

# The evolution of head structures in Diptera and the phylogeny of the order



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von Dipl. Biol. Katharina Schneeberg

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Gutachter:

1. Prof. Dr. Rolf G. Beutel (Friedrich-Schiller-Universität Jena)
2. Prof. Dr. Steffen Harzsch (Ernst Moritz Arndt Universität Greifswald)
3. PD Dr. Thomas Hörnschemeyer (Georg-August-Universität Göttingen)

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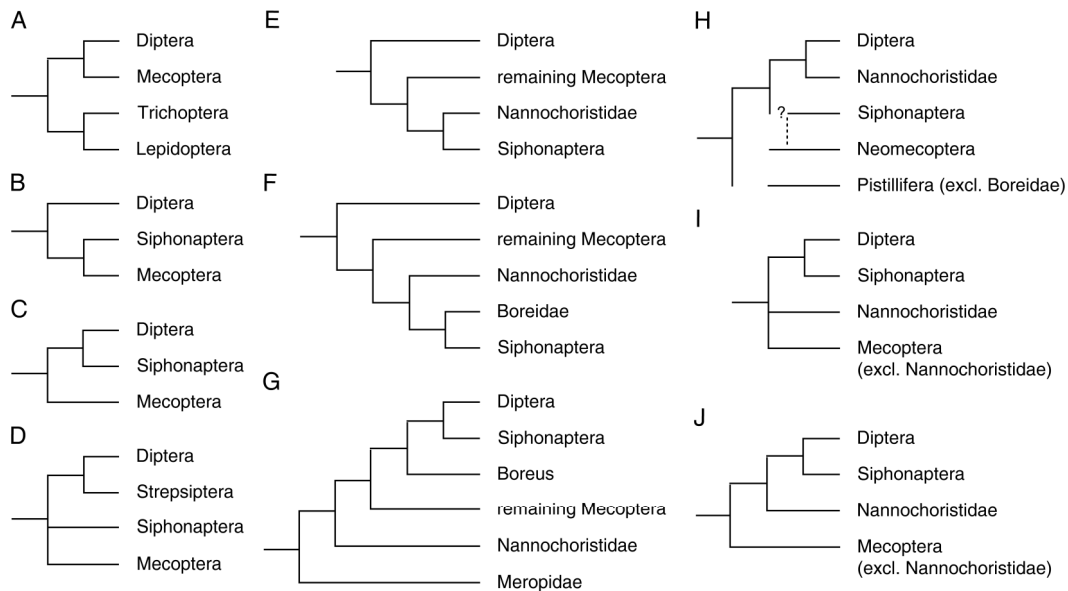
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# 1 Introduction

With approximately 170 000 described species Diptera (true flies) are one of the extremely species-riches groups of Holometabola (Pape et al. 2011). Fossil dipterans are known since the Triassic (Krzemiński & Krzemińska 2003). Recent species occur worldwide, on all continents and in all zoogeographic regions (Alexander & Byers 1981). The number of individuals is often extremely high.

Diptera are very versatile in their ecology and they also show a considerable variation in their anatomy, especially in their immature stages (Grimaldi & Engel 2005). Nevertheless, their monophyly has never been questioned (see Schneeberg & Beutel 2013). It is supported by the transformation of the hind wings into halteres (Hennig 1973) correlated with a strong enlargement of the mesothorax, which bears the functional wings, whereas the pro- and metathorax are distinctly reduced in size (Hennig 1973). The halteres are equipped with a numerous sensilla and serve as gyroscopic sense organs and stabilize the flight (Faust 1952; Pringle 1948). Another apomorphy of the group is the transformation of the two-segmented labial palps into labellae (Matsuda 1965; Hennig 1973). They are thickened and used to take up liquid food. Male dipterans bear a maximum of seven abdominal spiracles (Hennig 1973). The number of chromosomes is reduced (maximum  $n = 10$ ,  $n = 3-6$  in most dipteran groups) (Hennig 1973). The larvae of Diptera are legless and called maggots (Hennig 1973).



**Fig. 1:** Proposed phylogenetic relationships among the antliophoran orders to: **A**, Hennig 1969, 1973, 1981 (morphological data); Kristensen 1999 (morphological data). **B**, Kristensen 1981, 1991, 1995 (morphological data); Beutel & Gorb 2001 (morphological data, attachment device); Sinclair et al. 2007 (morphological data, male genitalia); Wiegmann et al. 2009 (molecular data, nuclear genes); McKenna & Ferrell 2010 (molecular data, nuclear genes); Ishiwata et al. 2011 (molecular data, nuclear genes). **C**, Beutel et al. 2011 (morphological data). **D**, Whiting & Wheeler 1994 (modified) (morphological data); Whiting et al. 1997 (modified) (molecular data, 18S and 28S rDNA); Wheeler et al. 2001 (morphological and molecular data, 18S and 28S rDNA); Whiting 2002a (modified) (molecular data, 18S rDNA). **E**, Grimaldi & Engel 2005 (morphological and paleontological data). **F**, Whiting 2002b (molecular data, nuclear genes, 18S and 28S rDNA). **G**, Friedrich & Beutel 2010a (morphological data, thorax). **H**, Beutel & Baum 2008 (morphological data, head morphology); Beutel et al. 2009 (morphological data, larva). **I**, Friedrich & Beutel 2010b (morphological data, thorax). **J**, Wood & Borkent 1989 (morphological data).



The morphology of Diptera is not well known and consequently the groundplan of the group is insufficiently established. This impedes interordinal comparisons and the understanding of the early evolution within the group. Diptera form a monophyletic unit Antliophora (= pump bearers) with Siphonaptera (fleas) and Mecoptera (scorpion flies and hanging flies) (Hinton 1958; Hennig 1969), but the phylogenetic relationships between these groups is still discussed controversially (Beutel & Baum 2008; Beutel et al. 2009; Wiegmann et al. 2009; Friedrich & Beutel 2010a; Beutel et al. 2011; Peters et al. 2014) (Fig. 1). Potential sistergroups of Diptera are Siphonaptera (Wood & Borkent 1989; Friedrich & Beutel 2010b; Beutel et al. 2011) (Figs. 1C, I, J), Mecoptera (Hennig 1969, 1973, 1981; Kristensen 1999) (Fig. 1A), Nannochoristidae (Beutel & Baum 2008; Beutel et al. 2009) (Fig. 1H) or a taxon comprising Mecoptera + Siphonaptera (Kristensen 1981, 1991, 1995; Beutel & Gorb 2001; Sinclair et al. 2007; Wiegmann et al. 2009; McKenna & Farrell 2010; Ishiwata et al. 2011) (Fig. 1B). While the monophyly of Siphonaptera is extremely well supported, the monophyly of Mecoptera was repeatedly questioned (Wood & Borkent 1989; Whiting 2002b; Grimaldi & Engel 2005; Beutel & Baum 2008; Friedrich & Beutel 2010a, b) (Figs. 1E-J). The “Halteria-hypothesis”, i.e. a sister group relationship between Diptera and Strepsiptera, which was mainly supported by 18S rDNA (Whiting et al. 1997; Whiting 1998; Wheeler et al. 2001; Whiting 2002a) (Fig. 1D), has been refuted in recent studies (Wiegmann et al. 2009; Friedrich & Beutel 2010b; Beutel et al. 2011; Ishiwata et al. 2011; Niehuis et al. 2012; Peters et al. 2014) (Figs. 1B, C, I).

Traditionally Diptera is subdivided into “Nematocera” and Brachycera (Hennig 1953). The former group is paraphyletic (lower Diptera) and contains all groups, which are not part of the monophyletic Brachycera (Hennig 1973). Most species of lower Diptera are slender and have long and slender legs, whereas brachycerans are usually (but not always) characterized by a more robust body (Hennig 1973). The main focus of this study lies on the lower Diptera.

Lower Diptera contain 37 extant families which were recently assigned to eight subgroups: Deuterophlebiomorpha, Nymphomyiomorpha, Tipulomorpha, Ptychopteromorpha, Psychodomorpha, Culicomorpha, Perissommatomorpha and Bibionomorpha (Pape et al. 2011). The composition and phylogenetic relationships of these groups are still discussed controversially (Fig. 2).

Deuterophlebiomorpha contains only the family Deuterophlebiidae and is doubtlessly monophyletic (e.g., Rohdendorf 1974; Courtney 1991b). Nymphomyiomorpha contains also a single family, Nymphomyiidae, and the monophyly is undisputed (e.g., Rohdendorf 1974; Courtney 1991b). In some older contributions Deuterophlebiidae and Nymphomyiidae were placed together with Blephariceridae into a suborder Blephariceromorpha (e.g., Wood & Borkent 1989; Oosterbroek & Courtney 1995; Yeates et al. 2007) (Figs. 2E, G, K, M). Possible synapomorphies of the three groups are the presence of a folded network, which is connected to the primary wing venation and some larval characters (Hennig 1973; Wood & Borkent 1989; Yeates et al. 2007). But these features are questionable as they are not applicable to the miniaturized Nymphomyiidae (Courtney 1994b; Oosterbroek & Courtney 1995).

Tipulomorpha contain Pediciidae, Limoniidae, Cylindrotomidae and Tipulidae (Wood & Borkent 1989; Friedrich & Tautz 1997; Yeates et al. 2007). After Hennig (1973), Stary (1992, 2008), Oosterbroek & Courtney (1995), Bertone et al. (2008), Wiegmann et al. (2011) and Pape et al. (2011) the group contains also Trichoceridae (Figs. 2B-D, G, J, K, N, O). Synapomorphies of these groups are for example characters of the wing venation, the abdomen, and long and slender legs. Their monophyletic origin is also tentatively supported by nuclear genes. Larval characters do not support an inclusion of Trichoceridae (Oosterbroek & Theowald 1991).

Ptychopteromorpha contain only Ptychopteridae after Pape et al. (2011) (Fig. 2O). Tanyderidae were included in studies of Hennig (1973), Wood & Borkent (1989), Oosterbroek & Courtney (1995) and Yeates & Wiegmann (1999) (Figs. 2B, E, K). Potential synapomorphies of these two groups are the specific formation of the tarsi and larval characters (Hennig 1973; Wood & Borkent 1989; Oosterbroek & Courtney 1995; Yeates & Wiegmann 1999). Molecular analyses do not support a close relationship of Ptychopteridae and Tanyderidae (Bertone et al. 2008; Wiegmann et al. 2011) (Figs. 2N, O). Characters of the wing venation and molecular studies support a close relationship between Tanyderidae and Psychodidae (Shcherbakov et al. 1995; Krzemiński & Krzemińska 2003; Bertone et al. 2008; Wiegmann et al. 2011) (Figs. 2G, N, O) and not the Ptychopteromorpha.

The subgroup Psychodomorpha contains Blephariceridae, Psychodidae and Tanyderidae (Bertone et al. 2008; Wiegmann et al. 2011; Pape et al. 2011) (Figs. 2N, O). Wood & Borkent (1989) suggested a broader concept, which also includes Perissommatidae, Trichoceridae, Anisopodidae, Scatopsidae and Synneuridae (Fig. 2E). However, studies of Oosterbroek & Courtney (1995), Friedrich & Tautz (1997) and Krzemiński & Krzemińska (2003) suggest the paraphyly of Psychodomorpha (Figs. 2F, K). In contrast to that, characters of the male genitalia support a close relationship between Anisopodidae, Psychodidae, Scatopsidae and Trichoceridae (Sinclair et al. 2007) (Fig. 2H).

Culicomorpha is a well-supported suborder, containing Dixidae, Corethrellidae, Chaoboridae, Culicidae, Thaumaleidae, Simuliidae, Ceratopogonidae and Chironomidae (Hennig 1973; Wood & Borkent 1989; Friedrich & Tautz 1997; Sæther 2000; Blagoderov et al. 2007; Yeates et al. 2007; Wiegmann et al. 2011; Pape et al. 2011) (Figs. 2B, E, F, I, J, M, O).

The clades Perissommatomorpha and Bibionomorpha are part of the Neodiptera, which also include Brachycera (Wiegmann et al. 2011; Pape et al. 2011) (Fig. 2O). Neodiptera were first introduced by Michelsen (1996b). He suggested a wide concept of this possible clade also including Axymyiidae and Psychodomorpha. Perissommatomorpha contains only the family Perissommatidae (Wiegmann et al. 2011; Pape et al. 2011) (Fig. 2O). According to Wood & Borkent (1989) Perissommatidae are part of Psychodomorpha (Fig. 2E). The subgroup Bibionomorpha comprises Anisopodidae, Canthyloscelidae, Scatopsidae, Valeseguyidae, Axymyiidae, Hesperinidae, Bibionidae, Pachyneuridae, Ditomyiidae, Diadocidiidae, Mycetophilidae, Bolitophilidae, Keroplatidae, Lygistorrhinidae, Rangomaramidae, Sciaridae and Cecidomyiidae (Wiegmann et al. 2011; Pape et al. 2011) (Fig. 2O). The composition of Bibionomorpha is discussed controversially in different studies, especially the inclusion or exclusion of

Axymyiidae. Axymyiidae were included in the analyses of Hennig (1973), Oosterbroek & Courtney (1995), Shcherbakov et al. (1995), Yeates et al. (2007) and Wiegmann et al. (2011) (Figs. 2B, G, K, M, O), but excluded in the analyses of Mamayev & Krivosheina (1966), Rohdendorf (1974) and Wood & Borkent (1989) (Figs. 2C, E).

The relationships between the different subunits of Diptera are discussed controversially. In older contributions Tipulomorpha were placed as the sistergroup of the remaining Diptera (Hennig 1973; Wood & Borkent 1989; Sinclair 1992; Michelsen 1996b; Beutel & Gorb 2001; Shcherbakov et al. 2005) (Figs. 2B, E, G). This hypothesis is not supported in most recent contributions (Oosterbroek & Courtney 1995; Yeates & Wiegmann 1999, 2005; Yeates et al. 2007; Bertone et al. 2008; Lambkin et al. 2013) (Figs. 2K-N). Only morphological characters of the wing venation of fossil and recent species (Blagoderov et al. 2007) (Fig. 2J) and features of the pretergite (Beutel & Gorb 2001) and wing stalk (Stary 2008) support the basal position of Tipulomorpha. In some studies Tipulomorpha form the sister group of Brachycera (Lindner 1949; Oosterbroek & Courtney 1995; Yeates & Wiegmann 1999, 2005; Yeates et al. 2007) (Figs. 2A, K, M), whereas they were placed as sister to the remaining lineages of Diptera excluding Deuterophlebiidae and Nymphomyiidae in the comprehensive study of Wiegmann et al. (2011) (Fig. 2O). Oosterbroek & Courtney (1995), Yeates & Wiegmann (1999, 2005) and Yeates et al. (2007) suggested a clade Ptychopteromorpha + Culicomorpha as most basal branch of Diptera (Figs. 2K, M). A basal placement of Deuterophlebiidae (Bertone et al. 2008; Wiegmann et al. 2011) (Figs. 2N, O) or Nymphomyiidae (Hackman & Väisänen 1982; Lambkin et al. 2013 [Deuterophlebiidae is not included in the taxon sampling]) (Figs. 2D, L) is mainly suggested by analyses of molecular data. Alternatively, Nymphomyiidae were grouped together with Axymyiidae as sistergroup of Culicomorpha (Bertone et al. 2008) (Fig. 2N).

The sistergroup of Brachycera possibly belongs to Psychodomorpha (Wood & Borkent 1989; Sinclair 1992) (Fig. 2E), which would imply the paraphyly of this lineage. After Woodley (1989), Oosterbroek & Courtney (1995) and Blagoderov et al. (2007) Anisopodidae is closely related to Brachycera (Figs. 2K, J). After Hennig (1973), Häckman & Väisänen (1982) and results of molecular analyses (Friedrich & Tautz 1997; Bertone et al. 2008; Wiegmann et al. 2011) Bibionomorpha is closely related to Brachycera (Figs. 2B, D, F, N, O). Investigations on the wing stalk indicate a clade Anisopodidae + Culicomorpha + Bibionomorpha as the brachyceran sister taxon (Stary 2008).

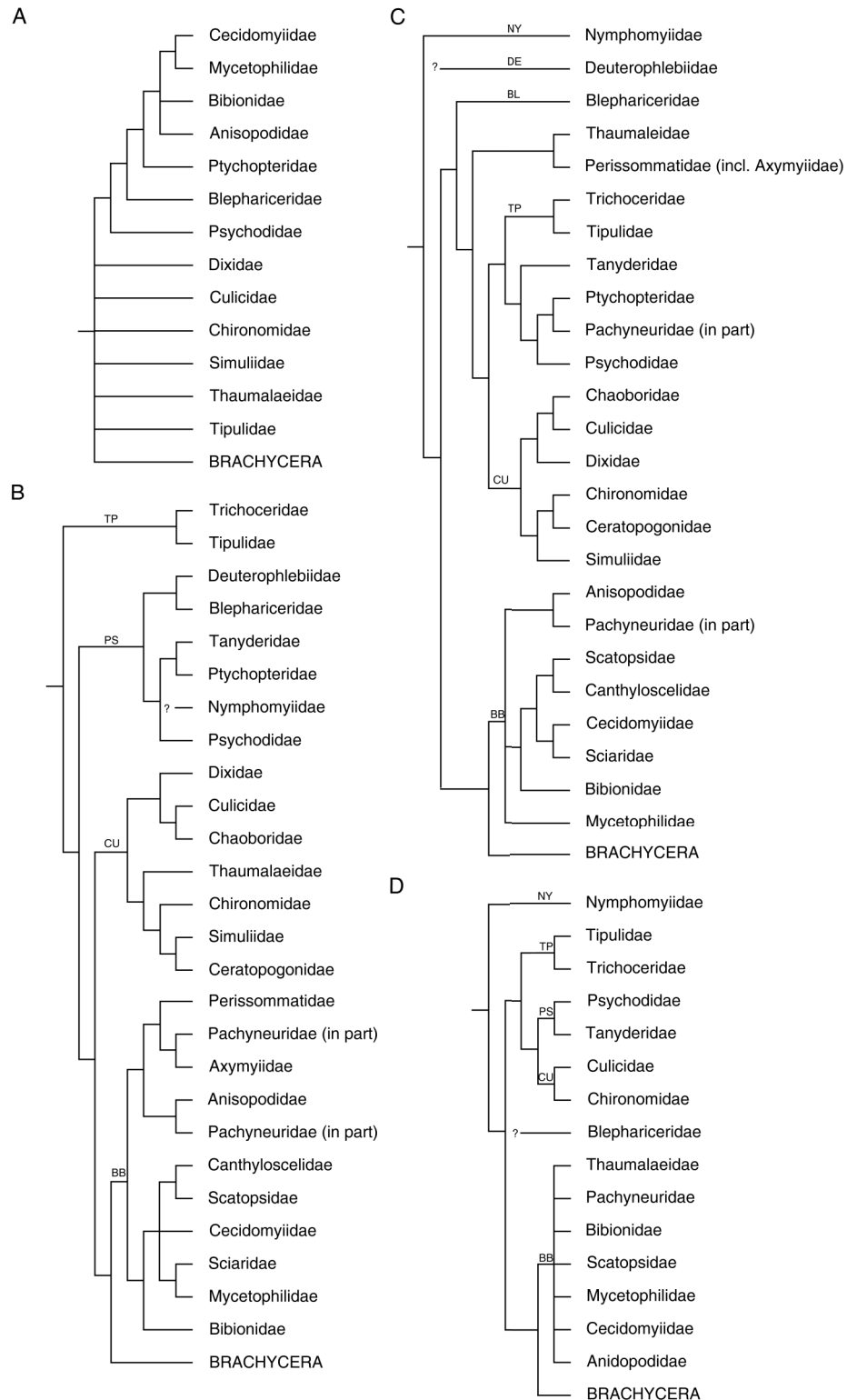
The reconstruction of the phylogeny and of the character evolution in Diptera is strongly impeded by the lack of morphological data. The comprehensive study of Wiegmann et al. (2011), for instance, was mainly based on molecular data. To facilitate an evaluation of the morphological data sets presented here, the taxon sampling of the present work was largely coordinated with the flytree-project (see Wiegmann et al. 2011). Only few taxa were not available for anatomical investigations.

Diptera are not only a challenging group in terms of the phylogenetic relationships. As vectors of numerous diseases the adults have a tremendous negative impact on the health of humans (e.g., Malaria [*Anopheles*]; e.g., Schneeberg & Beutel 2013) and life stock (Onmaz et al. 2013). Head structures play a

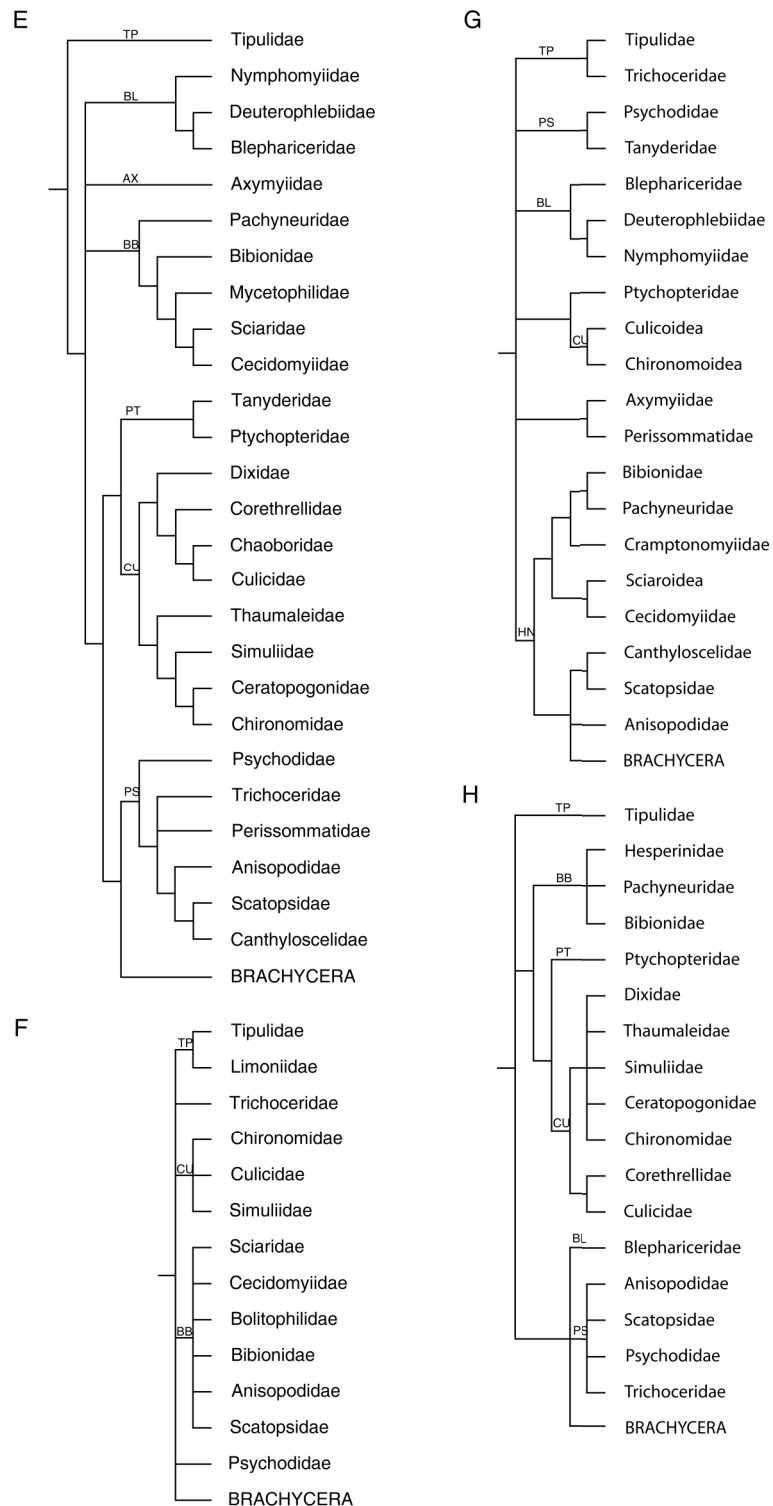
major role in this context. They are comparatively well studied in blood sucking groups (e.g., Culicidae; Thompson 1905; Schiemenz 1957; Snodgrass 1959; Christophers 1960; Owen 1985; Simuliidae; Wenk 1962; Ceratopogonidae; Gad 1951; Tabanidae: Bonhag 1951). More comprehensive morphological studies on the adult head morphology were published by Peterson (1916), Crampton (1942), Madsuda (1965) and Hennig (1973). However, contributions dealing with external and internal head structures of other dipteran groups are very rare (e.g., Gad 1951; Schiemenz 1957; Szucsich & Krenn 2000).

