DIPLOMARBEIT

Elongated mouthparts in nectar feeding Nemognathinae (Meloidae, Coleoptera)

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1. Introduction

The mutualistic associations between insects and flowers – often summarized as pollination biology – are as multifaceted as they are essential for the global existence of present-day terrestrial ecosystems since they ensure the reproductive success of plants, mainly angiosperms, and provide adult insects with valuable foodstuffs. Paleontological records indicate that this evolutionary interaction started in the Cretaceous period (Labandeira 1997). The evolutionary onset of angiosperms was due, in part, to the frequent visits of beetles, which are among the earliest pollinating insects. The co-radiation of angiosperms and flower-visiting insects has culminated in manifold modifications on both the zoological and the botanical sides. Adaptations to flower visitation in insects have produced new learning strategies, feeding behaviors, sensory systems and mouthpart morphologies.

Today the majority of flower visiting insects belong to the Hymenoptera (47%), Diptera (26%), Lepidoptera (10%) and Coleoptera (15%). The mouthparts of flower visiting insects are highly integrated structural units which manipulate the food source (Betz et al. 2003). Two basic types can be distinguished: the biting-chewing type for solid food like petals and pollen, and the sucking type for taking up nectar. This liquid food source has a sugar concentration that varies from 5 to 75% and contains glucose, fructose, sucrose, amino acids along with other components (Krenn et al. 2005). In contrast to pollen as a food source, nectar has no resistant cell wall that must be overcome to obtain the nutrients (Barth 1991). Generally, there are two options for nectar uptake i.e. suction and adhesion. Specialized mouthparts for nectar uptake have independently evolved in many Hymenoptera, Diptera and Lepidoptera. They are often composed of elongated mouthparts that form a tubular proboscis for sucking nectar along a pressure gradient from long and/or narrow floral tubes (Krenn et al. 2005).

Flower visiting beetles may consume petals, pollen and nectar. Although nectar feeding by adhesion is widespread among beetles of various families, special adaptations are inconspicuous. Usually these beetles consume nectar in addition to pollen and their prognathous mouthparts are only slightly modified. Their moist surfaces function according to the principle mechanism of adhesion and capillarity. To transport nectar to the mouth, they perform licking, sponging or sweeping movements, e.g. as in some Scarabaeid beetles (Krenn et al. 2005). In general, beetles feed on nectar normally by licking it from exposed surfaces and shallow flowers (Barth 1991).

An exceptional case is found in the beetle family Meloidae. Some taxa in the tribe Nemognathini (subfamily Nemognathinae) possess mouthparts that are adapted for nectar uptake from deep flowers with concealed nectaries (Barth 1991). The family Meloidae contains more than 2500 species worldwide in approximately 120 genera (Bologna and Pinto 2001). Except for some genera in which the adults do not feed, the adults of all four subfamilies (Eleticinae, Meloinae, Tetraonycinae and Nemognathinae) are phytophagous and possess prognathous mouthparts of the biting-chewing type (Pinto and Bologna 1999). The mouthparts of some genera within the tribe Nemognathini are unique among beetles in that they are modified to use nectar as the main food source. A few species of the genera *Leptopalpus*, *Nemognatha*, *Gnathium* and *Zonitis* possess greatly extended mouthparts which form a food canal for nectar uptake.

The genus *Leptopalpus* GUERIN DE MENEVILLE 1844 probably contains only a single representative, *L. rostratus*, which is distributed in the Mediterranean region of Europe and northern Africa. Its proboscis is composed of the elongated four-segmented maxillary palps which possess a median row of short setae on the distal segments. To feed, both palps are brought together, forming a food canal in combination with the bristles of the galea and lacinia (Handschin 1929).

In contrast, the proboscis of species of *Nemognatha* ILLIGER 1807 (distributed worldwide except Australia, Oceania and the cold high latitudes of the New World) is formed by the elongated galeae which vary in length from 1.5 - 10 mm (Pinto and Bologna 1999). In some species it is longer than the body (Lovell 1915). The short galeae are equipped with relatively long bristles which form a pointed brush-like structure (Schremmer 1961), while long galeae are filiform and have short bristles on their concave inner margins which come together to form a true food canal (Schremmer 1961; Kaszab 1962).

In representatives of *Gnathium* KIRBY 1818 (limited to the western and southern United States) the galeae are elongated and loosely connected, to produce a filiform proboscis or sucking tube. The proboscis length is variable but usually at least as long as the head (Pinto 2009). With an average size of 4 or 5 mm, species of *Gnathium* are among the smallest of Meloidae (Pinto and Bologna 1999).

In addition to the adaptations for nectar uptake, all genera named above possess structural adaptations for pollen feeding. These structures include mandibular modifications, such as a soft lacinia mobilis and a postmola for kneading and conveying pollen grains. Harvesting structures, i.e. specially shaped bristles to which pollen grains adhere, are common in many other beetle families, e.g. Malachiidae, Cetoniidae and Cerambycidae (Fuchs 1974; Krenn et al. 2005). Probably the best scrutinized feeding mechanisms in Coleoptera are found in protea beetles and monkey beetles. The maxillae tips are densely covered with setae to mop up nectar and sweep pollen into the mouth with the aid of nectar or saliva to improve pollen adherence (Johnson and Nicolson 2001; Karolyi et al. 2008).

Although the unique elongation of mouthparts in beetles has been known since the work of Handschin (1929), detailed studies of proboscis morphology and function in nectar-feeding Meloidae are lacking. The aim of this study is to investigate the proboscis and mouthpart morphology, as well as the microanatomy of the heads in three different species of flower visiting Meloidae: *Leptopalpus rostratus* (FABRICIUS 1792), *Gnathium nitidum* Horn 1870 and *Nemognatha chrysomelina* (FABRICIUS 1775). Functional aspects, such as the formation of the food canal, and surface structures of the mouthparts, which are used for nectar adhesion, were studied using SEM technique for the first time. Furthermore, the musculature of the head and mouthparts were examined using serial semithin sections and MicroCT to discover structural modifications which are related to nectar feeding.

2. Materials and Methods

2.1. Studied species

The mouthparts and head musculature of three species of Nemognathinae LAPORTE 1840 were studied (Tab. 1). Since alcohol material of *Nemognatha chrysomelina* was not available, the internal structures were investigated in an undetermined species of the genus *Nemognatha* possessing a similar galeal length. In the following study the name *N. chrysomelina* is used for both.

Tab. 1 Studied species, number of individuals, origin and preservation medium

Specimens	Origin	Preservation
Nemognatha chrysomelina (FABRICIUS 1775) (n = 5)	Turkey (Collection A. Link), Natural History Museum Vienna	dry
Nemognatha sp. (n = 3)	Spain, Mario Garcia-Paris	70% Ethanol
Leptopalpus rostratus (FABRICIUS 1792) (n = 2)	Natural History Museum Vienna	dry
Leptopalpus rostratus (FABRICIUS 1792) (n = 3)	Spain, Mario Garcia-Paris	70% Ethanol
Gnathium nitidum HORN 1870 (n = 2)	USA, John Pinto	70% Ethanol

2.2. Light microscopy

2.2.1. Preparation of mouthparts and guts

Mouthparts of *L. rostratus* fixed in 70% ethanol were prepared under a stereo microscope and separately mounted in Polyvinyllactophenol on glass slides. The dry material of *N. chrysomelina* was soaked with diluted lactic acid (1:1 with Aqua bidest) overnight before preparation. In addition, gut content of all species was studied to examine ingested pollen grains. Guts were removed and dissected under a stereo microscope and fore-, middle-, and hindgut separately mounted in Polyvinyllactophenol on glass slides. All images were taken with an Olympus CX41Mikroskop equipped with an Olympus E330 digital camera. Line drawings were arranged with CorelDRAW X3®. All line drawings and images were edited with Adobe®Photoshop® CS4.

2.2.2. Semithin section technique

The head musculature was examined by using serial semithin section technique. The heads of *N. chrysomelina* and *L. rostratus* plus one of the removed maxillary palps were dehydrated in an ethanol series (70-100%). The head and one galea of *G. nitidum* were previously stored in 100% ethanol due to the preparation for MicroCT. The heads and the removed mouthparts were embedded in Agar Low viscosity Resin and polymerized at 60 ℃ for four days (Pernstich et al. 2003). Serial semithin sections with a thickness of 1 µm were made using a Leica EM UC6 microtome with diamond knife and stained with 1: 9 Richardson´s blue for 30 sec on a heating plate at 60 ℃ (Blumer et al. 2002). One head of each specimen was cut in sagittal section up to the half of the head. The galea of *G. nitidum* was cut in cross section with a span of about 50 µm at the basis, the middle and the tip region. The second segment of the maxillary palp of *L. rostratus* was completely cut in cross section.

2.3. Scanning electron microscopy (SEM)

Heads of all specimens were dissected under the stereo microscope. Mouthparts were dehydrated with ethanol (70-100%) and Hexamethyldisilazan as an intermedium for 15 min. After overnight air drying, the mouthparts were glued on stubs using doubled-sided carbon-containing adhesive tape and sputter-coated with gold (Agar sputtercoater B7340 for 240 sec). All images were taken at the Core Facility of Cell Imaging and Ultrastructure Research (University of Vienna, Austria) using a Philips XL 20 SEM.

2.4. Micro computerized tomography (Micro CT)

Additional to conventional semithin section technique, the head musculature was investigated using MicroCT. This technique is less time-consuming and enables a stereoscopic examination using 2D images and 3D reconstructions. Furthermore, it is a nondestructive technique, so that the same individual of *G. nitidum* used for MicroCT was available for semithin sectioning. Heads of *L. rostratus*, *N. chrysomelina* and *G. nitidum*, which were stored in 70% ethanol, were rinsed with 70% ethanol and transferred to 100% ethanol for 1 h on a tumbling station. Subsequently, the ethanol was replaced by 1% iodine solution, and the heads were stained overnight and returned to 100% ethanol again. Previously the galeae of *G. nitidum* and the maxillary palps of *L. rostratus* were removed to be scanned separately. These objects were fixed in specially prepared Eppendorf tubes filled with ethanol (Metscher 2009 a,b). Scans were taken overnight with an Xradia MicroXCT system at the Department of Theoretical Biology (University of Vienna, Austria). Objects were scanned in cross section with different resolutions (pixel size*: L.*

rostratus 4.599 µm, *G. nitidum* 3.057 µm, *N. chrysomelina* 5.0443 µm), each scan consisting of staples of single images (in *L. rostratus* 512 images, *G. nitidum* 495 images, *N. chrysomelina* 714 images). The recombination of the single cross sections into 3D models of the objects was done with the ImageJ® software. This program enables one to rotate the model on all three axes and to manipulate it in various ways.

