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Evolution of proboscis musculature in Lepidoptera

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Abstract. The suctorial proboscis of adult Lepidoptera represents a key morphological innovation that enabled these insects to gain access to new food sources. In the ancestral condition of the lepidopteran proboscis only extrinsic galeal muscles are present in the basal joint region. The presence of additional muscles (i.e., the intrinsic galeal muscles) is regarded as a morphological novelty of the Myoglossata that evolved after the galeae were modified to form suctorial mouthparts. The present comparative investigation of the galeal anatomy in representatives of all major taxa revealed that the intrinsic galeal muscles are derived from the basal galeal musculature. In the examined Neopseustoidea, Exoporia, Nepticuloidea, Incurvarioidea, and Tischerioidea all galeal muscles have their origin in the stipes-galea joint and/or in the proximal region of the galea. Two muscle units form the basal galeal musculature of the joint region and one to three longitudinal muscles extend into the galea lumen. Multiple intrinsic galeal muscles, of which both the origin and attachment sites are markedly distal from the basal joint region are regarded as a groundplan autapomorphy of the Ditrysia. Some slightly oblique muscles may occur along the lateral wall; these were lost in species with extremely slender galeae. In most investigated Obtectomera two series of intrinsic galeal muscles occur; these are the (1) oblique lateral intrinsic galeal muscles, which are arranged one upon the other along the lateral proboscis wall and (2) the median intrinsic galeal muscles, which run more or less longitudinally along the ventral wall. Oblique muscle arrangement probably evolved in concert with the functional demands of a long lepidopteran proboscis. A likely evolutionary pathway to account for the serial arrangement of galeal muscles is propo sed.

INTRODUCTION

The mouthparts of insects lend themselves well to evolutionary studies in particular when mouthpart structure and function as well as the environmental challenges they encounter are well understood. In flower-visiting insects, modification of the mouthpart design is correlated with adaptation to specialized modes of feeding. For example, the suctorial proboscis of Lepidoptera can be traced back to the ancestral biting/chewing mouthparts retained in the most basal taxa, viz., the Micropterigidae, Agathiphagidae and Heterobathmiidae (Tillyard, 1923; Hannemann, 1956; Kristensen & Nielsen, 1979; Nielsen & Kristensen, 1996). A coilable proboscis was evolved in the stem lineage of the clade Glossata, which comprises the vast majority of extant Lepidoptera. While the feeding habits of most ancestral Glossata remain unknown, some members of the most basal extant lineages drink from water droplets and sap seeping from injured leaf tissue (Kristensen, 1984). None of them is known to be a flower visitor even though extant Glossata predominantly feed on nectar. Indeed, the evolution of the lepidopteran proboscis may be regarded as a key innovation, since it enables the bulk of the Glossata to exploit nectar which is a high-energy food source.

Although the anatomical design of the proboscis in higher Glossata, such as butterflies (e.g., Eastham & Eassa, 1955; Krenn, 2000), and its homology with the maxillary galeae are well-known (Philpott, 1927; Scoble, 1992), almost no studies have addressed the problem of the evolutionary origin of its components.

In the non-glossatans, the galea is a diminutive, bristled part of the maxilla with only few sensilla and one small stipes-galea muscle (Hannemann, 1956; Chauvin & Faucheux, 1981; Krenn & Kristensen, unpubl.). During evolution the galeae became decoupled from their role in the functioning of biting/chewing mouthparts in the ancestors of the Glossata and underwent considerable modification. The two very elongated galeae, which are interlocked to form a food canal for suction, are hallmarks of "higher" Lepidoptera (Krenn & Kristensen, 2000). Compared to the paucity of structures in the ancestral galeae, this suctorial proboscis boasts a number of morphological innovations, such as the galeal linking structures, the complexly textured galeal wall, the numerous internal muscles, the modifications of the proboscis tip and the unusually shaped sensilla (Krenn, 1990; Krenn & Kristensen, 2000). The tiny proboscises of the most basal Glossata, viz., the Eriocraniidae, Acanthopteroctetidae and Lophocoronidae, contain only extrinsic galeal muscles (Kristensen, 1968; Nielsen & Kristensen, 1996). The intrinsic galeal musculature was found in previous studies to consist either of a few longitudinal strands in monotrysian moths (Kristensen & Nielsen, 1981a; Kristensen, 1984) or two series of obliquely arranged muscles in Ditrysia (e.g., Eastham & Eassa, 1955; Krenn, 1990, 2000; Krenn & Mühlberger, 2002).

Most studies of proboscis anatomy are confined to single species (e.g., Pradhan & Aren, 1941; Eastham & Eassa, 1955; Vasudeva, 1956; Krenn, 2000), and comparative studies are restricted to the Macrolepidoptera (Krenn, 1990; Krenn & Mühlberger, 2002). The present study focuses on the diversity of proboscis anatomy in the monotrysian Heteroneura and the "lower" Ditrysia. For this purpose, the anatomy of the mouthparts of 55 species representing all major taxa of Lepidoptera are examined, primarily with the use of semithin sections. The aim is to elucidate the evolution of the intrinsic galeal musculature, which in those species with a long proboscis may comprise up to several hundred bundles of small muscles. The reconstruction of the evolutionary pathway provides a hypothesis for the development of the novel and complex galeal musculature, starting from a single muscle offshoot.

MATERIAL AND METHODS

Serial semithin section technique was employed for the light microscopical examination of the internal features of the proboscis, i.e. muscles, nerves, trachea and septa. The specimens were fixed in 70% ethanol or, preferably, in Duboscq-Brazil solution. The heads and the isolated proboscises were dehydrated and embedded in ERL-4206 epoxy resin under vacuum impregnation. Serial semithin sections were cut using diamond knives. The sections were stained with a mixture of 1% azure II and 1% methylene blue in an aqueous 1% borax solution for approximately 1 min at 80°C. A detailed description of fixation, embedding, and section technique is given in Pernstich, Krenn & Pass (2003). The anatomy of the basal galeal joint was investigated by means of longitudinal semithin section series of the head including the joint region of the proboscis base in selected species (see list below). Cross sections of the proboscises were taken at least in the proximal and distal half. In most specimens cutting the spirally coiled proboscis resulted in several cross sections at once. Prior to sectioning, the proboscis spirals were sketched by means of a drawing tube device attached to the light microscope to determine the plans for the cross sections. Some supplementary observations were made on whole mount preparations examined by brightfield and polarization microscopy.

Galea anatomy was studied in the following Lepidoptera. An asterisk following a species name indicates that the basal galeal joint was also examined; two asterisks indicate that existing paraplast section series were reinvestigated. Systematic arrangement follows usage in the "Handbook of Zoology" (Kristensen, 1998); current placement of individual superfamilies in the high-rank hierarchy within the order is shown in Fig. 12.