Many dipteran larvae play an important role as scavengers of carrion and excrements, as parasites or pest insects. The morphology of the larval head is treated in a considerable number of contributions (e.g., Anthon 1943a, b; Cook 1944a, b, 1949; v. Lieven 1998; Wipfler et al. 2012a). However, detailed anatomical studies on immature stages of key taxa (Axymyiidae, Tipulidae, Nymphomyiidae) are still missing.

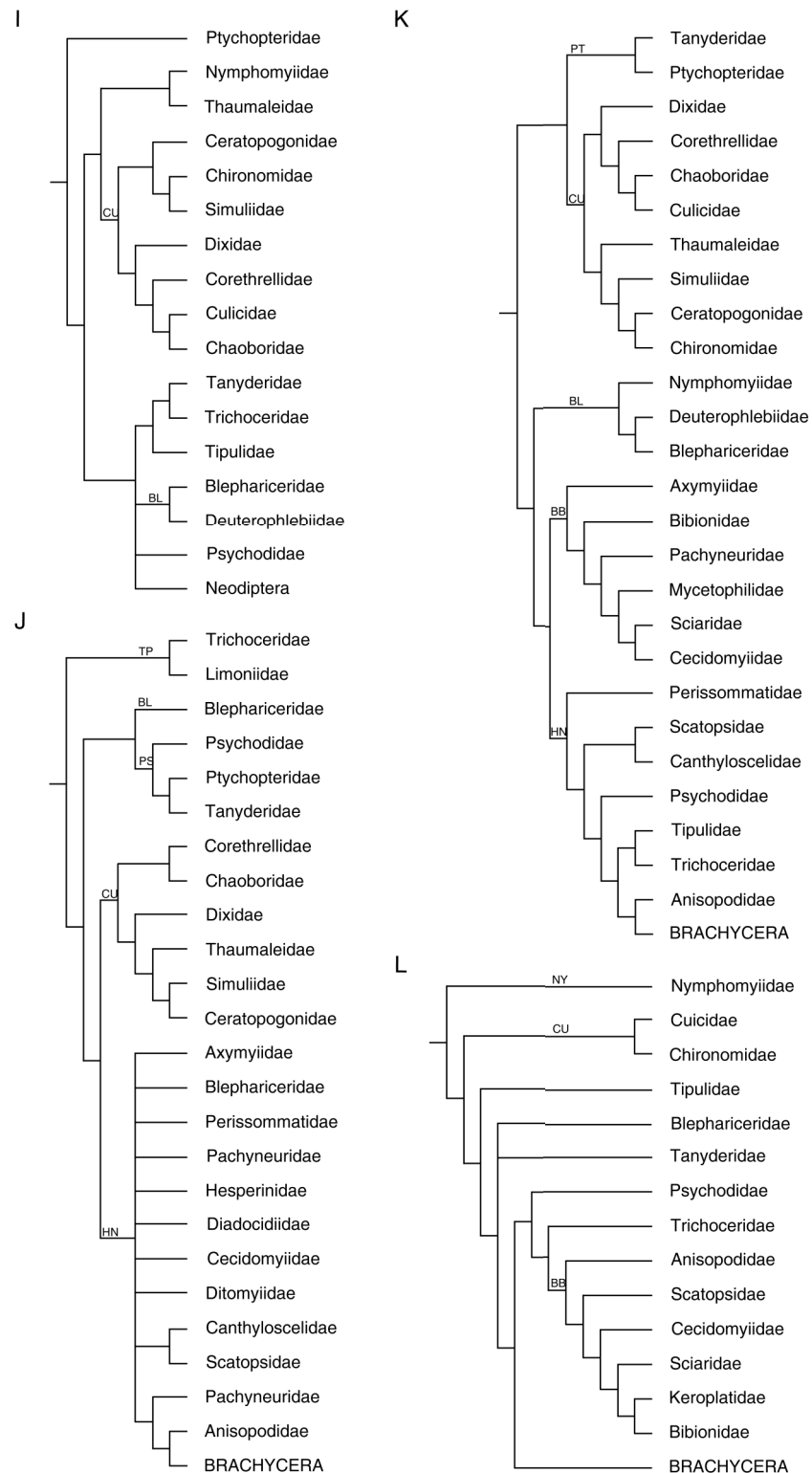
The knowledge of adult and larval anatomy in general is still very fragmentary. The understanding of crucial evolutionary events is impeded by a severe lack of morphological data. Consequently, the primary aim of this contribution was a documentation of morphological characters using innovative techniques, which were optimized by the Entomology Group in the Phyletisches Museum Jena in the last years (e.g., Friedrich et al. 2013b) (1). An extensive morphological character set was compiled (adults and larvae) (2). Morphological characters were mapped on the phylogenetic tree based on the most comprehensive data set analyzed so far (Wiegmann et al. 2011). (3) An evolutionary scenario was developed, with emphasis on the adult head and feeding apparatus and larval features and habits.



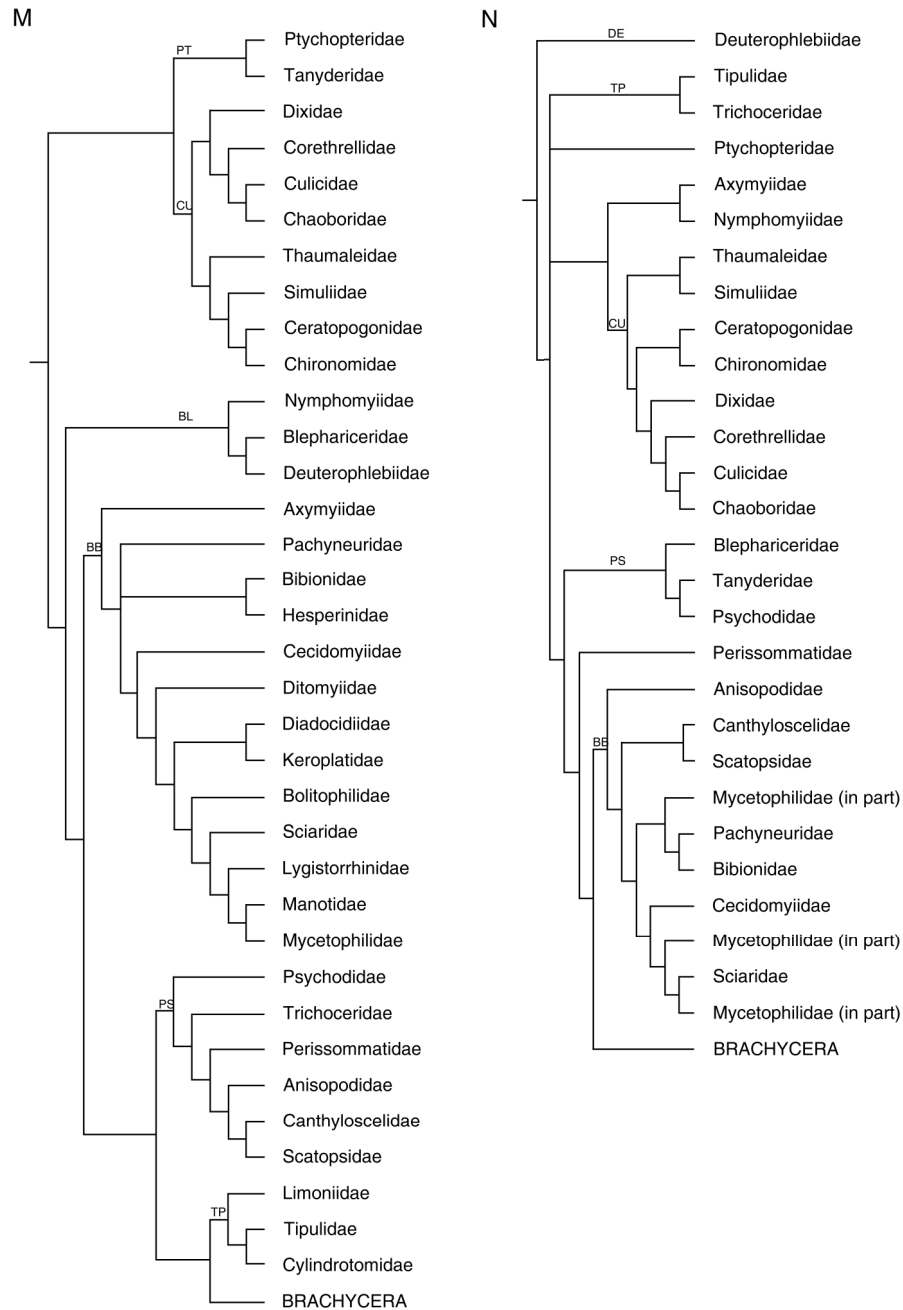
**Fig. 2:** Proposed phylogenetic relationships among the orders of lower Diptera to: **A**, Lindner 1949 (morphological data). **B**, Hennig 1968, 1973 (morphological data). **C**, Rohdendorf 1964, 1974 (morphological data). **D**, Hackmann & Väisänen 1982 (morphological data). Abbreviations: BB, Bibionomorpha. BL, Blephariceromorpha. CU, Culicomorpha. DE, Deuterophlebiomorpha. NY, Nymphomyiomorpha. PS, Psychodomorpha. TP, Tipulomorpha.



**Fig. 2:** Proposed phylogenetic relationships among the orders of lower Diptera to: **E**, Wood & Borkent 1989 (morphological data). **F**, Friedrich & Tautz 1997 (molecular data, 28S rDNA). **G**, Shcherbakov et al. 1995 (morphological data, wingmorphology, fossil and recent). **H**, Sinclair et al. 2007 (morphological data, male genitalia). Abbreviations: AX, Axymyiomorpha. BB, Bibionomorpha. BL, Blephariceromorpha. CU, Culicomorpha. HN, higher Nematocera + Brachycera. PS, Psychodomorpha. PT, Ptychopteromorpha. TP, Tipulomorpha.

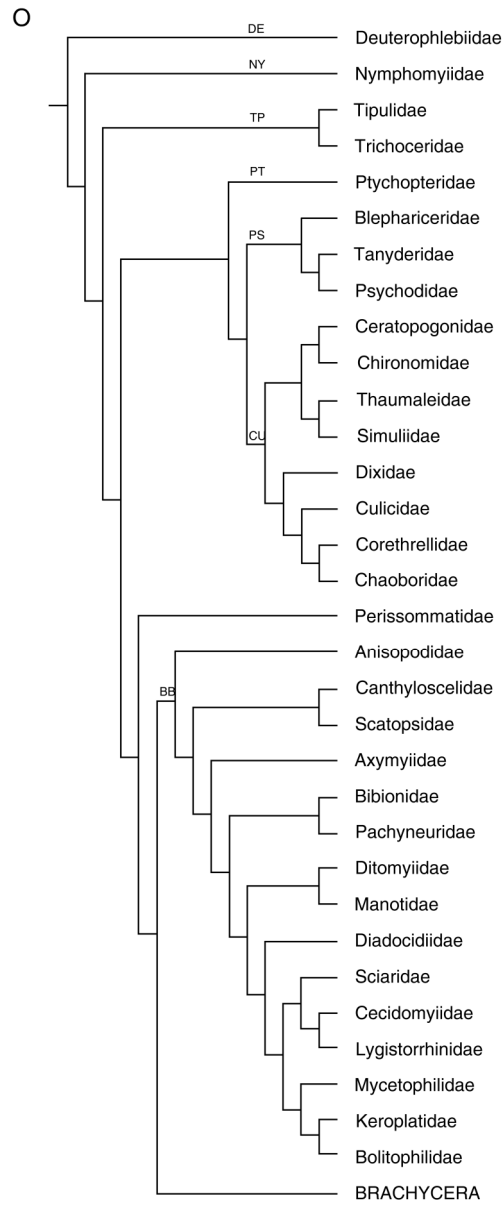


**Fig. 2:** Proposed phylogenetic relationships among the orders of lower Diptera to: **I**, Sæther 2000 (morphological data). **J**, Blagoderov et al. 2007 (morphological data, fossil and recent). **K**, Oosterbroek & Courtney 1995 (morphological data, mainly larvae and pupae). **L**, Lambkin et al. 2013 (morphological data). Abbreviations: BB, Bibionomorpha. BL, Blephariceromorpha. CU, Culicomorpha. HN, higher Nematocera + Brachycera. NY, Nymphomyiomorpha. PS, Psychodomorpha. PT, Ptychopteromorpha. TP, Tipulomorpha.



**Fig. 2:** Proposed phylogenetic relationships among the orders of lower Diptera to: **M**, Yeates et al. 2007 (supertree analysis). **N**, Bertone et al. 2008 (molecular data, nuclear genes). Abbreviations: BB, Bibionomorpha. BL, Blephariceromorpha. CU, Culicomorpha. DE, Deuterophlebiomorpha. PS, Psychodomorpha. PT, Ptychopteromorpha. TP, Tipulomorpha.





**Fig. 2:** Proposed phylogenetic relationships among the orders of lower Diptera to: **O**, Wiegmann et al. 2011 (molecular data, nuclear genes, 18S and 28S rDNA, complete mitochondrial genome). Abbreviations: BB, Bibionomorpha. CU, Culicomorpha. DE, Deuterophlebiomorpha. NY, Nymphomyiomorpha. PS, Psychodomorpha, PT, Ptychopteromorpha. TP, Tipulomorpha.

## 2 Material and techniques

### 2.1 List of taxa examined

In the present study 43 adult and 31 larval specimens were examined. The taxon sampling includes at least one representative from most dipteran families and was coordinated with the flytree-project (see taxon sampling in Wiegmann et al. 2011). External and internal head structures of adults of 28 species and larvae of 10 species were examined in detail. The list of species used in the studies is given in tables 1 and 2.

Table 1: List of adult Taxa examined. Abbreviation: EtOH – ethanol, FAE – formaldehyde-ethanol-acetic acid 3:6:1).

Taxon		Fixation	Origin of material
<b>Diptera</b>			
Anisopodidae	<i>Sylvicola fenestralis</i> (Scopoli, 1763)	70% EtOH	Germany, Thuringia, Jena
Asilidae	<i>Silpnogaster aemula</i> (Meigen, 1920)	70% EtOH	Germany, Thuringia, vicinity of Jena
Axymyiidae	<i>Axymyia furcata</i> McAtee, 1921	95% EtOH	USA, North Carolina, Haywood County, Great Smoky Mountains National Park
Bibionidae	<i>Bibio marci</i> Linnaeus, 1758	70% EtOH	Germany, Thuringia, Jena
Blephariceridae	<i>Edwardsina gracilis</i> Edwards, 1929	70% EtOH	Chile, Las Lagos, Parque National Puyehue, Rio Anticura
Bombyliidae	<i>Bombylius major</i> Linnaeus, 1758	data taken from Szucsich & Krenn (2000)	
	<i>Hemipenthes mario</i> (Linnaeus, 1758)	data taken from Szucsich & Krenn (2000)	
Cecidomyiidae	<i>Mayetiola destructor</i> (Say, 1817)	70% EtOH	USA, North Dakota, in culture (Entomology Department, North Dakota State University)
Ceratopogonidae	<i>Culicoides impunctatus</i> (Goetghebuer, 1920)	data taken from Gad (1951)	
Chaoboridae	<i>Chaoborus crystallinus</i> (De Geer, 1776)	70% EtOH	Germany, Thuringia, Jena, in culture (Institut für Spezielle Zoologie und Evolutionsbiologie)
Corethrellidae	<i>Corethrella appendiculata</i> Grabham, 1906	70% EtOH	USA, Florida, Vero Beach, in culture (Florida Medical Entomology Laboratory)
Culicidae	<i>Aedes aegypti</i> (Linnaeus, 1962)	data taken from Christophers (1960)	
	<i>Anopheles maculipennis</i> Meigen, 1818	70% EtOH	Germany, Nordrhein-Westfalen, Höxter
	<i>Culex pipiens</i> Linnaeus, 1758	70% EtOH	Germany, Thuringia, Orlamünde, near Riverside
	<i>Culiseta annulata</i> (Schrank, 1776)	data taken from Schiemenz (1957)	
Cylindrotomidae	<i>Cylindrotoma distinctissima</i> (Meigen, 1818)	70% EtOH	Italy, Piemonte, Cuneo Marora, San Sebastiano
Deuterophlebiidae	<i>Deuterophlebia coloradensis</i> Pennak, 1945	95% EtOH	USA, Colorado, Boulder County, Boulder Creek

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Drosophilidae	<i>Drosophila melanogaster</i> Meigen, 1830	FAE	Germany, Thuringia, Jena, in culture (wildtype Canton S) (Max-Planck-Institute for Chemical Ecology)
Glossinidae	<i>Glossina palpalis gambiensis</i> Vanderplank, 1911	FAE	Austria, Wien, in culture (International Atomic Energy Agency)
Limoniidae	<i>Limonia</i> sp. Meigen, 1803	70% EtOH	Germany, Thuringia, vicinity of Jena
Keroplastidae	<i>Macrocera phalerata</i> Meigen, 1818	70% EtOH	Germany, Thuringia, vicinity of Jena
Mycetophilidae	<i>Exechia separata</i> Lundström, 1912	70% EtOH	Germany, Thuringia, vicinity of Jena
Nymphomyiidae	<i>Nymphomyia dolichozeza</i> Courtney, 1994	95% EtOH	USA, North Carolina, Macon County, Coweeta Hydrologica Lab
Pediciidae	<i>Pedicia rivosa</i> (Linnaeus, 1758)	70% EtOH	Italy, Piemonte, Torino, Villar Péllice
Psychodidae	<i>Psychoda alternata</i> Say, 1824	70% EtOH	Germany, Hamburg, Eimsbüttel
Ptychopteridae	<i>Ptychoptera contaminata</i> (Linnaeus, 1758)	70% EtOH	Germany, Thuringia, Jena
Scatopsidae	<i>Coboldia fuscipes</i> (Meigen, 1930)	95% EtOH	USA, specific location unknown
Sciaridae	<i>Spathobdella falcifera</i> (Lengersdorf, 1933)	70% EtOH	Germany, Thuringia, Jena
Simuliidae	<i>Wilhelmia equina</i> (Linnaeus, 1746)	data taken from Wenk (1962)	
Stratiomyiidae	<i>Pachygaster atra</i> (Panzer, 1798)	70% EtOH	Germany, Thuringia, Schinditz
Syrphidae	<i>Eristalis arbustorum</i> (Linnaeus, 1758)	data taken from Schiemenz (1957)	
Tabanidae	<i>Tabanus sulcifrons</i> Macquart, 1855	data taken from Bonhag (1951)	
Tanyderidae	<i>Mischoderus</i> sp. Handlirsch, 1909	70% EtOH	New Zealand, Mount Arthur, Flora Hut, 920m
	<i>Protoplasa fitchii</i> Osten Sacken, 1860	data taken from Williams (1933)	
Thaumaleidae	<i>Androprosopa</i> sp. Mik, 1898	95% EtOH	USA, Oregon, Marion County, Squirrel Creek 1035m
Tipulidae	<i>Tipula paludosa</i> Meigen, 1830	70% EtOH	Germany, Thuringia, Jena
Trichoceridae	<i>Trichocera saltator</i> (Harris, 1776)	FAE	Germany, Thuringia, Jena
<b>Mecoptera</b>			
Boreidae	<i>Caurinus dectes</i> Russel, 1979	data taken from Beutel et al. (2008a)	
Meropidae	<i>Merope tuber</i> Newman, 1838	data taken from Friedrich et al. (2013a)	
Nannochoristidae	<i>Nannochorista neotropica</i> Navás, 1928	data taken from Beutel & Baum (2008)	
Panorpidae	<i>Panorpa communis</i> Linnaeus, 1758	data taken from Heddergott (1938)	
<b>Lepidoptera</b>			
Micropterigidae	<i>Micropterix calthella</i> (Linnaeus, 1761)	data taken from Hannemann (1956)	
<b>Siphonaptera</b>			
	<i>Ctenocephalus canis</i> (Curtis, 1826)	data taken from Wenk (1953)	

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Table 2: List of larval Taxa examined. Abbreviation: EtOH – ethanol, FAE – formaldehyde-ethanol- acetic acid 3:6:1).

Taxon		Fixation	Origin of material
<b>Diptera</b>			
Anisopodidae			data taken from Anthon (1943a, b)
Axymyiidae	<i>Axymyia furcata</i> McAtee, 1921	95% EtOH	USA, North Carolina, Macon County
Bibioniadae	<i>Bibio marci</i> Linnaeus, 1758	70% EtOH	Germany, Thuringia, Jena
	<i>Bibio</i> sp. Geoffroy, 1962		data taken from Cook 1949
Blephariceridae			data taken from Anthon & Lyneborg (1968)
Ceratopogonidae	<i>Dasyhelea</i> sp. Kieffer, 1911		data taken from v. Lieven (1998)
Chironomidae	<i>Chironomus</i> sp. Meigen, 1803		data taken from Cook (1944b); Gouin (1959)
Corethrellidae	<i>Corethrella appendiculata</i>	70% EtOH	USA, Florida, in culture (Florida Medical Entomology Laboratory; University of Florida)
Culicidae	<i>Culex</i> sp. Linnaeus, 1758	70% EtOH	Germany, Thuringia, Jena
	<i>Culiseta</i> sp. Felt 1904		data taken from Cook (1944a)
	<i>Anopheles</i> sp. Meigen, 1818	70% EtOH	Germany, Thuringia, Jena
	<i>Anopheles</i> sp. Meigen, 1818		data taken from Cook (1944a); Schremmer (1949)
Deuterophlebiidae	<i>Deuterophlebia</i> sp. Edwards 1922		data taken from Courtney (1990a)
Dixidae	<i>Dixa</i> sp. Meigen, 1816		data taken from Felix (1962); Schremmer (1950b)
Limoniidae	<i>Limonia</i> sp. Meigen, 1803		data taken from Lindner (1949); Oosterbroek & Theowald (1991)
Mycetophilidae	<i>Exechia separata</i> Lundström, 1912	70% EtOH	Germany, Thuringia, Jena
Nymphomyiidae	<i>Nymphomyia dolichozeza</i> Courtney, 1994	95% EtOH	USA, North Carolina, Macon County
Pediciidae	<i>Dicranota</i> sp. Zetterstedt, 1838		data taken from Cook (1949)
Psychodidae			data taken from Anthon (1943a)
Ptychopteridae	<i>Bittacomorpha</i> sp. Westwood, 1835		data taken from Kramer (1954)
Sciaridae	<i>Phaenobremia aphidimyza</i> (Rondani, 1847)		data taken from Solinas (1968)
Simuliidae	<i>Simulium</i> sp. Latreille, 1802		data taken from Cook (1949)
Stratiomyiidae	<i>Odontomyia</i> sp. Meigen, 1803		data taken from Cook (1949)
Tabanidae	<i>Tabanus</i> sp. Linnaeus, 1758		data taken from Cook (1949)
Tanyderidae	<i>Protanyderus</i> sp. Handlirsch, 1909		data taken from Wipfler et al. (2012a)
Thaumaleidae	<i>Androprosopa</i> sp. Mik, 1898	95% EtOH	USA, Oregon, Benton County
Therevidae			data taken from Cook (1949)
Tipulidae	<i>Tipula irrorata</i> Marquart, 1926	70% EtOH	Germany, Thuringia, Jena
	<i>Tipula montium</i> Egger, 1863	70% EtOH	Germany, Thuringia, Jena
Trichoceridae	<i>Trichocera</i> sp. Meigen, 1803		data taken from Karandikar (1931); Anthon (1943a); Brindle (1958)
<b>Mecoptera</b>			
Nannochoristidae	<i>Nannochorista</i> sp. Tillyard, 1917		data taken from Beutel et al. (2009)
Panorpidae	<i>Panorpa communis</i> Linnaeus, 1758		data taken from Bierbrodt (1942)
<b>Siphonaptera</b>			
Ceratophylidae	<i>Nosopsyllus fasciatus</i> (Bosc, 1800)		data taken from Sharif (1937)

## 2.2 Morphological techniques

### Scanning electron microscopy (SEM)

Scanning electron microscopy was used to visualize external features. Specimens were dehydrated in ascending ethanol series (70% - 100% ethanol and 99.9% acetone) and dried at the critical point (EmiTech K850 Critical point Dryer, Quorum Technologies Ltd., Ashford, Kent, UK). They were glued on a fine pin, sputter coated with gold (EmiTech K500 sputter coater, Quorum Technologies Ltd., Ashford, Kent, UK) and mounted on a rotatable specimen holder (Pohl 2010). Images were taken with a Philips XL 30 ESEM (Philips, Amsterdam, Netherlands) using Scandium software (Olympus, Münster, Germany). The figure plates were processed with Adobe Photoshop<sup>®</sup> (Adobe, San José, USA) and Adobe Illustrator<sup>®</sup> (Adobe, San José, USA).