3. Results

3.1. *Leptopalpus rostratus* (Fig. 1)

The prognathous head is triangulate in shape (without mandibles) with a broad proximal head capsule and a strongly pronounced postgenal region. The smooth emarginated eyes are located laterally. The antennae insert above the dorsal joint of the mandibles between the eyes. The mouthparts, consisting of labrum, mandibles, maxillae and labium, are directed forward parallel to the body axis. The maxillary palps are conspicuously elongated – nearly as long as the body – and are the main organs for nectar uptake (Fig. 1A, B).

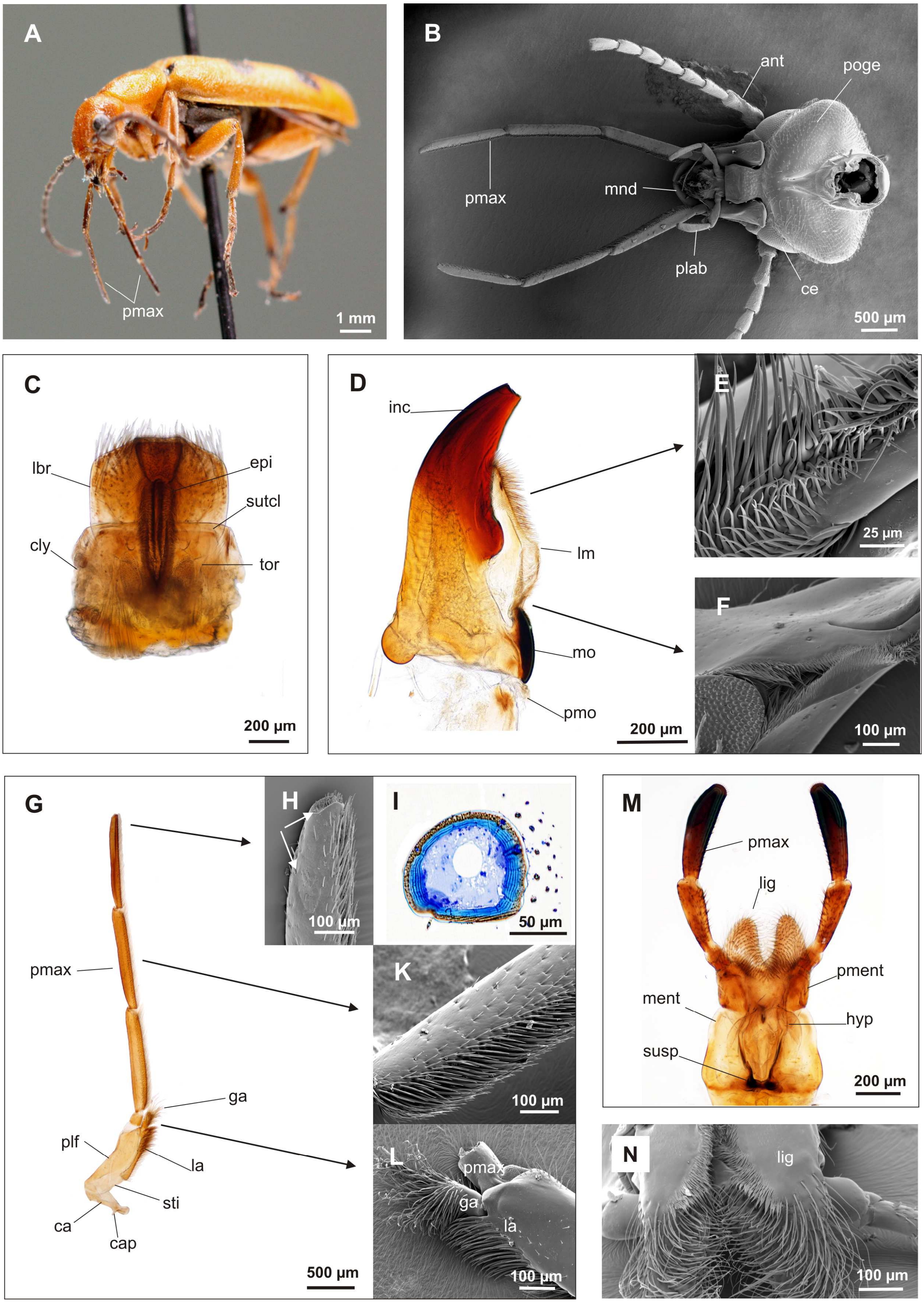
Labrum (Fig. 1C): The labrum is more or less quadratic in shape. It extends nearly to the apex of the mandibles and closes the praeoral cavity dorsally. It is hinged to the distinct clypeus at the sclerotized sutura clypeolabralis. The outer surface and the inner margins are equipped with long bristles. At the distal edge of the inner surface, a glabrous pore plate is located medially. Proximally is a distinct epipharynx that nearly extends to the posterior edge of the clypeus and is located on the median axis. The epipharynx slopes down proximally and possesses three rows of fine setae. The setae of the lateral row are vertically erected, followed by a second row of setae which is directly median to the inner groove. The setae of the third row are directed caudally to the pharynx. Next to the epipharynx are two sclerotized bars (tormae) which originate from the sutura clypeolabralis (Fig. 1C).

Mandible (Fig. 1D): The left and right mandible are symmetrical, each mandible consists of an incisor part, a lacinia mobilis, a mola plate and a postmola. The sclerotized incisor part is shovel-like with a relatively acute apex that is curved to the body axis. The discoid mola plate is sclerotized and its grinding surface is covered with small teeth that are posteriorly directed. In the proximal area, these teeth were intact in one individual, however, completely worn down in another individual. The soft lacinia mobilis extends between the mola plate and the mid incisor part. This lobe-like structure possesses two different types of bristles at the free margins. The distal half bears rows of dense bristles (30 – 100 µm) with a bulb-like basis forming a comb-like structure (Fig. 1E). The proximal edges are covered with small rod-like bristles of different length (5 – 15 µm). Groups of three to seven parallel setae that originate at the edge of small scales form triangular structures that are arranged in tiles (Fig. 1F). The space in front of the mola plate is covered with similar bristle units of greater length. The postmola, which is also a setose lobe, originates at the proximal margin of the mola and extends into the oesophagus.

Maxilla (Fig. 1G): The paired maxillae consist of cardo, stipes, lacinia, galea and a four-segmented maxillary palp. The three distal segments of the maxillary palp are greatly elongated, each measuring approximately 1.3 mm. These segments possess a median row of distally directed, unruffled and smooth curved bristles with arcuate tips (Fig. 1K). All bristles have the same length (diameter about 130 µm; n = 10) except those of the distal ends of the 2nd and 3rd segment which measure double length. The distal bristles of the 4th segment are shorter than all others, they extend to the rods of the sensory pit at the apical end (Fig. 1H). A second sensory pit is located ventrally in the distal region of the 4th segment (Fig. 1H). All three segments are more or less round in cross section (Fig. 1I). The first segment is clearly shorter than the three others and nearly as long as the galea, measuring about 200 µm. This segment is glabrous except for a single long and thin seta on the ventral side that points distally.

The bulb-shaped galea (Fig. 1L) possesses long and corkscrew-like bristles in the distal region that form a brush-like structure positioned next to the first segment of the maxillary palp. The bristles of the basal region are shorter and less twisted and resemble those of the lacinia. The median distal half of the lacinia is densely covered with thick and smooth bristles that curve backward (Fig. 1L). The proximal half is less densely covered with thin and short bristles.

Fig. 1 Mouthparts of *Leptopalpus rostratus* (photograph, scanning electron micrographs, light microscope images and semithin section); **A** Body of *L. rostratus*. **B** Ventral view of the head capsule. **C** Inner surface of the labrum. **D** Mandible. **E** Distal part of the lacinia mobilis of mandible. **F** Proximal part of the lacinia mobilis. **G** Maxilla. **H** Tip of the distal segment with two sensilla regions (arrows). **I** Palp segment in cross section. **K** Part of a palp segment with median bristle row. **L** Vestiture of the brush-like galea and bristle arrangement on the comb-like lacinia. **M** Inner surface of the labium. **N** Ligula and v-shaped bristle rows. *Ant* antenna, *ca* cardo, *cap* cardo apodeme, *ce* compound eye, *cly* clypeus, *epi* epipharynx, *ga* galea, *hyp* hypopharynx, *inc* incisor part, *la* lacinia, *lbr* labrum, *lig* ligula, *lm* lacinia mobilis, *ment* mentum, *mnd* mandible, *mo* mola plate, *papo* praementum apodeme, *plab* palpus labialis, *plf* palpifer, *pmax* maxillary palp, *pment* praementum, *pmo* postmola, *poge* postgena, *sti* stipes, *susp* suspensorium, *sutcl* sutura clypeolabralis, *tor* tormae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Labium (Fig. 1M): The labium consists of submentum, mentum and the praementum with glossae, paraglossae and the three-segmented labial palps. The submentum is fused to the head capsule. The mentum is as long as wide and jointed to the praementum. Glossae and paraglossae are fused to the ligula which is bifid forming two large lobes. The notch between the lobes extends to the base of the labial palps at the half of the praementum. Both lobes are covered with long and individual bristles forming a brush-like structure. Two rows of dense and short setae on these lobes are v-shaped (Fig. N) and extend to the hypopharynx. The hypopharynx extends medially over the whole length of the mentum and consists of two large lobes covered with small setae at the distal edges. At the proximal edge of the praementum is a median apodeme which projects into the mentum and on which the labial musculature inserts. The dorsal arms of the suspensorium reinforce the hypopharynx and are fused to form a sclerotized bridge at the proximal edge of the mentum. Each labial palp bears two regions of sensilla on the clubbed distal segment, similar to the maxillary palps.

Pollen grains on mouthparts and gut content: Pollen grains of various species of Asteraceae were found between the bristles of the ligula and galea, on the one hand, and in the gut, on the other hand. Inside the gut, pollen grains were evenly spread over fore, mid, and end gut. The majority of pollen grains was intact or only exhibited a small pore at the inner wall (intine). Even if some gains were visibly damaged their content seemed to remain inside the pollen grain. Some pollen grains in the end gut had two vesicular structures alternately positioned.

3.2. *Nemognatha chrysomelina* (Fig. 2)

The prognathous head is as broad as wide (without mandibles). The postgenal area is moderately pronounced. The strongly emarginated eyes are located laterally. The antennae insert between the eyes above the dorsal joint of the mandibles. The mouthparts, consisting of labrum, mandibles, maxillae and labium, are directed forward and lie parallel to the body axis. The galeae are noticeably elongated, measure about the half the length of the head capsule and form the main organ for nectar uptake (Fig. 2A, B).