Micropterigoidea, Micropterigidae: *Micropterix aruncella* (Scopoli, 1763)*

Eriocranioidea, Eriocraniidae*: Eriocrania (Heringocrania) unimaculella* (Zetterstedt, 1839)*, *Eriocrania cicatricella* (Zetterstedt, 1839)

Acanthopteroctetoidea, Acanthopteroctetidae: *Acanthopteroctetes unifascia* Davis, 1978**

Lophocoronoidea, Lophocoronidae: *Lophocorona pediasia* Common, 1973**

Neopseustoidea, Neopseustidae: *Synempora andesae* Davis & Nielsen, 1980*

Exoporia, Mnesarchaeoidea, Mnesarchaeidae: *Mnesarchaea paracosma** Meyrick, 1885; *M. fusilella* (Walker, 1864)*

Exoporia, Hepialoidea, Hepialidae: *Fraus minima* Nielsen & Kristensen, 1989**;

Nepticuloidea, Opostegidae: *Opostega salaciella* (Treitschke, 1883)*, Nepticulidae: *Ectoedemia septembrella* (Stainton, 1849)*, *E. argyropeza* (Zeller, 1839)*

Incurvarioidea, Adelidae: *Nemophora degeerella* (L., 1758)*, *Cauchas fibulella* ([Denis & Schiffermüller], 1775)*; Prodoxidae: *Prodoxus quinquepunctella* (Chamb., 1875)*, *P. decipiens* Riley, 1880*, *Tegeticula yuccasella* (Riley, 1872)*

Tischerioidea, Tischeriidae: *Tischeria ekebladella* (Bjerkander, 1795)*

Tineoidea, Tineidae: *Euplocamus anthracinalis* (Scopoli, 1763), *Morophaga choragella* ([Denis & Schiffermüller], 1775)*

Gracillarioidea, Gracillariidae: *Cameraria ohridella* Daschke and Dimic, 1986

Yponomeutoidea, Yponomeutidae: *Yponomeuta cagnagella* (Hübner, 1813); Plutellidae: *Plutella xylostella* (L., 1758); Glyphipterigidae: *Glyphipterix thrasonella* (Scopoli, 1763)

Gelechioidea, Coleophoridae: *Coleophora* sp.; Elachistidae: *Ethmia dodecea* (Haworth, 1828); Oecophoridae: *Bisigna procerella* ([Denis & Schiffermüller], 1775); Blastobasidae: *Blastobasis phycidella* (Zeller, 1839); Gelechiidae: *Exoteleia dodecella* (L., 1758)

Sesioidea, Sesiidae: *Synathedon vespiformis* (L., 1761), *Bembecia pavicevici* Tosevski, 1989

Zygaenoidea, Zygaenidae: *Zygaena trifolii* (Esper, 1783), *Z. exulans* (Hohenwarth, 1792), *Procris* sp.

Choreutoidea, Choreutidae: *Anthophila fabriciana* (L., 1767)

Tortricoidea, Tortricidae: *Cydia pomonella* (L., 1758)*, *Agapeta zoegana* (L., 1767)

Alucitoidea, *Alucita hexadactyla* L. 1758

Pterophoroidea, Pterophoridae: *Pterophorus pentadactylus* (L., 1758), *Merrifieldia tridactyla* (L., 1758)

Obtectomera, Pyralioidea, Pyralidae: *Eurrhypara hortulata* (L., 1758), *Plodia interpunctella* (Hübner, 1813)*; *Pionea damastesalis* (Walker, 1859); Crambidae: *Platytes alpinella* (Hübner, 1813).

Obtectomera, Bombycoidea, Brahmaeidae: *Brahmaea tancrei* Austant, 1896; Sphingidae: *Sphinx ligustri* (L., 1758), *Macroglossum stellatarum* (L., 1758), *Manduca sexta* (L., 1763).

Obtectomera, Geometroidea, Geometridae: *Camptogramma bilineata* (L., 1758), *Psodos quadrifarius* (Sulzer, 1776), *Plagodis dolabraria* (L., 1767).

Obtectomera, Noctuoidea, Arctiidae: *Syntomis phaegea* (L., 1758)*; Noctuidae: *Hypena proboscidalis* (L., 1758), *Autographa* sp., *Mamestra brassicae* (L., 1758)

RESULTS

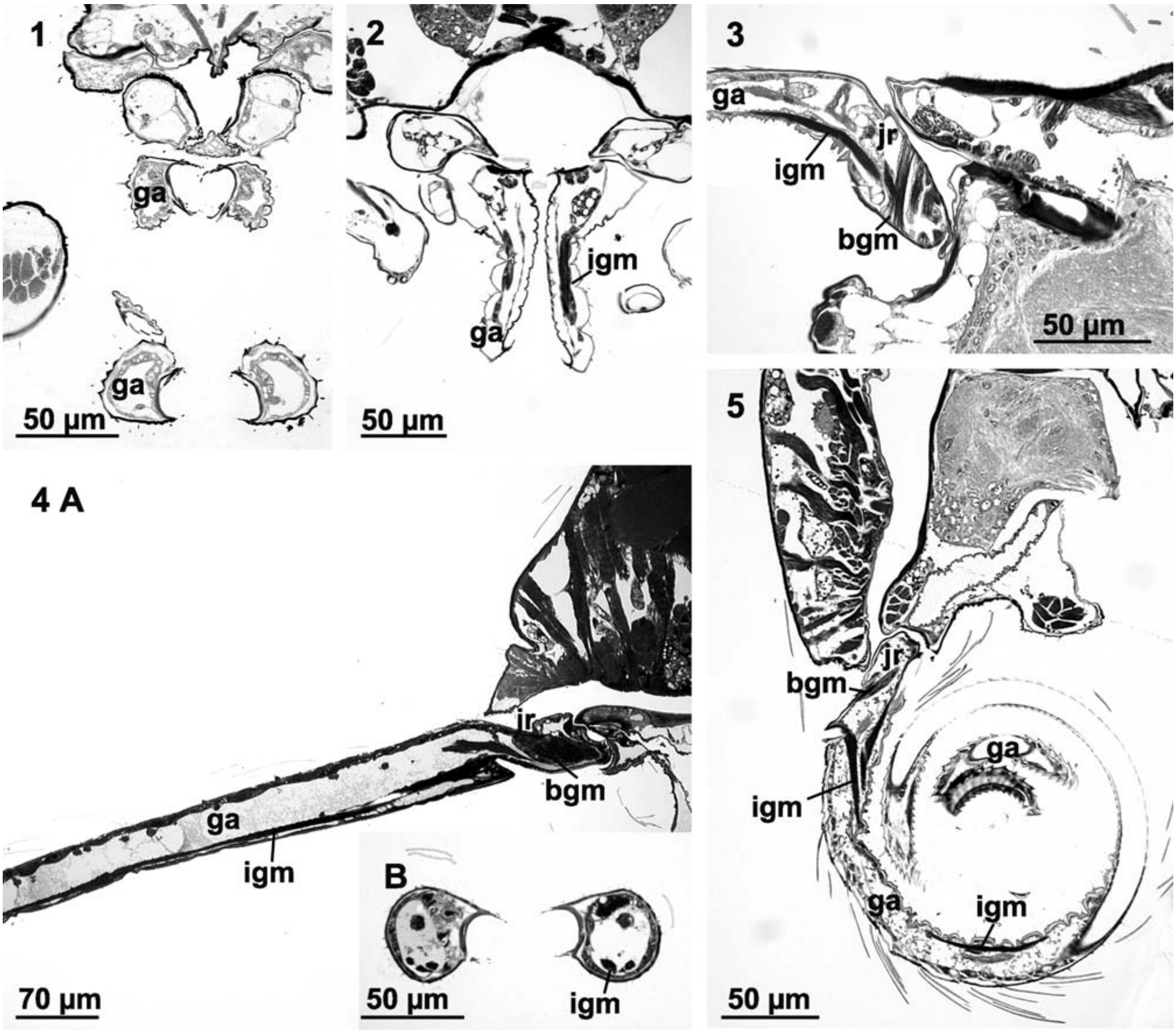
Regardless of proboscis length, the internal composition of the galeae is similar in all glossatan Lepidoptera except for internal musculature. At least in the proximal parts, the galeal lumen contains a delicate longitudinal septum, a trachea, and a nerve which enters at the basal joint to the stipes. The presence, number and particular arrangement of the galeal muscles varies considerably in the examined representatives at a high-taxonomical level (Table 1; Figs 1–11).