### Confocal Laser Scanning Microscopy (CLSM)

For the investigation of the skeletal elements and for an overview of the arrangement of muscles images with a confocal laser scanning microscope were taken. The specimens were mounted between two cover glasses with a drop of glycerine. Images were taken with an argon laser (488 nm) on a LSM 510 (Carl Zeiss AG, Göttingen, Germany). As insect cuticle, musculature and nerves are autofluorescent, a treatment with antibodies is not necessary (Klaus et al. 2003).

### Histology

Histological sections were used to investigate internal features. Specimens were fixed in FAE (formaldehyde-ethanol-acetic acid 3:6:1) and dehydrated with ethanol (80% - 100% ethanol) and acetone (99.9%). They were embedded in Aradite (CY 212, Agar Scientific, Stansted/Essex, UK), sectioned (1  $\mu\text{m}$  / 1.5  $\mu\text{m}$ ) with a diamond-knife (Elementsix) on a microtome (HM 360, Microm, Walldorf, Germany) and stained with Toluidine blue and Pyronin G (Waldeck GmbH and Co. KG/Division Chroma, Münster, Germany). The sections were digitalized with a Zeiss Axioscope (Carl Zeiss AG, Jena, Germany) with a PixeLINK PL-A622C and PixeLINK PL-B686 (PixeLINK, Ottawa, Canada) digital camera, using the software PixeLINK Capture OEM 7.12 (PixeLINK, Ottawa, Canada). The alignment of the image stack was calculated by AutoAligner<sup>®</sup> 6 (Bitplane AG, Zürich, Suisse) and Amira<sup>®</sup> 5.3 (Visage Imaging GmbH, Berlin, Germany) software.

### Micro-Computer-Tomography ( $\mu\text{CT}$ )

Micro-Computer-Tomography was used to investigate internal structures. Specimens were dehydrated with an ascending ethanol series (70% - 100% ethanol) and acetone (99.9%), dried at the critical point (EmiTech K850 Critical point Dryer, Quorum Technologies Ltd., Ashford, Kent, UK) and mounted on a metal rod with super glue or specific dough. The scans were performed at Beamline BW2 of the DESY

(Deutsches Elektronen-Synchrotron, Hamburg; see Beckmann et al. 2006) using a stable low photon energy beam (8kV) and absorptions contrast (see Friedrich et al. 2008). Further scans were performed on a Skyscan 1172 micro-CT scanner (Bruker, Billerica, Massachusetts, USA).

### **Computer based 3-dimensional reconstruction**

The arrangement of external and internal structures was visualized using computer based 3d-reconstructions, based on digitalized and aligned section series. Image stacks of head structures were reconstructed using Imaris<sup>®</sup> 6.2.0 (Bitplane AG, Zürich, Suisse) and Amira<sup>®</sup> 5.3 (Visage Imaging GmbH, Berlin, Germany) software. The surfaces were polished, smoothed and rendered with Maya<sup>®</sup> 2012 (Autodesk, San Rafael, USA).

## **2.3 Cladistic analyses**

The data matrix comprises 76 characters of adults of 42 terminal taxa (species) and 70 larval characters for 30 species. For the character coding, tree calculation and cladistic character analysis WinClada (Nixon 1999-2002) software was used (Rachet Island Hopper, 1000 replicates). All characters were treated as non-additive and equally weighted.

In the analyses of the combined data set (adult and larval head structures) in some families, different adult and larval genus's of the same family were combined (Ptychopteridae, Simuliidae, Ceratopogonidae, Blephariceridae, Anisopodidae, Psychodidae, Pediciidae), otherwise the same genus were used.

For the evaluation of the character evolution the characters of adult and larval heads were mapped onto the cladogram of Wiegmann et al. (2011) using the function implemented in Mesquite (trace characters over trees, reconstruction method parsimony ancestral states; Maddison & Maddison 2011).

### 3 Published results

#### Study I

**Schneeberg K**, Courtney GW, Beutel RG. 2011.

Adult head structures of Deuterophlebiidae (Insecta), a highly derived “ancestral” dipteran lineage. *Arthropod Structure and Development* 40: 93-104.

#### Study II

**Schneeberg K**, Polilov A, Harris MO, Beutel RG. 2013.

The adult head morphology of the Hessian fly *Mayetiola destructor* (Diptera, Cecidomyiidae). *Journal of Morphology* 274: 1299-1311.

#### Study III

**Schneeberg K**, Krause K, Beutel RG. 2013.

The adult head morphology of *Axymyia furcata* (Axymyiidae, Diptera). *Arthropod Systematics and Phylogeny* 72: 91-102.

#### Study IV

Neugart C, **Schneeberg K**, Beutel RG. 2009.

The morphology of the larval head of Tipulidae (Diptera, Insecta) – the dipteran groundplan and evolutionary trends. *Zoologischer Anzeiger* 248: 213-235.

#### Study V

**Schneeberg K**, Friedrich F, Courtney GW, Wipfler B, Beutel RG. 2012.

The larvae of Nymphomyiidae (Diptera, Insecta) – ancestral und highly derived? *Arthropod Structure and Development* 41: 293-301.

#### Study VI

Wipfler B, **Schneeberg K**, Löffler A, Hünefeld F, Meier R, Beutel RG. 2013.

The skeletomuscular system of the larva of *Drosophila melanogaster* (Drosophilidae, Diptera) – a contribution to the morphology of a model organism. *Arthropod Structure and Development* 42: 47-68.

#### Study VII

Friedrich F, Hünefeld F, Pohl H, Beckmann F, **Schneeberg K**, Herzen J, Beutel RG. 2010.

Reconstructing the evolution of Holometabola (Hexapoda) using SR $\mu$ CT-based morphological data. *Hasylab Annual Review* 2009.

### 3.1 Study I

**Schneeberg K**, Courtney GW, Beutel RG. 2011.

Adult head structures of Deuterophlebiidae (Insecta), a highly derived “ancestral” dipteran lineage. *Arthropod Structure and Development* 40: 93-104.

#### **Abstract**

Adult head structures of *Deuterophlebia coloradensis* were examined, 3-dimensionally reconstructed and described. The results are compared to those of other representatives of basal dipteran lineages, primarily *Nymphomyia dolichozeza* (Nymphomyiidae) and *Edwardsina gracilis* (Blephariceridae). The head structures are extremely simplified. The labrum, mandibles, maxillae and labium are completely reduced. Only eight muscles are present. These modifications are possibly linked with the extremely short life span and non-feeding of adults. Possible synapomorphies of Deuterophlebiidae + Nymphomyiidae are the loss of all mouthparts, the elongation of the terminal antennal segment, and the loss of *M. tentoriobuccalis anterior*. A clade Blephariceroidae (Deuterophlebiidae + Blephariceridae) is only suggested by the origin of *M. tentorioscapalis posterior* on the vertex. Blephariceromorpha (Deuterophlebiidae, Nymphomyiidae, Blephariceridae) is only weakly supported by features of the adult head. The missing frontoclypeal and clypeolabral suture and the origin of *M. tentorioscapalis* on the head capsule are potential autapomorphies. Our results do not support a basal placement of Deuterophlebiidae. A reliable reconstruction of basal dipteran relationships is impeded by missing morphological data for many potential key taxa.

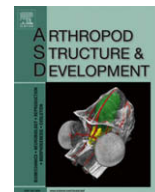
#### **Significance in the present thesis**

Deuterophlebiidae are highly specialized group and obviously a key taxon for understanding dipteran phylogeny. Recent studies suggest that Deuterophlebiidae form the sister group of all remaining lineages of Diptera. A study on the head morphology was not available so far.

#### **Own contribution**

80%





## Adult head structures of Deuterophlebiidae (Insecta), a highly derived “ancestral” dipteran lineage

Katharina Schneeberg<sup>a,\*</sup>, Gregory W. Courtney<sup>b</sup>, Rolf G. Beutel<sup>a</sup>

<sup>a</sup>Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, Erbertstraße 1, 07743 Jena, Germany

<sup>b</sup>Department of Entomology, 432 Science II, Iowa State University, Ames, Iowa 50011-3222, USA

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### ABSTRACT

Adult head structures of *Deuterophlebia coloradensis* were examined, 3-dimensionally reconstructed and described. The results are compared to those of other representatives of basal dipteran lineages, primarily *Nymphomyia dolichozepe* (Nymphomyiidae) and *Edwardsina gracilis* (Blephariceridae). The head structures are extremely simplified. The labrum, mandibles, maxillae and labium are completely reduced. Only eight pairs of muscles are present. These modifications are possibly linked with the extremely short life span and non-feeding of adults. Possible synapomorphies of Deuterophlebiidae + Nymphomyiidae are the loss of all mouthparts, the elongation of the terminal antennal segment, and the loss of *M. tentoriobuccalis* anterior. An alternative placement of Deuterophlebiidae as sister group of Blephariceridae is only suggested by the origin of *M. tentorioscapalis* posterior on the vertex. Blephariceromorpha (Deuterophlebiidae, Nymphomyiidae, Blephariceridae) is only weakly supported by features of the adult head. The missing frontoclypeal and clypeolabral suture and the origin of *M. tentorioscapalis* on the head capsule are potential autapomorphies. Our results do not support a sister group relationship between Deuterophlebiidae and the remaining Diptera. A reliable reconstruction of basal dipteran relationships is impeded by missing morphological data for many potential key taxa.

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### 1. Introduction

Deuterophlebiidae are a highly specialised dipteran group comprising only 14 species (Courtney, 1994a; Wagner et al., 2008). The larvae are aquatic and always associated with cool, running water. The adults live only a few hours (Courtney, 1991a; Wagner et al., 2008).

A close relationship between Deuterophlebiidae and Blephariceridae was suggested by Hennig (1973), Wood and Borkent (1989), Courtney (1990b, 1991b) and Oosterbroek and Courtney (1995). Hennig (1973) placed the Deuterophlebiidae and Blephariceridae in the Psychodomorpha. Cutten and Kevan (1970) pointed out that Deuterophlebiidae and Nymphomyiidae may form a monophyletic group. Wood and Borkent (1989) suggested a clade Blephariceromorpha comprising Deuterophlebiidae, Blephariceridae and Nymphomyiidae, and the monophyly of the lineage was also supported by Courtney (1990b, 1991b) and Oosterbroek and Courtney (1995). In contrast to this concept, analyses of 28S rRNA

and protein-coding (CAD, PGD, TPI) genes sequence data suggest that Deuterophlebiidae form the sister group of all remaining lineages of Diptera (Bertone et al., 2008). A basal placement was also suggested for Nymphomyiidae (Rohdendorf, 1974; Hackman and Väisänen, 1982; Bertone et al., 2008 [analyses of reduced data set]), Culicomorpha + Ptychopteromorpha (Oosterbroek and Courtney, 1995; Yeates and Wiegmann, 1999, 2005; Yeates et al., 2007) and Tipulomorpha (or Tipulidae s.l.) (Hennig, 1973; Wood and Borkent, 1989; Beutel and Gorb, 2001; Blagoderov et al., 2007).

The larval morphology of Deuterophlebiidae was treated in detail in Courtney (1990a,b) but morphological data on adults were quite limited. Courtney (1990a) described external features of the adult head, but a study on the internal head anatomy including musculature, endoskeleton, digestive tract and nervous system was not available so far. Consequently, the primary aim of this study was to document the head morphology of this potentially basal dipteran taxon in detail. In addition to *Deuterophlebia* Edwards, 1922, we examined external and internal head structures of potentially related groups, notably Nymphomyiidae and Blephariceridae. To create a solid framework for a discussion of morphological features we compiled an extensive list with potentially relevant phylogenetic characters. Based on these data

\* Corresponding author. Tel.: +49 3641 949149; fax: +49 3641 949142.

E-mail address: [katharina.schneeberg@gmx.de](mailto:katharina.schneeberg@gmx.de) (K. Schneeberg).

we discuss whether the highly specialised features have evolved as autapomorphies of Deuterophlebiidae in correlation with a specialised life style, or at least some of them indicate a common ancestry with Nymphomyiidae, Blephariceridae or other groups. We also evaluate whether some of the head features we identified support a clade Blephariceromorpha or alternatively the concept of Deuterophlebiidae as “the most ancestral” lineage of Diptera. A formal phylogenetic evaluation of the presented characters was not performed, as phylogenetic conclusions based on only one limited character system are questionable. However, our data will serve as a starting point for future comprehensive phylogenetic evaluations including characters of other body parts and life stages, as well as molecular data.

## 2. Materials and methods

### 2.1. Material examined

Diptera, Deuterophlebiidae: *Deuterophlebia coloradensis* Pennak, 1945 (both sexes) (USA, Colorado, Boulder Co.; 95% Ethanol, SEM (scanning electron microscopy), microtome sections [ms], micro-computer tomography [ $\mu$ CT]).

Nymphomyiidae: *Nymphomyia dolichozepea* Courtney, 1994 (undetermined sex) (USA, NC, Macon Co.; 95% Ethanol, SEM, ms) Blephariceridae: *Edwardsina gracilis* Edwards, 1929 (male) (Chile, Las Lagos, Parque National Puyehue; 95% Ethanol, SEM, ms).

Tipulidae: *Tipula paludosa* Meigen, 1930 (both sexes) (Germany, vicinity of Jena; 70% Ethanol, FAE [=formaldehyde–ethanol–acetic acid], SEM, confocal laser scanning microscopy [CLSM], ms). Limoniidae: *Limonia* sp. (female) (Germany, vicinity of Jena; FAE, SEM, ms).

Trichoceridae: *Trichocera saltator* Harris, 1776 (both sexes) (Germany, Jena; FAE, SEM, ms).

Tanyderidae: *Mischoderus* sp. (male) (New Zealand, New Zealand Arthropod Collection; 70% Ethanol,  $\mu$ CT)

Culicidae: *Culex pipiens* Linnaeus, 1758 (female) (Germany, Orlamünde; 70% Ethanol, SEM, CLSM, ms), *Anopheles maculipennis* Meigen, 1818 (female) (Germany, Orlamünde; 70% Ethanol).

### 2.2. Methods

Drawings were made using a stereo microscope MZ 125 (Leika). Figures were processed with Adobe Photoshop<sup>®</sup>, Adobe Illustrator<sup>®</sup> and Image J 1.410 (freeware: <http://rsb.info.nih.gov/ij>). Specimens were embedded in Araldit CY 212<sup>®</sup> (Agar Scientific, Stansted/Essex, England) for sectioning. Longitudinal sections (1  $\mu$ m) and cross section (1  $\mu$ m) series were carried out with an HM 360 (Microm, Walldorf, Germany) microtome. The sections were stained with Toluidine blue and Pyronin G (Waldeck GmbH & Co. KG/Division Chroma, Münster, Germany), documented with a PixelINK PL-A622C digital camera and examined with a light microscope Leica DME. The alignment of the image stack was calculated by AutoAligner<sup>®</sup> 6 (Bitplane AG, Zürich, Suisse) software. Three-dimensional reconstructions were carried out using Imaris<sup>®</sup> 6.2.1 (Bitplane AG, Zürich, Suisse) and MAYA<sup>®</sup> 7.0 (Alias Wavefront, Toronto/Ontario, Canada) software based on the  $\mu$ CT-image stack. For the synchrotron radiation based micro-computed tomography the specimens were dehydrated with ethanol (70%–100%) and acetone, critical point dried (EmiTech K850 Critical Point Dryer; Ashford, Kent, UK) and mounted with superglue on a metal rod. The scans were performed at Beamlane BW2 on the German Electron Synchrotron Facility (DESY, Hamburg) using a low photon energy beam (8 kV) and absorption contrast (see Friedrich et al., 2008). For

scanning electron microscopy specimens were dehydrated with ethanol (70%–100%) and acetone, critical point dried, glued on a fine pin and sputter coated. Images were taken with an FEI (Philips) XL 30 ESEM on a specimen holder after Pohl (in press). One specimen was examined with a confocal scanning microscope LSM 510 (Zeiss, Göttingen, Germany). As insect cuticle, musculature and nervous system are autofluorescent, treatment with antibodies was not necessary (see Klaus et al., 2003).

Muscles are named following the nomenclature of Kéler (1963).

## 3. Results

The head structures are described in detail for the male, whereas the treatment of the female is focussed on sexually dimorphic features.

### 3.1. Head capsule

The head is held in an unusual position on the ventral side of the thorax (Fig. 1). Its ventral part is closely adjacent to the fore-legs. The head capsule is sclerotised and all parts except the ventral side are densely covered with microtrichia. More widely spaced longer setae are present on the clypeal region. The head is broader than long in frontal view, flattened in lateral view and ventromedially concave. The nearly rounded compound eyes are placed on the ventrolateral head region. On their mesal side they are delimited by a circumocular ridge. The antennae insert on the frontal side, dorsally between the compound eyes. The articulation fossae are widely separated. A deep furrow is present below the antennal bases (Fig. 1A). The anterior tentorial grooves are distinctly recognisable between the compound eyes (atg, Fig. 1A). Ocelli are absent.

The coronal-, frontal-, frontoclypeal-, subgenal- and occipital-sutures are missing, and also the median frontal apodeme. The head regions can only be vaguely identified by their position and the muscle insertion areas. The genal regions lie frontally between the compound eyes and reach the clypeus. The insertion area of M. clypeobuccalis (M. 43) and the anterior tentorial grooves can be considered as landmarks identifying the clypeal region. The frontal area can be identified by the insertion area of M. frontobuccalis posterior (M. 46) (46, Fig. 4D). The ventral closure of the head is completely sclerotised and corresponds with the postgenal regions. They are fused medially thus forming a postgenal or hypostomal bridge. Laterally on the ventral side, laterad the “smooth” region, the distinct posterior tentorial grooves are present (ptg, Fig. 2A). The mouth opening lies also on the ventral side of the head (mo, Fig. 2A). Movable mouthparts are completely missing. Posterad the mouth opening there is a second small opening, probably representing opening of the vestigial salivary duct (sd, Fig. 2A). A field with five sensilla is present anterior to the mouth opening.

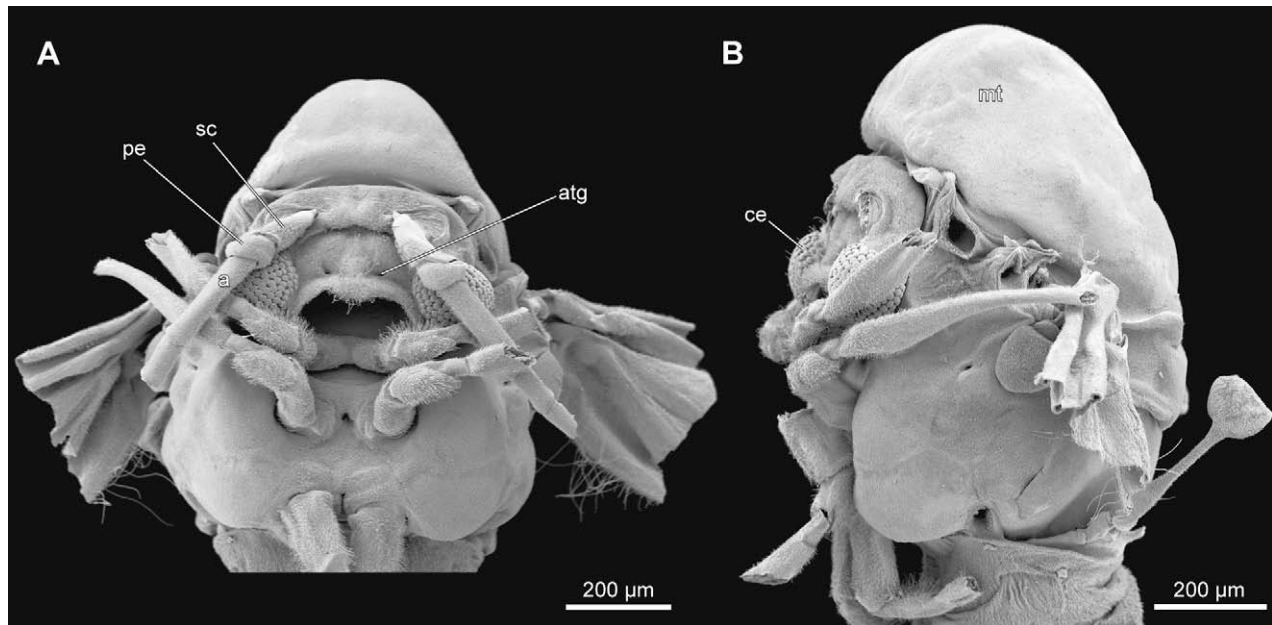
The head capsule of the female is less sclerotised and less flattened in lateral view but otherwise very similar.

### 3.2. Tentorium

The tentorium is represented by a simple hollow rod connecting the ventral and dorsal side of the head capsule (tnt, Fig. 4B). The internal surface of the tentorium is covered with short microtrichia like most parts of the external head capsule.

### 3.3. Labrum

Absent or completely fused with the head capsule.  
Musculature: absent.



**Fig. 1.** *Deuterophlebia coloradensis*, head and thorax, SEM images. (A) ventral view, frontal view of the head; (B) lateral view. Abbreviations: a, antenna; atg, anterior tentorial groove; ce, compound eye; mt, mesothorax; pe, pedicel; sc, scape.

### 3.4. Antenna

The antenna is composed of 6 segments, the scape, pedicel and 4 flagellomeres. They insert frontally between the compound eyes. All antennomeres are densely covered with short microtrichia (Fig. 2B).