Labrum (Fig. 2C): The labrum is oval in shape and extends to the apex of the mandibles. It is movably connected to the distinct clypeus at the sutura clypeolabralis and covers the mouthparts dorsally to form a praeoral cavity. The outer surface bears long and individual bristles. The distal edge of the inner surface bears a slender and glabrous pore plate medially. A slender epipharynx extends medially over the whole length of the labrum, nearly reaching the proximal edge of the clypeus. The epipharynx possesses two rows of fine setae. The setae of the lateral row point vertically upward and those of the median row are directed toward the pharynx. Adjacent the epipharynx are tormae which originate from the sclerotized sutura clypeolabralis (Fig. 2C).

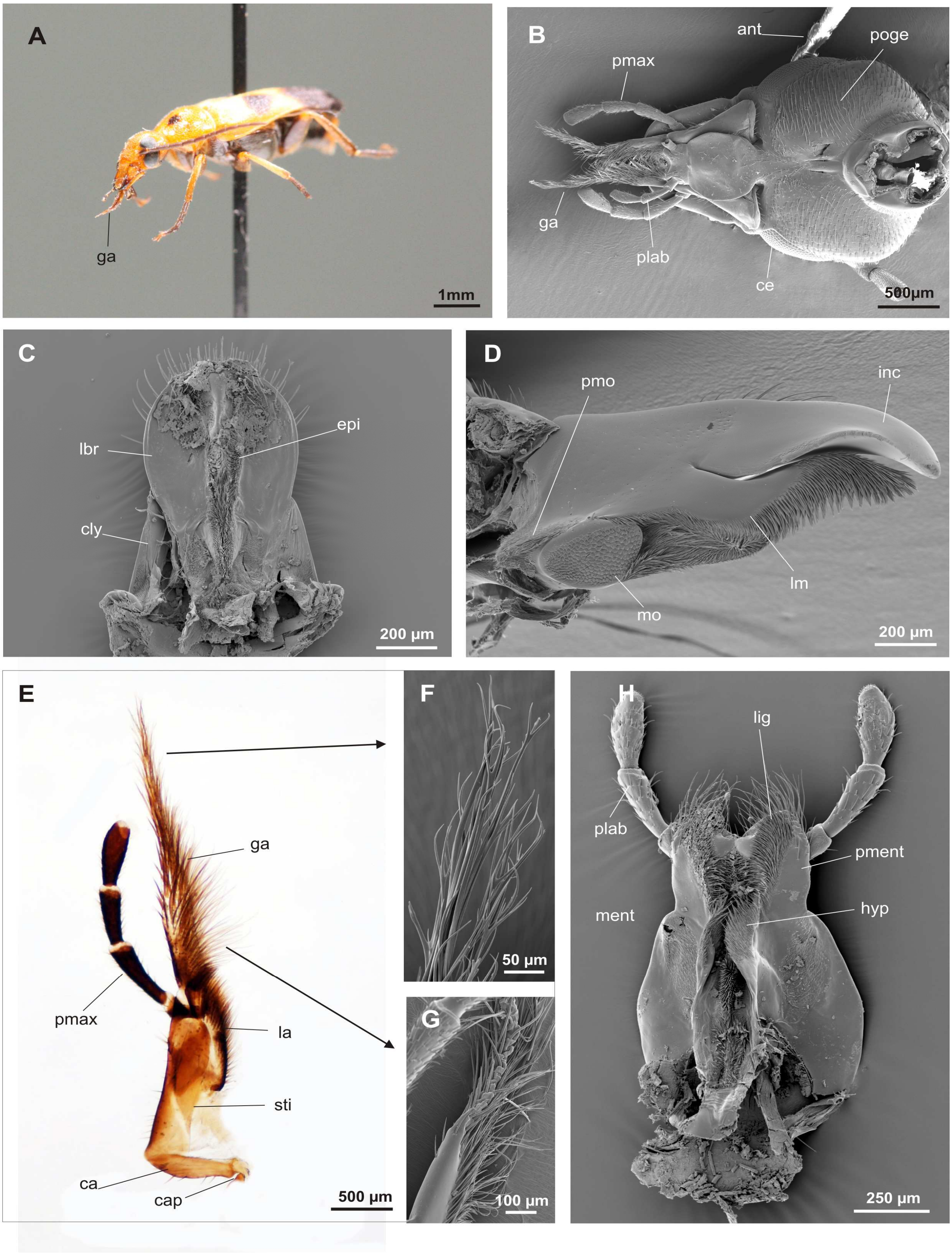
Mandible (Fig. 2D): The symmetrical left and right mandibles consist of incisor part, lacinia mobilis, mola plate and postmola. The sclerotized incisor part is moderately shovel-like with an acute apex that is strongly curved with respect to the body axis. The discoid mola plate possesses a smooth sculptured surface. The soft lacinia mobilis extends between the mola plate and the tip of the mandible. The distal half of this structure bears rows of dense bristles with a bulb-like basis forming a comb-like structure. The proximal edges of the lacinia mobilis are covered with small rod-like bristles of different lengths forming triangular bristle units that are arranged in tiles. The space in front of the mola plate is covered with similar ring-like bristle units in which the setae are all directed toward the center. The soft and setose postmola originates behind the mola plate and extends into the oesophagus.

Maxilla (Fig. 2E): The paired maxillae consist of cardo, stipes, lacinia, the four-segmented maxillary palp and a conspicuous elongated galea measuring about 1.5 mm. The basal region of the galea is medially equipped with bristles that are long and wavy, while the rest of the surface is completely covered with short and straight bristles. All bristles are smooth with acute tips, except for the distal ones which are branched up to five times and form a slender brush-like tongue (Fig. 2F). The surface structure of the galea is very rough at the tip region (Fig. 2G). The lacinia is densely covered with smooth backward curving bristles in the distal half to form a comb-like structure. These bristles measure about half the length of the proximal galea bristles. The bristles of the proximal part are less dense, thinner and shorter than the distal bristles. The maxillary palps measure about half the length of the galea and bear two regions of sensilla on each distal segment.

Labium (Fig. 2H): The labium consists of a submentum, a mentum and the praementum with glossae, paraglossae and the three-segmented labial palps. The submentum is fused to the head capsule. The mentum is more or less round and is jointed to the praementum that is about four times smaller than the mentum. The glossae and paraglossae are fused to form the ligula which is bilobed. The groove does not reach the base of the labial palps. The lobes are covered with long and individual bristles. Two rows of dense and short seta form a v-shape and lead to the strongly developed hypopharynx that extends to the proximal end of the mentum. A row of caudally facing bristles is located in the median axis between the two lobes of the hypopharynx that are setose in the distal half. The labial musculature inserts on the apodeme which faces caudad from the proximal edge of the praementum. The dorsal arms of the suspensorium are not fused to a sclerotized bridge. Each labial palps bear two regions of sensilla on the distal segments.

Pollen grains on mouthparts and gut content: Pollen grains of Asteraceae were found between the bristles of ligula and galea, as well as in the dissected gut. Most pollen grains were found in the end gut where only a few were cracked in the middle which is the thinnest part of the exine. The majority of pollen grains was intact or exhibited a small pore at the inner wall (intine).

Fig. 2 Mouthparts of *Nemognatha chrysomelina* (photograph, scanning electron micrographs and light microscope image); **A** Body of *Nemognatha chrysomelina*. **B** Ventral view of the head capsule. **C** Inner surface of the labrum. **D** Mandible. **E** Maxilla. **F** Brunched bristles at the galea tip. **G** Rough surface structure of the galea in the proximal region. **H** Inner surface of the labium. *Ant* antenna, *ca* cardo, *cap* cardo apodeme, *ce* compound eye, *cly* clypeus, *epi* epipharynx, *ga* galea, *hyp* hypopharynx, *inc* incisor part, *la* lacinia, *lbr* labrum, *lig* ligula, *lm* lacinia mobilis, *ment* mentum, *mnd* mandible, *mo* mola plate, *plab* palpus labialis, *pmax* maxillary palp, *pment* praementum, *pmo* postmola, *poge* postgena, *sti* stipes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3.3. *Gnathium nitidum* (Fig. 3)

The prognathous head is prolate and nearly two times longer than wide. The postgenal area is barely pronounced. The eyes are located laterally and are not emarginated. The antennae insert above the dorsal joint of the mandibles between the eyes. The mouthparts are directed forward parallel to the body axis. The conspicuous elongated galeae, measuring nearly half of the body length, are the main organs for nectar uptake (Fig. 3A, B).