The proximal end of the galea forms a joint with the stipes, and it accommodates the insertion of the basal galeal musculature in all examined species.

Reexamination of the galeal anatomy of non-glossatans and Eriocraniidae confirmed previous findings (Hannemann, 1956; Kristensen, 1968) that both groups have common galeal features although the length, external morphology and function of the galeae differ fundamentally. In these taxa, the lumen of the galea contains a septum and a nerve, which innervates the sensilla; however there are no intrinsic galeal muscles (Fig. 1). Apart from the musculature of the maxillary palp, two small muscles originate on the stipes. In *Micropterix* one

Table 1. Systematic distribution of various galea musculature in studied Lepidoptera. Data from present study in bold letters, data from previous studies in normal print. Classification follows Kristensen & Skalski (1998). Basal galeal muscles (bgm) present in all taxa. The most basal groups of the Myoglossata have one to three intrinsic galeal muscles (igm). The monotrysian Heteroneura, plus basal groups within the Ditrysia, have few longitudinal intrinsic galeal muscles. Almost all Ditrysia with a functional proboscis have at least a few obliquely arranged lateral intrinsic muscles (lim) in addition to the primitive longitudinal muscles; exceptions are regarded to be secondary. The presence of two oblique muscle series (a median intrinsic muscles, mim, and the lim set) is characteristic of the Obtectomera as well as of the Tortricidae.

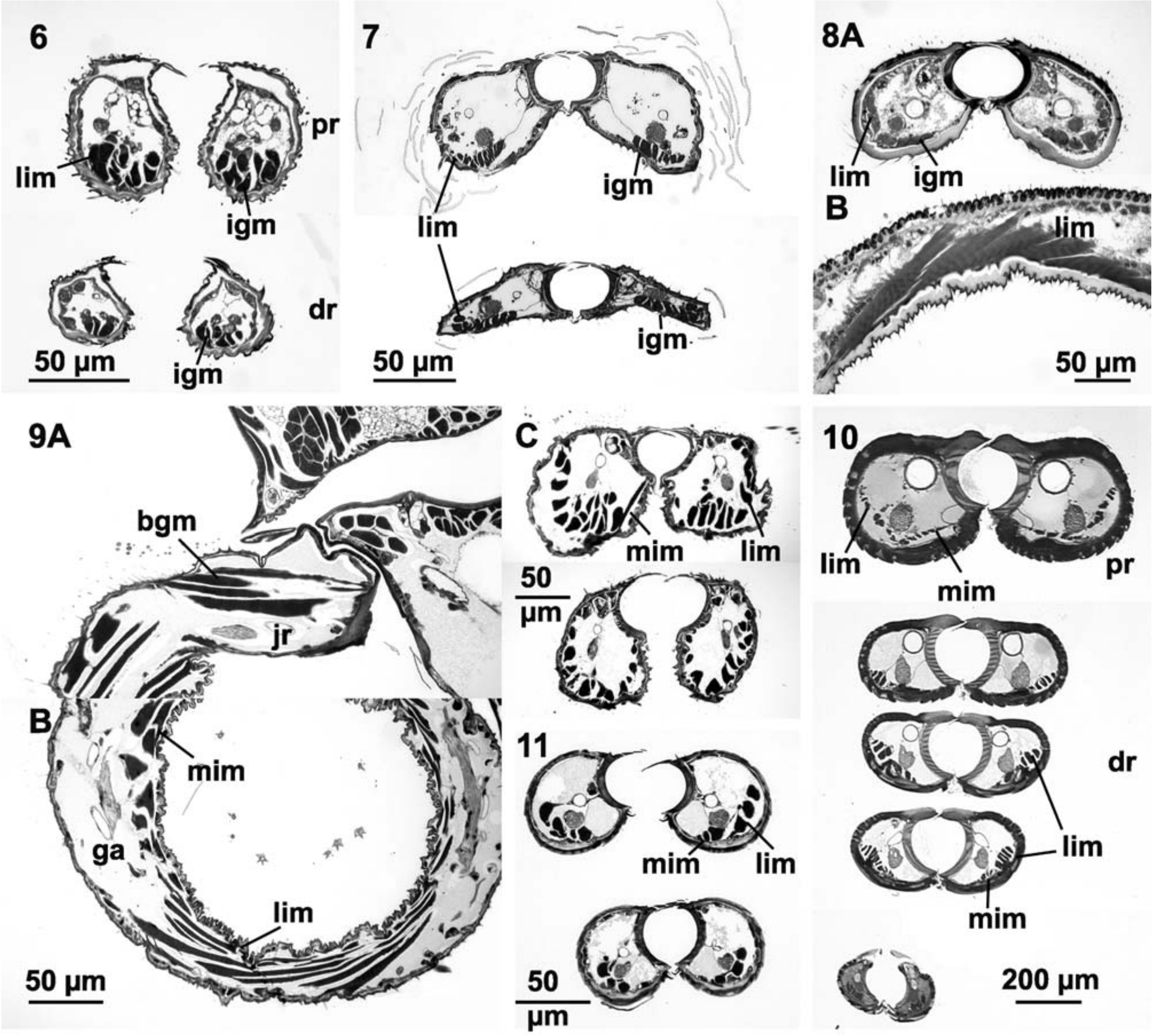
Taxon	Muscles of the galea in			Previous studies
	basal joint	proximal galea	distal galea	
Micropterigoidea				
Micropterigidae	**bgm**	—	—	Hannemann, 1956
GLOSSATA				
Eriocranioidea			—	
Eriocraniidae	**bgm**	—		Kristensen, 1968
Acanthopteroctetoidea				
Acanthopteroctetidae	**bgm**	—	—	
Lophocoronoidea				Nielsen & Kristensen, 1996
Lophocoronidae	**bgm**	—	—	
MYOGLOSSATA				
Neopseustoidea				Kristensen & Nielsen, 1981a,b
Neopseustidae	**bgm**	**igm**	**igm**	
Hepialoidea				Nielsen & Kristensen, 1989;
Hepialidae	**bgm**	igm		Kristensen & Nielsen, 1981b
Mnesarchaeoidea				
Mnesarchaeidae	**bgm**	**igm**	**?**	
HETERONEURA				
Nepticuloidea				
Opostegidae	**bgm**	**igm**	—	Kristensen & Nielsen, 1981b
Nepticulidae	**bgm**	**igm**	—	
Incurvarioidea				
Adelidae	**bgm**	**igm**	**igm**	Kristensen & Nielsen, 1981b
Incurvariidae	?	igm	?	
Prodoxidae	**bgm**	**igm**	**igm**	
Tischerioidea				Kristensen & Nielsen, 1981b
Tischeriidae	**bgm**	**igm**	—	
DITRYSIA				
Tineoidea				
Tineidae	**bgm**	**lim, igm**	**igm**	
Gracillarioidea				
Gracillariidae	?	**igm**	**igm**	
Yponomeutoidea				
Yponomeutidae	?	**lim, igm**	**lim, igm**	Krenn, 1990
Plutellidae	?	**lim, igm**	**igm**	
Glyphipterigidae	?	**igm**	**igm**	
Gelechioidea				
Coleophoridae	?	**igm**	**igm**	Krenn, 1990
Elachistidae	?	**lim, igm**	**lim, igm**	
Oecophoridae	?	**lim, igm**	**lim, igm**	
Blastobasidae	?	**lim, igm**	**igm**	
Gelechiidae	?	**lim, igm**	**igm**	
APODITRYSIA				
Sesioidea				
Sesiidae	**bgm**	**lim, igm**	**lim**	
Zygaenoidea				
Zygaenidae	?	**lim, igm**	**lim**	
Choreutoidea				
Choreutidae	?	**lim, igm**	**lim**	
Tortricoidea				
Tortricidae	**bgm**	**lim, mim**	**lim, mim**	
Alucitoidea				
Aluctidae	?	**lim, igm**	—	
Pterophoroidea				
Pterophoridae	**bgm**	**igm**	**lim**	
OBTECTOMERA				
Pyralioidea				
Pyralidae	**bgm**	**lim, mim**	**lim, mim**	Krenn, 1990
Crambidae	?	**lim, mim**	**lim, mim**	
MACROLEPIDOPTERA				
Bombycoidea				
Brahmidae	?	**lim, mim**	**lim, mim**	
Sphingidae	**bgm**	**lim, mim**	**lim, igm**	Krenn, 1990
Geometroidea				
Geometridae	**bgm**	**lim, mim**	**lim, mim**	Krenn & Mühlberger 2002
Uraniidae	bgm	lim, mim	lim, mim	
Noctuoidea				
Arctiidae	**bgm**	**lim, mim**	**lim, mim**	Krenn, 1990; Bänziger, 1970
Noctuidae	**bgm**	**lim, mim**	**lim, mim**	
Hedyloidea				Krenn & Mühlberger 2002
Hedylidae	bgm	lim, mim	lim, mim	
Hesperioidea				Krenn & Mühlberger 2002
Hesperiidae	bgm	lim, mim	lim, mim	
Papilionoidea				Krenn, 1990; Krenn & Mühlberger, 2002
Papilionidae	bgm	lim, mim	lim, mim	
Pieridae	bgm	lim, mim	lim	Eastham & Eassa, 1955
Lycaenidae	bgm	lim, mim	lim, mim / lim	
Nymphalidae	bgm	lim, mim	lim, mim / lim	Krenn, 2000