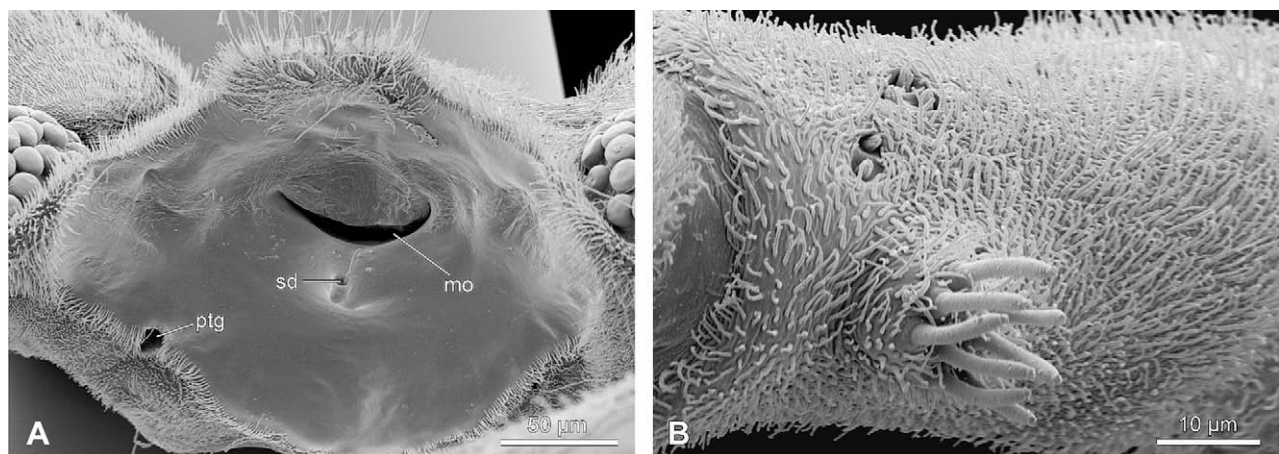
The scape is cylindrical and about three times as long as the small, globular pedicel (sc, pe, Fig. 1A). Flagellomere 1 is about  $\frac{1}{4}$  longer than the scape, but narrower. The diameter of the following antennomeres is similar to flagellomere 1, but they differ in their length. Flagellomeres 2 and 3 are about  $\frac{1}{2}$  as long as flagellomere 1. The last segment is greatly enlarged, distinctly longer than the entire body and narrows from its base to the conical apex.

Widely spaced sensilla are regularly distributed between the microtrichia of all antennal segments. A tuft of about eight sensilla is on the ventral side of the proximal part of flagellomere 1. Two additional sensilla are inserted distally. Several sensilla sunk in

mesal grooves are present lateral of the sensilla tuft. Tufts of eight sensilla are also present on the middle region of flagellomeres 2 and 3, and five countersunk sensilla on the lateral side of both segments (Fig. 2B). The extremely elongated flagellomere 4 bears two rows of hook-shaped sensilla on the mesal side. Their size decreases from the base to the apex of the segment.

The antenna of the female is distinctly shorter. The apical segment is not elongated.

**Musculature:** (1, 2, 5, 6, Fig. 4A, B, D) M. 1: M. tentorioscapalis anterior: a muscle with multiple areas of origin; O (origin) – genal region, laterally of the tentorial base (1), dorsal side of the tentorium (2), on the clypeal region mesally of the first two bundles (3), dorsolaterally of the third bundle (4); I (insertion) – ventromedial margin of the scapus; F (function) – depressor of the antenna. M. 2: M. tentorioscapalis posterior: a very broad and flat muscle on the dorsal side of the head; O – vertex; I – dorsal margin of the scapus; F – levator, antagonist of M. tentorioscapalis anterior. M. 3:



**Fig. 2.** *Deuterophlebia coloradensis*, head, SEM images. (A) ventral view; (B) antenna, flagellomere 2. Abbreviations: mo, mouth opening; ptg, posterior tentorial groove; sd, vestigial salivary duct.



M. tentorioscapalis lateralis: absent. M. 4: M. tentorioscapalis medialis: absent. M. 5: M. scapopedicellaris lateralis: long and slender muscle; O – ventrolateral wall of the scape; I – ventrally on the basal margin of the pedicel; F – extensor of the flagellum. M. 6: M. scapopedicellaris medialis: compact muscle; O – dorsomesally on the basal margin of the scape; I – dorsally on the basal margin of the pedicel; F – flexion and mesal rotation of the flagellum.

### 3.5. Mandible

Absent.

Musculature: Absent.

### 3.6. Maxilla

Absent.

Musculature: Absent.

### 3.7. Labium

Absent.

Musculature: Absent.

### 3.8. Epi- and hypo-pharynx

The anterior epipharynx is completely reduced. The posterior epipharynx is largely membranous and devoid of microtrichia. The hypopharynx forms the U-shaped floor of the cibarium (cib, Fig. 5). Posteriorly the cibarium is continuous with the pharynx. At the anatomical mouth opening the hypopharynx forms two dorsolateral apophyses.

Musculature: (43, Figs. 3, 4A, D, 5) M. 41: M. frontohypopharyngalis: absent. M. 42: M. tentorihypopharyngalis: absent. M. 43: M. clypeopalatalis: compact muscle; O – mesal clypeal lobe; I – palatum; F – cibarial dilator. M. 44: M. clypeobuccalis: absent. M. 48: M. tentoriobuccalis anterior: absent.

### 3.9. Pharynx

The position of anatomical mouth opening is marked externally by the antennal insertions. The precerebral part of the pharynx is U-shaped in cross section, with the upper edges forming

dorsolateral extensions. The following part of the pharynx between the brain and the suboesophageal complex and also the postcerebral part of the pharynx are Y-shaped in cross section, with distinct drawn out edges (ph, Fig. 4C). The spaces between these extensions are the attachment areas of the muscles forming the postpharyngeal pumping apparatus (51, 52, Figs. 3, 4C, 5). In the postoccipital region the pharynx is continuous with the oesophagus.

Musculature: (46, 51, 52, 68, Figs. 3, 4A, C, D, 5) M. 45: M. frontobuccalis anterior: absent. M. 46: M. frontobuccalis posterior: the only dorsal precerebral dilator; O – posterior frontal region; I – anterior pharynx, immediately anterad of brain; F – widens the lumen of the precerebral pharynx. M. 47: M. frontobuccalis lateralis: absent. M. 49: M. tentoriobuccalis lateralis: absent. M. 50: M. tentoriobuccalis posterior: absent. M. 51: M. verticopharyngalis: long and slender muscle; O – postoccipital region, dorsad of foramen occipitale; I – dorsal wall of the postcerebral pharynx, immediately posterad the brain. M. 52: M. tentoriopharyngalis: strongly developed muscle; O – extensive region of the postgena; I – ventrolateral part of the posterior pharynx; F – dilator of the posterior pharynx, together with M. verticopharyngalis. M. 67: M. transversalis buccae: a thin muscle band, above the dorsal wall of the anterior pharynx, anterad the insertion of M. frontobuccalis posterior. M. 68: M. anularis stomodaei: ring muscles around the oesophagus, pharyngeal elements attached to the lateral folds, enclosing only the ventral and lateral pharyngeal wall. A complete ring muscle is present posterad the postcerebral pharynx pump (M. 51 & M. 52). M. 69: M. longitudinalis stomodaei: absent.

### 3.10. Salivarium

The salivary duct opens ventrally of the mouth opening on the ventral side of the head (sd, Fig. 2A). It is only a short vestigial duct, without a connection to salivary glands or any other structure.

Musculature: Absent.

### 3.11. Nervous system

The brain and suboesophageal complex are very large in relation to the size of the head capsule (Figs. 3 and 4). Both units form a compact complex around the posterior pharynx (Fig. 4C). It lies within the posterior half of the head capsule and occupies a large proportion of the lumen. Proto-, deuto- and trito-cerebrum are completely fused, without externally recognisable borders. A tritocerebral commissure is not recognisable as a separate structure. The optic lobes are very large (Fig. 4). They comprise about one third of the brain volume. The thin antennal nerves (Nn. antennales) arise at the frontal region on the head. The frontal ganglion lies above the palatum and thick frontal connectives connect it with the brain (fg, Fig. 3). The N. frontale, N. connectivus and N. recurrens are absent.

### 3.12. Tracheal system

Four pairs of tracheae enter the head, two of them in the dorsal region and the two others laterally. The dorsolateral and the lateral pairs split into many thin tracheae, which mainly supply the brain with oxygen. The branches arising from the dorsomedian pair mainly supplies the musculature of the antennae.

### 3.13. Fat body

A larger assemblage of fat body tissue is posterior to the brain.

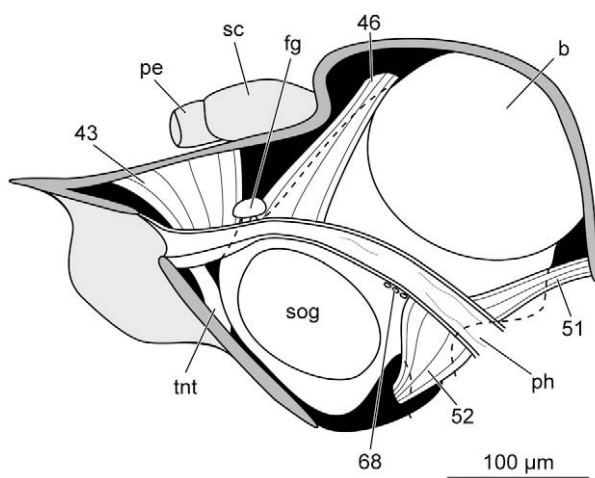
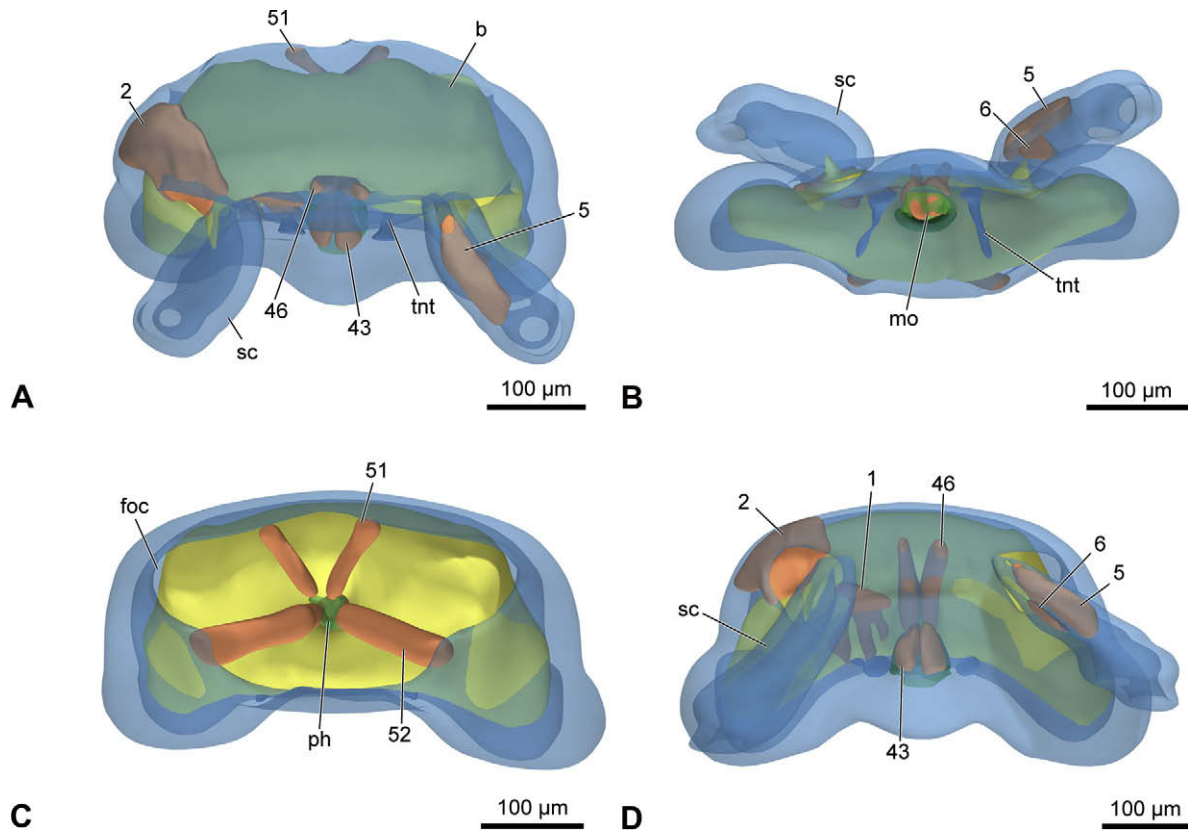


Fig. 3. *Deuterophlebia coloradensis*, head, sagittal section. Abbreviations: b, brain; fg, frontal ganglion; pe, pedicel; ph, pharynx; sc, scape; sog, suboesophageal ganglion; tnt, tentorium; 43, M. clypeopalatalis; 46, M. frontobuccalis posterior; 51, M. verticopharyngalis; 52, M. tentoriopharyngalis; 68, M. anularis stomodaei.



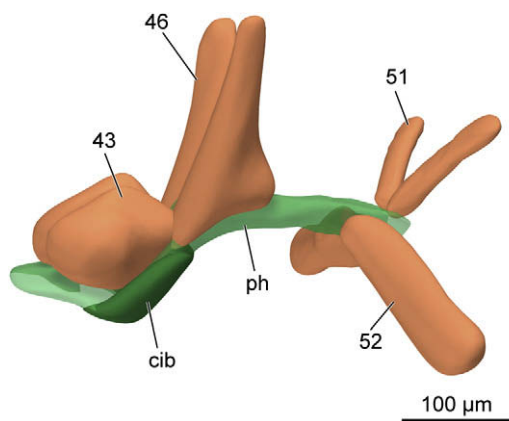
**Fig. 4.** *Deuterophlebia coloradensis*, head, three-dimensional reconstruction (skeleton, blue; musculature, orange; nervous system, yellow; digestive tract, green). (A) dorsal view; (B) ventral view; (C) posterior view; (D) frontal view. Abbreviations: b, brain; foc, foramen occipitale; mo, mouth opening; ph, pharynx; sc, scape; tnt, tentorium; 1, M. tentorioscapalis anterior; 2, M. tentorioscapalis posterior; 5, M. scapopedicellaris lateralis; 6, M. scapopedicellaris medialis; 43, M. clypeopalatalis; 46, M. frontobuccalis posterior; 51, M. verticopharyngalis; 52, M. tentiopharyngalis.

### 3.14. Phylogenetically relevant characters

(see Table 1, 2)

#### 1. Frontal apodeme (*fap*): (0) present; (1) absent

A frontal apodeme between the antennal bases is absent in *Deuterophlebia* and also missing in *Nymphomyia* Tokunaga, 1932 (Tokunaga, 1935), *Tipula* Latreille, 1802 (Tipulidae) (Schneeberg and Beutel, in press), *Biblio* Geoffroy, 1762 (Bibionidae) (Skanda,



**Fig. 5.** *Deuterophlebia coloradensis*, head, digestive tract. Abbreviations: cib, cibarium; ph, pharynx; 43, M. clypeopalatalis; 46, M. frontobuccalis posterior; 51, M. verticopharyngalis; 52, M. tentiopharyngalis.

2008), *Eristalis* Latreille, 1804 (Syrphidae) (Schiemenz, 1957), *Hemipenthes* Loew, 1861, *Bombylius* Linnaeus, 1758 (Bombyliidae) (Szucsich and Krenn, 2000), *Ctenocephalus* Curtis, 1826 (Siphonaptera) (Wenk, 1953), and also in members of all groups of Mecoptera with the notable exception of Nannochoristidae (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Fig. 5 [fap]). A small apodeme-like structure is present in *Trichocera* Meigen, 1803 (Trichoceridae) dorsally between the antennal bases (Schneeberg and Beutel, in press). It is distinctly developed in *Edwardsina* Alexander, 1920, *Limonia* Meigen, 1803 (Limoniidae), *Mischoderus* Handlirsch, 1909 (Tanyderidae) (Schneeberg and Beutel, in press), in representatives of Culicidae (Schiemenz, 1957; Christophers, 1960; Owen, 1985; Harbach and Kitching, 1998), and in *Wilhelmia* Enderlein, 1921 (Simuliidae) (Wenk, 1962).

#### 2. Frontoclypeal-/epistomal suture (*fcs*): (0) present as a strengthening ridge; (1) present as a joint; (2) absent

Both regions are fused in *Deuterophlebia* (Fig. 1A), *Edwardsina*, *Nymphomyia* (Tokunaga, 1935), *Simulium* Latreille, 1902 (Wenk, 1962, Fig. 1) and *Ctenocephalus* (Wenk, 1953). The suture is present in *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Eristalis* (Schiemenz, 1957), *Bombylius* (Szucsich and Krenn, 2000), *Tabanus* Linnaeus, 1758 (Bonhag, 1951), *Mischoderus* (Schneeberg and Beutel, in press) and *Protoplasa* Oosten Sacken, 1859 (Williams, 1933). It is distinctly developed in *T. paludosa* (Schneeberg and Beutel, in press), whereas it appears to be absent in *Tipula reesi* Alexander, 1939 (Rees and Ferris, 1939) and *Tipula* sp. (Bitsch et al., 1973). The

**Table 1**  
Data matrix. Presumptive groundplan features of Diptera are coded as 0, regardless whether they are plesiomorphic or apomorphic compared to the corresponding character states in the other antliophoran taxa.

Taxa/character	1 fap	2 fcs	3 cly	4 ais	5 lae	6 oce	7 cos	8 pgb	9 tnt	10 dta	11 ata
<i>Deuterophlebia</i>	1	1	1	2	1	1	1	0	0	2	0
<i>Nymphomyia</i>	1	1	1	0	1	0	0	0	1	–	–
<i>Edwardsina</i>	0	1	1	0	0	0	1	1	0	2	0
<i>Tipula</i>	1	0/1	1	0	0	1	1	0	1	–	–
<i>Limonia</i>	0	0	1	0	0	1	1	0	0	2	1
<i>Trichocera</i>	1?	0	1	1	0	0	1	1	0	2	0
<i>Culex</i>	0	0	0	0	0	1	0	?	0	2	0
<i>Anopheles</i>	0	?	?	0	0	1	?	?	0	?	0
<i>Culiseta</i>	0	0	0	0	0	1	0	1	0	1	0
<i>Aedes</i>	0	?	0	0	0	1	0	0	0	2	0
<i>Wilhelmia</i>	0	1	1	0	0	1	1	1	0	1	0
<i>Biblio</i>	1	0	1	–	0	0	1	0	0	2	0
<i>Mischoderus</i>	0	0	1	0	0	1	1	1	0	?	0
<i>Protoplasa</i>	?	0	1	0	0	1	1	1	0	?	?
<i>Eristalis</i>	1	0	1	1	0	0	1	0	0	1	2
<i>Hemipenthes</i>	1	0	1	0	?	?	1	?	0	?	?
<i>Bombylius</i>	1	0	1	0	?	?	1	?	?	?	?
<i>Tabanus</i>	?	0	1	0	0	1	1	0	0	0	?
<i>Nannochorista</i>	0	0	0	0	0	0	0	1	0	1	0
<i>Caurinus</i>	1	0	0	2	0	1	1	0	0	0	2
<i>Panorpa</i>	1	0	1	0	?	0	0	0	0	2	?
<i>Ctenocephalus</i>	1	1	1	2	0	1	1	0	0	2	–

suture is also present in members of Mecoptera (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008). A joint between the clypeus and frons in adults of Culicidae (e.g. Schiemenz, 1957; Christophers, 1960; Hennig, 1973) is a potential autapomorphy of the family. The increased mobility of the clypeus in relation to the anterior frontal margin is likely correlated with the formation of a fulcrum (see character 13).

3. *Clypeus (cly)*: (0) subdivided into ante- and postclypeus; (1) undivided

A transverse suture separating an anterior anteclypeus from the posterior postclypeus is present in the groundplan of Diptera according to Crampton (1942). However, the division is absent in *Deuterophlebia*, *Edwardsina* and *Nymphomyia* (Courtney, 1994b), and is also almost generally missing in other groups (e.g. Williams, 1933; Tokunaga, 1935; Bonhag, 1951; Hoyt, 1952; Schiemenz, 1957; Wenk, 1962; Szucsich and Krenn, 2000; Skanda, 2008; Schneeberg and Beutel, in press). A transverse clypeal furrow is present in adults of Culicidae (e.g. *Culex* Linnaeus, 1758, *Culiseta* Felt, 1904, *Aedes* Meigen, 1818; Schiemenz, 1957; Christophers, 1960; Owen, 1985), and also in *Nannochorista* Tillyard, 1917 (Beutel and Baum, 2008) and *Caurinus* Russel, 1979 (Beutel et al., 2008). It is absent in other groups of Mecoptera (Heddergott, 1938; Hepburn, 1969) and also in Siphonaptera (Wenk, 1953) and some other groups of endopterygote insects (Beutel and Baum, 2008).

4. *Insertion of antennae (ais)*: (0) frontally, not adjacent medially; (1) frontally, adjacent in midline; (2) laterally, widely separated

The antennae insert on the dorsal side of the head and are widely separated in *Deuterophlebia* (Fig. 1A) and *Biblio* (Skanda, 2008, Fig. 2A). They are also widely separated in *Caurinus* (Beutel et al., 2008) and *Ctenocephalus* (Wenk, 1953). The antenna insertions lie frontally between the compound eyes in *Edwardsina*, *Nymphomyia* (Tokunaga, 1935), *Tipula*, *Limonia*, *Mischoderus* (Schneeberg and Beutel, in press), *Protoplasa* (Williams, 1933), in representatives of Culicidae (Schiemenz,

1957; Christophers, 1960; Owen, 1985; Harbach and Kitching, 1998), Simuliidae (Wenk, 1962), Tabanidae (Bonhag, 1951), Bombyliidae (Peterson, 1916), and also in *Nannochorista* (Beutel and Baum, 2008) and *Panorpa* Linnaeus, 1758 (Heddergott, 1938). This is also the case in *Trichocera* (Schneeberg and Beutel, in press) and *Eristalis* (Schiemenz, 1957, Fig. 31), where they are medially adjacent.

5. *Terminal segment of the antenna (lae)*: (0) not elongated; (1) elongated

The last antennal segment is extremely elongated in males of *Deuterophlebia* and also elongated in *Nymphomyia* (Courtney, 1994b, Figs. 16, 26, 29). In *Deuterophlebia* only the terminal segment is elongate, whereas the enlarged element in *Nymphomyia* is probably formed by several fused flagellomeres. Nevertheless, a distinct increase in size of the true terminal segment has apparently taken place but the last part might be the elongated last flagellomere (see Courtney, 1994b, Fig. 79). The segment is not elongated in members of most other groups of Diptera (Williams, 1933; Bonhag, 1951; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Skanda, 2008; Schneeberg and Beutel, in press), and this condition is also unknown in Mecoptera (Beutel et al., 2008; Beutel and Baum, 2008).

6. *Ocelli (oce)*: (0) present; (1) vestigial or absent

Ocelli are absent in adults of *Deuterophlebia* (Courtney, 1990a, 1991a), *Tipula*, *Limonia*, *Culex*, *Anopheles* Meigen, 1816 *Mischoderus* (Schneeberg and Beutel, in press), *Culiseta* (Schiemenz, 1957; Owen, 1985), *Wilhelmia* (Wenk, 1962), *Protoplasa* (Williams, 1933), *Caurinus* (Beutel et al., 2008), and also in *Ctenocephalus* (Wenk, 1953). Three ocelli are present on the vertex in *Edwardsina*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Eristalis* (Schiemenz, 1957), *Exoprosopa* Marcquart, 1840 (Peterson, 1916), *Nannochorista* (Beutel and Baum, 2008) and *Panorpa* (Heddergott, 1938). Christophers (1960) described a pair of degenerated ocelli on the frons of *Aedes*. A pair is present posterolaterad of the large compound

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
lbr	flc	M.1	M.2	M.4	mdb	max	lbn	M.41	M.43	Mm. 45/46	M.48	Mm. 51/52	M.68	M.37
1	0	0/1	1	–	1	1	1	1	2	1	1	0	0	1
1	0	1	1	2	1	1	1	–	0?	0/1	1	0	1	0
0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
0	0	1	1	2	1	0	0	0?	0	1	0	0	0	0
0	0	1	0	0?	1	0	0	1	0	1	0	0	1	1
0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
0	?	0	0	–	0	0	0	0	0	0	0	0	?	0
0	?	0	0	–	0	0	0	0	0	0	0	0	?	0
0	1	0	0	–	0	0	0	0	0	0	0	0	?	0
0	1	0	0	–	0	0	0	0	1	0	0	0	1	0
0	0	0	0	–	0	0	0	1	1	0	0	0	1	0
0	0	0	0	1	1	0	0	1	0	1	0	0	?	0
0	?	?	?	?	1	0	0	?	0	0	?	0	?	0
0	1	?	?	?	1	0	0	?	?	?	?	?	?	?
0	1	0	1?	0?	1	0	0	0	0	2	1	1	?	0
0	1	0	1	0	1	0	0	1	0	1	1?	1	?	0
0	1	0	1?	0?	1	0	0	1	0	1	1?	1	?	0
0	0	0	0	1	0	0	0	0?	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0	1	2	1	0	0	0
0	0	0	0	0	0	0	0	0	2	0	1	0	?	1
0	0	0	0	3	0	0	0	1	0	2	1	0	1	1
0	0	1	1	(–)	1	0	0	0	0	0	1	0	?	?

eyes of *Nymphomyia* (Tokunaga, 1935; Courtney, 1994b). Bonhag (1951) described three rudimentary ocelli on the vertex of *Tabanus*.