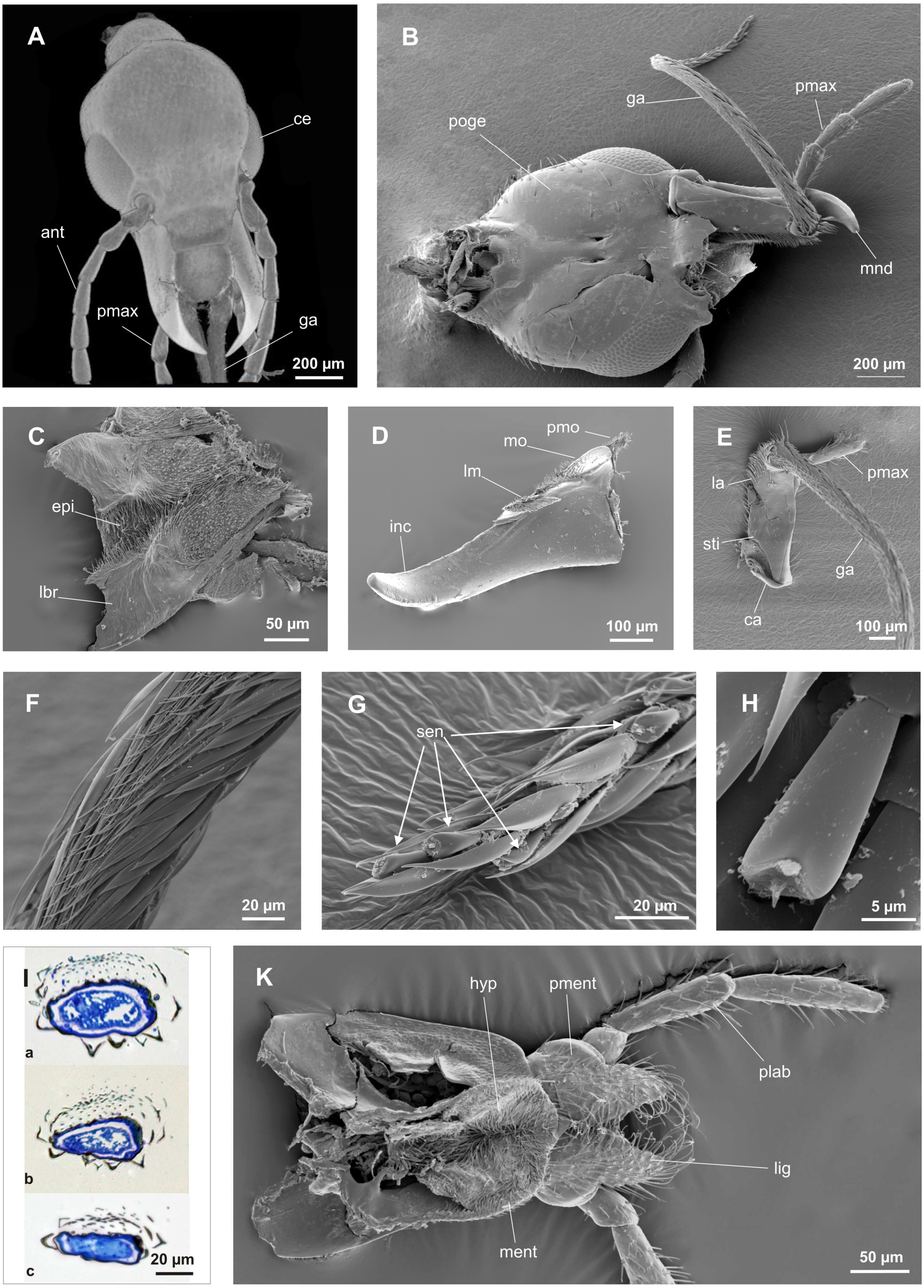
Labrum (Fig. 3C)**:** The labrum is round in shape extending to the half of the mandible length. It is movably connected to the slightly smaller clypeus at the sutura clypeolabralis. The outer surface bears few long and individual bristles. The epipharynx on the inner side is relatively broad and shallow. The bristles are directed toward the median groove.

Mandible (Fig. 3D): Left and right mandible are symmetrical. Each consists of incisor part, lacinia mobilis, mola plate and postmola. The mandibles are as long as the head capsule possessing a slender and prolate incisor part with a moderate acute tip smooth curved to the body axis. The discoid mola plate possesses small grinding teeth directed posterior in the distal half. The soft lacinia mobilis extends from the mola plate to the half of the mandible length. The distal half of the lacinia mobilis forms a comb-like structure with rows of bristles possessing a bulb-like basis. The edges of the proximal half possess small rod-like bristles. They form triangulate units that are arranged in tiles. The space in front of the mola plate is covered with longer bristles. The soft and setose postmola is relatively small and originates behind the mola plate.

Maxilla (Fig. 3E): The paired maxillae consist of cardo, stipes, lacinia, the four-segmented maxillary palps and the galeae which form the most conspicuous parts of the maxillae. The galeae are greatly elongated, filiform and measure about 1.8 mm. The whole surface of the galea is densely covered with two types of long and distally oriented bristles. The median bristles are thin and smoothly flattened. The rest of the surface is covered with overlapping bristles possessing an acute tip and a keeled dorsal side (Fig. 3F). The thick bristles are as long as the thin median ones over the whole galea length. The length of both bristle types changes from about 85 µm at the galea base, to 75 µm in the middle region and 50 µm in the tip region. In the tip region, the bristles are less dense and the rough galea surface is visible. In addition, the tip region is equipped with several club-like sensilla (length about 20 µm) which point distally (Fig. 3G, H). The galeal surface is round in cross section in the proximal area, whereas it flattens more and more to a nearly rectangular cross section in the distal region (Fig. 3I, a - c). The galea does not possess a concave inner surface. The surficial area formed by the bristles of the galeae is also more or less round in cross section over the whole length. Thus, a food canal can neither be formed by the galeal surfaces nor by the bristles. The lacinia forms a comb-like structure near the base of the galea which is equipped with a median row of dense bristles in the distal region. The maxillary palps are nearly as long as the mandibles and each possess two regions of sensilla at the tip of the distal segment.

Labium (Fig. 3K): The labium consists of the submentum, the mentum and the praementum with glossae, paraglossae and the three-segmented labial palps. The submentum is fused to the head capsule. The mentum is two times longer than wide with a rectangular shape. It is jointed to the praementum which is as broad as the mentum but measures only half its length. The glossae and paraglossae are fused to the ligula which is bilobed. The groove between the lobes reaches the base of the labial palps. Both lobes possess long and individual bristles that are curved inward. The hypopharynx consists of two large lobes, the inner surfaces of which are densely covered with fine setae. Each labial palp bears two regions of sensilla on the distal segment similar to the maxillary palps.

Pollen grains on mouthparts and gut content: No pollen grains were found between the bristles. In the gut of one individual few Asteraceaen pollen grains were present evenly spread over fore, mid and end gut. Two pollen grains in the end gut were clearly damaged, the rest of the pollen grains showed no sign of mechanical deformation.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K
hyp
pment
plab
lig
ment
50 µm

3.4. Musculature of heads and mouthparts (Fig. 4)

No differences were apparent among *L. rostratus*, *N. chrysomelina* and *G. nitidum* concerning the composition and the course of head and mouthpart muscles. The following description of the origins and insertions of the single muscles is, in principle, similar for all three species. One special feature was found in the head of *L. rostratus*, a muscular lobe-like structure located between cerebrum and suboesophageal ganglion (Fig. 4A, B). This structure is absent in *G. nitidum* and *N. chrysomelina*. The probable functions of the described muscles are mentioned in table 2. The nomenclature and numeration of the muscles in parentheses follow Kéler (1963).

Labrum (Fig. 4C)

Musculus labroepipharyngalis (M. 7): This pair of muscles originates medially at the dorsal labrum wall and inserts laterally at the ventral labrum wall. The origin area is always smaller than the insertion area.

M. frontoepipharyngalis (M. 9): These two large muscles originate on the frons above the cerebrum. In *Leptopalpus* they originate in front of M. 41 and in *Nemognatha* and *Gnathium* they insert beside M. 41. They insert on the caudad oriented tips of the tormae.

Mandible (Fig. 4D)

M. craniomandibularis internus (M. 11): This three-portioned muscle is the largest head muscle and originates over the whole posterior head capsule behind the eyes. It inserts on a slender tendon plate directly behind the postmola.

M. craniomandibularis externus (M. 12): This muscle is much smaller than M. 11, originates on the posterior lateral head capsule and inserts on a tendon plate below the articulation joint of the mandible. The first half of the muscle is widespread, and the second half is narrowly constricted.

Fig. 3 Mouthparts of *Gnathium nitidum* (3D reconstruction based on MicroCT, scanning electron micrographs and semi thin sections); **A** Dorsal view of the head capsule. **B** Ventral view of the head capsule. **C** Inner surface of the labrum (fragmented). **D** Mandible. **E** Maxilla. **F** Frontal view of the thin median bristles of the galea. **G** Tip region of the galea with sensilla. **H** Bottle-shaped sensilla of the galea. **I** Galea in cross section in the tip region (a), in the middle (b) and the proximal region (c). K Inner surface of the labium. *Ant* antenna, *ca* cardo, *ce* compound eye, *cly* clypeus, *epi* epipharynx, *ga* galea, *hyp* hypopharynx, *inc* incisor part, *la* lacinia, *lbr* labrum, *lig* ligula, *lm* lacinia mobilis, *ment* mentum, *mnd* mandible, *mo* mola plate, *plab* palpus labialis, *pmax* maxillary palp, *pment* praementum, *pmo* postmola, *poge* postgena, *sti* stipes, *sen* sensilla.

M. tentoriomandibularis (M. 13): This very small muscle originates on the anterior tentorial arm where all antennal muscles originate. The muscle inserts on an apodeme on the ventral inner wall of the mandible.

Maxilla (Fig. 4E)

M. craniocardinalis externus (M. 15): This large muscle originates broadly on the ventral posterior head capsule and inserts laterally on the cardo apodeme.

M. tentoriocardinalis (M. 17): This muscle is smaller than M. 15 and consists of a dorsal and a ventral portion (1 + 2), both of which originate on the posterior tentorial arm. The muscle inserts median on the cardo apodeme.

M. craniolacinialis (M. 19): This very large muscle originates on the posterior tentorial arm and extends along the body axis to the lateral basis of the lacinia.

M. stipitolacinialis (M. 20): A relatively thick muscle that originates ventrolateral on the stipes and inserts on the lateral basis of the lacinia.

M. stipitogalealis (M. 21): This slender muscle has its origin medially on the ventral stipital wall and inserts at the base of the galea.

M. stipitopalpalis externus (M. 22): This large muscle originates on the inner wall of the palpifer in front of M. 20 and inserts on the lateral basal ridge of the 1st palpal segment.

M. stipitopalpalis internus (M. 23): This muscle is as large as M. 22 and originates on the ventral stipital wall below M. 20. The insertion is medially on the inner basal ridge of the 1st palpal segment.

M. palpopalpalis tercius (M. 26): This muscle originates dorsally at the base of the 2nd palpal segment in *Nemognatha* and *Gnathium* (Fig. 4E right side). In *Leptopalpus* it originates dorsally on the distal third of the 2nd palpal segment (Fig. 4E left side). The insertion is on the median basal ridge of the 3rd palpal segment in all three genera.

M. palpopalpalis quartus (M. 27): This muscle is similar to M. 26 and originates dorsally at the base of the 3rd palpal segment in *Nemognatha* and *Gnathium.* In *Leptopalpus* it originates dorsally on the distal third of the 3rd palpal segment. The insertion is medially on the basal ridge of the 4th palpal segment in all three genera.

Labium and Hypopharynx (Fig. 4F)

M. submentopraementalis (M. 28): This pair of muscles originates at the proximate edge of the submentum and inserts lateral on the median apodeme of the praementum.

M. tentoriopraementalis superior (M. 30): A pair of large muscles that originates on the proximal tentorial arm and inserts at the tip of the median apodeme of the praementum.

M. frontohypopharyngalis (M. 41): These short and large muscles originate on the frons. In *Leptopalpus* the origin is directly in front of M. 9, in *Gnathium* and *Nemognatha* beside M. 9. In all three genera they insert on the dorsal arms of the suspensorium which reinforces the pharynx.