Figs 1–5. Proboscis anatomy of monotrysian Glossata. Evolution of intrinsic galeal muscles. 1 – *Eriocrania (Heringocrania) unimaculella* (Eriocraniidae). Cross section of the coiled proboscis. The lumen of the galea (ga) contains one nerve, a septum and no muscles. Absence of intrinsic galeal muscles represents the plesiomorphic condition of the anatomy of the lepidopteran galea. 2 – *Mnesarchaea paracosma* (Mnesarchaeidae). Horizontal section through the proboscis showing a single longitudinal intrinsic galeal muscle (igm) in the lumen of the galea (ga). 3 – *Opostega saliciella* (Opostegidae). Sagittal section through the head and basal proboscis reveals that the intrinsic galeal muscle (igm) is derived from the basal galeal muscle (bgm) in the basal joint (jr) which connects the stipes and galea. The micrograph is composed of two consecutive cuts of the same series. 4 – *Nemophora degeerella* (Adelidae). A – Sagittal section through the head and basal proboscis shows basal galeal muscle (bgm) in the joint region (jr) and the intrinsic galeal muscles (igm) in the lumen of the galea (ga) distal from the joint region. B – Cross section through the galeae in the proximal half of the proboscis. Intrinsic galeal muscles (igm) extend along the ventral galeal wall. 5 – *Tischeria ekebladella* (Tischeriidae). Sagittal section through the head and proboscis; the basal galeal muscle (bgm) attaches at the dorsal/anterior wall of the basal joint region (jr); some longitudinal intrinsic galeal muscles (igm) originate distal from the basal joint. The muscles do not run exactly in the plane of cutting, hence only short sectors are visible in this section.

extends in longitudinal direction to the base of the galea and the second runs obliquely to the base of the lacinia. Although the lacinia is reduced in *Eriocrania*, the muscles follow similar courses (Fig. 13). The first muscle has an oblique longitudinal course from the proximal part of the posterior/ventral stipes wall to the anterior/dorsal basis of the galea. The second, which originates in the distal part of the stipes, similarly extends from its posterior/ventral wall to the anterior/dorsal basis of the galea, with a course that is near-perpendicular to that of the first. A somewhat similar arrangement of the basal galeal musculature occurs in the acanthopteroctetid *Acanthopteroctetes unifascia* and the lophocoronid *Lophocorona pediasia* (in which all stipital muscle fibres to the galea base were collectively designated as "H15" by Nielsen & Kristensen, 1996).