#### 7. Coronal-/epicranial-suture (cos): (0) present; (1) absent

A coronal suture is absent in *Deuterophlebia*, *Edwardsina*, *Tipula*, *Limonia*, *Trichocera*, *Mischoderus* (Schneeberg and Beutel, in press), *Protoplasma* (Williams, 1933), *Biblio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), *Tabanus* (Bonhag, 1951), *Eristalis* (Schiemenz, 1957), *Hemipenthes* and *Bombylius* (Szucsich and Krenn, 2000). It is also missing in *Ctenocephalus* (Wenk, 1953), *Caurinus* (Beutel et al., 2008) and many other representatives of Mecoptera (Hepburn, 1969). It is present in *Culex* (Schneeberg and Beutel, in press), *Culiseta* (Schiemenz, 1957; Owen, 1985, Fig. 1), *Aedes* (Christophers, 1960, Fig. 53/1), *Nymphomyia* (Tokunaga, 1935), and in *Nannochorista* (Beutel and Baum, 2008). The presence is apparently a plesiomorphic condition preserved in Nymphomyiidae and Culicidae.

#### 8. Postgenal bridge (pgb): (0) present; (1) absent

A postgenal bridge is present in *Deuterophlebia* (Courtney, 1990a), *Nymphomyia* (Tokunaga, 1935; Courtney, 1994b), and also in *Tipula* (Schneeberg and Beutel, in press), *Limnophila* Macquart, 1834 (Hoyt, 1952), *Aedes* (Christophers, 1960), *Biblio* (Skanda, 2008), *Tabanus* (Bonhag, 1951), *Eristalis* (Schiemenz, 1957), *Exoprosopa* (Peterson, 1916), *Rhagio* Fabricius, 1775 (Hoyt, 1952), *Ctenocephalus* (Wenk, 1953), and in representatives of Mecoptera (e.g. Heddergott, 1938; Beutel et al., 2008) (with the possible exception of *Nannochorista*, Beutel and Baum, 2008). The ventral closure of the head capsule is largely membranous in *Limonia*, but a narrow postgenal bridge is present anterior to the foramen occipitale (Schneeberg and Beutel, in press). The bridge is absent in *Edwardsina*, *Erioptera* Meigen, 1803, *Dicranomyia* Stephens, 1829 (Hoyt, 1952), *Trichocera*, *Mischoderus* (Schneeberg and Beutel, in press), *Protoplasma* (Williams, 1933), *Culiseta* (Schiemenz, 1957, Fig. 4 [gu]; Owen, 1985), *Ptychoptera* Meigen, 1803, *Mycetophila* Meigen,

1818, *Mycomya* Rondani, 1856 (Hoyt, 1952), *Wilhelmia* (Wenk, 1962) and *Symphoromyia* Frauenfeld, 1867 (Hoyt, 1952). The head is largely membranous on its ventral side in adults of these taxa.

#### 9. Tentorium (tnt): (0) present; (1) absent

The tentorium is present as a more or less straight tube-like rod in *Deuterophlebia* (tnt, Fig. 4B), *Edwardsina* and many other groups of Diptera (e.g. Thompson, 1905; Peterson, 1916; Williams, 1933, Fig. 3; Hoyt, 1952; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Skanda, 2008). A similar condition is found in representatives of Mecoptera (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008) and Siphonaptera (Wenk, 1953). The tentorium is completely absent in *Nymphomyia* (Tokunaga, 1935) and *Tipula* (Schneeberg and Beutel, in press).

#### 10. Dorsal tentorial arm (dta): (0) present or present as a thin thread-like structure; (1) short vestigial structure; (2) absent

The dorsal arm is completely missing in *Deuterophlebia* and *Edwardsina* (Courtney, 1991a), and is also absent in *Limonia* (Schneeberg and Beutel, in press), *Culex* (Thompson, 1905), *Aedes* (Christophers, 1960), *Biblio* (Skanda, 2008) and *Ctenocephalus* (Wenk, 1953). It is present as a short vestige in *Culiseta* (Schiemenz, 1957, Fig. 5 [d.Ta]; Owen, 1985, Fig. 4), *Eristalis* (Schiemenz, 1957, Figs. 38, 39), *Exoprosopa* (Peterson, 1916) and *Nannochorista* (Beutel and Baum, 2008, Fig. 2d [dta]). Wenk (1962) described a tentorial ridge for *Wilhelmia*, extending dorsad towards the antennal foramen (Wenk, 1962, Fig. 2). A typical, well developed dorsal arm is apparently almost always absent in Antliophora (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008), but a thin, sclerotised structure is present and connected to the head capsule in *Caurinus* (Beutel et al., 2008, Fig. 5C, D). It is noteworthy that dorsal arms are also present in *Tabanus* (Bonhag, 1951, Fig. 5). However, considering the position of Tabanidae, it appears likely that the presence is due to reversal in this case.



**Table 2**  
Insect taxa mentioned in the text (classification mostly after Oosterbroek and Courtney, 1995).

Genus	Family	Suborder	Order
<i>Deuterophlebia</i>	Deuterophlebiidae	Blephariceromorpha	Diptera
<i>Nymphomyia</i>	Nymphomyiidae	Blephariceromorpha	Diptera
<i>Edwardsina</i>	Blephariceridae	Blephariceromorpha	Diptera
<i>Culex</i>	Culicidae	Culicomorpha	Diptera
<i>Culiseta</i>	Culicidae	Culicomorpha	Diptera
<i>Aedes</i>	Culicidae	Culicomorpha	Diptera
<i>Anopheles</i>	Culicidae	Culicomorpha	Diptera
<i>Wilhelmia</i>	Simuliidae	Culicomorpha	Diptera
<i>Simulium</i>	Simuliidae	Culicomorpha	Diptera
<i>Ptychoptera</i>	Ptychopteridae	Ptychopteromorpha	Diptera
<i>Mischoderus</i>	Tanyderidae	Ptychopteromorpha	Diptera
<i>Protoplasa</i>	Tanyderidae	Ptychopteromorpha	Diptera
<i>Tipula</i>	Tipulidae	Tipulomorpha	Diptera
<i>Limonia</i>	Limoniidae	Tipulomorpha	Diptera
<i>Limnophila</i>	Limoniidae	Tipulomorpha	Diptera
<i>Erioptera</i>	Limoniidae	Tipulomorpha	Diptera
<i>Dicranomyia</i>	Limoniidae	Tipulomorpha	Diptera
<i>Trichocera</i>	Trichoceridae	Tipulomorpha	Diptera
<i>Bibio</i>	Bibionidae	Bibionomorpha	Diptera
<i>Mycetophila</i>	Mycetophilidae	Bibionomorpha	Diptera
<i>Mycomyia</i>	Mycetophilidae	Bibionomorpha	Diptera
<i>Tabanus</i>	Tabanidae	Tabanomorpha	Diptera
<i>Rhagio</i>	Rhagionidae	Tabanomorpha	Diptera
<i>Symphoromyia</i>	Rhagionidae	Tabanomorpha	Diptera
<i>Eristalis</i>	Syrphidae	Muscomorpha	Diptera
<i>Hemipenthes</i>	Bombyliidae	Asilomorpha	Diptera
<i>Bombylius</i>	Bombyliidae	Asilomorpha	Diptera
<i>Exoprosopa</i>	Bombyliidae	Asilomorpha	Diptera
<i>Nannochorista</i>	Nannochoristidae		Mecoptera
<i>Caurinus</i>	Boreidae		Mecoptera
<i>Panorpa</i>	Panorpidae		Mecoptera
<i>Ctenocephalus</i>			Siphonaptera
<i>Osmylus</i>			Neuroptera

11. Shape of the anterior tentorial arm (*ata*): (0) thick, approximately round in cross section, hollow; (1) partly hollow; (2) massive

The tentorium of *Deuterophlebia*, *Edwardsina*, *Mischoderus*, *Trichocera* (Schneeberg and Beutel, in press), *Bibio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962) and *Nannochorista* (Beutel and Baum, 2008) is a thick, hollow tube, and this is also the case in Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985), where the lumen is wider anteriorly. A recognisable lumen is not present in *Eristalis* (Schiemenz, 1957), *Exoprosopa* (Peterson, 1916) and *Caurinus* (Beutel et al., 2008). The lumen of the anterior part of the tentorium is narrow in *Limonia*. It widens at the level of the brain and the posterior hollow part is approximately round in cross section (Schneeberg and Beutel, in press). The anterior tentorial arms of *Ctenocephalus* are missing (Wenk, 1953).

12. Labrum (*lbr*): (0) present; (1) absent

With the exception of *Deuterophlebia* (Courtney, 1990a, 1991a) and *Nymphomyia* (Tokunaga, 1935), the labrum is present in all members of Diptera (e.g. Thompson, 1905; Williams, 1933; Bonhag, 1951; Hoyt, 1952; Schiemenz, 1957; Wenk, 1962; Christophers, 1960; McAlpine, 1981; Owen, 1985; Courtney, 1991a; Skanda, 2008; Schneeberg and Beutel, in press), Mecoptera (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008) and Siphonaptera (Wenk, 1953) examined.

13. Fulcrum (*flc*): (0) absent; (1) present

A fulcrum with lateral fulcral plates, which join the external clypeal wall distally, is absent in *Deuterophlebia*, *Edwardsina*, and

*Nymphomyia* (Tokunaga, 1935), and is also missing in *Tipula*, *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Bibio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), *Tabanus* (Bonhag, 1951), *Nannochorista* (Beutel and Baum, 2008), *Caurinus* (Beutel et al., 2008), *Panorpa* (Heddergott, 1938) and *Ctenocephalus* (Wenk, 1953). It is generally present in adults of Culicidae (Schiemenz, 1957; Christophers, 1960; Owen, 1985), and does also occur in Syrphidae (Schiemenz, 1957), Tanyderidae (Williams, 1933; *Protoplasa*) and Bombyliidae (Szucsich and Krenn, 2000; *Hemipenthes*, *Bombylius*).

14. Origin of *M. tentorioscapalis anterior* (*M. 1*): (0) tentorium; (1) head capsule

In *Deuterophlebia* the muscle has multiple origins, three on the head capsule and one on the dorsal side of the tentorium (1, Fig. 4D). It originates on the prefrontal region of the head capsule in *Tipula*, *Limonia* (Schneeberg and Beutel, in press) and *Nymphomyia* (Tokunaga, 1935). The muscle originates on the clypeus in *Ctenocephalus* (Wenk, 1953), and on the tentorium in *Edwardsina*, *Trichocera* (Schneeberg and Beutel, in press), *Bibio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), *Tabanus* (Bonhag, 1951), *Eristalis* (Schiemenz, 1957), and also in representatives of Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985), Bombyliidae (Szucsich and Krenn, 2000) and Mecoptera (Heddergott, 1938; Beutel and Baum, 2008; Beutel et al., 2008).

15. Origin of *M. tentorioscapalis posterior* (*M. 2*): (0) tentorium; (1) head capsule

*M. 2* originates on the genal region of the head capsule in *Tipula*, between the clypeus and the margin of the compound eye (Schneeberg and Beutel, in press). It also originates on the head capsule in *Deuterophlebia* (2, Fig. 4D), *Edwardsina*, *Nymphomyia* (Tokunaga, 1935), *Hemipenthes* (Szucsich and Krenn, 2000) and *Ctenocephalus* (Wenk, 1953). It is difficult to distinguish between *M. 2* and *M. 4* in *Limonia*. Both muscles lie very closely together and have a nearly identical point of insertion on the scapus and closely adjacent areas of origin on the tentorium. In *Trichocera* the origin lies dorsally on the anterior tentorial arm (Schneeberg and Beutel, in press) and this is also the case in *Aedes* (Christophers, 1960) and *Wilhelmia* (Wenk, 1962). In *Culiseta* (Schiemenz, 1957; Owen, 1985) it originates laterally on the anterior tentorium and it arises on the dorsal arm in *Tabanus* (Bonhag, 1951). The muscle also originates on the tentorium in *Bibio* (Skanda, 2008) like in all adults of Mecoptera examined (e.g. Heddergott, 1938; Beutel et al., 2008; Beutel and Baum, 2008). The area of origin lies on the circumocular ridge in *Eristalis* (Schiemenz, 1957) and a similar *M. orbitoscapalis* is also present in *Bombylius* (Szucsich and Krenn, 2000). Schiemenz (1957) interpreted this muscle as *M. tentorioscapalis posterior* (*M. 2*). However, it cannot be excluded that it is in fact *M. tentorioscapalis medialis* (*M. 4*).

16. Origin of *M. tentorioscapalis medialis* (*M. 4*): (0) tentorium; (1) frontal region of head capsule; (2) genal region of head capsule; (3) on the vertex

*M. tentorioscapalis medialis* originates on the genal region in *Tipula* (Schneeberg and Beutel, in press) and *Nymphomyia* (Tokunaga, 1935), and on the frons in *Trichocera*, *Bibio*, *Tabanus* and *Nannochorista* (Bonhag, 1951; Skanda, 2008; Beutel and Baum, 2008; Schneeberg and Beutel, in press). It originates on the vertex in *Panorpa* (Heddergott, 1938), and on the clypeus in *Ctenocephalus* (Wenk, 1953). The muscle is absent in representatives of Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985) and Simuliidae (Wenk, 1962).



It originates on the anterior tentorium in *Hemipenthes* (Szucsich and Krenn, 2000) and *Caurinus* (Beutel et al., 2008). As pointed out above, the homology of the muscle is not entirely clear in *Limonia*, *Eristalis* and *Bombylius* (see character 15).

17. Mandible (*mdb*): (0) present; (1) absent

The mandibles are absent in males and females in *Deuterophlebia* (Courtney, 1990a, 1991a), *Nymphomyia* (Tokunaga, 1935; Courtney, 1994b), *Tipula*, *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Protoplasa* (Williams, 1933), *Eristalis* (Schiemenz, 1957), in representatives of Bombyliidae (Szucsich and Krenn, 2000), and also in *Ctenocephalus* (Wenk, 1953). They are only developed in females of *Edwardsina* and *Symphoromyia* (Rhagionidae) (Hoyt, 1952), whereas they are always present in adults of Culicidae (Schiemenz, 1957; Christophers, 1960; Owen, 1985), Simuliidae (Wenk, 1962), Tabanidae (Bonhag, 1951) and Mecoptera (e.g. Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008).

18. Maxilla (*max*): (0) present; (1) absent

The maxilla is absent in *Deuterophlebia* (Courtney, 1990a, 1991a), *Nymphomyia* (Tokunaga, 1935; Courtney, 1994b) and also missing in some chironomids (Neumann, 1976). It is present in all other members of Diptera (e.g. Thompson, 1905; Williams, 1933; Bonhag, 1951; Hoyt, 1952; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Skanda, 2008; Schneeberg and Beutel, in press), Mecoptera (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008) and Siphonaptera (Wenk, 1953).

19. Labium (*lbm*): (0) present; (1) absent

In all dipterans (e.g. Thompson, 1905; Williams, 1933; Bonhag, 1951; Hoyt, 1952; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Skanda, 2008; Schneeberg and Beutel, in press), mecopterans (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008) and siphonapterans (Wenk, 1953), with the exception of *Deuterophlebia* (Courtney, 1990a, 1991a), *Nymphomyia* (Tokunaga, 1935; Courtney, 1994b) and some Chironomidae (Neumann, 1976), a labium is present.

20. *M. frontohypopharyngalis* (*M. 41*): (0) present; (1) absent

The muscle is absent in *Deuterophlebia*, *Edwardsina*, *Nymphomyia* (Tokunaga, 1935), *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), and in representatives of Bombyliidae (Szucsich and Krenn, 2000). A small bipartite muscle is present in *Tipula*. It extends from the lateral clypeal wall to the lateral wall of the hypopharynx (Schneeberg and Beutel, in press). The homology with *M. frontohypopharyngalis* is questionable. A muscle connecting the postfrontal ridge and the dorsal fulcral apophyses is present in representatives of Culicidae (Thompson, 1905; Schiemenz, 1957; Owen, 1985) and in *Eristalis* (Schiemenz, 1957). It is likely that it mainly stabilises the cibarium and it is probably homologous with *M. frontohypopharyngalis*. However, Schiemenz (1957) assumed its homology with *M. frontobuccalis lateralis* (*M. 47*) (*Culiseta* and *Eristalis*) and a similar muscle is present in *Aedes*. It originates on the median frontal ridge and is inserted on the lateral horn of the cibarium (Christophers, 1960, Fig. 67/1 [21]). A muscle, which connects the clypeo-frontal ridge and the anterior surface of the pharynx, is present in *Tabanus* (Bonhag, 1951, Fig. 10 [22]), but the homology with *M. 41* is also questionable. *M. frontohypopharyngalis* is present in *Ctenocephalus* (Wenk, 1953), *Nannochorista* and *Caurinus*, but

absent in other mecopterans (Heddergott, 1938; Hepburn, 1969; Beutel and Baum, 2008; Beutel et al., 2008).

21. Size of *M. clypeopalatalis* (*M. 43*): (0) long series of bundles; (1) bipartite, strongly developed; (2) not enlarged

The muscle is not enlarged in *Deuterophlebia* (43, Figs. 3, 5). *M. clypeopalatalis* is a long series of bundles in *Edwardsina* and also in *Tipula*, *Limonia*, *Trichocera*, *Mischoderus* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Tabanus* (Bonhag, 1951), *Eristalis* (Schiemenz, 1957), *Hemipenthes*, *Bombylius* (Szucsich and Krenn, 2000), and most members of Culicidae (Thompson, 1905; Schiemenz, 1957; Owen, 1985). A similar condition is found in *Panorpa* (Heddergott, 1938) and *Ctenocephalus* (Wenk, 1953). Christophers (1960) described a bipartite muscle for *Aedes*, and a similar condition is present in *Wilhelmia* (Wenk, 1962) and *Nannochorista* (Beutel and Baum, 2008). It is composed of three subcomponents in *Caurinus* and is not enlarged (Beutel et al., 2008). Tokunaga (1935) described a large muscle with three subcomponents for *Nymphomyia* (Tokunaga, 1935, Figs. 1, 7).

22. *Mm. frontobuccalis anterior/posterior* (*Mm. 45/46*): (0) both present; (1) one muscle; (2) absent

*M. frontobuccalis posterior* (*M. 46*) is the only dorsal precerebral dilator in *Deuterophlebia* (46, Figs. 3, 5), *Tipula*, *Limonia* (Schneeberg and Beutel, in press) and representatives of Bombyliidae (Szucsich and Krenn, 2000). Both precerebral dorsal dilators are present in *Edwardsina*, *Trichocera* (Schneeberg and Beutel, in press) and *Mischoderus*, in representatives of Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985), and in *Tabanus* (Bonhag, 1951), *Caurinus* (Beutel et al., 2008) and *Ctenocephalus* (Wenk, 1953). *M. frontobuccalis anterior* is present in *Biblio* according to Skanda (2008). Both muscles are absent in *Eristalis* (Schiemenz, 1957), *Nannochorista* (Beutel and Baum, 2008) and *Panorpa* (Heddergott, 1938). A series of bundles is present between the frontal ganglion and the brain in *Nymphomyia* (Tokunaga, 1935). It probably comprises both muscles.

23. *M. tentoriobuccalis anterior* (*M. 48*): (0) present; (1) absent

The muscle is absent in *Deuterophlebia*, *Nymphomyia* (Tokunaga, 1935), *Eristalis* (Schiemenz, 1957), *Nannochorista* (Beutel and Baum, 2008), *Panorpa* (Heddergott, 1938), *Caurinus* (Beutel et al., 2008) and *Ctenocephalus* (Wenk, 1953). Szucsich and Krenn (2000) described a protractor of the fulcrum in *Hemipenthes* and *Bombylius*, which is possibly homologous with *M. 48* (*mgc*). A muscle that extends from the ventrolateral wall of the anterior pharynx, below the frontal ganglion, to the circumocular ridge is present in *Edwardsina*. This muscle is probably homologous with *M. tentoriobuccalis anterior*. *M. 48* is present in *Tipula*, *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), *Tabanus* (Bonhag, 1951), and in representatives of Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985).

24. Postcerebral pharyngeal pump (*Mm. 51/52*): (0) present; (1) absent

A strongly developed postpharyngeal pump is present in *Deuterophlebia* (51, 52, Fig. 4C), *Edwardsina*, *Nymphomyia* (Tokunaga, 1935) and also in *Tipula*, *Limonia*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008), *Wilhelmia* (Wenk, 1962), *Mischoderus*, *Tabanus* (Bonhag, 1951), and generally occurs in Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985). It is also present in *Nannochorista* (Beutel and Baum, 2008), *Panorpa* (Heddergott, 1938)

and Siphonaptera (Wenk, 1953), but is missing in *Eristalis* (Schiemenz, 1957) and representatives of Bombyliidae (Szucsich and Krenn, 2000). A bipartite *M. verticopharyngalis* and a moderately developed *M. tentoriopharyngalis* posterior are present in *Caurinus* (Beutel et al., 2008). The presence is a potential synapomorphy of Diptera, Nannochoristidae and Siphonaptera. The postcerebral pharyngeal pump is functionally replaced by the labro-epipharyngeal pump in Cyclorrhapha (Gouin, 1949).

25. *M. anularis stomodaei* (M. 68): (0) enclosing the lateral and ventral wall of the posterior pharynx; (1) comprising a ring muscle

Complete ring muscles are restricted to the oesophagus in *Deuterophlebia* (68, Fig. 3) and *Edwardsina*, and also in *Tipula*, *Trichocera* (Schneeberg and Beutel, in press) and *Nannochorista* (Beutel and Baum, 2008). The bundles enclose only the ventral and lateral walls of the posterior pharynx and of the anterior pharynx in *Edwardsina*. Postpharyngeal ring muscles are present in *Limonia* (Schneeberg and Beutel, in press), *Aedes* (Christophers, 1960), *Wilhelmia* (Wenk, 1962), *Tabanus* (Bonhag, 1951), *Nymphomyia* (Tokunaga, 1935) and *Panorpa* (Heddergott, 1938).

26. *M. hypopharyngosalivariialis* (M. 37): (0) present; (1) absent

A salivary pump is absent in *Deuterophlebia* and *Limonia* (Schneeberg and Beutel, in press). *M. hypopharyngosalivariialis* is present in *Edwardsina*, *Tipula*, *Trichocera* (Schneeberg and Beutel, in press), *Biblio* (Skanda, 2008) and *Mischoderus*. It connects the ventral wall of hypopharynx with the dorsal wall of the salivary duct. An extrinsic dilator is also present in adults of Culicidae (Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985), Simuliidae (Wenk, 1962), Tabanidae (Bonhag, 1951), Syrphidae (Schiemenz, 1957), Bombyliidae (Szucsich and Krenn, 2000), and *Nannochorista* (Beutel and Baum, 2008). *M. hypopharyngosalivariialis* is bipartite in *Nymphomyia*. It originates on the ventral membrane of the basipharynx and inserts on the salivarium (Tokunaga, 1935, Fig. 1). A typical *M. hypopharyngosalivariialis* is absent in Mecoptera (excluding Nannochoristidae, e.g. Beutel and Baum, 2008), but a strong intrinsic muscle of the wall of the salivary duct (Heddergott, 1938; Beutel et al., 2008) may be a derivative of this muscle.