Cibarium and Pharynx (Fig. 4A)

M. clypeopalatalis (M. 43): The origin of this muscle is directly in front of the sutura epistomalis and its insertion is on the dorsal wall of the cibarium. In *Nemognatha* the insertion area is slightly broader than the origin area. In *Gnathium* it is more expanded and in *Leptopalpus* the insertion area is nearly twice as large as the origin area. Here, the first muscle strand after the sutura epistomalis is oriented backwards under the sutura epistomalis and inserts between two strands of M. 67 near the insertion of M. 44.

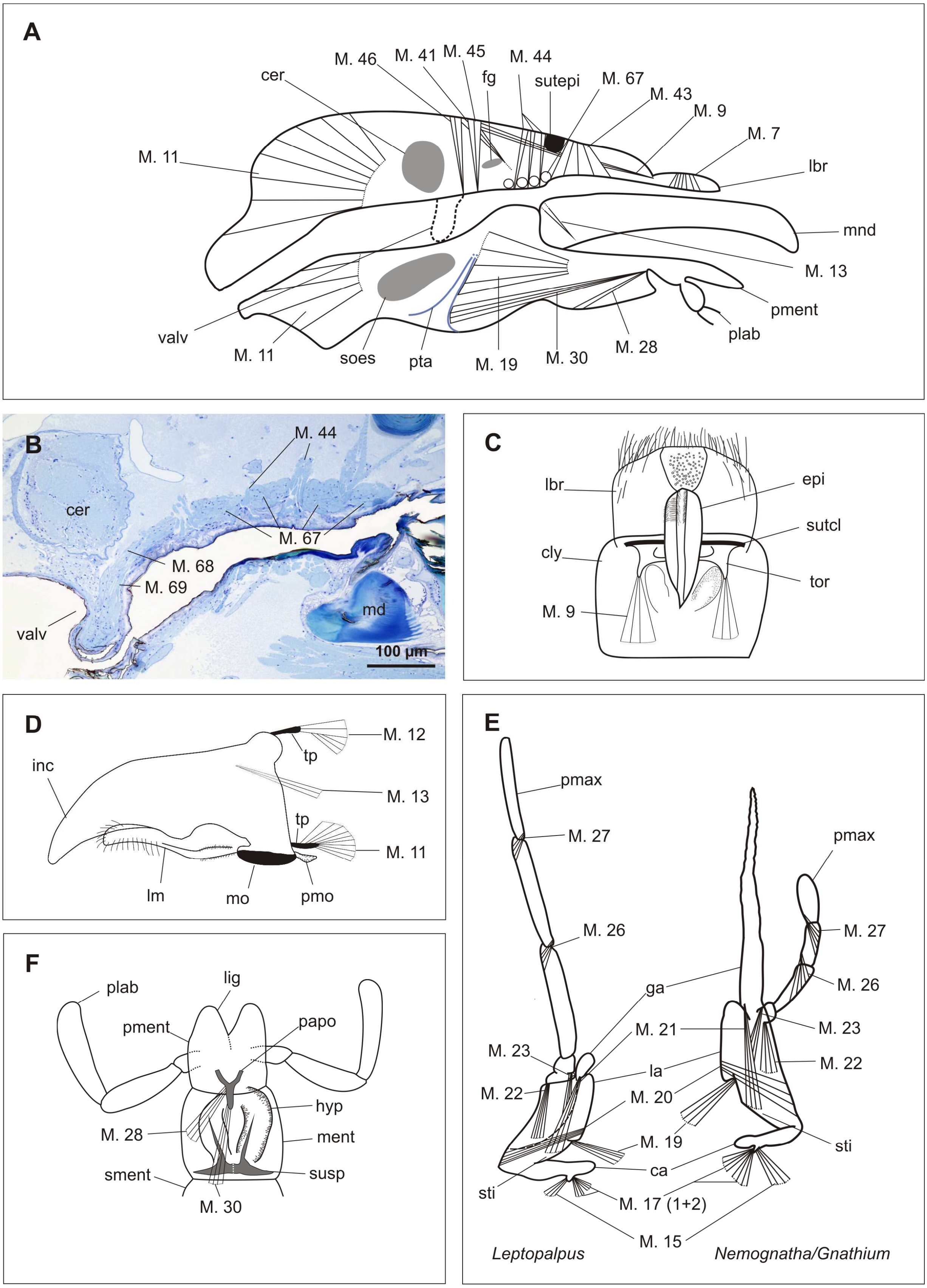
M. clypeobuccalis (M. 44): This muscle consists of three strong portions that origin one after another directly after the sutura epistomalis. They insert on the dorsal praepharyngeal wall in front of the frontal ganglion between the muscle strands of M. 67.

M. frontobuccalis anterior/posterior (M. 45/ M. 46): These two similarly strong muscles originate on the frons. M. 45 directly after M. 44 and M. 46 directly after M. 45. Both insert on the dorsal praepharyngeal wall behind the frontal ganglion.

M. tentoriobuccalis anterior (M. 48): This pair of muscles originates on the ventral head capsule near the posterior tentorial arm and inserts on the ventral praepharyngeal wall.

M. transversalis buccae (M. 67): The four portions of these large muscles run dorsally over the praepharynx and insert on the anterior tentorial arms.

M. anularis/longitudinalis stomodaei (M. 68/ M. 69): The circular musculature M. 68 begins behind M. 67 and surrounds the whole pharynx together with the longitudinal musculature M. 69, which lies under M. 68. In *L. rostratus* a lobe-like structure originates on the dorsal pharyngeal wall and extends vertically down the ventral pharyngeal wall. The lobe is surrounded by M. 68 and M. 69 (Fig. 4B). In *Gnathium* and *Nemognatha* the lobe structure is absent.


A
cer
M. 46
M. 41
M. 45
fg
M. 44
sutepi
M. 67
M. 43
M. 9
M. 7
lbr
M. 11
mnd
M. 13
pment
plab
valv
M. 11
soes
pta
M. 19
M. 30
M. 28
B
M. 44
cer
M. 67
M. 68
M. 69
valv
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100 µm
C
lbr
epi
sutcl
cly
tor
M. 9
D
inc
M. 12
tp
M. 13
tp
M. 11
lm
mo
pmo
E
pmax
M. 27
M. 26
pmax
M. 27
M. 26
ga
M. 21
M. 23
M. 23
la
M. 22
M. 22
M. 20
M. 19
sti
ca
sti
M. 17 (1+2)
M. 15
Leptopalpus
Nemognatha/Gnathium
F
plab
lig
pment
papo
hyp
M. 28
ment
sment
susp
M. 30

Fig. 4 Mouthpart and head musculature of Nemognathinae (schematic line drawings and semithin section). **A** Sagittal section of a head with the muscular valve of *L. rostratus* (broken line). **B** Sagittal section of the head of *L. rostratus* shows the pharyngeal region with the valve in detail. **C** Inner surface of labrum. **D** Mandible. **E** Left maxilla of *Leptopalpus* (left side) and right maxillae of *Nemognatha* and *Gnathium* (right side). **F** Inner surface of labium. *Ca* cardo, *cap* cardo apodeme, *cer* cerebrum, *cly* clypeus, *epi* epipharynx, *fg* frontal ganglion, *ga* galea, *hyp* hypopharynx, *inc* incisor part, *la* lacinia, *lbr* labrum, *lig* ligula, *lm* lacinia mobilis, *ment* mentum, *mnd* mandible, *mo* mola plate, *papo* praementum apodeme, *plab* palpus labialis, *plf* palpifer, *pmax* maxillary palp, *pment* praementum, *pmo* postmola, *pta* posterior tentorial arm, *sment* submentum, *soes* suboesophageal ganglion, *sti* stipes, *susp* suspensorium, *sutcl* sutura clypeolabralis, *sutepi* sutura epistomalis, *tor* tormae, *valv* valve. The abbreviations for the muscles are described in the text and in table 2.

Tab. 2 Musculature of heads and mouthparts of *Leptopalpus rostratus*, *Nemognatha chrysomelina,* and *Gnathium nitidum*, origins, insertions and probable functions. The symbol (+) indicates presence of a slender muscle, (++) presence of a moderately developed muscle, (+++) presence of a very well-developed muscle. Nomenclature and numeration of muscles follow Kéler (1963).

Muscle	*Leptopalpus rostratus*	*Nemognatha chrysomelina*	*Gnathium nitidum*	Origin	Insertion	Function
Labrum						
M. labroepipharyngalis (M. 7)	+	+	+	dorsal labrum wall	ventral labrum wall	raise epipharynx
M. frontoepipharyngalis (M. 9)	++	++	++	frons	proximale tormae	depressor of labrum, moves epipharynx
Mandible						
M. craniomandibularis internus (M. 11)	+++	+++	+++	posterior head capsule	tendon plate behind postmola	adductor mandible
M. craniomandibularis externus (M. 12)	+++	+++	+++	lateral posterior head capsule	tendon plate ventral of joint head	abductor mandible
M. tentoriomandibularis (M. 13)	+	+	+	anterior tenorial arm	ventral inner wall of mandible	adductor of mandible?
Maxilla						
M. craniocardinalis externus (M. 15)	++	++	++	lateroventral head capsule	lateral cardo apodem	adductor of cardo
M. tentoriocardinalis (1+2) (M. 17)	++	++	++	posterior tentorial arm	median cardo apodem	adductor of cardo
M. craniolacinialis (M. 19)	++	++	++	posterior tentorial arm	lateral lacinia basis	adductor of lacinia
M. stipitolacinialis (M. 20)	++	++	++	ventrolateral stipes basis	lateral lacinia basis	adductor of lacinia
M. stipitogalealis (M. 21)	+	+	+	ventromedial stipes wall	lateral lacinia basis	abductor of galea
M. stipitopalpalis externus (M. 22)	+	+	+	inner palpifer wall	lateral basis 1st palpal segment	abductor of palpus maxillaris
M. stipitopalpalis internus (M. 23)	+	+	+	ventral stipital wall	inner median basis 1st palpal segment	adductor of palpus maxillaris

