per

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galea in *Myscelia orsis* and *Callicore texa titania*. The sensilla are densely arranged, forming a brush-like structure in all species.

Four different types of sensilla styloconica are found within the subfamily. Proboscides can be equipped with platyform/equicoronate, platyform/paucispinate, platyform/semicoronate or platyform/aspinate sensilla. The sensory cones are short and situated centrally on the apical part of the styli. The styli are of intermediate length, only *Biblis hyperia* has sensilla with long sensilla styloconica.

Most of the species have a food canal with a smooth surface. Only in *Eurytela dryope* and *Callicore texa titania* the surface of the food canal is equipped with small spines. The density of sensilla basiconica in the food canal ranges between 3.2 sensilla per mm galeal length in *Eurytel dryope* and 4.6 sensilla per mm galeal length in *Dynamine mylitta*.

Nymphalinae

All species have areas of dark cuticle along the entire proboscis. *Araschnia burejana* and *Kallimoides rumia* have continuous stripes of dark cuticle shaped like strings of pearls in the proximal and the distal region of the proboscis. *Chlosyne lacinia* and *Eresia lansdorfi* have stripes of dark cuticle shaped like strings of pearls in the proximal region which change into corrugated stripes of dark cuticle in the distal region (Figure 2e, 2d). *Vanessa myrinna* has straight stripes of dark cuticle in the proximal region and corrugated stripes in the distal region. *Vanessa atalanta* has straight stripes of dark cuticle in the proximal region and stripes shaped like strings of pearls in the distal proboscis region.

All species have slender and acute cuticular spines. In *Araschnia burejana* and *Vanessa atalanta* spines occur beyond the whole proboscis length. In the remaining species the spines extend over the mid region of the proboscis.

All species have short sensilla trichodea in all three proboscis regions. The average length of the sensilla does not change much between the three proboscis regions except for *Vanessa myrinna*, where the average sensilla length at 25 % proboscis length is three times higher than at 50 % proboscis length. On the proboscis of *Chlosyne lacinia* the average sensilla length increases from the proximal to the distal region. In the remaining species the average sensilla length decreases from the proximal to the distal region.

The relative tip region is short in all species and ranges between 5.5 % of the total proboscis length in *Eresia lansdorfi* and 14.9 % of the total proboscis length in *Araschnia burejana*. Actually *Eresia lansdorfi* has the shortest relative tip region length within the investigated Nymphalidae.

The extension of sensilla styloconica corresponds to the length of the tip region. In *Araschnia burejana* and *Vanessa atalanta* the sensilla styloconica are arranged in two rows, which are restricted to the dorsal side of the tip region. The other species have one row of sensilla styloconica per galea. Sensilla are densely arranged in *Araschnia burejana* and *Vanessa atalanta* and more sparsely arranged in the remaining species. *Araschnia burejana* and *Vanessa atalanta* have short sensilla styloconica, the other species have sensilla styloconica of intermediate length. All species have short sensory cones.

Five different types of sensilla styloconica occur within the investigated species. *Araschnia burejana* has cylindriform/equicoronate sensilla, *Vanessa myrinna* platyform/ inequicoronate sensilla, *Vanessa atalanta* platyform/equicoronate sensilla, *Kallimoides rumia* platyform/paucispinate sensilla and *Chlosyne lacinia* and *Eresia lansdorfi* have platyform/aspinate sensilla. The platyform/aspinate sensilla have eccentrically sensory cones, the other sensilla types have centrally situated sensory cones.

The surface of the food canal has a smooth appearance. The density of sensilla basiconica ranges between 2.1 sensilla per mm galeal length in *Vanessa myrinna* and 3.7 sensilla per mm galeal length in *Araschnia burejana*.

4.3 Comparison of feeding guilds

Nymphalid butterflies can be divided into two feeding guilds, flower-visiting and non-flower-visiting species (DeVries 1987, DeVries 1988, Fermon et al. 2000, Fermon et al. 2001, Krenn et al. 2001, Kawahara 2006, Mollemann et al. 2006). The investigated species, classified after primary food source, are listed in Table 2. Several proboscis characters were tested statistically in order to find out if they are related to feeding habits.

Extension of cuticular spines

Extension is expressed as percentage of total proboscis length. The average extension is 59.1 % (S.D. = 30.7) in flower-visiting species and 57.9 % (S.D. = 37.6) in non-flower-visiting species. No significant difference was detected between feeding guilds (Mann-Whitney *U* test, $N = 53$, $Z = -0.101$, $P = 0.920$).

Length of sensilla trichodea

The average length of sensilla trichodea at 25 % proboscis length is 21.7 μm (S.D. = 14.4) in flower-visiting species and 34.7 μm (S.D. = 34.3) in non-flower-visiting species. At 50 % proboscis length the average length is 12.3 μm (S.D. = 4.7) in flower-visiting species and 29.4 μm (S.D. = 39.2) in non-flower-visiting species. At 75 % proboscis length the average length is 10.1 μm (S.D. = 3.0) in flower-visiting species and 19.2 μm (S.D. = 22.5) in non-flower-visiting species. The average length of sensilla trichodea at 25 % and 75 % proboscis length do not differ significantly between the feeding guilds. But the average length of sensilla trichodea at 50 % proboscis length is significantly longer in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney *U* test, $N = 52$, $Z = -2.351$, $P = 0.019$).

Relative tip region length

The relative tip region length is expressed as percentage of total proboscis length. The average relative length in flower-visiting species is 9.3 % (S.D. = 2.8) and in non-flower-visiting species 15.5 % (S.D. = 5.6). The relative tip region length is significantly longer in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney *U* test, $N = 53$, $Z = -4.447$, $P = 0.0001$).

Extension of sensilla styloconica

The extension of sensilla styloconica is expressed as percentage of total proboscis length. In flower-visiting species the average extension is 8.9 % (S.D. = 4.4) and 14.1 % (S.D. = 5.6) in non-flower-visiting species. The extension of sensilla styloconica is significantly longer in the

non-flower-visiting species than in the flower-visiting species (Mann-Whitney U test, $N = 53$, $Z = -3.392$, $P = 0.001$).

Number of sensilla styloconica rows per galea

The number of rows did not differ significantly between the two feeding guilds (Mann-Whitney U test, $N = 53$, $Z = -1.239$, $P = 0.215$).

Density of sensilla styloconica

The average density in flower-visiting species is 7.4 sensilla per 100 μm (S.D. = 4.2) and 9.7 sensilla per 100 μm (S.D. = 2.9) in non-flower-visiting species. The density of sensilla styloconica is significantly higher in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney U test, $N = 53$, $Z = -2.483$, $P = 0.013$).

Sensilla styloconica - stylus length

The average stylus length in flower-visiting-species is 44.4 μm (S.D. = 27.5) and 90.7 μm (S.D. = 31.2) in non-flower-visiting species. The average stylus length is significantly longer in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney U test, $N = 53$, $Z = -7.728$, $P = 0.001$).

Sensilla styloconica - sensory cone length

The average sensory cone length in flower-visiting species is 6.9 μm (S.D. = 2.8) and 12.3 μm (S.D. = 5.9) in non-flower-visiting species. The average sensory cone length is significantly longer in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney U test, $N = 53$, $Z = -4.375$, $P = 0.001$).

Density of sensilla basiconica

The average density in flower-visiting species is 2.9 sensilla per 1 mm (S.D. = 0.7) and 3.8 sensilla per 1 mm (S.D. = 0.8) in non-flower-visiting species. The density of sensilla basiconica is significantly higher in the non-flower-visiting species than in the flower-visiting species (Mann-Whitney U test, $N = 52$, $Z = -3.663$, $P = 0.0001$).

Table 2. Classification of investigated species arranged according to food preference (DeVries 1987, DeVries 1988, Fermon et al. 2000, Fermon et al. 2001, Krenn et al. 2001, Kawahara 2006, Mollemann et al. 2006).

Primarily flower-visiting species	Primarily non-flower-visiting species
Libytheinae	Charaxinae
<i>Libythea celtis</i>	<i>Charaxes varanes</i>
<i>Libytheana carinenta</i>	<i>Agrias sardanapalus</i>
Danainae	<i>Archaeoprepona chalciope</i>
<i>Amauris niavius</i>	Apaturinae
<i>Lycorea cleobaea</i>	<i>Apaturopsis cleochares</i>
<i>Tellervo zoilus</i>	<i>Doxocopa agathina</i>
<i>Mechanitis lysimnia</i>	Satyrinae
<i>Ithomia drymo</i>	<i>Morpho achilles</i>
Satyrinae	<i>Taenaris onolaus</i>
<i>Maniola jurtina</i>	<i>Zeuxidia aurelius</i>
<i>Melanargia galathea</i>	<i>Caligo oileus</i>
Heliconiinae	<i>Dasyophthalma creusa</i>
<i>Altinote dicaeus</i>	<i>Narope cyllastros</i>
<i>Dione junio</i>	<i>Bia actorion</i>
<i>Dryadula phaetusa</i>	<i>Pierella lamia</i>
<i>Heliconius erato</i>	<i>Melanitis leda</i>
<i>Euptoieta hegesia</i>	<i>Gnophodes chelys</i>
Cyrestinae	<i>Bicyclus mandanes</i>
<i>Cyrestis thyodamas</i>	<i>Magneptychia libye</i>
Biblidinae	<i>Godartiana muscosa</i>
<i>Dynamine mylitta</i>	<i>Eteona tisiphone</i>
Nymphalinae	Calinaginae
<i>Araschnia burejana</i>	<i>Calinaga buddha</i>
<i>Vanessa myrinna</i>	Limenitidinae
<i>Vanessa atalanta</i>	<i>Limenitis populi</i>
<i>Chlosyne lacinia</i>	<i>Cymothoe herminia</i>
<i>Eresia lansdorfi</i>	<i>Neptis rivularis</i>
	<i>Aterica galene</i>
	Pseudergolinae
	<i>Dichorragia nesimachus</i>
	Biblidinae
	<i>Biblis hyperia</i>
	<i>Eurytela dryope</i>
	<i>Catonephele numilia</i>
	<i>Myscelia orsis</i>
	<i>Epiphile iblis plusios</i>
	<i>Callicore texa titania</i>
	Nymphalinae
	<i>Kallimoides rumia</i>