#### 4. Discussion

Deuterophlebiidae are an extremely specialised group of Diptera (e.g., Kennedy, 1958; Courtney, 1991b). The monophyly of the family is well supported by features of the larvae, pupae and characters of the postcephalic body of adults (see Courtney, 1991a, chars. 46–57), and also by characters of the adult head described here. A conspicuous potential autapomorphy is the widely separated insertion of the antennae on the dorsal side of the head (Fig. 1A). This condition, which is very unusual for Diptera, is found in some Bibionidae (Hoyt, 1952; Skanda, 2008), but is absent in other groups of Bibionomorpha such as Sciaridae and Mycetophilidae (Peterson, 1916), and also in Axymyiidae (Wihlm, 2009, Figs. 3.43, 3.44). An anterior extrinsic antennal muscle (M. 1) with multiple origins (1, Fig. 4D) and the loss of *M. hypopharyngosalivariialis* (M. 37) are further potential autapomorphies of Deuterophlebiidae. *M. hypopharyngosalivariialis* (M. 37) is also missing in *Limonia* (Schneeberg and Beutel, in press), but considering the presence in other members of Tipuloidea this is certainly due to parallel evolution. Adults of Deuterophlebiidae display an exceptional degree of muscle reduction in the head. They possess only eight muscles (excluding intrinsic muscles of the fore gut), five of which belong to the antenna. Eleven head muscles are present in

adults of Nymphomyiidae (Tokunaga, 1935), which are also characterised greatly reduced head structures (see below), 19 in Nannochoristidae (Nannomecoptera) (Beutel and Baum, 2008), and 40 in *Osmylus* Latreille, 1802 (Neuroptera) (Beutel et al., in press), a number that may come close to the holometabolan groundplan.

Despite the high degree of specialisation and numerous derived features, a basal position of Deuterophlebiidae within Diptera was suggested by Bertone et al. (2008) based on analyses of multiple nuclear genes. This hypothesis is not supported by characters of the adult head. There is not a single retained plesiomorphic feature in Deuterophlebiidae with a corresponding apomorphic condition in the other dipteran lineages.

Interestingly, the results of our study suggest close phylogenetic affinities of Deuterophlebiidae with Nymphomyiidae, another family considered as a candidate for the most basal lineage of Diptera (Rohdendorf, 1974; Hackman and Väisänen, 1982; Bertone et al., 2008 [analyses of reduced data set]). Like Deuterophlebiidae, Nymphomyiidae are a highly specialised group of Diptera. The adults are also very small (<2 mm) and the larvae live in a similar type of habitat (Courtney, 1991a, 1994b). Nymphomyiids are doubtlessly a monophyletic group (see Courtney, 1991a, chars. 14–32), with autapomorphies such as the loss of the unpaired ocellus, the shift of the paired lateral ocelli posterior to the compound eyes (Tokunaga, 1935; Courtney, 1994b), the complete reduction of the tentorium (parallel loss in Tipulidae; Schneeberg and Beutel, in press), and the correlated shift of the origin of all extrinsic antennal muscles to the head capsule.

Adults of Deuterophlebiidae and Nymphomyiidae display an entire array of shared derived features of the adult head. In both groups and differing from nearly all other groups of Diptera (e.g., Hennig, 1973), the labrum is completely reduced. The maxillae, which are usually modified but distinctly developed, are also completely absent. The same applies to the labium, which usually plays a vital role in the food uptake. The loss of these structures (also missing in some chironomids with short-lived adults; Neumann, 1976) is associated with a dramatic degree of muscle reduction in both groups (see above). All labral, labial, mandibular and maxillary muscles are absent. Another potential synapomorphy is the loss of the anterior ventral dilator of the pharynx (M. 48). This muscle is also missing in *Eristalis* and in members of Bombyliidae (and some other groups), but is certainly present in the groundplan of Diptera (e.g., Heddergott, 1938; Wenk, 1953; Schiemenz, 1957; Szucsich and Krenn, 2000; Beutel and Baum, 2008; Beutel et al., 2008). Another feature supporting a clade Deuterophlebiidae + Nymphomyiidae is the elongation of the ultimate segment of the antenna of males. However, as pointed out above, the conditions in both groups differ distinctly. The antenna of male deuterophlebiids is extremely long (ca. 9.5 mm, body length ca. 2 mm; Pennak, 1951: Fig. 1), with an extremely elongated ultimate flagellomere (see Courtney, 1994a: char. 11), whereas the female antenna is distinctly shorter and lacking an elongated apical segment. In nymphomyiids only the apical element is elongated in males and females, but the antenna as a whole is short (Courtney, 1994b).

Some other affinities concerning the morphology and life history were pointed out by Courtney (1991a). In both families females shed their wings after or during oviposition, the femur and tibia are subdivided by a membranous zone, all abdominal spiracles are vestigial, and the spermatheca is absent (see Courtney, 1991a: chars. 29–32). These similarities were interpreted as convergences, related to an independent evolution of a short-lived adult stage (Courtney, 1991a).

In contrast to the potential sister group relationship between Nymphomyiidae and Deuterophlebiidae discussed here, Hennig (1973), Wood and Borkent (1989), Courtney (1990b, 1991a), and

Oosterbroek and Courtney (1995) proposed a clade Blephariceroidae, with Deuterophlebiidae as sister group of Blephariceridae. Thirteen synapomorphies for these two groups were suggested by Courtney (1991a). None of them was a feature of the adult head, and in this study we found only one character supporting Blephariceroidae, the origin of *M. tentorioscapalis* posterior (*M. 2*) on the vertex (*Deuterophlebia* and *Edwardsina*). The monophyly of Blephariceroidae would imply that an elongated apical flagellomere, a unique suite of reductional features of the adult head, larval features such as abdominal prolegs (in later instars) and multi-toothed apical mandibular combs (also present in some Blephariceridae; Oosterbroek and Courtney, 1995), miniaturisation, and very specific life habits have evolved independently in Deuterophlebiidae and Nymphomyiidae.

A clade Blephariceromorpha comprising Deuterophlebiidae, Nymphomyiidae and Blephariceridae (e.g., Courtney, 1991a; Oosterbroek and Courtney, 1995; Yeates and Wiegmann, 1999) is suggested by the specific habitat preference and associated features. The immature stages of all three families are aquatic and display features likely associated with life in cool, fast-flowing mountain streams, such as the presence of abdominal prolegs (only in first-instar larvae of Blephariceridae; Oosterbroek and Courtney, 1995). Blephariceromorpha is not well supported by features of the adult head. The missing frontoclypeal suture is a potential synapomorphy of the three families, but the frontoclypeal suture is also missing in members of Simuliidae (Wenk, 1962), and also in *Ctenocephalus* (Wenk, 1953). The absence of the clypeolabral suture in Blephariceromorpha is a questionable argument, as the labrum is entirely reduced in deuterophlebiids and nymphomyiids, and the suture is also absent in Siphonaptera (e.g., Wenk, 1953) and most groups of Mecoptera (Heddergott, 1938; Hepburn, 1969). Another potential autapomorphy of Blephariceromorpha is the origin of *M. tentorioscapalis* posterior (*M. 2*) on the head capsule. However, the muscle originates on the vertex in *Deuterophlebia* and *Edwardsina* (see above) and in *Nymphomyia* on the genal region (Tokunaga, 1935), as is also the case in *Tipula* (Schneeberg and Beutel, in press). The origin of the muscle has apparently shifted several times. It lies on the frons in *Hemipenthes* (Szucsich and Krenn, 2000) and on the circumocular ridge in *Eristalis* (Schiemenz, 1957: Fig. 41 [M. orb.-sc.]).

The crucial question of the sister group of the remaining Diptera remains unanswered. Features of the adult head do not support a basal position of either Nymphomyiidae or Deuterophlebiidae (or both), and characters suggesting other “ancestral candidates” such as Culicomorpha (Oosterbroek and Courtney, 1995; Yeates and Wiegmann, 1999, 2005; Yeates et al., 2007) or Tipulomorpha (Hennig, 1973; Wood and Borkent, 1989; Beutel and Gorb, 2001; Blagoderov et al., 2007) where not found. Likewise, the systematic affinities of Deuterophlebiidae must still be considered as uncertain. A placement in a clade Blephariceromorpha as sister group of Blephariceridae (e.g., Oosterbroek and Courtney, 1995) would imply that a considerable number of unusual morphological features and a very similar aquatic life style have evolved independently in Deuterophlebiidae and Nymphomyiidae. It is evident that analyses of a comprehensive data set for a representative sample of Diptera are required to solve these questions. Molecular data acquired in the Flytree project (NSF, see Bertone et al., 2008) may provide insights into the relationships of some taxa. Unfortunately, morphological data are still missing for many potential key taxa such as Tanyderidae, Ptychopteridae, and Axymyiidae. The documentation and evaluation of morphological data for adults and immature stages, and analyses of extensive combined data sets will likely lead to a solid reconstruction of basal dipteran relationships in the near future.

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## 3.2 Study II

**Schneeberg K**, Polilov A, Harris MO, Beutel RG. 2013.

The adult head morphology of the Hessian fly *Mayetiola destructor* (Diptera, Cecidomyiidae). Journal of Morphology 274: 1299-1311.

### Abstract

The adult head of the Hessian fly *Mayetiola destructor* was examined and described in detail. Morphological features are evaluated with respect to phylogenetic implications and possible effects of miniaturisation. Preserved groundplan features of Diptera are the orthognathous orientation of the head, the vestiture of small microtrichia (possible autapomorphy), filiform antennae inserted frontally between the compound eyes, the presence of a clypeolabral muscle (possible autapomorphy), the presence of labellae (autapomorphy), and the presence of only one premental retractor. Potential synapomorphies of the groups assigned to Bibionomorpha are the origin of *M. tentorioscapalis* medialis on the frons and the loss of *M. craniolacinalis*. Further apomorphies of Cecidomyiidae identified in *Mayetiola* are the unusually massive anterior tentorial arm, the absence of the labro-epipharyngeal food channel, the absence of the lacinia, and the presence of antennal sensilla connected by a seta, a feature not known from any other group of Diptera. The very large size of the compound eyes (in relation to the entire head surface) and the complete loss of ocelli are possible effects of miniaturisation. The large size of the brain (in relation to the cephalic lumen), the unusual shape of the optic lobes, and the absence of the frontal ganglion as a separate structure are probably also linked with size reduction.

### Significance in the present thesis

*Mayetiola destructor* is a major pest insect. The head structures reflect key features in the context of the reproductive strategy of males and females. The internal and external morphology of the cecidomyiid head was largely unknown. The results are compared with conditions found in other groups of Bibionomorpha.

### Own contribution

60%

# The Adult Head Morphology of the Hessian Fly *Mayetiola destructor* (Diptera, Cecidomyiidae)

Katharina Schneeberg,<sup>1\*</sup> Alexey Polilov,<sup>2</sup> Marion O. Harris,<sup>3</sup> and Rolf G. Beutel<sup>1</sup>

<sup>1</sup>Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, Jena 07743, Germany

<sup>2</sup>Department of Entomology, Biological Faculty, Lomonosov Moscow State University, Leninskie gory 1-12, Moscow, Russia

<sup>3</sup>Department of Entomology, North Dakota State University, NDSU Dept. 7650, Fargo, North Dakota 58108-6050

**ABSTRACT** The adult head of the Hessian fly *Mayetiola destructor* was examined and described in detail. Morphological features are evaluated with respect to phylogenetic implications and possible effects of miniaturisation. Preserved groundplan features of Diptera are the orthognathous orientation of the head, the vestiture of small microtrichia (possible autapomorphy), filiform antennae inserted frontally between the compound eyes, the presence of a clypeolabral muscle (possible autapomorphy), the presence of labellae (autapomorphy), and the presence of only one premental retractor. Potential synapomorphies of the groups assigned to Bibionomorpha are the origin of *M. tentorioscapalis medialis* on the frons and the loss of *M. craniolacinalis*. Further apomorphies of Cecidomyiidae identified in *Mayetiola* are the unusually massive anterior tentorial arm, the absence of the labro-epipharyngeal food channel, the absence of the lacinia, and the presence of antennal sensilla connected by a seta, a feature not known from any other group of Diptera. The very large size of the compound eyes (in relation to the entire head surface) and the complete loss of ocelli are possible effects of miniaturization. The large size of the brain (in relation to the cephalic lumen), the unusual shape of the optic lobes, and the absence of the frontal ganglion as a separate structure are probably also linked with size reduction. *J. Morphol.* 274:1299–1311, 2013. © 2013 Wiley Periodicals, Inc.

**KEY WORDS:** *Mayetiola*; Diptera; Cecidomyiidae; head morphology; miniaturization

## INTRODUCTION

The Hessian fly, *Mayetiola destructor* Say, 1817 is a major pest of wheat (*Triticum aestivum* L.) throughout the cereal-growing regions of Europe, North Africa, and North America (Harris et al., 2003). Its common name was given during a devastating pest outbreak that threatened North America wheat production in the years following the Revolutionary War (Hunter, 2001). The newly independent colonists chose a name that invoked the memory of the despised mercenary soldiers from the German state of Hesse that fought for the British during the Revolutionary War (Hunter, 2001).

*M. destructor* is a member of the family Cecidomyiidae (lower Diptera), which contain 6.131

described species (Gagné, 2010). It is doubtlessly a monophyletic group and assigned to Bibionomorpha (Hennig, 1973; Oosterbroek and Courtney, 1995; Bertone et al., 2008; Wiegmann et al., 2011; Lambkin et al., 2013). However, the monophyly and composition of this lineage is still disputed (e.g., Hennig, 1973; Wood and Borkent, 1989; Oosterbroek and Courtney, 1995; Michelsen, 1996), and the precise position of Cecidomyiidae within Bibionomorpha is not clarified yet. Hennig (1973) suggested a sistergroup relationship between Cecidomyiidae and Scatopsidae + Hyperoscelididae. A close relationship to the Sciaridae was suggested by Wood and Borkent (1989), Blaschke-Berthold (1994), Oosterbroek and Courtney (1995), and Amorim and Rindal (2007). Molecular data suggest a close relationship to Lyrgistorrhinidae (Wiegmann et al., 2011) or to a clade comprising Sciaridae and Mycetophilidae (Bertone et al., 2008).

The family Cecidomyiidae is evolutionarily successful relative to other families in the infraorder Bibionomorpha, many of which are considered to be moribund (Mamaev, 1968; Gagné, 1989). The success of the Cecidomyiidae is attributed to the innovation of feeding on living plants, which has arisen independently several times. Taxa containing plant-feeding species are extremely diverse and rich in species, contrasting with taxa having the ancestral

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\*Correspondence to: Katharina Schneeberg, Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, Jena 07743, Germany. E-mail: Katharina.Schneeberg@gmx.de

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habit of feeding on dead or decaying organic material or the fungi that are associated with such material.

The gall-making habit of cecidomyiids also has contributed to adaptive radiation (Rohfritsch, 1992). After encasing from the egg, the tiny larva (<0.5 mm) attacks undifferentiated plant cells found in zones of active growth (e.g., buds, expanding leaves). Effectors produced in the larva's extremely large salivary glands (Stuart and Hatchett, 1987; Stuart et al., 2012), in conjunction with specialized attack behavior (Rohfritsch, 1992), allow the larva to manipulate the developmental pathway of plant cells, creating a "nutritive" tissue that provides an enriched diet for the larva at the expense of plant growth (Harris et al., 2012). Often associated with the microscopic nutritive tissue is a macroscopic gall, which comes in a wide variety of forms (Gagné, 1989). The manipulation that results in gall formation is assumed to be the culmination of a highly evolved relationship with host plants (Stone and Schönrogge, 2003). Most plant feeding cecidomyiids are monophagous, feeding on one or only a small number of plant species.

Life history theory proposes that insects have limited resources, and therefore, must focus on a limited number of traits that make the greatest contributions to fitness (Price et al., 2011). Life history strategies of insects are extremely variable, reflecting the organism's environment as well as phylogenetic constraints. Like all insects with complete metamorphosis, cecidomyiids allocate functions of growth and reproduction to the larval and adult stages, respectively. What makes cecidomyiids different from many other insects with complete metamorphosis is how little time they have for reproduction, typically only 1–2 days (Barnes, 1956; Gagné, 1989). As well as having little time, adult cecidomyiids are constrained by their small size (2–3 mm long), delicate body, and simple wings (Gagné, 1989). Having such constraints, it is generally assumed that behavioural adaptations associated with a longer adult life (e.g., learning and predator avoidance) are absent (Roseenheim et al., 2008), along with the structures and physiology that support these behavioral adaptations.

The head is an essential feature of adult insects, bearing a number of important sensory organs as well as containing the brain, which organizes sensory inputs and makes reproductive decisions. We investigated the adult head of the Hessian fly *M. destructor*, arguably the cecidomyiid that provides the most complete picture of adult reproductive behavior (Harris et al., 2003). The behavior that makes the greatest contribution to male fitness is the ability to rapidly find virgin females, this depending on olfactory perception by the antennae of a volatile sex pheromone produced by virgin females (Andersson et al., 2009). In addition to release of the sex pheromone, the behavioral trait that makes the greatest contribution to female fitness is the ability to find suitable hosts for as

many as 450 eggs, this depending on olfaction and vision (Harris et al., 1993). We hypothesized that morphology of the adult Hessian fly head reflects key features of the reproductive strategy of males and females, as well as the constraints that come from short lifespan, small size, and evolutionary history. Comparisons were made with species belonging to evolutionarily less successful families within the infraorder Bibionomorpha.

## MATERIALS AND METHODS

### Material Examined

Cecidomyiidae: *Mayetiola destructor* Say, 1817 (70% Ethanol; USA, North Dakota State University, Department of Entomology, in culture)

Scatopisidae: *Coboldia fuscipes* Meigen, 1930 (95% Ethanol; USA, precise location unknown)

Sciaridae: *Spathobdella falcifera* Lengersdorf, 1933 (70% Ethanol; Germany, Jena)

Bibionidae: *Bibio marci* Linnaeus, 1758 (70% Ethanol; Germany, Jena)

### Methods

**Histology.** An Adult male and an female were fixed in 70% ethanol. They were embedded in Araldit M, cut at 1  $\mu\text{m}$  with a Leica microtome RM2255 (Leica Biosystems, Nussloch, Germany), and stained with toluidine blue. The serial sections were photographed with an AxioCam digital camera on a Zeiss Axioskop 40 (Carl Zeiss Microscopy, Göttingen, Germany).

**Three-dimensional reconstruction (3D).** A cross-section series was used for 3D-reconstructions. We used every section and the images have a resolution of 3 pixels per  $\mu\text{m}$ . Based on the aligned image stacks, structures of adults were reconstructed with Bitplane Imaris (Bitplane AG, Zürich, Suisse). The data files were then transferred to Autodesk Maya (Autodesk, San Rafael), in order to use the smoothing function, the specific display, and rendering options of this software.

**Scanning electron microscopy (SEM).** After cleaning with ultrasonic sound, the specimens (3 males, 3 females) were critical point dried (Hitachi HCP-1; Hitachi, Tokyo, Japan) and coated with gold (Hitachi IB-3; Hitachi, Tokyo, Japan). Pictures were taken with a Jeol JSM-6380 (JEOL, Tokyo, Japan).

## RESULTS

### Head Capsule

The orthognathous head is well sclerotized in both sexes and all parts are densely covered with short microtrichia. Longer setae are also present. They are widely spaced on the clypeus and vertex (Fig. 1). The head is oval in shape in frontal and lateral view. The compound eyes are very large and occupy a large proportion of the surface of the head capsule (ce, Figs. 1A,C). Dorsally, above the insertion of the antenna, they are connected to each other by a transverse eye bridge (eb, Figs. 1B and 2). On their mesal side they are delimited by an internal circumocular ridge. The conspicuous antennae insert on the frontal side of the head capsule, directly adjacent to the compound eyes (Figs. 1B,D). The articulatory fossae are closely adjacent. In males, they reach each other medially and laterally the margin of the compound eyes (Fig. 1B). In females, they are separated medially

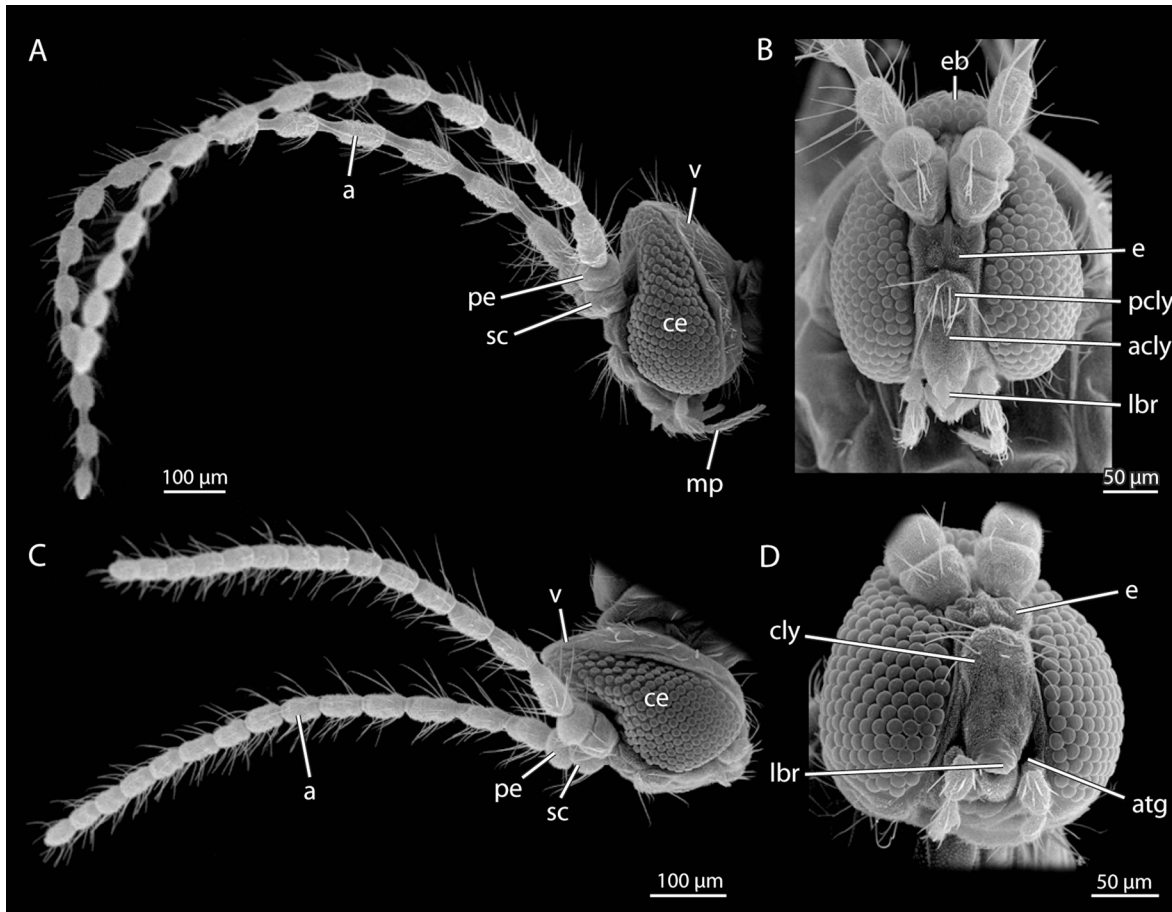


Fig. 1. *M. destructor*, head, SEM images. (A) Male, lateral view. (B) Male, frontal view. (C) Female, lateral view. (D) Female, frontal view. a, antenna; acly, anteclypeus; atg, anterior tentorial grooves; ce, compound eye; cly, clypeus; e, elevation on the prefrons; eb, eye bridge; lbr, labrum; mp, maxillary palp; pcl, postclypeus; pe, pedicellus; sc, scapus; v, vertex.

by a small bar (Fig. 1D). Beneath the antennal insertion area, two small elevations of the cuticle are recognizable on the prefrons of both sexes (e, Figs. 1B,D). Ocelli are absent. The coronal and frontal sutures are missing, whereas the frontal apodeme is well developed (fap, Figs. 2 and 3B).

