Evolution of head structures in Coleoptera with special emphasis on the feeding apparatus and miniaturized forms

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1 Introduction

Coleoptera is the largest order of the megadiverse Holometabola (Kristensen, 1999; Beutel et al., 2011; Misof et al., 2014; Beutel & McKenna, 2016). It includes approximately one quarter (~ 400000 spp.) of all described species of this lineage, which makes it also the largest known group of Metazoa of comparable rank and age. According to four different independent methods of estimations the total diversity of Coleoptera can reach 1.5 million species (Stork, 2015).

All representatives of the order have strongly sclerotized bodies with a shield-like pronotum, shortened mesothorax and forewings transformed into sclerotized elytra. The head is almost always prognathous and the antennae of extant species primarily consist of 11 segments (Beutel et al., 2014).

Considering the enormous size and complexity of the order (ca. 180 families), it is not surprising that its subgroups are highly diverse regarding their ecology and morphology. Beetles have managed to colonize all types of environment on our planet, apart from the polar region, the highest mountainous regions, and the open sea (e.g. Crowson, 1981). The body size of beetles varies between 0.3 mm (Ptiliidae, Nanosellini) and a maximum of 160 mm (Titanus giganteus L.; Cerambycidae). This increases the ecological versatility and small and very small species were apparently able to make use of a great variety of microhabitats as immatures and adults (Crowson, 1981). The tremendous diversity of Coleoptera and their appearance has made them attractive for entomologists for centuries. It has also inspired the Scottish-Indian geneticist and evolutionary biologist John B.S. Haldane to ascribe an “inordinate fondness for beetles” to the creator (e.g. Beutel et al., 2009).

Head structures and the feeding apparatus are extremely important, as they determine the biology and ecology of the species. Insect mouthparts, expressing a big variety and being an adaptation to various feeding preferences, can be an ideal model system for studying the effect of body structure on animal evolution and ecology (Betz, 2004).

Coleoptera consist of four extant suborders: Archostemata, Myxophaga, Adepagha and Polyphaga (Fig. 1). Regarding the feeding types of the adults, Archostemata are either aphagous, or feed on pollen or liquid substrates. Adepagha are almost exclusively predaceous as adults and larvae. Myxophaga are mostly saprophagous and feed on small plant particles or algae. Not surprisingly, Polyphaga – by far the largest suborder – is most diverse in terms of feeding habits, as already implied by the name. The majority of its representatives have are saprophagous, mycophagous or phytophagous, but various specialized feeding have developed in the group, for instance in the well-known fireflies. In contrast to other megadiverse or smaller groups of Holometabola, ecto- or endoparasitism play a marginal role in Coleoptera (e.g. Crowson, 1981).
Despite of the enormous diversity, the structure of the mouthparts of adults is relatively conservative – they are almost always orthopteroid, with biting mandibles. Within the framework of this presumptive groundplan condition, changes occur depending on the feeding type. For instance, the mandibles lack a mola in Archostemata and Adepsha, but it is well developed in Myxophaga and the majority of Polypag. Beetles that feed on small particles often display a membranous process behind the apical mandibular teeth called prosthca and other various mechanisms for filtration and directing the particles from the preoral cavity.
to the pharynx (e.g. Lawrence et al., 2011). One specific adaptation is the development of dense rows of microtrichia along the midline of the posterior region of the epipharynx and hypopharynx, called LEP (longitudinal epipharyngeal process) and LHP (longitudinal hypopharyngeal process) (Anton & Beutel, 2004, 2006, 2012).

It is worth mentioning that beetle larvae display an enormous variability of their mouthparts and feeding habits compared to the adults. For instance, aquatic immatures of different groups have channeled sucking mandibles (De Marzo, 1976; Beutel, 1993). Carabid larvae of Loricerae have unusually modified elongated antennae and maxillary palps as an adaptation to catching springtails (Goulet, 1999). Another unusual example of larval head modifications was found in the cucujoid family Corylophidae. Two species of Holopsis have phenotypically similar adults, but the larvae of one of the species is characterized by an elongate weevil-like snout, which is a unique feature in larval beetles. This structure is an adaptation to feeding on fungal spores inside the tubules of Ganoderma sp. (Kadowaki, 2010; Yavorskaya et al., 2014).

Studying the head morphology has played an important role in collecting data for analysis of phylogenetic relationships in Coleoptera and other groups of insects (e.g. Beutel et al., 2011). Mouthparts structure, cephalic exoskeletal structures, tentorium, head chaetotaxy and other character systems play an important in distinguishing larval and adult beetles. In the last decades numerous studies were published on larval and adult head structures of different groups of Coleoptera and also on phylogenetic implications (Anton, 2006, 2012; Beutel, 1986, 1993; Beutel & Haas, 1998; Dressler & Beutel, 2010; Hörnschemeyer et al., 2002, 2006). However, a very remarkable study was already published by Korschelt (1924) in the beginning of the 20th century, a monograph on the structure of different body parts and organs of larvae and adults of Dytiscus marginalis (Dytiscidae) with very detailed descriptions of anatomy, complicated drawings and schematic representations of different organs. Head morphology was also covered very thoroughly in this study, with numerous detailed drawings of the mouthparts and head musculature. All the results were obtained without modern techniques now available to us, just using light microscopy, histological sections, and simple dissections. Nevertheless, the quality and thoroughness of this study still impresses and has certainly set an example.

An essential and complex part of the insect head with its appendages is the set of cephalic muscles. Different nomenclatures have been applied in different studies over the years, but the one based on v. Keler (1963) has proved as most convenient for the holometabolous head. Wipfler et al. (2011) introduced a new set of muscle designations which is applicable for the entire ectognathous insects (see also Beutel et al. 2014).

Mycophagy, i.e. feeding on fungal mycelia or spores, is considered to as the ancestral feeding type of Coleoptera (Lawrence, 1989). It was suggested by Newton (1984) that it has evolved independently at least 18 times within the staphylinoid families Priliidae, Leiodidae
and Staphylinidae. Sporophagy in Staphylinioidea is a type of feeding that is particularly well suited for investigating the evolution of function and form of insect mouthparts (Betz et al., 2003). Sporophagous habits in this case means feeding on fungal spores, in contrast to consumption of other fungal materials (e.g. mycelia) or saprophagous habits, i.e. feeding on decaying substrates.

Mycophagous beetles can vary strongly in body size. Relatively large species have been investigated already, either with a focus on functional morphology (Betz, 2004; Betz et al., 2003; Weide et al. 2010) or on ecomorphology and evolution (Lawrence & Newton, 1982; Leschen, 1993). However, detailed data on the morphology and biology of very small mycophagous staphylinoids are very scarce. Ptiliidae (featherwing beetles), a family of Staphylinioidea closely related to the aquatic Hydraenidae and the terrestrial Leiodidae and Agyrtidae (Beutel & Leschen, 2005; Mckenna et al., 2015), includes extremely small species, some of which have become comparable in size to unicellular organisms (Fig. 2). *Scydosella musawensis* is the smallest representative of the family, with a body length of 0.325 mm, less than half the size of an amoeba. This makes the ptiliids the smallest free-living insects.

![Fig. 2. Body size of Ptiliidae compared with common American cockroach and a unicellular organism: A-C – SEM-micrographs. A – the head of Periplaneta americana, modified from Wipfler et al. (2016); B – Acrotrichis sericani (Ptiliidae, Acrotrichini); C – Scydosella musawensis, the smallest known free-living insect; D – Paramecium caudatum.](image-url)
Ptiliidae, which are in the main focus of this dissertation, consist of 3 subfamilies (Acrotchinae, Ptiliinae and Cephaloptectinae), approximately 80 genera and over 600 described species (Hall, 2016). Very little specific information is available about their feeding preferences. Most ptiliids are considered as microphagosous (Lawrence, 1989), feeding on spores and hyphae of fungi (i.e. a part of the family is sporophagous), but also on decaying plant parts and similar organic substrates. Two strictly sporophagous groups are also part of the family – *Nossidium* (and presumably closely related genera; Kilian & Burakowski, 2000) and the extremely small Nanosellini (Dybas, 1976; Hall, 1999) (Fig. 3). Almost all known species of the latter group inhabit basidiomycete fungi, particularly Polyporaceae and Stereaceae (Dybas, 1961; Hall, 1999). Their body size varies from 0.3 to 0.9 mm, fitting with the very small spore size of the fungi they inhabit (3–9 µm x 1–4.5 µm). There is also very limited detailed information on the structure of the mouthparts of Ptiliidae (Betz et al., 2003; Weide & Betz, 2009; Polilov & Beutel 2009; Polilov 2016a) and almost no information on the head musculature.

Associations with fungi have also played an important role in the evolution of very small cucujiform beetles, for instance in Corylophidae which have been already investigated in detail (Polilov & Beutel, 2010; Yavorskaya et al., 2014; Yavorskaya & Polilov, 2016; Polilov, 2016a). Considering the very distant relationship to Ptiliidae and other staphylinoid groups, this family is well suited for a comparative analysis of phenomena related to sporophagy.

Other groups of Coleoptera are distantly related with featherwing beetles, but have similar saprophagous habits and are close in size to larger Acrotchinae (1–1.5 mm). Clambidae is a family of Scirtoidea, which is the most basal lineage of Polyphaga (McKenna et al., 2015). They inhabit leaf litter and rotten wood and most likely feed on microfungi (Crowson & Crowson, 1955). Sphaerisidae (Myxophaga) are tiny (0.5–1.2 mm) beetles that inhabit moist substrate and are presumably saprophagous (most likely feeding on particles of algae). Therefore, these two groups are also suitable for comparisons with Ptiliidae.

Miniaturization, a tendency towards extremely small body size, is a common trend of animal evolution (Hanken and Wake, 1993). It was also described as one of the principal directions of insect evolution (Chetverikov, 1920). Body size reduction of vertebrates has been studied quite thoroughly (McMahon & Bonner, 1983; Schmidt-Nielsen, 1984), although invertebrate groups have gone much further in terms of miniaturization (Hanken and Wake, 1993; Polilov, 2016). There are studies on the morphology of tiny annelids (Westheide, 1984; Worsaae & Rouse, 2008, 2010), mollusks (Turner & Yakovlev, 1983; Strong & Glaubrecht, 2008; Brenzinger et al., 2013), arthropoda (Serban, 1960; Hartmann, 1973; Noodt, 1974; Quesada et al., 2011; Petrunina & Kolbasov, 2012), tardigrades (Kristensen, 1976, 1978, 1979; Schmidt-Rhaesa & Kulessa, 2007; Zantke et al., 2008; Halberg et al., 2009; Schulze and Schmidt-Rhaesa, 2011), and micrognathozoans (Kristensen & Funch, 2000; Kristensen,
However, almost none of them has a main focus on effects of miniaturization on different structures and organs. Miniaturized insects have reached a stunning level of size reduction. Recently, this field of entomology has become quite popular, with an increasing number of studies on different aspects of morphology, anatomy and biology of minute adults or larvae (Dybas, 1976; Beutel & Haas, 1998; Pohl, 2000; Grebennikov & Beutel, 2002; Beutel et al., 2005; Osswald et al., 2010; Jałoszyński, 2015; Polilov, 2004, 2005, 2008; Makarova et al., 2015; Knaute et al., 2016). Most of the studies are dedicated to minute Coleoptera and Hymenoptera, as these two orders contain more than 50% of all insect species under 2 mm (Polilov, 2016). Parasitic wasps of the genus *Megaphmigma* (Trichogrammatidae) probably have reached the highest known level of insect miniaturization. *Megaphmigma* cariabea are just 0.17 μm long, males of some species have no eyes and their antennae are reduced to a single segment.

It is known today that miniaturization can strongly affect different character systems of insects, in some cases causing far-reaching reductions. In many microinsects it leads to oligomerization and condensation of ganglia and brain reorganization: in Corylophidae (Coleoptera), for instance, the brain and subesophageal ganglion are completely moved into the prothorax.
It was already shown that despite extreme body size reduction, the mouthparts and head musculature of Ptiliidae and Corylophidae (Cucujoida) remain complex and retain most of the features found in larger relatives with similar habitats and feeding preferences (Polilov & Beutel, 2009, 2010; Yavorskaya et al., 2014; Yavorskaya & Polilov, 2016, Polilov, 2016). Since even the mouthparts of the tiniest beetles remain complex and do not undergo major reductions, studying and describing them can also be relevant and useful for analyzing phylogenetic relationships of coleopteran groups with miniaturized species.

The minute size of the studied objects became one of the main challenges of this project. The width of the ptiliid heads ranges between 50 and 200 µm. This makes it almost impossible to use traditional methods, such as mouthparts dissection and sagittal sections of the head carried out with razor blades. Micro-CT has become a popular technique in insect anatomy in the last ten years and it has yielded good results for studying inner structures of insects. However, the resolution of most available µ-CT facilities is not enough for extremely small insects such as Ptiliidae. Histological sections have proved to be much more informative and useful for obtaining information even about fine details such as thin nerves and muscle fibers. Given the fact that some of the studied material (e.g., Sycosella musawensis and Micromalthus) was very rare and difficult to obtain, one of our goals was to find an alternative way to scan specimens efficiently without damaging them, with the option of keeping the specimen afterwards or even use them for other techniques. Confocal Scanning Microscopy (CLSM) is often used for visualizing exoskeletal structures of arthropods with auto-fluorescence or coloring (Michels, 2007). However, if the object is small and transparent enough, it is actually possible to visualize the inner structures. The resolution of these images is sometimes almost comparable to histological sections. By combining images, CLSM can theoretically visualize insects of all size, but it is especially suitable for smaller objects. This technique gives an opportunity to scan through whole objects and obtain stacks of perfectly aligned images, which can be used for 3D-reconstructions based on both volume- and surface rendering (Zill et al., 2000; Klaus, 2003, 2006). The use of different clarifying and bleaching solutions has proved to improve the results and make it easier to visualize inner structures of arthropods (Zucker, 2006; Friedrich et al., 2014; Stöckl & Heinze, 2015).

Considering the scarcity of anatomical data, the primary aim of this study is to document the head morphology of several representatives of Ptiliidae with different feeding preferences (saprophyte and sporophagy), with a main focus on the mouthparts structure and musculature. The morphological results are compared with conditions found in larger relatives with similar feeding types and with small or very small more distant relatives, also including members of the three other coleopteran suborders. The phylogenetic and functional interpretations are discussed with respect to their implications for the evolution of sporophagy in Ptiliidae and other groups of Coleoptera. In order to analyze the main results in a broader
In the phylogenetic context, they are also compared to cephalic conditions in Sphaeriusidae (Myxophaga), Gyrinidae (Adephaga) and Micromalthidae (Archostemata).

The main aims of the present work can be summarized as follows:

- Thorough documentation of head structures of different genera of Ptiliidae with different feeding preferences and body size using a combination of modern morphological techniques.

- Optimization of anatomical techniques and assessing the suitability for studying extremely miniaturized insects.

- Comparison of the obtained data with cephalic conditions in other miniaturized and moderately sized forms (Sphaerusidae, Clambidae, Leiodidae) with feeding preferences similar to the ones of Ptiliidae.

- Study the evolution of sporophagy within Ptiliidae and discuss the obtained data in context of Staphylinioidea.

- Discuss the evolution of the feeding apparatus of Polyphaga with conditions observed in Myxophaga, Adephaga and Archostemata.
2 Material and techniques

2.1 Material examined
See “Material and methods” chapters of studies I and III–VIII of the present work.

2.2 Morphological techniques

Scanning electron microscopy (SEM)
For SEM specimens were cleaned with Potassium hydroxide (KOH), dehydrated in ethanol in several steps, dried either at the critical point (Emitech K850; Emitech / Model E4850, BioRad) or using acetone, and thensputter-coated with gold (Emitech K500). Larger specimens such as whole beetles and mouthparts of larger objects were fixed on a rotatable specimen holder (Pohl, 2010). Samller samples were mounted on a specimen holder with conductive tape Scandium software (Soft Imaging System, Münster, Germany) was used for obtaining high resolution images.

Histology
The specimens were dehydrated in an ethanol series and embedded in Araldite CY 212R, sec- tioned at 1 µm with a microtome (Microm HM360 or Leica RM2255) equipped with a diamond knife and stained with methylene blue and acid fuchsin. Subsequently the sections were photographed using a Zeiss Axioskop with a Pixellink PL686CO digital camera or a Motic BA410 light microscope and aligned with Amira 6 software (Visage Imaging, Berlin, Germany).

Light microscopy
Dissected mouthparts and whole heads of some species were mounted in Euparal according to a standard protocol and photographed using a Zeiss Axioskop with a Pixellink PL686CO digital camera.

Confocal laser microscopy
For CLSM specimens were dissected and dehydrated with ethanol and acetone. BABB (mixture of benzyl alcohol and benzyl benzoate 1:2) was used as a clearing solution, according to a standard BABB protocol. The heads were mounted in small droplets of BABB between two coverslips and scanned with a Zeiss LSM 510 in two channels – red 633 nm and green 488 nm and from both (ventral and dorsal) sides. Series of digital slices were produced providing information on all internal structures including muscles. They were imported in Amira and used for 3D reconstruction.
Micro Computer-Tomography (µCT)
Specimens for µ-CT were dried at the critical point (EmiTech K850 Critical Point Dryer) and mounted with super glue or clear nail polish on special sample holders depending on the synchrotron facility. Appendages like antennae were clipped in order to adapt the specimens to the beam. Scans were performed at the Deutsches Elektronen Synchrotron (DESY / Hamburg), Berliner Elektronen Synchrotron (BESSY / Berlin), Advanced Photon Source (APS / Chicago), and the Zoological Institute (Functional Morphology and Biomechanics) of Kiel University (SkyScan 1172 desktop µCT, RJL Micro & Analytic GmbH, Karlsdorf-Neuthard, Germany).

Computer based 3-dimensional reconstruction
Reconstructions were based on µCT data or serial sections. Segmentation was performed manually with Visage Imaging® Amira 5.3 by labeling each discrete structure. Segmented material was exported to Bitplane® Imaris 5 where surfaces were generated. Autodesk® Maya 2011 was used for final surface polishing, smoothing and rendering.

Image processing
All figures were assembled and labeled in Adobe Photoshop® and in Adobe illustrator® (San Jose, California, USA).

Live observations
Adults of Acrotrichis, Nephanes and Ptenidium were collected and held in petri-dishes (setting similar to the one described by Jaloszyński 2015). Their behavior and mouthparts movements were documented using a digital microscope Keyence VHX-2000.

2.3 Cladistic analyses
For the phylogenetic analysis data were entered in Winclada (Nixon1999-2002) and parsimony analyses were carried out with NONA (ratchet, 1000 replicates) (Goloboff 1995) and TNT using the exact search algorithm (implicit enumeration) (Goloboff et al. 2008). The Bremer support values were calculated with NONA (Goloboff 1995).

2.4 Terminology
The nomenclature of v. Kéler (1963) is used for cephalic muscles but the muscle designations of Wipfler et al. (2011) are given in brackets in most studies included in this thesis.
The terminology of Larsén (1966) is used for thoracic muscles of the larva of Tenomerga mucida. To avoid confusion only numbers are used for head muscles in illustrations, whereas an “M” (e.g., M9) is added in the case of muscles of the thorax.
3 Published results

Study I: Yavorskaya MI, Beutel RG, Polilov AA (2017)
Head morphology of the smallest beetles (Coleoptera: Ptiliidae) and the evolution of sporophagy within Staphyliniformia. *Arthropod Systematics & Phylogeny* accepted, in press.

Study II: Wipfler B, Pohl H, Yavorskaya MI, Beutel RG (2016)

Study III: Yavorskaya MI, Anton E, Jaloszynski P, Polilov A, Beutel RG (subm.)
The head morphology of *Sphaerius* (Coleoptera: Sphaeriusidae) and the phylogeny of Myxophaga from the morphological perspective. *Systematic Entomology* submitted.

Study IV: Anton E, Yavorskaya MI, Beutel RG (2016)

Cephalic anatomy and three-dimensional reconstruction of the head of *Catops ventricosus* (Weise, 1877) (Coleoptera: Leiodidae: Cholevinae). *Organisms Diversity & Evolution* 17(1): 199–212.


Study VIII: Yavorskaya MY, Hörnschemeyer T, Beutel RG (subm.)
3.1 Study I

Yavorskaya MI, Beutel RG, Polilov AA (2017)

Head morphology of the smallest beetles (Coleoptera: Ptileidae) and the evolution of sporophagy within Staphyliniformia. *Arthropod Systematics & Phylogeny* accepted, in press.

**Abstract:** Ptileidae include the smallest known beetles (0.3 mm). External and internal head structures of species with different body sizes and feeding preferences were examined and described in detail. Saprophagous and sporophagous species are compared. The observed features are evaluated with respect to their phylogenetic and functional significance, and their correlation with extreme size reduction. A putative autapomorphy of Staphyliniformia is an unusual extrinsic maxillary muscle, which among ptillids is only present in the saprophagous species. Synapomorphies of Ptileidae and their sister group Hydraenidae are a lateral mandibular process forming a unique locking device with a lateral groove of the labrum, and mandibles divided into a main body and a mesal molar part, both connected by a membrane. Extreme body size reduction is a presumptive autapomorphy of Ptileidae that probably resulted in the following derived features: the loss of cephalic sutures and ridges, a simplified tentorium, and a brain modified in shape and very large in relation to the head size. The ptillid species with saprophagous and sporophagous feeding habits show only subtle differences in their cephalic structures, notably in details of the epipharynx and galeae and in the configuration of maxillary muscles. Two alternative scenarios are suggested for the evolution of feeding habits, based on the morphological results and presently available information on phylogenetic relationships. One option is to assign saprophagy to the groundplan of the family, with switches to sporophagy in the basal *Nasidium*, and then a second time in the very small Nanosellini, which are characterized by feeding habits we address as microsporophagy. An alternative scenario is that feeding on spores is ancestral for Ptileidae, with reversal to saprophagy in several branches, and a specialization on very small spores in the extremely miniaturized nanoselline species.

**Significance in the present thesis:** This study provides detailed data on the head morphology of members of Ptileidae, including extremely miniaturized forms. The findings are compared with conditions in other groups and evolutionary patterns linked to adult feeding habits (saprophagy/sporophagy) are evaluated for the first time.

**Own contribution:** 70%
Head morphology of the smallest beetles (Coleoptera: Ptiliidae) and the evolution of sporophagy within Staphyliniformia

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Abstract

Ptiliidae include the smallest known beetles. External and internal head structures of species with different body sizes and feeding preferences were examined and described in detail. Saprophagous and sporophagous species are compared. The observed features are evaluated with respect to their phylogenetic and functional significance, and their correlation with extreme size reduction. A putative autapomorphy of Staphyliniformia is an unusual extrinsic maxillary muscle, which among ptiliids is only present in the saprophagous species. Synapomorphies of Ptiliidae and their sister group Hydrophilidae are a lateral mandibular process forming a unique locking device with a lateral groove of the labrum, and mandibles divided into a main body and a molar molar part, both connected by a membrane. Extreme body size reduction is a presumptive autapomorphy of Ptiliidae that probably resulted in the following derived features: the loss of cephalic structures and roges, a simplified ventricle, and a brain modified in shape and very large in relation to the head size. The ptiliid species with saprophagous and sporophagous feeding habits show only subtle differences in their cephalic structures, notably in details of the epipharynx and galeae and in the configuration of maxillary muscles. Two alternative scenarios are suggested for the evolution of feeding habits, based on the morphological results and presently available information on phylogenetic relationships. One option is to assign saprophagy to the groundplan of the family, with two switches to sporophagy: first in the basal Nasonelini and then a second time in the extremely small Nasonellini, which are characterized by feeding habits that we address as microsporophagy. An alternative scenario is that feeding on spores is ancestral for Ptiliidae, with reversals to saprophagy in several branches of the family, and a specialization on very small spores in the strongly miniaturized monoline species. A well-founded species level phylogeny of Ptiliidae with a dense taxon sampling will help to clarify this issue.

Key words

Staphylinoidea, Ptiliidae, sporophagy, head morphology, phylogeny.

1. Introduction

Mycophagy, i.e. feeding on fungal mycelia or spores, was considered as the ancestral feeding type of Coleoptera (Lawrence 1969). Alternatively, it was suggested by Newton (1984) that this feeding type has evolved independently at least 18 times within the staphylinoid families Ptiliidae, Lesodidae and Staphylinidae. Sporophagy in Staphylinoidea is a mode of feeding that is particularly well suited for investigating the evolution of function and form of insect mouthparts (Beetz et al. 2003). Saprophagous habits in this case means feeding on fungal spores, in contrast to consumption of other fungal materials (e.g. mycelia) or saprophagous habits, i.e. feeding on decaying material.
Mycophagous beetles can vary strongly in body size. Relatively large species have been investigated already, either with a focus on functional morphology (Betz 2004; Betz et al. 2003; Weide et al. 2010) or on ecomorphology and evolution (Lawrence & Newton 1982; Lescchen 1993). However, detailed data on the morphology and biology of very small mycophagous staphylinoids are very scarce. Associations with fungi have also played an important role in the evolution of very small cucujiform beetles, for instance in Coryphinae which were already investigated in detail (Polilov & Brütel 2010; Yavorskaya et al. 2014; Yavorskaya & Polilov 2016; Polilov 2016a). Considering the very distant relationship to Ptilidae and other staphylinoid families, this family is well suited for a comparative analysis of phenomena related to sporophagy.

Ptilidae (featherwing beetles), a family of Staphylinoidea closely related to the aquatic Hydraenidae and the terrestrial Leiodidae and Agyridae (Brütel & Lescchen 2005; McInerney et al. 2015), includes extremely small species. The minimum body length is 0.325 mm, less than half the size of an amoeba. The group consists of approximately 80 genera and over 600 species (Hall 2016). Very little specific information is available about their feeding preferences. Most ptilids are considered to be microphagous (Lawrence 1989), feeding on sporophores and hyphae of fungi (i.e. a part of the family is sporophagous), but also on decaying plant parts and similar organic substrates. Two strictly sporophagous groups are also part of the family – Nossidium (and presumably closely related genera; Killian & Burakowski 2000) and the extremely small Nanosellini (Dybas 1976; Hall 1999). Almost all known species of the latter group inhabit basidiomycete fungi, particularly Polyporaceae and Stelchennaceae (Dybas 1961; Hall 1999). Their body size varies from 0.3 to 0.9 mm, fitting with the very small spore size of the fungi they inhabit (3–9 μm × 1–4.5 μm). There is also very limited detailed information on the structure of the mouthparts of Ptilidae (Betz et al. 2003; Weide & Betz 2009; Polilov & Brütel 2009, Polilov 2016a) and almost no information on the head musculature. Presently available studies show quite complicated structures, only minimal muscle reductions and many features found in larger relatives with similar feeding types.

Considering the scarcity of anatomical data, the primary aim of this study is to document the head morphology of several representatives of Ptilidae with different feeding preferences (saproxyphagy and sporophagy), with a main focus on mouthpart structure and musculature. The morphological results are compared with conditions found in larger relatives with similar feeding types. The phylogenetic and functional interpretations are discussed with respect to their implications for the evolution of sporophagy in Ptilidae and other groups of Coleoptera.
2. Material and methods

2.1. List of Ptiliidae adults examined


2.2. Anatomy

Microtome sectioning, scanning electron microscopy (SEM), confocal laser microscopy (CLSM) and light microscopy were used. Several specimens of Acrotrichis sericans, Ptenidium pustulum, Mikado sp. and Nanoella russia were fixed in FAA, embedded in araldite and cut at 1 mm using a Leica RM2255 microtome equipped with a diamond knife. The sections were stained with toluidine blue and pyronin G. Pictures were taken of every section using a Motic BA410 light microscope and Zeiss Axioplan. The images were aligned using Amira 6 software (Visage Imaging, Berlin, Germany) and used for 3D reconstruction.

All other examined specimens were fixed with 70% ethanol. For CLSM heads of Porophilla, Mikado, Nephanes and Scydosella were dehydrated with ethanol (20–100%) and acetone. BABF (mixture of benzyl alcohol and benzyl benzoate 1:2) was used as a clearing solution, according to a standard BABB protocol. The heads were mounted in small droplets of BABF between coverslips and scanned with a Zeiss LSM 510 in two channels – red 633 mm and green 488 mm and from both (ventral and dorsal) sides. Series of digital slices were produced providing information on all internal structures including muscles. They were imported in Amira and used for 3D reconstruction.

All structures were manually outlined and surfaces of each head structure were created separately for them. The raw surfaces were converted and scaled with Transform2 64 bit software (freeware, Hoko Stark, FSU Jena, Germany; URL: http://starkrats.de) Afterwards, Autodesk MAYA 2016 (Alias Wavefront, Toronto-Ontario, Canada) was used for smoothing and coloring the 3D models.

SEM (Philips XL 30 ESEM) was used to document surface structures of all examined species. Specimens were dehydrated in alcohol with increasing concentration (70–80–90–96–100%) and 100% acetone (two changes), sputter-coated with gold (Emitech K500) and mounted on the tip of a fine needle and fixed on a rotatable specimen holder (POHL 2010). Several heads of Acrotrichis, Ptenidium, and Mikado were dissected and the mouthparts examined. The single available specimen of Noscidium pilosellum was dried and glued onto a paper triangle. It was removed using warm distilled water and KOH solution, transferred to 70% ethanol, then dehydrated and prepared for SEM.

In order to understand the feeding process more thoroughly, living beetles were observed. Acrotrichis, Nephanes and Ptenidium were collected and held in petri-dishes (method similar to the one described by JAKOBSZEWSKI 2015). Their behavior and mouthparts movements were documented using a digital microscope Keyence VHX-2000.

The heads of Acrotrichis sericans and Porophilla myastae are described in detail, but in the case of other ptiliids under consideration only features that distinguish them from these two species.

2.3. Terminology

The terminology used for the musculature is based on V. KÈLE (1963) but muscle designations of the new system of WIFFLER et al. (2011) are given in brackets.

3. Morphological results

3.1. Acrotrichinae

Acrotrichis sericans

Body length 0.7–0.9 mm.

External features of head capsule. Head inclined, subprognathous, broad (ca. 0.25 mm wide) and laterally rounded, not flattened (Figs. 1A, 2C). Coloration of cuticle dark brown. Setae yellowish with slight silvery shine. Cuticle with fairly rough surface structure dorsally and regular scale-like reticulation on ventral side. Sutures absent. Clypeus and gula not separated by ridges from rest of head capsule. Entire dorsal surface with dense vestiture of setae with increasing length towards anterior margin of head capsule. Maximum length of setae 0.035 mm. Compound eyes large and round, only slightly protruding, consisting of 55–60 large ommatidia with slightly convex lenses. Ocelli absent. Posterior and anterior tentorial grooves not recognizable externally.

Tentorium with widely separated nearly parallel anterior and posterior arms, the latter connected by a thin tentorial bridge slightly curved in the middle region. Posterior arms broad and flattened, with large surface for muscle attachment, shorter than anterior and dorsal arms. Elongated anterior arms fairly thin, round in cross-section, mesally connected with apical part of posterior arms,
slightly curved laterad towards anterior end. Dorsal arms of similar shape, originating on middle part of anterior arms, dorsally attached to head capsule (Fig. 3C). Labrum approximately rectangular, movable structure differentiating into several bundles of shorter and thicker digitiform sensilla. **Musculature** (Fig. 3C,D): M1 – M. tentoriocapsulatus anterior, O proximal part of anterior arms and ventral surface of posterior arms, I: ventrally on base of scapus with a long tendon, M2 – M. tentoriocapsulatus posterior, two bundles merging on a common tendon, O: proximal surface of posterior tentorial arms, I: very close to M1, M4 – M. tentoriocapsulatus medialis (Om4), antagonist of M1 and M2, O: distal half of lateral surface of dorsal tentorial arms, I: posterodorsal scapal base. **Maxillae** distinctly retracted, symmetrical, short and broad, almost completely concealed by labrum (Fig. 2D,E). Molae large, flattened, enclosing longitudinal epipharyngeal process (LEP), connected with mandibular body by membranous zone, not firmly fused with it; dorsal molar surface parallel to cibarial roof, with parallel transverse rows of posteriorly directed microtrichia, corresponding with very similar structures of the epipharyngeal surface (Fig. 2F). Anterior mandibular margin slightly elongated. Prothorax present, ventromedially oriented. Distinct peg at lateral margin (lateral process) present as part of lateral locking device (Fig. 2E). Meso molar surface differentiated into several areas with
different surface properties: small smooth central area surrounded by several rows of prominent grinding cones and rows of trichomes (Fig. 2E). **Musculature**: M11 – M. craniomandibularis internus (0mus1), largest head muscle, O: dorsolateral and lateral areas of posterior head capsule, I: adductor tendon; M12 – M. craniomandibularis externus (0mus2), moderately large, O: lateral areas of posterior head capsule, I: lateral mandibular base; M13 – M. tentonocarinalis (0mus3), very thin, accompanied by a very indistinctly visible nerve, O: anterior tentorial arm, I: dorsally on base of mandible (Fig. 3C).

**Maxillae** composed of cardo, stipes, galea, lacina and 4-segmented palp (Fig. 2C). Cardo and stipes triangular, distinctly separated from each other, with one long set (10 µm) each. Maxillary palp 4-segmented; palpomere 3 much thicker than other segments, oval, with three long setae and several folds on apical margin; palpomere 4 long and slender. Galea moderately long and slender.

Distal part slightly bent outwards, with 4 parallel rows of curved microtrichia and several longer setae inserted on apical region. Lacina much shorter and thinner, apical part with several bundles of setae of different length and a row of short teeth on lateral margin. **Musculature** (Fig. 3B–D): M15 – M. cranioocularis (0mus1), O: ventro-laterally on posterior margin of head capsule, I: ventral surface of cardo; M17 – M. tentoniocarinalis (0mus3), composed of two subcomponents, M17a, O: anterior and anterior tentorial arm (two bundles), I: ventral surface of cardo; M17b, consists of two bundles that fuse into one tendon, O: anterior tentorial arm (2/3ds of its length) very close to M17, I: ventral surface of stipes; M19 – M. craniolacinalis (0mus2), O: postero-lateral part of head capsule, I: base of lacina. Mx – M. cranio
maxillaris (ANTON & BEUTEL 2012). O: laterally on the genital region of the head capsule; I: membrane linked to maxillary base (Fig. 3D).

Labium. Mentum large, sclerotized, rectangular, posterior edge fused with anterior edge of the submental region of the head capsule, apical margin straight, with row of five long setae (Fig. 2C). Ten additional short setae scattered on surface of mentum. Prementum smaller and semimembranous, with asymmetrical angular anterolateral process. Two-segmented thin palps inserted on premental processes separated by narrow median gap (Fig. 2C), distal segment with row of short setae on inner side.

Lateral walls of prementum transformed into pair of thin cylindrical processes to which M29 is inserted and which also serve as origin for M34 (Fig. 2G). **Musculature** (Fig. 3A,B): M28 – M. submentoprementalis (0la8), premental retractor, O: anterior surface of submentum, I: medially on posteroventral premental edge; M29 – M. tenontoprementalis inferior (0la5), retractor, O: ventral part of posterior head capsule, I: posterior process of prementum; M30 – M. tenontoprementalis superior (0la6), two long thin bundles fuse into one short tendon, O: ventral part of posterior head capsule near M29, I: posterior margin of prementum, on border with hypopharynx; M34 – M. prementopalpalis externus (0la14), O: ventral side of posterior process of prementum, I: basal margin of palponere I.

**Epipharynx.** Anterior part, i.e. ventral labral wall, semimembranous, with sparse short microtrichia. Intermediate epipharyngeal part with well-developed longitudinal epipharyngeal process (LEP) formed by dense groups of microtrichia along midline (Fig. 2A). Posterior part connected with hypopharynx at attachment area of M. frontohypopharynx, posteriorly reaching anatomical mouth. Cibarial roof (cr) with 9 parallel transverse rows of posteriorly directed microtrichia that match with similar rows on dorsal moa surface. Several rows of longer trichia present between two sides of cibarial roof (Fig. 2B). **Musculature:** M43 – M. clypeopalatalis (0S1); O: frontoclypeal region, I: posterior medial region of epipharynx; M44 – M. clypeobuccalis, two closely adjacent thick bundles, O: frontoclypeal region I: posterolateral region of epipharynx (Fig. 3B,C).

**Hypopharynx** fused with interior labium. Anterior part sclerotized, V-shaped in cross-section, continuous with short dorsal premental wall (Fig. 3C). Posterior hypopharynx laterally connected with posterior epipharyngeal part (see epipharynx), thus forming prepharyngeal tube, adjacent with ventral edge of anatomical mouth. **Musculature** (Fig. 3B): M41 – M. hypopharyngalis (0hy1), O: frons, I: laterally on epipharynx and M43, with short thin tendon. M42 – M. tentorihypopharyngalis (0hy3), absent. Transverse hypopharyngeal muscle absent.

**Pharynx** almost circular in cross-section, with decreasing diameter towards its posterior end (Fig. 3A). Pharyngeal wall quite thin. Oesophagus separated from pharynx by thin transverse fold. **Musculature** (Fig. 3B): M45 – M. frontobuccalis anterior (0ba2), one bundle, M46 – M. frontobuccalis posterior (0ba3), three thin bundles, O: anterior part of frontal region, I: dorsilaterally on pharynx, directly posterior to frontal ganglion; M48 – M. tentoribuccalis anterior (0ba5), unpaired muscle between tritocerebral commissure and suboesophageal ganglion, O: anteromedially on tentorial bridge, I: medially on ventral pharynx; M51 – M. verticopharyngalis absent, M52 – M. tentoripharyngalis (0ph2), O: tentorial bridge, I: ventral pharyngeal wall; M68 – M. analaris stomodaei (0st1), present; M69 – M. longitudinialis stomodaei (0st2) absent.

Pair of relatively large glands associated with labium, adjacent to each other over most of their length, open on dorsolateral corners of posterior hypopharynx, secretions released into preoral cavity (Fig. 3A).

### 3.2. Ptiliinae: Ptenidiini

**Nossidium pilosum**

Body length 1.1 – 1.2 mm; head 0.37 mm wide.

Antenna 10-segmented, with 2-segmented club. Labrum trapezoidal. Grooves of labral locking mechanism...
quite indistinct, but lateral mandibular pegs long and pointed. Stipes also with small pointed process on distal margin. Mentum large, sclerotized, rectangular, posterior edge fused with anterior edge of submental region (Fig. 4).

3.3. Ptiliniae: Nanosellini

Porophila mystacea

Body length 0.55–0.6 mm (Fig. 1C). External features of head capsule. Head inclined, sub-prognathous, broad (maximum width 0.13 mm) and laterally rounded, not flattened (Fig. 5). Coloration light brown with darker regions along edges of head capsule. Cuticle with regular scale-like reticulation on ventral side. Sutures absent. Clypeus and gula not separated by ridges from rest of head capsule (Fig. 5A). Fronto-lateral region sparsely covered with erect setae of medium length (0.02–0.05 mm). Compound eyes large and round, only slightly protruding, consisting of ~45 ommatidia with strongly convex lenses (Fig. 3B). Ocelli absent. Posterior and anterior tentorial grooves not recognizable externally. Tentorium distinctly simplified, lacking dorsal arms and laminatentorna, with widely separated, nearly parallel posterior and anterior arms (Fig. 5B). Tentorial bridge connects widely separated posterior arms, curved in middle region. Posterior arms strongly developed but short, broad and flattened, with large surface for muscle attachment. Elongated anterior arms distinct but fairly thin, round in cross-section, connected to apical part of posterior arm, slightly curved laterad towards anterior end.

Labrum of trapezoidal shape, moveably attached to head capsule by internal membranous fold (Figs. 5B, 6D). Pair of large grooves (sockets) fitting with lateral mandibular pegs (described below) present near lateral lral base (Fig. 5B). Three setae inserted in posterior corner, one directly above grooves on distinct tubercle; several dense rows of setae present on central and anterior region. Surface structure similar to that of ventral side of head capsule. Musculature: M7 – M. labroepipharyngalis (0b5). O: posterior margin of dorsal wall of labrum, I: paramedially on epipharynx (Figs. 6A, 7B). M9 – M. frontoepipharyngalis (0b2), retractor of labrum. O: posterior frons, J: with short tendon on tormae, near posterior corners of labrum (Fig. 6D, 7A).

Antennae 11-segmented, with 2-segmented club (Fig. 5A). Scapus and pedicellus large and cylindrical, much larger than proximal flagellomeres, pedicellus with small notch anteriorly on apical margin. Flagellomere 1 short and conical, narrowing distally, 2 ovoid; flagellomeres 3–10 pedunculate, with visible narrowed basal part, 3 cylindrical, with straight distal edge, flagellomeres 4–7 short, cup-shaped, 7 distinctly widened apically. All antennomeres with long thin setae, apical two with several bundles of shorter and thicker digitiform sensilla. Musculature (Figs. 6C, 7B–F). M. tentoriocapalis, 3 adjacent bundles with same insertion site on ventral cephalic margin. O: anterior and posterior tentorial arms.

Mandibles distinctly retracted, slightly asymmetrical, short and compact (Fig. 6B–D). Molae large, with several teeth, slightly extended dorsal, enclosing longitudinal epipharyngeal process (LEP) between them; connected with mandibular body by membranous zone,

not firmly fused with it; insertion slightly different on left and right mandible, dorsal molar surface parallel to cibarial roof (Fig. 7B,C). Anterior mandibular margin slightly elongated and curved, without any prominent apical tooth. Distinct peg present at lateral margin (lateral process), pointing towards lateral surface, closing preoral cavity tightly when interlocked with postero-lateral labral grooves. Condyle of ventral mandibular joint large, bulb-shaped; dorsal joint with mandibular groove (Figs. 6D, 7C). **Musculature** (Figs. 6C, 7C,D): M11 – M. craniomandibulares internus (0md1), largest head muscle; O: dorso-lateral and lateral areas of posterior head capsule, I: adductor tendon; M12 – M. craniomandibulares externus (0md2); moderately large; O: lateral areas of posterior head capsule, I: adductor tendon; M13 – M. tentorionmandibularis (0md3) not recognizable.

Maxillae composed of cardo, stipes, galea, lacina and 4-segmented palp (Fig. 5). Cardo and stipes triangular, distinctly separated from each other, each with one long seta (10 µm). Palpifer not distinct, maxillary palp 4-segmented; palpomere 3 much thicker than other segments, oval, with stout apical sensilla and several long setae; lateral surface with several sparse rows of microtrichia; palpomere 4 long and slender. Galeae moderately long, slender, filiform, with 3 parallel rows of short, curved microtrichia inserted on apical region. Basistipes and mediostipes fused; lacina separated from stipes by thin fold, barely reaching base of apical part of galea; distal part of lacina with several rows of teeth and short setae. **Musculature** (Figs. 6B,C, 7D–F): M15 – M. craniocardinalis (0mn1), O: ventromedially on posterior margin of head capsule, I: ventrolaterally on cardinal base; M17 –
Epipharynx divided into anterior part equivalent with ventral labral wall, intermediate section with longitudinal process (LEP), and posterior part connected with posterior hypopharynx and reaching anatomical mouth posteriorly (Fig. 6A,D). Anterior part largely semimembranous, devoid of recognizable surface structures; lateral sclerotized strengthening rods anteriorly continuous with spike-like processes of anterolateral labral margin. Intermediate epipharyngeal part with well-developed longitudinal epipharyngeal process (LEP) formed by dense groups of microtrichia along midline (Fig. 7B). Complex posteriorly most epipharyngeal part connected with intermediate region by lateral rod-like sclerotizations, firmly connected with hypopharynx at attachment area of M. frontohypopharyngalis; in dorsal view with large anteriorly rounded lateral projections and small, triangular process in deep anteromedian incision; large paired deep concavities form insertion site of M. clypeobuccalis (Fig. 7B); small posterolateral projection present above attachment site of M. frontohypopharyngalis; postero medial cone-like extension seemingly with narrow connection to anteromedial dorsal wall of pharynx, below anterior part of frontal ganglion (Fig. 6D). Musculature (Fig. 6B,D): M43 – M. clypeopalatinalis (0m31). O. frontolypeal region. I, posterior medial region of epipharynx; M44 – M. clypeobuccalis, consists of two closely adjacent bundles (not reconstructed separately). O. frontolypeal region I: posterolateral region of epipharynx. Hypopharynx fused with anterior labium and forming complicated three-dimensional structure with posterior epipharynx (Figs. 6C, 7C,D). Anterior part sclerotized, V-shaped in cross-section, continuous with short dorsal premental wall. Posterior hypopharynx laterally connected with posterior epipharyngeal part (see epipharynx), reaching ventral edge of anatomical mouth. Musculature: M41 – M. hypopharyngalis (0lh1). O. frons, I: laterally on epipharynx and M43, with short tendion, M42 – M. tentorohypopharyngalis (0lh3). Absent. Transverse hypopharyngeal muscle absent. Prepharynx present as short closed tube, formed by posterior epophageal hypopharynx, anteriorly continuous with preoral cavity between anterior epipharynx, paired mouthparts and anterior labium. Pharynx almost circular in cross-section anteriorly but flattened towards foramen occipitale, with longitudinal folds for muscle attachment (Fig. 6A). Pharyngeal wall thin. Oesophagus separated from pharynx by thin transverse fold. Musculature (Fig. 6D): M45 – M. frontobuccalis anterior (0bh2) and probably M46 – M. frontobuccalis posterior (0bh3), several thin closely adjacent bundles (not reconstructed separately), O. anterior part of frontal region, I: dorsolaterally on pharynx, directly posterior to frontal ganglion; M51 – M. verticopharyngalis absent; M52 – M. tentorohypopharyngalis (0ph2). O. tentorial bridge, I: ventral pharyngeal wall; M68 – M. mandibulari stomatodei (0zm2), present, M69 – M. longitudinalis stomatodei (0zm2) absent. Cephalic central nervous system and stomatogastric nervous system mainly composed of brain, suboesoph-
The cephalic morphology and set of muscles of species of *Mikado, Nanosella* and *Scydosella* are similar to the conditions observed in *Porophila*, but with the following distinguishing features:

*Mikado* sp.

Body length 0.4–0.45 mm, head width 0.16–0.17 mm (Figs. 1D, 8A).

All three antennal muscles (*M. tentorioscapalis*) are present and well separated from each other.

*Nanosella russica*

Body length 0.4 mm, head width 0.09–0.1 mm. Head more compact, compound eyes larger, and more convex, with ~30 ommatidia (Figs. 9, 10A).

Antennae 10-segmented. Antennal musculature (Fig. 10D): three thin separate extrinsic muscles. M1 – *M. tentorioscapalis* anterior (0am1), O: ventrally on anterior tentorial arm (base and 2/3 of the length), I: medially on base of scapus; M2 – *M. tentorioscapalis* posterior (0am2), short and compact, O: anterior arm, dorsoad and apicad of M1, I: dorso-laterally on scapal base; M4 – *M. tentorioscapalis medialis* (0am4), largest antennal muscle, antagonist of M1 and M2, O: ventral side of posterior tentorial arms, I: with long tendon ventrally on scapal base.

Maxillary musculature (Fig. 10B C): M15, M18 and M19 similar to *Porophila*. M17 with shifted origin, O: postero-lateral wall of head capsule, I: ventral surface of cardo. Labial palps very short and with indistinct segmentation. M43 absent.

*Scydosella musawasensis*

Body length 0.32–0.35 mm, head width ~0.06 mm (Fig. 1E).

Compound eyes large, with 25–27 convex ommatidia (Fig. 1I). Antenna 10-segmented. *M. tentorioscapalis*; only one bundle, like in *Porophila mystacea*. Mentum distinctly separated from submental region of head capsule, labial palps scarcely recognizable. Muscle set: see Table 1.

4. Discussion

4.1. Phylogenetic interpretations

The cephalic morphology of *Ptiliidae* is affected by three different but interrelated phenomena, the phylogenetic background, i.e. the sistergroup relationship with *Hydraenidae* within large clades *Staphylinoidae* and *Staphyliniformia*, functional constraints linked with the specific feeding habits, and finally different degrees of mummification, with some species belonging to the smallest known beetles and free-living insects.

A potential synapomorphic feature of *Staphyliniformia* + Scambaeoidea (or *Staphyliniformia* inc. Scambaeoidea) (see *McKenna et al.* 2015) is a characteri-
...corresponding to the maxilla shared by the two groups is the fimbrate galea with regularly arranged rows of curved microtrichia (Brütel & Leschen 2005). This condition has probably evolved independently in Hydrophiloidea (e.g. Brütel 1994) and some groups of Staphylinidae (Betz et al. 2003), but it cannot be excluded that it is ancestral for Staphyliniformia, linked to primarily microphagous feeding habits.

Even though all species of Hydrophilidae are small or very small (size range 0.8–3.3 mm; Jäch et al. 2016), it is likely that an even stronger degree of miniaturization (size range 0.3–1.5 mm; Hall 2016) is an autopomorphy of Philiniidae. Miniaturization can cause distinct modifications and rearrangements of organ systems (Polilov 2015, 2016a). The very high degree of size reduction apparently had a considerable impact on the general morphology and also on cephalic structures. Ec dysial sutures and strengthening ridges are completely lacking. Whereas the former are generally missing in beetles, the absence of the latter is apparently linked with the extremely small size of the head, which makes mechanical reinforcement by internal ridges superfluous. The loss or partial reduction of the clypeofrontal suture is quite common in Coleoptera (e.g. Lawrence et al. 2011), whereas the absence of the ridge separating the gula from the head capsule and the lack of lateral delimitation of the postlabium are very unusual features. Correlation of the reduced cephalic sutures and ridges with miniaturization is indicated by the occurrence of the same derived condition in non-related groups with very small species (0.8–1.1 mm). This applies to Corylophidae (Polilov & Brütel 2010; Yavorskaya & Polilov 2016) and Clambidinae (Anton et al. 2016), but also to groups of Hymenoptera such as Mymaridae (Polilov 2016b) or Trichogrammatidae (Polilov 2016c, 2017), and also to other groups of insects with very small species (Polilov 2016a).

An autopomorphy of Philiniidae, which is possibly related with miniaturization, is the simplified structure of the tentorium, with thin and nearly parallel posterior and anterior arms and missing laminaentorium. Dorsal arms, as well as the laminaentorium, are present in the groundplan of the family (Weide et al. 2014) but missing in Nanosellini, the smallest representatives of the group (0.3–0.7 mm). In Acrotrichidae, Naphanes and Pleinidium (0.6–1.1 mm) they are present but much shorter and slightly thinner than the anterior arms. A similar tendency was described for larvae and adults of Corylophidae, where the tentorium is more simplified in smaller representatives, and is completely absent in Orthoperus (0.8 mm) (pers. obs. M. Yavorskaya). Dorsal arms are...
also absent in adults of miniaturized Hymenoptera (Pollov 2016b, 2017).

The configuration of the antenna of Ptiliidae is certainly autapomorphic, with large cylindrical scapus and pedicellus, and a flagellum which appears very slender in comparison. The pleisiomorphic number of 11 antennomeres is preserved in the groundplan, but only 10 are present in Nanoseila, and a minimal number of 8 is reached in some Cephaloplectinae (Seegers & Dybas 1943). Reduced numbers of antennomeres and palpmomeres have been described for many minute insects (Pollov & Beutel 2016) including Coleoptera, for instance in Hydroscaphidae (Lawrence et al. 2011), Coryphophlaeidae (Pollov & Beutel 2010, Yavorskaya & Pollov 2016) and in Clambidae (Anton et al. 2016). However, reduced numbers can occur in comparatively large beetles as for instance in Hydrophilidae (Aursangel et al. 2016), and the full number is present in the very small Sphaeriusidae (Lawrence et al. 2011).

### 4.2. Effects of miniaturization

A general tendency towards simplification of major skeletal elements can be observed in very small beetles, where structural complexes like the head are simplified and compact but still maintain their functionality. This applies only to a lesser degree to the muscular system. Miniaturization apparently does not affect the general configuration of the muscle set of the mouthparts in Ptiliidae, even though it can lead to reductions of subunits and fibers in single muscles. Even in the smallest known non-parasitic insect Scydosella musawasenensis, the set of cephalic muscles does not show a distinct degree of reduction (Table 1). This suggests that minor differences to larger species may be due to the food preferences of extremely small ptiliids, rather than to effects of body size reduction. However, analyses of muscle variation between members of the family with different feeding habits also revealed a surprisingly homogeneous picture. The set of muscles of saprophagous species is almost identical to the one in the spore-feeding Nanoseila (Table 1). Only the number of bundles of some of the head muscles can vary: only a single extrinsic antennal muscle is present in Perophila, whereas the normal set of three muscles is present in Mikado and Acromica. The anterior prepharyngeal dilator M. clypeopalatalis (M43) is missing in Mikado and Nanoseila, but is present in larger species, and also in the extremely small Scydosella. The number of bundles of M. frontopharyngalis posterior (M46) is also variable within the family. The variation of the unusual extrinsic maxillary muscle Mx is discussed below.

Miniaturization can lead to distinct changes in the nervous system of insects. Detailed investigation of the brain was not a goal of this work, but data are available for the ptilid genera Acromica and Nanoseila (Markova & Pollov 2016a). Typical tendencies observed in the majority of micro-insects (Markova & Pollov 2016a)
2016a,b; Polilov & Makarova 2017) are also apparent in the examined Ptiniidae: macroscopic deformation of the brain, increase in size relative to the head capsule, partial shift into the prothorax, brain asymmetry, and fusion of the suboesophageal complex with the prothoracic ganglion.

4.3. Characters related to food uptake and shifts of feeding habits

The feeding apparatus of saprophagous, algophagous or sporophagous members of Myxophaga and Polyphaga is very complex (e.g., Anton & Beutel 2004, 2006, Anton et al. 2016, Antunes-Carroll et al. 2016) compared to that of predaceous Adephaga (e.g. Dressler & Beutel 2010; Beutel et al. 2017) or members of the “ancestral” Archostemata with largely unknown feeding habits (Hönschemeyer & Staff 2001; Beutel et al. 2008). It comprises epipharyngeal longitudinal bulges set with microtrichia, complicated mandibles with molae and brushes, and in some cases fimбриate galeae (see above). A noteworthy phenomenon observed in Ptiniidae is that the complexity of this apparatus is even increased, at least in some members of the family. Although sporophagy occurs in many species of Staphylinoidae (Bethz et al. 2003), extremely small body size as it is typical for Ptiniidae apparently requires specific adaptations. In some cases, this apparently results in an increase in complexity rather than in simplification. The epipharynx, for instance, is more complicated than in examined species of related groups, such as Hydrophilidae (Jäch et al. 2000), Leiodidae (Antunes-Carroll et al. 2016), Staphylinidae (Bethz et al. 2003), or Hydrophilidae (Anton & Beutel 2004). It is divided into an anterior part corresponding with the ventral labral wall, an intermediate section with the longitudinal process (LEP), and a posterior part connected with the posterior hypopharynx and adjacent with the anatomical mouth. An additional feature in this context was observed in all examined ptiniid species, the composition of M44 of two thick bundles inserted in deep concavities of the epipharyngeal wall. The preomentum bears slightly asymmetrical angular lateral processes at its anterior edge, separated by a narrow median gap. Another feature apparently unique to ptiniid beetles is the structure of the maxillary palp: palpomere 3 is much thicker and longer than the proximal two and often set with several rows of short microtrichia on its lateral surface, palpomere 4 is long, slender, and conical. It is likely that the palp with its specific modifications is involved in the process of collecting food particles.

Sporophagous feeding habits were assigned to the entire family Ptiniidae by some authors (Bethz et al. 2003). However, this specialization is in fact restricted to species of Nastidium (and presumably some closely related genera) and Nanosellini. All other representatives of the family should be considered as saprophagous.

Observations of living beetles (Nephares, Acrotrichis) provided information about feeding preferences and feeding mechanisms of saprophagous ptiniid species. The beetles consumed rotten plant materials and mold, and collected droplets of condensed liquid on the walls of the petri-dish in which they were held. They also consumed liquid yeast solution and droplets containing mold spores. During the feeding process, regardless of the consistency of the substrate, the maxillary palp and galeae are the main or even exclusive tools used for grasping and collecting food particles. The mandibles are concealed and apparently not involved in gathering food. Their main function is to push the food particles gathered by the galeae into the space between the molae with their elongate apical part. The substrate is processed between
the wide molar surfaces and presumably also between the 
molae and epipharyngeal lobes. The structures involved 
in these processes are very similar in the sporoportunous 
Nanosellini. A rather surprising observation was that all 
spores in the osesporus and anterior midgut appear intact 
(Fig. 3B). This suggests that they are not perforated 
and not noticeably deformed or broken by the activity of 
the molae. The function of these prominent structures is 
probably the transport of the substrate towards the 
epipharynx and anatomical mouth, and possibly cleaning of 
distal maxillary elements and of the spores. Whether 
the minute molar surface structures leave very fine traces 
on the surface, which may facilitate infiltration of 
digestive enzymes, is presently unknown. In any case, a 
sexual functional interpretation of the concerted activity 
of all involved complex and extremely small structural 
elements is a great challenge.