In the neopseustid *Synempora andesae* the galea base contains two discrete muscle sets. One originates on the



Figs 6–11. Proboscis anatomy of Ditrysia. Evolution of oblique intrinsic galeal muscles. 6 – *Euplocamus anthracinalis* (Tineidae). Cross section through proximal (pr) and distal region (dr), intrinsic galeal muscles (igm) run along the ventral galeal wall throughout the whole proboscis; some lateral strands of intrinsic galeal muscles (lim) insert at the lateral wall. 7 – *Ethmia dodecea* (Elachistidae). Intrinsic galeal muscles (igm) extend along the ventral wall in longitudinal direction; some lateral strands of intrinsic galeal muscles (lim) extend from the lateral wall to the ventral galeal wall. 8 – *Synathedon vespiformis* (Sesiidae). A. Cross section. Lateral intrinsic galeal muscles (lim) extending along lateral galeal wall in addition to ventrally extending intrinsic galeal muscles (igm). B. Sagittal section illustrating the course of the lateral intrinsic muscle series (lim). 9 – *Cydia pomonella* (Tortricidae). A. Sagittal section through the head and basal proboscis showing the basal galeal muscle (bgm) and both the lateral (lim) and median intrinsic galeal muscles (mim) distal from the basal joint region (jr). B. Sagittal section through the proboscis shows the direction of the lateral intrinsic galeal muscles (lim). Both sections are from the same semithin series. C. Cross sections through the proboscis in the proximal and distal half of the proboscis. Two oblique muscle series are present, the lateral intrinsic galeal muscles (lim) extend between the lateral and ventral walls; the median intrinsic galeal muscles (mim) extend between the median and ventral walls, muscle fibers with intermediate courses are also present. 10 – *Manduca sexta* (Sphingidae). Cross section through a coiled proboscis. Sections from various regions show that the arrangement of the muscle series slightly shifts from proximal to distal. In the proximal region (pr) the median intrinsic galeal muscles (mim) run distinctly from the median wall to the ventral wall, while in the distal region of the proboscis (dr) their course is more longitudinal with origin and insertion on the ventral wall. Lateral intrinsic muscles (lim) keep their course. 11 – *Hypena proboscidalis* (Noctuidae). Cross section through various regions of the proboscis. Two sets of oblique muscle series, lateral intrinsic muscles (lim) and median intrinsic muscles (mim) exist in the galeal lumen throughout the whole proboscis.

ventral/posterior stipes wall (adjacent to the insertion of the large tentorio-stipital muscle) and extends obliquely mediad, inserting frontally on the galea base. The other originates more distally in the stipes; some of its fibres insert in the galea base, while others run into the galea itself, extending right up to the apical region.

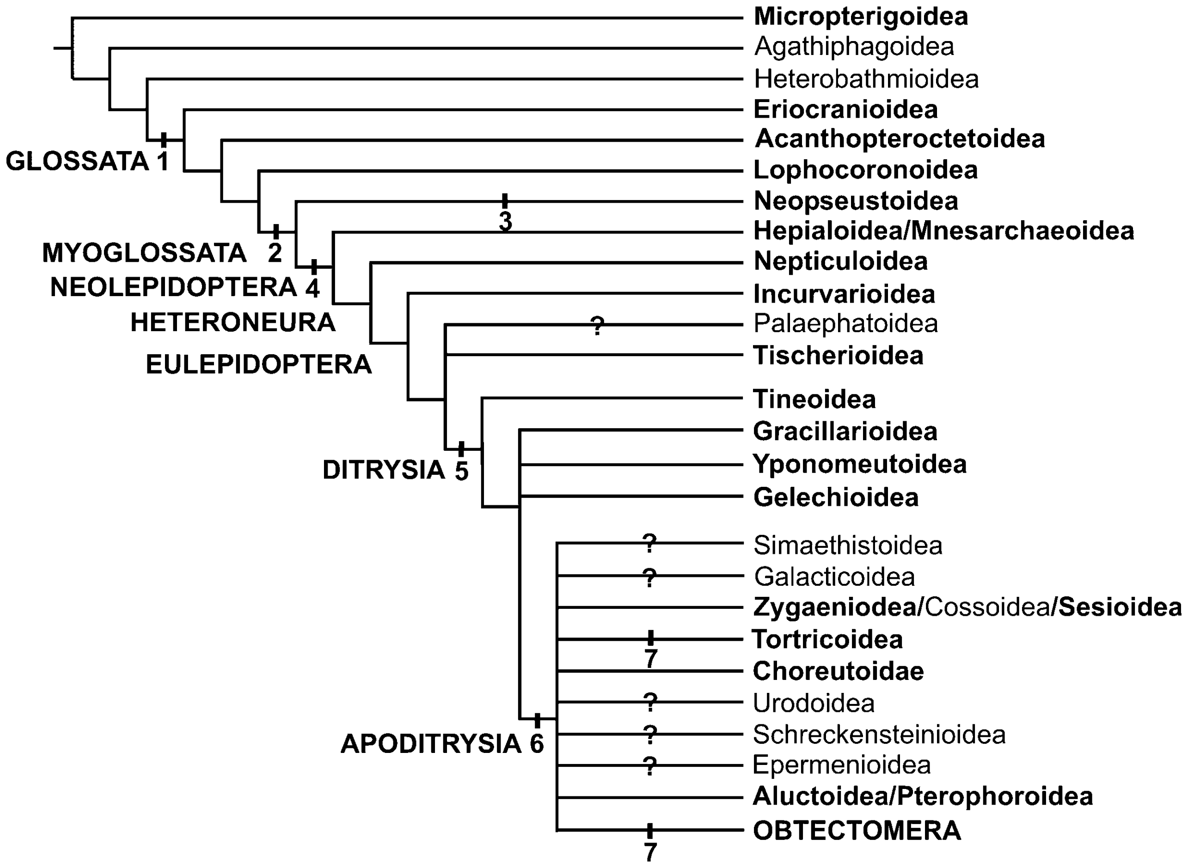


Fig. 12. Presence of apomorphic character states of proboscis anatomy mapped onto a cladogram of the Lepidoptera based on a recent review (Kristensen & Skalski, 1998). Plesiomorphic condition of Lepidoptera include two extrinsic galeal muscles, i.e. *musculus stipito-lacinialis* and *musculus stipito-galealis*. Numbers refer to derived character states: (1) galeae form proboscis; (2) intrinsic galeal muscles extending from the basal region; (3) double-tubed proboscis (Kristensen & Nielsen, 1981b); (4) loss of discrete *m. stipito-lacinialis* homologue; (5) intrinsic galeal muscles with both origins and insertions markedly distal from the basal region, at least some of them oblique; (6) series of pronouncedly oblique lateral intrinsic muscles; (7) two series of oblique lateral and median intrinsic galeal muscles. Taxon names in bold letters indicate present investigation; (?) indicates unknown character state.

In the Neolepidoptera, the basal galeal muscle normally consists of two divergent muscle units. Both muscle units originate near the stipes-galea junction and extend obliquely to the dorso-anterior wall of the basal galeal region. The proximal one is characterized by fibers that are shorter and run slightly more obliquely than those of the distal muscle, which extend more longitudinally. In general, the arrangement is uniform in all examined species (Figs 3, 4, 5, 9); however, the distinctness of the two separate muscle units is variable (Figs 3, 5, 9).