4.4. Character polarity

Twelve morphological characters have been selected for the character matrix (Appendix Table 3). The plesiomorphic character states were determined by the condition of the outgroup species. In order to find out if derived character states developed convergently or not, character states were mapped onto a cladogram based on the latest nymphalid phylogeny (Wahlberg et al. 2009) (Figure 7). The plesiomorphic character states are assigned with 0, derived character states are assigned with 1 to 3 and are unordered. Species are listed for the respective character states, the outgroup species are in parentheses. Characters with ambiguous plesiomorphic character states, such as the extension of cuticular spines, the length of the tip region, the number of rows of sensilla styloconica and the structuring of the stylus surface were excluded from character coding, since these characters varied highly in the outgroup species.

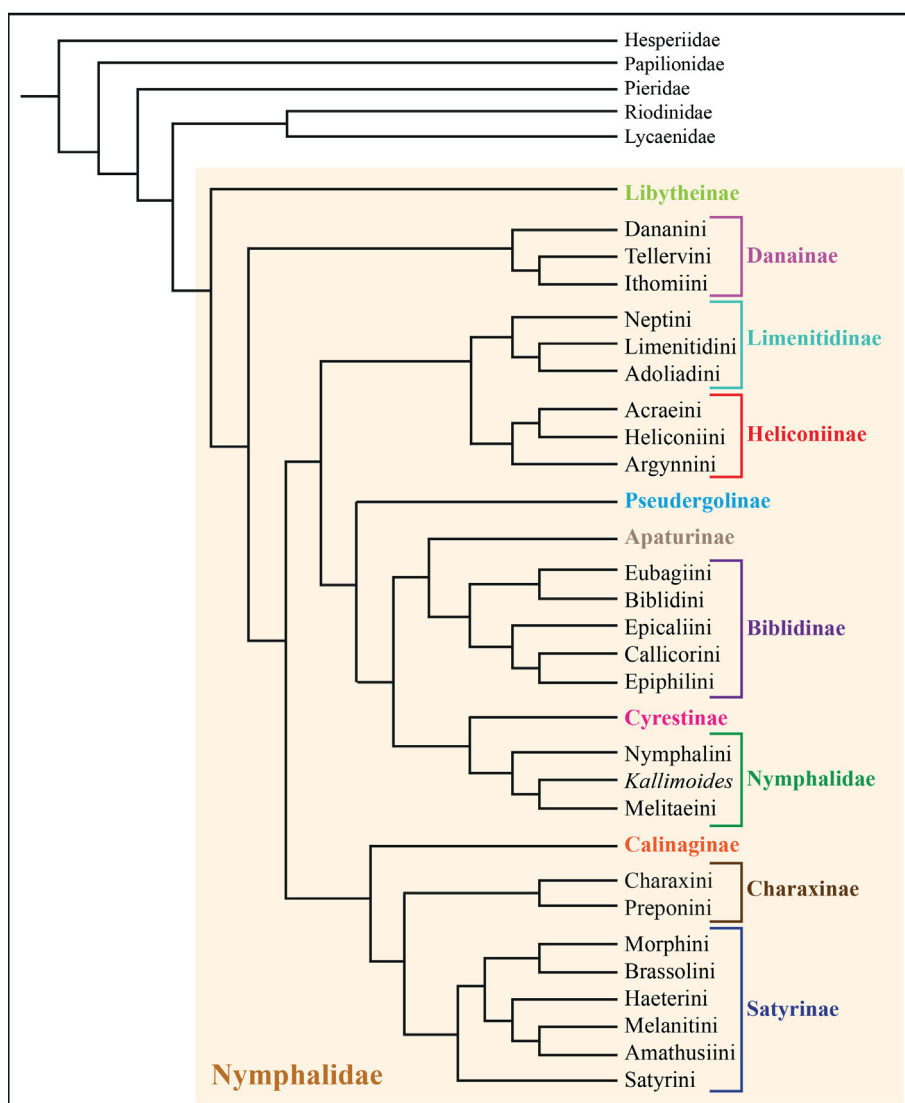


Figure 7. Cladogram of phylogenetic relationship of subfamilies (colour-coded) and tribes of Nymphalidae and outgroups (modified after Wahlberg et al. 2009).

Character 1. Composition of the outer galeal wall:

0: vertical and straight stripes of dark cuticle along the entire proboscis

Libytheinae, Danainae, Apaturinae, Cyrestinae, Pseudergolinae, Biblidinae, Nymphalinae (Lycaenidae, Pieridae, Papilionidae, Hesperidae)

1: vertical stripes of dark cuticle along the entire proboscis with a characteristic bend in the distal region

Heliconiinae

2: dark cuticle restricted to the proximal region of the proboscis

Limenitidinae

3: proboscides without dark cuticle

Charaxinae, Satyrinae, Calinaginae

Character 2. Shape of cuticular spines:

0: cuticular spines slender and acute

Libytheinae, Danainae, Charaxinae, Apaturinae: *Doxocopa agathina*, Satyrinae, Calinaginae, Heliconiinae, Limenitidinae, Cyrestinae, Pseudergolinae, Nymphalinae (Lycaenidae, Pieridae, Papilionidae, Hesperidae)

1: cuticular spines broad and triangular

Biblidinae, Apaturinae: *Apaturopsis cleochares*

Character 3. Average length of sensilla trichodea at 25 %, 50 % and 75 % proboscis length:

0: short sensilla trichodea (mean value under 50 µm) in all three proboscis regions

All species except those with character state 1 (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: average length of sensilla trichodea, at least in one proboscis region, longer than 50 µm

Satyrinae: *Morpho achilles*, *Zeuxidia aurelius*, *Pierella lamia*, Limenitidinae: *Aterica galene*, Cyrestinae, Biblidinae: *Myscelia orsis*

Character 4. Variability of average sensilla trichodea length between proboscis regions:

0: average sensilla length is less than three times longer between neighbouring proboscis regions

All species except those with character state 1 (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: average sensilla length is more than three times longer between neighbouring proboscis regions

Heliconiinae: *Dione juno*, *Dryadula phaetusa*, Biblidinae: *Catonephele numilia*, *Myscelia orsis*, Nymphalinae: *Vanessa myrinae*

Character 5. Length of the tip region in comparison to the extension of sensilla styloconica:

0: extension of sensilla styloconica approximately the length of the tip region

All species except those with character state 1 (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: extension of sensilla styloconica half the length of the tip region

Charaxinae: *Charaxes varanes*

Character 6. If sensilla styloconica are arranged in two or three rows on one galea, the rows are:

0: distributed on the dorsal and lateral side of the galea

Libytheinae, Danainae: *Amauria niavius*, *Lycorea clyobea*, *Mechanitis lysimnia*, *Ithomia drymo*, Satyrinae: *Maniola jurtina*, *Melanargia galathea*, Cyrestinae (Lycaenidae, Riodinidae: *Riodina refrecta*, Pieridae, Papilionidae)

1: restricted to the dorsal side of the galea

Apaturinae, Satyrinae: *Taenaris onolaus*, *Caligo oileus*, *Dasyophthalma creusa*, Calinaginae, Limenitidinae: *Limenitis populi*, *Neptis rivularis*, *Aterica galene*, Biblidinae: *Biblis hyperia*, *Eurytelia dryope*, *Catonephele numilia*, *Epiphile iblis plusios*, *Dynamine mylitta*, Nymphalinae: *Araschnia burejana*

Character 7. Density of sensilla styloconica:

0: sensilla styloconica are arranged sparsely

Libytheinae, Danainae, Charaxinae, Satyrinae: *Narope cyllastros*, *Melanitis leda*, *Maniola jurtina*, *Melanargia galathea*, Heliconiinae, Cyrestinae, Nymphalinae: *Vanessa myrina*, *Chlosyne lacinia*, *Eresia lansdorfi*, *Kallimoides rumia* (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae).

1: sensilla styloconia are arranged densely by touching each other and forming a brush-like structure

Apaturinae, Satyrinae: *Morpho achilles*, *Taenaris onolaus*, *Zeuxidia aurelius*, *Caligo oileus*, *Dasyophthalma creusa*, *Bia actorion*, *Pierella lamia*, *Gnophodes chelys*, *Bicyclus mandanes*, *Magneptychia libye*, *Godartiana muscosa*, *Eteona tisiphone*, Calinaginae, Limenitidinae, Pseudergolinae, Biblidinae, Nymphalinae: *Araschnia burejana*, *Vanessa atalanta*.