The sporoportunous Nassidium likely belongs to the 
first branch separating from the remaining Philiidae 
(Hall 1999; McKenna et al. 2015) (Fig. 12). Although its 
spores are strongly associated with Polyproporus squamo-
as (spores 13 × 4.5 µm), they were also found on other 
Polyporaceae fungi and once on the agaric Russula integr.
gra (Kilian & Burakowski 2000; Newton 1984). Due to 
lack of well-fixed material only external structures of 
Nassidium pilosellum could be examined. All its head 
features are similar to those of the other representatives 
of the family, including the lack of sutures and ridges, 
the presence of the lateral mandibular peg, and the 
labro-mandibular interlocking mechanism. Although 
Nassidium is sporoportunus, its body size is much larger 
(1–1.1 mm) than in all known Nanosellini, and also the 
size of the spores it is feeding on. Despite the sporoportunus 
of Nassidium, it is conceivable that this feeding type does 
not belong to the groundplan of Philiidae. It is found 
either in the majority of this family, nor in its sister group 
Hydraenidae or, more generally, in closely related out-
group taxa (e.g. Beutel & Leschen 2005; McKenna et al. 2015). Most species of Agyriziidae feed on dung, rotten 
fungi and similar decaying substances, and sporoporous 
feeding habits are also common in Leotidae and 
Hydraenidae. This suggests that sporoporous is ancestral for 
Philiidae, and that feeding on spores evolved once in 
Nassidium (and probably some related genera), and indepen-
dently in the distinctly smaller Nanosellini. Sporoporous 
as a groundplan feature of Philiidae cannot be completely 
excluded presently. However, it would imply that several 
pholid branches evolved sporoporous secondarily, which 
would be less parsimonious than the alternative.

The following features, previously described for 
spore-feeding Staphylinoidae (summarized by Betz 2003 
for the first time), are present in all studied Philiidae and 
are also characteristic for some sporoporous beetles 
e.g. Anton & Beutel 2004).

- cebarnial roof with rows of parallel microtrichia 
- gales with brushes and rows of long microtrichus, 
the main instrument for gathering spore masses and other 
food particles
- mandibles with well-developed molae

epiphrayx, prementum and hypopharynx with me-
dial longitudinal bristle-troughs bordered by hairs or 
spines, involved in concentating and directing the 
food stream in the median line (this and the previous 
feature are arguably groundplan characters of Coleop-
tera (Beutel et al. 2001, Anton & Beutel 2004, Anton 
et al. 2016, Antunes-Cardoso et al. 2016)) but a robust 
interpretation requires a robust inter-subordinal phylo-
genetic pattern, which is presently not available (e.g. 
McKenna et al. 2015)).

Our comparison of pholid species with sporoporous or 
sporoporous feeding habits surprisingly yielded only 
subtle differences in the involved cephalic structures. 
The galeae of sporoporous species usually bear 4 rows 
of longer setae and additional teeth on their apical ends. 
In sporoporous species the setae are shorter and not 
aranged in rows in all cases. In Scydosella the apical part 
of the galea is flat and bears several parallel rows of short 
teeth, which are apparently better suited for gathering dry 
particles, whereas longer setae are used to filter and grasp 
moist clumps of mold, spores and rotting plant materials 
after the liquid substrate.

An unusual maxillary muscle (Mx) consisting of one long 
bundle has been described earlier for some scarabaeoid 
representatives and for different staphyliniform beetles 
(Anton & Beutel 2004, 2012; M. cramosessimi; 
Beutel et al. 2001, 2003; Jäch et al. 2000; Weide & Betz 
2009). It was also found in all examined sporoporous 
Philiidae (Table 1). It originates laterally on the genal 
region and inserts on a membraneous fold between the 
maxillary basis and the lateral hypopharyngeal wall. The 
precise function is unclear. Due to lack of suitable mate-
rial the presence or absence in Nassidium could not be 
verified. However, our investigation revealed that it is 
probably generally absent in sporoporous Nanosellini.

Nanosellini is the pholid subgroup with extremely small 
species, most of them inhabiting basidioomycete fungi, 
particularly Polyporaceae and Stechmennaceae (Dy-
ras 1961, Hall 1999). Some of them can also inhabit 
Muriophaceae (Polyporales), Hymenochaetales (Schizo-
poraceae and Hymenochaetaceae) and Ascomyeetes 
(Valsacea) (Pollanov 2008). Their only source of food 
are fungal spores, with a size (diameter 2–6 µm) appa-
rently compatible with the size of the mouthparts (approx.
head width 50–130 µm). It is evident that their feeding 
mechanism differs distinctly from what is found in larger 
sporoportunous staphylinoids, where the mouthparts are 
at least hundred times larger than the spores. Therefore, it 
is appropriate to call their type of feeding necrosporo-
phy. Although nanosellines preserve all main fea-
tures of the feeding apparatus commonly found in larger 
spore-feeding staphylinoids (and also sporoporous 
philiids and sporoporous beetles of other families), they 
have evolved some new features to adjust to this modi-
ified feeding mode. The mandibles are more compact than 
those of larger pholid species, with a smaller molar sur-
face more tightly attached to the main mandibular body. 
The unusual basal maxillary muscle Mx, which is usually 
present in staphyliniform beetles including sporophago-
us ptihids, is missing. The extremely complicated epi-
pharyngeal hypopharyngeal structures could be also part of
the adjustment to more specialized feeding habits.

Our study suggests that switches between saprophagy
and more specialized sporophagous habits require only
minimal modifications of the mouthparts and other in-
volved cephalic structures, compared for instance with a
change to predacious habits (e.g. DRESSELL & BEUTEL
2010). This makes switches between these feeding types
relatively easy in Staphyliniformes and other groups of
beetles. The most parsimonius explanation for the ev-
olution of feeding habits in Staphylinidae (based on phy-
logenetik patterns in MCKENNA et al. 2013) is to assume
saprophagy for the groundplan of the superfamily and
also Ptihids, and one secondary switch to sporophagy in
Neasthina (see related: its implications for the phylogeny
of microsporophagy in Nanoselini, in this case linked with
extremely small size and life inside the fruiting bodies
of basidiomycete fungi. In a possible alternative scenario
feeding on spores would be ancestral for Ptihidae, with
reversal to saprophyphy in several branches of the family.
A solid phylogeny for the family will help to clarify this
issue.

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3.2 Study II

Wipfler B, Pohl H, Yavorskaya MI, Beutel RG (2016)

**Abstract**: Techniques currently used in insect morphology are outlined briefly. Scanning electron microscopy (SEM) and microphotography are used mainly for documenting external features. For anatomical studies a broad spectrum of methods is available now: histological serial sections, confocal laser scanning microscopy (CLSM), light-sheet fluorescence microscopy (LSFM), serial block-face scanning electron microscopy (SBFSEM), dual beam scanning electron microscopy (FIB-SEM), nuclear magnetic resonance imaging (NMRI), and m-computed tomography (micro-CT). The use of SBFSEM and FIB-SEM is restricted to extremely small samples. NMRI is used mainly in in vivo studies. Micro-computed tomography, in combination with computer-based reconstruction, has greatly accelerated the acquisition of high quality data in a phylogenetic context. Morphology will continue to play a vital role in phylogenetic and evolutionary investigations. It provides independent data for checking the plausibility of molecular phylogenies and is the only source of information for placing extinct taxa. It is the necessary basis for reconstructing character evolution on the phenotypic level and for developing complex evolutionary scenarios. Computer-based anatomical ontologies are an additional future perspective of morphological work.

**Significance in the present thesis**: Modern anatomical techniques were evaluated with respect to their usefulness in entomology, notably for the study of extremely small species. Scanning electron microscopy is ideal for surface structures. Digital confocal laser microscopy, microtome sectioning, and µ-computed tomography are very useful for documenting internal features and 3D reconstruction.

**Own contribution**: 25 %
A review of methods for analysing insect structures — the role of morphology in the age of phylogenomics
Benjamin Wipfler, Hans Pohl, Margarita I Yavorskaya and Rolf G Beutel

Introduction
Until the last decades of the 20th century light microscopy was the only method for examining external structures of insects. Histological serial sections and simple dissections were the only available anatomical techniques. Drawings were used for illustrating the morphological findings. Outstanding anatomical studies were already performed in the first two thirds of the 20th century with these techniques and even earlier, some of them used for phylogenetic interpretations (e.g. [1,2]). However, these approaches strongly depended on the skill and experience of the researcher. Precise and detailed three-dimensional line drawings such as those of Weber [2] require a high degree of artistic proficiency. Additionally, detailed morphological work was extremely time consuming, usually restricting a PhD thesis to one body part of a single insect species (e.g. [3]). In the last decade of the 20th century, systematics became more and more dominated by molecular analysis (e.g. [4]). This discipline made tremendous progress since then and today analyses of transcriptomes or genomes are almost standard (e.g. [5,6**,7**]).

With the rise of molecular techniques, morphology and its impact in systematics witnessed a standstill or even decline in the last decades of the century [8–11]. However, in the new millennium, insect morphology and systematics regained momentum with new technologies becoming available, especially but not only micro-computed tomography and computer-based 3D reconstruction (e.g. [12]). Technical innovations opened new ways to present anatomical data very attractively including animated and interactive 3D reconstructions. They also greatly accelerated the process of data acquisition and processing and made it possible to analyze morphological data sets of previously unknown size, as for instance 356 coded characters in a study on Holometabola [13]. Even though this is not comparable to the numbers of characters in recent molecular analysis (e.g. [7**]), the importance and specific role of morphology in the context of phylogeny and evolution is generally accepted today [14**,15,16*].

The aim of the present study is to give a brief outline of the techniques introduced (or optimized) in insect morphology during the last years, and to discuss the role of insect morphology in an era dominated by extensive molecular data sets. Obviously, morphology will continue to play an important role in disciplines such as developmental biology or functional morphology. However, in this study we...
focus on its relevance in a taxonomic and phylogenetic context. In this short review we will not focus on technical details of different approaches. The aim is rather to inspire new anatomical investigations and innovations leading to further progress in this field of research.

**External morphology: exoskeletal structures and surfaces**

In addition to conventional light microscopy and line drawings, which still play a role in taxonomy and some phylogenetic studies, scanning electron microscopy (SEM) and photomicrography are excellent tools for the documentation of surface structures.

Scanning electron microscopy (SEM) (Figure 1b) is frequently used by entomologists since the 1970s (e.g., [17]), shortly after the first commercial SEM was delivered by Cambridge Scientific Instrument Company. The technique is based on raster scanning of the object surface with a focused electron beam. In entomological samples secondary electrons emitted by molecules excited by the electron beam are used to obtain images of the surface. SEM can reach resolutions below 1 nm (see Figure 2). Larger samples can be documented by combining images of smaller surface areas. A useful tool is a rotatable specimen holder developed by Pohl [18]. With this device all standard views can be obtained with a single specimen and a homogenous black background.

A method increasingly used in entomological studies (e.g., [19]) is photomicrography (Figure 1a), that is, the combination of light microscopy or microscopic lenses and digital photography. Today all major microscope manufacturers offer adequate systems with automatic focus stacking. Less cost intensive systems can be obtained by combining microscope lenses, a digital camera, a stepper motor or an automated macro rail, a computer and suitable software. The achieved maximum resolution can be less than 1 μm depending on the lens.

Important characters in insect morphology are coloration, degree of sclerotization and transparency of the cuticle. This is a major advantage of photomicrography compared to SEM, which does not provide information about these properties (see comparison in Figure 1).

The strongly increased depth of focus was a strong argument in favor of SEM compared to light microscopy. However, this is largely compensated by focus stacking, that is, the combination of various images with different focal planes. Today, this technique is widely used (e.g. Figure 1a) and different software programs such as CombineZP, Helicon Focus or Zerene Stacker are available (or already included in commercial setups).
Anatomical methods: the documentation of endoskeletal structures and internal soft parts

In addition to histological sections and simple dissections, a broad spectrum of anatomical techniques is available today. The size of the specimen and the related issue of resolution are the main factors determining the method of choice. Large size generally implies lower resolution. Figure 2 provides an overview over these parameters for the different presented methods.

In traditional histology, a specimen is cut into semithin sections between 0.5 μm (embedding in araldite and similar materials) and 7 μm (paraffin). The maximum sample diameter is ca. 8 mm due to the maximum width of microtome knives (e.g. Diatom Histio Jumbo). The resolution in the X-axis and Y-axis is limited by the microscope used for examining or digitalizing the sections, with a maximum of ca. 1 μm. Common problems are deformations or the loss of parts or entire sections, especially in the case of strongly sclerotized insects. These limitations are largely compensated by harder resins as embedding medium, which were earlier used for ultrathin sections for transmission electron microscopy (e.g. [20,21]), and by elastic alignment algorithms (e.g. [22]) facilitating 3D reconstruction (Figure 3a). Combining these techniques, largely artifact free 3D objects can be created based on histological section series.

The fluorescence used in confocal laser scanning microscopy (CLSM) is detected by a photodetector camera including a large unfocused background part. Parts of the specimen with different material properties (e.g. chitinous structures, muscles) are excited by different wavelengths. The density and coloration of the sample determine to which depth the light penetrates. Aberration artifacts, mainly among the Z-axis, can occur in large specimens [23]. In order to visualize internal sclerotized structures different bleaching and staining agents can be used (see below). However, if the object is small enough and the cuticle is thin and not strongly pigmented, information on internal structures can be obtained without additional treatment (e.g. Figure 4a). By combining images, CLSM can theoretically visualize insects of all size. The maximum spatial resolution depends on the light and wavelength used, but reaches up to 400 nm. In insect morphology, CLSM can also be used to visualize the degree of sclerotization and the chemical composition of the cuticle (e.g. [24,25]) or internal soft parts (e.g. Figure 4a).

Light-sheet fluorescence microscopy (LSFM) is a laser-based approach like CLSM. However, light is bundled in a very thin plane to section a transparent sample optically from the side. The obtained two-dimensional images of single planes are stacked to create a 3D data set. The basic idea for this method was already theoretically proposed by Siedentopf and Zasignondy in 1902 but the first functional set-up was presented in 1998 [26]. Similar to CLSM, the sample has to be transparent or treated with bleaching agents and/or a fluorophore. Since the light
exposure is limited to a thin plane, more light-prone samples can be studied. The lateral resolution is limited by the length of the light wave while the axial one is usually lower at least by the factor 4. The sample size strongly depends on the available equipment but specimens up to several mm in diameter can be scanned with some instruments. LSFM was used in insect embryology (e.g. [27]) but not yet in studies with a phylogenetic focus.

Serial block-face scanning electron microscopy (SBFSEM) is a method used for very small samples (<1 mm in every direction) (Figure 4c). It was suggested
as a concept in 1981 [28] and introduced in 2004 [29]. The chamber of an SEM with a backscatter electron detector is equipped with a microtome for ultrathin sections. In contrast to transmission electron microscopy (TEM) the surface of the block is scanned and not the actual sections. Thus deformations caused by sectioning are irrelevant. The spatial resolution in the X-axis and Y-axis are limited by the capacity of the electron microscope which usually ranges between 1 and 5 nm (see above), and for entomological samples in the Z-axis between 15 and 25 nm. Owing to the size limitation, this method is only relevant for minute insects or isolated body parts (e.g. [30]). FIB-SEM or dual beam SEM is based on the same concept as SBFSEM. However, an ion column (mostly gallium or helium) is used instead of a microtome to manipulate or mill samples in a specific way [31]. This technique is used extensively in material sciences (e.g. [32]) but was also applied to biological samples (e.g. [33]). With less than 10 nm in Z-direction (X and Y are limited by the SEM), the FIB-SEM has the lowest currently available spatial resolution of all anatomical methods. However, it is only suitable for extremely small samples. It has been used to examine ultrastructural features of stridulatory organs [34] and antennal sensilla [35].
Nuclear magnetic resonance imaging (NMRI) is used for samples with a large diameter (see [36,37] for a review). A strong magnetic field is used to stimulate certain atomic nuclei (mostly protons and H-atoms) in liquid phase molecules. The measured stimulation provides information on tissue properties. Owing to its low resolution (between 15 and 100 μm: [37]) and the difficult access to suitable equipment, NMRI plays only a minor role in insect anatomy in a phylogenetic context. However since no harmful radiation is involved it is widely used in in vivo studies (e.g. [38]).

In micro-computed tomography (micro-CT) (Figure 3b) a sample is rotated while X-ray images are taken from different angles. From these projections, virtual sections are calculated. It was used for the first time in entomology in 2002 [12]. Since then, it has become the most widely used technique in insect anatomy (e.g. [59]). It greatly accelerates the data acquisition (a scan taking from a few seconds to several hours) and yields perfectly aligned image stacks. Two different types are used: commercial desktop micro-CTs or synchrotron particle accelerators (SR-micro-CT), which differ in energy range and beam geometry. An advantage of SR-micro-CT is the faster data acquisition. Additionally scanning with desktop equipment is almost exclusively based on absorption contrast, that is, detecting differences in X-ray attenuation to distinguish between structures and tissues. In contrast, SR-micro-CT is also suitable for scans in phase contrast. In this mode the distance between the sample and the detector is increased. Thus not only the absorption is detected, but also the phase of the wave affected by the refractive index of the medium. As a result, the borders between two tissues with different refractive index appear as black and white fringes in the images, which facilitates the distinction of structures and tissues in samples with poor absorption contrast as for instance insects (see e.g. [40]). During the last decade, the resolution in both desktop and SR-micro-CT has strongly improved reaching up to 0.5 μm and in the case of specialized nano-CTs even 80 nm. Micro-CT was also successfully used to investigate external and internal structures of amber fossils, including preserved softparts (e.g. [18]).

An additional important criterion for selecting techniques is invasiveness. Histological sectioning, FIB-SEM and SBFSEM do not leave samples intact, a disadvantage if specimens should be retained for additional investigations (see correlative microscopy below) or museum or type material is involved. Though use of micro-CT, CLSM, NMRI, or LSFM, specimens remain largely unaffected, even though clearing may be necessary in some cases (see below), which alters specimens in their chemical composition and optical properties. For good contrast and a clear distinction of internal structures (especially soft parts), samples examined with micro-CT should be dried at the critical point (e.g.[9]), a procedure which can be easily reversed by re-hydration.

Another criterion is the applicability of different staining techniques. Among the techniques presented here only histological sectioning provides images with specific colorations for different tissue types. In general, a broad set of staining agents is available in histology [41]. However, this is limited when resin (e.g. araldite) is used to obtain thinner sections. A combination of toluidine blue, borax and pyronin G is widely used as staining agents for these sections [9]. In CLSM and LSFM bleaching with various agents [42-45] is required for non-transparent samples. Additionally, the fluorescence of certain tissues can be increased or altered with different staining techniques or by combining bleaching with standard whole-mount immunocytochemistry (e.g. [42,46]). These staining options are a major advantage of these techniques. However, autofluorescence (e.g. [44]) or weakly sclerotized insects can be studied without staining (Figure 4a). As stated above, dehydration is necessary to distinguish between tissues in insects in micro-CT scans. Additionally agents like inorganic iodine and phosphotungstic acid help to increase the contrast (see [47] for a review). Contrasting is not required in NMRI, even though a broad set of different agents is available (e.g. [48]).

Most anatomical studies in systematic entomology are based on dead specimens in 70% or 100% ethanol or preserved in better fixatives like Bouin or Dubosq Brazil. However, in some contexts in vivo studies may be of interest. On principal this is possible with all non-invasive techniques described here. Micro-CT was used to scan live insects but due to the low contrast of tissues containing water, almost no internal structures apart from air filled trachea and the digestive system can be visualized [49,50]. Another major problem is harmful radiation, which kills insects within a more or less short time of exposure. LSFM and CLSM avoid harmful effects of radiation but can cause damage to tissues by high phototoxicity. LSFM is less harmful since only a very thin plane of the specimen is exposed at a time. However, for both methods non-transparent samples require bleaching, which is usually incompatible with maintaining normal life functions. Nevertheless both techniques are widely used for in vivo studies (e.g. [51,52]). NMRI lacks these limitations and is free of any hazard for the examined animal [36,37].

3-Dimensional reconstructions

Computer based 3D reconstructions are used in entomology since the late 1990s [53], by that time still extremely time-consuming and technically demanding. Today established as a standard technique, it allows fast and detailed reconstructions based on histological sections or data obtained with other techniques treated here (Figures 3 and 4). Different commercial (e.g. Imaris) and open-source...
(e.g. Reconstruct) software programs are available but Amira is used by most insect morphologists. Rendered 3D-models can be either based on surfaces (hollow structures enclosed by a thin membrane) or volume (tint colored particles forming a cloud-like three-dimensional shape). The latter resembles a low quality SEM image and allows to illustrate details such as individual muscle fibers, whereas surface renders mostly create solid but rather undetailed objects. Since the use of elastic alignment (see above), histological sections can also be used to create high-quality volume renders (Figure 3a). Figure 4 shows examples of both approaches. Both techniques can be used to create animated movies but only surface renders can be transformed to interactive PDFs (e.g. [54]).

Correlative microscopy
Two or more of the above mentioned approaches can be applied to the same specimen and images produced by combining the obtained data to a single data set. Combined different qualities of individual methods (e.g. high resolution, differentiated staining) can yield highly detailed and informative illustrations. Software programs like Amira provide packages for integrating various types of data. Protocols for almost all combinations are available (e.g. [55,56]).

The role of insect morphology in systematics
The rapid progress in molecular systematics (e.g. [41]) has prompted the question of whether morphology will be replaced or reduced to a marginal role in phylogenetic investigations (e.g. [8,10,11]). Indeed, 72.6% of all systematic studies between 1992 and 2007 and 66.8% of all entomological contributions in journals with a broad systematic spectrum (and relatively high IF) were based on molecular data, with the relative abundance of molecular studies increasing over these years [57]. However, the picture changes markedly with a focus on journals specialized on insect phylogeny. Among all articles published during that period, 71.9% were based on morphology [57], probably due to a long tradition [57], an unusual richness of easily accessible external characters, and the introduction of innovative techniques in the last decade that triggered a ‘renaissance’ in insect morphology [58].

Morphology will remain indispensable in systematics for several reasons [14*,15,16*]. Morphological data are an independent source of information for critically evaluating molecular phylogenies, as an approach Hennig [59] would have called ‘reciprocal illumination’, today applied as ‘plausibility check’ by molecular systematists (e.g. [7*]). In contrast to standard molecular data sets, morphology also provides specific arguments in terms of more or less complex synapomorphic features. In cases where molecular data cannot be obtained, analyses of morphological characters are obviously the only option. This may apply to older museum material and very rare species, but especially to fossils. Phylogenetically placing extinct taxa is essential for dating molecular trees, and more importantly for reconstructing evolution in the dimension of time, for analyzing communities of life forms of past time horizons. Morphology is and will very likely remain the only source of information in this important field of insect phylogeny and evolutionary biology.

Morphology is also essential for understanding evolutionary changes on the phenotypic level. Without knowing transformations of morphological structures, the main targets of natural selection, it is not possible to develop complex and meaningful evolutionary scenarios. A formal reconstruction of character transformations and ground-plans using Mesquite [60] was presented in a recent transcriptomics study on Holometabola [64*].

An additional future perspective of systematic morphological work is building computer-based anatomical ontologies, as it was done by Yoder and co-workers for the megadiverse Hymenoptera [61]. Despite of the ‘perceived pain’ [61] of creating such data sets, this concept has several advantages. It eliminates or reduces nomenclatural ambiguities and homology problems, which is obviously important in a taxonomic and phylogenetic context. It facilitates data mining and the referencing of anatomical concepts in insects and other groups of organisms [61]. Finally it helps to integrate anatomical data in studies focused on ontology and genetics (e.g. [62]).

Conflict of interest
The authors declare that there is no conflict of interest regarding this publication.

Acknowledgements
We are grateful to Pieter Kraaijeveld and David Neubert for 3D reconstructions, Thomas Hirschmeyer for the use of a serial block-face scanning electron microscope, Frank Friedrich for an aligned section series and a reconstruction, and Thomas Kleinteich for providing the CT-seas of a sphingid mouth. We are also grateful to Gregory W. Gourley and Brian M. Wegmann for critically reviewing and improving our manuscript. The present study was supported by grants to M. Yavorskaya (DAAD, grant number 9153492) and B. Weppler (DFG, grant number WI 4249/1-1) which is also gratefully acknowledged.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
• of special interest
◆ of outstanding interest


Transcription and an extensive morphological data are evaluated in this study. The phylogenetic branching pattern obtained with molecular data is used for reconstructing ground plans and the character evolution on the (pre)evolutionary tree.


This major study from ITKE (www.itke.org) presents a phylageny which can presently be considered as state-of-the-art in insects systematics. The project is a cooperation between molecular systematists, morphologists, developmental biologists, bioinformaticians and palaeontologists.


The author emphasizes the importance of morphological research programs for understanding the form and function, ecology and evolution of organisms.


The role of innovative morphology in *MorphoEvoDevo*, phylogenetics and evolutionary biology is discussed.


31. A technique allowing the reconstruction of extremely small structural features of insects and other arthropods is presented in this contribution.


68 Special section: Insect phylogeny


47. Metacher BD: MicroCT for comparative morphology; simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC Physiol 2009, 9.11.


The authors describe an innovative method to document structural features of living insects with X-ray microtomography.


A highly attractive method to present complex three-dimensional data interactively in publications is presented in this study.


3.3 Study III

Yavorskaya MI, Anton E, Jaloszyński P, Polilov A, Beutel RG (subm.)

The head morphology of Sphaerius (Coleoptera: Sphaeriusidae) and the phylogeny of Myxophaga from the morphological perspective. Systematic Entomology submitted.

Abstract: External and internal structures of the minute adults of Sphaerius were investigated using SEM and microtome sections. They are described and illustrated in detail including 3D reconstructions. The results are discussed with respect to effects of miniaturization and also under functional aspects, especially microphagous feeding habits. The head of Sphaerius is less affected by size reduction compared to other beetles of the same size class (e.g. larger Ptiiidae, Corylophidae). Features related to very small size are the absence of externally visible ridges and a partial shift of the brain into the prothorax; the cephalic musculature is apparently not affected. The feeding apparatus has features similar to those found in microphagous species of Polyphaga, especially in Scirtoidea and Staphyliniformia. However, in contrast to polyphagans with similar feeding habits, the hypopharyngeal longitudinal ridge (or process) of Sphaerius is strongly reduced and a fimbriate galea is lacking. The phylogenetic implications of the structural features are evaluated in a cladistic analysis of larval and adult characters. The results are distinctly in conflict with branching patterns suggested by analyses of molecular data, but in agreement with previous morphological studies. In contrast to a pattern obtained in a recent molecular study - (Hydroscaphidae + (Toriidincolidae + (Sphaeriusidae + Lepiceridae))) - our analyses yielded Lepiceridae as sister to the remaining Myxophaga (branch support 9), and Sphaerius as sister taxon of Hydroscaphidae (branch support 5). The monophyletic origin of the latter two taxa is supported by unusual synapomorphies of adults and larvae. Sphaerius is characterized by numerous autapomorphies of the head: a labro- mandibular locking device, a bipartite M. frontoepipharyngalis (M9) with subcomponents oriented in opposite direction, a deep antennal furrow, an intercalary antennomere with a structure resembling a sucking disc, a strongly elongated flagellomere 1, a compact 3-segmented antennal club, strong bundles of M. tentoriocranalis (M4) originating on the posterior head capsule, a concave anterior side of maxillary palps, and an elongated second pair of toriæ posteriorly connected with a process of the hypopharyngeal suspensorium.

Significance in the present thesis: Even though Sphaerius (Myxophaga) is distantly related to the group in the main focus of the thesis, it is of similar size as larger saprophagous Ptiiidae and has similar feeding preferences. Comparing these objects helps to understand the effects of miniaturization and evolution of the feeding apparatus within Coleoptera. Additionally, the largest morphological data set ever presented for Myxophaga is analyzed cladistically.

Own contribution: 60 %
The head morphology of *Sphaerius* (Coleoptera: Sphaeriusidae) and the phylogeny of Myxophaga from the morphological perspective

MARGARITA I. YAVORSKAYA, ERIC ANTON, PAWEŁ JAŁOSZYNSKI, ALEXEY POLILOV AND ROLF G. BEUTEL

Abstract. External and internal structures of the minute adults of *Sphaerius* were investigated using SEM and microtome sections. They are described and illustrated in detail including 3D reconstructions. The results are discussed with respect to effects of miniaturization and also under functional aspects, especially microphagous feeding habits. The head of *Sphaerius* is less affected by size reduction compared to other beetles of the same size class (e.g. larger Ptillidae, Coryphidae). Features related to very small size are the absence of externally visible ridges and a partial shift of the brain into the prothorax. The cephalic musculature is apparently not affected. The feeding apparatus is similar to what is found in microphagous species of Polyphaga, especially in Scirtolidae and Staphyliniformia. However, in contrast to polyphagans with similar feeding habits, the hypopharyngeal longitudinal ridge (or process) of *Sphaerius* is strongly reduced and a fimbriate galea is lacking. The phylogenetic implications of the structural features are evaluated in a cladistic analysis of larval and adult characters. The results are distinctly in conflict with branching patterns suggested by analyses of molecular data, but in agreement with previous morphological studies. In contrast to a pattern obtained in a recent molecular study - *(Hydroscaphidae + (Torridincolidae + (Sphaeriusidae + Lepiceridae))))* - our analyses yielded Lepiceridae as sister to the remaining Myxophaga (branch support 9), and *Sphaerius* as sister taxon of Hydroscaphidae (branch support 5). The monophyletic origin of the latter two taxa is supported by unusual synapomorphies of adults and larvae. *Sphaerius* is characterized by numerous autapomorphies of the head: a labro-mandibular locking device, a bipartite M. frontoepipharyngals (M9) with subcomponents oriented in
opposite direction, a deep antennal furrow, an intercalary antennomere with a structure resembling a sucking disc, a strongly elongated flagellumere 1, a compact 3-
segmented antennal club, strong bundles of M. tentorioscapalis (M4) originating on the
posterior head capsule, a concave anterior side of maxillary palptomere 2, and an
elongated second pair of tormae posteriorly connected with a process of the
hypopharyngeal suspensorium.

Introduction
Sphaeriidae is a monogenic family of the small beetle suborder Myxophaga. It presently
contains only 19 known species (Beutel & Arce-Perez, 2016) but has a very wide distribution,
including Europe (3 spp.), North-, Central and South America (4 spp.), Asia (8 spp.),
Madagascar (1 spp.), Australia (2 spp.) and Africa (1 spp.) (Endrödy-Younga, 1997a; Beutel
& Raffani, 2003; Beutel & Arce-Perez, 2016). Sphaerius acaroides Wattl, 1838 (Figs 1, 2),
which is in the main focus of this study, is the only species of the family and the suborder
known from Central Europe.

Species of Sphaerius Wattl, 1838 usually live in moist substrate at edges of different
water bodies, primarily in sandy substrate or humid gravel among roots of plants,
occasionally only at 10–20 cm distance from aquatic larvae and adults of another
myxophagan taxon, Hydroscapha LeConte, 1874 (pers. obs. R.G. Beutel: Sycamore
Canyon, Arizona, USA). However, species discovered in Asia (Löbl, 1995; Jäch, 1998) and
also S. africanus Endrödy-Younga, 1997 from the southeastern part of Africa (Endrödy-
Younga, 1997a) are apparently strictly terrestrial. The food preferences of larvae and adults
are not well known, but detailed field notes of R. Arce-Perez suggest that at least some
species are algophagous (Beutel & Arce-Perez, 2016).

The cephalic morphology of myxophagan larvae is relatively well known (e.g. Beutel &
Haas, 1998; Beutel et al., 1999). In contrast, a detailed treatment of adult head structures
was only available for Lepicerus Motschulsky, 1855 (Anton & Beutel, 2006) so far, the only
genus of Lepiceridae, a taxon considered as extremely rare for a long time (e.g. Reichardt,
1973; Lawrence et al., 2013; Arce-Perez et al., 2016; Jaloszynski et al., 2017). This inspired
us to examine and describe the adult head of Sphaerius in detail and to compare the results
with new data obtained for Hydroscapha (Hydroscaphidae) and Satonius Endrödy-Younga,
1997 (Torrinidcolidae). The findings are discussed with respect to miniaturization and
microphagous feeding habits, but mainly aiming at a clarification of the relationships within
the suborder. Incompatible results were suggested by morphological and molecular data.
Analyses of morphological characters of larvae and adults suggest a sistergroup relationship
between Lepiceridae and a clade comprising Sphaeriidae and Hydroscaphidae (Beutel et
et al., 1999; Beutel, 1999a), whereas analyses of single genes yielded a clade Lepiceridae + Sphaeriusidae and a sistergroup relationship between Hydroscaphidae and the remaining three myxophagan families (McKenna et al., 2015). The new data presented here are combined with larval and adult characters from earlier studies (Beutel & Haas, 1998; Beutel et al., 1999; Beutel, 1999a, b) and analyzed cladistically. The data set is partly congruent with the one presented in Beutel (1999a) (see also Ge et al., 2010). However, it was strongly modified in terms of characters (and taxon sampling) and extensive new data were incorporated in the matrix, based on the results presented here and more recent studies (e.g. Beutel, 1999b; Beutel & Haas, 2000; Hajek & Fikáček, 2008; Lawrence et al., 2011, 2013; Anton & Beutel, 2004; Short et al., 2015). The phylogenetic results are discussed with respect to character evolution and recent results of molecular investigations.

Material and methods

Examined taxa

Myxophaga, Sphaeriusidae: adult of *Sphaerius acaroides* Waltt., 1838 (53.4719N/22.7445E, Góra Babia Dupa (Poland), south of Goniądz gravelpit, leg. M. Wanat [fixed in FAE (formaldehyde-ethanol-acetic acid: 3:6:1)] (specimen with interrupted tentorial bridge and slight asymmetry of ventral pharyngeal dilator); Moscow region (Russia), leg. A. Pollov, [FAE] (specimen with complete tentorial bridge and symmetrical ventral pharyngeal dilators); vicinity of Mosina (Poland), leg. Sz. Konwerski [dried specimen without preserved internal soft parts]); *Sphaerius* sp. (undetermined species from North America, USA, Arizona, Sycamore Canyon) [fixed in FAE]; Lepiceridae: *Lepicerus inaequalis* (Sharp, 1882) (Mexico, precise locality unknown); Hydroscaphidae: *Hydroscapha natans* LeConte, 1874 (USA, Arizona, Sycamore Canyon) [fixed in FAE]; Torridincolidae: *Satonius kurosawai* (Satō,1982) (Japan, precise locality unknown) [preserved in ethanol]; *Ytuzeus* Reichardt, 1973 (Brazil, São Paulo, precise locality unknown) [preserved in ethanol].

Archostemata, Micromalthidae: adults of *Micromalthus debilis* LeConte, 1878, donated by T. Hörnschemeyer (from laboratory culture, preserved in 70% ethanol).

The specimens are kept in the research collection of RGB (Phyletisches Museum Jena).

Anatomy

Two specimens of *Sphaerius acaroides* (fixed in FAE) were embedded in araldite CY 212® (Agar ScientWc, Stansted/Essex, England) and sectioned with a microtome HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. The specimen from Poland
(with slight asymmetries) was sectioned at 1 μm thickness, the other one at 2 μm. The sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany). Pictures were taken of every second section using a light microscope (Zeiss Axioplan, Germany) equipped with a camera (PixeLink Capture OEM). The images were aligned using Amira 5.3.1 software (Visage Imaging, Berlin, Germany). Based on the aligned image stacks, internal structures were traced manually to reconstruct three-dimensional images. MAYA7 (Alias Wavefront, Toronto/Ontario, Canada) was used for smoothing and coloring.

SEM

The dried specimen was used for scanning electron microscopy (SEM). It was cleaned in warm water first and then for a few hours in KOH. After this it was dried at the critical point (Emitech K850 critical point dryer), sputter-coated with gold (Emitech K500), and fixed on a rotatable specimen holder (Pohl, 2010). Images were taken with a FEI (Phillips) XL 30 ESEM at 10 kv.

The muscular terminology is based on v. Keler (1963) but muscle designations of the recently introduced system of Wipfler et al. (2011) are given in brackets.

Phylogenetic analyses

The data (96 characters of adults and larvae: electronic appendage 1) were entered in a matrix with Winclada (Nixon 1999-2002) and parsimony analyses were carried out with NONA (ratchet, 1000 replicates) (Goloboff, 1995) and TNT using the exact search algorithm (implicit enumeration) (Goloboff et al., 2008). All characters had equal weight and were treated as unordered. The Bremer support values were calculated with NONA (Goloboff, 1995). The length of trees with enforced topology (Hydroscaphidae + (Torridincolidae + (Sphaeriusidae + Lepiceridae))) was calculated with Mesquite (Maddison & Maddison, 2001).

Results

External features of the head capsule (Fig. 3)

The head of Sphaerius acaroides is prognathous, moderately flattened and transverse, distinctly wider than long (~0.25 mm width, ~0.17 mm length) (Figs 3A, B). It is distinctly retracted into the prothorax (Figs 2, 3B), especially on the dorsal side, nearly flat ventrally and moderately convex dorsally. The cuticle is smooth. The setation of the head capsule is very sparse, largely restricted to the clypeal and pre-antennal frontal region. The well-
developed compound eyes with a relatively low number of large ommatidia (ca. 60) are only slightly protruding laterally; their posterior margin is adjacent to the anterolateral pronotal edge. Ocelli are absent. The coronal suture and frontal sutures (dorsal ecdysial lines) are absent. The clypeofrontal strengthening ridge is not visible externally but distinctly developed internally. A very distinct bead is present on the mesal edge of the deep frontal antennal furrow (Fig. 3B). The insertion area of the scapus lies anterior and slightly above the anterior ocular margin; it is delimited by a sharp edge, which is continuous with the lateral edge of the trapezoidal frontoclypeal region anterior to the compound eyes; this proximal part of the antennal furrow is continuous with a deep groove between the ventral margin of the compound eye and the dorsal mandibular surface (Figs 3A, B); a small group of normally sized and very short setae below the posterior ocular margin corresponds with a similar group on the lateral mandibular base, both enclosing antennomeres 4 and 5 in their resting position. The anterior clypeal margin is slightly convex laterally and concave medially; three pairs of medium-length setae (ca. 20 µm) are inserted on the clypeal surface close to the slightly bulged anterior edge. The region of the vertex is covered by the pronotum and not separated from the frontal and genal areas. A narrowed neck region is not present. On the ventral side a very broad and unusually short gular region is enclosed by fissure-shaped, longitudinally oriented posterior tentorial grooves; anteriorly it is completely fused with the submental region, which is laterally continuous with the genal areas without a recognisable border; the posterior portion of the gula including the posterior parts of the tentorial fissures are covered by the anterior prosternum (Fig. 3A). Cervical sclerites are not developed.

Endoskeleton (Figs 3, 4D, F, 6D)
Gular ridges are not visible as external surface modifications or very short and indistinguishable from the fissure-shaped posterior tentorial grooves at the posterior end of the head capsule (Fig. 3A). The strongly developed but short and flattened proximal part of the posterior tentorial arm bears distinctly developed lateral extensions (Fig. 6D, right side). The broad tentorial bridge was medially interrupted in one specimen examined (visible on microtome sections) but connected in the other one. The posterior arms are followed by moderately long, well-developed central tentorial bodies (as defined in Dressler & Beutel, 2010); these divide into the dorsal and anterior arms, which form an approximately right angle with their proximal portions (Fig. 4D, F). Laminatentoria are completely absent. The dorsal arms are thinner than the central bodies and the anterior arm; they are connected with the posterior region of the vertex by epidermal cells. The anterior arms are flattened proximally and more cylindrical anteriorly; they slightly increase in diameter towards their origin on the epistomal region. Anterior tentorial pits or grooves are not recognizable externally (Fig. 3B).
Labrum (Figs 3B, C, 4)
The large labrum forms a lid-like structure largely covering the distal parts of the paired mouthparts (Figs 3B, C). It is movably connected with the anteromedian clypeal concavity by an internal membranous fold. The proximolateral labral edges fit with a dorsal ridge of the mandible, both forming a locking mechanism. The dorsal surface bears a rather dense vestiture of setae, similar to those of the clypeal region. An unusually broad inflected ventral portion is sclerotized. Tooth-like structures are present along the distal edge (Fig. 3C). Short paired processes resembling typical tornae, each with a rounded apex, are present posterolaterally. A second unusual pair of tornae is present mesad these structures: these elongated sclerotized processes extend posteriorly alongside the lateral epipharyngeal edges, but are not connected with them (Figs 4B, 6A–C); the apical regions form a complex structure with a short but very distinctly developed, dorsally directed process; this part is connected with an elongate, sclerotized suspensorial process of the hypopharynx.

Musculature (Fig. 4A, C): M7 - M. labroepiphasaryngalis (0lb5 of Wipfler et al., 2011), epipharyngeal levator, well-developed, composed of two bundles; Origin (O): posterior labrum, close to the median line, Insertion (I): anterior epipharynx, close to the median line; M9 - M. frontoepiphasaryngalis (0lb2), composed of two strongly developed subcomponents oriented in opposite directions, M9/1, probably functioning as protractor and depressor of labrum, slightly converging towards insertion; O: frons, immediately posterolateral frontoclypeal ridge, I: anteriorly on dorsal process of elongate mesal tornae, above posterodorsal edge of prepharynx; M9/2, retractor of labrum, O: dorsally on the postoccipital ridge, laterad M4, I: very close to insertion of M41/1 but on posterior side.

Antennae (Figs 3B, C, 6)
The 11-segmented antennae are short and compact, not reaching the hind margin of the pronotum posteriory. In their resting position, the part proximad the club is inserted in the deep antennal groove (Fig. 3B). The scapus is short, with a bulb-shaped enlarged proximal part articulating with the head capsule and a short, curved distal part with a single seta; the pedicel is large, inflated proximately and tapering distally; it bears a seta close to its laterodistal edge. A small seemingly separate distal portion (intercalary antennomere) bears a round structure resembling a suction disc (Fig. 6B). Antennomere 3 is strongly elongated, about six times as long as its apical width; it is slender proximally and widening distally; a seta is inserted on the posterior edge at about two thirds of the length; antennomeres 4-6 are about equally sized, less than one third as long as 3 and each distally widening, 7 with an oblique distal edge, and each of them with a single seta; antennomere 8 is smaller than the preceding ones, cylindrical and lacking setae; antennomere 8 is also small but distinctly
widening distally, enclosing the base of the large first club-segment as a bowl-shaped structure; it bears a single seta; segment 9 is about 1.5 times as long as each of 4-7 and 221

cup-shaped, strongly widened distally; like the two following segments it bears several long 222

setae, some of them thin but others distinctly broadened; segment 10 is cylindrical, as wide 223
as 9 at its distal end but distinctly shorter; the apical antennomere 11 is conical, slightly, 224
smaller than 9, it bears more setae than the other two club segments.

Musculation (Figs 4D, 6C): M1 - M. tentorioscapalis anterior (0an1), three bundles 227
with the same insertion, O: laterally on middle and posterior region of anterior tentorial arms, 228
mid region of dorsal tentorial arms, I: posteroventrally on base of scapus; M2 - M. 229
tentorioscapalis posterior (0an2), relatively small, consists of two bundles with the same 230
insertion, O: distal end of dorsal arms and adjacent area of head capsule, I: anterodorsally 231
on scapal base; M4 - M. tentorioscapalis medialis (0an4), very strongly developed, three 232
bundles with the same insertion, O: two strongly developed bundles dorsally on the 233
postoccipital ridge, one bundle laterally on the proximal region of the dorsal tentorial arm, I: 234
posterodorsally on scapal base; M5/M6 - Mm. scapopedicellares lateralis/medialis 235
(0an6/0an7), present, connecting scapus and pedicellus, precise areas of origin and insertion 236
not recognizable on available microtome section series.

Mandibles (Figs 3A, 4F, 6A, B)
The basal part of the nearly symmetrical mandibles is rather robust, roughly triangular in 239
cross section. In contrast, the distal part is flattened and blade-like. The mandibles are 240
inserted in a typical dicondylic manner; the proximal part forming the ventral (primary) 241
articulation is distinctly extended ventrolaterad as a rather flat projection before it connects 242
with the head capsule. The lateral wall of the proximal part of the mandibles is very strongly 243
sclerotized and concave (Fig. 6A). A distinct ridge on the dorsal side forms a locking 244
mechanism with the basolateral labral edge. A large mola is present at the mesal mandibular 245
base; its outer contour appears nearly square in cross section and it is strongly sclerotized, 246
with a grinding surface. The mola is followed by an asymmetric semimembranosus lobe-like 247
structures on the ventral side; a posteriorly directed projection is present on the left side; the 248
rounded mesal parts of these structures interact in the median line like the molae. A movable 249
prostheca is not developed. The distal blade-like mandibular part is curved along its external 250
margin; it bears a thin-walled, strongly flattened apical tooth which interacts with the median 251
hypopharyngeal rim with its ventrally directed sharp apex. Two smaller subapical teeth are 252
present, the distal one bent downward with its sharp mesal edge. The three distal teeth are 253
separated from the main lumen of the mandible by a nearly vertical internal sclerotized 254
septum.
Musculature (Figs 4C, E, 6C, D): M11 - M. craniomandibularis internus (0md1), very large adductor, O: extensive posterolateral areas of dorsal head capsule, I: with very long and thin adductor tendon, inserted on short process posterior to mola; M12 - M. craniomandibularis externus (0md3), O: posterolateral corner of head capsule below M11, I: laterally on mandibular base with very long and thin tendon. M14 (M. zygomaticus mandibulae of Kéler (1963)), very thin, represented by two thin fibres that fuse right before the insertion point. O: distal ventral region of anterior tentorial arm, I: very thin tendon, posterior to insertion of M11. Its very small size suggests a function as proprioceptor (Honomichi, 1976, 1978).

Maxillae (Figs 3A, 4D, 6A, B)
The small transverse cardo is inserted in the groove at the lateral submental edge (fossa maxillaris); it bears a seta close to its posterior margin; its straight anterior edge is connected with the posterior edge of the triangular basitipes, which bears several setae; the mediotipes is fused with the palpifer; the palp is inserted distally on this scirite, which is also equipped with several setae and bears a shallow parabolic recess on its distal part; the first palpomere is not visible; palpomere 2 is elongated and distinctly deformed, with a large, irregular concavity along its anterior side; on its distal part it bears two setae; palpomere 3 is strongly inflated and bears more than ten setae; a ring-shaped distal bulge encloses the articulatory area of the small apical palpomere 4; the proximal part of the apical segment is roughly cylindrical and its distal part extended in a mushroom-like manner; one fairly large conical sensillum is present on the somewhat flattened apical region, together with several minute additional structures, probably also with sensorial function. The galea is very slender and elongated; its apical region is fusiform and lacks a recognizable microsculpture and also setae, spines or microtrichia. The lacinia is present but strongly reduced, short and triangular in cross-section.

Musculature (Figs 4D, 6C, D): M15 - M. craniocardinalis externus (0mx1), promotor of the maxilla, well developed muscle composed of two bundles, O: dorsal surface of posterior tentorial arm, I: laterally and mesally on a process at the base of the cardo; M17 - M. tentoriocardinalis (0mx3), promotor of the maxilla, O: two thin bundles laterally on tentorium, mesally bordered by origin of M18, I: inner surface of cardo, mesad M15, M18 - M. tentoriostitpitalis (0mx4), adductor of stipes, consisting of four bundles, O: mesal surface of tentorium, I: ventral base of stipes, close to the mesal margin; M19 - M. craniolacinialis (0mx2), well developed slender muscle, O: posterolateral corner of head capsule, above origin of M12, I: base of lacinia; intrinsic muscles were observed, but their precise areas of origin and insertion and origin points could not be identified with the available section series.
Labium (Figs 3A, 4, 6A, B)

The submental region is completely fused with the gula posteriorly and the genal area laterally; its surface is almost completely smooth, bearing only very few minute setae; the anterior submental portion widens and forms a prominent transverse bulge, which laterally bears the relatively small semicircular maxillary grooves; the anterior submental edge is straight except for very small lateral tooth-like projections; it articulates with the straight posterior edge of the large, trapezoid and plate-like mentum (Fig. 3A). The mentum is about as long as the exposed part of the gula and the submentum and slightly converging anteriorly; its surface bears a distinct vestiture of setae about as long as those on the dorsal side of the head; the anterior edge of the mentum is straight, with a very distinct bead. The main body of the strongly modified prementum is very short and laterally ear-shaped and unsclerotized; medially it bears a large ligula with a sclerotized ventral wall and a dense distal field of papillae, each of them with a centrally inserted sensillum; the papillary field (Fig. 3C) is medially divided by a vertical membranous bulge and unusual spatulate hair-like structures originate on its ventral edge; the short palps are inserted laterad the ligular base (Fig. 3C); the proximal palpomere is cylindrical and sclerotized, whereas the distal one is very short, membranous, with a folded structure and some knob-like sensilla. The posterior end of the prementum bears a slender, internal median process, posteriorly directed and serving as an attachment area for M28/2.

Musculature (Figs 4B, E, 6A-C): M28 - M. submentopalpae (0la8), premental retractor, consists of two paired subcomponents; M28/1, O: posteroventral region of head capsule, behind the posterior margin of submentum, I: medially on posteroventral premental edge; M28/2, O: medially from the origin of M28/1, I: process of prementum; M29 - M. tentoriopraementalis inferior (0la5), paired, consists of two subcomponents that merge on a long thin tendon, I: paramedially on posterior margin of prementum, laterad the labial gland, O: M29/1 – two smaller bundles between the origins of two subcomponents of M28, M29/2: mesoventrally on posterior tentorial arm. Considering the insertion, it cannot be fully excluded that this muscle is in fact M30 (M. tentoriopraementalis inferior (0la5)).

Epipharynx (Figs 4, 6A, B)

The semimembranous anteriormost part of the epipharynx forms the ventral wall of the labrum. Most parts of the epipharyngeal surface are smooth and glabrous and lack any surface structures. The midline of the middle region of the epipharynx bears a layer of microtrichia which form a longitudinal epipharyngeal process (LEP) (Fig. 6A). The posterior epipharynx is laterally fused with the posterior hypopharynx; it forms the roof of a short prepharyngeal tube.
A short closed prepharyngeal tube is present; it appears crescent-shaped in cross section, with the narrow lateral edges directed upwards and closely adjacent with the elongated tormae (Fig. 6C); its anterior opening, i.e. the functional mouth, is roughly quadrangular in cross section; its position is marked by the well-developed frontal ganglion (Fig. 6C). The pharynx has a wide lumen; the longitudinal folds for attachment of dilators are well developed (Fig. 6D).

Musculature (Figs 4B, 6C, D): M45 - M. frontobuccalis posterior (0bu3), two subcomponents, one moderately developed and four slender bundles, diverging towards insertion, M46/1, O: anterior frontal region, laterad M41/1, I: laterally on pharynx, distinctly...
posteral M45; M46/2, four very slender bundles, O: posterior frontal region, distant from M46/1, I: paramedially on dorsal pharynx, anterior to brain; M48 - M. tentoriobuccalis anterior (Obu5), pair of bundles with insertion (in asymmetrical specimen appearing unpaired, with single point of origin and insertion), between tritocerebral bridge and suboesophageal ganglion, O: mesal part of tentorial bridge (bridge interrupted in asymmetric specimen), I: ventral prepharyngeal wall, directly posteral anatomical mouth opening; M50 - M. tentoriobuccalis posterior (Obu6), O: dorsal surface of lateral areas of tentorial bridge, I: ventrally on anterior pharynx; M51 - M. verticopharyngalis (Oph1), absent; M52 - M. tentoriopharyngalis (Oph2), several well developed bundles, O: dorsally on tentorial bridge, I: ventrolaterally on posterior pharynx; M67 - M. transversalis buccae (Ohy9), several transverse cibarial muscle bundles; M68 - M. annularis stomodael (Ost1), pharyngeal ring musculature, enclosing the entire pharynx from the insertion of M41; M69 - M. longitudinalis stomodael (Ost2), layer of longitudinal muscles, covered by M68, very well developed on the dorsal side of the anterior pharynx.

Brain and suboesophageal ganglion (Figs 5, 6D)
The moderately flattened brain is large in relation to the size of the head capsule; it is located in the upper half of the lumen of the head and in the anterodorsal prothoracic region; the well-developed optic lobes connect the brain with the compound eyes. A distinct separate tritocerebral bridge is present. The suboesophageal ganglion is also large and partly shifted to the anteroverentral prothoracic region. In one of the examined specimens the tritocerebral bridge and the suboesophageal ganglion are slightly asymmetrical and shifted to the same side as M48 and M50.

Glands (Figs 4A, 6A)
Well-developed tubular glands in the anterior head region are associated with the labium (Fig. 6A: Ig1) and maxillae (Fig. 4A). The labial glands consist of two tubules that fuse into one channel with an opening in the premental region. A pair of two connected maxillary glands have two separate openings but nearly adjacent openings between the maxillary base and hypopharynx. They apparently release their secretions into the preoral cavity.

Adult characters used in the analysis (matrix see electronic appendage 1)
Cephalic characters
1. Head shape: (0) without elongated narrowed peristome; (1) elongate, conical, peristome present. A head with a narrow conical peristome is characteristic for Torridincolinae (Beutel, 1999a). The head is more or less rounded anteriorly and not
elaborated and narrowed in *Sphaerius* and the other groups (Beutel, 1999a; Anton & Beutel, 2006; Haječek & Fikáček, 2008: fig. 1; Short et al., 2015: fig. 5).

2. **Vertex:** (0) without keel; (1) keel present. Flattened keel present on the vertex of *Torridincolinae* (Beutel, 1999a). Absent in *Sphaerius* and the other groups (Beutel, 1999a; Anton & Beutel, 2006; Short et al., 2015: fig. 5).

3. **Shape of compound eyes:** (0) convex anteriorly; (1) slightly concave anteriorly; (2) distinctly elongated and concave anteriorly. Compound eyes convex anteriorly in *Sphaerius* and most other groups of Myxophaga (Beutel, 1999a; Anton & Beutel, 2006; Short et al., 2015). Slightly convex anteriorly in *lapir Py-Daniel, Fonseca & Barbosa, 1993* and *Yu Reichardt, 1973*, and distinctly kidney-shaped and elongated in *Torridincola Steffan, 1964* and *Incoltorrida Steffan, 1973* (Reichardt, 1973; Beutel, 1999a).

4. **Posterolateral extension of posterior tentorial arms:** (0) absent; (1) present. The posterior tentorium bears distinctly developed lateral processes in *Sphaerius* (Fig. 6D: Ippta) and *Hydroscapha*. They are missing in *Satonius* and *Lepicerus* (Anton & Beutel, 2006).

5. **Labro-mandibular locking device:** (0) absent; (1) present. Present in *Sphaerius* but missing in the other groups of Myxophaga (Anton & Beutel, 2006).

6. **Number of antennomeres:** (0) eleven; (1) ten; (2) nine; (3) eight; (4) five; (5) four.

   The antennae are composed of eleven segments in *Sphaerius*, *Delevea Reichardt, 1976* and *Satoni*, like in most groups of beetles (e.g. Lawrence et al., 2011). Nine are present in *Torridincolinae*, eight in *Hydroscaphidae* (excl. *Scaphydra Reichardt, 1973*), five in *Scaphydra*, and four in *Lepicerus* (Reichardt, 1973; Anon & Beutel, 2006; Short et al., 2015).

7. **Distal part of scapus and pedicellus:** (0) not urn-shaped; (1) urn-shaped. Scapus and pedicellus together form a stout urn-shaped structure in *Torridincola* (e.g. Reichardt, 1973; Endrödy-Younga, 1997b; Beutel, 1999a). This condition does not occur in *Sphaerius* or the other groups of Myxophaga (e.g., Beutel, 1999a; Anton & Beutel, 2006).

8. **Base of pedicellus:** (0) straight; (1) deflected. Deflected in *Sphaerius* and *Hydroscapha*.

9. **Separate intercalary antennomere with sucker-like appendage:** (0) absent; (1) present. Present in *Sphaerius* between the pedicellus and the basal flagellenmère. Absent in the other groups (Reichardt, 1973; Anton & Beutel, 2006).

10. **Shape of flagellomere 1:** (0) not distinctly elongated; (1) strongly elongated and distinct from remaining flagellum. A strongly elongated basal flagellenmère distinct from the remaining flagellum is characteristic for *Sphaerius*. This condition is not found in the other groups of Myxophaga (Reichardt, 1973, 1976; Anton & Beutel, 2006; Short et al., 2015: fig. 5).

11. **Three-segmented antennal club:** (0) absent; (1) present. Present in *Sphaerius* (Fig. 1, 2; Reichardt, 1973; Anton & Beutel, 2006).
12. Enlarged terminal antennomere: (0) absent; (1) enlarged, club-shaped, rounded; (2) very large, blade-like. An enlarged, rounded, club-shaped terminal antennomere is present in Hydroscaphidae and also Torridincolidae, even though to a lesser degree in Delevea (Reichardt, 1973; Endrödy-Younga, 1997b). The terminal segment of Lepicerus forms a very large blade-like structure with sharp anterior and posterior edges. Antennomere 11 of Sphaerius is part of the terminal club, not larger than the preceding two segments, conical, and apically pointed (coded as 0).

13. Origin of M. tentorio-scapalis medialis (M4): (0) tentorium; (1) partly on posterior head capsule. Two large bundles originate on the posterior head capsule in Sphaerius (Fig. 4D) but not in the other groups examined (e.g. Anton & Beutel, 2006).

14. Exposure of paired mouthparts: (0) largely or completely visible externally; (1) largely concealed between labrum and mentum. The apical parts of the mandibles and maxillae are largely concealed between the labrum and proximal parts of the labium in Sphaerius (Fig. 3B) and other groups of Myxophaga (Reichardt, 1973, 1976; Anton & Beutel, 2006; Short et al., 2015: fig. 5).

15. Mandibular mola: (0) absent; (1) present. Generally present in Myxophaga (gigs 4F, 6B) and also in many groups of Polyphaga (e.g. Anton & Beutel, 2004, 2006; Lawrence et al., 2011).