Frons and clypeus are separated by an indistinct frontoclypeal ridge. A subdivision into an anterior anteclypeus and a posterior postclypeus is not recognizable in females, whereas this region is distinctly divided in males (acly, pcl, Fig. 1B). The labrum is separated from the clypeus by an indistinct clypeolabral fold in both sexes.

The genal region can only be defined by its position, as the frontal suture and the subgenal- and occipital ridges are absent. Anteriorly, it reaches the frons and the clypeus and posteriorly, the compound eyes. The region occupied by the frons and vertex is subdivided by the dorsal eye bridge. The frontal region is subdivided into a preantennal and postantennal area (prefrons, postfrons) by the antennal fossae. A coronal suture medially divid-

ing the vertex is missing. The lateral part of the posterior head region corresponds with the gena and the dorsal part with the vertex.

Anterior tentorial grooves are only visible in females. They have a very unusual position laterad of the anterior clypeal margin (atg, Fig. 1D). Posterior tentorial grooves are always absent. The head capsule is flattened posterior to the compound eyes (Figs. 1A,C). The occipital and postoccipital region are not recognizable as separate areas. The foramen occipitale is large in relation to the size of the head capsule. A postgenal bridge or any other type of partial closure is lacking.

### Tentorium

The tentorium is a short, straight tube arising from the anterior tentorial grooves. It is vestigial and not connected to the posterior head region (t, Fig. 4). Posterior tentorial arms and grooves are missing and dorsal arms and the tentorial bridge are also absent.



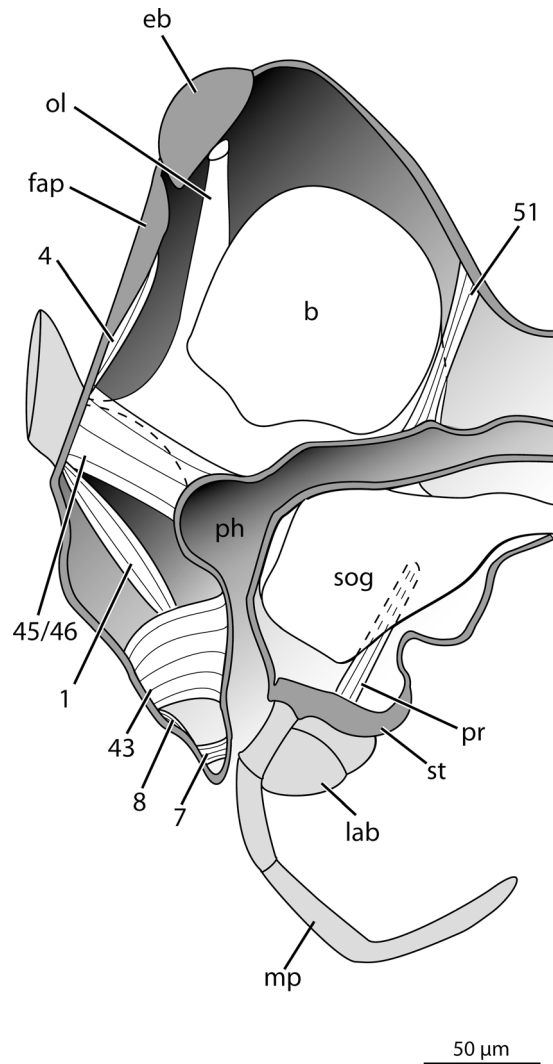


Fig. 2. *M. destructor*, head, male, sagittal section. b, brain; eb, eye bridge; fap, frontal apodeme; lab, labella; mp, maxillary palp; ol, optic lobes; ph, pharynx; pr, premental retractor; sog, subesophageal ganglion; st, stipes; 1, *M. tentorioscapalis* anterior; 4, *M. tentorioscapalis* medialis; 7, *M. labroepipharyngalis*; 8, *M. clypeolabralis*; 43, *M. clypeopalatalis*; 45/46, *Mm. frontobuccalis* anterior/posterior; 51, *M. verticopharyngalis*.

### Labrum

The labrum is completely membranous. It is connected with the clypeus by an internal clypeolabral membrane. The dorsal wall is tapering towards the apex (lbr, Fig. 5B). The ventral wall, that is, the distal part of the epipharynx, is weakly sclerotized and slightly bent upward.

**Musculature (7, 8).** M. 7: *M. labroepipharyngalis*: O (=origin)- dorsal labral wall; I (=insertion)- anterior epipharynx; F (=function)- levator of the anterior epipharynx. M. 8: *M. clypeolabralis* (of Schiemenz, 1957): O- anterior clypeal region; I- dorsal wall of labrum and hind margin of labrum; F- levator of the labrum (Fig. 2).

### Antenna

The well developed antenna is composed of the scapus, the pedicellus, and 14 flagellomeres (a, Figs. 1A,C). They insert frontally between the compound eyes (see above). The scapus and pedicellus are globular and the largest segments (sc, pe, Figs. 1A,C). The flagellum is narrower. All flagellomeres except for the apical one consists of a wider proximal part and a narrow distal portion ("neck" after Harris, 1964) (Figs. 1A and 6B). The proximal part is densely covered with small microtrichia and more widely spaced longer setae, whereas the distal part is glabrous (Fig. 6B). Both subdivisions are approximately equal in length in males, whereas in females the distal part is distinctly shorter and only visible on the first five flagellomeres (Figs. 1C and 6A). In both sexes, a bifurcate sensillum circumfila is connected by a seta, which extends around the proximal part of the flagellomere (Fig. 6C). Additionally, some sensilla trichodea are surrounded by a dome-like structure with small teeth on their opening (Figs. 6C,D). On the antennae of males, they are more numerous than on those of the females.

**Musculature (1, 2, 4).** M. 1: *M. tentorioscapalis* anterior: long and slender, without tendon; O- circumocular ridge; I- anterolateral margin of the scapus; F- depressor of the antenna. M. 2: *M. tentorioscapalis* posterior: long and slender; O- circumocular ridge, ventrad M. 1; I- posterior margin of the scapus; F- levator, antagonist of *M. tentorioscapalis* anterior. M. 3: *M. tentorioscapalis* lateralis: absent. M. 4: *M. tentorioscapalis* medialis: shorter than the other extrinsic antennal muscles; O- frontal apodeme; I- posteromesal margin of the scapus; F- rotation of the antenna toward the median line, together with *M. tentorioscapalis* posterior. M. 5: *M. scapopedicellaris* lateralis: strongly developed; O- ventrolateral wall of the scapus; I- basal lateral margin of the pedicellus; F- extensor of the pedicellus and flagellum. M. 6: *M. scapopedicellaris* medialis: divided into two subunits; M. 6a: O- ventromesal wall of the scapus; I- mesally on the basal margin of the pedicellus; F- flexion of the pedicellus and flagellum; M. 6b: larger part; O- frontomesal wall of the scapus; I- frontomesally on the basal margin of the pedicellus; F- flexion of the pedicellus and flagellum (Figs. 2,4A,B, 7, and 3B-D).

### Mandible

Absent.

### Maxilla

The rod-shaped stipites are fused with the cardines, and these are fused with each other on the ventral side of the head, approximately at the level of the anterior margin of the compound eyes (st, Fig. 2). The long maxillary palp articulate with the distal part of the stipites (mp, Fig 2).

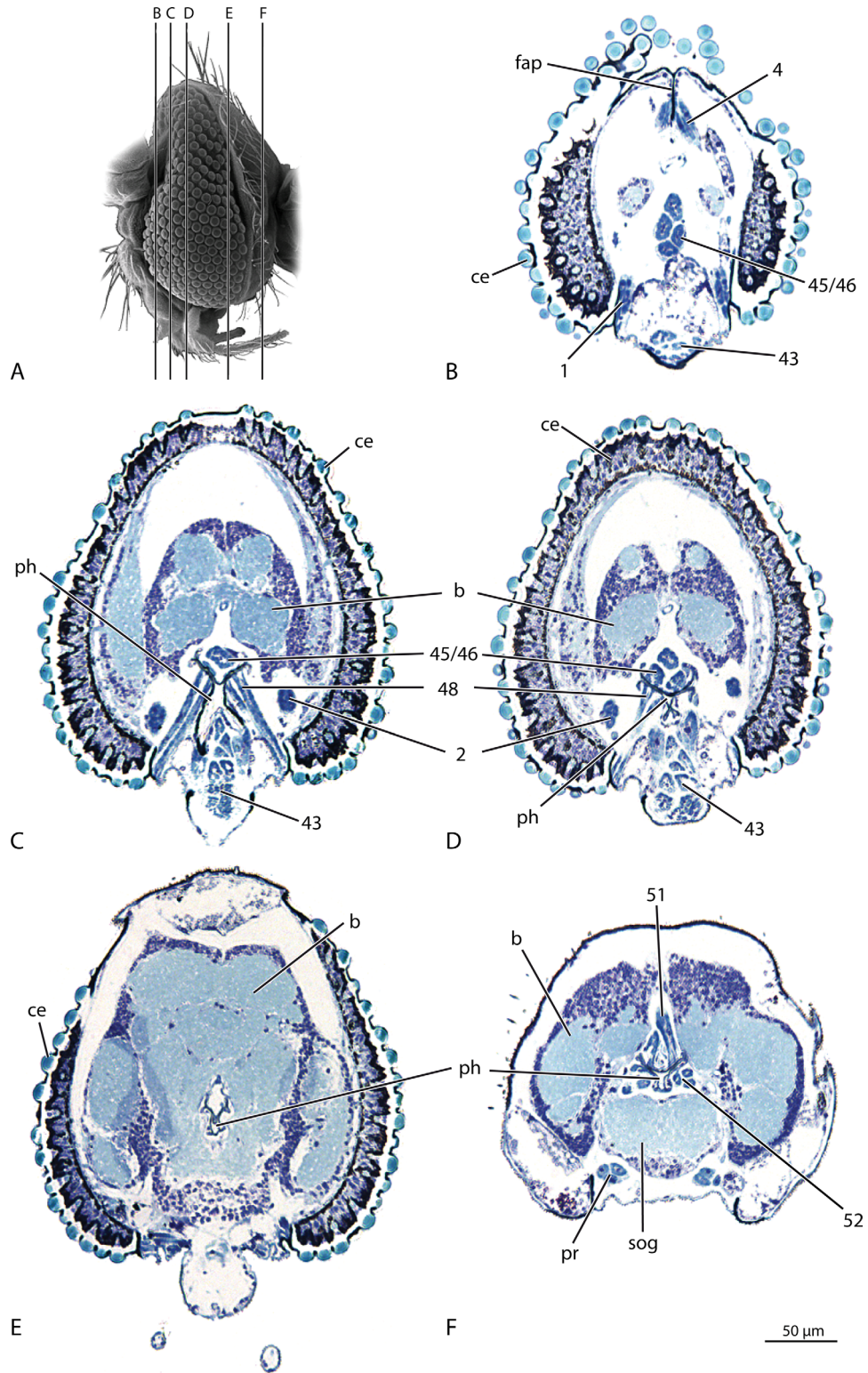


Fig. 3. *M. destructor*, head, male, cross sections. (A) Overview of the sections. (B–F) Cross sections. b, brain; ce, compound eye; fap, frontal apodeme; ph, pharynx; pr, premental retractor; sog, suboesophageal ganglion; 1, *M. tentorioscapalis anterior*; 2, *M. tentorioscapalis posterior*; 4, *M. tentorioscapalis medialis*; 43, *M. clypeopalatalis*; 45/46, *Mm. frontobuccalis anterior/posterior*; 48, *M. tentoriobuccalis anterior*; 51, *M. verticopharyngalis*; 52, *M. tentoriopharyngalis*.

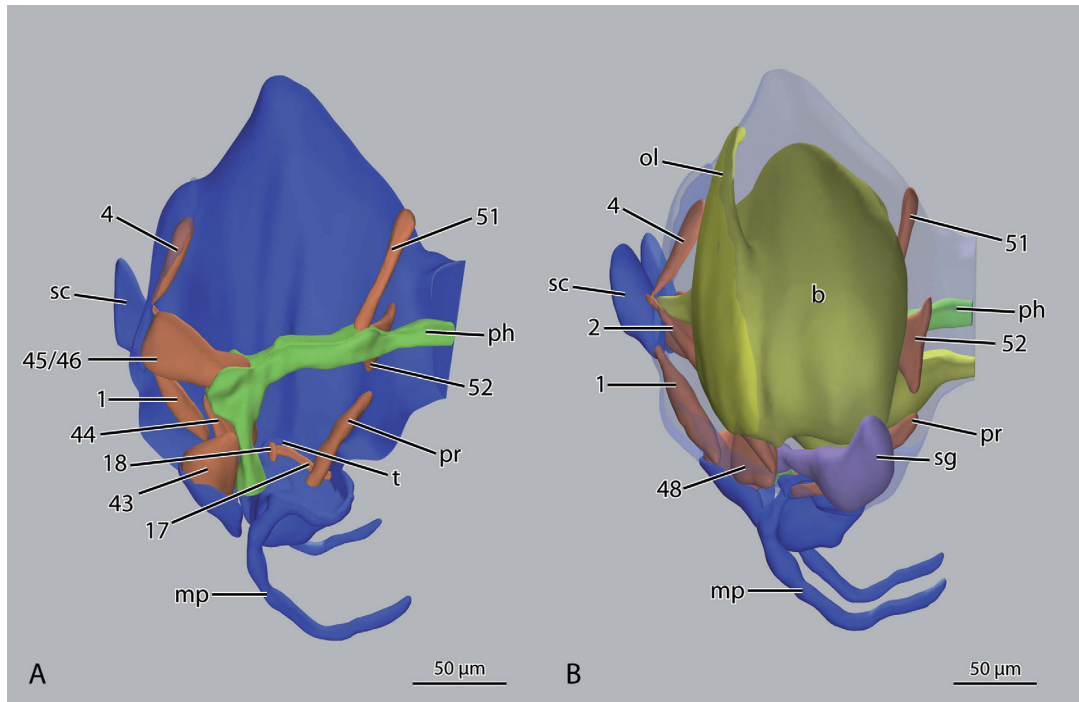


Fig. 4. *M. destructor*, head, male, 3D-reconstruction. Green, gut; orange, musculature; blue, skeletal elements; yellow, nervous system; violet, salivarium. (A) Sagittal section. (B) Lateral view (head capsule transparent). b, brain; mp, maxillary palp; ol, optic lobes; ph, pharynx; pr, premental retractor; sc, scapus; sg, salivary gland; t, tentorium; 1, *M. tentorioscapalis anterior*; 2, *M. tentorioscapalis posterior*; 4, *M. tentorioscapalis medialis*; 17, *M. tentoriocardinalis*; 18, *M. tentoriostipitalis*; 43, *M. clypeopalatalis*; 44, *M. clypeobuccalis*; 45/46, *Mm. frontobuccalis anterior/posterior*; 48, *M. tentoriobuccalis anterior*; 51, *M. verticopharyngalis*; 52, *M. tentoriopharyngalis*.

They are three-segmented and densely covered with short microtrichia (mp, Fig. 5). Segments 1 and 2 are approximately equal in size. The dorsal side of segment 2 is set with long and straight setae. The ultimate segment is two times as long as the previous one and slender (Fig. 5A). It is set with a widely spaced vestiture of curved setae. Lacinia and galea are absent.

**Musculature (17, 18).** *Mm.* 15/16: *Mm. craniocardinalis externus/internus*: absent. *M.* 17: *M. tentoriocardinalis*: long and slender; O- anterior part of the vestigial tentorium; I- cardo; F- protractor of the maxilla. *M.* 18: *M. tentoriostipitalis*: small muscle; O- mesal part of the vestigial tentorium; I- stipital ridge; F- stipital retractor. *M.* 19: *M. craniolacinalis*: absent. *M.* 20: *M. stipitolacinalis*: absent.

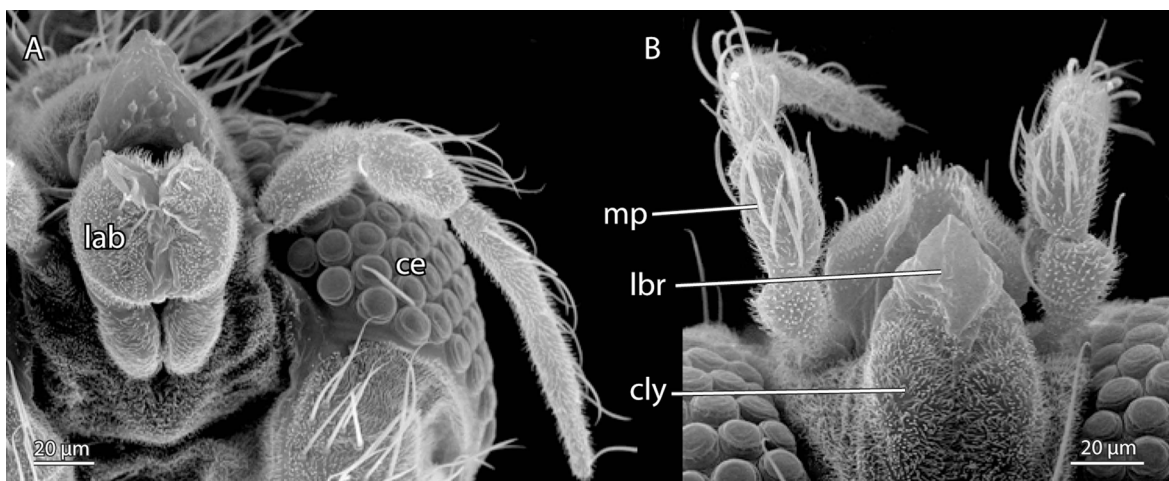


Fig. 5. *M. destructor*, head, mouthparts, male, SEM images. (A) Ventral view. (B) Dorsal view. ce, compound eyes; cly, clypeus; lab, labella; lbr, labrum; mp, maxillary palp.



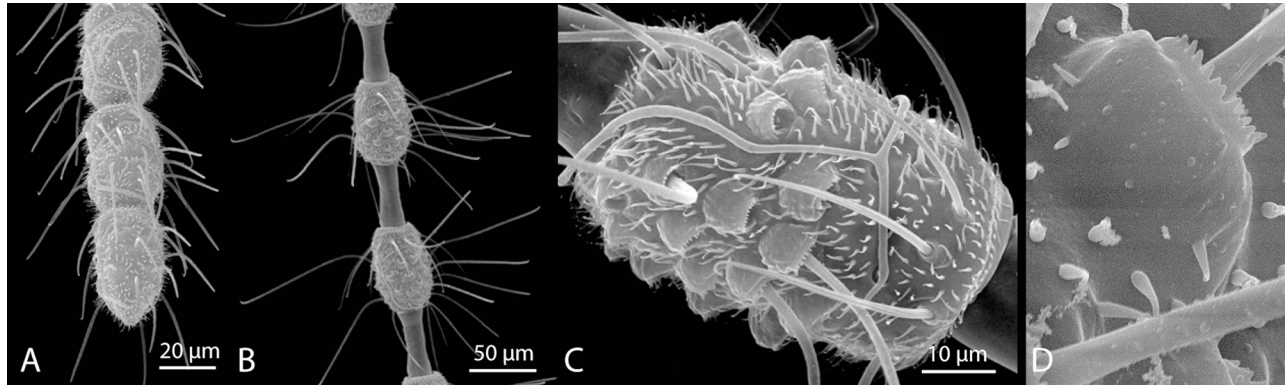


Fig. 6. *M. destructor*, head, antenna, SEM images. (A) Female. (B) Male. (C) Male, detail. (D) Male, dome-like structure.

M. 21: *M. stipitogalealis*: absent. M. 22: *M. stipitopalpalis externus*: absent. M. 23: *M. stipitopalpalis internus*: not identified. M. 24: *M. palpopalpalis maxillae primus*: not identified. Mm. 25–27: *Mm. palpopalpalis secundus/tertius/quartus*: not identified (Fig. 4A).

### Labium

The postmentum is completely reduced. The prementum is also greatly reduced except for a small process bearing the labellae. The labellae are two-segmented and incompletely fused with each other (lab, Figs. 5A and 2). The proximal segment is slightly less voluminous than the distal one. Both are completely covered with short microtrichia, except for the membranous inner surface of the second segment (Fig. 5A). The tip of the second

segment is equipped with setae. Paraglossae and glossae are absent.

**Musculature (pr).** Mm. 28/29/30: *M. submento-praementalis*, *Mm. tentoriopraementalis inferior/superior*: only one premental retractor is present; O- postgena; I- hind margin of the prementum; F- retractor of the prementum. M. 31: *M. praementoparaglossalis*: absent. M. 32: *M. praementoglossalis*: absent. Mm. 33/34: *Mm. praementopalpalis internus/externus*: both are merged or very indistinctly separated; O- prementum; I- lateral margin of palpomere 1. M. 35: *M. palpopalpalis labii primus*: not identified. M. 36: *M. palpopalpalis labii secundus*: absent (Figs. 2,4A,B, and 3F).

### Epi- and Hypopharynx

The distal part of the epipharynx is posteriorly continuous with the roof of the cibarium, which is

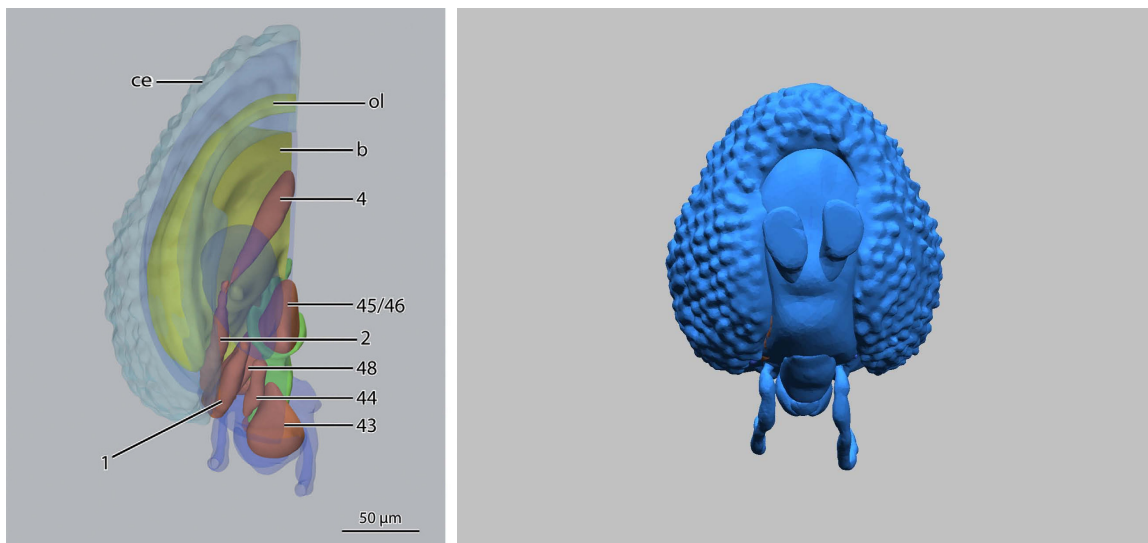


Fig. 7. *M. destructor*, head, male, 3D-reconstruction, frontal view. Green, gut; orange, musculature; blue, skeletal elements; yellow, nervous system; b, brain; ce, compound eye; ol, optic lobes; 1, *M. tentorioscapalis anterior*; 2, *M. tentorioscapalis posterior*; 4, *M. tentorioscapalis medialis*; 43, *M. clypeopalatalis*; 44, *M. clypeobuccalis*; 45/46, *Mm. frontobuccalis anterior/posterior*; 48, *M. tentoriobuccalis anterior*.