Character 8. Sensilla styloconica - length of the sensory cone:

0: average sensory cone length is lower than 19.5 μ m

Libytheinae, Danainae, Charaxinae, Apaturinae: *Apaturopsis cleochares*, Satyrinae: *Morpho achilles*, *Taenaris onolaus*, *Zeuxidia aurelius*, *Caligo oileus*, *Dasyophthalma creusa*, *Narope cyllastros*, *Bia actorion*, *Melanitis leda*, *Gnophodes chelys*, *Bicyclus mandanes*, *Magneptychia libye*, *Godartiana muscosa*, *Maniola jurtina*, *Melanargia galathea*, *Eteona tisiphone*, Heliconiinae, Limenitidinae: *Cymothoe herminia*, *Neptis rivularis*, *Aterica galene*, Cyrestinae, Pseudergolinae, Biblidinae, Nymphalinae (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: average sensory cone length is higher than 19.5 μ m

Apaturinae: *Doxocopa agathina*, Satyrinae: *Pierella lamia*, Calinaginae, Limenitidinae: *Limenitis populi*

Character 9. Sensilla styloconica - shape of the stylus:

0: cylindriform

Danainae, Satyrinae: *Maniola jurtina*, *Melanargia galathea*, Nymphalinae: *Araschnia burejana* (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: platyform

Charaxinae, Apaturinae, Satyrinae: *Morpho Achilles*, *Taenaris onolaus*, *Zeuxidia aurelius*, *Caligo oileus*, *Dasyophthalma creusa*, *Narope cyllastros*, *Bia actorion*, *Pierella lamia*, *Melanitis leda*, *Gnophodes chelys*, *Bicyclus mandanes*, *Magneptychia libye*, *Godartiana muscosa*, *Eteona tisiphone*, Calinaginae, Heliconiinae, Limenitidinae, Pseudergolinae, Biblidinae, Nymphalinae: *Vanessa myrinna*, *Vanessa atalanta*, *Cholsyne lacinia*, *Eresia lansdorfi*, *Kallimoides rumia*

2: asymmetriform

Libytheinae, Cyrestinae

Character 10. Sensilla styloconica - position of the sensory cone:**0: the sensory cone is situated at the centre of the apical part of the stylus:**

All species except those with character state 1 (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae).

1: the sensory cone originates lateral from the longitudinal axis of the apical part of the stylus

Heliconiinae, Nymphalinae: *Chlosyne lacinia*, *Eresia lansdorfi*

Character 11. Surface of the food canal:**0: surface of the food canal without spines**

All species except those with character state 1 (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: surface of the food canal is equipped with small spines

Biblidinae: *Eurytela dryope*, *Callicore texa titania*

Character 12. Density of Sensilla basiconica in the food canal:**0: lower than 3.6 sensilla per mm**

Libytheinae: *Libythea celtis*, Danainae: *Amauris niavius*, *Lycorea cleobaea*, *Tellervo zoilus*, *Mechanitis lysimnia*, Charaxinae, Apaturinae, Satyrinae: *Zeuxidia aurelius*, *Caligo oileus*, *Dasyophtalma creusa*, *Gnophodes chelys*, *Melanargia galathea*, Heliconiinae, Limenitidinae: *Cymothoe herminia*, *Aterica galene*, Cyrestinae, Biblidinae: *Eurytela dryope*, *Callicore texa titania*, Nymphalinae: *Vanessa myrinna*, *Vanessa atalanta*, *Chlosyne lacinia*, *Eresia lansdorfi*, *Kallimoides rumia* (Lycaenidae, Riodinidae, Pieridae, Papilionidae, Hesperidae)

1: higher than 3.6 sensilla per mm

Libytheinae: *Libytheana carinenta*, Danainae: *Ithomia drymo*, Satyrinae: *Taenaris onolaus*, *Narope cyllastros*, *Bia actorion*, *Pierella lamia*, *Melanitis leda*, *Bicyclus mandanes*, *Magneptychia libye*, *Godartiana muscosa*, *Maniola jurtina*, *Eteona tisiphone*, Calinaginae, Limenitidinae: *Limenitis populi*, *Neptis rivularis*, Pseudergolinae, Biblidinae: *Biblis hyperia*, *Catonephele numilia*, *Myscelia orsis*, *Epiphile iblis plusios*, *Dynamine mylitta*, Nymphalinae: *Araschnia burejana*

5. Disussion

By mapping the character states onto a cladogram, based on the latest nymphalid phylogeny (Wahlberg et al. 2009) (Figure 7), the results indicate that the composition of the outer galeal wall and the shape of the cuticular spines may be useful for phylogenetic analysis. The most likely plesiomorphic condition for the composition of the outer galeal wall of the Nymphalidae is a proboscis with vertical stripes of dark cuticle along the entire proboscis (Figure 2a-2f). This condition was also found in all examined outgroup species except for the Riodinidae. Other studies on proboscis morphology show that this, as plesiomorphic regarded galeal wall composition also occurs in other Papilionoidea and Hesperidae (Eastham and Eassa 1955, Hepburn 1971, Paulus and Krenn 1996). Derived character states are proboscides where the dark cuticle shows a characteristic bend on the lateral side of the distal galea, a potential autapomorphy of Heliconiinae (Figure 2h); a progressing loss and break-up of the dark cuticle resulting in proboscides where the dark cuticle is restricted to the proximal region, which is a potential autapomorphy for the Limenitidinae (Figure 2i, 2j), and a total loss of dark cuticle resulting in a uniform surface structure which is a possible synapomorphy for Calinaginae, Charaxinae and Satyrinae (Figure 2k, 2l). Based on the latest study on nymphalid phylogeny (Wahlberg et al. 2009), the derived character state of a proboscis with no dark cuticle as found in Calinaginae, Charaxinae and Satyrinae has evolved convergently to the character state found in Limenitidinae, where the distal region is composed of light cuticle. Therefore it is assumed that a loss of dark cuticle has evolved two times independently within the Nymphalidae. It is assumed that the differently composed galeal walls lead to a different degree of proboscis flexibility. The dark cuticle is interpreted as hard exocuticle and the light cuticle as a combination of more flexible endo- and mesocuticle (Hepburn 1971).

Even though the composition of the outer galeal wall seems to reflect the phylogenetic relationship within the Nymphalidae, a correlation with food preferences cannot be ruled out. It is suggested that a larger amount of dark cuticle causes an inelastic proboscis which is more suitable for butterflies foraging nectar and therefore have to insert the proboscis into narrow floral tubes. On the other hand, feeding from exposed fluids does not require a very stiff proboscis and the presence of dark cuticle is not necessarily needed (Krenn et al. 2001). A connection between food sources and galeal composition is reported in Noctuidae, where eye-frequenting and sweat-feeding species show a different morphology than skin- and fruit-piercing species (Büttiker et al. 1996). However the present study also shows that butterflies feeding on nectar can have proboscides composed of light cuticle, e.g. in *Melanargia galathea*

and *Maniola jurtina*. Whereas in non-flower-visiting butterflies (e.g. Biblidinae) proboscides with dark cuticle along the entire length can be found.

Because the musculature of the galeae has not been investigated in this study, and studies on the mechanical properties of the cuticle are lacking, functional aspects of the different arrangements of dark- and light-coloured proboscis cuticle cannot be answered satisfactorily.

Slender and acute spines are regarded as the plesiomorphic condition in Nymphalidae because they occur in nearly all investigated outgroup species. Broad and triangular spines distributed over the entire proboscis are reported for Pieridae, which probably represents an autapomorphy for this taxon (Paulus and Krenn 1996). Within the Nymphalidae broad and triangular spines, restricted to the ventral side of the proboscis are a derived character state and occur in all Biblidinae and *Apaturopsis cleochares* (Apaturinae). These two subfamilies have been proposed as sistergroups (Wahlberg et al. 2009). Since only two species of Apaturinae were investigated it is not clear if broad and triangular spines are a potential synapomorphy of Biblidinae and Apaturinae. Under this assumption slender and acute spines must have evolved a second time within the Apaturinae. On the other hand broad and triangular spines could have evolved convergently within Biblidinae and Apaturinae. Further taxon sampling of basal apaturine species would be necessary for clarification.

Cuticular spines on the ventral side of the proximal galea are regarded the plesiomorphic condition within the Rhopalocera (Paulus and Krenn 1996). In this study the assessment of the plesiomorphic condition was not possible, because the extension of cuticular spines was inconsistent among the outgroup species. No cuticular spines are probably a derived character state within the Nymphalidae. Based on the latest study on nymphalid phylogeny (Wahlberg et al. 2009), each character state obviously developed several times independently. Statistical analyses indicate that there is no connection between the extension of cuticular spines and food preference. It is assumed that the cuticular spines are responsible for the stabilization of the proboscis in the resting position by anchoring into surface structures of the consecutive coil (Krenn 1990, Krenn et al. 2005, Krenn 2010). If no spines are present, the resting position might be secured by cuticular spines on the ventral side of the head (Krenn 1990).

The results show that broad and triangular spines are always distributed along the entire proboscis length. This may indicate that in these taxa the spines not only stabilize the proboscis but have an unknown additional function.