M. palpopalpalis tercius (M. 26)	+	+	+	dorsal 2nd palpal segment	basis 3rd palpal segment	flexor 3rd segment
M. palpopalpalis quartus (M. 27)	+	+	+	dorsal 3rd palpal segment	basis 4th palpal segment	flexor 4th segment
Labium/Hypopharynx						
M. submentopraementalis (M. 28)	+	+++	++	submentum	transition mentum/ praementum	retractor of praementum
M. tentoriopraementalis superior (M. 30)	++	++	++	proximal tentorial arm	transition mentum/ praementum	adductor of praementum
M. frontohypopharyngalis (M. 41)	++	++	++	frons median of M. 9	hypopcharyngeal suspensorium	raise hypopharynx
Cibarium/Pharynx						
M. clypeopalatalis (M. 43)	+++	+	++	distal sutura epistomalis	dorsal cibarium wall	dilator of cibarium
M. clypeobuccalis (M. 44)	++	++	++	proxilmal sutura epistomalis	dorsal pharyngeal wall	dilator of praepharynx
M. frontobuccalis anterior (M. 45)	++	++	++	frons, distal of M. 46	dorsal pharyngeal wall	dilator of praepharynx
M. frontobuccalis posterior (M. 46)	++	++	++	frons, proximal of M. 45	dorsal pharyngeal wall	dilator of praepharynx
M. tentoriobuccalis anterior (M. 48)	+	+	+	ventral head capsule	ventral pharyngeal wall	dilator of praepharynx
M. transversalis buccae (M. 67)	+++	+	++	anterior tentorial arm, dorsally attached to the praepharynx		compressor of the praepharynx
M. anularis stomodaei (M. 68)	++	+	+	circular musculature of the pharynx		peristaltic movements of the pharynx
M. longitudinalis stomodaei (M. 69)	+	+	+	longitudinal musculature of the pharynx		peristaltic movements of the pharynx

4. Discussion

Adult flower visiting insects of numerous orders possess various mouthpart adaptations to take up liquid and/or solid food. In the subfamily Nemognathinae (Coleoptera; Meloidae) special mouthpart modifications for nectar uptake are found in *Leptopalpus*, *Nemognatha* and *Gnathium.* Elongated mouthpart structures of the maxillae evolved independently and enable these beetles to feed on nectar from deep corolla of flowers. Astonishingly, different parts of their maxillae are modified to produce the proboscis-like structures. In *Leptopalpus* the maxillary palps are elongated, while in *Nemognatha* and *Gnathium* the galeae are greatly increased in length. The present study yields new insights into our understanding of mouthpart morphology in Nemognathinae and, as a consequence, to the kind of feeding mechanism used by these flower visiting beetles.

4.1. Morphological comparison of the mouthparts

The **maxillae** are the main organs for nectar uptake in all Meloidae. However, as mentioned above, it is either the elongated maxillary palps as in *Leptopalpus* or the elongated galeae as in *Nemognatha* and *Gnathium* which forms the proboscis. A suctorial mode of nectar uptake can be excluded because both the maxillary palps and galeae are only loosely connected for nectar uptake. They do not form a closed food canal, thus nectar cannot be taken up by a pressure gradient from the proboscis tip to the mouth opening. In short, they do not possess sucking mouthparts. Nevertheless, previous authors have stated that the concave inner margins of the filiform galeae come together to form a true food canal or sucking tube (Schremmer 1961, Kaszab 1962, Bologna and Pinto 2001). However, the present study, which is the first to investigate the mouthparts using SEM and semithin sections, shows that neither the maxillary palps of *Leptopalpus* nor the long galeae of *Gnathium* possess concave inner margins which could form a true food canal.

The maxillary palps of *Leptopalpus* are round in cross section and the median bristles are directed distally not medially and not caudally (Handschin 1929; Kaszab 1962). The bristles at the tips of the 2nd and 3rd segments are twice as long as the rest. Possibly, they bridge the distance between the segments at the articulations. The function of the single bristle on the short 1st segment is unclear. It could function as mechano-sensillum since it was assumed that this segment causes the movement of the whole palp (Handschin 1929). However, the ventral position of the sensillum does not allow contact between the sensillum and the palps when the palps are brought together for nectar uptake. Instead it maybe useful when the palps are flexed under the body in resting position.

The galea of *G. nitidum* is filiform and more or less round in cross section but never possesses a median groove. Only the tip region with its rough surface structure is similar to the whole galea of *N. chrysomelina*. In general, the bristles of the galeae in *Nemognatha* and *Gnathium* were described as long in short galeae and short in very long galeae (Kaszab 1962), however the long galeae of *G. nitidum* are densely covered with relatively long bristles. Furthermore, the galeal tips bear several club-like sensilla which have been discovered for the first time in beetles. Their external shape indicates that they function not only as mechano-sensilla but could be a combined mechano-chemosensitive sensilla which is a kind of sensillum known as sensillum styloconica and is otherwise found at the proboscis tip in Lepidoptera. In Lepidoptera, the elongation of the galeae for nectar uptake encompasses the evolution of novel sense organs in the tip region e.g. sensilla styloconica. They are crucial for detecting the opening of the corolla tube and localizing the nectar source using chemical and mechanical cues (Krenn et al. 2000, Krenn 2010). In contrast, the elongation of the maxillary palps in *Leptopalpus* does not necessitate the evolution of new sensory organs, since these beetles already possess sensilla in the tip region of the maxillary palp.

The short galeae of *N. chrysomelina* are likewise covered with two types of long bristles. The basal ones are straight and the distal ones are branched probably to enlarge the surface area and to improve their function as a brush-like tongue during nectar feeding. Sensilla in the tip region, as in *G. nitidum*, are absent. The probable presence of those sensilla in *Nemognatha* specimens with long galeae and the probable absence of them in *Gnathium* specimens with short galeae must be verified in further investigations, as well as their actual functioning.

The **labrum** of *L. rostratus*, *N. chrysomelina* and *G. nitidum* are similar in shape and close the preoral cavity dorsally. They possess a strongly developed epipharynx that spans over the labrum and clypeus and is not absent in *Leptopalpus* as described by Handschin (1929).

The fused glossa and paraglossa of the **labium** and the prominent median groove between both lobes is regarded as typical for flower visiting Meloidae. In the genus *Mylabris* a small indentation is present, in *Leptopalpus* the apical margin is cleft and in *Nemognatha* the ligula is deeply cleft (Handschin 1929). However, SEM and light microscope images show that the groove between both lobes extends to the articulation of the labial palps in *L. rostratus* but not in *N. chrysomelina*. Handschin (1929) described the labium of *L. rostratus* as simple and without a hypopharynx. However, findings of the present study clearly show that a strongly developed hypopharynx is present in all three species. The dorsal arms of the suspensorium, which support the pharynx, are separated in *Nemognatha*, similar to non-nectar feeding meloid beetles (Schneider 1981) but are fused to a sclerotized plate in *Leptopalpus*.

The **mandibles** of all studied species are similar and possess a soft lacinia mobilis, a postmola and a sclerotized mola plate. These modifications, as well as the variously shaped bristles, are characteristic features of pollen feeding beetles from various families (Fuchs 1974). The biting capacity is not reduced as the sclerotized incisor part is curved and has a somewhat acute tip. Kaszab (1962) assumed that they use their mandibles solely to bite the pupae shells since beetles with such elongated mouthparts need not bite a flower’s calyx to reach the concealed nectaries.

The head and mouthpart **musculature** of specialized nectar feeding beetles was investigated for the first time in this study using MicroCT and semithin section techniques. The study shows that the muscles are similar in all three genera regarding their presence and course. In fact, their conditions correspond largely to the muscles found in the non-nectar feeding meloid beetle *Lytta vesicatoria* (Meloinae) (Schneider 1981). Nevertheless, it is difficult to interpret potential differences of the muscles regarding functional aspects for feeding mechanisms in the three genera.

One special muscular structure was found in *L. rostratus* between cerebrum and suboesophageal ganglion which may be used to close the cibarium proximally and to separate it from the postpharynx. This may enable the cibarium to effect changes in pressure, and thus may function as a sucking pump. The fused dorsal arms of the suspensorium, which form a ventral plate to the praepharynx, may support this hypothesis.

Most likely, the musculus tentoriomandibularis, the third mandibular muscle, is generally present in Meloidae and is assumed to function as a part of the proprioceptive system measuring the position relative to the head capsule (Schneider 1981) and not as adductor of the mandible (Kéler 1963).

The maxillary musculus stipitopalpalis internus inserts at the inner median basis 1st palpal segment in all three genera and may function as a flexor of the palp, similar to the observation of Sexena (1953) in the meloid beetle *Mylabris pustulatus* (Meloinae). In contrast, Schneider (1981) described this muscle as only just reaching the distal border of the palpifer.

The present study shows that four portions of musculus transversalis buccae extend dorsally over the praepharynx. This condition differs from *L. vesicatoria* where only three of these compressor muscles are found. The larger number and obvious larger size of these muscles, especially in *Leptopalpus* and *Gnathium*, are probable adaptations for nectar feeding in Nemognathinae.

4.2. Feeding mechanisms

Since detailed studies of feeding behavior in flower visiting Nemognathinae are lacking, the present morphological study corrects several older assumptions and provides new insights into the feeding mechanisms of these beetles.

Nectar feeding of ***Leptopalpus rostratus*** has been simply described as strange shaking movements of the body when the proboscis is withdrawn from the inflorescence of a *Centaurea* (Asteraceae) flower. The assumption that nectar ascends to the mouth between the median bristles of the loosely connected maxillary palps by adhesion (Handschin 1929) is still remaining but this study indicates that nectar uptake is supported by a sucking pump-like structure similar to insects with sucking mouthparts. When the maxillary palps are brought together and inserted into the corolla tube, nectar ascends upward between the median bristles of the palps, the galeae and laciniae and accumulates in the preoral cavity. Dilator muscles of the cibarium and prepharynx may cause a change in pressure and accelerate nectar ingestion from the preoral cavity. The contraction of the massive compressor muscle of the praepharynx may force the nectar into the oesophagus working together with the sclerotized ventral plate of the praepharynx.

In addition to nectar, it has been known since the work of Handschin (1929) that *L. rostratus* consume pollen, as well. He assumed that pollen is transported to the mouth along the median row of bristles permitting contrary movements with the maxillary palps, and that the pollen grains are manipulated by the mandibles.