16. Mandibular prostheca: (0) absent; (1) present on left mandible; (2) present on both mandibles. Present on left mandible of Lepicerus (Anton & Beutel, 2006) and other myxophagans. Missing in Sphaerius.

17. Lacinia: (0) distinctly developed as separate endite lobe; (1) vestigial or absent. The lacinia is strongly reduced in Sphaerius (Fig. 6A, B) and not recognizable as a separate element in all other examined myxophagans (Reichardt, 1973; Beutel, 1999a; Anton & Beutel, 2006). The remaining endite lobe is the galea and should not be addressed as mala (e.g. Beutel, 1999a).

18. Apical part of maxilla: (0) without row of flattened teeth; (1) row of fixed, flattened teeth present. Present in Satonius, Ytu and Torridincola (Satō, 1982: fig. 12; Beutel, 1999a).

19. Size of penultimate maxillary palpmere: (0) not distinctly larger than preceding palpmeres; (1) strongly inflated. Strongly inflated in Sphaerius (Fig. 3A).

20. Apical maxillary palpmere: (0) not distinctly smaller than preceding palpmeres; (1) apical palpmere strongly reduced in size, with very small distal appendage. Normally sized in Lepicerus (Anton & Beutel, 2006) but small and subulate in Sphaerius (Fig. 3A) and the other groups of Myxophaga (Reichardt, 1973; Reichardt & Hinton, 1976; Satō, 1982; Endrödy-Younga, 1997b; Beutel, 1999a).
21. Muscle “Mx”: (0) Absent; (1) present. An unusual muscle originating at the gena and inserting on the membrane connecting the maxillary base and the hypopharynx is characteristic for Staphylinoidae (Anton & Beutel, 2004; Antunes Carvalho et al., 2017). It is missing in Myoxophaga (Figs 4, 6; Anton & Beutel, 2006).

22. Connection of mentum and submentum: (0) separate; (1) fused. Mentum and submentum usually separated in Myoxophaga (Fig. 3A; Beutel, 1999a; Anton & Beutel, 2006). Fused in Hydroscapha and Scaphydra (Reichardt, 1973: ffg 107), but not in Yara and Confossa Short, Joly, García & Maddison, 2015 (Short et al., 2015).

23. Shape of mentum: (0) parallel-sided, roughly quadrangular; (1) narrow posteriorly, with two anterior lobes; (2) transverse, with large, rounded lateral lobes. Narrow posteriorly and extending into two anterior lobes in Torridincolidae excl. Deleva (Beutel, 1999a; Reichardt, 1973; Sató, 1982; Endrödy-Younga, 1997b). Transverse, with large, rounded lateral lobes in Adephaga.

24. Papillae on ligula: (0) absent; (1) present. The ligula of Sphaerus (Fig. 3C), Hydroscapha and Lepicerus (Reichardt, 1973; Beutel, 1999a; Anton & Beutel, 2005) is densely set with papillae. At least few are present in adults of Torridincolidae (Reichardt, 1973). Ligular papillae are also present in Hydraenidae.

25. Size of apical labial palpmere: (0) absent; (1) present. Greatly reduced in size in Sphaerus (Fig. 3A, C), Hydroscaphidae and Torridincolidae (Reichardt, 1973; Reichardt & Hinton, 1976; Beutel, 1999a). About as wide as the proximal palpmere and only slightly shorter in Lepicerus (Anton & Beutel, 2006).

26. Lateral appendage of apical labial palpmere: (0) absent; (1) present. Present in Hydroscapha and Scaphydra, but missing in Yara (Reichardt & Hinton, 1976; Beutel, 1999a).

27. Insertion of M. submentopraementialis (M. 28): (0) not on median apodeme of mentum; (1) on median apodeme. An unusual insertion of M. 28 on a strongly developed median apodeme of the mentum occurs in Sphaerus (Fig. 4C, 6C) and Satoni, and probably also in other torridincolids (apodeme visible on slide preparations of Ytu). This condition is absent in Hydroscapha and Lepicerus (Anton & Beutel, 2006).

28. Shape of labio-hypopharyngeal complex between maxillary bases: (0) not strongly narrowed; (2) strongly narrowed. Strongly narrowed and hourglass-shaped in most groups of Staphyliniformia including Hydraenidae and Leiodidae (Anton & Beutel, 2004; Beutel & Leschen, 2005; Antunes-Carvalho et al., 2017). This condition is unknown in Myoxophaga (Fig. 6a, B).

29. Cephalic glands: (0) absent; (1) present. Present in the labio-hypopharyngeal region and proximad the maxillae in Sphaerus (Fig. 4A), Lepicerus (Anton & Beutel, 2006) and Hydroscapha. Absent in Satonius.
30. Width of prosternal process: (0) narrow or moderately wide; (1) strongly broadened. Moderately wide in Lepicerus (coded as 0) (Beutel, 1999a: fig. 1) and narrow in Sphaerius and Hydroscaphidae (Beutel, 1999a; Short et al., 2015: fig. 9). Unusually broad in Torridicolinae (Sátó, 1982; Endrödy-Younga, 1997b: pl. 5A, B; Reichardt, 1973; Beutel, 1999a).

31. Apex of prosternal process: (0) not truncated; (1) truncated. Truncated in Torridicolinae (Sátó, 1982; Endrödy-Younga, 1997b: pl. 5A, B; Reichardt, 1973; Beutel, 1999a: fig. 5).

32. Exposure of propodeon: (0) free; (1) concealed. Exposed in Myxophaga like in Archostemata and Adephega. Completely concealed in Polyphaga (e.g. Lawrence et al., 2011).

33. Number of separate tarsomers: (0) five; (1) four; (2) three; (3) all fused into one element. Five are present in Delevea (Endrödy-Younga, 1997b), four in Satonius and Torridicolinae, and three in Sphaerius and Hydroscaphidae (Beutel, 1999a; Short et al., 2015). All five are fused into one distal element in Lepicerus (Beutel, 1999a: fig. 1).

34. Connection of pterothoracic ventrites: (0) connected by membrane or articulated; (1) firmly connected. Rigidly connected in Polyphaga with very few exceptions (e.g. Beutel & Haas, 2000; Friedrich & Beutel, 2006; Ge et al., 2007) and generally fused in Myxophaga (Beutel, 1999a; Beutel & Haas, 2000).

35. Separation of mesocoxae: (0) contiguous or separated by less than coxal diameter; (1) widely separated. Widely separated in Myxophaga (Endrödy-Younga, 1997b: pl. 5; Beutel, 1999a; Lawrence et al., 2011) with the exception of Confossa (Short et al., 2015: fig. 4C).

36. Pronoto-elytral angle: (0) distinct; (1) indistinct or absent. Obsolete or absent in Sphaerius, Hydroscaphidae, Delevea and Satonius (Endrödy-Younga, 1997a, b; Beutel, 1999a: figs 6, 7; Hájek & Fikáček, 2008: fig. 1; Short et al., 2015). Conspicuous in Lepicerus (Beutel, 1999a: fig. 1) and also distinct in Torridicolinae (Reichardt, 1973; Beutel, 1999a).

37. Elytral apex: (0) not truncated; (1) truncated. Truncated in Hydroscaphidae (e.g. Reichardt, 1973; Reichardt & Hinton, 1976; Short et al., 2015).

38. Longitudinal suture and ridge of metaventrite (discrimen): (0) present; (1) absent. Present in Lepicerus (Beutel, 1999a: fig. 1) but missing in all other groups of Myxophaga (Endrödy-Younga, 1997b: pl. 5; Beutel, 1999a; Lawrence et al., 2011; Short et al., 2015).

39. Transverse suture and ridge of metaventrite: (0) present; (1) absent. Only preserved in Lepicerus (Beutel, 1999a: fig. 1; Endrödy-Younga, 1997b: pl. 5; Beutel, 1999a: fig. 1; Lawrence et al., 2011).
40. Separation of metacoxae: (0) medially adjacent or narrowly separated; (1) widely separated by median piece of sternite II; (2) widely separated by broad posteromedian portion of metaventrite with straight posterior edge. Widely separated in Lepicerus by median piece of sternite II (Beutel, 1999a: fig. 1). Separated by broad posteromedian process of metaventrite in Hydroscaphidae (Short et al., 2015: fig. 4; Vanin et al., 2016).

41. Metacoxal plates: (0) very narrow or absent; (1) distinctly developed medially; (2) broad and concave posteriorly; (3) very large, covering large parts of the abdomen. Absent or extremely narrow in Lepicerus and Torridincolidae (e.g. Steffan, 1964, 1973; Reichardt, 1973; Beutel, 1999a). Concave posteriorly and overlapping with at least 40% of the width of the metafemur in Sphaerius, Hydroscapha and Scaphydra (Reichardt, 1973: fig. 64; Beutel, 1999a). Obsolete in Yara and Confossa (Short et al., 2015) (coded as 0). Very large and posteriorly rounded in Halipidae.

42. Origin of metafurca: (0) with single stem; (1) distinctly separated. Widely separated metafurcal bases are an unusual shared feature of Sphaerius and Hydroscapha (Beutel, 1999a: figs 14-15). The condition in the other hydroscaphid genera is unknown.

43. Margin of hind wing: (0) without fringe of long microtrichia; (1) fringe on hind margin; (2) fringe along anterior and posterior margin. Microtrichia with a coiled surface structure are present along the posterior wing margin of Lepicerus (Kukalová-Peck & Lawrence 1993) and almost along the entire margin in the other groups of Myxophaga (e.g. Reichardt, 1973; Kukalová-Peck & Lawrence 1993; Beutel, 1999a; Short et al., 2015). Wing polymorphism is described for species of Satonius (Hajek et al., 2011) (coded as 2 for the genus).

44. Oblongum: (0) present; (1) absent. Absent in Hydroscaphidae but present in the other groups (e.g. Reichardt, 1973; Kukalová-Peck & Lawrence, 1993: figs 23-29).

45. Configuration of subcosta: (0) not distinctly curved, separated from radius anterior (RA+); (1) curved, connected with RA+ after Short distance. Clearly separated from RA+ in Lepicerus and not distinctly curved. Curved and connected with RA+ in the other groups with distinctly developed wings (Kukalová-Peck & Lawrence, 1993: figs 23-28; Beutel, 1999a).

46. Plastron on terminal abdominal ventrite (stermite VII): (0) absent; (1) present. A plastron with curved microtrichia is present on sternite VII of Lepicerus bufo (Reichardt, 1976) (coded as 0&1 for the family) and Torridincolinae (Hinton, 1969; Reichardt, 1973; Endrödy-Younga, 1997b; Beutel, 1999a).

47. Recumbent pilosity of abdominal tergites: (0) absent; (1) present on tergites III and IV; (2) present on tergite VII. Present on tergites III and IV in Hydroscapha and...
48. **Shape of abdominal segments V-VIII:** (0) not narrowed and ring-like; (1) narrowed and ring-like. A strongly narrowed, posteriorly tapering and movable posterior abdomen is a characteristic of Hydroscaphidae (Reichardt, 1973; Beutel, 1999a; Short et al., 2015).

49. **Exposure of ventrite 2 (sternite IV):** (0) not covered by ventrite 1; (1) covered by ventrite 1. Sternite IV is completely hidden below the first visible sternite in *lapir* and *Claudia* Reichardt & Vanin, 1976; Reichardt & Vanin, 1976; Beutel, 1999a).

50. **Sternite VIII:** (0) not exposed; (1) exposed. Exposed in Hydroscaphidae (Short et al., 2016; Vanin et al., 2016).

51. **Median carina of abdominal sternites:** (0) absent; (1) present. Present on the first abdominal ventrite of *Ytu* and on all abdominal sternites in *lapir* and *Claudia* (Reichardt, 1973; Reichardt & Vanin, 1976; Beutel, 1999a).

52. **Tubular fields of abdominal segments VI and VII:** (0) absent; (1) present. Present on the pleurites of abdominal segments VI and VII of *Delevea, Satonius, lapir* and *Torrincola* (Endrödy-Younga, 1997b; Beutel, 1999a).

53. **Parallel-sided incisions of posterior abdominal sternites:** (0) absent; (1) present. Distinct emargination are present on the hind margins of the posterior abdominal sternites of *Torrincola* and *Incoltorrida* (Beutel, 1999a).

54. **Anterior extension of tergite VIII:** (0) absent (1) present: Present in *Sphaerius* (Beutel, 1999a).

55. **Deep posterior emargination of abdominal tergite VIII:** (0) absent (1) present: Present in Torridincolidae with the exception of *Delevea* (Reichardt & Vanin, 1976; 1977; Sató, 1982; Endrödy-Younga, 1997b; Beutel, 1999a).

56. **Parameres:** (0) present; (1) absent. Parameres are usually present (e.g., Reichardt, 1973; Lawrence et al., 2011) but absent in Hydroscaphidae. The aedeagus is vestigial and membranous in *Sphaeriusidae* (coded as inapplicable).

57. **Longitudinal split of aedeagus:** (0) absent; (1) present. Present in *Lepicerus horni* (Reichardt, 1976) and generally in Torridincolidae (e.g. Reichardt, 1973; Reichardt & Vanin, 1976, 1977).

58. **Forked apophysis with elongated stalk associated with basal bulb of aedeagus:** (0) absent; (1) present. Present in *Yara, Scaphydra* (Reichardt, & Hinton, 1973) and *Confossa* (Short et al., 2015).

59. **Habitat of adults:** (0) terrestrial; (1) semiaquatic; (2) hygroecopic; (3) aquatic. Adults of most species of *Lepicerus* and *Sphaerius* live in moist substrates close to water bodies (coded as 1) (e.g. Beutel & Arce-Perez, 2016; Arce-Perez et al., 2016). Adults of
Hydrosaphidae and Torridincolidae live in hygropteric habitats (e.g. Steffan, 1964, 1973; Reichardt & Costa, 1967; Reichardt, 1973; Beutel, 1999a; Hajek & Fikáček, 2008; Fikáček & Šípková, 2009).

Larval characters used in the analysis

This list of larval characters is mainly based on the characters analysed in Beutel et al. (1998), Beutel (1999) and Ge et al. (2010), but also on data in a description of a presumptive larva of Lepicerus (Lawrence et al., 2013).

60. **Length of ultimate larval instar:** (0) longer than 2.7 mm; (1) 2.7 mm - 1.5 mm; (2) 1.5 mm or less. The presumptive final instar of Lepicerus is 3 mm long (Lawrence et al., 2013), whereas larvae of Torridincolidae measure less than 2.7 mm (Lawrence & Reichardt, 1991; Beutel et al., 1999; Beutel, 1999b). The total length of mature larvae of Hydrosaphidae and Sphaeriidae does not exceed 1.5 and 1.2 mm, respectively (e.g., Britton, 1966; Costa et al., 1988; Lawrence & Reichardt, 1991; Beutel et al., 1999).

61. **Body shape:** (0) parallel-sided or subparallel; (1) thorax subparallel, abdomen distinctly tapering posteriorly; (2) body ovoid. The body of larvae of Sphaerus (Britton, 1966), Delevea, and larvae of many other families of Coleoptera (e.g. Cupedidae, Trachypinchidae, Hydraenidae, Lelodidae) is more or less parallel-sided or only slightly rounded laterally. A parallel-sided thorax and a distinctly tapering and moveable abdomen is characteristic for hydrosaphid larvae (Costa et al., 1988). The body is distinctly broadened and ovoid in larvae of Torridincolidae excl. Delevea (Reichardt, 1973; Beutel et al., 1999; Beutel, 1999b; Hajek & Fikáček, 2008: fig. 2).

62. **Tergites:** (0) without distinct lateral extensions; (1) lateral extensions present. Distinct lateral tergal duplicatures are present in all final-instar larvae of Myxophaga examined (Costa et al., 1988; Britton, 1966; Beutel et al., 1999; Beutel, 1999b; Hajek & Fikáček, 2008: fig. 2) with the exception of Lepicerus (Lawrence et al., 2013: fig. 1).

63. **Length of thorax:** (0) distinctly shorter than abdomen; (1) thorax and abdomen about equally long. The thorax of larvae of Satonis and Torridincolinae is roughly semicircular and about as long as the abdomen (Beutel et al., 1999; Beutel, 1999b; Hajek & Fikáček, 2008: fig. 2).

64. **Shape of head:** (0) not distinctly broadened; (1) head transverse. Broader than long in larvae of Torridincolidae (Reichardt, 1973; Beutel, 1999b; Hajek & Fikáček, 2008: fig. 12), Hydrosaphidae (Reichardt, 1974; Reichardt & Hinton, 1976; Costa et al., 1988), and Sphaerus (Britton, 1966). The head is also broadened in larvae of Cupedidae (e.g. Yavorskaya et al., 2015).

65. **Stemmata:** (0) six; (1) five; (2) four; (3) three; (4) stemmata vestigial or absent. Six stemmata are usually present in Adephaga, five in Hydrosaphidae (Costa et al., 1988)
and many groups of Polyphaga (e.g. Hydraenidae, Lelodidae part.; Newton, 1991), and four in Sphaeriidae (Britton, 1966), Delevea and Sationiidae (Beutel et al., 1999; Hájek & Fikáček, 2008). Three stemmata are present in other known torridicolid larvae (Costa et al., 1988). Stemmata are absent or vestigial in larvae of Cupedidae (Lawrence, 1991; Yavorskaya et al., 2015).

66. Insertion area of stemmata: (0) flat or only slightly elevated; (1) strongly pronounced elevation. The stemmata of torridicolid larvae are inserted on a conspicuous lateral elevation (Beutel et al., 1999; Beutel, 1999b; Hájek & Fikáček, 2008: fig. 12). The insertion area is only slightly elevated or flat in other myxophagan larvae including Lepicerus (Lawrence et al., 2013).

67. Dorsal body surface: (0) smooth; (1) tuberculate, with more or less distinct cuticular scales. A tuberculate dorsal surface is characteristic for larvae of Myxophaga excl. Lepicerus (Beutel et al., 1999; Lawrence et al., 2013).

68. Hemispherical projection of head capsule between insertion of antenna and maxilla: (0) absent; (1) present. Only occurring in Leiodidae, Hydraenidae, Philiidae and Agyridae (Beutel & Leschen, 2005).

69. Tentorial bridge: (0) not distinctly broadened; (1) distinctly broadened, origin of extrinsic maxillary muscles; (2) absent. The tentorial bridge is distinctly broadened in all myxophagan larvae examined (Beutel & Haas, 1998) with the exception of Lepicerus (Lawrence et al., 2013: moderately broad).

70. Labral extension: (0) labrum or anterior clypeolabral area not extended; (1) strongly extended. A distinctly extended labrum which covers large parts of the mouthparts is present in larvae of Hydroscaphidae and Sphaeriidae (Britton, 1966; Beutel et al., 1999).

71. Flattened disc-shaped labral sensilla: (0) absent; (1) present, separated; (2) present, fused. Flattened, disc-shaped sensilla are present on the anterior margin of the larval labrum of Hydroscapha, Sphaeriidae, Delevea, Torridincola, Sationiidae, Iapir, and Ytu (Beutel et al., 1999). They are probably absent in Lepiceridae (Lawrence et al., 2013) but this needs confirmation (coded as ?). They are fused in larvae of Torridincolinae excl. Delevea (Beutel et al., 1999; Beutel, 1999b).

72. Flattened, frayed labral setae: (0) absent; (1) present. A large flattened and apically frayed seta is inserted on the anterolateral labral margin of larvae of Torridincolinae (Beutel et al., 1999).

73. Fusion of genal lobes with lateral clypeolabral margin: (0) absent; (1) present. Triangular genal folds are fused with the posterolateral clypeolabral margin in larvae of Hydroscapha and Sphaeriidae, resulting in a semi-entognathous condition (Beutel & Haas, 1998; Beutel et al., 1999).
74. **Number of antennomeres of later instars:** (0) four; (1) three; (2) two. Usually 4-segmented in Archostemata (Lawrence, 1991) and Adephaga, but three-segmented in most polyphagan larvae and in *Lepicerus* (Lawrence *et al*., 2013). Two-segmented in Torridicolidae, *Sphaerius* and Hydroscaphidae (Costa *et al*., 1988; Beutel *et al*., 1999; Hájek & Fikáček, 2008).

75. **Length of antennomeres of later instars:** (0) moderately elongate; (1) well developed, distinctly elongate; (2) very slender but short in relation to head width. The distal antennomere is at least five times as long as the proximal segment in all torridicolid larvae examined (Costa *et al*., 1988; Beutel *et al*., 1999; Beutel, 1999b). The distal antennomere is very slender in Hydroscaphidae (Beutel & Haas, 1998; Costa *et al*., 1988) but very short in relation to the head width.

76. **Length of sensorial appendage of subapical antennomere:** (0) less than five times longer than basal width; (1) more than five times longer than basal width; (2) flattened, embedded in an apical excavation of antennomere 2; (3) absent. The sensorial appendage is distinctly elongated in larvae of Hydroscaphidae, *Sphaerius*, and Hydraenidae, whereas the appendage is embedded in a distal concavity of antennomere 2 and strongly flattened in Torridicolidae (Costa *et al*., 1988; Beutel *et al*., 1999; Beutel, 1999b; Hájek & Fikáček, 2008).

77. **Rounded subapical prostheca:** (0) prostheca absent or slender and articulated; (1) present. A subapical, rounded prostheca with posteriorly directed small spines is present in larvae of Hydroscaphidae and Torridicolidae (Reichardt, 1973; Costa *et al*., 1988; Beutel *et al*., 1999; Hájek & Fikáček, 2008: fig. 21). It is absent in *Lepicerus* (Lawrence *et al*., 2013). The condition in *Sphaerius* is still unclear.

78. **Distal appendage of maxillary palp:** (0) absent; (1) present. A small and slender distal appendage is present on the apical palpmere of larvae of Hydroscapha and *Scaphydra* (Costa *et al*., 1988; Beutel & Haas, 1998).

79. **Galea and lacinae:** (0) separate and distinctly developed; (1) only endite lobe; (2) lacina vestigial. Separate endite lobes are absent from larvae of Myxophaga (Britton, 1966; Lawrence, 1991; Costa *et al*., 1988; Beutel *et al*., 1999; Lawrence *et al*., 2013).

80. **Apex of mala or lacinae:** (0) simple or with several fixed teeth; (1) with external fixed tooth and several flattened and articulated teeth. Maxillary apex with one fixed lateral tooth, several flattened and articulated teeth, and a mesal row of spines in larvae of Torridicolidae (Costa *et al*., 1988; Beutel *et al*., 1999; Beutel, 1999b).

81. **Labial palpomeres:** (0) three; (1) two; (2) one. Palp one-segmented in Hydroscaphidae (Costa *et al*., 1988; Beutel *et al*., 1999).
82. Ligular papillae: (0) absent; (1) present. Sensorial papillae are inserted on the ligula of larvae of Myxophaga including Lepicerus (Costa et al., 1988; Beutel et al., 1999; Lawrence et al., 2013). Numerous minute sensilla are present on the ligular area of Satonius (Hájek & Fikáček, 2008: fig. 22) and at least one pair of larger papillae.

83. Internal sacs: (0) absent; (1) present. Voluminous internal sacs are present in the labium of larvae of Torridincolaides. It is likely that they are gland reservoirs. They are absent in larvae of Sphaerius and Hydroscapha (Beutel & Haas, 1998; Beutel et al., 1999).

84. Posterior tergal rows of lanceolate setae: (0) absent; (1) present. Larvae of Hydroscaphidae and Sphaerius with dense rows of lanceolate setae along posterior margins of thoracic terga and abdominal terga I-VII (Britton, 1966; Lawrence, 1991; Beutel et al., 1999).

85. Lateral rows of contact hairs: (0) absent; (1) present on thoracic terga; (2) present on thoracic and abdominal terga I-IX. Lateral rows of very long contact hairs or friction setae (Reichardt, 1973) present on thoracic and abdominal terga I-IX of larvae of Hydroscaphidae (Costa et al., 1988) and Sphaerius (Beutel et al., 1999). Similar setae inserted on thoracic segments of final-instar larvae of Delesea, Satonius, and Ytu. Absent in other known larvae of Torridincolaides and also in Lepicerus (Beutel et al., 1999; Lawrence et al., 2013).

86. Leg segmentation: (0) six; (1) five. Six-segmented in larvae of Archostemata and Adephaga, but composed of only five segments in Myxophaga and Polyphaga.

87. Base of claws: (0) without strong and flattened spines; (1) two strong spines present. Two strong and flattened spines present at base of single claws of larvae of Hydroscaphidae and Sphaeriusidae (Beutel et al., 1999). Absent in Torridincolaides and Lepicerus (Costa et al., 1988; Lawrence et al., 2013).

88. Spiracular gills: (0) absent; (1) present, slender and segmented; (2) unsegmented, balloon-like. Larval spiracular gills are a unique feature of Myxophaga excl. Lepicerus (Hinton, 1967; Beutel et al., 1999; Lawrence et al., 2011, 2013). They are segmented in torridincolaids and unsegmented and balloon-like in Sphaerius (abdominal segments I-VIII), Hydroscapha (prothorax, abdominal segments I and VIII), and Scaphydra (segment VIII) (Reichardt, 1973; Costa et al., 1988; Beutel et al., 1999; Hinton, 1967; Lawrence & Newton, 1982). The spiracular gills of the prothorax and abdominal segment I are tuft-like structures in Scaphydra (Costa et al., 1988).

89. Mesothoracic spiracles: (0) present; (1) absent. Reduced in Torridincolaides and Sphaerius (Hinton, 1967; Beutel et al., 1999).

90. Abdominal segment VIII: (0) without lateral process; (1) lateral process present. The spiracular gills of abdominal segment VIII are inserted on a posterolateral process in

91. Sternite IX: (0) well developed, broad; (1) strongly reduced, triangular; (2) absent. A distinctly reduced, triangular sternite IX is present in larvae of *Satonius* and Torridincolinae, (Costa *et al.*, 1988; Hájk & Fikáček, 2008), whereas the sternite is broad and more or less rectangular in larvae of *Delevea*, Hydroscaphidae, *Sphaerius*, and in the majority of other coleopteran larvae (Costa *et al.*, 1988; Beutel *et al.*, 1999).

92. Opercular plate of segment X: (0) absent; (1) present. Present in larvae of Hydroscaphidae (Beutel *et al.*, 1999).

93. Paired anal flaps of abdominal segment X: (0) absent; (1) present. Abdominal segment X transformed into paired anal flaps in larvae of Torridincolidae (Beutel *et al.*, 1999; Beutel, 1999b; Hájk & Fikáček, 2008).

94. Terminal abdominal hooks: (0) absent; (1) segment X with one pair of hooks; (2) with two pairs of hooks; (3) with three pairs of hooks. One pair of slender, terminal abdominal hooks is inserted on the opercular plate of larvae of Hydroscaphidae (Costa *et al.*, 1988). Three pairs are attached to segment X in larvae of *Sphaerius* (Britton, 1965). Terminal abdominal hooks are absent in larvae of Torridincolidae and *Lepicerus* (Lawrence *et al.*, 2013).


96. Abdominal glands: (0) absent; (1) present. Paired, segmentally arranged abdominal glands are present in larvae of Hydroscapha, *Sphaerius*, *Satonius*, *iapir*, *Torrindincola*, and *Ytu* (Beutel *et al.*, 1999). The condition in *Lepicerus* is unknown.

97. Pupation: (0) pupae leave last larval exuviae; (1) within last larval exuviae. Hydroscaphidae and Torridincolidae pupate within the last larval exuviae (Costa *et al.*, 1988). No observations are available for *Sphaerius* but the shape and ecdysial cleft of 3rd instar larval skins suggest a similar behaviour (Beutel *et al.*, 1999). The condition is unknown in *Lepicerus*. Pupation within larval exuviae in an aquatic environment is not known in other families of Coleoptera (Beutel *et al.*, 1999).

98. Habitat of larvae: (0) terrestrial; (1) semiaquatic; (2) hygropteric; (3) aquatic. Larvae of *Sphaerius* were collected in moist substrates close to water bodies. Larvae of Hydroscaphidae and Torridincolidae live in hygropteric environments (e.g. Reichardt, 1973; Hájk & Fikáček, 2008).

Results of the cladistic analysis (Fig. 7)
The analysis with NONA yielded 14 minimum length trees with 175 steps. Eight trees with
the same number of steps were obtained with TNT (implicit enumeration). A pattern
Hydroschaphidae + (Torridincolidae + (Sphaeriusidae + Lepiceridae)) required 21 additional
steps with Mesquite.

List of apomorphies (strict consensus tree with apomorphies mapped on it see electronic
appendage 2)

Only apomorphies of myxophagan clades supported in the strict consensus tree are
presented. Homoplasious changes in italics.

Mesocoxae widely separated (reversal in Confossa); 79.1. Larval maxillary mala.

Additional potential autapomorphies (ambiguous, not verified for most groups of
Hydroschaphidae and Torridincolidae): 29.1. Glands in labio-hypopharyngeal and
postmaxillary regions (missing in Satonius).

Apopomorphies that very likely evolved independently in Hydraenidae and Myxophaga:
14.1. Mouthparts largely concealed between labrum and mentum; 24.1. Ligular papillae;
43.1. Incomplete fringe of microtrichia at hind wing margin.

Myxophaga excl. Lepiceridae: 20.1. Apical maxillary palpomere much smaller than
penultimate segment; 25.1. Apical labial palpomere much smaller than penultimate segment;
36.1. Pronoto-elytral angle indistinct or absent; 45.1. Subcosta distinctly curved and
connected with RA+ after short distance; 62.1. Larval tergites with distinct lateral extensions;
64.1 Larval head broader than long; 67.1. Larvae with tuberculate dorsal surface with scale-
like surface structures; 69.1. Larval tentorial bridge strongly broadened; 74.2. Two-
segmented larval antenna; 77.1. Larval mandible with rounded subapical protheca
(condition in larvae of Sphaerius still unknown); 90.1. Lateral process of larval segment VIII
(present in Torridincola).

Additional potential apomorphies (ambiguous): 40.1. Discrmen of metaventrite absent;
39.1. Transverse suture of metaventrite absent; 43.2. Complete fringe of microtrichia at hind
wing margin; 86.1. Larval spiracular gills.

Sphaeriusidae + Hydroschaphidae: 4.1. Posterolateral extensions of tentorium; 8.1. base of
pedicellus bent; 33.2. Three tarsomers; 42.1. Bases of metafurca widely separated; 70.1.
Larval labrum strongly extended; 73.1. Triangular genal fold of larvae fused with lateral
clypeolabral margin; 84.1. Larval terga with posterior rows of lanceolate setae; 87.1. Larval
legs with strong and flattened spines at base of claws.
Additional potential synapomorphies: 41.2. Broad and posteriorly concave metacoxal plates (implying secondary loss in Yara and Confossa); 76.1. Larval sensorial appendage of antenna distinctly elongated; 85.1. Larval thoracic and abdominal terga with long lateral contact hairs (also on thoracic terga of torridincolid larvae); 88.2. Balloon-shaped larval spiracular gills.


Additional potential autapomorphies: 94.3. Larval abdominal segment X with three pairs of hooks.


Additional potential autapomorphies (adult anatomy and larvae of Yara and Confossa unknown): 43.2. Broad and posteriorly concave metacoxal plates (implying secondary loss in Yara and Confossa); 56.1. parameres reduced; 58.1. Stalked apophysis with elongated stalk associated with basal bulb of aedeagus; 61.1. Larval thorax subparallel, abdomen tapering posteriorly; 65.1. Five sternmata; 75.2. Distal larval antennomere very slender and elongated; 78.1. Distal appendage of larval apical maxillary palp present; 81.1. One labial palpomere; 92.1. Opercular plate of larval segment X present; 94.1. Larval abdominal segment X with pair of hooks.


Additional potential autapomorphy: 76.2. Larval antennal sensorial appendage flattened, embedded in an apical excavation of antennomere 2.

Torridincolidae excl. Deleva: 33.1. Four tarsomeres of forelegs and middle legs; 55.1. Deep posterior emargination of tergite VIII; 61.2. Larval body broadly ovoid; 63.1. Thorax
semicircular and about as long as the abdomen; 71.1. Disc-shaped labral sensilla of larvae fused; 91.1. Larval sternite IX strongly reduced and triangular.


**Torrindicola** and **Incolotrida:** 3.2. Compound eyes distinctly kidney-shaped; 53.1. Parallel-side incisions of posterior abdominal sternites.

**Iapir and Claudiella:** 49.1. Abdominal ventrite 2 completely hidden below ventrite 1.

**Discussion**

**Phylogeny** (Fig. 7)

Sphaeriusidae are supported by numerous apomorphies of different body parts and life stages, including characters of the adult head. Unusual derived features are the presence of a deep frontal antennal furrow (Fig. 3B), a fronto-epipharyngeal muscle with two subcomponents extending in opposite directions (Fig. 4A, E: 9/1, 9/2), an elongated second pair of tormae posteriorly connected with a process of the hypopharyngeal suspensorium (Fig. 6A-C), the origin of two strongly developed bundles of M. tentoriocapsalis medialis (M4) from the posterior head capsule (Fig. 4D), the presence of an intercalary antennomere with a structure resembling a suction disc (Fig. 6B), and a deep concavity on the anterior side of maxillary palpomere 2 (Fig. 3A). The presence of a compact three-segmented antennal club is a unique feature within the suborder. A labro-mandibular locking device does also occur in Ptiliididae and Hydraenidae, however in the case of *Sphaerius* with a mandibular ridge instead of a process. The loss of the mandibular prostheca is very likely a secondary feature, differing from the groundplan condition of Myxophaga with a movable tooth inserted on the left mandible (e.g. Lawrence et al., 2011). Additional apomorphies of Sphaeriusidae are the large metacoxal plates (Fig. 2), the presence of only three exposed abdominal sternites, and the unsclerotized male genital apparatus (Beutel, 1999a). Further apomorphies of the immature stages are discussed in Beutel et al. (1998), for instance the stout, posteriorly directed antennae, the presence of three pairs of terminal abdominal hooks, and also the
slug-like locomotion of the minute larvae (R.G. Beutel pers. obs. [laboratory culture of D.R. Maddison], not coded here).

Like in earlier morphology-based studies (Beutel et al., 1999; Beutel, 1999a; Ge et al., 2010) but in conflict with analyses of molecular data (McKenna et al., 2015), Sphaeriusidae are placed as sistergroup of Hydroscaphidae (Fig. 7). An unusual cephalic feature is the presence of posterolateral extensions of the tentorium (Fig. 6D: lptt). Additional synapomorphies are the presence of only three tarsomeres and widely separated bases of the metatarsus (Beutel, 1999a). A complex larval feature is a strongly extended larval labrum, laterally fused with triangular genal folds (Beutel et al., 1999). This results in a semi-entognathous condition, with largely concealed paired larval mouthparts (Beutel & Haas, 1998; Beutel et al., 1999). Other very unusual or unique synapomorphies of larvae are the presence of rows of lanceolate setae at the posterior tergal margins, balloon-shaped spiracular gills (e.g. Lawrence & Newton, 1982), and strong and flattened spines at base of the unpaired claws (Beutel et al., 1999). Even though data for larvae of two genera of Hydroscaphidae are presently not available, the clade Sphaeriusidae + Hydroscaphidae appears very strongly supported (branch support value 5), especially but not only by characters of the immature stages.

Hydroscaphidae are also supported by a series of unusual features. This includes the very movable and posteriorly tapering abdomen of adults and larvae (e.g. Beutel et al., 1999; Beutel, 1999a; Short et al., 2015), metacoxae very widely separated by a posteromedian projection of the metaventrite, an exposed sternite VIII, an apophysis with an elongated stalk associated with the basal bulb of the aedeagus, and the loss of the parameres.

Like in the earlier morphology-based studies, the clade Sphaeriusidae + Hydroscaphidae is robustly placed as a sister taxon of Torridicolidae (Fig. 7, branch support value 9). Cephalic synapomorphies are the size reduction of the apical maxillary and labial palpomeres. Other shared derived features are the complete fringe of microtrichia at the hind wing margin, a modified subcostal vein, and a metaventrite without discrinen and transverse suture. Larval apomorphies are a very broad head, two segmented antennae, tergites with distinct lateral duplicatures, and a tuberculate surface structure on the dorsal side (e.g. Beutel et al., 1999). A unique larval feature is the presence of spiracular gills, elongated and segmented in Torridicolidae, but short and balloon-shaped in the other two families (see above) (Hinton, 1967; Reichardt, 1973; Lawrence & Newton, 1982; Beutel et al., 1999).

The monophyly of Torridicolidae is well supported (Fig. 7, electronic appendage 2), and also a pattern with Dedevea as sister taxon of a clade comprising Satonius and the remaining genera, i.e. Torridicolinae. Autapomorphies of the family are an urn-shaped structure formed by the scapus and pedicellus, a very broad prosternal process, and a tubular field of abdominal segments VI and VII. Derived larval features are an elevated ocular
region, a strongly elongated distal antennomere, a maxillary mala with a specific armature of teeth, internal labial sacs, and a larval segment X transformed into anal flaps. A conspicuous transformation characterizes larvae of Satorius and Torridincolinae, a flattened and ovoid body with the thorax about as long as the abdomen (Beutel, 1999b; Hajek & Fikacék, 2008). Characteristic apomorphies of Torridincolinae are the elongated head with a conical peristome and paired dorsal keels, a very broad and truncated prosternal process, and a pleuron on abdominal sternite VII (Hinton, 1969; Reichardt, 1973; Beutel, 1999a).

Myxophaga as a whole are moderately well supported. Shared derived features of adults are the far-reaching reduction or absence of the lacinia (vestigial in Sphaerius) and widely separated mesocoxae. Largely concealed adult paired mouthparts, ligular papillae in adults and immatures, cephalic glands, and hind wings with a fringe of specialized microtrichia are probably additional apomorphies. A recent study based on molecular data (McKenna et al., 2015) suggested a pattern with Sphaeriidae as sister group of Lepiceridae, both groups as closest relatives of Torridincolidae, and consequently a placement of Hydrosaphididae as sister taxon of the remaining suborder. This would imply independent acquisition of several unusual features in Sphaerius and Hydrosaphididae: semi-entognathous larval mouthparts, balloon-shaped larva spiracular gills, and rows of lanceolate tergal setae. This appears highly unlikely, like the alternative option, secondary modification or loss in Lepicerus and Torridincolidae. This scenario would also imply a series of reversals in Lepiceridae, for instance a re-acquisition of a transverse ridge of the metaventrite, a secondary enlargement of the apical maxillary and labial palpomeres, and the loss of larval spiracular gills and tergal duplicatures. As a whole, the phylogenetic scenario suggested by molecular data (McKenna et al., 2015) would require 21 additional steps with the data analysed here, and obviously imply an unlikely pattern of character evolution.

Feeding apparatus
Direct observations on the feeding process of Sphaerius are not available, but detailed field notes (R. Arce-Pérez) suggest that at least some of them are algophagous (Beutel & Arce-Pérez, 2016). This is compatible with a preference for moist substrates at river edges, even though some species also occur in much drier habitats (e.g., Endrödy-Younga, 1977a; Beutel & Arce-Pérez, 2016). It appears likely that various rotting plant materials also belong to the food spectrum of the family.

The feeding apparatus of Sphaerius is similar to that of Lepiceridae (Anton & Beutel, 2005) in its general configuration (Figs 4A-C, 6), but also to that of polyphagous groups with saprophagous or sporophagous feeding habits, notably in the Scirtoidea (Anton et al., 2016) and Staphyliniformia (e.g., Anton & Beutel, 2004; Antunes-Carvalho et al., 2017; Yavorskaya
et al., 2017). An arrangement with mandibles with a large grinding mola, a longitudinal ridge (or process) of the epipharynx more or less densely set with microtrichia (Anton & Beutel, 2006: lep), and a similar corresponding structure of the hypopharynx (e.g., Anton & Beutel 2006: hlp) is very likely a groundplan feature of Polyphaga, and arguably also of Coleoptera, if polyphagans are confirmed as sistergroup of the other suborders (e.g. Kukalová-Peck & Lawrence, 1993, 2004; McKenna et al., 2015).

This potentially ancestral feeding apparatus is found in Lepiceridae (Anton & Beutel, 2006) and with some variation also in Sphaerulusidae. In contrast to the typical condition, the epipharyngeal longitudinal ridge of *Sphaerius* is rather low (Fig. 6A) and the microtrichia short. The hypopharyngeal longitudinal process is indistinct (Fig. 6A) and posteriorly continuous with a median longitudinal rim, which interacts with the distal mandibular cutting edge.

An unusual feature linked with the feeding apparatus has apparently evolved independently in Sphaerulusidae, in the staphylinid families Hydraenidae and Ptiliidae, and in some Scyphidaeinae specialized on orbilid mites, a locking device connecting the labrum and the mandibles in resting position. The mandibular part is formed by a ridge in *Sphaerius* and by a process in Hydraenidae and Ptiliidae, with a matching labral concavity present in both cases. In the scyphidaeinae species a mandibular process forms a concavity which fits with the lateral labral edge (Jaloszynski, P. & Olszanowski 2016). The grinding mola of *Sphaerius* (Fig. 6B) is enclosed in the preoral cavity and firmly connected with the main body of the mandible, as it is the case in most other groups of beetles where it is present. In contrast, it is separated from the lateral and apical parts of the mandible by a membranous zone of weakness in Hydraenidae and Ptiliidae, a presumptive synapomorphy of the two families. Like in other microphagous or saprophagous beetles, the mola of *Sphaerius* is used for grinding the food substrate in interaction with the longitudinal epipharyngeal ridge, after it was processed between the distal cutting edges of the mandibles and the median hypopharyngeal rim.

The maxillary endite lobes of *Sphaerius* and other myxophagans differ distinctly from those of fungivorous or saprophagous staphyliniform beetles (e.g. Beutel et al., 2003; Anton & Beutel, 2004; Beutel & Leschen, 2005; Antunes-Carvalho et al., 2017). The lacinia is largely reduced in *Sphaerius* and missing as a defined structure in the other myxophagan groups (Reichardt, 1973; Beutel, 1999; Anton & Beutel, 2006). In Myxophaga the galea always lacks regular rows of curved microtrichia (fimbriate condition), which are used for collecting food particles in Hydrophilidae, Hydraenidae, Ptiliidae and other groups of Staphylinoformia (Anton & Beutel, 2004; Beutel et al., 2003; Antunes-Carvalho et al., 2017; Yavorskaya et al., 2017). A noteworthy evolutionary parallel with *Ptiliidae* is the configuration of the maxillary palp, with an enlarged penultimate palpomere, and a narrow, peg-like apical
segment (Fig. 3A). The apical palpomere is used by adult ptillids to gather small food particles (e.g. spores) with rapid movements (M. Yavorskaya, pers. ob.). Whether this also applies to Sphearius is questionable. The apical palpomere is shorter, and rather flat apically, with a larger conical sensillum and several very tiny structures. It appears likely that it is mainly or exclusively used as a sensorial organ.

**Miniaturization**

Insects, which include some of the smallest multicellular animals, are likely the most complex of all miniature organisms (Poliiov, 2016). Therefore, they are obviously crucial for the study of effects of extreme size reduction. The order Coleoptera is of special interest in this context, as it includes the smallest free-living insects (Poliiov, 2015). A considerable number of studies have been published on effects of miniaturization in beetles. However, almost all of them exclusively deal with representatives of the suborder Polyphaga (Grebennikov & Beutel, 2002; Poliiov, 2005, 2008; Poliiov & Beutel, 2009, 2010; Makarova & Poliiov, 2013; Yavorskaya et al., 2014, 2017; Yavorskaya & Poliiov, 2016). In contrast, in the suborder Myxophaga, which also includes some very small species, only the larval head structures of Hydroscapha natans have been discussed in this context (Beutel & Haas, 1998). With a body length ranging between 0.5 and 1.2 mm (Fig. 2), species of Sphaeridae are on average slightly larger than those of Ptillidae (Polyphaga, Staphylinoidae), and comparable in size to Corylophidae (Polyphaga, Cucujoidea).

As it generally the case in adult beetles, the head capsule of Sphearius lacks sutures in the sense of ecdysial lines. However, in contrast to the typical condition in Coleoptera, externally recognizable ridges are also missing (Fig. 3B), even though the clypeofrontal ridge is distinctly developed as internal structure, and gular ridges are also discernible internally. Simplifications of the cephalic skeleton including an almost complete lack of distinctly developed ridges has been observed in small beetles of different families, especially in the strongly miniaturized Ptillidae (Poliiov & Beutel, 2009; Poliiov, 2016; Yavorskaya et al., 2017), but also in Corylophidae (Poliiov & Beutel, 2010; Yavorskaya & Poliiov, 2016) and Clambidae (Anton et al., 2016). Similar conditions also occur in microinsects belonging to other groups (Poliiov, 2016).

The tentorium of Sphearius Fig. 4 D, F, 6D) comprises all principal elements typical for Coleoptera except for the laminatentorium. In contrast, it is strongly simplified in some other miniaturized coleopterans: the dorsal arm is missing in many ptillids (Poliiov, 2016; Yavorskaya et al., 2017) and both the dorsal and anterior arms absent in some examined species of Corylophidae (Poliiov & Beutel, 2010; Yavorskaya & Poliiov, 2016). Simplifications of the tentorium were also observed in miniaturized adult hymenopterans (Poliiov, 2017). It is completely reduced in corylophid species of the genus Orthoperus Stephens, 1829, in the
moderately small archostematan species *Micromalthus debilis* (*Micromalthidae*) (ca. 2 mm), and also in the extremely small (average length ca. 200 μm) primary larvae of Strepsiptera (Knauthe et al., 2016).

Like in adults (and immatures) of other groups of beetles with small species, for instance in Hydraenidae and Ptiliidae (e.g. Yavorskaya et al., 2017), the mandibles of *Sphaerius* are largely concealed in their resting position (Fig. 3B), mainly covered by the labrum. This feature is even more advanced in larvae of Sphaeriidae and Hydroscaphidae: the basolateral part of the large labrum is connected with a genal fold, thus forming a condition referred to as semi-entognathous (Beutel & Haas, 1998; Beutel et al., 1999). This feature is arguably linked with microphagous feeding habits in beetles (see above), but very likely also with size reduction. The mandibles are also largely internalized in the extremely small primary larvae of Strepsiptera (Knauthe et al., 2016).

Another feature commonly found in miniaturized insects is an increased relative size of the cephalic elements of the central nervous system (Fig. 5), and a shift of the brain and suboesophageal ganglion into the posteriormost head region or even into the prothorax. A dislocation of these structures into the thorax is quite common in small larvae, likely linked with a limited movability of the larval head (e.g. Grebennikov & Beutel, 2002; Beutel & Hörnschemeyer, 2002). An extreme condition is found in first instars of Strepsiptera, where both structures are located in the middle body region and fused with the other elements of the central nervous system (Beutel et al., 2005). In *Sphaerius*, a considerable part of the posteriorly inclined protocerebrum reaches into the prothorax (Fig. 4A), a rare feature in adult beetles. In Ptiliidae, for instance, most of the brain is still located in the cephalic lumen, with only a small portion of it reaching the anterior prothorax (Makarova & Poliiov, 2013). As far as known at present, Corylophidae is the only group where the entire adult brain is shifted into the thorax (Poliiov & Beutel, 2010; Yavorskaya & Poliiov, 2016).

In contrast to miniaturized beetles of other families and other very small insects (Poliiov, 2016), and also in contrast to other groups of Myxophaga (e.g. Reichardt, 1973; Beutel, 1999a), a full number of eleven antennomers is preserved in *Sphaerius*. Likewise, the musculature of the head is apparently not distinctly affected (Figs 4, 6). In contrast to other groups of Myxophaga with larger species (e.g. Anton & Beutel, 2006), a strongly developed bipartite external labral retractor (M. fronto-epipharyngalis, M9) is present (Fig. 4A, E: 9/1, 9/2). The head appendages and the digestive tract are equipped with the normal set of muscles. The extrinsic antennal muscles are strongly developed. The unusual areas of origin on the head capsule are probably due to the large size of the extrinsic muscles, exceeding the available space on the tentorium. Apparently, the cephalic muscle system is rarely affected by reduction, as investigations of different groups have shown. This includes Ptiliidae (Poliiov & Beutel, 2005; Poliiov, 2016; Yavorskaya et al., 2016), Corylophidae
also groups outside of Coleoptera (Polilov, 2016). However, the head of the extremely small first instars of Strepsiptera forms a noteworthy exception: only nine cephalic muscles are preserved in the primary larva of Styllops (Knaus et al., 2016). In contrast to this, the muscular complexity of the thorax is scarcely affected by the extreme miniaturization (Osswald et al., 2009).

The morphological data presented in this study show that species of Sphaerius are less affected by miniaturization than other beetles of the same size group. Moreover, they are less modified in different cephalic features than the larger species of other groups of Myxophaga. Miniaturization in insects can result in a distinct reorganization of almost all organ systems, including even the complete disappearance of some organs (reviewed in Polilov, 2015, 2016). However, structures of the head are apparently relatively conservative, due to essential functions related to sense organs, the feeding apparatus, and the cephalic central nervous system. A far-reaching reorganization of the head does apparently not take place in the majority of microinsects including Sphaerius. However, a high degree of simplification and structural transformation is found in the head of extremely small and endoparasitic larvae of Strepsiptera (Beutel et al., 2005; Knaus et al., 2016).

Acknowledgements

We are very grateful to Martin Fikaček for the photo of Satonius used in the tree. A DAAD grant to MIY is gratefully acknowledged. The work of AAP has been supported by the Russian Science Foundation (project no. 14-50-00029).

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Electronic appendage 1: Appendix 1. Matrix_Myxophaga.nex

Electronic appendage 2: Appendix 2. Consensus tree (with all apomorphies mapped on tree, empty circles homoplasious apomorphies)
Fig. 1. Dorsal view of *Sphaerius acaroides*, habitus, microphotography.

106x140mm (300 x 300 DPI)
Fig. 2. Lateral view of *S. acaroides*, scanning electron micrograph. Scale bar: 200 µm.

52x34mm (300 x 300 DPI)
Fig. 3. S. acaroides, head, scanning electron micrographs. (A) ventral view, (B) flat lateral view, (C) flat anterior part of labrum. Abbreviations: ca, cardo; ey, eye; lb, labrum; ld, labial gland; md, mandible; mt, mentum; mx, maxillary gland; ph, pharynx; pgh, prepharynx; pl, labial palp; pp, premental process; pta, posterior tentorial arm; saes, suboesophageal ganglion; tb, tentorial bridge; 1, M. tentorioscapalis anterior; 2, M. tentorioscapalis posterior; 4, M. tentorioscapalis medialis; 7, M. labroepipharyngalis; 9, M. frontoepipharyngalis; 11, M. craniomandibularis internus; 12, M. craniomandibularis externus; 14, M. zygomaticus mandibulae; 15, M. craniocardinalis externus; 17, M. tentorioscardinalis; 18, M. tentoriostipitalis; 19, M. craniolacinalis; 28, M. submentopraementialis; 29, M. tentoriorostralalis; 41, M. frontohypopharyngalis; 43, M. clypeoepistomalis; 44, M. clypeobuccalis; 45, M. frontobuccalis ant.; 46, M. frontobuccalis post.; 48, M. tentoriobuccalis ant.; 50, M. tentoriobuccalis post.; 52, M. tentoriopharyngalis.
Fig. 5. Brain and suboesophageal ganglion of Sphaerius acaroides, 3D reconstructions. (A) Frontal view, (B) lateral view, (C) posterior view. Abbreviations: ane, antennal nerve; dcer, deuterocerebrum; olob, optic lobe; pcer, protocerebrum; soes, suboesophageal ganglion; tcer, tritocerebrum; trcom, tritocerebral commissure.
Fig. 6. Cross sections of head and prothorax of S. acaroides, position of sections indicated in the drawing. Abbreviations: am3, antennomere 3; bsc, base of scapus; dta, dorsal tentorial arms; epi, epipharynx; eye, eye; fg, frontal ganglion; ga, galea; hyp, hypopharynx; ia, intercalary antennomere; lc, lacinia; lgl, labial gland; md, mandible; lpta – lateral process of posterior tentorial arm; mo, mola; mt, mentum; mx, maxilla; mxg, maxillary gland; ped, pedicellus; ph, pharynx; pnt, pronotum; pp, premental process; psh, prepharynx; pt, posterior tentorial arm; lep, longitudinal epipharyngeal process; lhp, longitudinal hypopharyngeal process; sees, suboesophageal ganglion; sti, stipes; tb, tentorial bridge; tor1, first pair of tormae; tor2, second pair of tormae; 1, M. tentrioroscapsis anterior; 2, M. tentrioroscapsis post.; 4, M. tentrioroscapsis med.; 9, M. frontoepipharyngalis; 11, M. craniomandibularis int.; 12, M. craniomandibularis ext.; 15, M. craniocardinalis ext.; 17, M. tentriorocardinalis; 19, M. tentriorostipatalis; 28, M. submentopraementialis; 29, M. tentrioropraementialis; 41, M. frontolypharyngalis; 43, M. clypeopalatalis; 44, M. clypeobuccalis; 46, M. frontobuccalis post.; 48, M. tentriorobuccalis ant.; 50, M. tentriorobuccalis post.. Scale bar: 50 µm.

101x61mm (300 x 300 DPI)
Fig. 7. Phylogeny based on parsimony analysis (NONA) of 98 characters of adults and larvae. Branch support values (Bremer support) below branches, Apomorphies see Results of the cladistic analysis.
3.4 Study IV

Anton E, Yavorskaya MI, Beutel RG (2016)


Abstract: External and internal structures of the head of adults of *Clambus* are described and illustrated in detail. The results are compared with structural features found in the clambid genus Calyptomerus, in representatives of other scirtoid families, and also in species of other coleopteran suborders, notably Myxophaga. The results tentatively support the monophyly of Scirtoidea and a close relationship between Clambidae and Eucinetidae is suggested by one shared derived feature of the mandible, a long and slender apical tooth with a serrate edge. The monophyly of Clambidae is very strongly supported and Acalyptomerus is probably the sistergroup of a clade *Calyptomerus* + *Clambinae*. Potential scirtoid autapomorphies are the loss of the dorsal tentorial arms, a bulging gula, a strongly transverse labrum, and a ridge separating the mediostipes from the lacinia. However, all these features are homoplasious. The monophyly of Clambidae is supported by modifications of the head capsule which is strongly flattened and broadened, by a deep clypeofrontal incision enabling vertical antennal movements, and a series of antennal features. Synapomorphies of Clambinae + *Calyptomerus* (Clambidae excluding *Acalyptomerus*) are the conglobate body form with the ventral side of the head capsule in contact with the mesocoxae, and compound eyes integrated in the contour of the head. The completely subdivided eye is an autapomorphy of *Clambus*. An entire series of features is shared by Clambidae (or Scirtoidea) and Myxophaga. Most of them are apomorphies that apparently evolved independently in both groups. However, the presence of well-developed maxillary and labial glands is arguably a retained groundplan feature of Coleoptera, with parallel loss in Archostemata, Adephaga and various groups of Polyphaga.

Significance in the present thesis: Scirtoidea, probably the most basal branch in Polyphaga, have arguably preserved the most ancestral feeding apparatus of all groups of beetles. Clambidae are similar in body size and feeding preferences to Ptiliidae. Therefore, a detailed knowledge of their cephalic anatomy is important for understanding the evolution of the feeding apparatus in Coleoptera.

Own contribution: 50 %
The Head Morphology of Clambidae and its Implications for the Phylogeny of Scirtoidea (Coleoptera: Polyphaga)

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ABSTRACT External and internal structures of the head of adults of Clambidae are described and illustrated in detail. The results are compared with structural features found in the cladid genus Calyptopeterus, in representatives of other scirtoid families, and also in species of other coleopteran suborders, notably Myxophaga. The results tentatively support the monophyly of Clambidae and a close relationship between Clambidae and Eucinetidae is suggested by one shared derived feature of the mandible, a long and slender apical tooth with a serrate edge. The monophyly of Clambidae is very strongly supported and Acalyptopeterus is probably the sistergroup of a clade Calyptopeterus + Clambidae. Potential scirtoid autapomorphies are the loss of the dorsal tergital arms, a bulging gula, a strongly transverse labrum, and a ridge separating the mediostipes from the lacina. However, all these features are homoplasious. The monophyly of Clambidae is supported by modifications of the head capsule which is strongly flattened and broadened, by a deep clypeofrontal incision enabling vertical antennal movements, and a series of antennal features. Synapomorphies of Clambidae-Calyptopeterus (Clambidae excluding Acalyptopeterus) are the conglutate body form with the ventral side of the head capsule in contact with the mesoscutum, and compound eyes integrated in the contour of the head. The completely subdivided eye is an autapomorphy of Clambus. An entire series of features is shared by Clambidae (or Scirtoidea) and Myxophaga. Most of them are apomorphies that apparently evolved independently in both groups. However, the presence of well-developed maxillary and labial glands is arguably a retained groundplan feature of Coleoptera, with parallel loss in Anostomatida, Adophaga and various groups of Polyphaga. J. Morphol. 277:615–633, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: Clambidae; Scirtoidea; head; morphology; phylogeny

INTRODUCTION

Clambidae are a small family of polyphagan beetles including five genera with about 150 described species and a world-wide distribution (Endrödy-Younga, 1995; J.L. Leschen, in press). Today the monophyly is generally accepted (e.g., Lawrence and Newton, 1995; J.L. Leschen, in press) even though Crowson (1955) erected a separate family for the genus Calyptopeterus Redtenbacher, 1849 (4 spp.) and included it in the suborder Myxophaga.