Short sensilla trichodea are probably the plesiomorphic condition within the Nymphalidae. Only six investigated species had an average sensilla length higher than 50 μm in at least one proboscis region. This elongation of sensilla obviously developed several times independently. In the mid region of the proboscis sensilla trichodea are significantly longer in the non-flower-visiting species than in the flower-visiting species. Additionally, in five species with mostly short sensilla trichodea, the average sensilla length in the proximal region was at least three times longer than the average sensilla length in the mid region of the proboscis. These derived states seem to be convergent developments as well. Sensilla trichodea are mechanoreceptors which may give information about the diameter of the corolla, the depth of proboscis insertion during flower probing and the correct resting position of the proboscis (Paulus and Krenn 1996, Krenn 1998, Krenn et al. 2005). In flower-visiting species longer sensilla trichodea in the mid region of the proboscis could hamper the insertion of the proboscis into narrow corollae. Krenn and Penz (1998) pointed out that pollen-feeding Heliconiinae have more numerous and long sensilla trichodea in the proximal and mid region of the proboscis than non-pollen-feeding species. This seems to be an adaptation to this special feeding habit by supporting the attachment of pollen grains for further processing (Krenn and Penz 1998, Gilbert 1972). The results indicate that elongated sensilla trichodea on the mid region of the proboscis are possible adaptations to special feeding habits in non-flower-visiting species and developed several times independently within Nymphalidae. If they are also necessary for the attachment of food particles, like in pollen-feeding Heliconiinae, is not yet known.

The relative tip region length, the extension and density of sensilla styloconica are significantly greater in the non-flower-visiting species. Also the length of the styli and the length of sensory cones are significantly longer in the non-flower-visiting guild. Various studies assumed that these features are adaptations for a more efficient exploitation of alternative food sources like decaying fruits, tree sap or dung (Petr and Stewart 2004, Krenn et al. 2001, Knopp and Krenn 2003). For efficient food intake, it is important that all intake slits of the tip region are immersed in the food source (Krenn et al. 2001, Krenn et al. 2005). In flowers often only a small amount of nectar is available, therefore a short tip region would be of advantage for flower-visiting species. However, on exposed food sources, with a larger and irregular surface area like rotting fruits, food can more efficiently taken up with a long tip region because all intake slits can be easily immersed into the fluid (Krenn et al. 2001).

The extension of sensilla styloconica matches the length of the tip region in most species. An exception represents *Charaxes varanes*, a fruit-piercing species where the tip region is

approximately double the length than the extension of sensilla styloconcia. Previous studies showed that in fruit-piercing species the region, where intake slits occur is much longer than the area that bears the sensilla styloconcia (Mollemann et al. 2005).

Sensilla styloconica are regarded as combined chemo/mechanosensilla that respond to sucrose solution and may also help to find the opening of corolla tubes due to tactile stimuli (Altner and Altner 1986, Krenn 1998, Walters et al. 1998). Long and densely arranged sensilla styloconia, giving the tip region a brush-like appearance, act in combination as a sensitive enlargement of the tip that provides, due to capillary action, the gathering of liquid from larger areas (Paulus and Krenn 1996, Petr and Stewart 2004). It has been shown that *Morpho peleides*, a non-flower-visiting species with long and densely arranged sensilla styloconica, can suck up fluids more efficiently from wet surfaces than the flower-visiting species *Vanessa cardui*. On the other hand *Morpho peleides* is not able to insert the proboscis into narrow corollae because of the long sensilla styloconica (Knopp and Krenn 2003). Therefore it is obvious that flower-visiting species have an advantage of short sensilla styloconica. *Charaxes varanes* has a different proboscis morphology than the remaining non-flower-visiting species. Their proboscides are characterized by short and sparsely distributed sensilla styloconica which are restricted to a particular short area on the tip region. These proboscis characters seem to be adaptations to the piercing-technique, where the butterfly penetrates fruits with its proboscis (Mollemann et al. 2005, Krenn et al. 2001). Highly modified structures in the tip region as in fruit- and skin-piercing Noctuidae (Büttiker et al. 1996, Mollemann et al. 2005, Zaspel et al. 2011) did not occur in Charaxinae.

An extreme reduction of the stylus is found in Ithomiini and Dananini, where the styli of the sensilla hardly protrude the surface of the galeal wall. Similar sensilla also occur in Papilionidae (Paulus and Krenn 1996, Petr and Stewart 2004), Riodinidae (Bauder et al. 2013) and some investigated Pieridae. It is assumed that the absence of prominent sensilla styloconica is an adaptation to foraging in narrow corollas even with a thick proboscis and evolved several times independently (Paulus and Krenn 1996, Petr and Stewart 2004). Differently sized sensilla styloconica on one proboscis, like in some Lycaenidae and Riodinidae (Paulus and Krenn 1996, Petr and Stewart 2004, Bauder et al. 2013) did not occur within the Nymphalidae. Based on the latest phylogeny of the examined taxa (Wahlberg et al. 2009) these extremely reduced sensilla styloconica evolved at least two times independently in the Dananinae.

Cylindriform/pluricarinate sensilla styloconica are regarded the plesiomorphic condition within the Papilionoidea (Paulus and Krenn 1996, Krenn 2010). This condition occurred only in the investigated *Maniola jurtina* (Satyrinae). The most common sensilla type within the

Nymphalidae was platyform/paucispinate. However, since this type did not occur in any outgroup species; it can be assumed that it is a derived character state within the Nymphalidae, which evolved several time independently. The assessment of the plesiomorphic condition was not possible because three different sensilla types occurred in the outgroup species, cylindriform/pluricarinate, cylindriform/pluridentate and cylindriform/aspinate. These results suggest that a cylindriform stylus is probably the plesiomorphic condition within the Nymphalidae and platyform and asymmetriform styli are derived characters. It can further be assumed, that sensilla with a circular (equicoronate) or elliptical crown (inequicoronate) of spines or lobe-shaped appendages (paucispinate) on the apical region of the stylus are derived characters within the Nymphalidae. Libytheinae, Charaxinae, Heliconiinae and Limenitidinae were characterized by one sensilla type each. The other subfamilies show a higher variability concerning the sensilla styloconica. Comparing the two feeding guilds, it is noticeable that flower-visiting species showed a greater variability of sensilla types than the non-flower-visiting species, which conforms to the study of Krenn et al. (2001). In all investigated non-flower-visiting species the sensilla styloconica belong to the platyform type, which is maybe the best shape for building the tight arrangement of a brush-like structure for a more efficient food uptake from exposed food sources. Ridges on the styli could be interpreted to facilitate fluid transport to the intake slits or giving the sensilla more stability (Paulus and Krenn 1996, Petr and Stewart 2004). Appendages on the apical part of the stylus may protect the sensory cone against mechanical damage (Petr and Stewart 2004). In the investigated flower-visiting species that have two or more rows of sensilla styloconica, the sensilla are usually spread over the entire tip region. In non-flower-visiting species with several rows of sensilla they are always arranged on the dorsal side of the tip region, enabling the formation of a brush-like structure.

Apart from their occurrence on the outer galeal wall, sensilla basiconica are present over the entire length of the food canal and are arranged in one row. They are considered as chemoreceptive sensilla that respond to sucrose and may also give information about the flow rate of the ingested fluid (Altner and Altner 1986, Krenn 1998, Paulus and Krenn 1996, Walters et al. 1998, Inoue et al. 2009). In *Vanessa cardui* the sensilla basiconica are more densely arranged in the basal and the tip region of the proboscis (Krenn 1998). This result is confirmed by at hand. Additionally the investigated non-flower-visiting species have a significantly higher density of sensilla basiconica in the food canal than flower-visiting species. A possible

explanation could be that the feeding on exposed food sources requires more information about the composition of the imbibed fluid than drinking nectar from a floral tube.

A uniformly composed food canal consisting of smooth plates is found in all Glossata with a functionally intact proboscis. Only in some Hepialoidea with a reduced and non-functional proboscis also the surface of the medial wall of the galea is equipped with spines (Krenn and Kristensen 2000). Two members of the subfamily Biblidinae have small spines on the surface of the food canal, although the proboscis is not reduced and fully functional. These spines seem to be convergent developments, because they were found in two species which belong to different tribes (Biblidini, Callicorini) of the Biblidinae, which are not considered to be sister taxa. The functional aspects of spines in the food canal remain unclear.

In conclusion the most probable plesiomorphic condition of the Nymphalidae is represented by a proboscis with straight stripes of dark cuticle across the entire length, which is equipped with slender and acute spines on the ventral side. The sensilla trichodea are short and the average length does not substantially change in the course of the proboscis. The sensilla styloconica have a cylindriform stylus with a short and centrally situated sensory cone. They are widely spaced and occur along the entire tip region. The food canal is smooth and has a low density of sensilla basiconica.