In the examined Mnesarchaeidae, Hepialidae and Nepticulioidea, all galeal muscles originate in the basal galea region. One or two muscle units compose the basal galeal musculature and one to three longitudinal muscle units extend into the lumen of the galea, exceeding its mid length. The latter are referred to as the intrinsic galeal muscles. In *Mnesarchaea* (Mnesarchaeidae) longitudinal muscle strands originate ventral to the basal galeal muscles near the proximal end of the galea and extend into the galeal lumen where they attach to the ventral galeal wall. These intrinsic galeal muscles consist of one to three muscle units, all extending longitudinally in the same direction (Fig. 2). In *Fraus minima* (Hepialidae) the basal region of the vestigial galea contains one muscle extending from the stipes into the galea base. Its intrinsic galeal muscles originate in the basal galeal region and extend into the galeal lumen in the median side. In *Opostega saliciella* (Opostegidae) some fibers branch off from the basal galeal muscle. While the bulk of the muscle attaches to the anterior/dorsal wall of the galeal base, these fibers extend further into the galea where they attach to the ventral galeal wall (Fig. 3). In *Opostega saliciella* (Opostegidae) a single intrinsic galeal muscle runs in the longitudinal direction along the ventral wall up to the distal third of the galea. In the examined Nepticulidae the basal galeal musculature is composed of two muscles and another two muscle units extend into the galea. These intrinsic galeal muscles run longitudinally, one attaches on the ventral wall, the second attaches below the food canal on the ventro-median wall of the galea at about midlength. The distal half of the galea does not contain muscles.

In the examined Adelidae, Prodoxidae and Tischeriidae, the galea contains two or more longitudinal

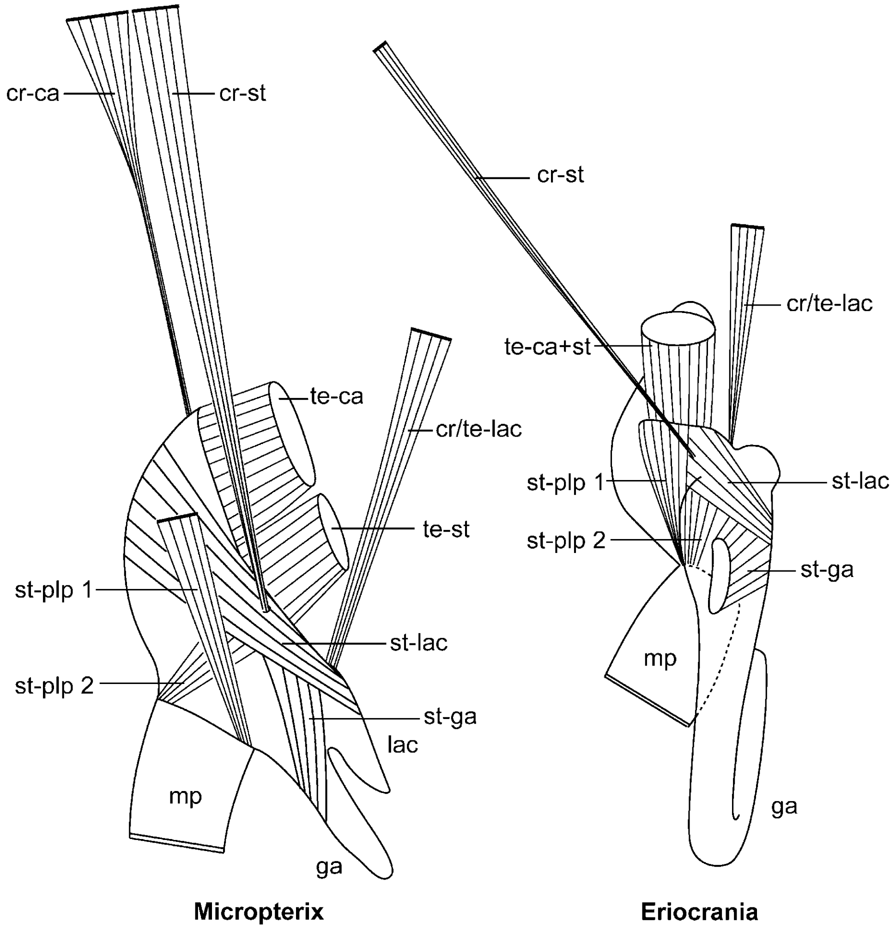


Fig. 13. Muscles of the maxillary bases of *Micropterix* (Micropterigidae) and *Eriocrania* (Eriocraniidae, Glossata); diagrams based on drawings by Hannemann (1956) and Kristensen (1968). The stipito-lacinial muscle (st-lac) in *Micropterix* is comparable to the most basal galeal muscle in *Eriocrania* in its course and spatial relations to adjacent muscles; hence the homology of these muscles is inferred. All other galeal muscles of the Glossata, including the intrinsic galeal musculature are concluded to be derived from the stipito-galeal muscle (st-ga). Abbreviations: cr-ca – cranio-cardinal muscle; cr-st – cranio-stipital muscle; cr/te-lac – cranio/tentorio-lacinal muscle; ga – galea; lac – lacinia; mp – maxillary palpus; st-pl 1 – stipito-palpal muscle 1; st-pl 2 – stipito-palpal muscle 2; st-ga – stipito-galeal muscle; te-ca – tentorio-cardinal muscle; te-st – tentorio-stipital muscle.

intrinsic muscles (Figs 4, 5). Their origins are restricted to the basal joint and the proximal region of the galea. At least one of the intrinsic galeal muscles has its proximal attachment on the ventral wall distal from the basal region (Fig. 4A). In *Tischeria ekebladella* (Tischeriidae) additional muscle units are found which originate at a small fold of the lateral galeal wall that delimits the basal region from the rest of the galea (Fig. 5). The distal attachment sites of all muscles are on the ventral galeal wall further distally. In cross sections, the muscles are found running side by side along the ventral wall (Fig. 4B). The muscles are very slender and occupy only a small area in the galeal lumen (Fig. 4). No muscles were detected near the tip region of the galeae. In *Nemophora degeerella* (Adelidae) the single muscle units are longer than 350 µm and measure 12 µm x 4 µm in cross section.

While longitudinal intrinsic galeal muscles also occur in basal Ditrysia, the presence of multiple muscle units with proximal attachments far beyond the galea base are a structural innovation encountered in this grade. In the galea of the examined Tineidae several muscle fibers run in a more or less longitudinal course in the lumen of the galea. There are 3–6 muscle units of the intrinsic galeal muscles, all of which extend along the ventral and lateroventral wall in the lower half of the lumen (Fig. 6). The rather lateral muscles were found to extend in a slightly oblique course at the midlength of the proboscis. Their proximal point of insertion lies somewhat dorsal and lateral to the distal point of attachment on the ventral wall, resulting in a slightly oblique course from the lateral to the ventral wall of the galea. In the examined gracillariid moth *Cameraria ohridella*, all intrinsic galeal muscles extend longitudinally. The galea is exceedingly small and contains only one or two longitudinal muscle units.