The biology of Clambidae, especially taxa outside of Europe, is insufficiently known (Crowson and Crowson, 1955; Johnson, 1966, 1992; Endrödy-Younga, 1995, 1998). Adults are usually collected from decaying vegetation, leaf litter and rotten wood, and sometimes flying at dusk (Young, 2002). At least some species feed on microfungi and this is possibly ancestral for the family. However, clambid species may also be found on larger fruiting bodies of Ascomycetes, Myxomycetes, and Basidiomycetes where they feed mainly on spores and hyphae (e.g., Lawrence and Newton, 1980; Lawrence, 1991; Wheeler and Hoebéke, 1984; Leschen, in press).

Clambidae were once considered as staphylinoid beetles and included in a broadly defined Silphidae (LeConte, 1861; see Leschen, in press) or close to Leiidiidae (Hatch, 1929; Endrödy-Younga, 1959). Crowson (1955, 1960, 1979) placed the family in Eucinetidae (= Scirtoidea; see Lawrence and Newton, 1995; J.L. Leschen, in press) based on morphological characters of adults and immatures (e.g., Crowson and Crowson, 1955; Hlavac, 1975; see also Lawrence and Newton, 1982; Beutel and Leschen, 2005; Leschen, in press). A placement in this superfamily (and series Sciriformia) together with Declinidae, Eucinetidae, and Sciridae (and possibly Derodontidae) is widely accepted today.
(e.g., Nikitey et al., 1993; Lawrence et al., 1996; Leschen, in press), even though the monophyly, composition and internal relationship of this group are not clarified (McKenna et al., 2015).

The discovery that Scirtoida (or Scirtiformia) are the most basal lineage in Polyphaga (Hunt et al., 2007; McKenna and Farrell, 2009; Beutel et al., 2014; McKenna et al., 2015) was a major breakthrough in beetle systematics. Nevertheless, the morphology is still very insufficiently known. A comparative study of the pterothorax (Friedrich and Beutel, 2006) is the only contribution with a detailed treatment including endoskeleton, muscles, and other softparts. A better knowledge of the anatomy of larvae and adults could be crucial for the understanding of the early evolution of Polyphaga. Consequently, a major aim of the present study is to narrow the gap in the morphological knowledge of Clambidae and Scirtoida. We chose the head as this has turned out as a rich source of phylogenetic information (e.g., Dressler and Beutel, 2010; Beutel et al., 2011; Lawrence et al., 2011: 91 cephalic characters). Moreover, the head with its concentration of sensorial structures, main elements of the central nervous system, and mouthparts reflects important aspects of the lifestyle of coleopteran species and insects in general. The morphological findings are documented in detail using a combination of traditional and innovative morphological techniques. They are discussed with respect to their phylogenetic implications for Clambidae and Scirtoida as a whole, and also with respect to functional aspects and miniaturization.

MATERIAL AND METHODS
Examined Taxa
Polyphaga, Scirtoida: Clambidae: Clambus nigricollis Steph., 1855 (cross sections "CS"); Calyptocephalidae dubius Mayr, 1802; Scirtidae: Cyphon coeruleus Paykull, 1799 (CS); Elodes pseudoancistroklamprobus (Dark, 1971; Ors sp.; Pseudomicrotoma sp.; Eucinetidae: Euchinesis haemorrhoidalis Germ., 1818
Derronolidae: Derodonatidae; Derodonatous maculatus Melsheimer, 1844 (CS)
Bauchrodenia: Dermonotidae: Dermonotus kaniarius Illiger, 1801 (CS)
Byrhididae: Byrhididae: Lampropylebyrhulcas nitidus (Schaller, 1783) (CS); Dryopidae: Dryops acrisylacculitus (Geoffroy, 1765) (CS); Elmididae: Elmis ornata (Muller, 1806) (CS); Heterocercidae: Heterocerus fenestristus (Tronche, 1784) (CS); Pulicolaegidae: Pulicolactyloides sp.

coroteridae: Electeridae: Atthis subsulcata (Muller, 1777)
Thomaeidae: Triasus dermcephaloides (Linsenmair, 1767) (CS); Lycidae: Lycidophorus sanguineus (Linsenmair, 1768) (CS); Oronalidae: Oronalis festibellavigna Troncho, 1785

Bythrobidae: Bythribidae: Anthaxia nitiduloides (Linsenmair, 1758) (CS)
Myxophagidae: Lepicera inquinata (Sjörgen, 1852) (CS); Hydraenidae: Hymenophora latest (Linné, 1758) (CS); Sphaeridiidae: Sphaeridius sp. (CS); Torridobatidae: Satorius kurowshii (Sito, 1969) (CS); Vutus successfully reared, 1979

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Fig. 1. Clambus nigricollis System, 1855, ventral view; (B) Calystocephalus dubius male, 1802, ventral view.

The taxonomic nomenclature used in this study follows Beutel and Leschen (2005) if not indicated otherwise. Cross section series and dissected specimens (SEM) are deposited in the Phylletisches Museum, Institut für Spezielle Zoologie und Evolutionistische, FSU Jena. Additional voucher specimens are stored in the collection of the first author.

Anatomy
One specimen of Clambus Fischer von Waldheim, 1822 was embedded in araldite CY 2128 (Agar Scient, W.e., Starned/Essex, England) and cut at 1 μm using a microtome RM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. The sections were stained with toluidine blue and pyronin G (Walex GmbH and Co.KG/Division Hema, Münster, Germany). Pictures were taken of every second section using a light microscope (Zeiss Axioskop, Germany) equipped with a camera (PikeLink Capture OEM). The images were aligned using Adobe 5.3.1 software (Vlange Imaging, Berlin, Germany). The raw surfaces were converted and scaled with Transform3D software (freeware, Heiko Stark, FSU Jena, Germany; URL: http://starkeins.de). Afterward, Autodesk MAYA 2013 (Alias Wavefront, Toronto/Ontario, Canada) was used for smoothing and coloring.

Scanning electron microscopy (SEM, Philips XL, 30 ESEM) was used to document surface structures of Clambus and Calyptocephalidae. Specimens were dried at the critical point (Emitech K550 critical point dryer), sputter-coated with gold (Emitech K500) and mounted on the tip of a fine needle and fixed on a rotating specimen holder (Pohl, 2010).

The terminology used for the musculature is based on v. Kéler (1963) but muscle designations of the recently introduced system of Wipfler et al. (2011) are given in brackets.

RESULTS
Clambus nigricollis
External features of the head capsule. The head is prognathous, strongly flattened and disc shaped, and wider than long (~800 μm width, ~360 μm length, ~160 μm maximum height); in a strongly deflected position (Fig. 1A) it fits with the anterior side of the mesocoxae, thus forming part of the defensive behavior of rolling into a ball.
The dorsoventral side and 8 mm fly, and a film of "Punktkäfer", also known as conglobation. The outline of the head capsule is semicircular in dorsal view (Fig. 1A) with a very deep incision between the frons and clypeus; in lateral view it appears wedge-shaped, strongly narrowing anteriorly, with an almost even ventral side and an evenly rounded dorsal surface (Fig. 2C). The coloration is mostly dark brown to blackish, with brown to orange areas along the edges of the clypeus and frons. The cuticle is distinctly sclerotized, with an average thickness of ca 15 μm on the dorsal side and 8 μm ventrally, and an almost equal thickness of the endocuticular and exocuticular layers; the surface is shiny and smooth dorsally but evenly rugose on the ventral side; most areas are glabrous; a thin but regular vestiture of long and erect setae is present on the clypeus and anterior frontal region (Fig. 1A).

The clypeal region is broad and elongate and not distinctly separated from the frons (Fig. 1A); the border between both areas is indicated by the origin of muscles (M43/44 on clypeus; M9/41/45 on frons; Fig. 5A) and a deep and narrow lateral incision which allows dorsal movements of the antenna; the clypeal surface is evenly curved in transverse and longitudinal direction; the anterior and lateral clypeal edges are sharp and form an extensive shield-like projection covering the labrum; the lateral clypeal margin is much longer than the lateral margin of the frons anterior to the eye; the lateral and anterior clypeal margins are evenly curved, forming approximately a quarter sector of a circle. The ventral clypeal surface is continuous with the anterior genal region which is very extensive and concave. The frons is evenly convex like the clypeus; its lateral edge forms a narrow epicanthus completely dividing the compound eye into a dorsal and ventral portion; the posterior region is continuous with the vertex (Fig. 2A,C); the posterior vertex is convex, without a neck-like constriction; a shallow transverse furrow is present posterior to the compound eyes; its rather indistinct but recognizable anterior border originates from the posterior ocular corner, suggesting its homology with the postocular ridge of other beetles (e.g., Anton and Beutel, 2012). The very short occipital region is dorsally separated from the vertex by a distinct furrow which is continuing along the posterior part of the ocular epicanthus; the posteriorly directed surface of the occiput is very distinctly separated from adjacent dorsal and ventral areas of the head by sharp ridges; in its dorsal half it is nearly vertical; in its ventral part it is concave, oblique, and anteromedially oriented; the occipital surface is smooth and glabrous; the occipital foramen is approximately rectangular, ca 1.5 times as broad as high; its ventral edge is completely formed by the postocular margin.

The genal region occupies a large part of the ventral head capsule; its surface is more or less even; posterolaterally, behind the compound eyes, its sides form nearly rectangular corners distinctly protruding from the outline of the head capsule in dorsal and ventral view; the posterior surface of the corners is adjacent with the occiput (Fig. 2A). The paramaxillare (i.e., the part of gena bordering the maxillae laterally, reaching from caudal edge
of maxillary cleft to articulation area for ventral mandibular condyle) are long and laterally adjacent with the maxillary corpus over most of its length (Fig. 2A). A narrow antennal furrow is very distinctly visible on the ventral side; it extends straight but obliquely toward the posterior corners of the gula; in the resting position of the antenna it receives the scapus, pedicellus, and two elongated proximal flagellomeres (Fig. 2A, C).

The well-developed trapezoid gula is approximately as long as broad at its base, and slightly narrowing anteriorly, due to a transverse bulge its posterior part distinctly protrudes from the contour of the adjacent genal and occipital areas; the gular surface is mostly rugose and uneven, with a pair of larger dints near the posterior border, separated from each other by a shallow median keel (Fig. 2A). The gula and submentum are separated by a straight transverse ridge.

The compound eyes are small and only slightly convex, almost completely embedded in the contour of the head capsule; they are completely separated by the frontal epicranium (see above) (Fig. 2A, C); the ventral part is smaller than its dorsal counterpart; the shape of both is broadly oval to rounded triangular; the number of the ocelli omatidia is relatively low (~40 omatidia in upper part; ~16 in lower part), with a diameter of the lens of ca. 16 μm and a length of ca. 40 μm (see also Covencey, 1986).

Endoskeleton. The long gular ridges are distinctly recognizable externally; corresponding internal ridges are missing, except for the region directly connected with the posterior tentorial arms (see below) and the posteriormost parts, which form a pair of strongly developed vertical carinae bordering the foramen occipitale laterally. The base of the posterior tentorial arms emerging from the gular ridges is very short; it is located at the level of the posterior border of the ocular epicranium; the rod-like distal parts of the posterior arms converge dorsally where they connect with the tentorial bridge, which is straight, thin and strut-like (Figs. 3A, C, 5B). Horizontal rod-like longitudinal arms originate from the connecting area; anteriorly they fuse medially thus forming a solid, plate-like laminatentorium (Fig. 3C). The vertically flattened broad anterior tentorial arms are attached to the anterolateral corners of the laminatentorium; they originate from the head capsule near the dorsal mandibular condyle; the anterior tentorial groove is not recognizable externally. Dorsal tentorial arms are absent (Fig. 3C).

Labrum. The labrum is enclosed in a cavity between the ventral clypeal surface and the dorsal surface of the mandible; dorsally it is completely covered by the clypeus and not visible from above (Figs. 1A, 5A). It is well developed, movably connected with the anterior clypeal margin by a membrane, and broad and short, ca. 2.5 times as wide...
as long; its anterior border is shallowly convex, with nearly straight edges; the lateral corners are rounded; the labrum is very slightly constricted posterior to the lateral corners. The torulae originating from the postcervical labral edges are very strongly developed (Supporting Information Fig. 1A); they are long and rod-like and slightly diverging in posterior direction; their apical part serves as attachment area of the tritocerebral retractor (M9); a pair of sclerotized transverse processes near mid-length supports the longitudinal epipharyngeal process (see below). The dorsal surface of the labrum is even and bears a more or less regular transverse row of setae; a transverse microreticulation is recognizable on the cuticular surface. Few short, spine-like sensilla are inserted on the anterior edge of the labrum.

Musculature (Figs. 3D,5,6): M7-M. labroepipharyngyal (0bb5 of Wipfler et al., 2011), epipharyngeal levator, pair of single bundles, closely adjacent on their origin, strongly diverging toward insertion, Origin (O): medially on posterior labrum, Insertion (I): lateral corners of epipharyngeal process; M9-M. frontoepipharyngyal (0bb2), retractor of labrum, O: posterior frons, anterior to brain, lateral of M9, I: anterior insertion on apex of tormatanterum.

Antennae. The 10-segmented antennae (Figs. 2A,C,3A; Supporting Information Fig. 1C–F) are short compared with the head length; distally they bear a distinct two-segmented club. The surface structure of antennomeres 1-4 is mostly smooth, whereas a very fine longitudinal microreticulation is present on all following segments. The scapus is approximately as long as broad, with one side flattened and the other convex; the external side is distally extended, forming a strongly developed and bluntly rounded process which conceals the scapo-pedicellar condyle; the basal condyle of the scapus is not distinctly separated from the rest of the segment; a deep proximal concavity fits with the antennifer. The pedicel is short and slightly broader than long; similar in its shape to the scapus, with one side flattened and the other convex; it is partly covered by the distal scapal process; the pedicellar base bears a strong, almost hook-shaped, posteriorly directed process which intersects with the distal process of the scapus; few long and scattered setae are inserted on the surface of the scapus and pedicellus. Flagellomeres 1-6 decrease in length and slightly increase in width toward the antennal apex; the distinctly elongated basal flagellomere is ca. 6 times as long as broad, nearly cylindrical, it is slightly curved in its proximal half, whereas the distal half is straight; a distinct constriction is recognizable near its insertion on the pedicellus; flagellomere 2 is cylindrical, ca. one-third shorter than flagellomere 1, and 3.5 times as long as broad; both proximal flagellomeres are placed in the antennal furrow on the ventral side of the head in their resting position; flagellomeres 3-6 are of nearly equal length, and ca. 0.4 times as long as flagellomere 1; flagellomeres 3-5 are ca. twice as long as broad; flagellomere 3 subcylindrical, and 4 and 5 slightly rounded on their mesal side but straight externally; flagellomere 6 is slightly longer than broad and ovoid. The flagellomeres bear a sparse vestiture of long setae, mainly inserted near the middle region, forming a complete whirl to flagellomere 6. The distinct antennal club is compact, elongate oval, flattened, and composed of flagellomeres 7 and 8; both are almost equally sized; the proximal one is broader but slightly shorter than the distal one; the broad sides are almost free of setae, whereas a dense vestiture of long setae is present on the narrow sides from the distal half of the first segment; additionally several long and curved peg-like sensilla are present and some dense sensorial grooves arranged in a more or less regular longitudinal row (Supporting Information Fig. 1E,F).

Musculature (Fig. 3A): M1-M. tentoriocapitellar anterior (0an1), two subcomponents, M1/1 relatively small, O: laterally on middle region of anterior tentorial arms, posteral of origin of M2, ventral origin of M3, I: anterior insertion on apex of tormatanterum; M1/1 very large, second largest head muscle; O: ventrolateral surface of proximal half of anterior tentorial arms, dorsal surface of laminatentorium and lateral surface of longitudinal tentorial arms, posteriorly reaching level of tentorial bridge, I: together with M1/1; M2-M. tentoriocapitellar posterior (0an2), relatively large, O: broadly on lateral surface of distal half of anterior arms, I: anterodorsally on scapal base, M4-M. tentoriocapitellar medialis (0an4), very thin, O: lateral surface of middle region of anterior arms, enclosed by origins of M1/1 (ventrally), M1/2 (posteroventrally) and M2 (anterodorsally), I: posterodorsally on scapal base, behind insertion of M2; M5/M6/Mm. scapopedicellares lateralis/medialis (0an6/0an7), O: extensive areas of proximal part of scapus, I: basal pedicellar condyle.

The compressor muscle of the antennal heart (M. frontoventralis, 0ah6; Wipfler et al., 2011) (see also Pass, 1980, 2000) is present but weakly developed. It is a flat muscle originating on the frons on the level of the anterior region of the compound eyes, lateral the origin of M9. It inserts on the ventral wall of the head capsule on the deepest point of the antennal furrow with a very thin and long tendon, which extends along the anterior surface of the protocerebrum.

Mandibles. The asymmetrical mandibles (Figs. 3C,4A,B) are slightly longer than broad, with a short and compact corpus with an even and glabrous dorsal and ventral surface; the dorsal surface is laterally bordered by a very distinct lamelliform longitudinal ridge (Supporting Information Fig. 1H), which is closely associated with a
longitudinal edge of the ventral clypeus enclosing the labrum (see above); the ventral surface of the corpus of the left mandible bears a distinct, curved ridge extending from the lateral edge distal the ventral articulation to the base of the apical part of the mandible; on the right mandible the ventral surface of the corpus bears a distinct furrow on its distal part, separating the distal mandibular region and the apical pointed process (see below); laterally the corpus is strongly rounded, with a sharp blade-like edge, distally continuous with a long, narrow and more or less pointed mesally directed process (sharply pointed on the left mandible; relatively blunt on the right one); two setae are inserted near the base of this structure, one dorsally, near the distal end of the longitudinal ridge, and one on its ventral side. The mesally directed apical part of the mandible is very distinctly detached from the corpus; it is long and slender, ca. 3 times as long as broad; on the left mandible its inner edge is strongly serrate over the entire length; the apex is flattened, oriented perpendicular to the longitudinal mandibular axis, and subdivided into multiple minute teeth; the inner edge of the apical part of the right mandible is smooth, but otherwise similar to its counterpart on the left side. The straight mesal edge of both mandibles bears a well-developed semimembranous lobe, with densely arranged long microtrichia on its distal half. A strongly developed, sclerotized and movable protheca is mesally inserted on the left mandible (Fig. 4a; Supporting Information Fig. 1l); it is long and slender, ca. 4 times as long as broad, and slightly curved; its tip is multipointed; in its appearance it is very similar to the apical tooth of the right mandible. The nearly symmetrical molae are large, occupying ca. 1/3 of the mandibular length; they are not distinctly separated from the mandibular corpus; a pattern of ca. 10 transverse rows of short microtrichia is present on the dorsal surface, whereas the ventral surface is glabrous; strongly developed asperities on the mesal side form a grading area; the proximal edge bears a dense brush of long, posteriorly directed microtrichia. The dorsal (secondary) mandibular joint is not very distinct; it is represented by a shallow concavity on the proximal lateral corner of the corpus, which is formed by a caudal extension of the dorsal mandibular carina; the ventral (primary) articulation is formed by a mandibular condyle at the external angle of the mandibular base; it is relatively large and bulb-shaped.

Musculature (Figs. 3C,5B): M11-M. craniomandibularis internus (0md1), largest muscle of head, adductor, O: posterolateral part of dorsal head capsule, behind level of compound eyes, laterad the brain (M11 pars verticalis) and lateroventral part of posteriormost head capsule, (M11 pars genalis), I: with very long struit-like adductor tendon below ventral side of M1, inserted on short process posterior to molae; M12-M. craniomandibularis externus (0md3), adducto, O: posterolateral corner of
head capsule behind compound eyes, mesally bordered by M11, 1: laterally on mandibular base with very long tendon.

An additional third mandibular muscle could be observed in Clambus, represented by few thin fibres (Fig. 3C). This muscle extends from the median surface of the anterior tentorial arm to the inner wall of the mandibular corpus. It is likely homologous with M14 (M. zygomaticus mandibulae) of v. Kéler (1963) (see also discussion).

Maxillae. The maxillae are largely exposed (Figs. 2A, 3B). The cardo is slender, with a long basal process with indistinct insertion points for M. craniofrontalis (M15) and M. tentoriofrontalis (M17), respectively; the cardinal surface is smooth and only a single long seta is inserted on it. The basistipes is large, subrectangular, and completely fused with the large and subrectangular pseudopalpi; laterally the combined structure forms a sharp longitudinal edge (Supporting Information Fig. 1L,M); its surface is smooth and largely glabrous, but two long setae are inserted on the mediodistal basistipital region; the smooth medioosteps is long, narrow, and subparallel; it is adjacent with the basistipes over almost its entire length but only very loosely connected to it; a longitudinal carina separates it from the lacina. The lacina is very long and slender and very distinctly projecting beyond the maxillary corpus; its mesal edge is sclerotized and bears a row of long and strong, apically pointed spines; the apical part is more or less acute with few spines inserted on it. The galea is two-segmented; the short basal galeomere is clasp-shaped and appears rectangular in ventral view; the long and slender distal galeomere is slightly curved mesad; some scattered long setae are inserted on its apical region and laterally on the distal half. The maxillary palp is four-segmented; the functional basal palpomere (see Anton and Beutel, 2012) is minute and clasp-shaped; palpomere 2 is large and club-shaped, with a straight inner side, a strongly convex lateral edge, and a distal articulation area almost perpendicular to the longitudinal axis; the subcylindrical palpomere 3 is ca. 2/3 as long as the preceding segment; the distal palpomere is conical, with a pointed apex. The setae of the palp are long and scattered; the ventral surfaces of the palpomeres are smooth, whereas the dorsal side is densely set with short and broad, apically pointed
Musculation (Figs. 3B, 5B): M15-M. craniocardinalis externus (0mx1), promoter of the maxilla, long muscle of moderate size, O: posteroventral head capsule, lateral the gular suture, dorsally bordered by M11, I: with short tendon on laterally directed cardinal process, adjacent to opening of tubular maxillary gland; M17-M. tentoriostitipitalis (0mx3), promoter of the maxilla, consisting of three subcomponents; M17/1-small and thin, positioned anterior to and clearly separated from other two subcomponents, O: ventral laminantentorium, frontally and mesally bordered by origin of M18/2, laterally bordered by origin of M1, I: carpo, near base of stem bearing cardinal processes; M17/2-O: ventral surface of posterior laminantentorium and anterior longitudinal tentorial arm, posterior to origin of M18 and ventrad M1, I: on medially directed process of carpo with short tendon; M17/3-O: lateral surface of posterior tentorial arms, I: same insertion as M17/2; M18-M. tentoriostitipitalis (0mx4), adductor of stipes, also consisting of three subcomponents; M18/1-O: anteroventral surface of laminantentorium, I: ventral base of stipes; M18/2-O: medially on ventral surface of laminantentorium, posterior to origin of M18/1, I: ventral base of stipes, medioposterad of insertion of M18/1; M18/3-O: ventral surface of posterior part of longitudinal tentorial arms, reaching level of tentorial bridge, extending along suboesophageal ganglion, I: same insertion as M18/2; M18-M. craniocardinalis (0mx2), small with long tendon, O: ventral head capsule, beside gular carina, slightly posterior to origin of M15 and anterad origin of M11, parallel to M16, I: with very long and thin tendon, between cardinal processes, inserting on base of labium; M20-M. stipitostitipitalis (0mx8): absent; M21-M. stipitostitipitalis (0mx7), extending longitudinally along basistipes, O: base of basistipes, anterior to insertions of M18, I: ventral wall of galea; M23-M. stipitostitipitalis internus (0mx10), connecting stipes and pseudopalpifer in Coleoptera (for a discussion and exact definition see Anton and Beutel, 2012); absent; M34-M. palpalpalpis maxillae primus (0mx12), extending longitudinally through pseudopalpifer, O: base of pseudopalpifer, I: base of functional palpomere 1; M35/M37-Mm. palpalpalpis tertius/quintus (0mx14/0mx15), O: lateral wall of (functional) palpomere 2 and 3, respectively, I: basal margin of (functional) palpomere 3 and 4, respectively.

Labium. The submentum is large and trapezoidal, nearly two times as broad as long, with its lateral edges straight and diverging anteriorly; its surface is even and shiny; a sparse vestiture of long anteriorly directed setae is arranged in a transverse patch (Fig. 2A). The small trapezoid mentum is fully sclerotized, with straight anteriorly converging lateral edges (Supporting Information Fig. 1G); the cuticle is smooth and largely glabrous, with a transverse row of few long and anteriorly directed setae; the sclerotized high lateral walls are present in the anterior half (Figs. 2A, 3D). The palpigers are well developed, with long posteriorly directed struts, serving as attachment areas for Mm. 29/34 (Supporting Information Fig. 1G); ventrally they are broadly fused with each other, the entire structure appearing U-shaped in cross section. The unpaired ligula is very large and subquadrangular, with rounded anterior corners and a rounded anterior edge (Supporting Information Fig. 1G); posteriorly a short median bridge connects the ligular sclerite with the suspensorium of the palpiger; the ventral surface of the ligula is largely glabrous but a transverse row of few long anteriorly directed setae is present near its anterior border; numerous small granular sensilla and few pairs of short spines are present along its anterior edge; the dorsal surface of the ligula is densely set with short, broad and pointed microtrichia; its lobe-like lateral sides are semimembranous (Fig. 4D). The labial palp is three-segmented (Supporting Information Fig. 1K); palpomere 1 is small and cup-shaped; palpomere 2 long and club-shaped; due to its curved distal half the distal articulatory area is nearly perpendicular to the longitudinal axis; the distal palpomere is slightly shorter than 2, thin, cone-shaped, and apically pointed. The surface of all palpomeres is mostly glabrous; some long setae and shorter spine-like sensilla are present from the distal third of segment 2 (Figs. 2A, 3D).

Musculation (Figs. 3D, 5A): M28-M. submentopraementialis (0la8), paired premental retractor, O: posteroventral corners of submentum, I: medially on posteroventral premental edge; M29-M. tentoriopraementialis inferior (0la5), paired, O: postoral on posterior submentum, I: posterior surface of mesally directed process of palpiger; M30-M. tentoriopraementialis superior: absent in C. lamellatus as well as in all Coleoptera (for a discussion see Anton and Beutel, 2012; see also M42); M33-M. praementialis externus (0la14), O: anterior surface of process of palpiger, anterior to insertion of M29, I: lateral wall of palpomere 1; M35/M37-Mm. palpalpalpis primus/secundus (0la16/0la17): not clearly identified.

Epipharynx. The epipharynx is semimembranous and its anterior part covers the entire ventral side of the labrum (Fig. 3D). Most parts of the anterior epipharyngeal half are smooth and glabrous and lack differentiated surface structures, except for a pair of parasagittal rows formed by short and broad mirotrichia and a distinct field of microtrichia laterad these rows (Supporting Information Fig. 1B); the parasagittal rows are connected with each other anteriorly by a transverse brush of mirotrichia or sensilla and posteriorly by a transverse field of pores, respectively. A
well-developed longitudinal epipharyngeal process (LEP) formed by a dense layer of microtrichia is present along the midline of the posterior part of the epipharynx. The epipharyngeal surface lateral to the LEP is densely set with distinct rows of microtrichia arranged in a ring-like pattern (Supporting Information Fig. 1B); posteriorly the LEP it is densely and regularly covered with microtrichia.

Musculature (Figs. 3A,6): M43-M. clypeolabialis (0c1), a single pair of bundles, O: paramedially on clypeus, diverging toward insertion, I: epipharynx, close to posterior end of epipharyngeal process; M44-M. clypeobuccalis (0b1), rather small, two paired bundles, O: clypeus, posteroventrally the origin of M43, posteroventrally oriented, converging toward insertion, I: paramedially on dorsal wall of prepharynx (cibarium), in front of frontal ganglion.

**Hypopharynx.** The hypopharynx is inconspicuous in its external appearance and anteriorly not distinctly separated from the labium (Figs. 3D,4D,5B); internally it is characterized by a complex configuration of sclerotized structures (Fig. 3D); it is short, broad and rectangular in cross section, and ventrally supported by the largely sclerotized palpigers (see above); the upper surface is semimembranous and slightly folded; medially a short and inconspicuous longitudinal hypopharyngeal process (LHP) is present; it bears a layer of microtrichia and lies between the mandibular molae; the LHP is internally supported by a pair of sclerotized paramedian struts, which are fused with each other anteriorly and form a strongly developed plate-like structure near the posterodorsal surface of the ligula. An isolated short and transverse sclerotized plate is present posterior to the LHP. The lateral wall of the hypopharynx is largely unsclerotized; small, thin lateral sclerites are present posteriorly, arising from a broad and extensive prepharyngeal suspensorium, connecting the hypopharynx with the anatomical mouth angles. The anterior edges of the epipharyngeal suspensorium are connected to the apical part of the labral tormae by long struts.

Musculature (Figs. 3D,5A,6): M41-M. frontohypopharyngalis (0hy1) (*retractor anguli oris*; Snodgrass 1935), levator of hypopharynx and mouth angles, two subcomponents; M41/1-O: frons, mesad M9, anterior to brain, converging toward insertion, I: with short tendon on angles of anatomical mouth and on posterior corners of epipharyngeal suspensorium, thus also connected to hypopharynx; M41/2-O: posterior frons, extending anterad, converging toward insertion, I: same as...
M41/M42-M. tentoriohypopharyngalis (M43-M. cranocephopharyngalis), often misinterpreted as M30 in studies on cephalic morphology of adult or immature beetles (see above) absent.

Prepharynx and pharynx. A closed prepharyngeal tube is present; its ventral side is formed by a well-developed prepharyngeal suspensorium (see above) (Figs. 5, 6). The lumen of the anterior pharynx is wide but it is narrowing toward the occipital region; in cross sections the pharynx appears almost circular; the longitudinal folds for attachment of dorsal and ventral dilators are very indistinct. The pharyngeal wall including its musculature (M68/69) is rather thin. The oesophageal muscle is not distinctly separated from the pharynx (Figs. 5, 6).

Musculature (Figs. 5, 6): M45-M. frontotubecalis anterior (0bu2), one pair of bundles, O: fons, mesal the origin of M41, at the level of the frontal ganglion, extending posteroverrad, converging toward insertion, I: paramedially on anterior pharynx, mesal the insertion of M41, posterior to frontal ganglion; M46-M. frontotubecalis posterior (0ba3), three or four pairs of bundles, converging toward insertion, O: fons, first bundle lateral the origin of M41/2, other bundles posteromesal the origin of M41/2, I: first bundle on dorsolateral pharynx, other bundles paramedially on dorsal pharynx, anterior to brain; M48-M. tentoriotubecalis anterior (0bu6): absent; M50-M. tentoriotubecalis posterior (0bu6), O: dorsal surface of lateral sides of tentorial bridge, dorsal of origin of M17/3, posterior to origin of M1, I: ventrolateral pharynx, level of circumoesophageal connectives; M51-M. vertigopharyngalis (0ph1): absent; M52-M. tentoriotopharyngalis (0ph2) not clearly distinguishable from M50, both muscles merged, O: small area on dorsal surface of posterior tentorial arms, posterior to tentorial bridge, I: ventrolateral pharynx; M67-M. transversalis buccae (0hy9), several transverse cibarial muscle bundles; M68-M. annularis stomodei (0st1), pharyngeal ring musculature, posterior to insertion of M41; M69-M. longitudinalis stomodei (0st2), layer of longitudinal muscles, covered by M68, very well developed on dorsal side of anterior pharynx.

An additional muscle of the digestive tract of unknown homology was visible on cross sections (Fig. 6A: apm). It originates over the entire width of the dorsal surface of the plate-like anterior laminatotumtum, extends dorsal with its very short fibres and inserts on the ventral surface of the pharynx, immediately behind the posterior edge of the epipharyngeal suspensorium and insertion of M41 (see also discussion).

Brain and suboesophageal ganglion. The distinctly flattened brain (Fig. 7) appears relatively large compared to the size of the head; it occupies a large part of the upper half of cephalic lumen from the level of the compound eyes to the occipital region; laterally it is adjacent with the mandibular adductor (M11) and its anterior part with the large extrinsic antennal muscle (M1). The tritocerebral commissure is present but not very distinctly separated from the tritocerebrum (absence of M48). The frontal ganglion above the antennal mouth is strongly enlarged; it is connected to the tritocerebrum by very thick frontal connectives. The suboesophageal ganglion does not show any specific modifications.

Glands. Two pairs of large tubular glands are associated with the maxillae and labium (Figs. 5, 8). The opening pore of the maxillary gland is situated on the base of the maxillary cleft between the
cardo and the proximal paramaxillare, near the insertions of M15 and M17 on the cardinal process. The gland extends to the posterior region of the compound eyes, between M11, M12 and M15. The labial tubular glands open on the dorsolateral corners of the posterior hypopharynx, releasing their secretions into the oral cavity; the glands are adjacent to each other over most of their length and enclosed by M29 and M18 (subcomponents 2 and 3); posteriorly they are adjacent with the suboesophageal ganglion.

Additionally diffuse glands of unknown function are associated with the cuticle of the head capsule (Fig. 6); they release their secretions through cuticular pores; only few and scattered gland units are present in the dorsal region of the head, whereas densely packed gland elements form a voluminous layer in the ventral cephalic region; it largely covers the ventral wall of the head capsule lateral to the mouthparts and gula and reaches the level of the posterior constriction of the head; M12, M15 and the maxillary gland are placed upon this layer.

**Calyptomerus dubius**

A detailed description of head structures is not included here as *Calyptomerus* is very similar to *Clambus* in most cephalic features. Important differences will be presented in the following as a list of characters.

**External features of the head capsule** (Figs. 1B,2B,D)

- clypeus with broad anterior incision containing the labrum
- frontal part of clypeofrontal incision broadly rounded, much larger than in *Clambus*
- ocular epicranium completely absent; compound eyes compact and undivided; convex but integrated in the contour of the head capsule
- shallow transverse postocular furrow absent; transverse postocular ridge distinct
- ventral antennal furrow less oblique than in *Clambus*, more longitudinally oriented
- gular surface rather smooth, without distinct dints or keels

**Labrum** (Fig. 1B; Supporting Information Fig. 2B,C)

- labrum partly exposed (see above)
- anterior labral border not convex, nearly straight
- cuticle of dorsal labral surface smooth, with dense and regular vestiture of moderately long setae
- tormae shorter, not projecting posteriorly beyond transverse LEP-supporting struts

**Antennae** (Fig. 2B; Supporting Information Fig. 2A)

- antennae shorter in relation to head size; in resting position nearly completely fitting with ventral surface of head, proximal part in anten nal furrow, distal part behind compound eyes
- scapus of regular ovoid shape (but scapo-pedicellar condyle also slightly oblique); densely setose
- scapal condyle small; distinctly separated from main body of scapus by very distinct constriction
- pedicel with regular ovoid shape; densely setose
- relative length of flagellomeres similar to *Clambus* (i.e., the first two the longest), but single segments shorter (ca. half as long as in *Clambus*)
- antennal club shorter and broader, very densely set with sensilla trichodea; deep sensory grooves arranged in a transverse row

**Mandibles** (Fig. 4E,F; Supporting Information Fig. 2D)

- lateral longitudinal carina on dorsal surface shorter and much less prominent
- ventral surface without a distinct curved ridge (on left mandible) or furrow (on right mandible)
- laterostial mandibular process much shorter and stouter than in *Clambus*
- conspicuous scale-like setae inserted at ventral apex of laterostial mandibular process (possibly homologous with the unmodified ventral setae on the base of the process in *Clambus*)
- mesal semimembranous lobe more prominent, with very short and sparse microtrichia along its edge
- apical tooth of right mandible more serrate, also on distal half of its inner edge
- prostheca smaller and much less conspicuous
- ventral mandibular condyle more distinct, knob-shaped

**Maxillae** (Fig. 2B; Supporting Information Fig. 2E,G)

- basitippe and pseudopalaipfer completely separated from each other; basitippe triangular, sharp lateral longitudinal edge absent; distal pseudopalaipfer with a single seta
- carina separating mediostipes from lacinia indistinct
- shape of maxillary palp similar as in *Clambus*, but segments shorter

**Labium** (Figs. 2B,4H; Supporting Information Fig. 2F)

- submentum much shorter, much wider than long, transverse, strip-like
- mentum much larger in relation, with rounded corners and anterior edge; distal two thirds even pubescent
- palpigers not broadly fused, connecting area absent or much less distinct than in *Clambus*
- palparse 2 nearly straight, subhyaloidal; palpomere 3 longer in relation to other palpomeres
### TABLE 1. Distribution of cephalic features of Clambidae (see Discussion) and other scirtoid taxa and selected taxa of other groups of Coleoptera, especially from Myxophaga and Elateriformia

<p>| Feature | Clambidae | Coleoptera | Echidna | Ophila | Cirrhida | Ptiliida | Staphylinidae | Hydrophilidae | Scolytidae | Sphenida | Anthidae | Lamyriphalidae | Heteroptera | Athalia | Omalus | Basilea | Descandia | Doremas |
|---------|------------|------------|---------|--------|---------|---------|---------------|---------------|------------|----------|----------|-------------|-------------|---------|-------|-------|-------|---------|--------|
| Clypeus shield-like and projecting antecervically | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Clypeus with deep lateral incision | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Distinct ventral anteocular groove separated by sharp angular edge | + | ? | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Galea convex (+) or even (-) | + | + | + | + | + | ? | - | - | - | - | - | - | - | - | - | - | - |
| Compound eyes within contour of head | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Dorsal antennal areas present | - | ? | - | - | ? | - | ? | + | + | ? | ? | - | + | + | + | + | + | + |
| Stridule tympanum compact &amp; plate-like | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Labrum &gt; 2.5 times as broad as long | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Antennal flagellum enlarged | + | - | - | - | - | ? | + | + | ? | ? | + | + | + | + | + | + | + | + |
| Number of antennomeres reduced (&gt;) | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Proximal pedicel with large process | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Distinct oval club | + | ? | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| First antennal flagellum strongly enlarged | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Mandibles with longitudinal interdental carina | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lateral mandible with distinct anteriorly directed process | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lateral mandibular surface glabrous (1) or pubescent (1) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Apical mand. tooth long, slender &amp; evenly curved | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |</p>
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"+" present; "-" absent; "+/-", probably present/absent but not fully confirmed; "?", condition unknown.
Epipharynx (Fig. 4G)

- different arrangement of vestiture on anterior epipharynx; central field of microtrichia shifted to lateral sides of epipharynx; parasagittal row of microtrichia much less developed; teeth on anterior edge larger
- arrangement of microtrichia on posterior epipharynx lateral LEP with a more regular patch-like arrangement (not ring-like)

DISCUSSION

Clambiidae and Scirotidae are small groups of inconspicuous beetles. Nevertheless, they are crucial for understanding the evolution of the megadiverse Coleoptera and Polyphaga. This is underlined by the currently proposed position of the superfamly (and series Scirotiformia) as the most basal branch of the polyphagan beetles (Hunt et al., 2007; McKenna and Farrell, 2009; Bocak et al., 2014; McKenna et al., 2015). Considering this phylogenetic placement, the polarity of characters found in Clambiidae (and other scirtoid families) appears ambiguous and open to new interpretations. These issues will be discussed in the following sections.

The monophyly of Scirotidae (and Scirotiformia) as presently defined (e.g., Beutel and Leschen, in press) is insufficiently supported and questionable, especially with respect to Derodontidae (McKenna et al., 2015), which were previously placed in the series Bostrichifoma (e.g., Lawrence and Newton, 1995) or in a series Derodontiformia together with Nosodendridae and Jacobsoniidae (Lawrence et al., 2010). The phylogenetic interpretation of the data presented here is impeded by a considerable lack of detailed information on head structures of potentially related groups. Nevertheless, some potential autapomorphies of Scirotidae were revealed by our study. A doubtlessly derived feature shared by Clambeae and other scirtoids (probably including Decliniidae; Lawrence et al., 1995: Fig. 1) is the loss of the dorsal tentorial arms. They are present in Myxophaga (rather indistinct in Lepicerus Motschulsky, 1855 [Lepiceridae] (Anton and Beutel, 2006); well developed in Saxionitus Endröw-Younga, 1997 [Torridicoleidae] and Hydroscapha LeCointe, 1874 [Hydroscaphidae]), in Archostemata (Hörnischmeyer et al., 2002; Beutel et al., 2008), Adephaga (Dressler and Beutel, 2010), and also in the majority of polyphagan groups (e.g., Anton and Beutel, 2004, 2012) (the distribution of characters in Scirotidae and selected other coleopteran taxa is shown in Table 1). Another potential autapomorphy of the superfamly is a bulging gula, distinctly raised from the contour of the head capsule, especially in the posterior region. It is usually more or less even in Archostemata (Hörnischmeyer et al., 2002; Beutel et al., 2008), Adephaga (Dressler and Beutel, 2010), and in most groups of Polyphaga. A distinctly convex gula also occurs in the non-related scarabaeoids and dasyclads (Anton and Beutel, 2012), in some other groups of Polyphaga (e.g., Byrrhidae, Omalisidae), and also in the myxophagan genera Hydroscapha and Sphaeriusculus, 1838 (Sphaeriuscidae). However, the phylogenetic relationships (e.g., McKenna et al., 2015) suggest that this is the result of parallel evolution. Another derived condition is the strongly transverse labrum, shared by Clambeae and other scirtoids such as Pseudomicrocora Armstrong, 1953, Ore Clarks, 1865, and Elodes Laparke, 1796 (all Scirotidae). A labrum with a width/length index of at least 2.5 occurs only in few other polyphagan taxa (Hydrophiloidea, some staphylinoids and scarabaeoids; Anton and Beutel, 2004, 2012).

Whether this is a groundplan apomorphy of Scirotidae remains ambiguous, as this condition is not found in Eucinetus Gémar, 1818 (Eucinetidae) and Cyphon Paykull, 1799 (Scirotidae). Another characteristic derived feature is the presence of a ridge separating the mediostipes from the lacina. However, this structure is absent or indistinct in Calyptotermes and the flea marsh beetle Oro (Scirotidae).

The characters we examined did not clearly indicate relationships between Clambiidae and other groups of Scirotidae (or Derodontidae). Shared characters and possible synapomorphies of Clambiidae and Decliniidae (Lawrence et al., 1995) are a distinct ventral antennal furrow (Fig. 2A,B) and a longitudinal carina on the laterodorsal surface of the mandibles (Supporting Information Fig. 1H). Eyes integrated in the contour of the head are also found in both families (Figs. 1, 2). However, they are distinctly protruding in the clambe genus Calyptotermes (Crowson, 1979). A potential synapomorphy of Clambiidae and Eucinetidae is the presence of a long and slender apical mandibular tooth with a serrate edge (Fig. 4A,B,E,F).

The monophyly of Clambiidae is strongly supported by many characteristic and partly unique features. The most conspicuous apomorphy is the strongly broadened and flattened head, closely fitting with the ventral side of the thorax, and apparently resulting in a broadened and dorsoventrally compressed brain (Figs. 1, 2, 5, 7). The peculiar head shape is also correlated with the enlarged shield-like, anteriorly and laterally projecting clypeus. An unusual autapomorphic feature is probably linked with this condition, the presence of a deep clypeofrontal incision, which allows dorsal and ventral movements of the antennae. A somewhat similar incision is described for Glareasis Emerson, 1848 (Glareidae, Scarabaeoidea) (Anton and Beutel, 2012). However, considering the widely separated systematic placements (e.g., McKenna et al., 2015), this is certainly due to parallel evolution.
A recently described very small beetle from Lower Cretaceous Lebanese amber was placed in a new family Lambyidae, in the series Staphyliniformia, and "hypothetically" in the superfamily Staphylinoidae (Kirejtshuk et al., 2016). The phylogenetic assessment was based on an informal character discussion and no convincing apomorphies linking the fossil with staphylinoid beetles were presented. The strongly deflected head flying with the venter of the anterior thorax, the abrupt two-segmented antennal club, and the broad and large metacoxal plates strongly suggest that the extinct taxon belongs in the family Clambidae.

The monophyly of Clambidae excl. Acalyptrome-rus (Calyptomerinae + Clambinae) is well supported. A convincing apomorphy is the conglobate form (Lawrence, 2005), the ability to roll the head and postcephalic body into a ball (Fig. 1). In Acalyptrome-rus and Clambus the ventral side of the head fits with the anterior surface of the meso- coxae and not with the procoxae as it is the case in Acalyptrome-rus. The former condition suggests a clad Calyptromerinae + Clambinae. Another apparent synapomorphy of these two groups is the strongly constricted head with conspicuous temples (Fig. 2; Lawrence, 2005), which are missing in Acalyptrome-rus. The protuberant compound eyes of Acalyptrome-rus are apparently plesiomorphic as a similar condition is found in other scirtoid taxa (Lawrence, 2005; Leschen, 2005). Consequently eyes more or less integrated in the contour of the head are an additional synapomorphy of Calyptromerinae + Clambinae (Fig. 1), and the complete division by a canthus an autapomorphy of the genus Clambus (Fig. 2A,C). The dividing canthus is incomplete in the genera Loricaster Motsant & Rix, 1861 and Dracanthi-chorax (Endrödy-Younga, 1959) (both Clambinae) (Endrödy-Younga, 1959).

A doubtlessly derived feature of Clambidae (or a subgroup, only verified for Clambus) is a compact plate-like laminatentorium (Fig. 3C). A similar condition occurs in members of Staphyliniformia and Searabaeoidea, and very rarely in other poly- phagan groups (Dermestes Linnæus, 1758 [Der- mestidae], Heterocerus Fabricius, 1792 [Heteroceridae]), obviously as a result of parallel evolution. A laminatentorium is also present in Sotonius, but in this case the longitudinal tentori- al arms are fused with each other over their entire length, which is a unique configuration resulting in the absence of a tentorial bridge as a separate structure. In the examined nonclambid scirtoids the basal section of the tentorial arms is also broadened. The arms are converging over a certain distance but are always clearly separated from each other. A similar condition occurs in Ade- phaga (Dressler and Beutel, 2010), in Lepiceridae (Anton and Beutel, 2006), and in a wide range of polyphagan taxa (e.g., Elateroidea s. l., Dascilli- dae, Byrrhidae, Dryopidae, Elmidae, but not in Heterocerus); Anton and Beutel, 2012). The presence of a short and broad muscle of unclear homol- ogy (Fig. 1A–C) is probably functionally correlated with the solid laminatentorium and the loss of the dorsal tentorial arms. It connects the laminatentorium and ventral pharynx in Clambus, shortly behind the prepharyngeal suspensorium. A possible function of this muscle is to fix or stabilize the (anterior) pharynx as an antagonist of the hypopharyngeal levator (M41). At present a similar muscle is only known in the non-related Dermestes.

Possible clambid autapomorphies of the antenna are a reduction to or fewer antennomeres (eight in Loricaster; Endrödy-Younga, 1959), a distinct two-segmented club, a strong elongation of the first two flagellomeres, and an oblique scapepedicellar condyle (Fig. 2; Supporting Information Fig. 1C.D). The shortened scapus and pediculus, together with a strongly developed distal scapal and proximal pedicellar projection are apomorphic conditions found in Clambus. An antennomere three distinctly longer than 4 is a possible autapo- morphy of the genus, in contrast to the condition in the other clambine genera (Endrödy-Younga, 1959).

The glabrous lateral surface of the mandible is arguably a further autapomorphy of Clambidae (Fig. 4A,B,E,F). It is densely pubescent in all other scirtoids, and also in the majority of other poly- phagan groups. However, it is also glabrous in most adephagans and also in the myoxophagan genera Hydroscapha and Sphaeriuss (but not in Lepicerus).

Another potential autapomorphy of Clambus is a mesally directed ridge on the ventral surface of the left mandible and a deep ventral furrow on the distal part of the right mandible. Both are absent in Acalyptrome-rus and all other examined scirtoids. Similar ridge-like structures occurring in different groups of Polyphaga (e.g., Anton and Beutel, 2004) are likely the result of parallel evolution.

An unusual feature found in Clambus is the ring-like arrangement of rows of microtrichia on the posterolateral epipharynx (lateral the LEF) (Supporting Information Fig. 1B). Rows of microtrichia showing a similar arrangement are also be present in the elaterid Atthus Escherschitz, 1829, but usually they form regular transverse rows, a condition found in other Elateroidea s. l., in Hydrophiloidea (e.g., Anton and Beutel, 2004) and in most staphylinoid subtaxa, especially those with a sporocephal or pollenphagous habit (Betz et al., 2008). This epipharyngeal region is glabrous or evenly set with microtrichia in the vast majority of other polyphagan taxa. In Calyp- tromerus the arrangement is not ring-like but rather in regularly arranged patches. Usually these rows of epipharyngeal microtrichia interact with a corresponding similar patch on the dorsal
surface of mandibular molae, forming a transport mechanism for the processed food (Betz et al., 2003). More data are required for a reliable phylogenetic interpretation of this character. However, it is likely that the specific arrangement of epi-
pharyngeal microtrichia found in Clambus is either an autapomorphy of the genus or of the family depending on the unknown condition in Axyspinopterus.

Despite the small size of Clambus, effects of miniaturization are minimal. Skeletal features of the head are apparently not affected or only to a very minor degree. Relatively few muscle reductions could be observed. The absence of M. stipitopalis (M20) and M. stipitopalpis internus (M23) is apparently a derived feature of Clambi-
da as both are present in Cyphon. However, the loss of M20 is common in Coleoptera (Adephypha, Myxophaga, Athous, Logistopterus [Lycidae], Byrrhidae, Dryopidae, Elmidae, Heteroceridae, Der-

mestes, etc.; Dressler and Beutel, 2010; Anton and Beutel, 2004, 2006, 2012). In contrast, the loss of M23 is an unusual feature, which may be corre-
lated with the fusion of basistipes and pseudopali-
fer in the case of Clambus. Other muscles reduced in Clambus are M. tentoriopharyngophary-

gialis (M42) and M. tentoriobuccalis anterior (M48) (Figs. 5, 6). The former is also missing in Myx-
ophaga (Anton and Beutel, 2006), whereas the latter is present in the examined species of this suborder (Anton and Beutel, 2005, 2006). Both are also

missing in Cyphon and probably also in Eucinetidae. It is conceivable that this reduction is a synapo-
morphy of several or all scirtoid families.

The small third mandibular muscle described for Clambus in this study has been identified in very few species of Coleoptera so far (Honomichl, 1975: Gyrius Geoffroy, 1762 [Gyrididae]; Hono-
michl, 1976: Deromestes; Homomichl, 1978: Oryzoe-

philus Ganglbauer, 1899 [Silvanidae]; Trachy-
pachus Motschulsky, 1844 [Trachypachi-
da], Dascillus Latreille, 1796 [Dascillidae], Gla-

raste]. Its size and position suggest a function as proprioreceptor (Honomichl 1975, 1976, 1978). Based on its origin and insertion points it is apparently homologous with M14 (M. xygomaticus mandibulae) of v. Kéler (1965), which is mainly known from hemimetabolous insects (e.g., Wippler et al., 2011, 2012).

A doubtlessly derived feature of the cephalic musculature of Clambus is an extremely enlarged distinctly separated subunit of M. tentoriocapsalis anterior (M1) (Fig. 3A). As this is likely correlated with the deep clypeofrontal incision and vertical antennal movements, it can be assumed that a similar condition is present in the other clambid genera. The anterior extrinsic antennal muscle is also subdivided in the scirtid genus Cyphon. One of the two bundles is also enlarged even though to a lesser degree than in Clambus. A highly unusual feature of Cyphon is the origin of this muscle from the gena, anterovertral the compound eyes. This condition is not described for any other group of Coleoptera and is also missing in Elyodes. Appar-
ently this is an apomorphic condition that evolved in a subgroup of Scirtoidea.

Interestingly an entire series of characters occurs in members of Scirtoidea and the small sub-
order Myxophaga. Considering the basal position of the scirtoid families within Polyphega and a possible sistergroup relationship between Polyphega and the remaining beetle suborders (e.g., McKenna et al., 2015), it is conceivable that some of these features belong to the groundplan ofCole-

optera. As the presently available morphological data are very fragmentary, the following interpreta-

tions should be considered as provisional.

A movable prosthca on the left mandible, as it is found in Clambidae (Fig. 4A,B,E,F), is unique among Polyphega, but is very likely a synapomorphic

of the four families of Myxophaga (Beutel, 1999). A movable prosthca is also present In

Hydraenidae and the hydrophil genus Berosus

Leach, 1817 (e.g., Anton and Beutel, 2004), how-


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The head morphology of Clambidae

The head morphology of Clambidae includes a characteristic feature of Clambus: elongated and strut-like tormae (Supporting Information Fig. 1A), connected to the epipharyngeal suspensorium and with additional transverse sclerites supporting the LEP. These structures are also typical for Myxophaga, even though detailed data are only available for Lepiceurus (Anton and Beutel, 2006). That the tormae are distinctly shorter and less complex in other scirtoidean taxa suggests that the condition found in Clambus and Myxophaga has evolved independently in both taxa.

A distinct and usually simple ridge or suture which separates the occipital and genal areas in the lateral and ventral head region is also present in Clambus (e.g., Dresler and Beutel, 2010: circular line), Lepiceurus (Anton and Beutel, 2006; absent in Hydroscapha and Sphaerius), and in many polyphagan taxa (Table 1). It is present as a sharp angular edge in the examined Scirtoideidae, and a similar condition occurs in Lepiceurus (Anton and Beutel, 2006) and in different polyphagan taxa (Ptilodactyloidea, most groups of Elateroidea (Table 1). The phylogenetic relationships suggest that this is very likely the result of parallel evolution.

A subterminal origin of M. tentorioprementalis inferior (M29) (Fig. 3D) is an unusual feature occurring in Clambus and Cyphon, in Myxophaga (not known for Sphaerius), and also in some staphyliniform and scarabaeoid beetles. It is likely that this derived condition has evolved several times independently. The muscle originates from the posterior tentorial arms in the vast majority of examined polyphagan groups and from the gula in Adephaga (e.g., Dresler and Beutel, 2010).

A phylogenetically ambiguous feature shared by the scirtoeid genera Clambus and Eucinetus is the presence of paired tubular glands associated with the maxillae (Fig. 5A). Interestingly these structures also occur in the myxophagan genera Lepiceurus and Hydroscapha, and are also present in Anthus Eschscholtz, 1839 (Buprestidae), Elmis Latreille, 1798 (Elmidae) and Dermestes. That similar glands occur in Neuroptera (Röber, 1942: Sialis Latreille, 1802 [Sialidae, Megaloptera]; Beutel et al., 2010; Osmius Latreille, 1802 [Osmiidae, Neuroptera]) suggests that the presence could belong to the groundplan of Coleoptera, with secondary loss in different lineages, notably Adephaga (Dresler and Beutel, 2010) and Archos- tendata (Hornschemeyer et al., 2003; Beutel et al., 2008), but also many groups of Polyphaga (e.g., Anton and Beutel, 2004, 2012). The compound eyes are probably used during flight but are of little use in habitats such as leaf litter (Leschen, 2006). The complete subdivision into an upper and a lower portion in Clambus (Fig. 2A,C) is difficult to explain. The mouthparts, especially the mandibles, are apparently used for a sort of filter feeding. It is likely that the adults feed on a more or less liquid suspension of mold, fungal spores and decaying plant materials. However reliable observations are lacking.

The present study underlines that the present knowledge of the morphology of Scirtoidea and potentially related groups is very insufficient, especially considering the phylogenetic key role of the group in Polyphaga. A detailed documentation of structural features of taxa like Declinidae, Eucinetidae, and Dermestidae should have high priority. This will likely not only elucidate the hitherto unclear phylogenetic relationships in Scirtoidea, but also help to understand the early evolution of Polyphaga, the starting point of one of the largest radiations in the animal kingdom.

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**Abstract:** Adult head structures are well known in the coleopteran suborders Archostemata and Adephaga, whereas the available information is very fragmentary in the megadiverse Polyphaga, including the successful superfamily Staphylinioidea. In the present study, the cephalic morphology of the cholevine species *Catops* ventricosus is described in detail and documented. The results were compared to conditions occurring in other polyphagan lineages, especially staphylinoid and scarabaeoid representatives. Specific external features documented in *Catops* and potential autapomorphies of Leiodidae include a five-segmented antennal club with a reduced eighth antennomere and the presence of periarticular grooves filled with sensilla on antennomeres 7, 9, and 10. The firm connection of the head and pronotum is possibly an apomorphy of Cholevinae. The monophyly of Cholevinae excluding Eucatopini and Oritocatopini is supported by the apical maxillary palpomere as long as or shorter than the subapical one, and the presence of cryptic pore plates on the surface of these palpomeres — a feature described and documented here for the first time. The internal cephalic structures of *Catops* are mostly plesiomorphic, as for instance the complete tentorium. The pattern of the muscles is similar to what is found in other staphylinoid taxa. The unusual maxillary muscle “Mx” is likely a groundplan apomorphy of the clade Staphyliniformia + Scarabaeoidea.

**Significance in the present thesis:** Leiodidae (Staphylinioidea) is a close relative of Ptiliidae, with similar feeding preferences (saprophagy), but larger body size. Besides this, they show multiple features that are presumably ancestral for Polyphaga and Coleoptera.