6. References

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7. Zusammenfassung

Bei 53 Vertretern aus allen Unterfamilien der Nymphalidae wurden Merkmale des Rüssels mittels Lichtmikroskopie untersucht. Der Aufbau der Galeawand, Sensilla trichodea, Rüsselspitzenlänge, Anzahl, Länge und Form der Sensilla styloconica sowie Merkmale des Nahrungskanals wurden für alle untersuchten Arten beschrieben und miteinander verglichen. Das Ziel der Arbeit war, Rüsselmerkmale zu identifizieren, die für phylogenetische Studien verwertbar sein könnten. Für den Außengruppenvergleich wurden 10 Vertreter von Riodinidae, Lycaenidae, Pieridae, Papilionidae und Hesperidae herangezogen. Der Aufbau der Galeawand und die Form von kutikulären Dornen, die auf der Außenseite des Rüssels vorkommen, kennzeichnen gewisse Taxa innerhalb der Nymphalidae und könnten Rückschlüsse auf verwandtschaftliche Beziehungen der Unterfamilien zueinander geben. Alle Vertreter der Libytheinae, Danainae, Apaturinae, Cyrestinae, Pseudergolinae, Biblidinae und Nymphalinae haben dieselbe Ausprägung der Galeawand, die über den ganzen Rüssel durchgehende und gerade Leisten von dunkler Cuticula aufweist, was wahrscheinlich auch den plesiomorphen Zustand der Nymphalidae repräsentiert. Eine potentielle Autapomorphie der Heliconiinae ist eine Galeawand mit dunklen Cuticulaleisten über den gesamten Rüssel, die jedoch im distalen Bereich einen scharfen Knick auf der lateralen Seite zeigen. Eine mögliche Autapomorphie der Limenitidinae sind dunkle Cuticulaleisten im proximalen Bereich und einen distalen Bereich der nur aus heller, einheitlicher Cuticula besteht und keine dunkle Cuticula aufweist. Die Vertreter von Charaxinae, Calinaginae und Satyrinae haben als gemeinsames Merkmal eine Galea, die nur aus heller und einheitlich geformter Cuticula aufgebaut ist. Schmale und sehr spitze Dornen sind wahrscheinlich der plesiomorphe Zustand der Nymphalidae. Davon leiten sich breite, dreieckige Dornen ab, die bei allen Vertretern der Biblidinae und einem Vertreter der Apaturinae vorkommen.

Der Großteil der untersuchten Rüsselmerkmale hängt wahrscheinlich mit der unterschiedlichen Nahrungsaufnahme der Arten zusammen. Blütenbesuchenden Schmetterlingen und Arten die andere Nahrungsquellen nutzen zeigen signifikante Unterschiede in ihrer Rüsselmorphologie. Nichtblütenbesuchende Arten haben längere Sensilla trichodea in der Rüsselmitte, eine längere Rüsselspitze, längere und dichter angeordnete Sensilla styloconica und dichter stehende Sensilla basiconica im Nahrungskanal.

8. Acknowledgements

First of all I am grateful to Harald Krenn for excellent and long-standing supervision. I thank Martin Lödl (Natural History Museum Vienna) for providing material. Thanks to Julia Bauder, Florian Karolyi, Kerstin Stejskal, Martin Hepner and Barbara Gereben-Krenn for their support and friendship. Very special thanks are dedicated to my parents Werner and Ursula, my sister Vera, and Rupert.

9. Curriculum vitae

Nora Lieskonig



Ausbildung

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2001 - 2006	Ferialpraktikum im Sommer als Tierpflegerin im Tiergarten Schönbrunn

10. Appendix

Table 3. Character matrix including 12 proboscis characters of investigated Nymphalidae and outgroup species. 0...plesiomorphic character state; 1, 2, 3...derived character states; –...character absent; x...missing data; *...character state only present in outgroup species.

Ingroup species	Characters											
	1	2	3	4	5	6	7	8	9	10	11	12
Libytheinae												
<i>L. celtis</i>	0	0	0	0	0	0	0	0	2	0	0	0
<i>L. carinenta</i>	0	0	0	0	0	0	0	0	2	0	0	1
Danainae												
<i>A. niavius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>L. cleobaea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>T. zoilus</i>	0	0	0	0	0	-	0	0	0	0	0	0
<i>M. lysimnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>I. drymo</i>	0	0	0	0	0	0	0	0	0	0	0	1
Charaxinae												
<i>C. varanes</i>	3	0	0	0	1	-	0	0	1	0	0	0
<i>A. sardanapalus</i>	3	0	0	0	0	-	0	0	1	0	0	0
<i>A. chalciope</i>	3	0	0	0	0	-	0	0	1	0	0	0
Apaturinae												
<i>A. cleochares</i>	0	1	0	0	0	1	1	0	1	0	0	0
<i>D. agathina</i>	0	0	0	0	0	1	1	1	1	0	0	0
Satyrinae												
<i>M. achilles</i>	3	-	1	0	0	-	1	0	1	0	0	x
<i>T. onolaus</i>	3	-	0	0	0	1	1	0	1	0	0	1
<i>Z. aurelius</i>	3	0	1	0	0	-	1	0	1	0	0	0
<i>C. oileus</i>	3	0	0	0	0	1	1	0	1	0	0	0
<i>D. creusa</i>	3	-	0	0	0	1	1	0	1	0	0	0
<i>N. cyllastros</i>	3	0	0	0	0	-	0	0	1	0	0	1
<i>B. actorion</i>	3	0	0	0	0	-	1	0	1	0	0	1
<i>P. lamia</i>	3	0	1	0	0	-	1	1	1	0	0	1
<i>M. leda</i>	3	0	0	0	0	-	0	0	1	0	0	1

Table 3. continued

Ingroup species	Characters											
	1 composition of outer galeal wall	2 shape of cuticular spines	3 average length sensilla trichodea	4 variability of sensilla trichodea length	5 extension sensilla styloconica on tip region	6 distribution of 2 or 3 rows of sensilla styloconica	7 sensilla styloconica/ density	8 sensilla styloconica/ length sensory cone	9 sensilla styloconica/ shape of stylus	10 sensilla styloconica/ position sensory cone	11 surface of food canal	12 sensilla basiconica/ density food canal
<i>G. chelys</i>	3	0	0	0	0	-	1	0	1	0	0	0
<i>B. mandanes</i>	3	0	0	0	0	-	1	0	1	0	0	1
<i>M. libye</i>	3	0	0	0	0	-	1	0	1	0	0	1
<i>G. muscosa</i>	3	0	0	0	0	-	1	0	1	0	0	1
<i>M. jurina</i>	3	0	0	0	0	0	0	0	0	0	0	1
<i>M. galathea</i>	3	0	0	0	0	0	0	0	0	0	0	0
<i>E. tisiphone</i>	3	0	0	0	0	-	1	0	1	0	0	1
Calinaginae												
<i>C. buddha</i>	3	0	0	0	0	1	1	1	1	0	0	1
Heliconinae												
<i>A. dicaeus</i>	1	0	0	0	0	-	0	0	1	1	0	0
<i>D. juno</i>	1	0	0	1	0	-	0	0	1	1	0	0
<i>D. phaeusa</i>	1	0	0	1	0	-	0	0	1	1	0	0
<i>H. erato</i>	1	0	0	0	0	-	0	0	1	1	0	0
<i>E. hegesia</i>	1	0	0	0	0	-	0	0	1	1	0	0
Limnitiidinae												
<i>L. populi</i>	2	0	0	0	0	1	1	1	1	0	0	1
<i>C. herminia</i>	2	0	0	0	0	-	1	0	1	0	0	0
<i>N. rivularis</i>	2	0	0	0	0	1	1	0	1	0	0	1
<i>A. galene</i>	2	0	1	0	0	1	1	0	1	0	0	0
Cyrestinae												
<i>C. thyodamas</i>	0	0	1	0	0	0	0	0	2	0	0	0
Pseudergolinae												
<i>D. nesimachus</i>	0	0	0	0	0	1	1	0	1	0	0	1
Biblidinae												
<i>B. hyperia</i>	0	1	0	0	0	1	1	0	1	0	0	1
<i>E. dryope</i>	0	1	0	0	0	1	1	0	1	0	1	0
<i>C. numilia</i>	0	1	0	1	0	1	1	0	1	0	0	1

Table 3. continued

	Characters											
	1 composition of outer galeal wall	2 shape of cuticular spines	3 average length sensilla trichodea	4 variability of sensilla trichodea length	5 extension sensilla styloconica on tip region	6 distribution of 2 or 3 rows of sensilla styloconica	7 sensilla styloconica/ density	8 sensilla styloconica/ length sensory cone	9 sensilla styloconica/ shape of stylus	10 sensilla styloconica/ position sensory cone	11 surface of food canal	12 sensilla basiconica/ density food canal
Ingroup species												
<i>M. orsis</i>	0	1	1	1	0	-	1	0	1	0	0	1
<i>E. iblis plusios</i>	0	1	0	0	0	1	1	0	1	0	0	1
<i>D. mylitta</i>	0	1	0	0	0	1	1	0	1	0	0	1
<i>C. texa titania</i>	0	1	0	0	0	-	1	0	1	0	1	0
Nymphalinae												
<i>A. burejana</i>	0	0	0	0	0	1	1	0	0	0	0	1
<i>V. myrina</i>	0	0	0	1	0	-	0	0	1	0	0	0
<i>V. atalanta</i>	0	0	0	0	0	-	1	0	1	0	0	0
<i>C. lacinia</i>	0	0	0	0	0	-	0	0	1	1	0	0
<i>E. lansdorfii</i>	0	0	0	0	0	-	0	0	1	1	0	0
<i>K. rumia</i>	0	0	0	0	0	-	0	0	1	0	0	0
Outgroup species												
Lycaenidae												
<i>L. phlaeas</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>L. phydela</i>	0	0	0	0	0	0	0	0	0	0	0	0
Riodinidae												
<i>R. refracta</i>	0	*	0	0	0	0	0	0	0	0	0	0
<i>C. calamisa</i>	0	*	0	0	0	-	0	0	0	0	0	0
Pieridae												
<i>D. belsama</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. bryoniae</i>	0	0	0	0	0	0	0	0	0	0	0	0
Papilionidae												
<i>G. sarpedon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. machaon</i>	0	0	0	0	0	0	0	0	0	0	0	0
Hesperiidae												
<i>E. montanus</i>	0	0	0	0	0	-	0	0	0	0	0	0
<i>E. rafflesia</i>	0	0	0	0	0	-	0	0	0	0	0	0