In the majority of examined Gelechioidea and Yponomeutoidea the proximal points of insertion of the more laterally extending intrinsic galeal muscles originate on the latero-ventral wall and attach more ventrally and medially, while both insertions of the more medially running muscles are on the ventral wall (Fig. 7). This results in a slightly oblique course to the lateral strands, in addition to the longitudinal course of the intrinsic galeal muscles in the median half of the cross section. However, the number of muscle units is dependent on the cross sectional area. This means that species with a very small galeal lumen have only one to two longitudinal muscles, while those with greater galeal volume, primarily in the proximal half of the proboscis, possess numerous muscle units, with slightly oblique courses of the lateral strands of the intrinsic galeal muscles.

In the slender galeae of the Aluctidae and Pterophoridae, only a few, small, intrinsic muscles are present. They run along the ventral wall in the proximal region and shift their courses to the lateral wall more distally. Muscles are missing in the distal half of the proboscis in the examined species of Aluctidae.

A more pronounced oblique course of the lateral muscles occurs in the examined Choreutidae, Zygaenidae and Sesiidae (Fig. 8). Particularly in the proximal region of the galeae where the lumen is more voluminous, two distinct muscle types are recognizable in most species. In the more slender distal regions of the proboscis, the intrinsic galeal muscles run obliquely along the lateral galea wall and overlap each other to form one series of oblique muscles (Fig. 8). They are referred to as lateral intrinsic galeal muscles. The term was coined for identically running muscles in butterflies (Krenn & Mühlberger, 2002).

The presence of two oblique muscle series within the entire proboscis is recognizable in the investigated Tortricidae (Fig. 9) and in most examined species of Pyraloidea, Bombycoidea (Fig. 10), Geometroidea and Noctuoidea (Fig. 11). There is a distinct lateral series of oblique muscles, as described above, and a second series of oblique galeal muscles, which are termed median intrinsic galeal muscles since they resemble those in Papilionoidea (Krenn & Mühlberger, 2002). The latter extend between the medio-ventral wall and the ventral wall; their points of insertion also lie on the ventral wall but median from those of the lateral intrinsic muscles (Figs 9–11).

Regardless of the area of the cross section, most examined Ditrysia-Obtectomera thus have a characteristic arrangement of two series of intrinsic galeal muscles, namely the oblique lateral and the more or less longitudinal median muscles. The strands of the single lateral intrinsic galeal muscle overlap each other in the lateral half of the cross section of the galea and run in an oblique course. Those of the median intrinsic muscles run slightly obliquely along the ventral galeal wall. In many taxa, e.g., the sphingid *Manduca sexta* (Fig. 11), the direction of the intrinsic muscle series changes throughout the proboscis length. Corresponding to the narrowing of the galeae, the muscles take a more longitudinal course and the separation of the lateral intrinsic galeal muscles from the median intrinsic galeal muscles is more pronounced in the first half of the proboscis. The number of single muscles in each series is correlated to the length and the diameter of the proboscis, such that the larger the galea, the greater the number of muscles (Figs 10, 11). The single units of the lateral intrinsic galeal muscles of *M. sexta* have a length of about 215 μm, they overlap up to 90% the preceding and following muscles. An estimate shows that the series of lateral intrinsic muscles may contain as many as 1500 separate units in one galea about 55 mm long.

DISCUSSION

The high-level systematics of the basal lineages of Lepidoptera is well-established (Minet, 1991; Kristensen & Skalski, 1998; Wiegmann et al., 2002) and forms the basis for the discussion of the evolution of the mouthparts of adult Lepidoptera (Fig. 12). The maxilla of adult Lepidoptera underwent a remarkable modification in length and shape in context with the shift from spore/pollen feeding to fluid feeding. The key innovation of the taxon Glossata was the evolution of the coilable proboscis, which is formed by the two elongated and modified galeae. It enables these insects to extract water or fluid nutrients hidden in relatively deep and narrow sources, yet it requires a minimum of space when not in use (Kristensen, 1984). Intrinsic galeal muscles are absent in those Lepidoptera with chewing maxillae, as well in the plesiomorphic condition of the galea in the Glossata (Kristensen, 1998). The presence of intrinsic galeal musculature characterizes the taxon Myoglossata, which includes the Neopseustidae, Mnesarchaeoidea and Hepialioidea plus the Heteroneura, and thus accounts for 99% of Lepidoptera species (Kristensen & Nielsen, 1981a; Kristensen & Skalski, 1998). Based on present information, the evolutionary pathway can be envisioned as starting from a galea with only extrinsic basal muscles (the plesiomorphic condition) and leading to the proboscis of “higher” Lepidoptera such as butterflies (Eastham & Eassa, 1955; Krenn & Mühlberger, 2002) with its complex arrangement of intrinsic musculature. The derived condition is characterized by hundreds of small muscle units arranged in two distinct series.

Our findings support the interpretation that the intrinsic galeal muscles are derived from the basal galeal musculature, which is retained in all examined Lepidoptera. In micropterigids and agathiphagids there is one stipito-galeal muscle and one stipito-lacinial muscle (Hannemann, 1956; Kristensen, unpubl.). All Glossata examined possess basal galeal muscles that extend between the stipes and the basal galea region. In Eriocraniidae, Acanthopteroctetidae and Lophocoronidae the two discrete muscle groups between the stipes sclerotizations and the base of the galea have courses similar to those of the endite muscles in *Micropterix* (Fig. 13) (Kristensen, 1968). Therefore, it seems likely that the primitive stipito-lacinial muscle is homologous with one of the two basal galeal muscles in the glossatan ground plan, where the lacinia is reduced. It is probably also homologous with the proximal stipito-galeal muscle in the Neopseustidae, while the more distal set with fibers partly extending into the proboscis itself then being the “true” stipito-galeal homologue. Judging from the course of the muscle fibers in the Myoglossata it is most probable that the intrinsic galeal muscles evolved from parts of the stipito-galeal muscle. Nonetheless it cannot be ruled out that the basal galeal musculature still comprises homologues of both of the primitive stipital endite muscles whereby the more proximal fibers stem from the lacinia "flexor" muscle, and the distal ones, which further evolved into the intrinsic galeal musculature, stem from the galeal "flexor". The plesiomorphic functional role of the extrinsic galea muscle may indeed have been flexion of this endite (Kristensen, 1968). However in the higher Lepidoptera, this muscle is considered to be an elevator of the whole proboscis (Eastham & Eassa, 1955; Krenn, 2000; Wannenmacher & Wasserthal, 2003).

In the plesiomorphic condition of the Myoglossata the intrinsic proboscis musculature consists of one or few longitudinal muscle strands (Kristensen & Nielsen, 1981a; Kristensen, 1998). Such longitudinal intrinsic galeal muscles are found in representatives of the Neopseustidae, Exoporia, and monotrysian Heteroneura. The present study demonstrates that the intrinsic proboscis muscles in the phylogenetically basal taxa of Myoglossata are just prolonged basal galeal muscles, since in *Synempora* (Neopseustidae), Mnesarchaeidae, Hepialidae, Opostegidae and Nepticulidae they originate in the galeal joint region next to the stipes sclerite.