**Own contribution:** 20 %
Cephalic anatomy and three-dimensional reconstruction of the head of *Catops ventricosus* (Weise, 1877) (Coleoptera: Leiodidae: Cholevinae)

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Abstract Adult head structures are well known in the coleopteran suborders Archostemata and Adephaga, whereas the available information is very fragmentary in the megadiverse Polyphaga, including the successful superfamily Staphylinoidae. In the present study, the cephalic morphology of the cholevine species *Catops ventricosus* is described in detail and documented. The results were compared to conditions occurring in other polyphagan lineages, especially staphylinoid and scarabaeoid representatives. Specific external features documented in *Catops* and potential autapomorphies of Leiodidae include a five-segmented antennal club with a reduced eighth antennomere and the presence of periantennal grooves filled with sensilla on antennomeres 7, 9, and 10. The firm connection of the head and pronotum is possibly an apomorphy of Cholevinae. The monophyly of Cholevinae excluding Eucatopinini and Ortioptopini is supported by the apical maxillary palps as long as or shorter than the subapical one, and the presence of cryptic pore plates on the surface of these palps—a feature described and documented here for the first time. The internal cephalic structures of *Catops* are mostly pleiomorphic, as for instance the complete tentorium. The pattern of the muscles is similar to what is found in other staphylinoid taxa. The unusual maxillary muscle “Mx” is likely a groundplan apomorphy of the clade Staphyliniformia + Scarabaeoidea. *M. hypopharyngomandibularis* (M13) was identified in *Catops* and is ancestral for Coleoptera, even though it is often missing. The same applies to *M. tentoriolympopharyngalis* (M42).

Keywords *Catops* · Leiodidae · Head morphology · 3D reconstruction · Musculature · Staphyliniformia · Staphylinoidae

Introduction

It was shown in previous studies on Archostemata (Beutel et al. 2008b) and Adephaga (Dressler and Beutel 2010) that the cephalic anatomy of Coleoptera is a complex and phylogenetically informative character system, which can also reveal important insights in life strategies, especially but not only in the context of feeding. The available information on adult head structures of Coleoptera has considerably increased in the last decade, facilitated by advanced morphological techniques (e.g., Beutel et al. 2008b; Anton et al. 2016). Head structures of Archostemata (Hörschtemeyer et al. 2002; Beutel et al. 2008b), Adephaga (e.g., Dressler and Beutel 2010) and the myxophagan family Lepiceridae (Anton and Beutel 2006) are very well known. In contrast, the available information on the cephalic morphology of the megadiverse Polyphaga is still very fragmentary. Earlier studies are those of Dönges (1954) on the weevil *Cius* or the study of Schneider (1981) on the Spanish fly *Lyttia* (Meloidae), both highly specialized taxa of Cucujiformia. Anatomical data on the
presumably basal Scitoidea (McKenna et al. 2015a) became available only recently (Anton et al. 2016) and detailed studies on the extremely species-rich Staphylinoidea are still very sparse. Interestingly, larval head structures are relatively well known (e.g., Beutel and Molenda, 1997; Beutel and Leschen 2005), whereas detailed data on adult cephalic structures are only available for few species of the aquatic Hydraenidae (Beutel et al. 2003), the strongly miniaturized Ptiliidae (Polilov and Beutel 2009), the small family Agyriidae (Weide and Betz 2009), and few species of the highly diverse Staphylinidae (Weide and Betz 2009; Weide et al. 2010, 2014).

With 435 described species (Newton 2016), Leiodidae are the second largest family of Staphylinoidea. Their distribution is worldwide, and they are able to explore an astonishing range of habitats and food resources. In general, leiodid beetles inhabit forested landscapes and are mycophagous relying on various groups of fungi or saprophagous and feeding on different kinds of decaying organic matter involving plant material (e.g., organic matter in the soil, leaf litter), animal matter (e.g., dung, carrion) or the yeasts, and bacteria associated with such substrates (Newton 2016). The family is currently organized into six subfamilies and 18 tribes. About half of the total species diversity belongs to Cholevinae, the most species-rich subfamily, encompassing seven tribes and 17 subtribes (Perreau 2000; Bouchard et al. 2011). There is no broad formal cladistic study available focused on Leiodidae or Cholevinae. The only phylogenetic contributions including these groups are broad-scale analyses with insufficient taxonomic sampling regarding subordinate taxa in Leiodidae, or more detailed studies on particular lineages, thus not providing a well-supported phylogenetic scheme at the family or subfamily level. In this context, Fresneda et al. (2011) is the most complete molecular study with emphasis on Cholevinae. Whereas some studies did not confirm the monophyly of Leiodidae (e.g., Lawrence et al. 2011; McKenna et al. 2015a) or Cholevinae (e.g., Fresneda et al. 2011; McKenna et al. 2015a), both have been supported as natural groups based on morphological characters evaluated by Newton (1998, 2016). Therefore, the higher level classification remains an important area of investigation in the systematics of Leiodidae.

The knowledge on the morphology of Cholevinae is predominantly limited to general external features and genitalia, mostly documented and described based on stereomicroscopy and light microscopy, and mainly aiming at taxonomic descriptions. The head morphology of Cholevinae has never been studied in detail, especially the internal soft parts. This contribution aims to explore the cephalic character complex of a representative of Cholevinae by providing a detailed description of the head of Catops ventricosus (Weise, 1877). The exo- and endoskeletal structures, musculature, nervous system, and digestive tract were studied and documented using scanning electron microscopy, micro-computed tomography, and computer-based three-dimensional reconstructions. The first 3D model of the head of Leiodidae is provided, and the morphological elements are discussed from a phylogenetic point of view. The morphological descriptions and documentation presented here offer the basis for future inferences on the higher level systematics of Leiodidae using cephalic structures as a source of characters.

Material and methods

This study is based on adults of C. ventricosus collected inside Anlı Mağarasi, a cave in Günlüganı, Turkey (1777 m, N 40° 26′ 50.8″ E 39° 19′ 19.1″). This species lacks sexual dimorphism associated with cephalic characters. Specimens were investigated using scanning electron microscopy (SEM) and synchrotron radiation micro-computed tomography (SR-μCT). For SEM, the specimen was dried at the critical point (Emitech K850 critical point dryer), sputter-coated with gold (Emitech K500), and fixed on a rotatable specimen holder (Pohl 2010). Images were taken with a FEI (Philips) XL 30 ESEM at 10kv. Specimens used for SR-μCT were dehydrated with ethanol (20–100 %) and acetone, dried at the critical point (Emitech K850 critical point dryer), and mounted on a standardized specimen holder. Micro-computed tomography was performed at the Deutsches Elektronen Synchrotron (DESY, beamline IBL P05 at PETRA III, operated by the Helmholtz-Zentrum Geesthacht, Hamburg, Germany) with a stable beam energy of 8 keV in attenuation contrast mode (Beckmann et al. 2008; Greving et al. 2014). We used an effective magnification of ×18 providing a resulting field of view of 2 mm × 2 mm, resulting in an effective pixel size of 1.33 × 1.33 μm in the two times binned reconstructed data set. Radiograms (n = 1200) were taken at equal intervals between 0 and π (exposure time of 6.3 s). Stacks of ≥899 slices were calculated from each set of radiograms using the tomographic reconstruction algorithm “back projection of filtered projections” (Huesman et al. 1977).

Three-dimensional models of head structures are provided. Uncompressed 16bit TIFF image stacks were imported into Amira 5.3.1 (Visage Imaging, Berlin, Germany) where the segmentation of individual structures was conducted. These were subsequently exported to VGStudio MAX 2.0.5 (Volume Graphics, Heidelberg, Germany) for volume rendering.

Detailed morphological studies on the head of staphyliniform and scarabaeoid beetles were used for comparison (e.g., Jäch et al. 2000; Beutel et al. 2001, 2003; Anton and Beutel 2004; Polilov and Beutel 2009; Weide and Betz 2009; Weide et al. 2010; Anton and Beutel 2012). The muscles are named following the terminology of von Kéler (1963).
Results

External head capsule

The posterior part of the prognathous head is abruptly narrowed thus forming a distinct neck region, which lies on a lower level dorsally and is retracted into the prothorax (Figs. 1 and 2). The posterodorsal border of the exposed part of the head is delimited by an occipital crest, which is firmly connected to the anterior edge of the pronotum (oc, Fig. 2a, e). The head capsule is mostly black, but the appendages vary from dark yellowish brown (antennae, maxillary palps, and most parts of cardo and stipes) to pale yellow (galea, lacinia, and labial palps) (Fig. 1). The dorsal surface of the head except for the neck region is microgranulated and densely covered with regularly distributed, medium length yellowish setae (Fig. 1a, c). The dorsal punctures are enclosed by a distinct rim (Fig. 2b). Setae are also present on most parts of the ventral side of the head, except for the gula (Fig. 2c). The ventral cuticular surface is weakly striated on the mentum and submentum, strongly striated on the genae, and strongly reticulated on the gula (Fig. 2c). The frontotemporal strengthening ridge is not visible externally (Fig. 2d). The clypeus is moderately sized. Its subparallel lateral margins are approximately one third as long as the straight anterior margin (Fig. 2d). The lateral and anterolateral borders are weakly demarcated by an indistinct ridge which obliterates in the middle region of the anterior border. The lateral surface of the frons anterior to the compound eyes is rounded excavated, and the antennal insertion is fully exposed in this region (Fig. 2a). Ocelli are absent. The compound eyes are moderately sized, with a distinctly convex surface and numerous small lenses of the individual ommatidia, and few scattered setae. The anterior edge is evenly rounded whereas the vertical posterior margin is nearly straight. Posteriorly, the compound eye is covered by a genal fold (pogf, Fig. 2c) and delimited by a large postocular ridge (por, Fig. 2d). The gula is large and has the shape of an elongated trapeziun. It is anteriorly delimited by a smooth transverse depression of its surface and appears convex in lateral view (Fig. 2c). The widely separated anteriorly converging gular sutures are very distinct externally and fissure-shaped. The posterior tentorial pits are not distinctly recognizable externally.

Cephalic endoskeleton

The paired anterior, dorsal, and posterior tentorial arms (ata, dta, pta, Fig. 5a) are well developed. The extensive gular ridges form wall-like structures and are fused with the posterior edge of posterior arms (“PTW” in Weide et al. 2014). They are connected by a thin tentorial bridge or corpotentorium, which is straight in posterior view, with a sinuously curved posterior margin, thus appearing W-shaped in dorsal view (th, Fig. 5a). The posterior tentorial arms converge anteriorly and merge medially forming a massive median tentorial body or laminatentorium (lt, Fig. 5a). This structure is composed of a median vertical plate dorsally continuous with a horizontal plate (“hp” in Anton and Beutel 2004), which is not directly connected with the tentorial arms. The median vertical plate produces a frontally projecting, median vertical lamella (mv, Fig. 5b). The elongate anterior and dorsal tentorial arms arise from the posterior arms, posterior to the horizontal plate of the laminatentorium. The apically narrow dorsal arms extend towards the dorsal wall of the head capsule at the level of the compound eyes, ending between the longitudinal midline of the head and the dorsal margin of the eyes (Fig. 6b). The anterior arms arise with a relatively robust proximal part at the edge of the frons, close to the antennal foramen and the sensory arista (Figs. 4d and 6b). The anterior tentorial pits are not visible externally.

Labrum

The transverse labrum is connected to the clypeus by an internal membrane. It is ca 2.5 times as wide as long and well visible from above (Fig. 3h). The anterolateral edges are rounded, whereas the median portion of the anterior margin is broadly margined and bears a dense fringe of setae (Fig. 3b). The dorsal surface is largely smooth, but a sparse vestiture of anteriorly directed setae is present. The dorsal surface is posteriorly delimited by a distinct transverse ridge (trdg, Fig. 3h), which is covered by the apical clypeal margin. The anteromedial region of the ventral side bears a cluster of blunt protuberances and a field of mesally directed microtrichia (Fig. 3i). It is followed posteriorly by a longitudinal epipharyngeal process densely covered with
Fig. 2 SEM micrographs of the head of Catops ventricosus. a Dorsal view. b Dorsal surface of vertex. c Ventral view. d Frontal view. e Lateral view. f Antenna. g Eighth antennomere. h Ninth antennomere. i Detail of the narrow slit opening on the distal surface of the ninth antennomere. by basistipes, ce compound eye, cl elytrum, ca cardo, ga galea, ge gena, gu gula, lc lacina, lp labial palp, lr labrum, md mandible, mp maxillary palp, ms mediostipes, mt mentum, oc occipital crest, pf palpifer, pmt prementum, pog postocular genal fold, por postocular ridge, smt submentum, oc epicsus.
Fig. 3 SEM micrographs of the mouthparts of *Catops ventricosus*. a Dorsal view of left mandible. b Ventral view of left mandible. c Dorsal view of left maxilla. d Ventral view of left maxilla. e Dorsal view of apical maxillary palpomere. f Lateral view of apical maxillary palpomere. g Lateral view of subapical maxillary palpomere. h Dorsal surface of labrum. i Ventral surface of labrum. j Dorsal surface of hypopharynx-prementum complex. k Ventral surface of hypopharynx-prementum complex, with arrows indicating areas of origin of selected cephalic muscles. The details in f and g show pore plates on the surface of the apical and subapical maxillary palpomere, respectively. avp mandibular accessory ventral process, bs basitipes, ca caridio, dgs digritiform sensilla, ga galea, hpp hypopharynx, ht tuft of hairs of the hypopharynx (or longitudinal hypopharyngeal process), lpp longitudinal epipharyngeal process, lc labial palp, mcnd mandibular condyle, ml mole, mp maxillary palp, ms mediostipes, pf palpifer, pmn prementum, prst prostheca, trdg transversal dorsal ridge. For muscle determination, see the main text.
microtrichia (lep, Fig. 3). Paired paramedian patches of sensilla are present close to the posterior portion of the fields of microtrichia.

Musculature: *Musculus labroepipharyngalis* (M7)—(origin = O) posteriorly on the dorsal wall of labrum; (insertion = I) posteriorly on the ventral wall of labrum (posterior to area of origin) (Figs. 4a and 6a). *M. frontoepipharyngalis* (M9)—absent.

**Antenna**

The insertion of the 11-segmented (Fig. 2f) antenna is clearly visible from above (Fig. 2a). The scapus is composed of a nearly globular articulatory piece and an elongated cylindrical shaft. The cylindrical pedicellus is shorter and also shorter than the elongated antennomere 3. The following segments are distinctly shorter and slightly widening distally. Segment 6 is wider than long. Antennomeres 7–11 form an indistinct club. Antennomere 7 is large and cupola-shaped and its apical part partly covers the small antennomere 8 (Fig. 2f). Segments 9 and 10 are almost as large as 7 and similarly shaped. The terminal antennomere 11 is elongate and subcomical on its distal half (Fig. 2f). A distal periarticular gutter bearing sensilla is present on antennomeres 7, 9, and 10, visible through a narrow slit-like opening on the apical surface of these segments (Fig. 2h, i, compare with Fig. 2g). All antennomeres are densely covered with setae. Some longer, curved setae are distributed laterally along the distal face of the antennomeres 7, 9, and 10, close to the apical border. On the distal antennomere, the setae are laterally inserted at the end of the most expanded part of the segment and also at the apex (Fig. 20). Numerous short peg-like sensilla are present at the distal margin of antennomeres 7, 9, and 10 (Fig. 2l). Some thinner, longer proprioceptive sensilla are also present on these antennomeres, oriented towards the central axis of the antennae (Fig. 2h, i).

Musculature: *M. tentorioscapalis anterior* (M1)—(O) the dorsal face of horizontal plate of laminantentorium, passing below the anterior portion of the anterior tentorial arm; (I) ventrally on articulatory piece of scapus (Fig. 4d, e). *M. tentorioscapalis posterior* (M2)—(O) the lateral side of the posterior tentorial arm; (I) medioposteriorly on articulatory piece of scapus, posterior to insertion of M4 (Fig. 4e). *M. tentorioscapalis medialis* (M4)—(O) lateral face of anterior tentorial arm and basal portion of dorsal tentorial arm; (I) medially on articulatory piece of scapus (Fig. 4e).

**Mandible**

The mandibles are largely symmetrical, with the lateral margins somewhat rounded (Fig. 3a, b). The external side is broad at the

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**Fig. 4** Transverse μCT sections through the head of *Catops ventricosus*. a–f Sections in anterior-posterior sequence. *ata* anterior tentorial arm, *gfr* frontal ganglion, *phr* pharynx. See the main text for muscle identifications.
base and narrows towards a moderately acute apex, which is proximally adjoined by a sinuous, dorsal cutting edge (Fig. 3a). A retinaculum, in the form of a very weakly developed blunt process, is present at midlength between the mandibular apex and the distal margin of the mola (Fig. 3a). It is slightly more prominent on the right mandible. The well-developed prosthoea bears a dense brush of long microtrichia along the mesal border of the ventral mandibular side, from the incisor area to the mola (prst, Fig. 3b). On the ventral face of the mandible, a weak elevation occupies a large area of the mesal region of the surface. A row of hairs arises from the distal and mesal limits of this area (Fig. 3b). A ventral accessory process is present mesally close to the basal mandibular edge (avp, Fig. 3b). Oblique rows of posteriorly directed microtrichia characterize the dorsal surface of the large mola (ml, Fig. 3b). The molar area is delimited by a slight depression of the mandibular surface and is therefore not firmly united to the rest of the mandible (condition defined as "loosely attached" in Betz et al. 2003). Its mesal grinding surface is densely covered by regularly distributed asperities or small denticles, while the ventral surface bears dense rows of short hairs.

Musculature: M. craniomandibularis internus (M11)—(O) posterolateral area of the head capsule, slightly posterior to the eyes, and from the posteriormost cephalic region lateral the occipital foramen; (I) medially on mandibular base, with strong adductor tendon (Figs. 4b, d–f and 5a). M. craniomandibularis externus (M12)—(O) genal region, and from the posteriormost part of the head capsule, close to the occipital foramen and attachment area of M11; (I) laterally on mandibular base with adductor tendon (Figs. 4b, d–f and 5a). M. hypopharyngomandibularis (M13), a thin muscle—(O) from region of posteroventral surface of laminatentorium but exact point of origin not recognizable; (I) medially on mandibular base, relatively close to insertion of M11 (Figs. 4e and 5a).

Maxillae

The moderately deep maxillary grooves anteromesad the compound eyes have a smooth surface. They are mesally bordered by the anterior third of the lateral edge of the submentum, and posteriorly and laterally by a distinct curved line. A distinct lateral genal edge is present above the lateral maxillary base and extends anteriorly forming an acute lobe below the ventral mandibular base. The short transverse cardio is rounded postero-laterally and has nearly straight anterior and mesal edges (ca, Figs. 2c and 3d). It is mostly glabrous, with only four setae on its basal part. The basitropes is elongate and triangular, with a single seta at each corner (bs, Fig. 3d). The mediostipes (ms, Fig. 3d) is continuous with the lacinia, which is long and narrow (lc, Fig. 3c). Its mesal edge bears a semimembranous seam densely covered with microtrichia, while the ventral surface is mostly covered with small cuticular protuberances. The apex is strongly sclerotized and hook-shaped. The subapical edge bears a dense set of thorn-like structures (Fig. 3c). The galea is composed of a short proximal segment, subtriangular in ventral view (ga, Fig. 3c). The distal segment is elongate, with a dense apical brush of setae and a fringe of long setae along the mesal edge of the dorsal side. Small finger-like cuticular protuberances are present on the anteromesal edge of the ventral face (Fig. 3c, d). The palpifer is elongate and subtriangular (pf, Fig. 3c). The palp is four-segmented (mp, Fig. 3c). Palpomere I is very short, whereas
the palpomeres II–IV are about twice as long as the maximum width. Palpomeres II and III are distinctly widening distally and slightly curved inwards. Palpomere IV is conical and apically rounded. A parallel arrangement of about 20 digitiform sensilla is present on its dorsal surface (dgs, Fig. 3c, f). Small pore plates are distributed over the surface of palpomeres III and IV (Fig. 3f, g), and a sensory field is present at the apex of palpomere IV.

Musculature. *M. craniocardinalis externus* (M15)—(O) anterolateral genae, at the level of the compound eyes (thin, single bundle), posterior head region, laterally on basal portion of gular ridge; (I) end of dorsally directed process of cardo base (Figs. 4d–f and 5b). *M. tentoriocardinalis* (M17)—(O) along lateral surface of posterior tentorial arms; (I) sclerotized process of cardo base (Figs. 4d–f and 5b). *M. tentoriostipitalis* (M18)—(O) lateral face of median vertical lamella and median vertical plate of laminotentorium, and from anterior extension of the posterior tentorial arm; (I) ventromesally on stipes (Figs. 4c–e and 5b). *M. craniolacinialis* (M19)—(O) posterior region of ventral surface of the head capsule, immediately posterior to main attachment area of M15; (I) on the membranous area close to the basal margin of lacinia (Figs. 4c–f and 5b).

*M. stipitostipitalis* (M20), arranged diagonally on base of maxillae—(O) lateral base of basistipes; (I) base of lacinia. *M. stipitostipitalis* (M21), arranged longitudinally on maxillary base—(O) lateral base of basistipes, anterior to origin of M20; (I) base of galea (Fig. 4a, b). *M. stipitopalpalis internus* (M23), short muscle arranged vertically in basal part of maxillae between insertions of M20 and M21—(O) medistipes; (I) palpifer (Fig. 4b). *M. palpopalpalpis maxillae primus* (M24), arranged longitudinally within palpifer—(O) base of palpifer; (I) base of basal maxillary palpomere (Fig. 4a, b).

Labium

The anteriorly widening submentum is about as large as the mentum and anteriorly delimited by a very slightly convex transverse suture. Its basal margin is not separated from the gula by a suture but the border is clearly indicated by the anterior transverse gular depression and a distinct angle formed by both areas (Fig. 2c). The anterior third of the lateral submental edge forms the mesial margin of the maxillary groove and is adjacent to the cardo. The broad plate-like mentum is trapezoidal and narrowing anteriorly. The converging lateral margins are almost straight (Fig. 2c) and adjacent with the medioptes. The posterolateral edges of the mentum are levellied with the anterior cardinal margin. The anterior margin is slightly convex. The submental and mental surface is transversely striated and bears a vestiture of short setae, with a slightly higher density on the submentum. In ventral view, the mentum covers part of the base of the prementum. The prementum is completely divided medially (pmt, Fig. 3j).

Epipharynx

An epipharyngeal process projects medially from the posterior margin of the ventral side of the labrum (lep, Fig. 3i). This structure is subtriangular, relatively broad at its base and narrowing towards its apex. It is densely covered with posteriorly directed microtrichia (Fig. 3i). The posterior part of the epipharynx (not shown in Fig. 3i) is laterally fused with the corresponding edges of posterior hypopharynx forming a short, closed prepharyngeal tube (Fig. 6a).

Musculature: *M. clypeopalpalaides* (M43), multiple short bundles—(O) along the clypeal area; (I) the dorsal wall of the cibarium (Figs. 4d and 6). *M. clypeobuccalis* (M44)—(O) frons, anterior to M45; (I) the dorsolateral wall of the posterior epipharynx.
Hypopharynx

The hypopharynx is firmly connected with the posterior part of the prementum (hph, Fig. 3). It is composed of a pair of elongated, posteriorly divergent lobes. The dorsal surface of each of them is densely covered with multiple oblique parallel rows of microtrichia. A conspicuous tuft of erect microtrichia is present medially on the posterior part of the dorsal surface (ht, Fig. 3). The concave lateral walls of the hypopharynx are sclerotized and medially fused (Fig. 3k).

Musculature: M. frontohypopharyngalis (M41)—(O) large area on posterior frons; (I) large attachment area on postero-lateral hypopharyngeal apodeme (Figs. 3k, 4e, f, and 6). M. tentoriohypopharyngalis (M42)—(O) paramedially on the submentum, between origin of M28 and M29; (I) medially on ventral prementum strut (Figs. 3k, 4e, and 6a). Additionally, an extrinsic muscle (“Mx” in Jäck et al. 2000; see details in the discussion) of unclear homology (not covered by v. Käfer, 1963) originates from the gena, approximately at the level of the hind edge of the compound eyes (Mx, Fig. 4d, c). It is laterally attached to the membranous area linked to the ventral hypopharyngeal surface.

Pharynx

The pharynx displays a typical pattern with longitudinal folds for muscle attachment in cross section (phr, Fig. 4i), with a circular to ovoid lumen gradually narrowing towards the posterior cephalic region, before it abruptly expands into a large esophagus. The pharyngeal wall is very thin.

Musculature: M. frontobuccalis anterior (M45)—(O) frons, anterior to M46; (I) dorsolaterally on the precerbral pharynx, anterior to M46 (Figs. 4e and 6). M. frontobuccalis posterior (M46), several bundles—(O) posterior frons; (I) dorsolaterally on precerbral part of the pharynx (Figs. 4f and 6). M. tentorio buccalis anterior (M48), long, thin-paired muscle stretching between tritocerebral commissure and subesophageal ganglion—(O) antemedially on tentorial bridge; (I) medially on ventral margin of the posterior hypopharynx (Figs. 3k, 4e, f, and 6). M. tentorio buccalis posterior (M50)—(O) tentorial bridge; (I) ventromedially on the anterior pharynx. M. verticopharyngalis (M51)—absent. M. tentorioropharyngalis (M52), three bundles—(O) mesally on gular ridges; (I) ventrolaterally on the posterior pharynx.

Brain and subesophageal ganglion

The medium-sized brain in the posterodorsal head region does not reach the occipital foramen posteriorly (cer, Figs. 4f and 6). It lies below the vertex dorsally and is enclosed by M11 laterally, the pharynx ventrally, and M46 anteriorly. The frontal ganglion is well developed (gfr, Figs. 4f and 6a) and also the frontal connectives and tritocerebral commissure. The subesophageal ganglion fills the entire space between the gula, posterior tentorial arms, and tentorial bridge. Anteriorly, it reaches the laminatentorium (see, Fig. 6a).

Discussion

Head capsule, appendages, and labiohypopharyngeal complex

A series of apparent plesiomorphies documented in Catops had already been identified as typical for microphagous Staphylinoidae, and possible as groundplan features of the entire superfamily (Betz et al. 2003). This includes a cibarial roof with parallel rows of microtrichia corresponding to similar elements on the dorsal molar surface (Fig. 3a), brush-, comb-, or rake-like maxillary structures (Fig. 3e), mandibles with a subapical hyalineous or hairy prostheca and a well-developed mola with grading surfaces (Fig. 3b), and epipharyngeal and hypopharyngeal median tufts of posteriorly directed hairs (Fig. 3i, j). Most of these characteristics were also observed in Myxophaga (Anton and Beutel 2006) and polyphagan groups outside Staphylinoidae (Beutel et al. 2001; Anton and Beutel 2004, 2012; Anton et al. 2016). The basal coleopteran interrelationships revealed in a comprehensive recent study (McKenna et al. 2015) suggest that a similar configuration may be a groundplan feature of the entire Coleoptera, with independent losses in Adephaga and Archostemata, as previously hypothesized by Beutel et al. (2001). In contrast to the apparently conservative entire complex, some components of the mandibles and maxillae (e.g., prostheca, mandibular apex, retinaculum, galea, lucinia) are very diverse, even between less inclusive taxa such as for instance Leiodidae (e.g., Betz et al. 2003; Moldovan et al. 2004). This variability probably reflects the strong selective pressures involving the paired mouthparts in the context of preferred food material (Betz et al. 2003). The high variability impedes the groundplan reconstruction of these structures for Leiodidae or Cholevinia. Nevertheless, unlike other leiodids, the species studied here is not specialized on a particular food substrate or habitat, which likely represents the plesiomorphic way of life among Cholevinia.

Another noteworthy character confirmed for Catops is the strong constriction of the labiohypopharyngeal complex (Fig. 3k), which occurs at the level of the basal maxillary portion. As a result, the labiohypopharyngeal complex appears hourglass-shaped in cross section. This feature has been found in many other staphyliniform beetles (e.g., Jäck et al. 2000; Beutel et al. 2001; Beutel et al. 2003; Anton and Beutel 2004; Weide and Betz 2009), and more recently in the scarabaeoid Giaresis (Anton and Beutel 2012). The absence in some subordinate taxa such as the leiodine genus
Agathidium (Weide and Betz 2009) is apparently due to secondary modification. The presence of this derived condition has been postulated as autapomorphy of a clade Staphyliniformia including Scarabaeoidea (Beutel and Leschen 2005), which is equivalent with Staphyliniformia in a narrower sense + Scarabaeoidea as recovered by McKenna et al. (2015a).

Based on molecular data, McKenna et al. (2015a) recovered Leiodidae as monophyletic except for the unexpected position of Colon as sister to Hydraenidae + Ptilidae. Likewise, Cholevinae was recovered as monophyletic with the exclusion of Afrocatops (Oriotocatops) (McKenna et al. 2015a), and with the exclusion of Eucatops (Eucatopini) in Fresneda et al. (2011). Specific external features documented here for the head of Catops have been considered as potential autapomorphies of Leiodidae (Newton 2016). This includes a five-segmented antennal club with the second club antennomere smaller than the first and third (Fig. 2k), and the presence of pericentral grooves filled with sensilla (Fig. 2h, i) on antennomeres 7, 9, and 10. An unusual derived feature of Cholevinae is the head shape firmly connected to the pronotum with the postdorsal border of the expanded part of the head abutting the anterior pronotal edge (Fig. 2c). In many species of Leptodirini, however, this characteristic is secondarily modified, presumably as a result of the morphological changes associated to subterranean habits. As described here for Catops (Fig. 2c), a conspicuous genal fold covers the posterior face of the compound eyes in cholevines as a whole, although it has been lost in several eyeless Leptodirini. This trait differentiates Cholevinae from most other leiodids, but a similar condition is found in the head of the few members of Platypsyllinae even though the eyes are missing. The presence of the postocular genal fold in the mentioned groups as well as its occurrence in Hydraenidae (Jäch et al. 2000; Beutel et al. 2003) and within Hydrophiloidea (Beutel 1994; Beutel et al. 2001; Anton and Beutel 2004) probably reflects independent gains.

A set of cryptic pore plates on the preapical and apical maxillary palpomere of Catops (see detail in Fig. 3r, g) has not been reported before in the literature. These structures are present in many species of Cholevinae, including representatives of the most diverse tribes, such as Anemadini, Cholevini, Plomaphagini, and Leptodirini (CAC pers. obs.). Similar structures were not found in Eucatopini and Oriotocatopini. They are also lacking in outgroup taxa such as in Camiariinae (Agytodes), Leiodinae (Colenisia, Decuria, Zeadiolopus), Platypsyllinae (Leptinus), and in Agyridinae (Zeucreophulus) (CAC pers. obs.). It is uncertain if the pore plates are associated to maxillary glands, since we could not detect any evidence based on the μCT scans. Due to its very small size, the pore plate may have been overlooked in some groups, but based on the available data, it seems to be apomorphic for a group inside the subfamily—i.e., Cholevinae minus Eucatopini and Oriotocatopini. The presence of an apical maxillary palpomere shorter than or at most as long as the subapical one, as documented in Catops (Fig. 2c, d), is a derived condition also shared by Cholevinae with the exception of Eucatopini and Oriotocatopini, whose apical maxillary palpomere is elongate. As mentioned above, Oriotocatopini and Eucatopini have been phylogenetically isolated from the remaining Cholevinae in recent molecular analyses (Fresneda et al. 2011; McKenna et al. 2015a). More data are required to understand their phylogenetic position within Leiodidae.

**Tentorium and cephalic musculature**

The internal cephalic structures of Catops are mainly pleiomorphic. The tentorium agrees with the configuration suggested for the groundplan of Staphylinidae (Weide et al. 2014), formed by paired anterior, dorsal and posterior arms, a fused laminentotrium, and an uninterrupted tentorial bridge. In contrast, in the related leiodid Agathidium and Agyrtdiae, the dorsal tentorial arms are missing in the former and the laminentotrium is unfolded in the latter (Weide et al. 2014). A laminentotrium is lacking in the highly miniaturized Philiidae (Polivov and Beutel 2009).

The pattern of the muscles is similar to what is found in other staphylinoid taxa (e.g., Jäch et al. 2000; Beutel et al. 2003) and is close to the hypothesized groundplan of Staphylinidae, which according to Weide et al. (2010) is composed by the following muscles: 1, 2, 4, 7, 9, 11, 12, 16, 17–19, Mx, 28–30, 34, 41, 43–46, 48, and 50. The muscle “Mx” was first described in Hydraenidae (Jäch et al. 2000) and later reported in other staphylinoid groups such as Agyrtdiae and Staphylinidae (Oxytelinae and Omalinae: Weide and Betz 2009; Proteininae: Anton and Beutel 2012). It originates on the genal region and inserts on the membranous area between the maxilla and hypopharynx. Whereas it is present in Catops, the muscle was not found in the leiodid Agathidium (Leiodinae, Agathidini) and is apparently also missing in Philiidae (Polivov and Beutel 2009) and in the staphylinid Tachyporinae and Ateocharinae (Weide and Betz 2009). The occurrence of “Mx” in Hydrophilidae (“M19a” in Spercheinae: Beutel et al. 2001; “Mx2” in Helophorinae: Anton and Beutel 2004; Hydrochidae: Anton and Beutel 2012) and Sphaeridae (Anton and Beutel 2012) suggests its presence in the groundplan of Staphyliniformia. However, the recent discovery of a likely homologue in Geotrupidae and Scarabaeidae (named as “M. craniobasimaxillaris”) by Anton and Beutel (2012) suggests that this unusual muscle has appeared even earlier in the evolution of beetles, with independent loss in several groups. The presence of “Mx” in the groundplan of Coleoptera can be ruled out as it was not found in Adephaga (Dressler and Beutel 2010), Archostemata (Hörnschemeyer et al. 2002, 2006; Beutel...
and Myxophaga (Anton and Beutel 2006) and is also absent in Dascillidae (Anton and Beutel 2012) and cecujiform taxa (Schneider 1981; Ge et al. 2015). Like the hypopharynx strongly narrowed between the maxillary bases, this is likely a groundplan apomorphy of a clade comprising Staphylinoidea and Scarabaeoidea.

The presence of *M. frontophasyngealis* (M9) is ancestral for Coleoptera (e.g., Weide and Betz 2009) and is preserved in the groundplan of Staphylinoidea. However, it is missing in *Catops* and also in *Agathidium* (Weide and Betz 2009), in the agyrtid *Necrophila* (Weide and Betz 2009), and in the ptilid *Mikado* (Polilov and Beutel 2009). A lineage formed by Leiodidae + Agyrtaeae together with Hydrenidae + Ptilidae has been placed as the sister group of the remaining Staphylinoidea (Beutel and Leesch 2005; McKenna et al. 2015a). The presence of *M. frontophasyngealis* in Hydrenidae (Jäch et al. 2000; Beutel et al. 2003) shows that it is present in the groundplan of this clade and was apparently reduced two or more times independently.

The presence of *M. hypopharyngomandibularis* (M13) in Cholevinae is apparently a pleiomorphic feature. Its exact point of origin could not be clarified in *Catops*, but it is inserted medially on the dorsal internal surface of the mandible (Fig. 5a). The muscle was not identified in detailed studies on the head of Adepaha (Dressler and Beutel 2010), Archosoma (Hörnschemeyer et al. 2002, 2006; Beutel et al. 2008b), Myxophaga (Anton and Beutel 2006), and of various lineages of Polyhaga (e.g., Beutel et al. 2003; Weide and Betz 2009; Anton and Beutel 2012; Anton et al. 2016). However, it is documented for *Gyrinus* and *Dermestes* (Honomichl 1975, 1976: as *M. tentoriomandibularis*) and also in *Lyto* (Schneider 1981) and is therefore very likely ancestral for Coleoptera. It also occurs in many other groups of insects, for instance in the primarily wingless *Archaognatha* and Zygontoma (Blanke et al. 2012), in Odomata and Ephemeroptera (Blanke et al. 2012), in polyneopteran orders (Wipfler et al. 2011), and in different groups of Holometabola including the basal Hymenoptera and Mecoptera (Beutel and Vielmersen 2007; Beutel et al. 2008a). It is usually extremely thin and may have been overlooked in some studies. Nevertheless, it was apparently reduced several or many times independently in Coleoptera.

Among the staphyliniform beetles hitherto investigated, *M. tentoriopharyngyalalis* (M42) is uniquely present in *Catops*. It was also identified in Hydrenidae (Hydraena: Jäch et al. 2000; Ochthebus and Limnebius: Beutel et al. 2003) with a submental origin, even though in this case the homology remains disputable. In any case, the presence in *Catops* is in contrast to the suggested absence in the groundplan of Staphylinoidea (Weide et al. 2010). Moreover, the muscle was also found in *Glanesia* (Anton and Beutel 2012), a basal representative of Scarabaeoidea (see McKenna et al. 2015a), suggesting its presence in the groundplan of Staphylinoformia + Scarabaeoidea. *M. tentoriobuccalis anterior* (M48) was misidentified in many studies as M42 (e.g., Beutel et al. 2001; Anton and Beutel 2004, 2006; Weide and Betz 2009), until this issue was clarified in Beutel et al. (2009) and Weide et al. (2010).

### Functional interpretation of the feeding apparatus

*C. ventricosus* is frequently found in caves or under leaf litter of forested landscapes. They forage on debris derived from different kinds of decaying organic matter such as carrion, dung, or plant material (Salgado 1985; Salgado-Costas and Vázquez-Blanco 1993; Salgado and Fernández 1998). However, facultative feeding on fungal spores does also occur. The functional configuration of the feeding apparatus is similar to the condition encountered in most other staphylinoids feeding on small particles (see Betz et al. 2003). The robust brush of hairs on the apex of the galea plays a major role in food acquisition, although the distal portion of the lacinia is probably also involved in this function. The interactions between the maxillary endite lobes of both sides transport food substrate towards the upper side of the labiohypopharyngyal complex and the proenal cavity. The mandibles likely support this process, sweeping food particles towards the galeae and laciniae with the dense prosthecal brushes, and presumably also onto the dorsal labiohypopharyngyal surface. In the median region of the transition zone of the dorsal prementum and anterior hypopharynx, an area surrounded by a dense field of microtrichia probably concentrates the collected food particles, which are subsequently transported into the proenal cavity. This process is supported by retraction of the prementum, induced by contractions of *S. submentopraenalis* (M28), *M. tentoriocapralimentalis inferior* (M29), and *M. tentoriopharyngyalalis* (M42). Grinding takes place between the molar surfaces of the mandibles. The epi- and hypopharyngyal tufts of microtrichia along with the prostheca keep the food particles within the proenal space while the material is processed. Parallel rows of microtrichia on the ventral and dorsal molar surfaces interact with similar elements on the cibarial roof and hypopharynx, respectively, transporting the fine particles towards the anatomical mouth. *M. frontopharyngyalalis* (M44) probably supports this process by elevating the hypopharynx, thereby narrowing the proenal space and bringing mandibles and hypopharynx in closer contact. In some staphylinids, this process probably results in a trituration of the food substrate by interaction of grinding structures on the ventral molar surface with correspondent structures of the hypopharynx (Weide et al. 2010). This is unlikely in the case of *Catops* as grinding structures are lacking on the hypopharynx. *M. tentoriobuccalis anterior* (M48) is also involved in the transport by retracting the hypopharynx, which supports the shifting of substrate towards the anatomical mouth (Weide et al. 2014).
Head morphology of Cholevinae

Conclusions

The muscle equipment of the head of Catops is largely plesiomorphic, probably close to the groundplan of Staphyliniformia. Our study shows that most cephalic muscles reported here are shared with other staphylinoid lineages. This suggests that the head anatomy is a rather conserved character system in the evolution of this highly diverse superfamily. The same applies to the general traits of the mouthparts and epipodid hypopharynx, mainly characterized by plesiomorphic traits correlated to feeding on small particles. However, Catops also displays typical apomorphy features that define the head of Leiodidae and Cholevinae, such as the interrupted five-segmented antennal club and the characteristic head capsule molded to fit with the anterior pronotal edge, respectively. Catops has been used as an outgroup representative in higher level phylogenetic inferences focused on head characters (e.g., Polilov and Beutel 2009; Beutel et al. 2010; Dressler and Beutel 2010; Randolf et al. 2014), although its cephalic morphology has not yet been documented or described in detail. The present contribution is the first to provide a complete characterization of the head structures of a representative of Cholevinae, offering a broad repertoire of characters potentially useful for future phylogenetic studies. When detailed information on cephalic structures of a broader spectrum of staphylinoid beetles becomes available, the recent molecular phylogeny of McKenna et al. (2015a) will be an excellent framework for tracing the character transformations and reaching a deeper understanding of the evolution of the head in this highly successful superfamily.

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References


3.6 Study VI


**Abstract:** External and internal structures of 1st instar larvae of *Tenomera mucida* are described and illustrated in detail. The larvae display previously identified archostematan autapomorphies such as the posterdorsal and posteroventral cephalic emarginations, the median endocarina, a distinctly reduced epicranial suture, the lateral cardinal sclerite, the loss of *M*. craniocardinalis, a sclerotized prominent ligula, a distinctly reduced tentorium, the transverse muscle of the posterior head capsule, tergal and sternal ampullae, and the strongly muscularized loop of the hind gut. Unusual plesiomorphies are the presence of a well-developed *M*. frontolabralis (M8) and a tentoriomandibular muscle. The presence of two extrinsic premental muscles and a subdivided postlabium are also plesiomorphic features. A derived character state described for the first time in an archostematan larva is the presence of glands in the anterior and posterior abdomen. The postcephalic musculature is similar to the condition found in *Micromalthus* and *Rhipsideigma*. The neck musculature is strongly developed, and also the dorsal and ventral longitudinal muscles, probably in correlation with wood-boring habits. The leg muscles are only moderately sized. Well-developed muscles of the eversibles lobes of segment IX are an apomorphy of Cupedidae. The cephalic central nervous system of the 1st instars is affected by the small size: the brain and suboesophageal ganglion are shifted to the prothorax. The postcephalic part is plesiomorphic, with three and eight distinctly separated ganglia in the thorax and abdomen, respectively. The phylogenetic results tentatively suggest a placement of Archostemata as sisteraxon of the other three beetle suborders. The monophyly of Archostemata is strongly supported but the interrelationships of the families are largely unresolved. Micromalthidae are placed as sistergroup of Crowsoniellidae in analyses of larval and adult morphological characters. Potential larval synapomorphies of Micromalthidae and Cupedidae are the transverse and laterally rounded head, the absence of stemmata, the shortened distal part of the mandibles, the presence of sternal asperities, the eversible lobes of segment IX and the caudal process of tergum IX. Cupedidae excl. Priacma and Paracupes are monophyletic. A cupedid subgroup well supported by larval features comprises the genera *Tenomera* and *Rhipsideigma*, and possibly also *Cupes* (larval features unknown). The reconstruction of the phylogeny and character evolution of Archostemata is still greatly impeded by the scarcity of data, especially the lacking information on internal features and larval characters.

**Significance in the present thesis:** Archostemata were often considered as ancestral beetles. The first detailed study of the larval anatomy of a species of Cupedidae closes an important gap of information. It elucidates structural transformations linked with feeding on wood in the immature stages. Adults of Archostemata are characterized by a tendency towards aphagy.

**Own contribution:** 60 %
Morphology of the first instar larva of Tenomerga mucida (Chevrolat, 1829) (Coleoptera: Archostemata: Cupedidae)

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Abstract
External and internal structures of 1st instar larva of Tenomerga mucida are described and illustrated in detail. The larvae display previously identified archostematan autapomorphies such as the postero-dorsal and posteroventral cephalic evaginations, the median endoconus, a distinctly reduced epiproct, the lateral cardinal sclerite, the loss of M. cranio-cardinalis, a sclerotized prominent ligula, a distinct reduced testitrus, the transverse muscle of the posterior head capsule, ventral and sternal aumberculae, and the strongly musculatered loop of the hind gut. Unusual plesiomorphies are the presence of a well-developed M. frontolabialis and a tentoioesomandibular muscle. The presence of two extrinsic preenatal muscles and a subdivided postabdomen are also plesiomorphic features. A derived character state described for the first time in an archostematan larva is the presence of glands in the anterior and posterior abdomen. The postephalic musculature is similar to the condition found in Litonotusidae and Rhipistegidae. The neck musculature is strongly developed, and also the dorsal and ventral longitudinal muscles, probably in correlation with wood-boring habits. The leg muscles are only moderately sized. Well-developed muscles of the eversibles lobes of segment IX are an autapomorphy of Cupedidae. The cephalic central nervous system of the 1st instar is affected by the small size the brain and suboesophageal ganglion are shifted to the prothorax. The postephalic part is plesiomorphic, with three and eight distinctly separated ganglia in the thorax and abdomen, respectively. The phylogenetic results tentatively suggest a placement of Archostemata as sister-group of the other three beetle suborders. The monophyly of Archostemata is strongly supported but the interrelationships of the families are largely unresolved. Micromaltidae are placed as sister-group of Cupedidae in analyses of larval and adult morphological characters. Potential larval synapomorphies of Micromaltidae and Cupedidae are the transverse and laterally rounded head, the absence of sternomata, the shortened distal part of the mandibles, the presence of a mental torus, the eversible lobes of segment IX and the caudal process of tegma IX. Cupedidae excl. Pronemata and Pronematon are monophyletic. A cupid subgroup well supported by larval features comprises the genera Tenomerga and Rhipistegia, and possibly also Cupes (larval features unknown). The reconstruction of the phylogeny and character evolution of Archostemata is still greatly impeded by the scarcity of data, especially the lacking information on internal features and larval characters.

Key words
Archostemata, Cupedidae, Tenomerga, larva, morphology, phylogeny.

1. Introduction

Species of Cupedidae and the closely related Ommatidae are the beetles with the maximum of preserved plesiomorphic features in the adult stage, corresponding derived conditions in all non-archostematan coleopteran groups (BEUTEL & HAAS 2000; BEUTEL et al. 2008; FREITRICH et al. 2009). The very small relic group Archostemata (ca. 40 spp.) also comprises Micromaltidae and Crowsonidae, and probably also Jaro-
Yavorskaya et al.: Larva of Tenomarga

idae (Hönschmeier 2005; Beutel et al. 2008). Micro- 
malthidae is monospecific and characterized by ma- 
aturity and a highly complicated life cycle (Pullock & 
Normark 2002). The single described species of 
Crocomellidae (pace 1975; Cronson 1975) is only 
known by the type series and the type locality in central 
Italy is debated (M. Ivie, pers. comm.). Only the holotype 
of the single extant species of Jurodidae is known. The 
female specimen of Sikhotaelinna fluidorum was found 
at the edge of a river in the Sikhoteal in Mountains in the 

Archostemata is strongly supported as a clade by lar- 
val features (e.g., Beutel et al. 2008). However, despite 
of considerable efforts to solve the problem, the posi- 
tion of the suborder Archostemata remains controversial. 
A basal placement among the four extant suborders of 
Coleoptera was suggested by Cronson (1953, 1981), and 
this was supported in analyses of extensive morphologi- 
cal data sets (Beutel & Haas 2000; Beutel et al. 2008; 
Friedrich et al. 2009). In contrast to this, based on char- 
acters of the wing base and wing venation, Kulâvová- 
Peck & Lawrence (1993, 2004) placed Polyphaga as the 
sistergroup of the remaining suborders, and Archostemata 
as sistergroup of a clade Myxophaga + Adephaga. The 
placement of Archostemata varies in molecular studies. 
Analyses of Hunt et al. (2007) (16S and 18S rRNA, cox1) 
yielded Archostemata as sistergroup of Myxophaga, 
and Adephaga as sistergroup of Polyphaga. Analyses of 
two nuclear and two mitochondrial genes (18S and 28S 
rRNA, cox1, rrl2) with a very extensive taxon sampling 
in the two large suborders resulted in basal Archostemata, 
and Myxophaga was placed as sistergroup of Adephaga 
+ Polyphaga (Bocak et al. 2014: 18S and 28S rRNA of 
ca. 8,000 spp., without outgroups). Hwone et al. (2006),

Fig. 1. SEM micrographs, 1st instar larva of Tenomarga mucida. A: dorsal view; B: lateral view; C: ventral view. Scale bar = 500 µm.

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based on analyses of transcriptions with a dense sampling in Adephaga and Polyphaga, but only Micromalthus debilis LeConte and Sphaerius sp. (Sphaeriusidae) as representatives of the two small suborders, suggested a pattern with basal Archostemata (without outgroup), followed by paraphyletic Adephaga, and a clade comprising the myzoplaugna genera Sphaerius and Polyphaga. The phylogeny of Archostemata and the family Cupedidae were analysed by Beutel et al. (2008) and Hornschmeyer (2009). In the latter study the genera Priscia and Paracupes were placed basally in Cupedidae and the genus Tonemerga was rendered polyphyletic.

The knowledge of the morphology of Archostemata has distinctly improved recently (e.g., Hornschmeyer et al. 2002, 2006; Beutel et al. 2008; Friedrich et al. 2009). However, the available information on immature stages is still very fragmentary (Boving & Craighead 1931; Fukuda 1938, 1941; Ross & Pothecary 1970, Lawrence 1991, 1999; Bresshnov 2004), even though a detailed knowledge of larval features, especially of 1st instars, is apparently very important for understanding the early evolution of beetles. Only two studies on the larval anatomy are presently known. The skeletonmuscular system of larvae of Micromalthus debilis (1st instar and cerambycid larva) was described by Beutel & Hornschmeyer (2002a) and external and internal features of mature larvae of the cupedid species Rhyssideigna raffreyi by Beutel & Hornschmeyer (2002b).

The very limited number of larval studies is largely due to the enormous difficulty of finding suitable material. The recent collection and successful breeding of immatures of the Japanese species Tonemerga mucida (Chevrolet, 1829) (Fig. 1) has stimulated us to carry out a detailed anatomical study of the 1st instar larvae. The detailed documentation of external and internal features is the primary aim of this study. However, the findings are also evaluated phylogenetically largely based on data sets published earlier (Beutel & Hornschmeyer 2002a,b; Beutel et al. 2008) and discussed with respect to their implications for the early evolution of Coleoptera, and the evolution in Archostemata and Cupedidae.

2. Material and methods

2.1. List of larva examined

Cupedidae: Rhyssideigna raffreyi Neboiss (last instar, Pampel’s fluid, ethanol), Tonemerga concolor (Westwood, 1830) (1st [ca. 20 specimens] and last instar [1 specimen], FAE).

Micromalthidae: Micromalthus debilis (Boëtius) (from decaying wood, collected in Madison, Wisconsin by D.K. Young).

Ommatidae: Ommat Newman sp. (ethanol, examined at the Australian National Insect Collection, Canberra – ANIC).

In late April of 2014 brown-rotted logs of a willow (Salix chaenomeloides) were collected at the riverbed of Edogawa river, Yoskawa, Saitama, Japan. The material was taken back to the laboratory of the Sugadaira Montane Research Center (Sugadaira, Nagano). In June many adults of Tonemerga mucida emerged from the logs incubated at 28°C. They were fed on diluted honey and water daily.

2.2. Anatomy

Microtome sectioning and scanning electron microscopy (SEM) were applied. Transverse semithin sections were made of the entire body. The specimens were embedded in araldite CY 2126 (Agar ScientifW, Stansted, Essex, England) and cut at 1 μm using a microtome RM 350 (Mecron, Walldorf, Germany) equipped with a diamond knife. Sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co KG). Chroma, Münster, Germany). Pictures were taken of every second section using a light microscope (Zeiss Axiosplan, Germany) equipped with a camera (PixelLink Capture OEM). The images were aligned using Avizo 5.3.1 software (Visage Imaging, Berlin, Germany). Based on the aligned image stack the arrangement of internal structures each element was traced manually to reconstruct three-dimensional images. MAYA? (Alias Wavefront, Toronto/Ontario, Canada) was used for smoothing and coloring.

Scanning electron microscopy (SEM, Philips XL 30 ESEM) was used to document surface structures. Larvae were dried at the critical point (Emitech K805 critical point dryer), sputter-coated with gold (Emitech K500) and mounted on the tip of a fine needle and fixed on a rotatable specimen holder (Pohl 2010).

2.3. Cladistic analyses

Features of 1st instars of T. mucida were coded and entered in a data matrix (see Electronic Supplement File 1) of 102 morphological characters (66 characters of adults from Beutel et al. 2008; see Electronic Supplement File 2). The larval part of the data set was also based on earlier studies (Beutel & Hornschmeyer 2002a,b; Beutel et al. 2008) but modified. The parsimony analysis was carried out with NONA (patchet, 1000 replicates) and TNT (traditional search) (Goloboff 1995; Goloboff et al. 2000). Bremer support values (Bremer 1994) were calculated with NONA.
thoracic muscles. To avoid confusion only numbers are used for head muscles in illustrations, whereas an "M" (e.g., M9) is added in the case of muscles of the thorax.

3. Morphological results

3.1. General appearance

The larvae are usually slender (Figs. 1, 2) but some specimens appear distinctly more compact, probably depending on the food uptake after hatching from the egg. Most larvae are ca. 1.8 mm long. The head is strongly sclerotized (Figs. 3–7), whereas the postcephalic body is largely semienambranous, with the exception of some prothoracic legs, the legs, and the terminal abdominal segment IX (Fig. 1). The postcephalic body is only slightly flattened dorsoventrally. Fairly indistinct tergal ampullae are present on most postcephalic segments and also sternal counterparts on the ventral side (Figs. 1, 8). Large parts of the unsclerotized surface of the thorax and abdomen are covered with minute spine-like asperities. The abdominal apex is formed by a tapering apically truncate process of the sclerotized tergite IX (Fig. 8B).
A defined cephalic neck region is absent. Posterior median exogastrations are present on the dorsal and ventral sides, the former rather shallow, whereas the latter is deep and triangular (Fig. 4). A vestiture of long, medium sized and very short setae is present. The distribution is shown in Figs. 4 and 7. Stemmata are absent. The labrum is movably connected with the anterior clypeal margin by an internal membraneous fold (Figs. 4, 5). The uniformly sclerotized clypeus is very wide posteriorly but distinctly narrowed and trapezoid anteriorly. It is separated from the frons by a transverse external furrow, which is distinct laterally but obliterated medially (Fig. 4). The frons is largely fused with the adjacent parts of the head capsule, but remnants of a V-shaped epicranial suture are present on the postero-dorsal cephalic region, anterior the dorsal exogastration. The short and strongly diverging vestiges of the frontal sutures are recognisable as distinct furrows (Fig. 7). The coronal suture is missing. Dorsomedially the head is divided by a strongly developed, unpaired and uniforked median mandibula (Fig. 5). The extensive sclerotized structure reaches the posterior clypeal margin anteriorly. The anterolateral cephalic region bearing the antennal insertion area is sclerotized and slightly protruding. An externally exposed articulatory membrane of the antenna is not present. The ventral side of the head is as long as the dorsal side. The maxillary grooves are very deep and separated from each other by the posterior labrum and the posteriorly adjacent sclerotized regions (Fig. 4B). Very faintly visible oblique impressions are present posterior to the insertion of the carico, but hypostomal and ventral epicranial ridges are missing. The ventromedian closure of the posterior head is formed by a para-
belic scleritization with unclear homology (Fig. 4B). It is
either a product of fusion of the postlabium and gula or
only the former element. The extensive ventral triangular
emargination of the head capsule is filled out with the
ventromedian cervical membrane.

3.3. Internal skeletal structures

Figs. 5, 6

The tentorium is distinctly reduced. Well-developed cynthia
drical anterior arms are present but end in the anterior third
of the head capsule. They are widely separated from the
robust ridge-like posterior arms. Dorsal arms and the ten-
torial bridge are missing and gular ridges are also absent.

Musculature: a strongly developed transverse muscle
connects both halves of the head capsule at the postero-
ventral edges (Fig. 5). The homology is unclear.

3.4. Labrum

Figs. 3A, 4A, 5

The sclerotized transverse labrum articulates with the
anterior clamped margin. The anterior margin is nearly
straight and the anterior lateral corners rounded. Two pairs
of setae are present on the dorsal side, the ones closer to
the midline ca. 3 times as long as the lateral ones. Three
pairs of strong and moderately long setae are inserted on
the anterior and anterolateral margin, and one pair of
broad, almost claw-like setae on the ventral side, close to
the anterolateral edge.

Musculature (Figs. 5, 6A): M7 (M. labroopharyngalis):
absent, M8 (M. frontolabralis): moderately sized muscle,
distinctly narrowing towards its origin, O: anterior edge
doorsal endocarina, I: upper posterior lateral margin; M9
(M. frontoopharyngalis): moderately sized, also nar-
rowing towards the origin, converging with M8, anterior
edge of dorsal endocarina, together with M8, I: laterally
on the surface of the ventral wall of the labrum (pharyn-
xis), but distinctly separated from the lateral edge.

3.5. Antenna

Figs. 4, 7

The very short two-segmented antenna is inserted on the
anterolateral prominence without an externally exposed
articulatory membrane. It is anteriorly directed and
slightly inclined towards the median line. The stout basal
antennomere is wider (maximum diameter) than long and
slightly compressed dorsoventrally. It is rounded along
its apical edge and longer mesally than laterally. The cy-
lindrical distal antennomere is only ca. half as long and
half as wide as the basal one. On its rounded apical part
it bears several short, stiff setae. A large hyalineous senso-

3.6. Mandible

Figs. 4–7

The mandibles are strongly developed, with three very
strong triangular teeth arranged in an oblique row and
a prominent basal molar area. The apical and subap-
cal teeth are longer than wide and apically acuminate,
whereas the proximal one is broader and less pointed.
Two long setae are inserted in deep articulatory pores on
the dorsolateral area. A penicillum is not present and a
lacinia mobilis is also missing.

Musculature (Figs. 5, 6B, C): M11 (M. cranioanalis
internus): largest muscle of the head, O: extensive
parts of the dorsal, dorsolateral, lateral and ventrolateral
areas of the head capsule, I: adductor tendon, M12 (M.
cranioanalis externus): moderately large, O: later-
ally from the head capsule, between bundles of M11,
I: adductor tendon. M13 (M. cranioanalis internus): finer,
accompanied by a very thin nerve, O: anterior tentorial
arm, I: dorsally on the base of the mandible, close to the
mesal margin.