Table 4. Morphometry of the proboscides and associated characters of all investigated Nymphalidae and outgroup species. %...percentage of total proboscis length.

species	Proboscis length (mm)	Extension of cuticular spines (mm)	Tip-region length (mm)	Extension of sensilla styloconica (mm)	Extension of cuticular spines (%)	Tip-region length (%)	Extension of sensilla styloconica (%)
Libytheinae							
<i>L. celtis</i>	9.48	6.52	1.31	1.29	68.8	13.8	13.6
<i>L. carinenta</i>	7.92	4.52	0.87	0.86	57.1	11.0	10.9
Danainae							
<i>A. niavius</i>	14.67	8.79	1.35	1.67	59.9	9.2	11.4
<i>L. cleobaea</i>	15.17	9.50	1.42	2.98	62.6	9.4	19.6
<i>T. zoilus</i>	12.19	6.13	0.94	0.56	50.3	7.7	4.6
<i>M. lysimnia</i>	10.06	8.87	0.98	0.58	63.1	7.0	4.1
<i>I. drymo</i>	10.04	7.73	0.71	0.50	77.0	7.1	5.0
Charaxinae							
<i>C. varanes</i>	17.96	6.00	1.52	0.75	33.4	8.5	4.2
<i>A. sardanapalus</i>	21.21	9.62	2.89	2.14	45.4	13.6	10.1
<i>A. chalciope</i>	21.21	10.50	2.81	1.87	49.5	13.2	8.8
Apaturinae							
<i>A. cleochares</i>	11.42	11.42	1.13	1.06	100.0	9.9	9.3
<i>D. agathina</i>	13.06	13.06	2.17	2.17	100.0	16.6	16.6
Satyrinae							
<i>M. achilles</i>	19.33	0.00	3.71	3.61	0.00	19.2	18.7
<i>T. onolaus</i>	18.50	0.00	3.23	2.89	0.00	17.5	15.6
<i>Z. aurelius</i>	27.44	1.46	3.75	3.31	5.30	13.7	12.1
<i>C. oileus</i>	19.13	0.77	2.94	2.14	4.00	15.4	11.3
<i>D. creusa</i>	17.88	0.00	3.03	2.46	0.00	16.9	13.8
<i>N. cyllastros</i>	10.42	5.86	1.40	0.95	56.2	13.4	9.1
<i>B. actorion</i>	9.10	2.45	2.21	2.14	26.9	24.3	23.5
<i>P. lamia</i>	12.79	10.35	3.46	3.02	80.9	27.1	23.6
<i>M. leda</i>	13.25	7.62	1.27	1.27	57.5	9.6	9.6
<i>G. chelys</i>	13.25	9.06	2.15	1.35	68.4	16.2	10.2
<i>B. mandanes</i>	8.15	2.44	1.19	1.19	29.9	14.6	14.6
<i>M. libye</i>	7.02	7.02	1.29	1.24	100.0	18.4	17.7
<i>G. muscosa</i>	8.92	1.83	2.31	2.31	20.5	25.9	25.9
<i>M. jurtina</i>	9.81	5.02	0.81	0.81	51.2	8.3	8.3
<i>M. galathea</i>	12.08	6.10	0.83	0.60	50.5	6.9	5.0
<i>E. tisiphone</i>	10.15	0.77	1.63	1.63	7.6	16.1	16.1
Calinaginae							
<i>C. buddha</i>	9.62	0.94	2.94	2.65	9.8	30.6	27.5
Heliconiinae							
<i>A. dicaeus</i>	8.31	2.44	0.77	0.56	29.4	9.3	6.7
<i>D. juno</i>	17.00	0.67	1.42	1.13	3.9	8.4	6.6
<i>D. phaetusa</i>	16.21	0.29	1.75	1.42	1.8	10.8	8.8
<i>H. erato</i>	16.06	0.44	1.12	0.88	2.7	7.0	5.5
<i>E. hegesia</i>	14.52	5.75	0.98	0.62	39.6	6.7	4.3

Table 4. continued

species	Proboscis length (mm)	Extension of cuticular spines (mm)	Tip-region length (mm)	Extension of sensilla styloconica (mm)	Extension of cuticular spines (%)	Tip-region length (%)	Extension of sensilla styloconica (%)
Limenitidinae							
<i>L. populi</i>	14.56	7.50	2.44	2.44	51.5	16.8	16.8
<i>C. herminia</i>	14.56	12.38	2.17	2.08	85.0	14.9	14.3
<i>N. rivularis</i>	9.31	7.73	1.40	1.40	83.0	15.0	15.0
<i>A. galene</i>	16.4	13.94	2.60	2.38	85.0	15.9	14.5
Cyrestinae							
<i>C. thyodamas</i>	12.85	12.85	1.54	1.42	100.0	12.0	11.1
Pseudergolinae							
<i>D. nesimachus</i>	15.81	10.54	1.96	1.96	66.7	12.4	12.4
Biblidinae							
<i>B. hyperia</i>	11.63	11.63	2.31	2.31	100.0	19.9	19.9
<i>E. dryope</i>	10.10	10.10	1.48	1.48	100.0	14.7	14.7
<i>C. numilia</i>	17.29	17.29	1.48	1.48	100.0	8.6	8.6
<i>M. orsis</i>	13.17	13.17	1.00	1.00	100.0	7.6	7.6
<i>E. iblis plusios</i>	12.88	12.88	1.31	1.31	100.0	10.2	10.2
<i>D. mylitta</i>	7.33	7.33	1.10	1.10	100.0	15.0	13.9
<i>C. texa titania</i>	9.56	9.56	0.94	0.94	100.0	9.8	9.8
Nymphalinae							
<i>A. burejana</i>	8.5	8.5	1.27	1.52	100.0	14.9	17.9
<i>V. myrinna</i>	13.31	10.19	0.94	0.94	76.6	7.1	7.1
<i>V. atalanta</i>	13.92	13.92	1.53	1.48	100.0	11.0	10.6
<i>C. lacinia</i>	8.37	5.67	0.62	0.60	67.7	7.4	7.2
<i>E. lansdorfi</i>	10.58	8.33	0.58	0.58	78.7	5.5	5.5
<i>K. rumia</i>	17.21	14.04	1.51	1.38	81.6	8.8	8.8
Lycaenidae							
<i>L. phlaeas</i>	8.06	8.05	0.60	0.63	100.0	7.4	7.8
<i>L. phydela</i>	5.98	5.98	1.20	1.20	100.0	20.1	20.1
Riodinidae							
<i>R. refracta</i>	9.64	4.03	1.54	1.39	41.8	16.0	14.4
<i>C. calamisa</i>	6.34	1.94	0.36	0.36	30.6	5.7	5.7
Pieridae							
<i>D. belisama</i>	15.71	3.80	1.67	1.19	24.2	10.6	7.6
<i>P. bryoniae</i>	11.19	11.19	0.71	0.64	100.0	6.3	5.7
Papilionidae							
<i>G. sarpedon</i>	13.13	5.40	2.06	3.98	41.1	15.7	30.3
<i>P. machaon</i>	17.42	6.10	3.13	2.48	35.0	18.0	14.2
Hesperiidae							
<i>E. montanus</i>	11.87	5.40	0.44	0.40	45.5	3.7	3.4
<i>E. rafflesia</i>	19.29	7.20	0.94	0.83	37.3	4.9	4.3

Table 5. Morphometrics of sensilla styloconica and sensilla basiconica of the food canal. x... missing data.