Although some pollen grains were also found in this study on the palps, the morphology of the mouthparts argues against a selective pollen uptake with the maxillary palps. Furthermore, flower morphology of Asteraceae causes the spatial separation of nectar and pollen. The small florets are densely clustered into so-called inflorescences. The anthers form a cylindrical tube around the style which grows through the anthers and pushes the pollen out so that it is presented above the surface of the florets, while the nectaries are located at the bottom of the corolla tube (Bresinsky et al. 2008). Thus, it can be assumed that the maxillary palps are inserted into the corolla tube for imbibing nectar and that pollen uptake occurs from above mainly with the mandibles. It can also be supposed that nectar and pollen feeding do not occur at the same time which is true of the lacewing *Nemoptera sinuate* (Nemopteridae), when it feeds on Asteraceaen flowers (Krenn et al. 2008). In the beetle, probably the galea and the lobes of the ligula with their long bristles are the main organs for harvesting pollen in addition to their function in nectar uptake. The brush shaped galeae with their wavy bristles resemble the galeae in pollen feeding protea beetles and monkey beetles (Cetoniidae) (Johnson and Nicolson 2001). Previously, it was assumed that pollen was acquired by outward sweeping movements of the brushy galeae in these beetles. However, Karolyi and Krenn (2008) observed that the mouthparts of *Cetonia aurata* (Scarabaeidae) are covered with liquid and therefore pollen is probably removed by dabbing the anthers with the wet brush rather than by a sweeping motion. It was also previously presumed that the liquid is extracorporeal and consists of regurgitated nectar, as known from other specialized flower visiting insects such as *Apis mellifera* workers which regurgitate nectar to enhance the adherence of pollen to the collecting structures (Hesse 1980). This liquid is likely not to be saliva since labial glands, although present in all insect orders, are allegedly absent in Coleoptera (Chapman 1998, Dettner and Peters 2003) except for some few families like Rhysodidae and Coccinellidae (Beutel 2003). The assumption that fresh or regurgitated nectar improves adhesion of pollen in *L. rostratus* seems obvious since nectar is present in the diet. Furthermore, other special adhering surface structures such as spoon and trumpet shaped bristles found in *Malachius bipustulatus* (Malachiidae) or apically broadened and sculptured bristles (Fuchs 1974, Schicha 1967) are lacking. Similar to other beetles, pollen is afterwards presumably conveyed over the robust lacinia comb between the mandibles where the soft and also comb-like lacinia mobilis transport the pollen to the mouth in conjunction with back-and-forth movements of the epi- and hypopharynx (Schremmer 1961). The hard sclerotized and partly worn molar plates suggest that intact ingested pollen grains are mechanically crushed with the mandibles, however this assumption has not been substantiated in other pollen feeding beetles (Krenn et al. 2005) and is unlikely to be the main method in Nemognathinae since the majority of pollen grains in the gut were intact. Johnson and Nicolson (2001) described several possibilities to overcome the resistant outer wall (exine) of pollen grains and suggested that the most likely method used by beetles is digestive enzyme penetration. Some intact pollen grains found in the gut of *L. rostratus* revealed two bubble-like and vesicular structures on opposite sides. Although it is reported that nectar in the gut can initiate pseudo-germination of pollen grains in other insects (Karolyi and Krenn 2008) this can be excluded here because pollen grains normally develop only a single pollen tube. Nevertheless, in the studied beetles, I conclude that a combination of mechanical and enzymatic techniques is used to open pollen grains, provided that pollen is actually taken up actively. If not, pollen may be passively taken up as a result of nectar feeding or grooming, as mentioned for *G. nitidum* (Pinto 2009).

Nemognatha chrysomelina was described to feed on nectar and pollen of thistle flowers (Schremmer 1961). This study supports the general assumption that nectar ascends between the bristles of the galea to the basal mouthparts and is transported further to the praeoral cavity. Nevertheless, it can be shown for the first time that the bristles of the galeal tip are branched several times such that they improve the function of the brush-like tongue due to the increased adherence of nectar. This effect may additionally be enhanced by the very rough surface structure of the galeae. Although no mouthpart movements are known to occur during nectar feeding, it should be clear that the galeae cannot remain motionless when taking up nectar, but sweeping or sponging movements are performed by the maxillae. Nectar feeding in *Nemognatha* species with short galeae may be akin to feeding behavior of Scarabaeid beetles (Johnson and Nicolson 2001) and is different from *Nemognatha* and *Gnathium* species with long galeae. Thus, nectar could be transported by mouthpart movements to the mouth. If so, that could explain why the cibarial musculature is less well developed than in *L. rostratus* and *G. nitidum*.

I found pollen grains lodged between the bristles of the galea, an observation which indicates that the brush-like galeae serve for both nectar and pollen feeding. Pollen may be transported to the mouth by parallel movements of the maxillae. Since the majority of the pollen grains in the gut were intact, it seems evident that the same mechanism also occurs in *L. rostratus*, as mentioned above.

Gnathium nitidum was observed under laboratory conditions walking over the inflorescences of *Chrysothamnus* (Asteraceae) and probing individual florets for a second or two before they bury their heads in a floret which last for about five minutes (Pinto 2009). The slender head enables them to enter deep into the florets and represents a further adaptation to nectar uptake from tubular flowers. The fact that they probe florets supports the assumption that the sensilla on the galeal tip are used to mechanically detect the corolla opening and to locate the nectar reservoir inside using chemical cues, as described above. The loosely connected galeae cannot produce a pressure gradient along the proboscis and there is no median food canal in which nectar can be sucked. Therefore it is probable that nectar ascends along the galeae by adhesion. In *G. nitidum* the galeal surface is densely covered with bristles forming a more or less sealed cavity whereupon the bristles with the dorsal blade could improve the connection between the galeal bristles. It is possible that the nectar ascends on the exterior surface between both loosely connected galeae and runs over the lacinia bristles to the mouth. Alternatively, nectar could ascend the inner surface between the bristles. Presumably, nectar uptake occurs in accord with both possibilities. Although a structure to close the pharynx proximally, as found in *Leptopalpus*, is lacking, the massive cibarial musculus transversalis buccae may suck or at least force the nectar from the praeoral cavity into the pharynx and thereby accelerate the nectar uptake.

Gnathium does not feed exclusively on nectar (Lovell 1915). Pollen ingestion was interpreted to be a consequence of cleaning behavior of galeae, legs and antennae to which pollen grains adhere (Pinto 2009). Pollen accumulates in the mouth and is transported and processed with mouthpart movements. Most likely, the main diet of *G. nitidum* is nectar but further food preferences remain unclear since the beetles have been observed to bite the stigma and anthers (Pinto 2009).

4.3. Evolutionary aspects

In insects, adaptations for consuming nectar and pollen are found predominately in the adults of holometabolous insects, probably because the metamorphosis permits a radical differentiation of the mouthparts between larva and adults. The mouthpart elongation in adult Nemognathinae is unique among beetles. The elongation of the galeae is only present in a few genera, all of which belong to Nemognathini (Bologna and Pinto 2001, Krenn et al. 2005). At least in *Gnathium* and *Nemognatha*, a great variety of different galeal lengths have developed ranging from very short forms to extremely long forms that exceed the length of the body (Kaszab 1962, Pinto 2009). Until present, it was assumed that the evolution of the proboscis composed of galeae was a single event in the history of Nemognathinae which probably occurred shortly after the diversification at the subfamily level of Meloidae during the Cretaceous period (125 – 100 Mya) (Kristensen and Beutel 2010). It was the second way in the convergent evolution of nectar feeding organs beside the elongation of the maxillary palps in *Leptopalpus*.

Previous conclusions held that the lengthening of the galeae, as mentioned for *Nemognatha* (Handschin 1929, Schremmer 1961, Kaszab 1962), applies to all Nemognathinae (Bologna and Pinto 2001, Krenn et al. 2005). As mentioned above the very long galea of *G. nitidum* differs from the described form of long galeae with respect to the bristle length and density, the concave median surface and the sensilla. This study shows that the proboscides, which are composed of lengthened galeae, must have evolved more than once.

Phylogenetic studies on the systematics of Meloidae based on morphological characters (Pinto and Bologna 2001) and molecular analyses (Bologna et al. 2008) strongly support the monophyly of the subfamily Nemognathinae. Nevertheless, the Nemognathini includes a heterogeneous assemblage of genera without clarified relationships (Kristensen and Beutel 2010). Together with the results of the present study, the hypothesis is evident that proboscides composed of elongated galeae evolved at least twice independently in the Nemognathine. Therefore, the evolution of a proboscis in nectar feeding Meloidae took place more than twice.

The status of *Leptopalpus* within the Nemognathinae still remains exceptional although it is confirmed by the systematic studies mentioned above. This genus, probably represented by a single present-day species, is the only one that possesses a sucking pump and a nectaring organ formed by elongated maxillary palps in contrast to several genera with elongated galeae. This development is astonishing and unique in Coleoptera and similar occurrences are rare in insects except among the Hymenoptera. In the Euryinae (Hymenoptera; Pergidae), for example, different elongated parts are involved in proboscis composition, namely glossa, paraglossa and galea in one representative and the maxillary palps in another representative (Krenn et al. 2005).

In general, nectaring proboscides have evolved convergently several times in multiple insect orders. Within the Hymenoptera long forms have developed about 25 times and moderately long forms over 100 times. Likewise, multiple taxa of Diptera have evolved elongated proboscides on several occasions. In contrast to the Hymenoptera and Diptera, the suctorial proboscis of Lepidoptera is regarded as a single morphological innovation of Glossata since it can be shown to have evolved only once (Krenn et al. 2005).

This study concludes that a convergent evolution of a nectaring proboscis involving recruitment from different components of the maxillae must have occurred in the Meloidae. Although a sucking pump-like structure is present in *Leptopalpus*, the uptake of nectar in all nectar feeding meloid beetles proceeds according to the principle of adhesion. In sum, this study hopes to stimulate further morphological and behavioral investigations of these exceptional beetles.

5. Abstract

Certain genera of meloid beetles in the tribe Nemognathini possess mouthparts that are adapted for nectar uptake from flowers with a deep corolla. Parts of the maxillae, the main organ for nectar uptake, are modified to form proboscis-like structures, some of which are longer than the body. These elongated structures have evolved several times independently in this tribe of beetles. In *Leptopalpus*, the four-segmented maxillary palps form the proboscis, in *Nemognatha* and *Gnathium* the galeae are elongated and loosely connected to enable nectar feeding. The first investigation of three representatives (*L. rostratus*, *N. chrysomelina* and *G. nitidum*) using SEM and MicroCT clearly demonstrates that neither of the two kinds of proboscis possess a median groove. Furthermore, the study shows that the filiform galea of *G. nitidum* is densely covered with long bristles and the tip region bears special sensilla. The investigation of the head and mouthpart musculature revealed that *L. rostratus* possess a muscular valve-like structure in the postoral region that may close the pharynx and thus function as a part of a sucking pump. Moreover, the cibarial and pharyngeal musculature appears to be more massive in Nemognathinae with long proboscides than in species with short proboscides. Nectar uptake could therefore function according to the principle of adhesion along the proboscis and be enhanced by a sucking pump. In addition to the adaptations for nectar feeding, the prognathous mouthparts of these beetles serve for pollen feeding, as well. Investigations of gut content indicates that ingested pollen grains are treated enzymatically rather than mechanically with the mandibles. Morphological and anatomical results yield new insights into the functional aspects of feeding behavior and indicate that a nectaring proboscis evolved at least three times convergently in the Nemognathinae, once by elongation of the maxillary palps and twice by elongation of the galeae.