3.7. Maxilla

Figs. 3B, 4B, 6

The maxilla is inserted in a very deep fossa maxillaris. It
is composed of cardo, stipes, gales, lacina and a three-
segmented palp. An exposed articulatory membrane is
not present but a large, ovoid pad-shaped sclerite is in-
serted between the cardo and basal stipes and the poste-
rior labium. The cardo is divided into a short and curved
main sclerite and a smaller rounded lateral piece. Setae
are missing. The stipeo-cardinal hinge is indistinct but
a deep notch is present between the slightly convex lat-
eral stiptal edge and the lateral cardinal sclerite. The
large stipes is not subdivided into a proximal basistipes
and a distal medistipes. It bears a short and a very long
setae on its proximal region. The palpifer is recognisable
but very indistinctly demarcated from the lateral stiptal
area. Anteriorly the stipes is completely fused with the
large lacina, which is straight along its glabrous mesal
edge and bears a group of short, stiff setae on the me-

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On its nearly straight apical edge it bears several medium sized stiff setae. The short palp is composed of a broad and very short basal palpomere, a slightly longer and broad intermediate segment, and an apical palpomere, which is distinctly longer, slightly narrower and cylindrical. Its slightly rounded apex bears a group of short stiff setae. Two medium sized setae are inserted ventrally and laterally on palpomere 2, close to its anterior edge.

Musculature (Figs. 5B, D, 6B-C): M15 (M. craniocephalum), absent; M17 (M. tentoriorostralis), short cone-shaped muscle, O: basal part of the posterior tentorial arm, I: ventral surface of the cardo; M18 (M. tentorioptistalis), short, similar to M17, posterior tentorial arm, close to M17, I: ventral stipital surface; M19 (M. cranialocelis), O: ventrally on the posterior margin of the head capsule, I: basis of labium, without a tendon, M22, 23 (M. stipitopalpis externus and internus), the palp muscles could not be clearly identified on the available sections.

3.8. Labium

Figs. 3B, 4B, 6

The interpretation of the posterior elements is difficult. The proximal parabolic sclerite is likely the submentum, possibly fused with a gula. A very short area anterior to it possibly represents the mentum but is only indistinctly separated from the prementum and immovably connected with it. At anterior edge it bears a pair of long setae. The prementum is large. Anteromedially it bears a strongly sclerotized triangular and apically pointed ligula with irregular edges. A transverse line and a pair of medium-sized setae are present on its basal region. The short two-segmented pals are inserted laterally, distinctly proximad the apex of the labium. A palpiger is not present. Several short sensilla are inserted on the rounded apex of palpomere 2, which is relatively long, but distinctly smaller than the apical maxillary palpomere.

Musculature (Figs. 5, 6B): M28 (M. submentoprorostralis), absent; M29 (M. tentorioptoporostralis inferior), relatively short muscle, O: ventral wall of head capsule, area of origin adjacent to posterior labial margin, I: laterally at the premental edge, M30 (M. tentorioptoporostralis superior), short muscle, O: not visible on available microtome section series, probably immediately posterior to the hind margin of the prementum, I: dorsolaterally on the premental edge, M34 (M. praementialis ext.), absent.

3.9. Epipharynx

Figs. 5, 6B, see also labrum

The anterior part including the ventral wall of the labrum is semimembranous and glabrous. It is reinforced
by paired longitudinal parmedian ridges. The middle epipharyngeal region is laterally fused with the mandibular bases and forms the dorsal wall of a short and wide preoral chamber, which is ventrally delimited by the hypopharynx but open ventrolaterally. The posteriormost epipharyngeal part forms a short prepharyngeal tube together with a short posteriormost hypopharyngeal section. The unsclerotized cuticle of the preoral chamber and prepharynx is unusually thick.

Musculature (Figs. 5C, 6B): M43 (M. Clypeopalatalis): a fan-shaped muscle with two areas of origin, O: anterior end of the median endocarina and dorsal wall of head capsule, I: dorsally on the roof of the anterior epipharynx. Transverse epipharyngeal muscles are absent.

3.10. Hypopharynx

Figs. 5, 6

The anterior part is fused with the anterior part of the labium. The hypopharyngeal surface is devoid of setae or macrochaeta. It is flat anteriorly and more convex posteriorly. Laterally it is enclosed by distinct sclerotized ridges. The posterior part is laterally fused with the posterior part of the epipharynx (Fig. 6, see above).

Musculature (Figs. 5, 6): M41 (M. Frontohypopharyngealis): an unusually large muscle with several areas of origin. O: frons lateral of the dorsal endocarina, laterally on the dorsal endocarina, posterior part of the ventral edge of the endocarina, I: posterolateral edge of the hypopharyngeal tube with a strong tendon, lateral the anatomical mouth; M42 (M. Tentorhynpharyngalis): absent. A muscle with unclear homology connects the ventral wall of the prepharyngeal tube with the ventral wall of the anterior pharynx (Fig. 5).

3.11. Pharynx

Figs. 5, 6

The pharynx is moderately wide, with distinct folds for muscle attachment dorsally, laterally and ventrolaterally. The dorsal folds are closely adjacent.

Musculature (Figs. 5, 6): M44, a well-developed bundle, O: anterior frons; I: dorsally on the prepharyngeal tube, immediately anterior to the anatomical mouth; M45
on the ventral fl folds of the posterior pharynx; M52b, O: composed of two bundles, M52a, O: posteriorly from the head capsule, I: two separate areas of insertion on the ventral folds of the posterior pharynx; M52b, O: posteroverentral edge of the head capsule, I: lateral fold of the posterior pharynx. A well-developed ring muscle layer encloses the pharynx.

3.12. Brain and suboesophageal complex

Figs. 5, 6

The elongate, pear-shaped brain is entirely located in the prothorax. The protocerebral hemispheres are only connected by a very narrow bridge. Optic lobes and optic nerves are missing. A pair of well-developed nerve stems arises from the narrow anteriormost part of the brain. They are likely the bases of antennal nerves but their distal parts could not be traced on the available sections. The circumoesophageal connectives are long and moderately wide. A separate tritocerebral commissure below the pharynx is missing. The suboesophageal complex is completely shifted to the prothorax, elongate, triangular and narrowing anteriorly like the brain. The frontal ganglion is unusually narrow and located in the anterior head region above the anatomical mouth. The nervous recurrent below the cerebral hemispheres is well developed. It is visible on the sections up to the middle region of the mesothorax.

3.13. Cephalic glands

Tube-like ventral glands or glands associated with the antennae or mouthparts are missing.

3.14. Prothorax

Figs. 6, 7, 9A, 10A, B

The prothorax is distinctly longer than the meso- or metathorax. Like on the following segments few very long and thin setae are inserted dorsally and dorsolaterally. The prothorax is connected with the head by a broad and thick cervical membrane densely covered with microspines. A dorsomedian rounded projection of this flexible collar fills out the dorsomedian excavation of the head capsule and a larger counterpart is present ventromedially. The anterior and posterior corners of the prothorax are rounded and the lateral margins approximately parallel. A well-defined dorsolateral edge and a clearly delimited pronotum are not present (Fig. 7), but considerable parts of the pronotal area are sclerotized and smooth (Fig. 3A). The anterior region is continuous with the cervical collar and like the central pronotal part semimembranous and covered with microspines. A rounded, smooth and sclerotized projection is present at the posterior margin. This structure resembling a scutellum reaches into the unsclerotized anterior part of the mesothorax (Figs. 1A, 3A). The ventral side of the prothorax is also largely semimembranous. The microspines are absent on two sclerotized rounded plaques lateral a large median projection, which is wide anteriorly and tapering towards the posterior margin of the segment (Fig. 3B). The vestiture of microspines is very dense on the projection but distinctly less so on two regions posterior to the smooth plaques, which bear two short setae each. The hind margin of the main ventral part of the scutellum between the hind coxal margins is almost straight and smooth. It is followed by a very short smooth area on a slightly deeper level (Fig. 3B). A division of the sternal region into well-defined regions is not recognisable and the furca and spinus are also absent. Defined pleural elements are not recognisable. The well-developed six-segmented legs are inserted posterolaterally, quite close to the lateral margins of the segment (Figs. 1B, C, 3B, 7). A raised semimembranous element of semilunar shape is recognisable anteromedial the costa. The coxae are very widely separated, moderately sized, and only slightly tapering towards their apical margin. They bear three
relatively short setae and the anterior surface is covered with short spines. The trochantur is almost half as large as the coxa and distinctly subdivided into a proximal and distal part, the latter bearing two setae of medium length and a very long one. The distal part is connected with the femur, which is about as long as the coxa, with parallel sides and an oblique distal margin. The cylindrical tibia is slightly shorter than the femur. Several medium sized setae insert close to its straight distal margin. The tarsus is about twice as long as the tibia and tapering towards its apex. A very long seta is inserted on its dorsal side and it also bears several shorter and thin setae. Apically a long single claw is inserted. It is slightly curved and slender in its distal region but extended basally where it bears a single fine claw seta.

Musculature (Figs. 2B, C, 6, 9A): The homologization of the thoracic and abdominal muscles is impeded by the weak sclerotization of the postepithelial body and the almost complete lack of well-defined sclerites. Due to the small size the precise location of the insertions of some muscles could not be identified.

Dorsal muscles. M2, M. pronoti secundus (LARSEN 1966, M55, v. KELER 1963), a very large fan-shaped muscle; M55, V. KELER 1963), a very large fan-shaped muscle; O, seven separate areas on the posteromost pronotal region, with the very strong mesal bundle arising on the mesosternum, I, two adjacent but separate areas of insertion in the dorsal emargination of the head capsule.

Ventral muscles. M5, M. prosterni primus (LARSEN 1966, M55, V. KELER 1963), a strongly developed bundle; M6, M. prosterni secundus (LARSEN 1966), two strongly developed bundles with completely separate areas of origin; I, ventral to the thick cervical membrane, close to the median line. M6, M. prosterni secundus (LARSEN 1966), two strongly developed bundles with completely separate areas of origin; O, two widely separated areas, posterior prosternal region and middle region of the metasosternum; I, ventrolaterally on the cervical membrane, adjacent with the posterior edge of the head capsule. M. prosterni tertius, a short muscle, O, anterior prosternal region; I, ventrally on the cervical membrane, mesal and below of the insertion of M. prosterni secundus.

Dorsoventralis muscles. M7, M. dorsi-ventralis primus (LARSEN 1966), O, posterior pronotal region, anterior edge of the phragma; I, ventrolaterally on the cervical membrane, together with M6, M8, M. dorsi-ventralis secundus (LARSEN 1966), a bipartite muscle; O, separate areas of origin on the posterior pronotal and posterior pleural region; I, ventral cervical membrane together with M5, M9, M. dorsi-ventralis tertius (LARSEN 1966), a bipartite muscle; O, two widely separated areas of origin, from the phragma and from the posterior pleural region; I, laterally on the cervical membrane.

Lateral muscles. M13, M. pronoto-episternalis (LARSEN 1966), a bifurcated muscle with widely separated areas of insertion; O, laterally on the posterior edge of the pronotal area, adjacent with the anterior mesosternal...

border, I: the shorter bundle on the mesopleural region, the larger and much longer bundle on the anterior mesosternum. M. pleuro-pleuralis (plp): the homology of this strongly developed lateral muscle is unclear. It connects the posterolateral prothoracic border with the posterolateral border of the mesothorax.

*Leg muscles:* M15, M. note-coxalis (Larsen 1966); O: posterolateral notal region; I: anterior coxal margin. M18, M. sterno-coxalis, M19, Mm. furca-coxales (Larsen 1966), three bundles with sternal origin due to the complete reduction of the furca. O: posteromost sternal region, one lateral and one close to the median line; I: coxal base, two posteriorly and one anteriorly.

*Intrinsic legs muscles:* not examined, largely constant in insects and larvae with well-developed legs.

**3.15. Mesothorax**

Figs. 1, 8A, 9B, 10

The mesothorax is distinctly shorter than the prothorax and most of its surface is unsclerotized and covered with microspines. A relatively short unsclerotized anterior part of the segment shows a rather irregular pattern of folds and the typical spiny surface structure. The main part of the tergal region is subdivided by two pairs of longitudinal folds. The largest part in the middle of the segment is elevated, thus forming a fairly inconspicuous but recognizable tergal ampulla. On the ventral side a short, laterally narrowing semimembranous bulge is present anteriorly, followed by the main part of the sternal region, which is also semimembranous and covered with microspines anteriorly and laterally. In the anterior part of the central region of the segment a distinctly raised bulging area forms the ventral ampulla, which is semimembranous and covered with posteriorly directed microspines. Two pairs of short setae are inserted at the posterior margin. Deep curved furrows separate the anterolateral part of the ampulla from the adjacent anterior sternal region. The transverse area posterior to the ampulla is smooth, sclerotized, and mediadly divided by a short longitudinal furrow. A distinct round spiracle is present laterally, posterior to the posterolateral margin of the sclerotized pronotal region. It is enclosed by a distinctly raised and sclerotized socket and this in turn by a small smooth and sclerotized pleural area. The legs are very similar to the prolegs.

*Senckenberg*
Musculature (Figs. 2B, C, 9B, 10). Dorsal muscles. M28, M. mesonotum primus (Larsén 1966), a strongly developed bundle; O: dorsomedially on the second phragm, I: laterally on the intersegmental membrane connecting the prothorax and mesothorax. M29, M. mesonotum secundus (Larsén 1966), two bundles; O: from an extensive lateral fold of the metathorax; I: laterally on the intersegmental membrane connecting the prothorax and mesothorax, below M28.

Lateral muscles. M30, M. mesosternum primus (Larsén 1966), a well-developed muscle; O: laterally on the intersegmental fold between the meso- and metathorax, I: on the intersegmental fold between the pro- and mesothorax, close to the median line. M31, M. mesosternum secundus (Larsén 1966), composed of two parallel bundles; O: laterad M30, I: laterad M30.

Lateral muscles. M43, M. noto-pleuralis (Larsén 1966), one longer and one relatively short bundle; O: postero lateral mesonotal region, I: posterior pleural region.

Leg muscles. M40, M. noto-coxalis, M47, M. noto-trochanteralis (Larsén 1965), two parallel bundles with two separate insertion areas; O: postero lateral edge of the mesonotal area, I: not exactly identified, probably on the coxal base and trochanter, respectively. M46, M. furca-convexalis posterior (Larsén 1965), a well-developed flat muscle; O: anterior part of lateral fold of mesothorax, I: posterior coxal base. Three moderately sized parallel bundles originate on the lower pleural region and insert on the basal part of the leg. The precise points of insertion could not be identified on the microtome sections. They likely comprise M. furca-coxalis anterior (M447), M. fur-
3.16. Metathorax

Figs. 1, 8A, 9A, 10

Similar to the mesothorax. The spiracle in the dorsolateral region of the anteromost part of the segment is much smaller than the mesothoracic one.

Musculature: similar to that of the mesothorax.

3.17. Abdominal segments I–VIII

Figs. 1, 9C, 10

The abdomen is about 2.5 times longer than the thorax. Segments I–VII are slightly longer than the meso- and metathorax and similar in their general shape, but more rounded along the lateral edge and with moderately distinct lateral bulges. Segment VIII is distinctly shorter. As in the thoracic segments the terminal region is indistinctly delimited and a sparse vestiture of very long and thin setae is present dorsally and dorsolaterally. Dorsal and ventral ampullae are present, similar to those of the meso- and metathorax. Rather indistinct longitudinal furrows are recognizable dorsally and ventrally. Small annular spiracles are present dorsolaterally, close to the anterolateral edge of the tergal regions.

Musculature (Figs. 2B, C, 9C, 10). Dorsal muscles. Several well-developed bundles (dim), some of them connecting the anterior and posterior dorsal intersegmental fold. A mesal bundle inserts on the middle region of the segment and a lateral one on a lateral fold distinctly posterior to the anterior segmental border.

Vebral muscles. Several well-developed bundles (vdm) connecting the anterior and posterior ventral intersegmental fold.

Lateral muscles. M. notopleuralis (glm1): one vertical bundle originates on the lateral notal region and is inserted on a longitudinal pleural fold. Several well-developed longer and shorter vertical bundles (glm2) connect the upper and lower edges of the longitudinal pleural bulge. A lateral longitudinal muscle (ilm) connects anterolateral and posterolateral epipodes of the abdominal segments. A well-developed muscle is present in abdominal segment V close to a gland (glm). Its position suggests that it is involved in releasing gland secretions but the precise function remains unclear.

3.18. Abdominal segments IX and X

Figs. 1, 8B, 9D, 10D

Segment IX is about as long as the prothorax and slightly narrower than the anterior abdominal segments at its anterior margin. It is a largely sclerotized and undivided structure and bears a vestiture of long and thin setae. It is distinctly tapering posteriorly. Its apex is formed by a parallel-sided and apically truncated tergal process. The tergal part including the lateral and ventrolateral areas is sclerotized and the surface is smooth. A short sternal region is semimembranous and bears scattered microsomes and two pairs of long setae. It forms a short triangular posterior median process. The greatly reduced segment X is inserted on the ventral side posterior to sternum IX. It is represented by two pairs of flattened, sclerotized and flaps. The larger lateral flap bears two long setae, the smaller ventromedial flap one long seta and several short ones along its rounded posterior edge. The latter are fused basally but divided by a deep median crest.

Musculature (Figs. 2B, 9D, 10). Lateral muscles. One well-developed almost vertical muscle is present in the posterolateral region of segment IX. Muscles of the eversible lobes. Three muscles originate from the wall of segment IX and insert on the base of the eversible lobes ventrally, dorsally and posteriorly. Muscles of the rectum. Five dilators are present. They originate dorsally, laterally and ventrally on the external wall of segment IX and insert on the rectum between the papillae.

3.19. Postcephalic central nervous system

Figs. 2, 5

The thoracic ganglia are comparatively large and distinctly connected by connectives. The abdominal ganglia are flattened. All of them including the ganglionic mass of segment VIII are distinctly separated from each other.

3.20. Postcephalic gut

Figs. 2A, C, 10

The pharyngeal-oesophageal transition area lies in the anterior prothoracic region. In contrast to the pharynx the oesophagus has a very wide lumen, the dorsal lateral and ventral folds are short and very narrow, and a ring-muscle layer is lacking. A well-developed proventriculus is present in the region of the meso- and metathorax, with a strongly developed muscle layer around it and six folds with thickened cuticle and a rather irregular surface structure. The anterior end of the long and straight mid gut lies in the metathorax and its posterior end in the posterior abdomen. Caeca are absent. The mid gut wall is formed by moderately high cells, partly appearing almost cylindrical but mostly more or less quadratic in cross section. The epithelium appears rather loose and contains numerous glandular elements. Some crypt-like cell aggregations are present in the basal part of the midgut epithelial layer (Fig. 10C). Active apocrine secretion is recognizable on microtome sections, especially in the anterior part. The border between the mid gut and hindgut is marked by a...
sharp bend in segment VIII which conceals the origin of the Malpighian tubes. The hind gut first turns forward and then again backward in segment VI. The fairly long loop is characterized by a very strongly developed muscular envelope. The hindgut itself is strongly folded longitudinally in this region and the intima is distinct. It ends in segment IX with the rectum with well-developed rectal papillae and a strongly developed apparatus of radially arranged dilator muscles.

3.21. Malpighian tubules

Fig. 2A,C

The Malpighian tubules form a complicated network around the gut. Two pairs of branches extend forwards and connect pairwise, with the joint flattened terminal part attached to the midgut wall dorsally and ventrally in the region of segment II. The area of origin in the loop at the midgut-hindgut border is not distinctly visible. A cryptonephric complex is not present.

3.22. Postcephalic glands

Figs. 2C, 9B,C

One postcephalic gland is present in the dorsal region of segment VI and a distinctly asymmetrical one in the apical region of segment IX.

3.23. Circulatory system

Fig. 2A

A well-developed dorsal vessel extends almost through the entire postcephalic body.

3.24. Fat body

The postcephalic body is largely filled with very loose fat body tissue.

4. Characters and character states

(matrix see Electronic Supplement File 1)

Larval features were mainly taken from Beutel & Hornschemeyer (2002a,b). Modified characters are marked with a asterisk and added characters with two. Features of adults (chars 38–103 in the matrix; see Electronic Supplement File 2) were taken from Beutel et al. (2008).

1. *Head shape of 2nd and later instars:* (0) parallel-sided or very slightly rounded or narrowing anteriorly; (1) transverse, strongly rounded laterally, greatest width near hind margin; (2) transverse, with distinctly protruding eye region. Like in other cupedid larvae the head of Tonomera mucida (also in 1st instar) is shortened, distinctly broader than long and strongly rounded laterally, with the greatest width close to the foramen occipitale (Figs. 3, 4; Beutel & Hornschemeyer 2002a,b; Lawrence 1991: fig. 34.6a−c; Ross & Pothecary 1970) (coded as mappicle [-] for 1st instar larva).

2. *Posteromedian emarginations of head capsule:* (0) absent; (1) present. Dorsal and ventral posteromedian emarginations are present in 1st instars of *T. mucida* like in all known other larvae of Archostenata (Figs. 3, 4, Beutel & Hornschemeyer 2002a,b; Boying & Craighead 1931; Ross & Pothecary 1970; Lawrence 1991, 1999).

3. *Endocarina:* (0) absent; (1) present, unforked; (2) present, forked. A distinct dorsal endocarina is present in 1st instars of *T. mucida* like in all other known larvae of Archostenata (Figs. 3, 5; Beutel & Hornschemeyer 2002a,b; Boying & Craighead 1931; Lawrence 1991, 1999). In contrast to the forked endocarina of *Ommia* it is unridged and extensive like in larvae of Micromalthus, Rhipsideigma and Distocarpus.

4. *Frontal suture:* (0) distinct and complete; (1) strongly shortened or absent. The frontal suture, which is absent from mature larvae of Archostenata (Figs. 3A, 4A; Lawrence 1999, Beutel & Hornschemeyer 2002a,b) and 1st instars of *Priacma serrata* (Ross & Pothecary 1970; fig. 3), is partly retained (coded 1) in 1st instars of *T. mucida* (Figs. 3, 4, 7).

5. *Stemmata:* (0) more than one pair of stemmata; (1) one eyespot or eyeless. Eyes are absent (Fig. 4) like in Micromalthidae and other known larvae of Cupedidae (Ross & Pothecary 1970; Lawrence 1991, Beutel & Hornschemeyer 2002a,b). Four pairs of stemmata are present in *Ommia* (Lawrence 1999).

6. *Endoskeleton:* (0) well-developed, with tentorial bridge and connected anterior and posterior arms; (1) tentorial bridge absent, anterior and posterior arms disconnected (Figs. 5, 6). The tentorium of 1st instars of *T. mucida* is distinctly reduced like in other archostenatan larvae (Beutel & Hornschemeyer 2002a,b).

7. *Length of antenna:* (0) at least 30% of greatest width of head capsule; (1) less than 20% of greatest width of head capsule. The antenna of cupedid larvae are generally shorter than 20% of the head width. They are extremely shortened in 1st instars of *T. mucida* and *P. serrata* and also in larvae of Micromalthus (Figs. 3, 4, 7; Boying & Craighead 1931; Ross & Pothecary 1970; Lawrence 1991;
**8. Number of antennomeres:** (0) 4 or more; (1) 3 or less. Four antennomeres are present in larvae of *Onnema* (Beutel & Hornschmeyer 2002a: fig. 11).

**10. Shape of distal part of larval mandible:** (0) less than 3 apices; (1) 3 apices or more. Three well developed mandibular apices are present in 1st instars of *T. mucida* like in all other known larvae of Archostemata (Boving & Craighed 1931; Lawrence 1991, 1999; Beutel & Hornschmeyer 2002a). The apical part is less slender than in larvae of *Omma* (Beutel & Hornschmeyer 2002a: fig. 11A).

**11. Retinaculum:** (0) present; (1) absent. A distinct hook-like retinaculum is absent like in other groups of Cupedidae and Micromalthus (Boving & Craighed 1931; Beutel & Hornschmeyer 2002a).

**12. Shape of mola:** (0) not quadrangular, not delimited by a distinct margin; (1) quadrangular and delimited by a distinct margin. The margin of the mola of 1st instars of *T. mucida* is less distinct than in 1st instars of *P. serrata* (Ross & Pottharck 1970), in mature cupedid larvae, and in larvae of Micromalthus (Boving & Craighed 1931; Lawrence 1991; Beutel & Hornschmeyer 2002a).

**13. Cardo:** (0) undivided; (1) divided, with separate lateral semmembraneous piece. A subdivided cardo as it is typical for archostematan larvae (Beutel & Hornschmeyer 2002a,b) is also present in 1st instars of *T. mucida* (Figs. 3, 4).

**14. Ligula:** (0) un sclerotized; (1) sclerotized, enlarged and wedge-shaped. In all other known larvae of Archostemata (Boving & Craighed 1931; Lawrence 1991, 1999; Beutel & Hornschmeyer 2002a,b) the ligula is strongly sclerotized and wedge shaped. In contrast to the typical condition it is apically pointed in 1st instars of *T. mucida* (Figs. 3, 4).

**15. Mentum and submentum:** (0) not fused; (1) fused and narrowed between maxillary grooves. The mentum and submentum of the 1st instar larva of *T. mucida* appear indistinctly separated from each other (Figs. 3, 4), but the homology of the anterior part remains uncertain (coded as ?). A completely undivided postmentum is present in the other known larvae of Archostemata.

**16. Labial muscles:** (0) present; (1) absent. Two pairs of extrinsic labial muscles are present in 1st instar larva of *T. mucida* (Fig. 5). They are absent in all other archostematan larvae examined (Beutel & Hornschmeyer 2002a,b).

**17. Transverse ventral muscle between posterior halves of head capsule:** (0) absent, (1) present. This unusual muscle with unclear homology is present in 1st instar larva of *T. mucida* (Figs. 5, 7) like in other archostematan larvae examined (Beutel & Hornschmeyer 2002a,b).

**18. Pronotricularia:** (0) absent; (1) present. A pronotriculus is present in 1st instars of *T. mucida* and also in larvae of Micromalthus, Rhipsideigna and Distocypes (Beutel & Hornschmeyer 2002a,b). It is equipped with distinct cuticular teeth in the cephalid larvae examined.

**20. Pronotal pseudocolletium:** (0) absent; (1) present. A distinct triangular posterozamidan proportion of the pronotum is present in 1st instars of *T. mucida* (Figs. 3A, 7) and also in larvae of *Rhipsideigna* (Boving & Craighed 1931; Fukuda 1938; Beutel & Hornschmeyer 2002a,b). They are absent in primary larvae of Micromalthus (Beutel & Hornschmeyer 2002a) and probably also in 1st instars of Priacma (Ross & Pottharck 1970: fig. 3).

**21. Prosternal glabrous patches:** (0) absent; (1) present. Distinctly delimited glabrous, shiny patches are present anterad to the prosternal field of aspenthes in 1st instars of *T. mucida* (Fig. 2B) and also in later instar larvae of *Cupedid*. They are absent in primary larvae of *Micromalthus* (Beutel & Hornschmeyer 2002a). The condition in 1st instars of *Priacma* is unclear (Ross & Pottharck 1970: fig. 4, coded as ).

**22. Legs of 2nd and following instars:** (0) present; (1) absent. The absence of legs in secondary larva is apparently an autapomorphy of Micromalthus (coded as — for 1st instars of *T. mucida*).

**23. Number of leg segments:** (0) 6; (1) 6. 6-segmented legs are present in 1st instars of *T. mucida* (Figs. 1, 3B, 7, 8A) like in larvae of other groups of Archostemata (present in 1st instars of *Micromalthus*, coded as 0).
24. Claws: (0) double; (1) single. Single claws are present in 1st instar larvae of *T. mucedora* (LAWRENCE 1991: fig. 3A, 8) and *Priaonica* (ROSS & POTHECY 1970). Double claws occur in primary larvae of *Micromalthus* (BEUTEL & HÖRNSCHEIMER 2002a,b) and in mature larvae of *Tenomorpha* and *Ommia* (LAWRENCE 1999).

25. Tergal ampullae of 2nd and later instars: (0) absent; (1) present. Tergal ampullae, which probably facilitate boring in fungus-infested wood (CROSON 1981; LAWRENCE 1991), are present in later instars of all archosstantan larvae (LAWRENCE 1999; BEUTEL & HÖRNSCHEIMER 2002a,b). They are possibly absent in 1st instars of *P. serrata* (ROSS & POTHECY 1970: fig. 3), but recognizable in the 1st instars of *T. mucedora* (Fig. 1, coded as – for 1st instars).

26. Abdominal segments I–III of 2nd and later instars: (0) shorter than thorax; (1) longer than thorax. The abdomen is cylindrical and unusually elongate in later instars of *Archosstantata* (BOUQUIN & CREAGHEAD 1991; FUKUDA 1983; VULCANO & PEREIRA 1975: fig. 47, LAWRENCE 1991: fig. 12). Segments I–III combined are longer than the thorax. They are slightly shorter than the thorax in the 1st instar larva of *T. mucedora* (Fig. 1, coded as – for 1st instars).

27. Sternal asperities: (0) absent; (1) present. A proventorial field of asperities is present in larvae of *Cupedidae* including 1st instars of *T. mucedora* (Fig. 3B). It is not recorded for 1st instar larvae of *Priaonica* (ROSS & POTHECY 1970) but this may be due to the lack of SEM micrographs (coded as –). They are present on all sternites in larvae of *Micromalthus* (BEUTEL & HÖRNSCHEIMER 2002a: fig. 4C) but absent in the larva of *Ommia* (LAWRENCE 1999).

28. Lateral longitudinal bulge of abdominal segments I–VIII: (0) absent; (1) present. A semi-membranous longitudinal bulge is present on segments I–VIII of larvae of *Tenomorpha* (Fig. 10, BOUQUIN & CREAGHEAD 1991) and also in larvae of *Rhizophagus*.

29. Sclerotized process of tergum IX: (0) absent; (1) present. A median posteriorly directed appendage of tergum IX is present in 1st instars of *T. mucedora* (Figs. 1, 8B) and also in other cupedid larvae and larva of *Micromalthus* (LAWRENCE 1991; BEUTEL & HÖRNSCHEIMER 2002a,b). It is absent in the larva of *Ommia* (LAWRENCE 1999).

30. Asperities on segment IX of mature larvae: (0) absent; (1) present. Asperities are present on abdominal segment IX of carinocyctid larvae of *Micromalthus* and also in examined mature larvae of *Cupedidae*. They are largely absent in 1st instar larvae of *T. mucedora* (Fig. 8D) and probably also in *Priaonica* (ROSS & POTHECY 1970: fig. 4) (coded as – for 1st instars).

31. Eversible lobes of segment IX: (0) absent; (1) present. Eversible ventral lobes of segment X are present in *C. flavicollis* (Fig. 1C, 8B) like in larvae of *Micromalthus* and larvae of other groups of *Cupedidae* (LAWRENCE 1991: fig. 34 D1e, BEUTEL & HÖRNSCHEIMER 2002a,b). In contrast to the usual condition the lobes are sclerotized in the 1st instar of *T. mucedora* (Figs. 1C, 8D). Specific information on this feature is not given in the description of the 1st instar larva of *P. serrata* (ROSS & POTHECY 1970) (coded as –).

32. Urogomphi: (0) absent; (1) present, fixed or articulated. Urogomphi are absent from tergum IX like in all other larvae of *Archosstantata* (Figs. 1, 8D).

33. Toothed process of sternum IX: (0) absent; (1) present. A sclerotized, toothed process of sternum IX is present in larvae *Micromalthus* but absent in 1st instar larvae of *T. mucedora* (Figs. 1, 8B) like in larvae of all other groups of *Archosstantata*.

34. Segment X: (0) exposed, pygopod-like; (1) not visible externally or extremely modified. Segment X is not visible externally like in larvae of all other groups of *Archosstantata* (Figs. 1, 8B). It cannot be excluded that the eversible lobes (char. 31) represent a strongly modified segment X, which is probably developed as a pygopod with terminal eversible membranous vesicles in the groundplan of Coleoptera (BEUTEL 1997).

35. Distal ends of Malpighian tubules: (0) not attached to hind gut; (1) attached to hind gut. The distal ends of the Malpighian tubules are not attached to the hindgut in primary larvae of *T. mucedora* (Fig. 2C), like in the mature larvae of *Rhizophagus* and *Diotocopus* (BEUTEL & HÖRNSCHEIMER 2002a,b).

36. Habitat: (0) not associated with wood; (1) associated with wood. First instar larvae of *T. mucedora* are associated with wood like other archosstantan larvae with the possible exception of *Ommatidiae* (LAWRENCE 1999) (coded as –).

37. Life cycle with hypermetamorphosis, parthenogenesis and viviparous larvae: (0) absent; (1) present. The unique complicated life cycle of *Micromalthus* (POLOKK & NORMARK 2002) is obviously autopoecyst

5. Results of the cladistic analyses

The analyses with NONA and TNT yielded 15 and nine minimum length trees, respectively, in both cases with 185 steps (consistency index: 0.67, retention index: 0.82). The branching pattern of the strict consensus trees was identical (Fig. 11). Archosstantata were placed as sistergroup of the three included non-archosstantan terminals representing *Adephaga* (Trachypachidae), *Myxophaga* (Torridicoleidae), and *Polyphaga* (Heliophoridae). The monophyly of *Archosstantata, Ommatidiae, and Cupedidae* was confirmed and a clade comprising the monospecific families *Crowniellidae* and *Micromalthidae*.
6. Discussion

Almost the complete set of previously identified archozan larval autapomorphies (BEUTEL & HÖRNSCHEMEYER 2002a,b) is already manifest in the 1st instar larvae of Tenonerga mucida. The postpedal and posteroventral emarginations of the head capsule are present (Figs. 3, 4) even though the one on the dorsal side is rather shallow. The dorsal endocarina, a groundplan autapomorphy of Archozenta, is strongly developed (Fig. 5). It is unforked as in Micromalthidae and other caped species examined (BEUTEL & HÖRNSCHEMEYER 2002a,b), in contrast to the forked endocarina of Ommatidae (LAWRENCE 1999; BEUTEL & HÖRNSCHEMEYER 2002a: fig. 11A).

Another autapomorphy of Archozenta is the far-reaching reduction or complete loss of the epcapral (frontal and coronal) suture. In contrast to mature larvae (BEUTEL & HÖRNSCHEMEYER 2002b) short frontal sutures are preserved in the 1st instar of T. mucida (Figs. 3A, 4A). Like in other archozan larval larvae several mandibular teeth are present and a distinct mola, whereas a penicillum or lacina mobilis is absent (Figs. 4, 5). The typical lateral cardinal sclerite is distinct and additionally a sclerotized, pad-like articulatory piece is present (Figs. 3B, 4B). The labrum is strongly modified like in other archozan larval larvae. The sclerotized prominent ligula is another autapomorphy of Archozenta. It is not apically truncate in 1st instars of Tenonerga but sharply pointed (Figs. 3B, 4B), possibly an autapomorphy of the genus, but the condition in almost all 1st larval larvae is unknown. Another archozan autapomorphy is the presence of tergal and sternal impalements on the posterocephalic segments. They are not very prominent but recognizable in the 1st larval instar of Tenonerga (Fig. 1), whereas they are apparently absent in 1st instars of Prriacma (ROSS & POSTECALI 1970: figs. 3, 4). Other potential autapomorphies of Archozenta are the distinctly reduced tergum, the transverse muscle of the posterior head capsule, the proventriculus, and the strongly muscled loop of the hind gut (Fig. 2). As internal structures of larvae of Ommatidae are still unknown and any information on immature stages of Tetraptera (Ommatidae), Croussonella (Croussonellidae), and Sikkhotesia (Jurudidae) is lacking completely, the phylogenetic interpretation of these features remains very uncertain. Information on the digestive tract of immature beetles is presently extremely scarce (e.g., KORSCHELT 1923). It cannot be excluded that both above mentioned features belong to the groundplan of the order.
The 1st instar larvae of *T. mucida* display some unusual plesiomorphies. The presence of a well-developed M. frontalabis (MS) is a unique feature in the entire Coleoptera (e.g., BEUTEL & HAAS 2000). Similarly, a ten- toriomandibularis muscle was identified for the first time in a coleopteran larva (e.g., BEUTEL, 1993; BEUTEL & HAAS 1990; BEUTEL & HÖRNSCHEMeyer 2002a,b, BEUTEL & FRIEDRICH 2005). In contrast to all other archosomatid larvae with available anatomical data (BEUTEL & HÖRNSCHEMeyer 2002a,b), the submentum and mentum appear still separated and two extrinsic labial muscles are preserved. A seemingly plesiomorphic feature, arguably due to reversal, is the lack of a distinct margin of the mandibular mola. It is present in 1st instars of *Micromalthidae* and *Prionacidae* and also in mature larvae of other genera of Cupedidae (BEUTEL & HÖRNSCHEMeyer 2002a,b). Another feature described here for the first time is the presence of glands in the anterior and posterior abdomen (Fig. 2). The function is presently unknown and the phylogenetic significance unclear.

The musculature of 1st instars of *T. mucida* is similar to what was described previously for larvae of *Micromalthidae* and *Rhyssideigina* (BEUTEL & HÖRNSCHEMeyer 2002a,b). Only minor reductions occur in the well-developed and strongly sclerotized head. This includes the presence of only one extrinsic antennal muscle (like in *Micromalthidae dolbelii*; BEUTEL & HÖRNSCHEMeyer 2002a), the absence of the maxillary extensor, and the absence of intrinsic labial muscles (all archosomatid larvae with available anatomical data). The neck musculature responsible for movements of the head is strongly developed. In correlation with wood-boring habits and a cylindrical and largely unscerotized procephalic body the dorsal and ventral longitudinal muscles are also well-developed, and some muscles associated with the body wall extend over more than one segment (Fig. 9) like in *Rhyssideigina* (BEUTEL & HÖRNSCHEMeyer 2002b). The leg muscles are rather weakly developed (Fig. 9A) compared to more active larvae as for instance those of *Dysticus or Tenodrius* (KORNBLUM 1925: figs. 11, 14; JOHNSON 1942). Well-developed muscles of the eversibles lobes of segment IX (Fig. 8B) are probably a derived feature of Cupedidae. The strongly developed muscle associated with the gland in the anterior abdomen (Fig. 2B) was not described previously.

The cephalic central nervous system of the 1st instar larva is obviously affected by the small size like in the primary larvae of *Micromalthus*: the brain and subesophageal ganglion are completely shifted to the prothorax and the hemispheres of the former are pout-shaped and distinctly elongated (Figs. 2A, 5). The postcephalic part is plesiomorphic, with three distinctly separated ganglia in the thorax and eight in the abdomen (Fig. 2B). The presence of a fully formed dorsal vessel, which extends through almost the entire procephalic body is also obviously a plesiomorphic feature.

Like in some other studies based on morphology (e.g., BEUTEL et al. 2006, FRIEDRICH et al. 2009) Archostemata were placed as sistergroup to the terminal taxa representing the other three suborders. However, this result should be taken with caution as the non-archosomatid sampling is very limited. Moreover, the available morphological information on Crowsoniellidae and Jurodiidae is very scarce and larvae of all families except for Cupedidae and Micromalthidae are unknown or insufficiently documented. The monophyly of Archostemata is very strongly supported (Fig. 11) but the interrelationships of the families are largely unresolved, mainly due to missing data. Like in HÖRNSCHEMeyer (2009: figs. 1A, 2) and BEUTEL et al. (2008) Micromalthidae + Crowsoniellidae were placed as sistergroup of Crowsoniellidae, but this result is weakened by missing data for the latter family. Several presumably apomorphic larval features are shared by Micromalthidae and Cupedidae. This includes the strongly transverse and laterally rounded head capsule, the complete reduction of sternomata, the distinctly shortened distal part of the mandibles, the loss of the retinacula (Figs. 3–5), the presence of sternal asperities and asperities on segment IX, and the presence of eversible lobes of segment IX and of a caudal sclerotized process of tergum IX (Fig. 1). Antennae shortened compared to the well-developed four-segmented antenna of *Omnia* are also a derived feature found in both families, but this mainly applies to the 1st instars. The monophyly of Ommatidae is well supported by adult features (FRIEDRICH et al. 2009) (larvae of *Tetrarcha* are unknown). The monophyly of Cupedidae is strongly supported, but like in the case of Ommatidae exclusively by apomorphies of adults. Like in HÖRNSCHEMeyer (2009: figs. 1A, 2) and BEUTEL et al. (2008) Cupedidae excl. *Prionacidae* and *Paracupes* (larvae unknown) are monophyletic, but again this is only supported by apomorphies in the adult stage (later instars of *Prionacidae* and larvae of *Paracupes* are unknown). A cupedid subgroup well supported by larval features comprises the genera *Tenebrogyna* and *Rhyssideigina*, and possibly also *Cupes* (larval features unknown). Shared apomorphies are the presence of glabrous prosternal patches (Fig. 3B, not described for 1st instars of *P. serrata*; ROSS & POTHECA 1970), a widened prothorax (less strongly but still distinct in 1st instars of *T. mucida*; Fig. 1), the protosternal pseudocutellum (Fig. 7), and the presence of lateral longitudinal bulge of abdominal segments I–VIII with specifically developed lateral muscles (Fig. 10).

Several characters are apparently related to the instar. A specific feature of 1st instar larvae is the extremely shortened antenna with a reduced number of antennomeres (Fig. 3A, ROSS & POTHECA 1970; BEUTEL & HÖRNSCHEMeyer 2002a). Another feature is the presence of well-developed and moderately long legs (Figs. 1, 7, 8A; ROSS & POTHECA 1970: figs. 3, 4), in contrast to strongly shortened or reduced legs in later larval stages (BEUTEL & HÖRNSCHEMeyer 2002a: fig. 3A,b; BEUTEL & HÖRNSCHEMeyer 2002a: figs. 3, 28).

Reconstruction of the phylogeny and character evolution of Archostemata is still greatly impeded by the scarcity of data, especially the lacking information on internal features and larval characters. The search for additional and unequivocally identified larva of Ommatidae
should have high priority. However, considering the rarity of adults and the presumably cryptic habits of the immature stages, this is obviously a great challenge. In the case of Crowsoniidae (only type series known) and Sikhatelatinia (only holotype known) this is definitely unrealistic, at least in the near future, and this applies also to tissue of these taxa for DNA extraction. Consequently, the phylogeny and evolution of the most mysterious and archaic group of beetles will remain enigmatic to a considerable extent.

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Yavorskaya et al.: Larva of Teneomelana

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics
(“Contents”)

File 1: yavorskaya@ltenomelana-asg/2015-electronic-supplement/ment-1.mex – Character state matrix.

File 2: yavorskaya@ltenomelana-asg/2015-electronic-supplement/ment-2.docx – Characters of adults (38–103) in matrix.
3.5 Study VII

Beutel RG, Richter A, Büss S, Miller KB, Yavorskaya M, Wipfler B, Yan EV


**Abstract:** Head structures of *Heterogyrus milloti* Legros, 1953 are described in detail and documented with different morphological techniques, including μ-computed tomography and computer-based 3D reconstructions. The results are compared with cephalic conditions found in other gyrrinid taxa and the observed characters are interpreted and analysed phylogenetically. Nine unambiguous cephalic apomorphies support the monophyly of Gyrinidae. In addition to well-known characters like the subdivided compound eyes and highly modified antennae, this includes the very tight connection of the dorsal surface of the head with the anterior pronotal margin, the absence of a clypeofrontal gland, the separation of a lateral portion from the clypeus, and the loss of the dorsal tentorial arm. Unambiguous synapomorphies of *Heterogyrus* and Gyrininae sensu Miller and Bergsten (2012) are the relatively widely separated dorsal and ventral ocular subunits, the absence of tactile setae on the head capsule, the shortened mesal mandibular edge, widely separated mandibular incisivi, three rows of setae on the labrum, the enlargement of the lateral mental lobes, and the loss of the stiptal muscle attached to the galea. The monophyly of Gyrininae (excl. *Heterogyrus*) is supported by the widened bridge between the dorsal and ventral ocular subunits, the reduced size and dorsal shift of the dorsal eye, its distinct separation from the anterior pronotal margin, the detachment of the lateral frontal ridge from the supraocular bead, the almost completely reduced setation of the antennal flagellum, and a one-segmented galea. The steep frontal side of the head, a transverse regular field of setae on the frontal region, and the fused laminaentatoria are autapomorphies of *Spanglergyrus* Folkerts, 1979. A field of sensilla on the interocular antenial groove is a potential cephalic autapomorphy of *Heterogyrus*. The cephalic characters we analyzed remain ambiguous about the interrelationships among the three tribes currently recognized in Gyrininae.

**Significance in the present thesis:** The suborder Adephaga is mainly characterized by predacious habits of larvae and adults, which has resulted in profound modifications of the feeding apparatus. The placement of the enigmatic *Heterogyrus* from Madagascar is evaluated. The genus is placed as second branch in Gyrinidae.

**Own contribution:** 20 %
The head of *Heterogyrus milloti* (Coleoptera: Gyrinidae) and its phylogenetic implications

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Abstract

Head structures of *Heterogyrus milloti* (Lagros, 1953) are described in detail and documented with different morphological techniques, including micro-computed tomography and computer-based 3D reconstructions. The results are compared with cephalic conditions found in other gyrinid taxa and the observed characters are interpreted and analysed phylogenetically. Nine unambiguous cephalic apomorphies support the monophyly of Gyrinidae. In addition to well-known characters like the subdivided compound eyes and highly modified antennae, this includes the very tight connection of the dorsal surface of the head with the anterior pronotal margin, the presence of a clypeofrontal gland, the separation of a lateral portion from the clypeus, and the loss of the dorsal antennal arm. Unambiguous synapomorphies of *Heterogyrus* and Gyrininae sensu Miller and Bergsten (2012) are the relatively widely separated dorsal and ventral ocular subunits, the absence of tactile setae on the head capsule, the shortened metatral mandibular edge, widely separated mandibular incisors, three rows of setae on the labrum, the enlargement of the lateral mental lobes, and the loss of the sigillate muscle attached to the galea. The monophyly of Gyrininae (excl. *Heterogyrus*) is supported by the widened bridge between the dorsal and ventral ocular subunits, the reduced size and dorsal shift of the dorsal eye, its distinct separation from the anterior pronotal margin, the detachment of the lateral frontal ridge from the suboccipital bead, the almost completely reduced scutum of the antennal flagellum, and a one-segmented galea. The steep frontal side of the head, a transverse regular field of setae on the frontal region, and the fused laminae are autapomorphies of *Sparganoxyrus* Fallkert, 1979. A field of sensilla on the interocular antennal groove is a potential cephalic autapomorphy of *Heterogyrus*. The cephalic characters we analysed remain ambiguous about the interrelationships among the three tribes currently recognized in Gyrininae.

Key words

Head, mouthparts, 3D reconstruction, musculature, *Heterogyrus*, Gyrinidae.

1. Introduction

Gyrinidae are a fascinating and intensively investigated family of highly specialized adephagous beetles (e.g., *HATCH* 1925a,b, 1926; *BRINK* 1955; *LARSEN* 1954, 1966; *BEUTEL* 1989a,b, 1990; *BEUTEL & ROUGLEY* 1988, 1994; *MILLER & BERGSTEN* 2012; *GUSTAFSON et al.* 2017). The distribution is worldwide. With ca. 750 described species they are a relatively small family compared to the aquatic Dytiscidae (ca. 5,000 spp.) or the terrestrial Carabidae (ca. 30,000 spp.) (*BEUTEL & ROUGLEY* 2016; *BAKE & HENDRICKS* 2016; *ARDNT et al.* 2016). However, with their highly specialized surface-swimming habits (e.g., *HATCH* 1925a; *OMER-COOPER* 1934; *NACHTRALL* 1961; *LARSEN* 1966) they are a unique group of Coleoptera. Numerous morphological modifications are linked...
2. Material and methods

Material. Heterogryus milloti Legros, 1953 (Heterogryinae, MILLER & BERGSTEN 2012), fixed and stored in 97% ethanol; collected at Fianarantsoa, small stream ~8 km W Ranomofana, Ranomofana NP, Madagascar, 21°14.992'S 47°24.332'E, 2 November 2014, Miller, Gustafson and Bergsten. — Spanglerogryus albiven- tris Folkerts, 1979 (Spanglerogryinae), fixed in FAE [formaldehyde-ethanol-acetic acid], stored in 75% ethanol, dissected specimens, SEM micrographs; collected at Evergreen, Conecuh county, Alabama, USA. — Gyretus substriatus Stephens, 1829 (Gyretinae), fixed in FAE and stored in ethanol. — Dinurus assimilis Kirby, 1837 (Enhydridina), fixed in Kahlhe’s fluid and stored in ethanol, collected at Evergreen, Conecuh county, Alabama, USA. — Macrogryus australis (Braüle, 1835) (Enhydridina), 70% ethanol; collected at Gveesten, Arve Forest Drive, SW Tasmania, Australia (donated by Zoologische Staatssammlung München). — Orectochilus villosus (Miller, 1776) (Orectochilina) fixed in FAE and stored in ethanol; collected in Saale river, 8 km south of Jena, Thuringia, Germany. — Orectogyus sp. (Orectochilina), fixed in Dubosq Brazil and stored in ethanol; collected in Maasai Mara National Reserve, Kenya by M.S. Fischer. — Gyretes tictoal Young, 1947 (Orectochilini), fixed in Kahlhe’s fluid and stored in ethanol; collected at Evergreen, Conecuh county, Alabama, USA.

Specimens of the two enhydridae species and of Gyri- nus substriatus, Gyretes tictoal and Orectogyus sp. were only minced dissected. Micro-CT data sets were available of Orectochilus and Heterogryus, and additionally a microtome sections series of the latter.

Micro-Computed Tomography (μCT). One female specimen of Heterogryus milloti was dehydrated in an ethanol series, dried at the critical point (EmTech K850 Critical Point Dryer) and scanned with a SkyScan 1172 (Bruker micro-CT, Kontich, Belgium) desktop μCT (Zoological Institute, Functional Morphology and Bio mechanics, Kiel University) at 40 kV and 250 μA with images taken every 0.25°. A female of Orectochilus villosus was also dehydrated and dried at the critical point (EmTech K850 Critical Point Dryer). It was scanned in a small Eppendorf tube at Beamlime BW2 of German Electron Synchrotron Facility (DESY, Hamburg) using a stable low photon energy beam (0 KVP) and absorption contrast.

The data sets are stored in the collection of the Physi lagenz, Jena, Germany and can be accessed by contacting the corresponding author.

Computer-based 3D-reconstruction. Based on the μCT-image stack the head of Heterogryus milloti was reconstructed using Amira 6.0 (Visage Imaging, Berlin, Germany). Musculature, nervous system and gut were manually outlined using the interpolation function. Subse- quently the individual materials were separated (using the algorithm function of Amira) and imported into VG studio Max 2.0.5 (Volume Graphics, Heidelberg, Germany) where volume rendering was performed with Scatter HQ. Image plates were assembled and arranged with Adobe Photoshop and Illustrator (Adobe Inc., California, USA).

Histology. One specimen of Heterogryus milloti (head and thorax) was embedded in araldite CY 2120 (Agar ScientificLc, Stansted/Essex, UK) and cut at 1 μm using a microtome HM 360 (Micron, Walldorf, Germany) equipped with a diamond knife. Sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co KG/Division Chroma, Münster, Germany). Selected images of the sections were photographed using a light
Scanning electron microscopy. Specimens of *Heterogyrus milleti* and *Cyrinus substrarius* were cleaned with ultrasonic sound, dehydrated in an ethanol series, dried and coated with gold (Emitech K500 sputter coater). SEM micrographs were taken with a Philips XL 30 ESEM equipped with Scandum software.

**Terminology.** The terminology for skeletal cephalic structures is mainly based on the detailed description of *Spargerogyrus* by Beutel (1989a) (see also Dressler & Beutel 2010). The nomenclature of v. Keler (1956) is used for cephalic muscles but the muscle designations of Wipller et al. (2011) are given in brackets.

**Phylogenetic analyses.** The data (44 characters of the head) were entered in a matrix with Winclada (Goloboff 1995) and parsimony analyses were carried out with NONA (ratchet, 1000 replicates) (Goloboff 1995) and TNT using the exact search algorithm (implicit enumeration) (Goloboff et al. 2008). All characters had equal weight, and all were treated as unordered, with the exception of character 6 (subdivision of compound eyes). The Bremer support values were calculated with NONA (Goloboff 1995).

### 3. Morphological results

3.1. **External head capsule**

Figs. 1, 2A, 3

The prognathous head is distinctly broader than long, with a maximum width (ca. 1.8 mm) at the lateral edge of the interocular bridge separating the upper and lower part of the compound eyes. A short posterooral part of the head capsule is retracted into the thorax. A deep lateral emargination of the anterior pronotal margin precisely fits with the posterior edge of the dorsal subunit of the compound eye, and a broad median pronotal projection reaches the middle dorsal ocular region. The nearly straight anterior edge of the projection appears almost merged with the dorsal surface of the frontal region, with the border almost obliterated. On the ventral side, the prosternum covers the posterior gular region and adjacent gular areas. Its lateral part almost reaches the head margin of the ventral subunit of the compound eye, which is laterally adjacent with the anteriorly pointed lateral protal projections. The colouration of the head capsule is almost black on the posterodorsal region, along the lateral frontal edges and between the subunits of the compound eyes, partly with a metallic greenish hue. It is brownish on the remaining dorsal region and posteroventral parts, but yellowish on most areas of the ventral side, like on most parts of the postocular body (Fig. 1). The surface is almost completely glabrous, without granulation, specific sculpture, pustulence, or long setae (Fig. 3) only an extremely fine wrinkled pattern is recognizable on the dorsal side at high magnification (SEM). The compound eyes are completely subdivided into a ventral and a dorsal subunit. Both are moderately convex and not protruding beyond the lateral edge of the head, which is formed by the sharp ventrolateral edge of the interocular bridge (Fig. 2A). The dorsal surface of this structure is distinctly concave, thus providing an elongate groove for reception of the antennal flagellum in posterior orientation, with a group of medium length sensorial setae (sensilla trichodes) on its anterior portion. It is less than half as wide as the dorsoventral diameter of the ventral ocular subunit. The dorsal eye is about as large as the ventral ocular subunit and not shifted to the dorsal surface of the head (Fig. 1A). The numerous small and slightly convex cuticular lenses are very similar on both subdivisions. The ventral edge of the dorsal eye is almost straight whereas the dorsal border is evenly rounded and delimited by an indistinct supraocular bead. The slightly convex upper margin of the ventral eye is adjacent with a sharp lateral edge of the interocular bridge. The anterior and ventral margins are more rounded. The exposed part of the dorsal side of the head is largely formed by the frons but frontal sutures are missing. The converging lateral edges form slightly rounded ridges (lfr in Fig. 2A) anterior to the compound eyes and a distinct lateral bead, continuous with the dorsal supraocular bead. The lateral edge delimits a shallow, roughly triangular groove between the lateral cephalus, the antennal foramen, and the anterior margin of the upper portion of the compound eye. The groove serves for reception of the basal part of the antennal flagellum in posterodorsal orientation. It is subdivided by a curved longitudinal furrow anteriorly and a nearly straight, oblique furrow posteriorly. The interocular suture (Hatch 1925b, 1926: exoculata) is continuous with the anterior curved furrow. It is quite indistinct dorsally and obliterated ventrally. The anterior frontal margin is nearly straight. A thin but distinct transverse furrow corresponds with an internal chyppo-fronital strengthening ridge. The transverse cephalus appears evenly rounded and slightly convex along its anterior edge in dorsal view, but is in fact slightly concave. A horizontal lateral part is very distinctly separated from the main middle cephalic portion. The lateral cephalic part bears a distinct bulge and a group of medium length setae is inserted on its surface. On the ventral side of the head the posterior tentorial grooves are visible as distinct fissure-shaped openings. The gula is...
anteriorly completely fused with and externally not delimitated from the labial submentum. Internally the gula gives rise to gular ridges that are visible through the partly transparent ventral cuticle.

3.2. Internal skeletal structures

Figs. 6, 7, 10

The transverse clypeofrontal (epistomal) strengthening ridge is low but distinct and complete. The flat longitudinal gular ridges are well-developed and high. Their upper edge is bent lateral. Posterolaterally they are continuous with the well-developed postoccipital ridge, which encloses the foramen occipitale laterally and dorsally and forms a strongly developed apodeme dorsomedially. The gular ridges are anteriorly indistinguishably fused with the equally flat posterior tentorial arms, which are continuous with the distinctly developed anterior arms, which are flat in this transition area but triangular in cross section closer to their origin on the head external capsule. Their anterior end is fused with the nearly vertical lateral wall of the head capsule at the border between the frons and clypeus. An anterior tentorial groove or pit is not recognizable externally. Mesally directed laminatentoria are distinctly developed but not fused medially. The dorsal arms are absent. A typical transverse tentorial bridge is not present but narrow tendons of M. frontobuccalis anterior are probably vestiges of it. A high and long triangular midgular apodeme is present. Extensive internal circumocular ridges are present around both ocular subunits, with separate dorsal and ventral openings for the bipartite optic lobes.