species	Sensilla styloconica							Sensilla basiconica (food canal)					
	Rows of sensilla on one galea	Number of sensilla on both galeae	Density (sensilla/100 μ m)	stylus length			sensory cone length			Number of sensilla on one galea	Density (sensilla/mm)		
Libytheinae													
<i>L. celtis</i>	3	162	12.6	56.6	67.9	45.3	6.9	4.2	5.7	3.8	0.8	35	3.7
<i>L. carinenta</i>	3	142	16.6	56.4	69.8	39.6	13.8	3.6	3.8	2.1	0.5	25	3.3
Danainae													
<i>A. niavius</i>	3	92	5.5	4.1	5.3	3.4	0.6	3.6	3.8	2.8	0.4	45	3.2
<i>L. cleobaea</i>	3	75	2.5	4.6	5.7	3.6	0.7	3.8	4.0	3.6	0.1	40	2.6
<i>T. zoilus</i>	1	17	3.0	10.4	12.3	7.5	1.4	5.2	5.7	3.8	0.7	36	3.0
<i>M. lysimnia</i>	2	15	2.6	3.5	3.8	2.8	0.5	2.8	4.0	1.9	0.7	28	2.1
<i>I. drymo</i>	2	17	3.4	3.6	4.7	2.8	0.6	2.5	3.8	1.7	0.8	38	3.8
Charaxinae													
<i>C. varanes</i>	1	52	6.9	27.5	30.2	24.5	1.6	5.9	7.9	5.3	0.7	61	3.4
<i>A. sardanapalus</i>	1	129	6.0	75.7	84.9	66.0	6.5	12.3	13.2	9.4	1.3	59	2.8
<i>A. chalciope</i>	1	112	6.0	68.1	75.5	56.6	4.8	11.7	15.1	9.4	1.9	69	3.3
Apaturinae													
<i>A. cleochares</i>	2	118	11.2	74.9	83.0	60.4	7.0	9.2	11.3	7.5	1.1	40	3.5
<i>D. agathina</i>	3	337	15.5	129.2	156.6	92.5	23.5	20.0	26.4	14.2	4.4	45	3.4
Satyrinae													
<i>M. achilles</i>	1	266	7.4	124.3	137.7	111.3	9.9	13.4	17.0	11.3	1.7	x	x
<i>T. onolaus</i>	2	240	8.3	73.4	94.3	64.2	12.7	10.3	11.3	9.4	0.9	70	3.8
<i>Z. aurelius</i>	1	214	6.5	140.6	164.2	120.8	16.4	13.8	17.0	11.3	2.0	59	2.2
<i>C. oileus</i>	2	147	6.8	120.4	137.7	103.8	13.9	9.5	11.7	7.5	1.3	58	3.0
<i>D. creusa</i>	2	202	8.2	129.6	141.5	113.2	9.6	9.7	13.2	7.5	2.1	62	3.5
<i>N. cyllastros</i>	1	66	6.9	50.0	54.7	41.5	3.7	9.6	11.3	7.5	1.1	54	5.2
<i>B. actorion</i>	1	194	9.1	69.7	81.1	58.5	7.4	10.0	11.3	7.5	1.3	46	5.1
<i>P. lamia</i>	1	280	9.3	92.3	109.4	75.5	9.8	36.6	50.9	18.9	12.1	48	3.8
<i>M. leda</i>	1	93	7.3	55.8	62.3	50.9	3.9	6.8	7.5	5.7	1.0	50	3.8
<i>G. chebys</i>	1	84	6.2	70.0	73.6	64.2	3.3	7.7	9.4	5.7	1.1	35	2.7

Table 5. continued

species	Sensilla styloconica					Sensilla basiconica (food canal)			
	Rows of sensilla on one galea	Number of sensilla on both galeae	Density (sensilla/100 μm)	stylus length			sensory cone length		
				mean (μm)	max (μm)	min (μm)	mean (μm)	max (μm)	S.D.
<i>B. mandanes</i>	1	141	11.8	64.6	68.9	59.4	8.7	10.4	1.1
<i>M. tibye</i>	1	127	10.2	61.4	67.0	54.7	11.6	14.2	1.5
<i>G. muscosa</i>	1	245	10.6	61.3	69.8	45.3	9.2	11.3	1.9
<i>M. jurtina</i>	2	73	9.0	26.2	28.3	15.1	6.8	7.5	1.3
<i>M. galathea</i>	2	55	9.2	44.5	47.2	41.5	7.4	7.5	0.6
<i>E. tisiphone</i>	1	142	8.7	66.6	75.5	60.4	11.7	13.2	1.5
Calinaginae									
<i>C. buddha</i>	2	334	12.6	125.7	147.2	101.9	19.6	22.6	2.2
Heliconinae									
<i>A. dicaeus</i>	1	26	4.7	48.0	51.9	45.3	7.5	9.4	1.0
<i>D. juno</i>	1	46	4.1	70.7	77.4	60.4	9.2	11.3	1.0
<i>D. phaetusa</i>	1	61	4.3	108.7	117.0	94.3	13.8	15.1	0.9
<i>H. erato</i>	1	45	5.1	62.5	65.1	60.4	10.8	12.3	1.0
<i>E. hegesia</i>	1	31	5.0	62.4	69.8	58.5	8.8	9.4	0.8
Limenitidinae									
<i>L. populi</i>	2	260	10.6	130.8	175.5	71.7	20.0	26.4	3.9
<i>C. herminia</i>	1	208	10.0	123.2	141.5	84.9	17.9	19.4	1.6
<i>N. rivularis</i>	2	234	16.7	141.9	160.4	126.4	15.7	19.2	3.1
<i>A. galene</i>	2	259	10.9	122.3	149.1	84.9	12.4	16.0	2.0
Cyrestinae									
<i>C. thyodamas</i>	3	162	11.4	45.8	52.8	39.6	6.6	7.5	1.0
Pseudergolinae									
<i>D. nesimachus</i>	1	146	7.4	107.8	126.4	73.6	15.1	18.9	2.8
Biblidinae									
<i>B. hyperia</i>	3	375	16.3	120.2	137.7	107.5	11.5	13.2	1.4
<i>E. dryope</i>	2	153	10.3	70.9	84.0	54.7	9.5	11.3	0.7
<i>C. numilia</i>	2	143	9.7	90.0	101.9	75.5	9.5	11.3	0.7

Table 5. continued

species	Sensilla styloconica							Sensilla basiconica (food canal)					
	Rows of sensilla on one galea	Number of sensilla on both galeae	Density (sensilla/100 μm)	stylus length			sensory cone length			Number of sensilla on one galea	Density (sensilla/mm)		
<i>M. orsis</i>	1	102	10.2	mean (μm)	max (μm)	min (μm)	S.D.	mean (μm)	max (μm)	min (μm)	S.D.	55	4.2
<i>E. ibilis plusios</i>	2	178	13.6	73.8	84.9	66.0	5.5	8.3	9.8	5.7	1.4	51	4.0
<i>D. mylita</i>	2	143	14.0	98.5	111.3	79.2	12.5	10.8	13.2	7.5	1.6	34	4.6
<i>C. texa titania</i>	1	89	9.4	64.5	73.6	52.8	7.5	8.9	11.3	5.7	2.0	32	3.3
Nymphalinae				56.7	60.4	54.7	2.0	6.4	7.5	5.7	0.7		
<i>A. burejana</i>	2	205	13.5	35.7	47.2	25.5	8.3	7.7	11.3	5.7	2.4	31	3.7
<i>V. myrinna</i>	1	62	6.6	42.9	50.9	34.0	4.6	8.5	10.4	7.5	1.0	28	2.1
<i>V. atalanta</i>	2	146	9.9	63.2	73.6	45.3	8.3	8.7	11.3	7.5	1.5	33	2.4
<i>C. lacinia</i>	1	43	7.2	51.7	54.7	49.1	2.0	6.8	9.4	5.7	1.2	24	3.1
<i>E. lansdorffi</i>	1	33	5.7	65.7	67.9	60.4	1.9	7.1	7.5	5.7	0.8	28	2.9
<i>K. rumia</i>	1	113	8.2	84.2	94.3	75.5	5.1	7.7	9.4	7.5	0.6	50	2.9
Lycaenidae													
<i>L. phlaeas</i>	2	58	9.1	27.0	39.6	16.0	9.1	3.8	4.7	2.8	0.6	20	2.4
<i>L. phydela</i>	2	117	9.7	27.9	44.3	11.3	14.2	3.3	3.8	1.9	0.7	19	3.2
Riodinidae													
<i>R. refracta</i>	2	86	6.2	24.1	27.4	17.9	2.8	5.4	5.7	3.8	0.6	22	2.3
<i>C. calamisa</i>	1	24	6.8	32.0	35.8	28.3	2.3	6.1	7.5	5.7	0.6	18	2.8
Pieridae													
<i>D. belisama</i>	2	40	3.4	9.2	11.3	7.5	1.1	6.5	7.9	5.3	0.9	24	1.5
<i>P. bryoniae</i>	2	34	5.5	8.5	9.8	6.6	1.2	6.5	7.5	4.2	1.1	25	2.3
Papilionidae													
<i>G. sarpedon</i>	2	83	2.1	8.8	11.3	4.7	1.8	4.4	5.7	3.4	0.8	31	2.7
<i>P. machaon</i>	2	58	2.3	7.9	9.4	4.7	1.5	4.7	5.7	3.8	0.6	23	1.3
Hesperiidae													
<i>E. montanus</i>	1	17	4.2	10.6	13.2	9.1	1.3	4.0	4.7	3.8	0.3	17	2.5
<i>E. rafflesia</i>	1	17	2.1	16.0	18.9	14.2	1.4	4.5	5.7	3.8	0.7	36	1.9

Table 6. Morphometrics of sensilla trichodea. x...missing data.