Intrinsic galeal muscles having both the origin and the attachment sites markedly distal from the basal galeal joint were found in all examined Ditrysia (Fig. 12); note that no representatives of the Palaephatoidea were included in the present study, and the newly described monotrysian Andesianidae mouthparts are highly reduced (Davis & Gentili, 2003). It is hypothesized that these muscles shifted their insertion sites to more distal regions of the galea. Perhaps they evolved by duplication of the single muscle unit, which originally branched off the basal galeal muscle. Subsequent evolution led to the side-by-side arrangement of several long but very slender muscles running along the ventral galeal wall.

Since series of longitudinal intrinsic galeal muscles are present in the investigated Tineidae, Gracillariidae, Gelechioidea and Yponomeutoidea, and since the approximately longitudinal fibers occur in many other ditrysian taxa, they are considered plesiomorphic for that clade (Fig. 12). A moderately high muscle number was found in the Tineidae examined. A shift in the points of origin of the more lateral muscles toward the lateral galeal wall could lead to the overlapping arrangement in the lateral area of the wider galeal lumen. This results in the slightly oblique direction of the muscles from the lateral wall to the ventral wall, while the remaining intrinsic galeal muscles retain their longitudinal extension along the ventral wall. Oblique lateral intrinsic galeal muscles could be found at least in the proximal half of the proboscis in all Ditrysia except in the examined species of Gracillariidae, Glyphipterigidae and Coleophoridae. In all of these, the particularly slender proboscis contains only one to two muscle strands, possibly due to the small galea lumen; the configuration is likely a secondary simplification.

The markedly oblique course of the lateral muscle strands is characteristic of the Apoditrysia. Two series of oblique muscles – one extending from the lateral wall to the ventral wall and the other from the medioventral wall to the ventral wall – are found in the examined species of Obtectomera. Such lateral and medial series of galeal muscles have been previously described only in a number of Macrolepidoptera (e.g., Eastham & Eassa, 1955; Bänziger, 1970; Krenn, 1990, 2000; Krenn & Mühlberger, 2002). Two similar muscle series are also present in the Tortricidae, which is the only taxon outside the Obtectomera in which the dual muscle arrangement can be found throughout the entire proboscis in most groups. Due to its wide distribution, the presence of two separate series of oblique intrinsic galeal muscles can be ascribed to the groundplan of the Obtectomera (Fig. 12). The two muscle series correspond to the lateral and the median intrinsic galeal muscles, respectively, with regard to their points of insertion and course (Krenn & Mühlberger, 2002). In previous studies they were referred to as the primary and secondary oblique muscles (Eastham & Eassa, 1955; Krenn, 2000). Alternative names were proposed by Krenn & Mühlberger (2002) since they provide more information about the distinction of the muscle series. The present analysis suggests that the median intrinsic muscles correspond to the longitudinal intrinsic galeal muscles of the monotrysian Heteroneura, and that the lateral and more pronounced oblique muscles are further derivations of the longitudinal ones.

The proboscis of all Myoglossata (in which the organ is not secondarily reduced) is equipped with the same set of internal organs. From recent studies (Krenn & Kristensen, 2000; Krenn et al., 2001; Krenn & Mühlberger, 2002) a comprehensive picture of the evolution of the lepidopteran mouthparts has emerged. In the context of uptake of floral nectar by suction, the recognized evolutionary key innovations encompass the galeal linking structures, which ensure an airtight food canal, and the modified sensory equipment such as a new and unique type of sensilla in the tip region (Krenn & Kristensen, 2000). The evolution of additional intrinsic galeal muscles can also be interpreted in context with the enormous elongation of the galea and the widening of its diameter that obviously is associated with flower probing abilities. The small proboscis of an eriocraniid moth measures about 0.7 mm in length. The elasticity of the galeal wall is obviously sufficient to enable the galeae to coil into more than one loop in the resting position (Kristensen, 1968). For biomechanical reasons, the longer the proboscis, the thicker the galeal wall must be; also, the larger the food canal, the more liquid which can be taken up per time unit. In conjunction with the general trend toward longer and larger proboscises, which is thought to reflect an adaptation to flowers with deep corollas, the development of a more powerful mechanism of proboscis movement can be seen in the Ditrysia. This is achieved by the series of oblique muscles permitting a dense packing of the intrinsic galeal muscles, which was recently shown to be responsible for the spiral coiling of the proboscis (Krenn, 2000; Wannenmacher & Wasserthal, 2003). The present study revealed that the length of the single muscle fibers ranges between approximately 200 and 400 µm, although the total length of the proboscis in the studied species varies hundredfold in length. This means that enlargement of the galea is not merely a process of elongation of all components but requires additional functional units, such as the muscles and their needed innervation.

We present a hypothetical pathway of the evolution of the intrinsic galeal muscles whereby (1) the "recruitment" of these muscles from the basal galeal muscles by the distal shift of the attachment site to the ventral wall beyond the basal region; (2) the subsequent shift of the muscle origin sites resulting in the individualization of the new (i.e., fully intrinsic galeal) muscle in the Eulepidoptera; (3) the multiplication of the intrinsic galeal muscle which accompanies the further elongation of the proboscis in the Ditrysia; (4) the rearrangement of these muscles into distinct series in most parts of the proboscis by shifts of the attachment sites to the lateral and median walls as found in most Apoditrysia and all Macrolepidoptera.

The present findings have some significance for phylogeny (Fig. 12). The organization of the intrinsic proboscis musculature into a multitude of short muscle units (with both points of attachment inside the proboscis itself and the lateral muscles slightly oblique) may indeed be a groundplan autapomorphy of the Ditrysia (see also Kristensen & Skalski, 1998), while a well-developed series of pronounced oblique intrinsic fibers would be an apomorphy characterizing at least a major subset of the Apoditrysia. Moreover, the presence of two sets (a lateral and a median) of oblique muscles in both the Obtectomera and the Tortricidae is intriguing and poses the question whether it is a parallelism – or are the tortricids really the closest relatives of the Obtectomera? In any case, the currently available evidence bearing on the resolution of the apoditrysian polychotomy is ambiguous, as evidenced e.g. by Fänger's (1999) findings on the occurrence of the "euphragma".

The absence of one of the muscle sets in particular proboscis regions is a puzzling specialization of some of the "basal" Apoditrysia. Presently it is not known how widespread this condition is. Examination of more basal plume moths (Pterophoridae) including Agdistinae and Macropiratinae is now an obvious challenge. So are inquiries into the presence of one or two oblique intrinsic muscle sets in the proboscis of several additional apoditrysian lineages, both outside (e.g. Schreckensteiniidae, Epermeniidae) and inside (e.g., Copromorphoidea, Thyrididae) the assemblage currently considered to constitute the monophylum Obtectomera. The presence of two extrinsic (stipital) proboscis muscles with very different courses, and indeed likely different morphological derivations, is clearly a glossatan ground plan trait. While, as noted above, both components are possibly retained in the basal galeal musculature of higher Glossata, it is characteristic of all Neolepidoptera here examined that even where two entities are topographically clearly separate neither is identifiable as a likely stipito-lacinial homologue.

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