3.3. Labrum

Figs. 1, 2A, 3A, 7

The transverse, short but thick labrum is connected with the clypeus by a membranous fold which is not visible externally. Its basolateral edges reach the postlateral clypeal edge posteriorly. They are concealed by the horizontal lateral clypeal areas in dorsal view. The basal part of the labrum is bulging except for the lateral area, and fits very closely with the interior clypeal margin. It is distinctly separated from the main part of the labrum and covered by medium length setae, similar to those of the horizontal lateral clypeal portions. A pair of small, round
semitransparent areas are present paramedially close to the labral base. A dense regular row of short setae is inserted in a furrow along the anterior labral edge. A second and third row of long setae are present anteroventrally. The ventral side of the labrum is flat and sclerotized but mainly formed by endocuticle. Internally loosely arranged fat body tissue is present.

**Musculation.** Intrinsuc (M. labroospharyngalis, M.7 = Olb5) and extrinsic (M. frontolabialis, M.8 = Olb1; M. frontoepipharyngalis, M.9 = Olb2) labral muscles are absent.

### 3.4. Antennae

Figs. 1, 2A, 3A,B, 6A,B, 8C

The 11-segmented antenna is inserted in the ventral part of the triangular lateral frontal groove, above the dorsal mandibular articulation. The scapus is large and cup-shaped, with a dorsal rounded lobe partly covering the basal part of the pedicellus, and shows a distinct basal construction and a comparatively small, elongated articulatory piece. It rests on a narrow triangular genital projection with a sharp anterior edge, which separates it from the dorsal maxillary surface. The large parabolic or shield-shaped pedicellus is broadly inserted into the wide distal calyx of the scapus. It bears a dense row of long sensorial setae on its sharp lateral edge. The Johnston’s organ is well developed (Fig. 9D). Flagellomere 1 is much smaller than the two basal segments and calyx-shaped, with a distinctly narrowed and curved basal part that articulates with a posteroventral orifice of the pedicellus. The entire 9-segmented flagellum is compact but still fairly elongate. The short, cylindrical segments fit very closely together, without externally visible articulatory membranes. Antennomeres 3–10 have a distinctly reticulate surface structure and are slightly decreasing in size towards the antennal apex. Setae of variable length.
are inserted on the ventral side of antennomeres 6 (one pair) to 10, increasing in number on the distal segments. The terminal antennomere 11 is cone-shaped and densely covered with short setae.

Musculature (Figs. 8C, 10B). M. tentorioscapalis anterior (M.1 = om3): (O) dorsolaterally on posterior tentorial arm; (I) anterolaterally on base of scapus; (F) depressor and rotator of the antenna. — M. tentorioscapalis posterior (M.2 = om2): (O) anterolaterally on gular ridges and dorsal surface of lamintentorium; (I) posteriorly on the base of the scapus; (F) retractor and rotator of the antenna. — M. tentorioscapalis lateralis/medialis (M.3/4 = om3/4): (O) basal part of anterior tentorial arm and mesally on the lamintentorium; (I) dorsally on the base of the scapus; (F) levator of the antenna. — M. scapopedicellaris lateralis (M.5 = om6): (O) posterodorsal wall of the scapus, narrow at the origin; (I) dorsally on the base of the pedicellus; (F) depressor of the pedicellus and flagellum. — M. scapopedicellaris medialis (M.6 = om7), a strongly developed cone-shaped muscle: (O) extensive area on mesal wall of the enlarged distal part of the scapus; (I) dorsally on the base of the pedicellus; (F) levator of the pedicellus and flagellum.

3.5. Mandibles
Figs. 4, 6C,D, 7, 9A–D

The slightly asymmetric mandibles are short and stout and almost completely concealed between the labrum and the ventral mouthparts in their resting position. They are articulated in a typical deicondylic manner. A strongly developed condyle on the ventral mandibular base fits into a corresponding socket of the head capsule, and a deep dorsal mandibular socket fits with a condyle of the head capsule. The two mandibular incisors are widely separated from each other, the mesal one forming the tip of an extensive triangular projection. The lateral apex is rather pointed on the right mandible but blunt on the left one. The anterior cutting edge connecting the two incisors is nearly straight on the left mandible, but irregular on the right one, and ending with a more pronounced mesal tooth. The shortened mesal edge proximal the triangular projection is set with a row of very short and fine setae. A mola and prostheca are lacking. The mesal edge of the basal part of the mandibles is short. The comparatively long external mandibular margin is strongly rounded and bears a flat lamella ventrally. The cuticle of the distal half of the lateral edge is perforated by thin channels, and also the anterior edge of the triangular projection.

Musculature (Figs. 6C,D, 9, 10). M. craniomandibularis internus (M.11 = om11), the largest muscle of the head: (O) extensive areas of the posterior half of the head capsule, interocular bridge and circumcerebral ridges, ventrolateral, postero lateral and posterodorsal regions; (I) mesal mandibular base with a strongly developed, sclerotized tendon; (F) adductor of the mandible. — M. craniomandibularis externus (M.12 = om12): (O) ventrolaterally on the posterior head capsule (I) lateral mandibular base with a sclerotized tendon; (F) abductor. — M. hypophrystego-mandibularis (M.13 = om4): present but very thin, composed of three thin fibres accompanied by a nerve, (O) anterior tentorial arm; (I) dorsoesmal inner surface of the mandible, very close to the basal margin.

3.6. Maxillae
Figs. 3B, 5

The maxillae are inserted in a shallow maxillary groove delimited by the lateral margin of the mentum and the anteriormost submentum, and a rounded edge on the gnathal region, which is anterolaterally adjacent to the anteroven tral margin of the ventral subunit of the compound eye. It is almost completely covered by large mental lobes. Only the lateral parts of the cardo and basitites and the pulp are exposed. The transverse cardo is well-developed. An obtuse rounded angle separates the posterior margin from the oblique lateral edge, which bears several short setae. At the basal articulation with the head capsule the cardo is divided into two projections for attachment of the cardinal extensor and flexor, respectively (M. craniocardinalis, M. tentoriocardinalis). The latter is more distinct and rounded, and separated from the nearly straight posterior cardinal margin by a distinct notch. The basitipes and medistipes are connected with the slightly rounded anterolateral cardinal edge, the former slightly overlapped by it. The basitipes is shaped like a narrow triangle in ventral view, with a rounded anterior tip. It is adjacent with the smaller palpifer, which is scarcely visible in ventral view between palpomere 1 and a separate scierite covering the base of the galea and palp. The nearly straight mesal edge of the basitipes is connected with the medistipes, which is completely fused with the strongly sclerotized lacina, both forming the largest part of the maxilla. The mesal edge of the lacina bears a fairly short ventral row of thin setae and dorsally a distinctly longer row of very strong, long setae, some of them distinctly curved. The hook-shaped distal part is tapering and rounded at its apex. The palp-like galea is slender, 2-segmented and inserted between the basal part of the rounded lateral edge of the lacina and the basal palpomere. The small scierite covering the base of the proximal galeomere is inserted in a lateral emargination of the lacina and posteriorly adjacent with the anterior part of the basitipes. Palpomere 1 is inserted between this scierite and the small palpifer on the dorsal side of the maxilla. It is slightly longer than wide and bears a short spine anteriorly at its distal edge. Palpomere 2 is about 1.5 times as long and slightly widening distally, palpomere 3 times as long as 2 and cylindrical. The terminal palpomere 4 is almost 3 times as long as 2 and 3, with a slightly convex posterior edge, and a nearly truncate apex with a membranous sensory field. Musculature (Figs. 6E,F, 7A, 9, 10). M. craniocardinalis externus (M.15 = om1), a fan-shaped muscle (O) ventrolaterally on the head capsule, between the ventral margin of the ventral eye and the gular ridges; (I) list-
eral branch of the internal cardinal process, with a short sclerotized tendon; (F) extensor of the cardo (inserts laterad the cardinal articulation pivot). — M. tentoriocardinalis (M.17 = 6×3), fan-shaped. (O) lateral surface of the gular ridge; (I) mesial branch of the internal cardinal process; (F) flexor of the cardo, adductor of the maxilla, antagonistic to M.13. — M. tentoriostipitalis (M.18a,b,c = 6×4, 5), a strongly developed muscle with a triple origin: (O) posterior margin of gula, mid-gular apodeme, and mesial surface of gular ridge; (I) ventral membrane between the cardo and the stipital base; (F) stipital adductor and retractor. — M. craniolacinalis (M.19 =
The median emargination enclosed by them contains the prementum. The mesal edges are nearly straight and a broadly rounded median projection is present proximally. A distinct bead is present along the entire margin of the emargination. The prementum is well-developed and approximately quadrangular, with a slightly convex anterior margin. Its base is covered by transverse membranous scales, which are closely covered by the median projection of the emargination of the mentum. The small palpsers are distinctly recognizable as individual structures, only incompletely fused with the premental surface. The lateral borders are clearly visible. Their anterior edge is slightly concave and a faintly impressed transverse line is present between them. An internal apodeme is present but firmly connected with the lateral premental wall. A ligula is not developed and vestiges of paraglossae are not recognizable. The short basal palpomere is curved downwards and slightly narrowing towards its distal edge, which is interrupted by a short, narrow slit dorsally. Palpomere 2 is more than twice as long, slender basally and widening distally. The terminal palpomere is similar to the apical maxillary palpomere but slightly shorter.

**Musculature** (Figs. 7A, 9). M. submentopermentalis (M.28 = 0.0a): (O) medially on the submentum, anterior to the mid-gular apodeme; (I) medially on the posterior edge of the prementum; (F) retractor of the prementum. — M. tentoriorostralis (M.29 = 0a5): (O) two separate areas of origin, submentum lateral M.28 and anterior edge of mid-gular apodeme; (I) apodeme of palpsper. — M. prementopalspalis externus (M.34 = 0a14): (O) posteriorly on internal apodeme of palpalse; (I) base of palpomere 1, (P) moves the labial palp.

### 3.7. Labium

**Figs. 1A, 2A, 3B, 9**

The submentum is firmly connected with the head capsule and posteriorly completely fused with the gula. Its lateral margin anterior to the posterior tentorial grooves is delimited a faintly impressed oblique line. Its anterior margin is slightly rounded laterally but nearly straight in the middle region. The large, plate-like mentum is connected with it by a membranous fold that is not visible externally as the posterior margin of the mentum is covered by the anterior submental edge. The rounded lateral lobes of the mentum are large, with their anterior margin projecting beyond the anterior premental margin.

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**Fig. 6. Heterogryus milleri**, head, 3D reconstructions, volume renders. A.B. cephalic nervous system and digestive tract in (A) dorsal view and (B) ventral view. C.D. mandibles and mandibular muscles in (C) dorsal view and (D) ventral view. E.F. maxillae and maxillary muscles in (E) dorsal view and (F) ventral view. **Abbreviations**: a.s. — anterior tentorial arm, bu — buccae, ca — caro, car — caruncular ridge, ca — galea, ex — gular ridge, fbr — labrum, le — lacina, lest — lamanastoma, md — mandible, mna — mandibular apodeme, ol — optic lobe, ped — pediculus, pmx — palpus maxillary, pph — prepharynx, sc — scapus, soe — suboesophageal ganglion, scg — subglossal ganglion. **Musculature**: 11 — M. craniomandibularis internus, 12 — M. craniomandibularis externus, 13 — M. tentoriohypopharyngalis, 13 — M. craniocardinalis, 17 — M. tentoriohypopharyngalis, 18 — M. tentoriohypopharyngalis, 19 — M. craniobuccalis.
flattened, pad-like structures. In the middle region of the epipharynx, a median concavity is enclosed by large lateral bulges, with a semimembranous cuticular structure, but reinforced by longitudinal sclerotized bars laterally. Epipharyngeal sensorial lobes or appendages are not developed and microtrichia are almost completely missing. The anterior hypopharynx is a transparent, ramp-like structure dorsally connected with the prelabium and continuous with the upper prementinal surface. Elongate-triangular lateral appendages are present, resembling those described for Spangleragus (Beutel 1989a) and Gyrius (Honnerich 1975). In its middle region the hypopharynx is strongly convex, slightly asymmetric, sclerotized, and roughly fitting with the median concavity of the middle epipharyngeal region. This part of the hypopharynx interacts with the mandibles.

A very short preoral chamber is enclosed by the posterior epipharynx and hypopharynx and the membrane adjacent with the mandibular bases. In this region the flat semimembranous epipharyngeal surface is enclosed by lateral folds. The corresponding hypopharyngeal surface is also flat. An internal thickening serves as attachment area of the strongly developed hypopharyngeal retractor, M. tentorio-buccalis anterior (M.48), and is posteriorly continuous with a strongly developed tendon. Two layers of a very strongly developed transverse muscle are present in this region and between them a thin layer of longitudinal muscles. The edges of the postmost parts of the epi- and hypopharynx are fused, thus forming a prepharyngeal tube, shaped like a transverse crescent in cross section, with distinctly upturned lateral edges. The posterior hypopharynx is reinforced by a distinct median sclerotization.

Musculature (Figs. 7B, 8A). M. frontohypopharyngalis (M.41 = 0by1), a well-developed bipartite muscle: (O) from two nearly adjacent areas at the level of the anterior margin of the dorsal eye, lateral M.45, (I) apically on dorsolateral edge of prepharynx, immediately anterior the anatomical mouth, (F) elevator of the anatomical mouth — M. clypeoporalis (M.43 = 0c11), a strongly developed muscle with many parallel fibers: (O) laterally on the clypeus, (I) lateral bulges of middle region of epipharynx, lateral the median concavity, (F) dilator of the preoral cavity, M. clypeobuccalis (M.44 = 0b11), an oblique, nearly horizontal muscle, composed of numerous very thin fibers which penetrate the strongly developed transverse epipharyngeal muscles: (O) broad area on anterior clypeus, between M.43; (I) immediately anterior to the anatomical mouth; (F) levator of the poste-

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3.9. Pharynx
Figs. 7A, B, 8A, 9, 10

Shaped like a flattened U in cross section at the anatomical mouth opening (Figs. 9F, 10A), then nearly quadrangular, with a relatively wide lumen (Fig. 10B–D). Dorsal, lateral and ventrolateral longitudinal folds for muscle attachment are low in the preccerebral pharyngeal region, but more distinct in the postcerebral section where Mn. verticopharyngalis (M.51) and tentoropharyngalis (M.52) insert (Figs. 9, 10).

Musculature (Figs. 7B, 8A, 9, 10). M. frontobuccalis anterior (M.45 – 0bu2) (O) paramedially on the anterior frons, mesad M.41; (I) laterally on the anatomical mouth, immediately behind the frontal ganglion, very close to the attachment of M.41; (F) dilator of the preccerebral pharynx. — M. frontobuccalis posterior (M.46 = 0bu3), composed of several parallel bundles. (O) frons, directly anterior to the anterior protocerebral margin; (I) dorsolaterally on the posterior preccerebral pharynx, (F) dilator of the preccerebral pharynx, together with M.50. — M. tentorobuccalis anterior (M.48 = 0bu5) (often misinterpreted as M. tentorichypopharyngalis [M.42], e.g., Dressler & Beutel, 2010), a strongly developed unpaired muscle (Fig. 9E). (O) on extremely thin, paired tendons posteriorly attached to gular ridges; (I) on the median thickening of the posterior hypopharynx, (F) retractor and depressor of the hypopharynx, dilator of the preoral cavity.

The muscle always stretches posteriorly between the protocerebral commissure and the suboesophageal ganglion (Fig. 5E) and anteriorly between the mesal margins of the lammmntentor (Fig. 5D). — M. verticopharyngalis (M.51 = op1), well developed. (O) dorsolaterally on postoccipital ridge, (I) dorsolaterally on the postcerebral pharynx, opposite to M.52. (F) dilator of the postcerebral pharynx, together with M.52. — M. tentorichypopharyngalis (M.52 = op2), relatively short but well developed. (O) posterior edge of gular ridge, (I) ventrolaterally on the postcerebral pharynx, opposite to M.51. (F) dilator of postcerebral pharynx, together with M.51. — A ring muscle layer encloses the entire pharynx.

3.10. Cephalic central nervous system
Figs. 6A, B, 7A, 8B

The brain (cerebrum) is moderately sized in relation to the head size and located slightly posterior to the middle region of the head, between the anterior parts of the dorsal compound eyes. The protocerebrum including the large optic lobes appears dumbbell shaped. The central body is clearly defined in the central protocerebral region and the optic neuropils are also distinct. The optic lobes are divided distally (Fig. 8B), with a smaller portion connected with the dorsal ocellar subunit and a larger one to the ventral eye, both passing through the relatively small openings of the extensive internal circumocular ridges. The protocerebral halves are continuous with relatively short and broad circumoesophageal connectives and connected...
with each other by a very distinct tritocerebral commissure below M. tentoribuccalis anterior (M.48). The large suboesophageal complex is enclosed by the gular ridges (Fig. 10D,E). It reaches the limnatinotorion anteriorly and extends into the anterior prothorax posteriorly, where it appears almost fused with the prothoracic ganglia.

3.11. Stomatogastric nervous system

Figs. 6A, 7A, 8B, 10E,F

The well-developed transverse frontal ganglion has a triangular shape in dorsal view, with an obtuse posterior angle. The nerve recurrents originates more medially from the frontal ganglion and the frontal connectives at its lateral edges.

3.12. Glands

Fig. 7A, 9D–F

A large, transverse and flattened structure is present in the clypeofrontal region, with a thick gland-like epithelium. Ducts are recognizable internally and some very limited lumina. A duct connecting it directly with the lumen of the mandible or with the exterior could not be found on the available section series. Glands in the labral and mandibular lumen and salivary glands are absent.
4. Characters of the adult head

A character state matrix in Winclada format is provided as Electronic Supplement File 2.

1. Contact between anterior pronotal margin and upper surface of head: (0) surfaces distinctly separated; (1) surfaces almost merging. The anterior pronotal margin and the dorsal surface of the head appear almost fused in the monospecific ancestral genus Spangler gyrus (Beutel 1989a), in Gymnus (Fig. 2B) and in Heterogyrus (Figs. 1A, 2A). This is likely a groundplan synapomorphy of Gymnidae, but this condition is very insufficiently documented in the family. The character is unknown in other groups of Adephaga (Beutel 1986; Belkacem 1991; Dressler & Beutel 2010).

2. Shape of clypeal region: (0) evenly sloping, head wedge-shaped; (1) clypeal region almost vertical. A nearly vertical clypeal region is likely an autapomorphy of Spangler gyrus (Fig. 11; Beutel 1989a: figs. 3, 4).

3. Tactile setae on head capsule: (0) present; (1) absent. Absent in Heterogyrus like in all genera of Gymnanae, but present in Spangler gyrus (Fig. 11; Beutel 1989a: figs. 1, 3). The absence is likely a synapomorphy of Heterogyrus and Gymnanae. Tactile setae are present in terrestrial groups of Adephaga.
4. Dense field of setae on frontal region: (0) absent; (1) present. Absent in *Heterogyrus* and all genera of Gymninae (Figs. 1, 2). The specifically arranged setae of *Spanglerogyrus* (BEUTEL 1989a: figs. 1, 3) are probably an autapomorphy of the genus (BEUTEL 1989a: figs. 1, 3).

5. Lateral frontal ridge: (0) joining suprioral bead along dorsal margin of dorsal eye; (1) not joining bead along dorsal margin of dorsal eye. The lateral frontal ridge is continuous with the bead along the dorsal margin of the dorsal eye in *Spanglerogyrus* (Fig. 11, BEUTEL 1989a: figs. 1, 3) and *Heterogyrus* (Fig. 2A). This is possibly a groundplan synapomorphy of Gymninae. The absence of this connection, probably linked with the dorsal shift of the dorsal ocular subunit, is a possible synapomorphy of Gymnini (Fig. 2B), Enhydridae and Orectochilinae (Gymninae).

In *Gyrinus* the frontal ridge joins the ventral margin of the ventral ocular subunit and is continuous with the interocular suture (HONOMICHE 1975: figs. 3, 4).

6. Division of compound eyes: (0) absent; (1) upper and lower portion divided by narrow stripe; (2) interocular bridge less than half as wide as dorsoventral diameter of ventral ocular subunit; (3) interocular bridge about as wide as dorsoventral diameter of ventral ocular subunit. The compound eyes are subdivided by a narrow stripe in *Spanglerogyrus* (Fig. 11). This is likely a groundplan synapomorphy of Gymninae (BEUTEL 1989a). A moderately wide interocular bridge as it is found in *Heterogyrus* (Fig. 2A, GUSTAFSON et al. 2017) is probably a derived groundplan feature of Gymninae excluding *Spanglerogyrus*, and a distinctly widened bridge (HATCH 1925b, 1926, HONOMICHE 1975) a potential synapomorphy of Gymnina (Fig. 2A), Enhydridae and Orectochilinae (Gymninae).

7. Flagellar groove of interocular bridge: (0) absent; (1) present. The bridge separating the ocular subunits in Gymnina bears a distinct longitudinal groove for reception of the antennal flagellum on its dorsal surface (Fig. 2). This is likely a synapomorphy of *Heterogyrus* and Gymninae.

8. Field of sensilla on flagellar groove: (0) absent; (1) present. A field of sensorial setae is present on the anterior region of the flagellar groove of *Heterogyrus* (Fig. 2A). This is likely an autapomorphy, as this condition has not been observed in other genera (HATCH 1925b, 1926, HONOMICHE 1975, BEUTEL 1989a).

9. Size of upper subunit of compound eye: (0) as large as ventral subunit; (1) distinctly smaller. Both subunits are approximately equally sized in *Heterogyrus* and *Spanglerogyrus* (Figs. 1, 2A, 11), where-
as the dorsal ocular subunit is distinctly smaller in Gyriniinae (Fig. 2B).

10. Position of dorsal ocular subunit: (0) not shifted onto dorsal surface of head capsule; (1) shifted onto dorsal surface of head capsule. The dorsal subunit is shifted onto the dorsal surface of the head in Gyriniinae (Fig. 2B). This apomorphic feature is apparently correlated with the broadened interocular bridge.

11. Contact of posterior margin of dorsal eye with anterolateral pronotal edge: (0) present; (1) absent. The hind margin of the dorsal ocular subunit is directly in contact with the anterolateral pronotal margin in Heterogyrus and Spanglerogyrus (Beutel 1989a) (Figs. 2A, 11). This is likely a groundplan feature of Gyriniidae, and the distinct separation a possible autapomorphy of Gyriniinae (Fig. 2B).

12. Separate lateral clypeal portion: (0) absent; (1) present. A small lateral portion is distinctly separated from the main part of the clypeus of Heterogyrus and the remaining gyniine genera including Spanglerogyrus (Hatch 1925b, 1926; Honomichl 1975; Beutel 1989a) (Figs. 1A, 2, 11). This is likely an autapomorphy of the family.

13. Field of setae on lateral clypeal portion and labral base: (0) absent; (1) present. Its presence in Heterogyrus is likely an autapomorphy (Fig. 2B). The lateral clypeal portion is smooth in Gyrini (Fig. 2B) and few setae are present in Spanglerogyrus (Honomichl 1975; Beutel 1989a) (Fig. 11).

14. Midgut apodeme: (0) absent; (1) present. Well-developed in Heterogyrus (Figs. 6E, F, 7C, 10) like in other gyniine genera (Hatch 1925b, 1926; Beutel 1989a). This structure probably belongs to the groundplan of Adephaga but it varies strongly within the suborder (e.g., Carabidae, see e.g., Dressler & Beutel 2010).

15. Anterior tentorial arms: (0) present; (1) absent. Present in Heterogyrus (Fig. 7C), Spanglerogyrus (Beutel 1989a), Enhydrina and Orectochilus (Hatch 1925b, 1926; Honomichl 1975). Almost generally present in Adephaga (Dressler & Beutel 2010) but absent in Gyrinus and Aulognys (Hatch 1925b, 1926; Honomichl 1975; Beutel 1989a).

16. Dorsal tentorial arm: (0) present; (1) absent. Absent in Heterogyrus (Fig. 7C) like in all Gyriniinae (Hatch 1925b, 1926; Beutel 1989a). Very likely an autapomorphy of the family.

17. Tentorial bridge: (0) present, (1) vestigial, present as thin tendons, (2) absent. Present in Spanglerogyrus and Enhydrina, but absent as a distinctly developed transverse bar in Heterogyrus (Fig. 7C), Gyriniinae and Orectochilus (Hatch 1925b, 1926; Beutel 1989a). The thin tendons connecting M. tentorio-buccalis anterior with the gular ridges in Heterogyrus are probably vestiges of the tentorial bridge.

18. Lamnagerronia: (0) not fused mediadly, (1) fused mediadly. The lamnagerronia are small but present in Gyriniinae (absence is an overstatement in Dressler & Beutel 2010). They are separate in Heterogyrus (Fig. 7C) and in all Gyriniinae examined (Hatch 1925b, 1926). Their median fissure in Spanglerogyrus albovirans (Beutel 1989a) is likely an autapomorphy of this species.

19. Labral setae: (0) one row, (1) three rows. Three rows are present in Heterogyrus (Fig. 1B) and the other Gyriniinae examined (Honomichl 1975; Beutel 1989a). This is apparently an autapomorphy of Gyriniinae (Beutel 1989a).

20. M. labroopharyngalis (M.7): (0) present; (1) absent. M. labroopharyngalis is absent in Heterogyrus (Fig. 8A, 9) and in all other Gyriniinae examined (Honomichl 1975; Beutel 1989a). Labral muscles are probably generally absent in Adephaga (e.g., Beutel 1986; Bleidacke 1991; Dressler & Beutel 2010), but occur in different groups of Polyphaga (e.g., Anton & Beutel 2004; Anton et al. 2018).

21. Scapus and pedicel: (0) cylindrical; (1) scapus distally cup-shaped, pedicel with large auricular lobe with dense fringes of long mechanoreceptive setae. Highly modified in Heterogyrus like in all other Gyriniinae (Honomichl 1975; Beutel 1989a).

22. Flagellum: (0) filiform; (1) flagellomeres short and wide, broadly connected. A stout flagellum with broadly connected flagellomeres is present in all Gyrini (Figs. 1, 2; Hatch 1926, Beutel 1989a). It is quite long in Heterogyrus but this is only a gradual difference to the condition found in other gyniine taxa. The length of the flagellum varies considerably within the family (e.g., Hatch 1926).

23. Number of flagellomeres: (0) nine; (1) less than nine. The number of nine free flagellomeres is certainly a groundplan feature of the family and of Adephaga (e.g., Beutel 1989a; Dressler & Beutel 2010). This number is found in Heterogyrus (Figs. 1, 2) and Spanglerogyrus (Fig. 11) and some other genera (Hatch 1925b, 1926; Beutel 1989a). Fuscos occur in Gyrini (1 free flagellomeres in Gyrinus and Aulognys), Enhydrina (6 in Enhydrina and Dinema, 8 in Macrogynus and Porphyrhynchus) and Orectochilus (6 in Orectochilus) (Hatch 1925b, 1926; Beutel 1989a).

24. Pubescence of flagellomeres: (0) present; (1) partly reduced but still distinct; (2) absent. A distinctly reduced but still distinct pubescence on flagellomeres, especially the apical one, is present in Heterogyrus and in Spanglerogyrus (Beutel 1989a) (Figs. 1A, 2A, 11). It is almost completely absent in the other Gyriniinae (e.g., Honomichl 1975; Fig. 2B).

25. Mesal side of mandibular base: (0) almost as long as total length of mandible, moderately long; (1) much shorter. Of normal length in Spanglerogyrus (Beutel 1989a: figs. 6–9). Distinctly shortened in Heterogyrus (Fig. 4) and Gyriniinae (Hatch 1926; Honomichl 1975; Beutel 1989a).

26. Area between apical mandibular incisivi: (0) narrow; (1) wide triangular area. A very wide triangular area is present between the mandibular incisivi of
Heterogyrus (Fig. 4) and the genera of Gymnaceae (Hatch 1926; Honomich 1975; Beutel 1989a). In contrast to the interpretation in Beutel et al. (1989a), this unusual condition (see e.g., Beutel 1986; Belkacem 1991; Dressler & Beutel 2010) is likely a synapomorphy of Heterogyrus and Gymnaceae. This character is possibly correlated with the previous one.

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rioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorioriorio
documented in other groups of Adephaga. Apparently different degrees of reduction of lateral appendages occur (Dresenser & Beutel 2010: figs. 3D, 9C).

40. Origin of M. tenotorostralis anterior (M.48): (0) on teotoral bridge; (1) on laminaoutoria; (2) attached to gular ridges with very thin, posteriorly diverging tendons. The highly unusual attachment on the gular ridges with very thin posteriorly diverging tendons is very likely an autapomorphy of Heterogyrus. The origin on the fused laminaoutoria is an autapomorphy of Spanglergyrus (Beutel 1989a).

41. Clypeofrontal glands: (0) absent; (1) present. A single flat gland-like structure with some small lumina in its interior is present in the clypeofrontal region of Heterogyrus (Figs. 7A, 9). Similar structures are probably generally present in Gymnidae (Beutel 1989a) and an autapomorphy of the family. However, the interpretation of this organ varies. It was hollow in a single sectioned specimen of Spanglergyrus and consequently described as an air sac (Beutel 1989a), like structures of Divenius with a similar placement (Tonnari 1978).

42. Position of the brain: (0) central region of the head; (1) posterior head region close to foramen occipitale. The posterior shift and relatively large size of the brain is likely an autapomorphy of Spangle
gyrus (Beutel 1989a). This condition is very likely correlated with a reduced head size.

43. Division of the optic lobes: (0) absent; (1) present. The optic lobes are subdivided into a dorsal and a ventral branch in Heterogyrus (Fig. 8C) and probably in all Gymninae (Honkowieli 1975). This is obviously correlated with the separate openings of the circumsagittal ridges, which are still united in Spanglerogyrus (Beutel 1989a: fig. 19). This is likely an autapomorphy of Gymninae.

44. Internal opening of circumsagittal ridge: (0) not separated; (1) separate openings for dorsal and ventral ocular subunits. Separate openings in Heterogyrus and all examined Gymninae (Fig. 7C) (Hatch 1926, Honkowieli 1975).

5. Results of the phylogenetic analyses

The analysis (16 terminal taxa, 4 outgroup terminals) yielded 12 minimum length trees of 55 steps with NONA (consistency index 0.67) and TNT. The monophyly of Gymnidae (branch support [BS] 0), Gymnidae excluding Spanglerogyrus (BS 10) and Gymninae (BS 6) was strongly supported. Within Gymninae only a clade Gy- rinas + Aulogyrus (Gymnina) was consistently resolved (BS 2). The strict consensus tree with apomorphies mapped on it is shown in Fig. 12.

6. Discussion

Not surprisingly, the results of the present study clearly confirm the monophyletic origin of Gymnidae (Fig. 12). This was already supported by numerous derived features in earlier studies (Hatch 1925b, 1926, Beutel 1989a, 1990, Miller & Bergsten 2012) and has never been seriously questioned. Some unique and complex apomorphies are directly related to the surface swimming habits (e.g., Bendenle 1986), like the far-reaching modification of the antenna or the complete subdivision of the compound eyes (Fig. 2). New or little known apomorphies are the very tight connection of the upper surface of the head capsule with the anterior pronotal edge (Figs. 1A, 2), the presence of a separate lateral clypeal portion (Figs. 2, 11), the loss of the dorsal tentorial arms (Fig. 7C), the complete absence of labral muscles, and the strong enlargement of the transverse muscles of the cibarium (M. 67). Another apomorphy is the presence of glands in the clypeofrontal region (Figs. 7A, 9), previously also described as mandibular glands (Honkowieli 1975: Gyrinus) or air sacs (Tokapi 1978: Dineina; Beutel 1989a: Spanglerogyrus). The histological properties in Heterogyrus clearly show that this structure is formed by glandular tissue. It is accompanied by tracheae but they do not enter it, and the internal hollow space is very limited. As a connection with the mandibles could not be found in Heterogyrus, it is named clypeofrontal gland according to its placement in this cephalic region. A re-examination of this structure in well-preserved specimens of Spanglerogyrus albiventris is desirable. The function is presently unclear.

Like the monophyletic origin of the family, the monophyly of a clade Heterogyrus + Gymninae (as defined in Miller & Bergsten 2012) is strongly supported by cephalic features (branch support 8). In contrast to Spanglerogyrus (Fig. 11), doubtlessly the sistergroup of the remaining family (Folkerts 1979, Beutel 1989a, b, 1990), the compound eyes are not only divided by a thin interocular stripe of cuticle, but by a more or less wide interocular bridge (Fig. 2). The openings of the internal circumsagittal ridges, which are still connected in Spanglerogyrus (Beutel 1989a: fig. 19), are distinctly separated. Tactile setae are absent from the surface of the head capsule (Figs. 1, 2). The mandibles are modified, with a distinctly shortened mesal edge (Beutel 1989a) and a wide triangular area enclosed between the widely separated incisivi. Another convincing cephalic autapomorphy is the presence of three rows of setae on the labrum, instead of only one in Spanglerogyrus, and possibly the loss of a triangular field of microtrichia on the anteroventral labral surface (Beutel 1989a). An apomorphic character of the labrum is the distinct enlargement of the rounded lateral mental lobes (Fig. 1B).

The data presented here clearly corroborate a sistergroup relationship between Heterogyrus and Gymninae (branch support 10), as already discussed by Miller & Bergsten (2012) as one of two possible options. In particular, Heterogyrus lacks a series of features related to the compound eyes that form a complex synapomorphy of Gymnina, Enhydrina and Oreotrichini. The bridge separating the dorsal and ventral ocular subunits is distinctly broader in species of Gymninae than in Heterogyrus (Fig. 2). The dorsal eye is smaller than its ventral counterpart and shifted onto the dorsal side of the head. Moreover, in contrast to Spanglerogyrus and Heterogyrus, it is distinctly separated from the anterior pronotal margin. The lateral frontal ridge, which is continuous with the supraocular bead in Spanglerogyrus and Heterogyrus, is completely separated from the upper margin of the dorsal eye in Gymninae (e.g., Hatch 1926; Honkowieli 1975). Even though these characters are likely more or less closely correlated, it is plausible to assume that they have evolved only once as a complex innovation of Gymninae. An independent character is the almost complete loss of the setation of the antennal flagellum, in contrast to Spanglerogyrus and Heterogyrus, where a relatively sparse but distinct vestiture is still present (Figs. 1, 2A, 11; Beutel 1989a: figs. 1–3, 21). Another argument for a placement of Heterogyrus as the second branch in the family is the presence of two galgomeres, in contrast to one or none in Gymninae. Additional apomorphies of Gymninae not shared by Heterogyrus are the reduction...
of the apodeme of the palpiger (premental apodeme) and the origin of the labial pulp muscles from the inner surface of the praepons.

Despite of its basal placement in Gymnidae, *Sponglerogyrus* has evolved some apomorphic features of the head. This includes the shortening of the head with a steep clypeal surface (Fig. 11) (Beutel 1989a), a partly reduced submento-mental suture, fused lamcartalorion, the origin of M. tentorioocularis anterior (M.45) from this structure, and the transverse field of regularly arranged setae on the front. The large size of the brain in relation to the size of the head is probably an effect of miniaturation (Beutel 1989a; Beutel & Haas 1998).

*Heterogyrus* is probably very close to the groundplan of Gymnidae excluding *Sponglerogyrus* in its cephalic features. A potential autapomorphy is the attachment of M. tentorioocularis anterior to the gular ridges by tendons. These very tiny structures are likely vestiges of a tentorial bridge, which is completely missing in Gymnus and Oreotrichini (Hatch 1925b, 1926). Another potential apomorphy of *Heterogyrus* is the field of setae on the antennal groove between the ocular subunits. This feature has not been described for other genera of Gymnidae thus far.

The results presented here, i.e. the monophyly of Gymnidae, Gymnidae excl. *Sponglerogyrus*, and Gymninae (excl. *Heterogyrus*) are in agreement with earlier studies based on morphological characters (e.g., Beutel 1989a,b, 1990) and a recent study based on molecular and morphological data (Miller & Bergsten 2012 [preferred topology]). However, as already pointed out by Beutel (1989a), characters of the adult head do not provide unambiguous evidence for the relationships between the tribes of Gymnidae. Only few cephalic features tentatively indicate relationships within this subfamily. The complete loss of the tentorial bridge is a shared derived feature of Gymnina and Oreotrichini, and the absence of the anterior tentorial arm is a synapomorphy of *Gymnus* and *Autonogyrus* (Gymnina). The loss of the galea is a potential synapomorphy of Oreotrichini and Enhydrini, which also share a number of larval features (Beutel & Rodgersley 1994). It is evident that a broader spectrum of characters is necessary for a clarification of this issue, including extensive molecular data.

7. Conclusions

Even though phylogenetic conclusions should be based on features of more than one body part and of different life stages, the head with its complex external and internal structures provides a rich character set for provisionally placing *Heterogyrus*. The cephalic characters clearly suggest that this Malagasy genus is the second branch in the phylogenetic tree of Gymnidae, the sistergroup of a clade comprising the three tribes Gymnini, Enhydrini and Oreotrichini.

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9. References


3.5 Study VIII

Yavorskaya MY, Hörn schemeyer T, Beutel RG (submitted)

The head of *Micromalthus debilis* (Micromalthidae, Archostemata, Coleoptera) – an “archaic” beetle with an unusual morphology and a unique life cycle.

**Abstract:** Cephalic features of *Micromalthus debilis* were examined with different morphological techniques and described in detail for the first time. The head displays several seemingly plesiomorphic features compared to other studied species of Archostemata, especially representatives of Cupedidae and Ommatidae. Cephalic protuberances characteristic for species of these two families are missing and antennal grooves are also absent. The surface of the head capsule is largely smooth, without tubercles as they are found in stemgroup beetles and ommatid and cupedid species. Cuticular scales, probably ancestral for Archostemata and possibly for Coleoptera, are also completely absent. The arrangement of three mandibular teeth in a vertical row and an immobilized labrum are derived features shared with Ommatidae. The complete loss of the maxillary endite lobe is shared with the small species *Crowsoniella reticata*. Like in all other examined archostemat species mandibular molae and prosthecae are missing. The simplified maxillae apparently play no role in the food uptake but rather function as accessory “ventral antennae”. Derived features are the partly reduced musculature of the maxillae and lacking extrinsic labial muscles. Apomorphies of the digestive tract are sclerotized median protuberances of the anterior epipharynx and hypopharynx, and the presence of a vertical loop of the anterior pharynx and a subcerebral postpharyngeal pouch. The tentorium is largely reduced. Consequently, all antennal muscles originate from the head capsule. A seemingly plesiomorphic feature is the presence of a short salivary tube and two associated muscles. This is a unique condition in Coleoptera as far as known at present. Structural features suggest that Micromalthus probably feeds on wood infested with fungi. A robust phylogenetic evaluation of anatomical features is presently not possible due to the lack of data for *Crowsoniella* (Crowsoniellidae) and *Sikhostalinina zhisloveae* (Jurodidae). Moreover, phylogenetic and evolutionary interpretations are impeded by possible effects of vestigialization of adults possibly resulting from endosymbionts (Wolbachia).

**Significance in the present thesis:** This study is focused on the adult head morphology of *Micromalthus debilis* (Archostemata). Its body size (2 mm) is comparable to other objects studied in this thesis. The feeding apparatus is evaluated in the context of vestigialization of adults and a reduced role of feeding. As a member of one of the four major coleopteran subgroups, *Micromalthus* is obviously important in the context of the evolution of feeding in adult beetles.

**Own contribution:** 50 %
The head of *Micromalthus debilis* (Micromalthidae, Archostemata, Coleoptera) - an “archaic” beetle with an unusual morphology and a unique life cycle

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Abstract

Cephalic features of *Micromalthus debilis* were examined with different morphological techniques and described in detail for the first time. The head displays several seemingly plesiomorphic features compared to other studied species of Archostemata, especially representatives of Cupedidae and Ommatidae. Cephalic protuberances characteristic for species of these two families are missing and antennal grooves are also absent. The surface of the head capsule is largely smooth, without tubercules as they are found in stemgroup beetles and ommatid and cupedid species. Cuticular scales, probably ancestral for Archostemata and possibly for Coleoptera, are also completely absent. The arrangement of three mandibular teeth in a vertical row and an immobilized labrum are derived features shared with Ommatidae. The complete loss of the maxillary endite lobe is shared with the small species *Crowsoniella relicta*. Like in all other examined archostematan species mandibular molae and prosthoeae are missing. The simplified maxillae apparently play no role in the food uptake but rather function as accessory “ventral antennae”. Derived features are the partly reduced musculature of the maxillae and lacking extrinsic labial muscles. Apomorphies of the digestive tract are sclerotized median protuberances of the anterior epipharynx and hypopharynx, and the presence of a vertical loop of the anterior pharynx and a subcerebral postpharyngeal pouch. The tentorium is largely reduced. Consequently, all antennal muscles originate from the head capsule. A seemingly plesiomorphic feature is the presence of a short salivary tube and two associated muscles. This is a unique condition in Coleoptera as far as known at present. Structural features suggest that Micromalthus probably feeds on wood infested with fungi. A robust phylogenetic evaluation of anatomical features is presently not possible due to the lack of data for *Crowsoniella* (Crowsoniellidae) and *Sikhotealinia zhititzovae* (Jurodidae). Moreover, phylogenetic and evolutionary
interpretations are impeded by possible effects of vestigialization of adults possibly resulting from endosymbionts (Wolbachia).

Keywords
*Micromalthus*, Micromalthidae, Archostemata, head, morphology

1. Introduction

*Micromalthus debilis* LeConte, 1878 is the only extant species of Micromalthidae, which is one of four or five families of the small beetle suborder Archostemata (e.g. Beutel et al. 2008; Hörnschemeyer 2016). This relict group, which has retained many ancestral features (e.g. Lawrence 1999; Beutel et al. 2008; Friedrich et al. 2009), comprises only approximately 40 extant species, and only one of them naturally occurs in Europe, the miniaturized and strongly flattened *Crowsoniella relicta* (Crowsoniellidae) (Pace 1975; Crowson 1975).

*Micromalthus debilis* is also miniaturized, highly modified structurally (e.g. Barlet 1996; Hörnschemeyer 2016), and has the most complicated life cycle of all beetles, including vivipary, hypermetamorphosis, different kinds of parthenogenesis, and paedogenetic larvae (Pollock & Normark 2002). The “ghost-sex life” was treated in a recent study by Perotti et al. (2016), who pointed out effects of endosymbionts likely resulting in a vestigialization of adults. Like other species of Archostemata, *Micromalthus debilis* is considered as rare and is only sporadically collected (e.g. Crowson 1962). Adults of *Micromalthus* were recently described from Eocene amber from France (Kirejtshuk et al. 2010), and larval specimens were found in Eocene Baltic amber, in Oligocene amber from Mexico and in Early Cretaceous Lebanese amber (e.g. Lawrence & Newton 1995; Hörnschemeyer 2010). The original area of distribution of *Micromalthus debilis* is the eastern part of North America, but today, resulting from transportation with timber, there are records from many parts of the world, including for instance Austria, Hong Kong, Hawaii, and South Africa (Hörnschemeyer 2016).

Like in some other groups of Coleoptera (e.g. Adephaga, Staphyliniformia, Myxophaga, Cupedidae; Beutel 1993, 1999; Beutel & Molenda 1997; Beutel et al. 1998; Yavorskaya et al. 2016), larval head structures of *Micromalthus* are described in detail (Beutel & Hörnschemeyer 2002). However, despite of the exceptionally
interesting biology and apparent phylogenetic importance, the morphology of the head and other body regions of adults is not well known. The external cephalic structures are treated briefly in a chapter of the Handbook of Zoology (HÖRNSCHEMHEYER 2016) and features of the head were discussed in phylogenetic studies focussed on Archostemata (BEUTEL et al. 2008; HÖRNSCHEMHEYER 2009). However, a detailed description and documentation was still lacking. Even though only two adult specimens of *Micromalthus debilis* were available for this study, and both of them were not optimally preserved for histological investigations, it appeared worthwhile to increase the knowledge of the cephalic morphology of this apparent key taxon. The observed features of the head and its appendages are compared to conditions found in other archostematan taxa, and discussed with respect to their functional, phylogenetic and evolutionary implications.

2. Material and methods

**Material.** Micromalthidae: *Micromalthus debilis* LeConte, 1878 (fixed and preserved in 70% ethanol; adults and larvae from laboratory colony, M.A. PEROTTI, Bangor, UK) Ommatidae: *Tetraphalerus bruchi* Heller, 1913 (fixed in FAE [formaldehyde, ethanol, acetic acid] and preserved in 70% ethanol; Argentina, Provincia de Mendoza, collected by Dra. Adriana Marvaldi) Cupedidae: *Priacma serrata* LeConte, 1861 (fixed and preserved in 70% ethanol; Montana, USA, collected by one of the authors [T.H.]).

**Anatomy.** One specimen of *Micromalthus debilis* was embedded in araldite CY 212® (Agar ScientWc, Stansted/Essex, England) and sectioned with a microtome HM 360 (Microm, Walldorf, Germany) equipped with a diamond knife. The sections were stained with toluidine blue and pyronin G (Waldeck GmbH and Co.KG/Division Chroma, Münster, Germany). The other specimen used for CLSM (confocal laser scanning microscopy) was dehydrated with ethanol (20–100 %) and acetone. BABB (mixture of benzyl alcohol and benzyl benzoate 1:2) was used as a clearing solution, according to a standard BABB protocol. The head was mounted in small droplets of BABB between two coverslips and scanned with a Zeiss LSM 510 in two channels – red 633 nm and green 488 nm and from both (ventral and dorsal) sides. Series of
digital slices were produced providing information on all internal structures including muscles. They were imported in Amira and used for 3D reconstruction.

All structures were manually outlined and surfaces of each head structure were created separately for them. The raw surfaces were converted and scaled with Transform2 64 bit software (freeware, Heiko Stark, FSU Jena, Germany; URL: http://starkrats.de). Afterwards, Autodesk Maya 2016 (Alias Wavefront, Toronto/Ontario, Canada) was used for smoothing and coloring the 3D models.

**Scanning electron microscopy (SEM).** Specimens for SEM investigation were dehydrated with ethanol, dried at the critical point and sputter-coated with gold (Balzers SCD050) and studied and imaged with a LEO 438 VP scanning electron microscope.

**Terminology.** The muscular terminology is based on v. Kéler (1963)

3. **Results**

3.1. **General features**

*Micromalthus debilis* is a small and comparatively weakly sclerotized species, varying in length between 1.5 and 2.5 mm (Hörnschemeyer 2016). The body surface is largely smooth, with a sparse vestiture of fine setae but lacking cuticular tubercles or scales. The elytra are shortened and lack window punctures. The abdomen comprises six or seven visible segments (Hörnschemeyer 2016).

3.2. **Head capsule**

(Figs. 1-3)

The head is prognathous, compact, only moderately flattened dorsoventrally, and posteriorly slightly retracted into the prothorax (Figs 1A, B). It is 0.38 mm long from the anterior clypeolabral margin to the hind margin of the head capsule, 0.51 mm broad at the ocular region, and 0.4 mm at the temporal region shortly behind the compound eyes (measurements based on male specimen examined with SEM). The cuticle of the head is largely smooth; it lacks tubercles and scales but the surface of the posterior genal region posterior to the compound eyes is wrinkled, and indistinct scale-like surface structures with slightly serrated edges are present on the clypeal
region. The head capsule lacks any dorsal protuberances and antennal grooves are also absent; it is nearly parallel-sided, with very slightly rounded posterolateral edges, which slightly converge towards the large foramen occipitale. A vestiture of medium length setae (ca. 30 µm) is present, with a higher density on the clypeal area (Fig. 2A) and below the compound eyes. The compound eyes are large and strongly protruding laterally, with ca. 160 ommatidia with distinctly convex cuticular lenses; the ommatidia are not separated by chitinous bridges and ocular setae are also lacking. Ocelli are absent. Any dorsal ecdysial sutures are absent and the transverse frontoclypeal strengthening ridge is also missing. External furrows enclosing the gula and posterior submentum on the ventral side of the head are very distinct (Fig. 1C); they are distinctly curved outwards and the entire enclosed gula-submental sclerite is widest at a level slightly posterior to the posterior ocular margin. Posterior tentorial grooves or pits could not be identified with certainty (see Fig. 1C) and the anterior pits are absent.
3.3. Cephalic endoskeleton
(Figs. 3, 4)

The tentorium is largely reduced. A pair of rudimentary, short posterior arms arises from the gula-submental furrows below the tritocerebral commissure; it is connected by a vestigial ligamentous tentorial bridge. Dorsal arms, anterior arms and laminatentoria are completely missing. The circumocular ridges are very well developed, with a relatively narrow passage for the optic lobes. Other internal cephalic ridges are absent including those enclosing the gula.

3.4. Labrum
(Figs. 1, 2A, 3B)

The labrum is completely fused with the clypeus. The anterior edge of the clypeofrons is slightly concave, without specific structural modifications; the lateral clypeolabral edges are slightly converging and the anterolateral corners are rounded. Ten long setae are inserted on the anterior clypeolabral surface.
Musculature: Musculus (=M.) labroepipharyngalis (7), probably absent (but see below); M. frontoepipharyngalis (8), absent; M. frontoepipharyngalis (9), absent. The homology of a non-skeletal structure in the clypeolabral region could not be clarified with the available CLSM images and the microtome section series.

3.4. Antenna
(Figs 2B, 3A)

The moniliform 11-segmented antennae are inserted anterolaterally. The antennal foramen is dorsally covered by a shallow, rounded anterolateral frontal projection. The scapus is large compared to flagellomeres 1-8 but is about as long as the pedicellus and the apical flagellomere; a deep constriction divides it into a proximal articulatory piece and an enlarged, rounded, cup-shaped distal part. Like the other antennomeres it bears a moderately dense vestiture of medium length setae (ca. 25 µm). A fine pubescence is missing on the entire antenna; the surface of the scapus is largely smooth on the distal part but a scale-like surface structure is recognizable on the proximal area of the cup-shaped portion; scale-like surface modifications are also present on the other antennomeres, most distinct on the distal 2/3 of the apical
The barrel-shaped pedicellus is slightly longer but narrower than the distal part of the scapus; it bears two circular rows of setae. Flagellomeres 1-8 are cup-shaped and each of them bears a loose whorl of setae on the widened distal part with the scale-like surface modifications; the apical flagellomere 9 is about twice as long as the preceding ones, almost cylindrical, slightly widening distally, and rounded apically; its medium length setae are less regularly arranged than on the other segments and stiff shorter setae are concentrated on the apical region.

Musculature (Figs 3A, 4A, B): strongly developed, M. tentorioscapalis anterior (1), M. tentorioscapalis posterior (2), M. tentorioscapalis medialis (4), O: all three from the central region of the head capsule (Fig. 3A), I: anteriorly, dorsally and posteriorly on the base of the scapus; M. scapopedicellaris lateralis/medialis (5/6), three bundles, O: two dorsally and one on the anterior wall of the scapus, I: dorsally and posterovertrally on the base of the pedicellus.

Fig. 2. SEM micrographs, head structures of M. debilis. A, clypeolabral region and mandibles, dorsal view; B, antenna; C, mouthparts, frontal view; D, maxillary palp. Abbreviations: ce – compound eye, cll – clypeolabrum, lp – labial palp, md –

3.5. Mandible
(Fig. 1A, D, 2A, C, 4A, B)

The robust, almost evenly curved mandibles are articulated in a typical dicondylic manner, with a strongly developed ventral condyle forming the mandibular part of the primary joint. The surface is largely smooth but the proximolateral area is wrinkled; this sculptured surface reaches the ventral condyle posteriorly. Approximately ten medium length setae are inserted dorsally, laterally and ventrally. The mandibular bases are relatively broad (ca. 60 µm) but widely separated and completely lacking molae (Fig. 4A); a retinaculum or moveable appendages (prostheca) are also missing. The curved distal part is concave on its inner side, which results in a spoon-like condition; three strongly developed and acuminate distal teeth are vertically arranged (Fig. 2C); the middle tooth is longer than the others.

Musculature (Fig. 3A, 4C, D): M. craniomandibularis internus (11), largest muscle of the head, filling out about 1/3rd of the cephalic lumen, composed of numerous thin bundles, O: extensive parts of the posterior head capsule, I: extensive, approximately horizontal adductor tendon; M. craniomandibularis externus (12), much smaller than M. 11, less than ten bundles, O: ventrolaterally on posterior head capsule, I: abductor tendon; M. tentoriomandibularis (13), distinctly developed, O: head capsule, close to the antennal insertion area, I: dorso-mesally on the basal part of the mandible.

3.6. Maxilla
(Figs. 1, 2C, 3B)

The distinctly simplified maxillae are inserted in very shallow maxillary fossae below the ventral mandibular bases and laterad the mentum. The large cardo is only indistinctly separated from the stipes mesally. The stipes is simple, almost tubular and undivided, with few short setae inserted on its surface. The galea and lacinia are completely missing (Fig. 2C). The palp is composed of four distinctly developed palpomeres (Fig. 2D). A palpiifer is missing. The short palpomere 1 is distally
extended and lacks setae. Palpomeres 2 and 3 are slightly larger but of similar shape. One medium length seta is mesally inserted on palpomere 2 and three setae are present on palpomere 3. The apical palpomere is distinctly enlarged, distally widening, with a distinct ventrolateral protuberance with extremely short apical sensilla and a slightly concave apical field with ca. 20 peg-shaped, hyaline sensilla (ca. 12 µm) (Fig. 2D). The proximal surface shows a very indistinct scale-like pattern. Three setae are inserted mesally on the apical segment, one dorsally and one laterally.

Musculation (Fig. 4): Only two extrinsic muscles are present; they likely function as extensor and levator or of the maxilla; the homology assessment is difficult as the maxillary base is strongly simplified; the origin and function tentatively suggests that the muscle with originating on the wall of the head capsule is M. craniocardinalis, O: ventrolaterally on the posterior head capsule, at the level of the posterior ocular margin, I: dorsally on the maxillary with a tendon. The second extrinsic muscle is either M. tentoriocardinalis (17) or M. tentoriostitipalis (18), O: vestigial posterior tentorial arm, I: laterally on the maxillary base; M. stipitopalpalis externus/internus (22/23), a single bundle, O: ventrally on maxillary base, I: base of proximal palpomere. Intrinsic palp muscles are present but the exact arrangement could not be reconstructed with the material at hand.

3.7. Labium
(Figs 1C, 2C, 4A, B)

The submentum is not present as a separate unit but completely integrated in the large and laterally distinctly delimited gula-submental plate (Fig. 1C). The mentum is a small element between the maxillary bases and the prementum, but distinctly separated from the anterior submental border by a very distinct transverse suture. The small prementum bears the three-segmented palps (Fig. 1C) on distinct palpigers; a sclerotized, roughly triangular structure resembling a ligula is present above the insertion areas of the palps; it is nearly vertically oriented, with paired ventrolateral emarginations and a slightly convex upper edge; it bears eight short setae on its surface. Palpomeres 1 and 2 are moderately widening distally; palpomere 2 is slightly longer than the proximal one and bears three or four setae on its apical region. The terminal palpomere 3 is spindle-shaped, slightly curved, and
slightly longer than the intermediate segment; it bears two setae on its dorsal side and a very small sensillum is present on its slender, apically rounded distal part; a second subapical projection also bears a similar sensillum.

Musculature (Figs 3B, 4): extrinsic muscles and muscles of labial endite lobes are absent. M. praementopalpalis externus (34), distinctly developed, O: lateral wall of prementum, I: laterally on the base of the proximal palpomere. Intrinsic labial palp muscles are probably present but could not be identified with certainty.


3.9. Epipharynx and anterior part of digestive tract
(Figs 3A, B, 4A, B)
The anteriormost part of the ventral wall of the clypeolabrum is slightly convex, glabrous and sclerotized. A strongly sclerotized but rather shallow median elevation with several setae and an irregular surface is present in the middle region. It is followed by a reverse V-shaped median rim with sclerotized wall. The posteriormost epipharyngeal section below the anteriormost pharynx is flat and semimembranous. The entire epipharyngeal region is devoid of microtrichia and a longitudinal epipharyngeal process (ANTON & BEUTEL 2004, 2006: lep) is not developed. A closed prepharyngeal tube is missing.

Musculation (Fig. 3B): M. clypealatalis (43), a V-shaped pair of medially converging bundles, O: clypealbral region, between areas of origin of extrinsic antennal muscles, I: medially on the rim of the middle epipharyngeal region. Transverse epipharyngeal muscles are completely lacking.

3.10. Hypopharynx
(Figs 4A, B)

The anterior hypopharyngeal part is fused with the prelabium and not visible as a protruding structure. A distinct, strongly sclerotized protuberance with a rough, irregular surface is present on the dorsal surface of the middle region of the hypopharynx, opposite to the sclerotized epipharyngeal elevation.

Musculation (Figs 3, 4): M. frontohypopharyngalis (41), two vertical and slender bundles, O: central area of frontal region, anterad of M. 44, I: laterally on the anatomical mouth.

3.11. Salivarium
(Fig. 4A, B)

A salivarium as a cavity between the prelabium and hypopharynx is not developed. A short salivary duct is present in the prelabio-hypopharyngeal region.

Musculation (Fig. 3B, 4A, B): Two well developed muscles arise from the lateral prelabio-hypopharyngeal wall and insert at the opening of the salivary duct. M.
hypopharyngosalivarialis (37), O: dorsolaterally on the anterior hypopharynx; M. prementosalivarialis anterior (38), O: laterally on anterior prepharynx, I: together with M. 37. A ring muscle layer of the salivary duct is missing.