species	Sensilla trichodea											
	25 % proboscis length			50 % proboscis length			75 % proboscis length			S.D.		
	mean (μm)	max (μm)	min (μm)	mean (μm)	max (μm)	min (μm)	mean (μm)	min (μm)	max (μm)			
Libytheinae												
<i>L. celtis</i>	8.1	9.4	5.7	1.6			9.6	11.3	7.5	1.1		
<i>L. carinenta</i>	12.8	34.0	8.5	7.6			9.3	17.9	5.7	3.8		
Danainae												
<i>A. niavius</i>	12.1	16.0	6.6	2.7			10.2	15.1	4.7	3.1		
<i>L. cleobaea</i>	9.4	13.2	6.6	1.9			8.7	13.2	5.7	2.0		
<i>T. zoilus</i>	19.6	56.6	11.3	13.9			8.3	9.4	5.7	1.6		
<i>M. lysinnia</i>	7.7	13.2	3.8	2.7			5.1	7.5	2.8	1.3		
<i>I. drymo</i>	15.7	26.4	9.4	4.5			10.9	15.1	7.5	2.6		
Charaxinae												
<i>C. varanes</i>	12.1	17.0	7.5	2.6			8.5	9.4	7.5	1.0		
<i>A. sardanapalus</i>	14.2	24.5	11.3	3.7			10.9	15.1	9.4	2.0		
<i>A. chalciope</i>	13.8	18.9	11.3	2.4			13.4	17.0	9.4	2.6		
Apaturinae												
<i>A. cleochares</i>	13.8	35.8	5.7	11.2			9.2	14.2	7.5	2.1		
<i>D. agathina</i>	19.8	28.3	13.2	4.7			26.8	45.3	15.1	11.0		
Satyrinae												
<i>M. achilles</i>	140.0	273.6	11.3	105.8			144.2	254.7	20.8	86.0		
<i>T. onolaus</i>	18.5	45.3	7.5	10.5			11.7	17.0	5.7	3.2		
<i>Z. aurelius</i>	127.2	279.2	15.1	122.3			145.1	277.4	11.3	112.3		
<i>C. oileus</i>	19.4	26.4	13.2	4.6			22.3	34.0	13.2	5.3		
<i>D. creusa</i>	21.5	37.7	13.2	7.2			16.8	28.3	9.4	6.1		
<i>N. cyllastros</i>	16.6	26.4	9.4	5.0			9.5	13.2	7.5	1.9		
<i>B. actorion</i>	19.6	26.4	11.3	4.5			16.9	20.8	13.2	2.1		
<i>P. lamia</i>	104.7	156.6	67.9	35.7			118.7	222.6	37.7	67.7		
<i>M. leda</i>	13.4	41.5	5.7	12.2			9.1	15.1	5.7	2.9		
<i>G. chelys</i>	8.7	13.2	3.8	2.8			11.7	20.8	5.7	4.3		

Table 6. continued

species	Sensilla trichodea											
	25 % proboscis length			50 % proboscis length			75 % proboscis length					
	mean (μ m)	max (μ m)	min (μ m)	SD	mean (μ m)	max (μ m)	min (μ m)	SD	mean (μ m)	min (μ m)	max (μ m)	SD
<i>B. mandanes</i>	17.5	32.1	9.4	6.6	11.7	17.0	7.5	3.5	9.1	11.3	7.5	1.5
<i>M. libye</i>	13.3	34.0	5.7	10.0	7.2	9.4	5.7	1.2	6.8	9.4	3.8	1.6
<i>G. muscosa</i>	47.4	88.7	28.3	19.0	38.3	83.0	13.2	25.1	32.6	52.8	15.1	12.3
<i>M. jurtina</i>	11.1	13.2	9.4	1.4	13.8	17.0	11.3	2.0	12.1	13.2	9.4	1.3
<i>M. galathea</i>	10.6	13.2	6.6	2.3	13.4	17.0	9.4	2.4	15.5	22.6	13.2	2.9
<i>E. tisiphone</i>	14.5	24.5	11.3	4.3	12.8	15.1	9.4	2.1	12.6	17.0	9.4	2.5
Calinaginae												
<i>C. buddha</i>	x	x	x	x	x	x	x	x	x	x	x	x
Heliconiinae												
<i>A. dicaeus</i>	14.2	18.9	9.4	3.5	14.0	24.5	5.7	5.4	11.5	17.0	7.5	2.5
<i>D. juno</i>	49.9	58.5	35.8	8.6	10.9	15.1	7.5	2.8	9.2	20.8	5.7	4.3
<i>D. phaeusa</i>	41.7	73.6	17.0	17.6	8.3	13.2	5.7	2.4	6.8	11.3	5.7	2.0
<i>H. erato</i>	42.1	56.6	22.6	12.7	24.0	45.3	13.2	10.0	14.0	20.8	7.5	4.6
<i>E. hegesia</i>	23.7	45.3	7.5	12.7	10.1	18.9	5.7	4.3	8.6	11.3	5.7	2.0
Limnitiidinae												
<i>L. populi</i>	47.7	71.7	32.1	12.9	31.3	41.5	24.5	6.1	20.9	41.5	13.2	10.0
<i>C. herminia</i>	31.3	41.5	18.9	7.1	21.5	28.3	13.2	6.3	11.9	17.0	9.4	2.5
<i>N. rivularis</i>	37.0	56.6	17.0	12.3	28.1	49.1	11.3	12.4	18.1	28.3	13.2	4.9
<i>A. galene</i>	78.1	203.8	13.2	59.3	104.9	226.4	37.7	72.0	63.2	141.5	18.9	41.1
Cyrestinae												
<i>C. thyodamas</i>	54.0	92.5	18.9	28.1	24.2	34.0	13.2	9.0	13.4	18.9	7.5	3.3
Pseudergolini												
<i>D. nesimachus</i>	40.6	84.9	7.5	31.3	19.4	66.0	9.4	16.9	10.6	19.8	5.7	3.9
Biblidinae												
<i>B. hyperia</i>	32.3	62.3	7.5	19.6	11.1	13.2	7.5	1.7	10.9	13.2	7.5	1.7
<i>E. dryope</i>	16.3	24.5	9.4	5.8	10.4	17.0	7.5	2.9	9.2	11.3	5.7	1.9
<i>C. numilia</i>	43.7	117.0	11.3	42.1	12.2	15.1	9.4	2.2	8.4	11.3	6.6	1.6

Table 6. continued

species	Sensilla trichodea											
	25 % proboscis length			S.D.	50 % proboscis length			mean (μm)	75 % proboscis length			
mean (μm)	max (μm)	min (μm)	mean (μm)		max (μm)	min (μm)	mean (μm)		min (μm)	max (μm)	S.D.	
<i>M. orsis</i>	71.5	141.5	17.0	55.7	15.4	22.6	9.4	5.3	10.8	16.0	7.5	2.9
<i>E. iblis plusios</i>	11.3	17.0	7.5	2.3	14.2	22.6	10.4	4.6	10.2	17.0	7.5	2.6
<i>D. mylitta</i>	20.6	43.4	5.7	14.1	16.2	26.4	5.7	6.4	13.8	24.5	7.5	7.8
<i>C. texa titania</i>	12.5	18.9	5.7	4.0	12.2	14.2	11.3	1.1	9.0	10.4	7.5	0.9
Nymphalinae												
<i>A. burejana</i>	17.7	22.6	13.2	3.2	13.3	19.8	9.4	2.8	12.1	13.2	9.4	1.3
<i>V. myrinna</i>	38.2	62.3	17.0	16.6	11.9	18.9	7.5	4.1	9.8	13.2	7.5	2.0
<i>V. atalanta</i>	21.4	45.3	11.3	12.4	13.4	20.8	9.4	3.1	9.8	11.3	7.5	1.2
<i>C. lacinia</i>	11.0	15.1	9.4	1.9	12.8	17.0	8.5	2.7	15.2	18.9	11.3	2.2
<i>E. lansdorffi</i>	14.2	18.9	9.4	3.2	9.0	13.2	5.7	2.5	7.9	9.4	5.7	1.4
<i>K. rumia</i>	32.5	56.6	9.4	19.2	14.9	41.5	5.7	13.6	7.4	9.4	5.7	1.7
Lycaenidae												
<i>L. phlaeas</i>	5.2	7.2	3.8	1.1	5.1	7.2	3.8	1.0	4.6	5.8	3.8	0.8
<i>L. phydela</i>	7.9	10.9	5.7	1.9	13.1	18.9	9.4	3.0	9.5	16.0	6.6	3.0
Riodinidae												
<i>R. refracta</i>	15.4	22.6	9.4	3.9	13.0	17.0	10.4	2.7	10.5	13.2	9.4	1.4
<i>C. calamisa</i>	12.2	18.9	7.5	4.2	9.5	15.1	7.5	2.5	10.9	13.2	9.2	1.1
Pieridae												
<i>D. belisama</i>	21.2	47.2	11.3	12.2	10.8	13.2	7.5	2.0	7.0	11.3	3.8	2.0
<i>P. bryoniae</i>	15.3	18.5	11.3	2.3	10.8	13.2	7.7	2.1	9.2	10.4	7.5	0.9
Papilionidae												
<i>G. sarpedon</i>	9.5	11.3	7.5	1.4	7.3	10.9	5.7	1.5	7.0	9.4	5.7	1.4
<i>P. machaon</i>	14.7	21.7	9.4	3.3	10.9	18.9	7.5	3.6	11.6	18.9	7.5	3.6
Hesperiidae												
<i>E. montanus</i>	9.1	13.2	7.5	1.8	8.1	11.3	5.7	1.6	6.7	9.4	5.7	1.3
<i>E. rafflesia</i>	12.0	17.0	7.5	2.5	5.5	7.5	3.8	1.1	8.5	11.3	5.7	1.6