6. Zusammenfassung

Einige Ölkäfer (Meloidae) innerhalb des Tribus Nemognathini besitzen Mundwerkzeuge, die an die Nektaraufnahme aus tiefen Blüten angepasst sind. Teile ihrer Maxillen, welche für die Nektaraufnahme verantwortlich sind, sind verlängert und bilden rüsselartige Strukturen, die länger als der Körper werden können. Solche verlängerten Strukturen sind mehrfach unabhängig entstanden. Bei *Leptopalpus* bilden die vier gliedrigen Maxillartaster den Rüssel, bei *Nemognatha* und *Gnathium* sind die Galeae verlängert und werden zur Nahrungsaufnahme lose zusammen gelegt. Die erstmalige morphologische Untersuchung dreier Stellvertreter (*L. rostratus*, *N. chrysomelina* und *G. nitidum*) mit REM und MicroCT konnte zeigen, dass keine der beiden Rüsselformen eine mediane Rinne besitzt. Außerdem ist die fadenförmige Galea von *G. nitidum* mit langen Borsten besetzt und in der Spitzenregion befinden sich spezielle Sensillen. Die Untersuchung der Muskulatur des Kopfes sowie der Mundwerkzeuge zeigte, dass *L. rostratus* im postoralen Bereich eine muskulöse Struktur besitzt, welche den Pharynx verschließen und somit den Teil einer Saugpumpe darstellen könnte. Zusätzlich scheint die cibariale und pharyngeale Muskulatur bei Nemognathinae mit langen Rüsseln ausgeprägter zu sein als bei solchen mit kurzen Rüsseln. Somit könnte die Nektaraufnahme entlang des Rüssels durch Adhäsion, unterstützt durch eine Saugpumpe funktionieren, welche den Nektar aus der Mundhöhle absaugt. Zusätzlich zu den Anpassungen an die Nektaraufnahme, sind die prognathen Mundwerkzeuge dieser Käfer für die Aufnahme von Pollen geeignet. Wie Untersuchungen des Darminhalts nahe legen, werden Pollenkörner eher enzymatisch im Darm aufgebrochen als mit den Mandibeln mechanisch zerstört. Die morphologischen und anatomischen Ergebnisse dieser Arbeit geben neue Hinweise auf funktionelle Aspekte bei der Nahrungsaufnahme und lassen den Schluss zu, dass sich der Nektarrüssel bei Nemognathinae mindestens dreimal konvergent entwickelt hat, nämlich einmal durch Verlängerung der Maxillarpalpen und zweimal durch Verlängerung der Galeae.

7. References

BARTH, F.G. (1991) Insects and Flowers. The Biology of Partnership. *Princeton University Press, Princeton, New Jersey.*

BETZ, O., THAYER, M.K., NEWTON, A.F. (2003) Comparative morphology and evolutionary pathways of the mouthparts in spore-feeding Staphylinoidea (Coleoptera). *Acta Zoologica (Stockholm)* **84**, 179 - 238.

BEUTEL, R.G. (2003) 26. Ordnung Coleoptera; Käfer. In: Dathe H.H Lehrbuch der speziellen Zoologie Band I: Wirbellose Tiere 5. Teil: Insecta, 2. Aufl. *Spektrum Akademischer Verlag Heidelberg. Berlin*, pp 426 – 526.

BLUMER, M.J.F., GAHLEITNER, P., NARZT, T., HANDL, C., RUTHENSTEINER, B. (2002) Ribbons of semithin sections: an advanced method with a new type of diamond knife. *Journal of Neuroscience Methods* **120:** 11 – 16.

BOLOGNA, M.A., PINTO, J.D. (2001) Phylogenetic studies of Meloidae (Coleoptera), with emphasis on the evolution of phoresy. *Systematic Entomology* **26**, 33 - 72.

BOLOGNA, M.A., OLIVERIO, M., PITZALIS M., MARIOTTINI P. (2008) Phylogeny and evolutionary history of the blister beetles (Coleoptera, Meloidae). *Molecular Phylogenetics and Evolution* **48:** 679 – 693.

BRESINSKY, A., KÖRNER, C., KADEREIT, J.W., NEUHAUS, G., SONNEWALD, U. (2008) Strasburger – Lehrbuch der Botanik. *Spektrum Akademischer Verlag Heidelberg. Berlin.*

CHAPMAN, R.E. (1998) The insects. Structure and function, 4th edition *Cambridge University Press, Cambridge.*

DETTNER, K., PETERS, W. (2003) Lehrbuch der Entomologie. 2. Aufl. *Spektrum Akademischer Verlag Heidelberg. Berlin*.

FUCHS, G.V. (1974) Die Gewinnung von Pollen und Nektar bei Käfern. *Natur und Museum* **104**, 45 - 54.

HANDSCHIN, **E.** (1929) Ein neuer Rüsseltypus bei einem Käfer. Biologische und morphologische Beobachtungen an *Leptopalpus rostratus* F. – *Zeitschrift für Morphologie und Ökologie der Tiere* **14**, 513-521.

HESSE, M. (1980) Zur Frage der Anheftung des Pollens an blütenbesuchende Insekten mittels Pollenkitt und Viscinfäden. *Plant Syst Evol Suppl* **133:** 135 – 148.

JOHNSON, S.A., NICOLSON, S.W. (2001) Pollen digestion by flower-feeding Scarabaeidae: protea beetles (Cetoniini) and monkey beetles (Hopliini). *Journal of insect Physiology* **47:** 725 – 733

KAROLYI, F., KRENN, H.W. (2008) Pollen grains adhere to the moist mouthparts in the flower visiting beetle *Cetonia aurata* (Scarabaeidae, Coleoptera). *Arthropod-Plant Interactions*. DOI 10.1007/s11829-008-9052-5.

KASZAB, **Z.** (1962) Merkmale der Adaption, Spezialisation, Konvergenz, Korrelation und Progression bei den Meloiden (Coleoptera). *Acta zoologica Academiae Budapest* **9**, 135-175.

KÉLER, **S.v** (1963) Entomologisches Wörterbuch. *Akademischer Verlag, Berlin.*

KRENN, H.W., KRISTENSEN, N.P. (2000) Early Evolution of the Proboscis of Lepidoptera (Insecta): External Morphology of the Galea in Basal Glossatan Moths Lineages, with Remarks on the Origin of the Pilifers. *Zool. Anz.* **239**: 179 – 196.

KRENN, H.W., J. PLANT & **N.U. SZUCSICH** (2005) Mouthparts of flower visiting insects. *Arthropod Structure and Development* **34**: 1-40.

KRENN, H.W., **GEREBEN-KRENN, B., STEINWENDER B.M., POPOV A.** (2008) Flower visiting Neuroptera: Mouthparts and feeding behaviour of *Nemoptera sinuata* (Nemopteridae). *Eur. J. Entomol.* **105**: 267 – 277.

KRENN, H.W. (2010) Feeding Mechanisms of Adult Lepidoptera: Structure, Function and Evolution of the Mouthparts. *Annu. Rev. Entomol.* **55**: 307 – 327.

KRISTENSEN, N.P., BEUTEL, R.G. (2010) Handbook of Zoology. Arthropoda: Insecta. Vol.2: Coleoptera: Beetles – Morphology and Systematics. *De Gruyter – Verlag, Berlin.*

LABANDEIRA, C.C. (1997) Insects mouthparts: ascertaining the paleobiology of insect feeding strategies. *Annual Review of Ecology and Systematics* **28**, 153 - 193.

LOVELL, J.H. (1915) The origin of anthophily among the Coleoptera. *Psyche* **22**, 67 - 84.

METSCHER, B.D. (2009 a) MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC Physiology* **9:**11 doi:10.1186/1472-6793-9-11.

METSCHER, B.D. (2009 b) MicroCT for Developmental Biology: A Versatile Tool for High-Contrast 3D Imaging at Histological Resolutions. *Developmental Dynamics* **238:** 632-640.

PERNSTICH, A., KRENN, H.W., PASS, G. (2003) Preparation of serial sections of arthropods using 2,2-dimethoxipropane dehydration and epoxy resin embedding under vacuum. *Biotechnic & Biochemistry. Biological Stain Commission* **78 (1):** 5 – 9.

PINTO, J.D. (2009) A taxonomic review of the genus *Gnathium* Kirby (Coleoptera: Meloidae). *Transactions of the American Entomological Society* **135**, 1 - 58.

PINTO, J.D., BOLOGNA, M.A. (1999) The New World genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural History* **33**, 569 - 620.

SCHICHA, E. (1967) Morphologie und Funktion der Malachiidenmundwerkzeuge unter besonderer Berücksichtigung von *Malachius bipustulatus* L. (Coleoptera, Malacodermata). *Zeitschrift für Morphologie und Ökologie der Tiere 60:* 367 – 433.

SCHNEIDER, W. (1981) Zur Kopfmorphologie der Imago des Ölkäfers *Lytta vesicatoria* (Coleoptera: Meloidae). – *Entomologia Generalis* **7(1)**, 69 - 87.

SCHREMMER, F. (1961) Morphologische Anpassung von Tieren-insbesondere von Insekten-an die Gewinnung von Blumennahrung. *Verhandlungen der Deutschen Zoologischen Gesellschaft* **25**, 375-401.

SEXANA, O.N. (1953) Musculature of *Mylabris pustulatus* (Thnb.) (Coleoptera). *Agra Univ. J. Res.* ***2:*** 285 – 302.

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9. Curriculum vitae

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EDUCATION

10/2009 – 02/2011	Diploma thesis: “Elongated mouthparts in nectar feeding Nemognathinae (Meloidae, Coleoptera)”
2004-2010	University of Vienna, Study of Biology Since 2006 study of Zoology (major: Evolutionary Biology) Completion of the second part of the study awarded with the highest possible grade, 1 (very good)
8/2002 – 8/2004	Graduated modern apprenticeship as farmer. Emphasis on biological crop raising, dairy farm and conventional pig breeding
7/2001 – 7/2002	Military service as medical corps soldier in the air force of the German armed forces
9/1992 – 6/2001	Secondary school in Nuremberg, Germany

WORK EXPERIENCE

10/2010 – 2/2011 and 10/2009 – 2/2010	Teaching assistant: Animal determination for teacher training students, *University of Vienna*
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Since 9/2008	Kindergarten and after-school teacher at Wiener Kinderfreunde, *Vienna*
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PROFESSIONAL ACTIVITIES

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	Course: entomological laboratory, determination and sorting of insects
	Field trip and course: Mediterranean habitats, Crete, *Greece* (1 week)
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Wien, am 16. Januar 2011

gezeichnet Andreas Wilhelmi