3.12. Pharynx and oesophagus
(Figs 3B, 4B-D)

The anteriormost pharyngeal section forms a vertical loop before connecting with the open preoral cavity. The precerebral part is moderately wide; indistinct dorso-lateral and Ventro-lateral folds serve as attachment areas of dilators. The postcerebral pharynx is narrow. A second vertical loop of the digestive tract is formed at the pharyngeal-oesophageal border (Fig. 3B). A voluminous dorsal pouch of the oesophagus appears very closely connected with the posteriormost part of the protocerebrum (histological sections: Fig. 4C); its walls are smooth, whereas the posteriorly directed main tract of the oesophagus is strongly folded; it is very thin-walled and completely lacks a layer or circular or longitudinal muscles.

Musculature (Fig. 3B, 4B-D): M. clypeobuccalis (44), a V-shaped pair of bundles immediately anterior to the frontal ganglion, converging towards its insertion; O: posterior clypeofrontal region, I: anterior to anatomical mouth, medially on rim of posterior epipharynx, between insertions of M. 41; M. frontobuccalis anterior (45), one slender vertical bundle, O: posterad of M. 44 and frontal ganglion, I: laterally on indistinct fold of anterior precerebral pharynx; M. frontobuccalis posterior (46), five thin bundles, O: posterad of M. 45, I: successively on dorso-lateral folds of posterior precerebral pharynx; M. tentoriobuccalis posterior (50), several very thin bundles, O: ventral wall of head capsule, along the gula-submentual furrows, I: ventrolaterally on pharynx, below tritocerebral commissure. M. tentoriopharyngalis (52), a series of very thin bundles, O: posterior part of the ventral head capsule, along the gula-submentual furrows, I: ventrolateral postpharyngeal folds. A thin layer of circularly arranged muscle fibres is present around the pharynx.

3.13. Brain, suboesophageal complex and frontal ganglion
(Fig. 3a; B, 4B, C)
4. Characters of the adult head

1. Tubercles: (0) absent or very indistinct; (1) present. Tubercles are absent in *Micromalthus debilis* (Figs. 1, 2), in the miniaturized *Crowsoniella relicita*, and in *Sikhotalinia zhitovae* Lafer, 1996 (LAFER 1996), as it is usually the case in non-archostematan beetles. They are present in Cupedidae and Ommatidae, and also in stem-group Coleoptera (PONOMARENKO 1969; BEUTEL et al. 2008; HÖRNSCHEMEYER 2009).

2. Scale-like setae: (0) absent; (1) present. Absent in *Micromalthus* (Figs. 1, 2), *Crowsoniella* (PACE 1975) and *Sikhotalinia*, and also in non-archostematan beetles. Present in Cupedidae, Ommatidae, and stem-group Coleoptera (PONOMARENKO 1969; BEUTEL et al. 2008; HÖRNSCHEMEYER 2009). The scale-like surface modifications occurring on some head regions of *Micromalthus* are possibly vestiges of distinct scales occurring in other archostematan groups.

3. Ocelli: (0) three; (1) absent. Absent in *Micromalthus* (Fig. 1A), like in species of Cupedidae, Ommatidae and *Crowsoniella* (BEUTEL et al. 2008). The presence of three true ocelli in *Sikhotalinia* (LAFER 1996) is unconfirmed. Paired ocelli or a single ocellus occur in very few groups of Polyphaga (LESCHEN & BEUTEL 2004).

4. Constricted neck and postocular extensions: (0) absent or indistinct; (1) present. The head of *Micromalthus* lacks a constricted neck region and postocular extensions (Fig. 1A, C), as they are present in the other groups of Archostemata (incl. *Sikhotalinia*) (BEUTEL et al. 2008; HÖRNSCHEMEYER 2009).

5. Dorsal cephalic protuberances: (0) absent; (1) present. Paired dorsal protuberances of the head are characteristic for Cupedidae and Ommatidae. They also occur in *Crowsoniella* and *Sikhotalinia* (PACE 1975; LAFER 1996; BEUTEL et al. 2008) but are completely absent in *Micromalthus* (Fig. 1A).

6. Cephalic antennal groove; (0) absent; (1) below compound eye; (2) above compound eye. Completely missing in *Micromalthus* (Fig. 1A-C), and also absent in *Omma* and Cupedidae (BEUTEL et al. 2008). Grooves are present
below the compound eyes in *Tetraphalerus*, and above it in *Crowsoniella* and *Sikhtotealinia* (Beutel et al. 2008; Hornschemeyer 2009).

7. Gular sutures: (0) complete, reaching hind margin of head capsule; (1) incomplete, not reaching hind margin of head capsule; (2) absent. Distinct and reaching hind margin of head in *Micromalthus* (Fig. 1C) and Cupedidae. Not reaching hind margin in *Omma* and obliterated in *Tetraphalerus* (Beutel et al. 2008; Hornschemeyer 2009).

8. Tentorial bridge: (0) present, sclerotized; (1) ligamentous; (2) absent. Only present as transverse ligamentous structure in *Micromalthus*. The bridge is present and sclerotized in *Tetraphalerus* but missing in Cupedidae (Beutel et al. 2008; Hornschemeyer 2009). The condition in *Omma, Crowsoniella* and *Sikhtotealinia* is unknown.

9. Anterior tentorial arms: (0) well developed; (1) distinctly reduced or absent, detached from posterior tentorium. Absent in *Micromalthus* (Fig. 4A, B). Distinctly or completely reduced in *Tetraphalerus* and in other adults of Archostemata examined (Hornschemeyer et al. 2002; Beutel et al. 2008).

10. Frontoclypeal strengthening ridge: (0) present; (1) absent. Absent in *Micromalthus* (Fig. 1A, D) and other extant Archostemata with the exception of *Sikhtotealinia* (Lafer 1996: fig. 137.1).

11. Labrum: (0) free, connected with clypeus by membrane; (1) indistinctly separated from clypeus, largely or completely immobilised; (2) fused with head capsule. Fused with clypeus in *Micromalthus* (Figs 1A, D, 2A), *Crowsoniella* and *Omma* (Lawrence 1999; Beutel et al. 2008; Hornschemeyer 2009). Free in Cupedidae and *Sikhtotealinia* (Lafer 1996). Not fused with head capsule but immobilised in *Tetraphalerus* (Beutel et al. 2008).

12. M. frontoepipharyngalis (M. 9): (0) present; (1) absent. Absent in *Micromalthus* (Figs 3, 4A, B), *Tetraphalerus* and *Priacma* (Hornschemeyer et al. 2002). Also missing in many other beetles (e.g., Dressler & Beutel 2010; Antunes-Carvalho et al. 2017). Present as a very thin bundle in *Ascioplaga* (Hornschemeyer et al. 2006).

13. Length of antenna: (0) not or scarcely reaching hind margin of head; (1) reaching middle region of prothorax; (1) reaching middle region of body. Short in *Micromalthus* (Fig. 2B), and *Crowsoniella*, reaching the middle region of the
prothorax in Ommatidae (e.g. Lawrence 1999) and Sikhotealinia (Lafer 1996), and strongly elongated in Cupedidae (e.g., Hörnschemeyer 2009).

14. Shape of antennae: (0) filiform; (1) moniliform; (2) with cup-shaped flagellomeres and one-segmented distal club. Moniliform in Micromalthus (Fig. 2B). With cup-shaped flagellomeres and one-segmented distal club in Crowsoniella (Pace 1975: fig. 6).

15. Location of antennal insertion on head capsule: (0) laterally; (1) dorsally. Laterally in Micromalthus (Fig. 1B), Ommatidae, and Crowsoniella. On dorsal side of head capsule in Cupedidae excl. Priacma (Hörnschemeyer et al. 2002, 2006) and in Sikhotealinia (Lafer 1996).

16. Ventro-mesal margin of sculptured mandibular surface: (0) not reaching position of mandibular condyle; (1) reaching mandibular condyle. The sculptured lateral surface of the mandibles of Micromalthus and Ommatidae reaches the posterior ventral condyle (Beutel et al. 2008; Hörnschemeyer 2009).

17. Cutting edge of mandible: (0) horizontal, (1) three vertically arranged teeth. Three apical teeth are arranged in a vertical row in Micromalthus (Fig. 2C) and Ommatidae (Beutel et al. 2008; Hörnschemeyer 2009). The cutting edge is horizontal in Cupedidae and Sikhotealinia like in most other beetles (Hörnschemeyer et al. 2002; Beutel et al. 2008; Lawrence et al. 2011). Mandible apparently vestigial in Crowsoniella (Pace 1975) but insufficiently documented.

18. Galea: (0) present; (2) absent. Completely reduced in Micromalthus (Fig. 2C). Apparently also missing in C. relicta (Pace 1975: fig. 6) but insufficiently documented.

19. Lacinia: (0) present; (1) absent. Absent in Micromalthus (Fig. 2C; Hörnschemeyer 2005) and also in Crowsoniella according to Pace (1975).

20. Number of extrinsic maxillary muscles: (0) four; (1) two. Four extrinsic muscles are almost generally present in adult beetles, two originating on the head capsule and two on the tentorium (Hörnschemeyer et al. 2002, 2006; Beutel et al. 2008; Dressler & Beutel 2010; Antunes-Carvalho 2017). Only two bundles are recognizable in Micromalthus (Fig. 4C), one originating on the head capsule and one on the vestigial tentorium.
Digitiform sensilla on apical maxillary palpomere: (0) absent, (1) present. Missing in *Micromalthus* (Fig. 2D) and other archostematan beetles (e.g. Hörnschemeyer 2009). Countersunk digitiform sensilla of the apical palpomere occur in the other extant lineages of Coleoptera (Honomichl 1980).

Pit containing sensilla of dorsolateral field of apical maxillary palpomere: (0) absent; (1) present. The sensilla of the dorsolateral field are exposed in *Micromalthus* (Fig. 2D) but placed in a deep pit in Ommatidae (Hörnschemeyer et al. 2002, 2006; Beutel et al. 2008; Hörnschemeyer 2009).

Basal cavity of prementum: (0) absent, (1) present. Absent in *Micromalthus* (Fig. 1C), *Crowsoniella* (Pace 1975: fig. 6) and *Silkotealinia* (Lafar 1996: fig. 2). The deep pit and a corresponding strongly developed apodeme for attachment of the median premental retractor are present in *Tetraphalerus*, *Omma* and Cupedidae (Beutel et al. 2008).

Lid-like ventral premental plate: (0) absent, (1) present. A large lid-like premental plate is absent in *Micromalthus* (Fig. 1C; Beutel et al. 2008; Hörnschemeyer 2009). The presence is characteristic for Cupedidae and Ommatidae (Beutel et al. 2008). It is also present in *Crowsoniella*, with a fairly short transverse part and a median spoon-shaped process (Pace 1975: fig. 6).

Anterior appendages of prementum: (0) present; (1) absent. Absent in *Micromalthus* (Fig. 2C) and also in *Crowsoniella* (Pace 1975: fig. 6; Beutel et al. 2008). Subdivided into many digitiform appendages in *Cupes*, *Asciplaga*, *Distocupes* and *Tenomerga* (Hörnschemeyer 2009), presumably for the uptake of liquid food like nectar.

Mentum: (0) distinctly developed; (1) vestigial or absent. Absent in *Micromalthus* (Fig. 1C) and most other representatives of Archostemata (Hörnschemeyer et al. 2002; Beutel et al. 2008). A short transverse sclerotized element is present in *Tetraphalerus* (Beutel et al. 2008) and *Crowsoniella* (Pace 1976: fig. 6).

Sclerotized protuberance of hypopharynx and corresponding sclerotized elevation of hypopharynx: (0) present; (1) absent. Both structures are present
in *Micromalthus* (Fig. 4A) and apparently involved in triturating food. Not described in other groups of beetles.


30. Transverse epipharyngeal muscles: (0) present; (1) absent. Absent in *M. debilis* (Figs. 3B, 4A) but almost generally present in other groups of beetles (ANTON & BEUTEL 2004, 2006; ANTON et al. 2016; ANTUNES-CARVALHO et al. 2017; HÖRNSCHEMHEYER et al. 2002, 2006; BEUTEL et al. 2008; DRESSLER & BEUTEL 2010).

31. Vertical loop of anterior pharynx: (0) absent; (1) present. So far only described for *Micromalthus* (Fig. 3B).

32. Subcerebral oesophageal pouch: (0) absent; (1) present. So far only described for *Micromalthus* (Figs. 3B, 4D).

33. Muscularis of oesophagus: (0) present; (1) absent. Almost generally present but missing in *Micromalthus*. The cuticle of the oesophagus of *M. debilis* is very thin and strongly folded (Fig. 4D: oes).

34. Salivary duct: (0) present; (1) absent. Present in *Micromalthus* (Fig. 4B). Not observed in any other group of beetles (e.g. ANTON & BEUTEL 2004, 2006; BEUTEL et al. 2008; DRESSLER et al. 2010; ANTON et al. 2016; ANTUNES-CARVALHO et al. 2017).

35. Glands associated with mouthparts: (0) absent; (1) present. Present in *M. debilis* (Figs. 3B, 4) and also in different representatives of Myxophaga and Polyphaga, and also in examined species of Ommatidae and Cupedidae (ANTON & BEUTEL 2004, 2006; ANTON et al. 2016; ANTUNES-CARVALHO et al. 2017; HÖRNSCHEMHEYER et al. 2002, 2006; BEUTEL et al. 2008). Cephalic
glands are absent in Adephaga (e.g. Dressler & Beutel 2010; Beutel et al. 2017).

36. Voluminous glands in the prothorax: (0) absent; (1) present. Strongly developed in *M. debilis*. Not described in other archostematan beetles (Baehr 1975; Beutel et al. 2008; Friedrich et al. 2009) and non-archostematan beetles (e.g. Beutel & Komarek 2006; Ge et al. 2007).

5. Discussion

The body organization of *Micromalthus* is likely affected by miniaturization, like for instance the weak sclerotization and the fused prothoracic sclerites (e.g. Barlet 1996; Lawrence et al. 2011). However, modifications, especially structural simplifications, may be also due to the sporadic appearance and vestigialization of adults, especially males (Pollack & Normark 2002; Perotti et al. 2016). The head shows some apomorphies, which are arguably linked with reduced size. Cephalic ridges are missing except for extensive circumocular ridges. The tentorium, which is also partly reduced in other archostematan species (e.g. Hörschemeyer et al. 2002, 2006; Beutel et al. 2008), is only preserved as vestigial posterior arms and a ligamentous bridge in *Micromalthus*.

*Micromalthus* differs in an entire series of features observed in other archostematan groups, especially Cupedidae and Ommatidae, which are arguably closest to the groundplan of the suborder and also show the greatest structural similarity with stem group beetles (Ponomarenko 1969; Beutel 1997; Beutel et al. 2008; Friedrich et al. 2009). This includes the lack of cuticular tubercles or scales, the absence of dorsal protuberances, and the absence of a narrowed neck region. These features probably evolved in the stem group of beetles (Ponomarenko 1969; Beutel 1997; Beutel et al. 2008). This and the subordinate position of *Micromalthus* within Archostemata, either as sistergroup of Cupedidae (Beutel & Hörschemeyer 2002) or of Ommatidae (Hörschemeyer 2009), implies secondary loss, even though the absence is consistent with the condition found in most other groups of Coleoptera (e.g. Anton & Beutel 2004; Dressler & Beutel 2010; Anton et al. 2016; Antunes-Carvalho et al. 2017).
*Micromalthus debilis* is characterized by numerous autapomorphic features. It is arguably one of the most aberrant species of the entire order, especially in its lifecycle, but also in some morphological traits. The distinctly moniliform antenna are probably autapomorphic, even though similar conditions occur in Ommatidae and also in *Sikhotaelinia*. What is highly modified in *Micromalthus* is the feeding apparatus including the mouthparts. The maxillae lack endite lobes completely, as it is probably also the case in the miniaturized *Crowsoniella*. The extrinsic musculature is distinctly simplified, largely restricting the maxilla to vertical movements. It is likely that it functions like an accessory ventral antenna like in larvae of Adephaga or Hydrophiloidea (e.g. BEUTEL 1993, 1999). Its structural configuration clearly shows that it is not involved in the food uptake (Fig. 2C). The same applies to the prementum, which in contrast to other beetles lacks extrinsic retractors (Figs. 3B, 4). The complete lack of the mola (Fig. 2C) is a feature shared with other archostematan groups, with Adephaga (DRESSLER & BEUTEL 2010), and with some groups of Polyphaga (LAWRENCE et al. 2011). This shows that grinding of food is not achieved by the mandibular bases. The shovel-like distal mandibular region of *Micromalthus* (Fig. 2C) and Ommatidae is equipped with three vertically arranged teeth. It is apparently suitable for scraping off wood particles and moving them towards the functional mouth opening, but not for intensive mechanical processing. The structural configuration of the sclerotized epi- and hypopharyngeal protuberances (Fig. 4B) and preoral dilators (Mm. 43, 44) indicate that trituration of food takes place in this area. Food pulp is probably diluted with gland secretions and then sucked back in the pharynx by coordinated contraction of the series of dorsal and ventral dilators (Mm. 45, 46, 50, 52) (Fig. 3B). The two vertical loops of the anterior digestive tract are a very unusual condition not known from other beetles. It is conceivable that the subcerebral pouch (Figs. 3B, 4D) functions as a fermenting chamber. The presence of cephalic glands (Fig. 3B, 4) is a feature shared with other non-adephagan beetles. The presence of large and branched glands in the prothorax and the presence of a well-defined salivary duct with salivary duct muscles (Fig. 4B) are very unusual features of *Micromalthus* and arguably plesiomorphic.

The reasons for the far-reaching modifications of the feeding apparatus remain unclear, as the feeding habits of adults are largely unknown (HÖRNSCHEMeyer 2016). A minor or obsolete role of food uptake linked with the vestigialization of adults can probably be ruled out. The entire configuration of the feeding apparatus is only partly
simplified and rather increased in complexity as far as the pharynx is concerned. As the larvae develop in wood (e.g. BEUTEL & HÖRNSCHEMEYER 2002) and considering the shape of the mandibles, it is plausible to assume that feeding of wood infested with fungi plays a major role.

The phylogenetic affinities of Micromalthus debilis remain ambiguous presently. Due to scarcity of material, analyses of molecular data with a sufficient archostematan taxon sampling have not been carried out yet (see e.g. McKENNA et al. 2015). However, the subordinate inclusion of Micromalthidae in Archostemata is largely undisputed (e.g. FORBES 1926; BÖVING & CRAIGHEAD 1931; BEUTEL & HÖRNSCHEMEYER 2002; BEUTEL et al. 2008; HÖRNSCHEMEYER 2009), even though the adults of the family lack characteristic features of the suborder, like for instance a lid-like enlarged prementum and a constricted neck region (see above). A close relationship between Micromalthus and Ommatidae is tentatively supported by features of the adult head. Supposedly derived conditions shared by the two taxa are mandibular teeth arranged in a vertical row and the immobilization of the labrum. Additional features of the male genitalia were pointed out by HÖRNSCHEMEYER (2009). A clade Micromalthus + Ommatidae is in conflict with larval features, which suggest a sistergroup relationship between Micromalthus and Cupedidae, for instance reduced stemmata, shortened antennae, a quadrangular mola with a distinct margin, asperities on segment IX, and a sclerotized project of tergum X. The reconstruction of the phylogeny of Archostemata is obviously impeded by fragmentary morphological information. The adult anatomy and larvae of Crowsoniella (only type series known) and Sikhoneula (only female holotype known) are completely unknown. The larvae of Tetraphalerus are also undiscovered and detailed information on internal structures of adults of Omma is not available. Another factor impeding phylogenetic and evolutionary interpretations is the difficulty to assessing effects of vestigialization of adults, which may have resulted from the association with endosymbiontic Wolbachia (PEROTTI et al. 2016).

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7. References


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4 Discussion

4.1 Anatomical techniques and optimization of the work flow

The following combination of techniques turned out as most effective for studying both external and internal head structures of small beetles.

Scanning electron microscopy (SEM) is optimal for documenting surface structures. For whole specimens and for mouthparts of larger objects (such as for instance Heterogyrus), a rotatable specimen holder developed by Pohl is very useful (2010). With this device all standard views can be obtained with a single specimen and a homogenous black background.

A disadvantage of SEM is that it does not provide information on the degree of sclerotization and transparency of the cuticle. However, this can be easily compensated using microscopy, or in case of the present thesis CLSM. Confocal laser microscopy does not only provide information on internal structures including softparts (see below), but also allows to obtain 3-dimensional images of the surfaces of the object. Volume renderings based on stacks of CLSM images look quite similar to SEM pictures, but provide more information on the cuticle properties.

Very small objects (<1,5 mm) are usually too fragile for ultrasonic sound, which is most commonly used for larger insects. However, it is mandatory to clean objects, as they are often covered with food particles and pieces of the substrate they live in. Therefore, Potassium hydroxide (KOH) is efficient for cleaning specimens and dissolving organic material on the cuticular surface (Schneeberg et al. 2017).

CLSM is very useful for visualizing internal structures of weakly sclerotized objects. This technique allows to obtain images just based on the auto-fluorescence of the cuticle. Specimens are not damaged and can be investigated with other techniques afterwards. Usually glycerin is used for mounting the specimens for CLSM. It is very easy to transfer the object from 70% ethanol and scan it right away, without any additional steps. However, different clarifying media such as BABB (benzyl alcohol + benzyl benzoate) or methylsalicylate help to obtain better images of internal structures (Friedrich et al., 2014; Wipfler et al., 2016). In the course of this Ph.D-project, BABB turned out as most effective for clarifying heads of tiny beetles. The longer the specimen is left in the solution before scanning, the clearer the images of the inner structures become.

It has to be noted that not all objects are suitable for visualizing inner structures with CLSM. For example, this technique does not work well with larger Ptiliidae. Apparently, their heads are too strongly pigmented and sclerotized to obtain signal from the deeper layers. Different bleaching techniques do not yield as good results as they do with either smaller or distinctly larger objects (Smolla et al., 2014). One possible reason why not all bleaching or coloring media work efficiently in the case of Ptiliidae might be that the fixator does...
not sink into it properly. Solving this problem, which is certainly not restricted to Ptilliidae, would help to obtain even better results with CLSM in the future.

Micro-computed tomography (μ-CT) is widely used in entomology since more than ten years. It has greatly accelerated the acquisition of high quality anatomical data in different projects (e.g. Beutel et al., 2011; Beutel et al., 2017). Advantages are the speed of data acquisition, nearly complete absence of deformation artifacts, the perfect alignment of image stacks for 3D reconstruction, and the non-invasiveness, with specimens intact after scanning. A disadvantage is insufficient information about tissue properties. Depending on the available equipment, resolution can be a problem if extremely small samples (e.g. Ptilliidae) are scanned. Therefore, this approach was only applied in one study of this PhD thesis (Beutel et al. 2017). Both surface renders and volume renders were applied in the study on Heterogyrus for an optimal visualization of internal and external structures, combined with SEM images.

Even though μCT and CLSM allow to visualize inner structures, traditional histological serial sections still provide additional information on the fine microstructure and histological properties of tissues, and should not be underestimated. This method is time consuming, but combining it with modern techniques provides maximum information about internal structures.

3D-reconstructions allow to visualize the obtained data on external and internal structures and facilitate the understanding of complex configurations. Sometimes even after histological sectioning and obtaining confocal or μCT datasets of the object, it remains challenging to comprehend the arrangement of all structures. Only a 3D-reconstruction brings these methods together and gives us full information on the arrangement of muscles, mouthparts and other structures and organs (Fig. 4). In this thesis almost all reconstructions are based on surface rendering, even though some CLSM datasets turned out to be detailed and complete enough for volume rendering of a complete head. Models based on manual outlines of each structure are not as detailed, but usually look clearer and contain a level of morphological interpretation like conventional drawings. This is important for visual perception and understanding complicated structural configurations.

All techniques listed above can help to obtain detailed and complex information on the head of a beetle. However, there is one more important approach to understand the function of structures – live observations. It can be very challenging to observe a tiny featherwing beetle consume food particles. Aside from the minute body size these beetles move fast and tend to jump or fly spontaneously. Nevertheless, it was possible to observe and even film the mouthpart movements of Acrostichis sericans and Nephantes titan. These observations helped to understand their feeding preferences and the coordinated movements and function of their mouthparts.
Fig. 4 Head of *Porofilla mystacea* (Ptiliidae) visualized with different techniques; A-B – 3D-reconstructions based on a CLSM dataset. A – cross-section of a 3D-reconstruction based on volume rendering; B – 3D-reconstruction based on surface rendering; C – SEM-micrograph.
4.2 Evolution of the feeding apparatus in Coleoptera

After evaluating the obtained data from representatives of all four major groups of Coleoptera and already existing studies on them, we suggest that saprophagy is the ancestral feeding type of Coleoptera (Fig. 5), with the presumably ancestral feeding apparatus (groundplan) similar to the one in Sphaeriusidae (Myxophaga) (Yavorskaya et al., subm. a) and Clambidae (Polyphaga) (Anton et al., 2016: Fig. 4). Archostemata (Yavorskaya et al., subm. b) and Adephaga (Beutel et al., 2017) adapted to different feeding habits with correlated modifications of the feeding apparatus: the life habits of Archostemata are not well known, but they apparently switched to more or less complete aphagy in the majority of adults, which is also the case in Strepsiptera, the sistergroup of Coleoptera in a different context (endoparasitism and very short-lived adults (e.g. Pohl & Beutel, 2008). Adephaga are carnivorous with very few secondary exceptions (e.g. Crowson, 1981; Beutel, 1997).

Fig. 5 Cladogram with selected taxa of Coleoptera: Archostemata (Archost.), Myxophaga (Myx.), Adephaga (Adeph.), Scirtiformia (Scirt.), Staphyliniformia (Hydraenidae (Hy), Pulillaed (Pt), Leiodidae (Lei), Agyrtidae (Ag)) and remaining Polyphaga; feeding habits mapped on tree.
4.2.1 Archostemata

*Micromalthus* differs in an entire series of features from the closely related Cupedidae and Ommatidae (Yavorskaya et al., subm. b). These to families are likely close to the groundplan of the suborder, and also similar to stem group beetles due shared ancestral features (Ponomarenko, 1969; Beutel, 1997; Beutel et al., 2008; Friedrich et al., 2009). *Micromalthus* lacks cuticular tubercles or scales, dorsal protuberances, and also a narrowed neck region, features regularly found in the stem group of beetles (Ponomarenko, 1969; Beutel, 1997; Beutel et al., 2008). The head shows some apomorphies likely linked with reduced size and a vestigialization of the adults (Perotti et al., 2016), for instance largely reduced cephalic ridges and a vestigial tentorium (e.g. Hörnschemeyer, 2002, 2006; Beutel et al., 2008).

The feeding apparatus is highly modified in *Micromalthus*. The maxillary endite lobes are absent. The distinctly simplified extrinsic maxillary musculature enables the maxilla mainly to carry out vertical movement, like in adephagan larvae where they function like accessory ventral antennae (e.g. Beutel, 1993). The structural configuration shows that it is not involved in the food uptake. This also applies to the prementum, which in contrast to other beetles lacks retractors. The complete loss of the mola is shared with other archostematian groups, with Adephaga (Dressler & Beutel, 2010), and also with some groups of Polyphaga (Lawrence et al., 2011). This and the wide separation of the proximal parts show that grinding of food between the mandibular bases does not take place. Like in Omatidae, the shovel-like distal mandibular region of *Micromalthus* is equipped with three vertically arranged teeth, apparently suitable for scraping off wood particles and moving them towards the functional mouth opening, but not for intensive mechanical processing. The presence of sclerotized epi- and hypopharyngeal protuberances and the arrangement of preoral dilators (Mm. 43, 44) indicate that trituration of food takes place in this region of the anterior digestive tract. It can be assumed that food pulp is diluted with gland secretions and then sucked back in the pharynx by coordinated contraction of dorsal and ventral dilators (Mm. 45, 46, 50, 52). The two vertical loops of the anterior digestive tract are an unusual condition not known from other beetles. The subcerebral pouch may possibly function as a fermenting chamber. The presence of well-developed glands is a feature shared with other non-adephagan beetles. The presence of large and branched glands in the prothorax and the presence of a well-defined salivary duct with salivary duct muscles are very unusual features of *Micromalthus* and arguably plesiomorphic.

The larvae of *Tenomera mucida* have almost the complete set of previously identified archostematian larval autapomorphies (Beutel & Hörnschemeyer, 2002 a,b). They also display some unusual plesiomorphies, such as the presence of M8 (M. frontolabralis), which absent in all other groups of Coleoptera (Beutel & Haas, 2000), and a tentoriomandibular muscle, not found in any other coleopteran larvae (Beutel, 1993; Beutel & Haas, 1998; Beutel &
Hörnschemeyer, 2002a,b; Beutel & Friedrich, 2005). In contrast to all other archostematan larvae with available anatomical data (Beutel & Hörnschemeyer, 2002a, b), the submentum and mentum appear still separated and two extrinsic labial muscles are preserved. A seemingly plesiomorphic feature, arguably due to reversal, is the lack of a distinct margin of the mandibular mola. It is present in 1st instars of Micromalthus and Priacma and also in mature larvae of other genera of Cupedidae (Beutel & Hörnschemeyer, 2002a, b).

Larvae of Micromalthus are wood-borers like immatures of Cupedidae (Hörnschemeyer, 2016; Yavorskaya et al., 2016). In contrast to the adults, food is mechanically processed and handled by strongly developed mandibular teeth and molae, and by the well-developed maxillary endite lobes.

4.2.2 Adephaga
The suborder Adephaga, mainly characterized by predaceous habits of larvae and adults (with very few exceptions; e.g., Crowson, 1981; Beutel, 1997), will not be treated in detail in this dissertation. The entire feeding apparatus of adults appears simplified (Dressler & Beutel, 2010) compared to saprophagous beetles, like for instance in Staphyliniformia (e.g., Anton & Beutel, 2004; Anton et al. 2016; Antunes-Carvalho, 2017) and or also in Myxophaga (Anton & Beutel, 2004; Yavorskaya et al. subm.). Mandibular molae and prothecae are completely missing. The galea does not bear fringes of regularly arranged hairs but is modified as a two-segmented palp like structures. Epi- and hypopharyngeal bulges or processes set with microtrichiae are also missing (Dressler & Beutel, 2010; Beutel et al., 2017). As in the case with the reduced role of feeding in adults of Archostemata, predacious habits and preoral digestion in Adephaga has apparently resulted in profound modifications of the feeding apparatus.

4.3 Evolution of saprophagy
General saprophagy and mycophagy are considered as ancestral feeding types of the two major groups of Polyphaga – Staphyliniformia and Cucujoida (Lawrence, 1989; Betz et al., 2003). They have developed different variations of this feeding type: apart from strict mycophagy and sporophagy, there were other multiple switches to various types of microphagy, such as feeding on pollen grains, dung, algae etc. Phytophagy was adapted by the majority of the remaining polyphagan beetles (e.g., Crowson, 1981). This requires other adaptations and modifications of the feeding apparatus such as dorsal cuticular glands that release defense chemicals (Reid, 2014), and will not be discussed further in this study.

The feeding apparatus of saprophagous, algophagous or sporophagous members of Myxophaga and Polyphaga is complex (e.g., Anton & Beutel, 2004, 2006; Anton et al., 2016; Antunes-Carvalho et al., 2016) compared to that of predacious Adephaga (e.g. Dressler & Beutel, 2010; Beutel et al., 2017) or Archostemata (Hörnschemeyer & Stapf, 2001; Beutel et al., 2008).
4.3.1 Characters related to food uptake and shifts of the feeding habits

According to present knowledge, mycophagy was adopted at least 18 times only in the superfamily Staphylinioidea, which includes Leiodidae and Ptiliidae (Newton, 1984) and other groups, among them the megadiverse Staphylinidae.

A potential synapomorphic feature of Staphyliniformia + Scarabaeoidea (or Staphyliniformia incl. Scarabaeoidea) (see McKenna et al., 2015) is an hourglass-shaped in cross section hypopharynx. Another apomorphic feature of this lineage is the presence of an unusual extrinsic maxillary muscle that originates laterally on the head capsule and is inserted on an internal membranous region proximad the mesal maxillary base which was also found in Catops (Leiodidae) (e.g. Anton & Beutel, 2004: Mx2; Anton & Beutel, 2012: M. craniobasis-maxillaris; Antunes-Carvalho, 2017). The former character is present in all examined species of Ptiliidae, whereas the latter is missing in some of them (see below). Aside from these two derived features, Staphylinioidea are mainly characterized by plesiomorphic conditions of the adult head, with a character combination likely coming close to the groundplan of the entire Polyphaga (and presumably Coleoptera) (e.g. Anton et al., 2016; Antunes-Carvalho et al., 2017).

A very unusual feature shared by Ptiliidae and their sistergroup Hydraenidae and apparently a synapomorphic condition is the subdivision of the mandible, with a membranous connecting zone between the mandibular main body and the mesal molar part. Another synapomorphy is a lateral process of the mandible, which is part of a unique mandibular-labral locking device (e.g. Jäck et al., 2000; Beutel & Leschen, 2005).

Other common features of the mandibles of both families are the well-developed grinding mola and the prosthca, probably ancestral conditions retained from the groundplan of Polyphaga. Whether the weakly developed mandibular apex is a synapomorphy of the two families (Betz et al., 2004; Beutel & Leschen, 2005) is debatable. A feature of the maxilla shared by the two groups is the fimbriate galea with regularly arranged rows of curved microtrichia (Beutel & Leschen, 2005). This condition has probably evolved independently in Hydrophiloidea (e.g. Beutel, 1994) and some groups of Staphylinidae (Betz et al., 2003), but it cannot be excluded that it belongs to the groundplan of Staphyliniformia, linked to primarily microphagous feeding habits. It comprises epi- and hypopharyngeal longitudinal bulges (or processes) set with microtrichiae, complicated mandibles with molae and brushes, and in some cases fimbriate galeae (e.g. Hydrophiloidea, Hydraenidae; Anton & Beutel, 2004). A noteworthy phenomenon observed in Ptiliidae is that the complexity of this apparatus is even increased, at least in some members of the family. Although sporophagy occurs in many species of Staphylinioidea (Betz et al., 2003), extremely small body size as it is typical for Ptiliidae apparently requires specific adaptations. In some cases, this apparently results in an increase in complexity rather than in simplification. The epipharynx, for instance, is
more complicated than in examined species of related groups, such as Hydraenidae (Jäch et al. 2000), Leiodidae (Antunes-Carvalho et al., 2016), Staphylinidae (Betz et al., 2003), or Hydrophiloidea (Anton & Beutel, 2004). It is divided into an anterior part corresponding with the ventral labral wall, an intermediate section with the longitudinal process (LEP), and a posterior part connected with the posterior hypopharynx and adjacent with the anatomical mouth. An additional feature in this context was observed in all examined ptilidiid species, the composition of M44 of two thick bundles inserted in deep cavities of the epipharyngeal wall. The prementum bears slightly asymmetrical angular lateral processes at its anterior edge, separated by a narrow median gap. Another feature apparently unique to ptilid beetles is the structure of the maxillary palp: palptomere 3 is much thicker and longer than the proximal two and often set with several rows of short microtrichia on its lateral surface, palptomere 4 is long, slender, and conical. It is likely that the palp with its specific modifications is involved in the process of collecting food particles.

The following features, previously described for spore-feeding Staphylinoidea (summarized by Betz, 2003 for the first time), are present in all studied Ptiliidae and are also characteristic for some saprophagous beetles (e.g. Anton & Beutel, 2004):

− cibarial roof with rows of parallel microtrichia
− galea with brushes and rows of long microtrichia, the main instrument for gathering spore masses and other food particles
− mandibles with well-developed molae
− epipharynx, prementum and hypopharynx with medial longitudinal bristle-troughs bordered by hairs or spines, involved in concentrating and directing the food stream in the median line.

4.3.2 Evolution of sporophagy and microsporophagy in Ptiliidae

The sporophagous Noscidium likely belongs to a first branch separating from the remaining Ptiliidae (Hall, 1999; Mckenna et al., 2015). Its species are strongly associated with Polyporus squamosus (spores 13 × 4.5 μm). All cephalic features are similar to those of other representatives of the family, including the lack of ridges, the presence of a lateral mandibular peg, and the labro-mandibular interlocking mechanism. Although Noscidium is sporophagous, its body size is much larger (1–1.1 mm) than in all known Nanosellini, and also the size of the spores it is feeding on. Despite the sporophagy of Noscidium, it is conceivable that this feeding type does not belong to the groundplan of Ptiliidae. It is found neither in the majority of this family, nor in its sister group Hydraenidae or, more generally, in closely related outgroup taxa (e.g. Beutel & Leschen, 2005; McKenna et al., 2015). Most species of Agyrtidae feed on dung, rotten fungi and similar decaying substances, and saprophagous feeding habits are
also common in Leiodidae and Hydraenidae. This suggests that saprophyagy is ancestral for Ptiliidae, and that feeding on spores evolved once in Nossidium (and probably some related genera), and independently in the distinctly smaller Nanosellini. Sporophagy as a groundplan feature of Ptiliidae cannot be completely excluded presently. However, it would imply that several ptiliid branches evolved saprophyagy secondarily, which would be less parsimonious than the alternative.

Sporophagous feeding habits were assigned to the entire family Ptiliidae by some authors (see Betz et al., 2003). However, this specialization is in fact restricted to species of Nossidium (and presumably some closely related genera) and Nanosellini. All other representatives of the family should be considered as saprophyagous.

Nanosellini is the ptiliid subgroup with extremely small species, most of which inhabit basidiomycete fungi, particularly Polyporaceae and Steccerinaeaceae (Dybas, 1961; Hall, 1999). Some of them can also inhabit Meripilaceae (Polyporales), Hymenochaetales (Schizoporaceae and Hymenochaetaeaceae) and Ascomycetes (Valsaeeae) (Polilov 2008). Their only source of food are fungal spores, with a size (diameter 2–6 μm) apparently compatible with the size of the mouthparts (approx. head width 50–130 μm). It is evident that their feeding mechanism differs distinctly from what is found in larger sporophagous staphylinids, where the mouthparts are at least hundred times larger than the spores. Therefore, it is appropriate to call their type of feeding microsporophagy. Although nanosellines preserve all main features of the feeding apparatus commonly found in larger spore-feeding staphylinoids (and also saprophyagous ptiliids and saprophyagous beetles of other families), they have evolved some new features to adjust to this modified feeding mode. The mandibles are more compact than those of larger ptiliid species, with a smaller molar surface more tightly attached to the main mandibular body. The unusual basal maxillary muscle Mx, which is usually present in staphyliniform beetles including saprophyagous ptiliids, is missing. The extremely complicated epipharyngeal-hypopharyngeal structures could be also part of the adjustment to more specialized feeding habits.

Observations of living beetles (Nephanes, Acrotrichis) provided information about feeding preferences and feeding mechanisms of saprophyagous ptiliid species. The beetles consumed rotten plant materials and mold, and collected droplets of condensed liquid on the walls of the petri-dish in which they were held. They also consumed liquid yeast solution and droplets containing mold spores. During the feeding process, regardless of the consistency of the substrate, the maxillary palp and galea are the main or even exclusive tools used for grasping and collecting food particles. The mandibles are concealed and apparently not involved in gathering food. Their main function is to push the food particles gathered by the galeae into the space between the molae with their elongate apical part. The substrate is processed between the wide molar surfaces and presumably also between the molae and epipharyngeal lobes.
The structures involved in these processes are very similar in the sporophagous Nanosellini. A rather surprising observation was that all spores in the oesophagus and anterior midgut appear intact. This suggests that they are not perforated and not noticeably deformed or broken by the activity of the molae. The function of these prominent structures is probably the transport of the substrate towards the prepharynx and anatomical mouth, and possibly cleaning of the distal maxillary elements and of the spores. Whether the minute molar surface structures leave very fine traces on the spore surface, which may facilitate infiltration of digestive enzymes, is presently unknown. In any case, a solid functional interpretation of the concerted activity of all involved complex and extremely small structural elements is a great challenge.

Our comparison of ptiliid species with saprophanous or sporophagous feeding habits surprisingly yielded only subtle differences in the involved cephalic structures. The galeae of sporophagous species usually bear 4 rows of longer setae and additional teeth on their apical end. In sporophagous species the setae are shorter and not arranged in rows in all cases. In Scydosella the apical part of the galea is flat and bears several parallel rows of short teeth, which are apparently better suited for gathering dry particles, whereas longer setae are used to filter and grasp moist clumps of mold, spores and rotting plant materials out of the half-liquid substrate.

An unusual maxillary muscle (Mx) consisting of one long bundle has been described earlier for some scarabaeoid representatives and for different staphyliniform beetles (Anton & Beutel, 2004, 2012: M. craniobasimaxillaris; Beutel et al., 2001, 2003; Jäch et al., 2000; Weide & Betz, 2009). It was also found in all examined saprophanous Ptiliidae and in Catops (Leiodidae). It originates laterally on the genal region and inserts on a membranous fold between the maxillary basis and the lateral hypopharyngeal wall. The precise function is unclear. Due to lack of suitable material the presence or absence in Nosidatum could not be verified. However, our investigation revealed that it is probably generally absent in sporophagous Nanosellini.

Analyses of muscle variation between members of the family with different feeding habits also revealed a surprisingly homogenous picture. The set of muscles of saprophanous species is almost identical to the one in the spore-feeding Nanosellini (Table 1). Only the number of bundles of some of the head muscles can vary: only a single extrinsic antennal muscle is present in Porophilla, whereas the normal set of three muscles is present in Mikado and Acrotrichis. The anterior prepharyngeal dilator M. clypeopalatalis (M43) is missing in Mikado and Nanosella, but is present in larger species, and also in the extremely small Scydosella. The number of bundles of M. frontopharyngalis posterior (M46) is also variable within the family.

4.3.3 Saprophagy in Myxophaga
Although Sphaerusiidae are not closely related to Staphylinoidea (Fig. 4), the studies on the head of Sphaerius (Yavorskaya et al., subm. a) and Scirtoidae has revealed many structural
affinities.

The feeding apparatus of *Sphaerius* is similar not only to that of other members *Myxophaga*, such as Lepiceridae (Anton & Beutel, 2006), but also to polyphagan groups with saprophagous or sporophagous feeding habits, notably in the Scirtoidea and Staphyliniformia (e.g. Anton et al., 2016; Antunes-Carvalho, 2017). An arrangement with mandibles with a large grinding mola, a longitudinal ridge (or process) of the epipharynx more or less densely set with microtrichia (Anton & Beutel, 2006: lhp), and a similar corresponding structure of the hypopharynx (e.g., Anton & Beutel, 2006: lhp) is very likely a groundplan feature of Polyphaga, and arguably also of Coleoptera, if polyphagans are confirmed as sistergroup of the other suborders (e.g. Kukalova-Peck & Lawrence, 1993, 2004; McKenna et al., 2015). An alternative interpretation would imply independent evolution of the complex configuration in Myxophaga, which appears very unlikely.

An unusual feature linked with the feeding apparatus has apparently evolved independently in Sphaeriusidae, in the staphylinoid families Hydreaenidae and Ptiliidae, and in some Scydmaeninae specialized on oribatid mites (Jaloszyński & Olszanowski, 2016), a locking device connecting the labrum and the mandibles in resting position. The mandibular part is formed by a ridge in *Sphaerius* and by a process in hydraenids and ptiliids, with a matching labral concavity present in both cases. In the scydmaenine species a mandibular process forms a concavity which fits with the lateral labral edge (Jaloszyński & Olszanowski, 2016). The grinding mola of *Sphaerius* is enclosed in the preoral cavity and firmly connected with the main body of the mandible, as in most other groups of beetles where it is present. In contrast, it is separated from the lateral and apical parts of the mandible by a membranous zone of weakness in Hydreaenidae and Ptiliidae, a presumptive synapomorphy of the two families. Like in other microphagous or saprophagous beetles, the mola of *Sphaerius* and *Lepicerus* (Anton & Beutel, 2006) is used for grinding the food substrate in interaction with the longitudinal epipharyngeal ridge, after it was processed between the distal cutting edges of the mandibles and the median hypopharyngeal rim.

### 4.4 Effects of miniaturization

A general tendency towards simplification of major skeletal elements can be observed in very small beetles, where structural complexes like the head are simplified and compact but still maintain their functionality. All beetles with body size smaller than 2 mm studied in the present work have shown this tendency. The same applies only to a lesser degree to the muscular system. Miniaturization apparently does not affect the general configuration of the muscle set of the mouthparts in miniaturized beetles, even though it can lead to reductions of subunits and fibers in single muscles. Certain muscle modifications or reductions and minor differences to larger species may be due to adaptation to certain feeding habits rather than minia-
Discussion

turization. Even in the smallest known non-parasitic insect *Scylosella musawasensis*, the set of cephalic muscles does not show a distinct degree of reduction. Similar tendencies were also earlier discovered in adults and larvae of tiny Corylophidae (Cucujoidea) (Polilov & Beutel, 2010; Yavorskaya et al. 2014; Yavorskaya & Polilov, 2016).

Even though all species of Hydraenidae are small or very small (size range 0.8–3.3 mm; Jäch et al., 2016), it is likely that an even stronger degree of miniaturization (size range 0.3–1.5 mm; Hall, 2016) is an autapomorphy of Ptiliidae. Miniaturization can cause distinct modifications and rearrangements of organ systems (Polilov, 2015, 2016a). The very high degree of size reduction apparently had a considerable impact on the general morphology and also on cephalic structures of Ptiliidae. Ecdysial sutures and strengthening ridges are completely lacking. Whereas the former are generally missing in beetles, the absence of the latter is apparently linked with the extremely small size of the head, which makes mechanical reinforcement by internal ridges superfluous. The loss or partial reduction of the clypeofrontal suture is quite common in Coleoptera (e.g. Lawrence et al. 2011), whereas the absence of the ridge separating the gula from the head capsule and the lack of lateral delimitation of the postlabium are very unusual features. Correlation of the reduced cephalic sutures and ridges with miniaturization is indicated by the occurrence of the same derived condition in non-related groups with very small species (0.8–1.1 mm). This applies to Corylophidae (Polilov & Beutel, 2010; Yavorskaya & Polilov, 2016) and Clamididae (Anton et al., 2016), but also to groups of Hymenoptera such as Mymaridae (Polilov, 2016b) or Trichogrammatidae (Polilov, 2016c, 2017), and also to other groups of insects with very small species (Polilov, 2016a).

An autapomorphy of Ptiliidae, which is possibly related with miniaturization, is the simplified structure of the tentorium, with thin and nearly parallel posterior and anterior arms and missing laminatentoria. Dorsal arms, as well as the laminatentorium, are present in the groundplan of the family (Weide et al., 2014) but missing in Nanosellini, the smallest representatives of the group (0.3–0.7 mm). In *Acrotrichis*, *Nephanes* and *Ptenidium* (0.6–1.1 mm) they are present but much shorter and slightly thinner than the anterior arms. A similar tendency was described for larvae and adults of Corylophidae, where the tentorium is more simplified in smaller representatives, and is completely absent in *Orthoperus* (0.8 mm) (pers. obs. M. Yavorskaya). Dorsal arms are also absent in adults of miniaturized Hymenoptera (Polilov, 2016b, c, 2017).

Miniaturization can lead to distinct changes in the nervous system of insects. Detailed investigation of the brain was not a goal of this work, but data are available for the ptiliid genera *Acrotrichis* and *Nanosella* (Makarova & Polilov, 2016a). Typical tendencies observed in the majority of micro-insects (Makarova & Polilov, 2016a, b; Polilov & Makarova, 2017) are also apparent in the examined Ptiliidae: macroscopic deformation of the brain, increase in size relative to the lumen of the head capsule, partial shift into the prothorax, brain asymmetry,
and fusion of the suboesophageal complex with the prothoracic ganglion.

4.5. Conclusions and outlook

The investigations carried out in this PhD project suggest that a complicated feeding apparatus has evolved early in Coleoptera, probably in the stemgroup. It is likely that mandibular grinding molae and epipharyngeal and hypopharyngeal bulges (processes) belong to the ground plan of Coleoptera. This condition is preserved in the small suborder Myxophaga and different subgroups of the megadiverse Polyphaga, notable in the basal branch Scirtoidea and in Staphyliniformia. Evolutionary shifts occurred in Polyphaga, notably linked with secondarily predacious habits and alternatively with a specialization on fresh plant material (e.g. Crowson, 1981; Beutel & Leschen, 2005; Leschen et al., 2010; Leschen & Beutel, 2014). Feeding plays a minor role in adults of Archostemata and accordingly the mouthparts are simplified, and differ strongly from those of larvae (Yavorskaya et al., 2016; subm. b). In Adephaga, a switch to predacious feeding habits and preoral digestion has also led to different modifications, notably the loss of the mola and epi- and hypopharyngeal bulges. Despite of very small size, the saprophagous feeding habits of Ptillidae does not differ strongly from that of Myxphaga or other groups of Polyphaga. Within the family, a shift from primarily saprophagy to sporophagous feeding took place in Nosidium and relatives, a basal branch (probably sistergroup of the remaining family), and then microsporophagy in the extremely miniaturized Nanosellini.

Presently detailed information on head structures of adult beetles are still scarce, especially in the megadiverse Polyphaga. Therefore, anatomical studies using suitable combinations of techniques outlined above are still important and necessary. A good coverage of polyphagan subgroups will likely a low a formal (numerical) character evaluation in the future, also with the background of new molecular hypotheses (e.g. McKenna et al., 2015; Coleoptera subdivision of the 1KITE project: http://www.1kite.org/). An important future perspective in investigating the feeding apparatus and feeding process of beetles and other insects, is the visualization of active mouthparts (and digestive tract) of life specimens. This may still be a great challenge with very small forms like Ptillidae, but preliminary investigations with larger insects are promising.

Better insights in functional aspects of feeding and more complete data for a representative taxon sampling will probably lead to a deeper insight in the evolution of food uptake, dietary adaptations and shifts in the preference of food substrates. This is quite obviously one important facet in the evolution of the extremely successful (in terms of species diversity) order Coleoptera, especially in the Polyphaga. As the name indicates, this megadiverse suborder has evolved a plethora of feeding habits, with saprophagy and a specific feeding apparatus as a starting point.
5 Summary

Aims of the present study were (1) a thorough documentation of head structures of different genera of Ptiliidae (Staphyliniformia) with different feeding preferences and body size, (2) an optimization of anatomical techniques for studying extremely miniaturized insects, (3) a comparison of the obtained data with cephalic conditions in other miniaturized and moderately sized forms (Sphaeriusidae, Clambidae, Leiodidae) with feeding preferences similar those of Ptiliidae, (4) reconstructing the evolution of sporophagy within Ptiliidae and in the broader context of Staphylinoidea, and finally (5) discuss the evolution of the feeding apparatus in the context of the basal splitting events in Coleoptera, i.e. the relationships of the suborders Polyphaga, Myxophaga, Adephaga and Archostemata.

External and internal head structures of 2 saprophagous and 3 sporophagous ptiliid species, 1 species of Leiodidae (Staphyliniformia), 1 species of Clambidae (Scirtiformia), a larva and an adult of Archostemata and 1 species of Adephaga (Gyrinidae) were studied in detail (studies I, III-VIII). Most of the studied objects are less than 2 mm long. Combinations of the following techniques were applied to obtain detailed morphological good results. Scanning electron microscopy was used for documenting fine surface structures. Digital confocal laser microscopy, microtome sections and μ-computed tomography were used for documenting internal structures and provided data for 3D reconstructions (study II). Live observations provided information on the feeding process of saprophagous Ptiliidae and the movements of their mouthparts.

The obtained data are discussed in the context of the evolution of the feeding apparatus of Coleoptera. The evaluation suggests that saprophyag is probably a groundplan feature of beetles (Fig. 5), with the presumably ancestral feeding apparatus (groundplan) similar to the one observed in Sphaeriusidae (Myxophaga) (study III) and Clambidae (Polyphaga) (study IV). Archostemata (study VIII) and Adephaga (study VII) adapted to different feeding habits, with correlated distinct modifications of the feeding apparatus.

Mandibular grinding molae and epipharyngeal and hypopharyngeal bulges (processes) probably belong to the ground plan of Coleoptera. This condition is preserved in the small suborder Myxophaga and different subgroups of the megadiverse Polyphaga, notably in the basal branch Scirtiformia (Scirtoidea) and in Staphyliniformia. These features are absent in adults of Archostemata and Adephaga, in the former case linked with reduced food uptake (or aphagia), and in the latter correlated with predacious habits and extraoral digestion.

The saprophagous feeding habits of Ptiliidae are apparently very similar to mechanisms and food preferences in Myxophaga or other groups of Polyphaga. Within the family, a switch from saprophyag to sporophagy took place twice: first in the basal branch with Nossidium and relatives, and then microsporophagy in the extremely miniaturized Nanosellini. Our
study suggests that switches between saprophagy and more specialized sporophagous habits require only minimal modifications of the mouthparts and other involved cephalic structures. This makes switches between these feeding types relatively easy in Staphyliniformia and other groups of beetles.

A far-reaching reorganization of the head does apparently not take place in extremely miniaturized species of Coleoptera. A tendency towards simplification of endoskeletal elements such as the tentorium can be observed in very small beetles. However, all head structures fully maintain their functionality and the overall complexity is not distinctly affected. It is noteworthy that the epi- and hypopharyngeal structures of studied species of Nanosellini can be even more complicated than in larger relatives in Ptiliidae. Miniaturization has only a minor effect on the cephalic musculature of miniaturized beetles, even though it can lead to reductions of subunits and fibers in single muscles. Certain muscle modifications or reductions and minor differences to larger species may be due to an adaptation to specific feeding habits or mechanisms rather than miniaturization.
6 Zusammenfassung

Ziele der des Promotionsprojekts waren (1) eine sorgfältige Dokumentation der Kopfstrukturen von unterschiedlichen Gattungen der Ptiliidae (Staphyliniformia) mit verschiedenen Nahrungspräferenzen und Körpergrößen, (2) eine Optimierung der anatomicen Techniken für die Untersuchung von extrem miniaturisierten Insekten, (3) ein Vergleich der erhobenen Daten mit der Kopfmorphologie von anderen sehr kleinen und mittelgroßen Arten (Sphaeriusidae, Clambidae, Leiodidae) mit ähnlichen Ernährungsweisen (4) eine Rekonstruktion der Evolution der Sporophagie innerhalb der Ptiliidae und im breiteren Kontext der Staphylinoida, und schließlich (5) die Diskussion der Evolution des Nahrungsaufnahmeapparates im Kontext der basalen Aufspaltungseignisse innerhalb der Coleoptera, d.h. in Bezug zu den Verwandtschaftsbeziehungen der Unterordnungen Polyphaga, Myxophaga, Adephaga und Archostemata.

Außere und innere Kopfstrukturen von zwei saprophagen und drei sporophagen Arten der Ptiliidae, einer Art der Leiodidae (Staphyliniformia), einer Art der Clambidae (Scirtiformia), einer Larve und einer Imago der Archostemata und einer Art der Adephaga (Gyrinidae) wurden detailliert untersucht (Arbeiten I, III-VIII). Die meisten der untersuchten Objekte sind weniger als 2 mm lang. Kombinationen der folgenden Techniken wurden angewandt um detaillierte Befunde zu gewährleisten. Rasterelektronenmikroskopie (SEM) wurde zur Documentation von Oberflächendetails angewendet. Digitale konfokale Lasermikroskopie (CLSM), Mikrotomschnitte und µ-Computer Tomographie wurden zur Dokumentation von inneren Strukturen verwendet und für 3D Rekonstruktionen (Arbeit II). Lebendbeobachtungen lieferten Informationen zum Vorgang der Nahrungsaufnahme bei saprophagen Ptiliden und zur Bewegung der Mundwerkzeuge.

Die erhobenen Daten wurden im Kontext der Evolution des Nahrungsaufnahmeapparates der Coleoptera betrachtet. Die Auswertung legt nahe, dass Saprophagie wahrscheinlich ein Grundplanmerkmal der Käfer ist (Abb. 5), wobei die wahrscheinlich plesiomorphe Ausprägung des Nahrungsaufnahmeapparates (Grundplan) ähnlich zu den Verhältnissen ist wie sie bei den Sphaeriusidae (Myxophaga) (Arbeit III) und Clambidae (Polyphaga) (Arbeit IV) beobachtet wurden. Die Archostemata (study VIII) und Adephaga (study VII) sind an verschiedene abweichende Ernährungsweisen angepasst, mit korrelierten deutlichen Modifikationen des Apparates.

Mandibuläre Molae mit Reibeflächen und dicht behaarte epipharyngeale und hypopharyngeale Vorwölbungen (Fortsätze) gehören wahrscheinlich zum Grundplan der Coleoptera. Diese Strukturen sind bei der kleinen Unterordnung Myxophaga erhalten und bei verschiedenen Teilgruppen der megadiversen Polyphaga, insbesondere bei den basalen Scirti-
formia (Scirtoidea) und den Staphyliniformia. Diese Merkmale fehlen bei den Imagines der Archostemata und Aedephaga, bei ersteren in Zusammenhang mit reduzierter Nahrungsaufnahme (oder Aphagie), und bei letzteren in Zusammenhang mit räuberischer Lebensweise und extraoraler Verdauung.


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8 Eigene Publikationen

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Yavorskaya MI, Beutel RG, Polilov AA (2017) Head morphology of the smallest beetles (Coleoptera: Ptiliidae) and the evolution of sporophagy within Staphyliniformia. Arthropod Systematics & Phylogeny accepted, in press.


Yavorskaya MI, Anton E, Jaloszynski P, Polilov A, Beutel RG (subm.). The head morphology of Sphaerius (Coleoptera: Sphaeriusidae) and the phylogeny of Myxophaga from the morphological perspective. Systematic entomology submitted.


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10 Eigenständigkeitserklärung


Margarita Yavorskaya

Ort, Datum