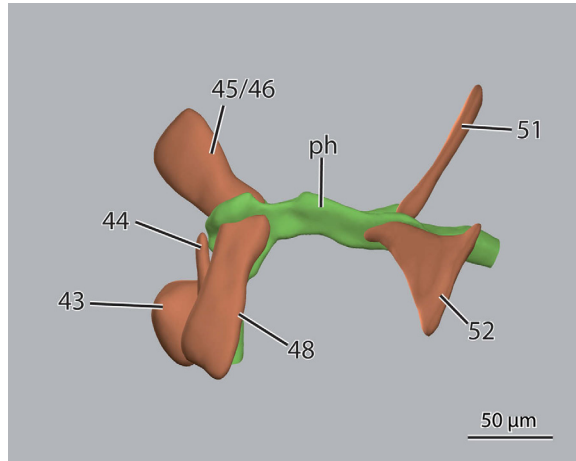


Fig. 8. *M. destructor*, head, male, 3D-reconstruction, digestive system, lateral view. Green, gut; orange, musculature. ph, pharynx; 43, *M. clypeopalatalis*; 44, *M. clypeobuccalis*; 45/46, *Mm. frontobuccalis anterior/posterior*; 48, *M. tentoriobuccalis anterior*; 51, *M. verticopharyngalis*; 52, *M. tentoriopharyngalis*.

completely sclerotized. In cross section the posterior epipharyngeal part (the palatum) appears concave. The U-shaped floor of the cibarium is formed by the proximal hypopharynx. It is sclerotized and laterally fused with the epipharynx, thus forming a closed prepharyngeal tube. The lumen of the cibarium is wide. Proximally, it is continuous with the pharynx (Fig. 2). The proximal hypopharynx forms two large lateral apophyses, which serve as attachment areas for *M. tentoriobuccalis anterior* (*M. 48*). The distal hypopharyngeal part forms a ventral plate, which bears the opening of the salivary channel.

**Musculature (43, 44, 48).** *M. 41*: *M. frontohypopharyngalis*: absent. *M. 42*: *M. tentoriohypopharyngalis*: absent. *M. 43*: *M. clypeopalatalis*: very strongly developed; O- extensive area of the clypeus; I- roof of cibarium (palatum); F- cibarial dilator. *M. 44*: *M. clypeobuccalis*: O- frontal apodeme; I- roof of bucca; F- cibarial dilator. *M. 48*: *M. tentoriobuccalis anterior*: strongly developed; O- circumocular ridge, anteriorly of the compound eyes; I- lateral apophyses of the posterior hypopharynx, immediately anterad the anatomical mouth; F- stabilizes the cibarium (Figs. 2,4A,B, 7,8, and 3B–D).

### Pharynx

The position of the anatomical mouth approximately corresponds with the level of the antennal insertion. The precerebral pharynx is U-shaped in cross section. The dorsal edges form large folds which appear Y-shaped in cross section (ph, Fig. 3D). They serve as muscle attachment areas. The following pharyngeal section between the brain and the suboesophageal complex is also U-shaped

in cross section, but narrower (ph, Fig. 3E). A ventral longitudinal pharyngeal fold originates below the middle region of the brain. It also appears Y-shaped in cross section and increases in size toward the oesophagus (ph, Fig. 3F). Its presence results in a triangular profile of the postcerebral pharynx. The folds of the postcerebral pharynx serve as attachment areas for the strongly developed postcerebral dilators (51, 52, Figs. 2,4A,B, 8, and 3F). In the postoccipital region, the sclerotized pharynx is continuous with the membranous oesophagus.

**Musculature (45/46, 51, 52).** *Mm. 45/46*: *Mm. frontobuccalis anterior/posterior*: there is no criterium to assess whether the single paired precerebral dilator is *M. frontobuccalis anterior*, *M. frontobuccalis posterior*, or a product of fusion of both; O- frontal region, at the level of the antennal insertion; I- anterior pharynx, immediately posterad the anatomical mouth opening; F- widens the lumen of the precerebral pharynx. *M. 47*: *M. frontobuccalis lateralis*: absent. *M. 49*: *M. tentoriobuccalis posterior*: absent. *M. 50*: *M. tentoriobuccalis posterior*: absent. *M. 51*: *M. verticopharyngalis*: long and strongly developed; O- on the vertex, anterior to the foramen occipitale; I- dorsal wall of the postcerebral pharynx, immediately posterior to the brain, left and right muscle intercrossing; F- dilator of the posterior pharynx. *M. 52*: *M. tentoriopharyngalis*: very strongly developed; O- on an extensive region of the postgena; I- ventrolateral part of the posterior pharynx; F- dilator of the posterior pharynx, together with *M. verticopharyngalis*. *M. 67*: *M. transversalis buccae*: absent. *M. 68*: *M. anularis stomodaei*: ring muscles around the oesophagus. *M. 69*: *M. longitudinalis stomodaei*: absent (Figs. 2,4A,B, 7,8, and 3B–F).

### Salivarium

The salivary duct opens ventrally on the anterior hypopharyngeal plate. At the level of the opening in the hypopharyngeal plate, the duct is sclerotized. In the further extending, the duct is more membranous. The duct runs posteriorly from the medial to the lateral part of the head, where the salivary glands are located (sg, Fig. 4B).

**Musculature.** *M. 37*: *M. hypopharyngosalivariialis*: O- ventral wall of the anterior hypopharynx; I- dorsal wall of the anterior salivary duct; F- dilator of the salivarium. *Mm. 38/39*: *Mm. praementosalivariialis anterior/posterior*: absent. *M. 40*: *M. anularis salivariialis*: absent.

### Nervous System

The brain and suboesophageal complex are very large and takes 42.5% of the head volume (Fig. 4B). Both form a compact structural unit around the posterior pharynx and occupy a large proportion of the lumen of the posteroventral head

capsule (Fig. 3E). External borders between the pro-, deuto-, and tritocerebrum are not recognizable. A separate tritocerebral commissure is also absent. The optic lobes are largely incorporated into the compact brain (ol, Figs. 2,4B, and 7). They appear as flat structures below the eyes and the dorsal eye bridge. The antennal nerves are thick and arise from the frontal region of the brain. The frontal ganglion is not present as a recognizable structure and apparently fused with the brain.

## DISCUSSION

### Function of the Feeding Apparatus

The interpretation of the function of feeding structures is deduced from the observed structures in this study. The mouthparts are reduced in the same way like in other dipteran groups (see e.g., Schneeberg and Beutel, 2011). But, the number of muscles associated with the structures with feeding function suggests that feeding is important for the Hessian fly. They have labella and no chewing mouthparts; this suggests that their diet is liquid food. Labella are the strongly broadened two segmented labial palps (Crampton, 1925, 1942), their function is the salivary distribution; they uptake the primary food. The cibarial dilator musculature is strongly developed (*M. clypeopalatalis* and *M. clypeobuccalis*). These muscles provide the transport of the food in the cibarium. The precerebral pharynx pump (*Mm. frontobuccalis anterior/posterior*) transports the food along the precerebral pharynx and the postcerebral pharynx pump (*M. verticopharyngalis* and *M. tentoriopharyngalis*) along the postpharynx into the oesophagus.

### Phylogenetic Interpretation of Cephalic Features

Despite of substantial modifications, the head of *M. destructor* has preserved many ancestral features of Diptera. Apomorphic character states belonging to the dipteran groundplan are the two-segmented labial palpi modified as thickened labellae and the presence of a clypeolabral muscle (e.g., *Tipula*, *Limonia*, *Trichocera*, *Biblio*, *Spathobdella*, *Coboldia*, *Mayetiola*, *Mischoderus*, Culicidae, *Tabanus*, *Hemipenthes*, *Bombylius*; Thompson, 1905; Bonhag, 1951; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Szucsich and Krenn, 2000; Schneeberg and Beutel, 2011), which does not occur in other insect orders. This muscle is unpaired in *Mayetiola* like in some other groups such as Ceratopogonidae, Bibionidae and Tipulomorpha (e.g., *Culicoides*; Gad, 1951; Schneeberg and Beutel, 2011). Matsuda (1965) considered this as a groundplan feature of the order. However, this interpretation appears unlikely, as the muscle is not only paired in most dipteran lineages (e.g., *Spathobdella*, *Mischoderus*;

TABLE 1. Insect taxa mentioned in the text

Genus	Family		Order
<i>Aedes</i>	Culicidae		Diptera
<i>Biblio</i>	Bibionidae		Diptera
<i>Bombylius</i>	Bombyliidae	Brachycera	Diptera
<i>Boreus</i>	Boreidae		Mecoptera
<i>Coboldia</i>	Scatopsidae		Diptera
<i>Culex</i>	Culicidae		Diptera
<i>Culicoides</i>	Ceratopogonidae		Diptera
<i>Deuterophlebia</i>	Deuterophlebiidae		Diptera
<i>Edwardsina</i>	Blephariceridae		Diptera
<i>Erioptera</i>	Limoniidae		Diptera
<i>Eristalis</i>	Syrphidae	Brachycera	Diptera
<i>Fucellia</i>	Anthomyiidae	Brachycera	Diptera
<i>Hemipenthes</i>	Bombyliidae	Brachycera	Diptera
<i>Limonia</i>	Limoniidae		Diptera
<i>Mayetiola</i>	Cecidomyiidae		Diptera
<i>Mengenilla</i>	Mengenillidae		Strepsiptera
<i>Mischoderus</i>	Tanyderidae		Diptera
<i>Mycetophila</i>	Mycetophilidae		Diptera
<i>Nannochorista</i>	Nannochoristidae		Mecoptera
<i>Nymphomyia</i>	Nymphomyiidae		Diptera
<i>Simulium</i>	Simuliidae		Diptera
<i>Spathobdella</i>	Sciaridae		Diptera
<i>Tabanus</i>	Tabanidae	Brachycera	Diptera
<i>Tipula</i>	Tipulidae		Diptera
<i>Trichocera</i>	Trichoceridae		Diptera
<i>Wilhelmia</i>	Simuliidae		Diptera

Schneeberg and Beutel, 2011; Culicidae; Thompson, 1905; Schiemenz, 1957; Christophers, 1960; Owen, 1985; *Tabanus*; Bonhag, 1951; *Eristalis*, Schiemenz, 1957), but also the *M. frontolabralis* of other groups of insects (von Kéler, 1963), which is likely homologous with *M. clypeolabralis*. Other dipteran groundplan features preserved in *Mayetiola* are the orthognathous orientation of the mouthparts, the filiform antennae inserted frontally between the compound eyes, the presence of only one premental retractor (probably *M. tentoriopraementalis inferior* [M. 29] or a product of fusion of both tentoriopremental muscles [Mm. 29 + 30]; Beutel and Baum, 2008), and an *M. clypeopalatalis* (*M. 43*) with its bundles arranged as a long series. The head capsule of *Mayetiola* is densely covered with microtrichia (Fig. 1). This is also the case in all other dipterans examined (e.g., *Spathobdella*, *Coboldia*, *Tipula*, *Limonia*, *Trichocera*, *Culex*, *Aedes*, *Nymphomyia*, *Deuterophlebia*, *Edwardsina*; Tokunaga, 1935; Schneeberg and Beutel, 2011; Schneeberg et al., 2011) and likely a groundplan feature. An apomorphic condition is found in *Biblio marci*, which is characterized by a dense vestiture of long setae, this is also the case in other bibionids (Duda, 1930). The presence of densely arranged microtrichia has evolved independently in Strepsiptera (Beutel and Pohl, 2006), which are not closely related with Diptera, but the sistergroup of Coleoptera (Wiegmann et al., 2009; Beutel et al., 2011; Niehuis et al., 2012) and also in some members Heteroptera (see e.g., Swart and Felgenhauer 2003; Weirauch 2012). It is

conceivable that the microtrichia have an effect on the water repellence of the head (see Table 1).

A feature with ambiguous polarity is the frontal apodeme (fap, Figs. 2 and 3B). It is also present in *Nannochorista* (Mecoptera; Beutel and Baum, 2008) and many other dipteran groups such as Tanyderidae, Ptychopteridae, Culicidae, Simuliidae, Ceratopogonidae, Blephariceridae, Limoniidae, and Trichoceridae (Gad, 1951; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Harbach and Kitching, 1998; Schneeberg and Beutel, 2011). However, it is apparently always absent in Brachycera (*Hemipenthes*, *Bombylius*, *Eristalis*; Szucsich and Krenn, 2000; Schiemenz, 1957), and also missing in *Coboldia*, *Biblio*, *Tipula* (Schneeberg and Beutel, 2011) and the presumably basal dipteran families Deuterophlebiidae (Schneeberg et al., 2011) and Nymphomyiidae (Tokunaga, 1935) (see Wiegmann et al., 2011).

Most of the derived features displayed by *Mayetiola* also occur in other dipteran groups, but without a distinct pattern revealing phylogenetic affinities. A possible cephalic autapomorphy of Bibionomorpha is the origin of *M. tentorioscapalis* medialis on the frons. This is also the case in *Spathobdella* and *Biblio*. However, the same derived feature also occurs in *Trichocera* (Schneeberg and Beutel, 2011), *Tabanus* (Bonhag, 1951), *Nannochorista* (Beutel and Baum, 2008), and other members of Mecoptera (Friedrich et al., 2013) at least in some of these cases correlated with a more or less far-reaching reduction of the tentorium (see below). The shift of origin obviously took place several times independently. A further apomorphy consistently found in Bibionomorpha is the loss of *M. craniolacinalis*. The muscle is present in the dipteran groundplan (Gad, 1951; Bonhag, 1951; Schiemenz, 1957; Wenk, 1962; Szucsich and Krenn, 2000), but is not only missing in members of the bibionomorph families, but also in Tanyderidae, Blephariceridae, Tipulomorpha (Schneeberg and Beutel, 2011; Schneeberg et al., 2011), and Syrphidae (Schiemenz, 1957) and also in *Boreus* (Friedrich et al., 2013).

Michelsen (1996) considered the loss of the mandibles as one of two apomorphies supporting Bibionomorpha. However, the loss of the mandibles apparently occurred several times among the lower dipteran lineages and this is apparently linked with the feeding habits. They are usually present in blood-feeders (e.g., females of Culicidae, Ceratopogonidae, Simuliidae, females of Tabanidae; Bonhag, 1951; Schiemenz, 1957; Christophers, 1960; Wenk, 1962; Owen, 1985; Blackwell, 2004) whereas they are missing in dipterans feeding on nectar (e.g., Cecidomyiidae, Scatopsidae, Sciaridae, Bibionidae, Tipulidae, Limoniidae, Trichoceridae, Chironomidae; Peterson, 1916; Schneeberg and Beutel, 2011). Mandibles are present, but modified in males of most Culicidae, whereas they feed on nectar (Snodgrass, 1959).

A potential autapomorphy of Cecidomyiidae or *Mayetiola* is the massive anterior tentorial arm. A similar condition occurring in *Eristalis* (Schiemenz, 1957) has apparently evolved independently as Syrphidae are deeply nested within Brachycera. The far-reaching reduction of tentorial elements is a complex derived condition, but a tendency to reduce the cephalic endoskeleton is quite common in Diptera. In the groundplan of the order anterior, posterior, and dorsal arms are present and a vestigial corpotentorium (see also Peterson, 1916; Hennig, 1973). This condition is described for *Simulium* (Peterson, 1916). In most groups with available data on internal structures the tentorium is a simple, more or less straight thick and hollow tube connection the anterior and posterior tentorial grooves or pits (e.g., Culicidae, Deuterophlebiidae, Trichoceridae, Bibionidae, Culicidae; Schiemenz, 1957; Schneeberg et al., 2011; Schneeberg and Beutel, 2011). Dorsal arms are usually completely missing but a short vestige is present in Tanyderidae (Schneeberg and Beutel, 2011), Ceratopogonidae (Gad, 1951), Chironomidae (Peterson, 1916), and Syrphidae (Schiemenz, 1957). Hennig (1973) suggest a correlation between the reduction of the tentorium and the increasing sclerotization of the head capsule, especially the development of a hypostomal bridge. In *Nymphomyia* and *Tipula*, where this structure is well developed, the tentorium is completely reduced (Tokunaga, 1935; Schneeberg and Beutel, 2011). However, it is also largely reduced in *Mayetiola*, where the ventral closure is largely membranous, probably a result of secondary reduction. The tentorium is comparatively well developed in other representatives of the lower dipteran lineages such as for instance *Coboldia*, *Spathobdella*, *Biblio*, *Deuterophlebia*, *Trichocera*, *Mischoderus*, *Culiseta*, *Culicoides*, *Wilhelmia*, *Tabanus*, *Eristalis* (Bonhag, 1951; Gad, 1951; Schiemenz, 1957; Wenk, 1962; Schneeberg et al., 2011; Schneeberg and Beutel, 2011). The presence of a hypostomal bridge is very likely a derived groundplan feature of Diptera as it is distinctly developed in the potentially basal families *Nymphomyia* and *Deuterophlebia* (Tokunaga, 1935; Schneeberg et al., 2011; Wiegmann et al., 2011; Lambkin et al., 2013).

Apomorphic features of Cecidomyiidae also include the absence of the labro-epipharyngeal food channel, and the absence of the lacinia. The anterior tentorial arm is approximately round in cross section and hollow in the groundplan of Diptera and this condition is found in most lineages (e.g., Peterson, 1916; Schiemenz, 1957; Schneeberg and Beutel, 2011). The labro-epipharyngeal food channel is very likely a groundplan feature of Diptera. It is present in all other dipterans examined so far and also in *Nannochorista* and Siphonaptera (e.g., Vogel, 1921; Bonhag, 1951; Gad, 1951; Wenk, 1953; Schiemenz, 1957; Snodgrass, 1959; Wenk, 1962; Sutcliffe, 1985; Szucsich and Krenn, 2000;

Beutel and Baum, 2008; Schneeberg and Beutel, 2011). The lacinia is present in the groundplan of Diptera (e.g., Imms, 1944; Schiemenz, 1957; Snodgrass, 1959; Wenk, 1962; Krenn et al., 2005; see also character 43 in Schneeberg and Beutel, 2011; referred to as galea in some older contributions; Peterson, 1916; Williams, 1933; Crampton, 1942), but it is also absent in *Tipula*, *Erioptera*, *Mycetophila*, and *Fucellia* (Hoyt, 1952). It is likely, that the reduction took place several times independently. An unusual autapomorphy of Cecidomyiidae is the presence of sensilla circumfila, which are connected by one seta, which extends around the flagellomere (Fig. 3A). These sensilla are also present in other cecidomyiid species (Slifer and Sekhon, 1971; Gagné and Marohasy, 1997; Madeira et al., 2002; Boddum et al., 2010). They are involved in the detection of pheromones (Boddum et al., 2010). Boddum et al. (2010) suggests that the sensilla circumfila may have evolved from sensilla basiconia, because they are absent on the antenna of Cecidomyiidae. This type of antennal sensilla has not been described for any other group of Diptera so far (e.g., Schiemenz, 1957; Schneeberg and Beutel, 2011). Due the fact that adults are short lived (1–2 days) the ability to rapidly find reproductive resources is essential. The antenna have not only many sensilla, they have also well-developed extrinsic and intrinsic musculature (Mm. tentorioscapalis anterior/posterior/medialis and Mm. scapopedicellaris lateralis/medialis). The latter play a role in bringing the tips of the antennae in contact with plant surfaces during the exploratory behavior that precedes egg laying (Harris and Rose, 1989).

### Effects of Miniaturization

With a size ranging between 2.7 and 3.7 mm *Mayetiola* belongs to the moderately small insects. Nevertheless, some features may be related to miniaturization. This applies very likely to the specific condition of the cephalic nervous system. A compact unit formed by the brain and subesophageal ganglion is a derived groundplan feature of Antliophora (e.g., Beutel et al., 2011). However, the unusually large size of the brain in relation to the size of the head capsule, the unusual shape of the optic lobes, and the absence of the frontal ganglion as a separate structure are likely features resulting from size reduction (see e.g., Polilov and Beutel, 2009). The very large extension of the compound eyes and the loss of ocelli are possibly also linked with small size. However, the large compound eyes also reflect that vision is very important for the success of adults. The main objective for males is to find virgin females, and for females to find suitable host plants (Harris et al., 2003), both of which occur in a short time span. Whether the secondary loss of the hypostomal bridge and of

the labral food channel are related to miniaturization is unclear. The cephalic musculature is apparently not affected. Seventeen pairs of muscles are present in the head of *Mayetiola*. The same number occurs in *Coboldia* (approximately 2 mm in length) and 20 muscle pairs are present in *Spathobdella*. In most adult dipterans including large species, the number of muscle pairs in the adult head ranges from 20 to 25 (Bonhag, 1951; Gad, 1951; Schiemenz, 1957; Wenk, 1962; Christophers, 1960; Schneeberg and Beutel, 2011). In contrast to this, the number of cephalic muscles is greatly reduced in *Nymphomyia* and *Deuterophlebia*, with only nine or eight preserved in these two presumably basal lineages (Tokunaga, 1935; Schneeberg et al., 2011). In these cases, the reduction is likely correlated with an unusual life cycle. Adults of Deuterophlebiidae and Nymphomyiidae are very short lived and do not take up food (Courtney, 1991; Tokunaga, 1935). The discovery of significant musculature in the Hessian fly suggests that the long-held assumption that adults do not take up food (Harris and Rose, 1989) should be reconsidered.

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### 3.3 Study III

**Schneeberg K**, Krause K, Beutel RG. 2013.

The adult head morphology of *Axymyia furcata* (Axymyiidae, Diptera). *Arthropod Systematics and Phylogeny* 72: 91-102.

#### **Abstract**

The external and internal cephalic morphology of males and females of *Axymyia furcata* is described and illustrated in detail. The documented features are compared with those of potentially related groups. *Axymyia* displays a number of apomorphic features of the adult head, like the subdivision of the compound eyes, the absence of several muscles (M. clypeolabralis, M. stipitopalpalis externus, M. palpopalpalis maxillae primus, Mm. palpopalpalis maxillae primus and secundus), the absence of both maxillary endites, and the loss of the salivary pump musculature (M. hypopharyngosalivarialis). Another apomorphic character is the origin of M. tentorioscapalis anterior on the head capsule. Some features are plesiomorphic and probably belong to the groundplan of Diptera: the orthognathous head, three ocelli, 5-segmented maxillary palps, 2-segmented labial palps transformed into medially fused labella, one premental retractor, a dense vestiture of microtrichia on all exposed parts of the head except the labrum, and the presence of an epipharyngeal food channel. The systematic position of Axymyiidae is discussed. Our findings do not provide support for the phylogenetic position of Axymyiidae.

#### **Significance in the present thesis**

Axymyiidae is a poorly known group among the nematoceran groups and the phylogenetic position is still unclear. External features of larvae and adults were described (Whilm 2012), but a description of internal structures was missing.

#### **Own contribution**

70%

# The adult head of *Axymyia furcata* (Insecta: Diptera: Axymyiidae)

KATHARINA SCHNEEBERG \*, KATRIN KRAUSE & ROLF G. BEUTEL

Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, 07743 Jena, Erbertstraße 1, Germany; Katharina Schneeberg\* [katharina.schneeberg@gmx.de]; Katrin Krause [krause-kat@web.de]; Rolf G. Beutel [rolf.beutel@uni-jena.de] — \* Corresponding author

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## Abstract

The external and internal cephalic morphology of males and females of *Axymyia furcata* is described and illustrated in detail. The documented features are compared with those of potentially related groups. *Axymyia* displays a number of apomorphic features of the adult head, like the subdivision of the compound eyes, the absence of several muscles (*M. clypeolabralis*, *M. stipitopalpalis externus*, *M. palpopalpalis maxillae primus*, *Mm. palpopalpalis maxillae primus and secundus*), the absence of both maxillary endites, and the loss of the salivary pump musculature (*M. hypopharyngosalivarialis*). Another apomorphic character is the origin of *M. tentorioscapalis anterior* on the head capsule. Some features are plesiomorphic and probably belong to the groundplan of Diptera: the orthognathous head, three ocelli, 5-segmented maxillary palps, 2-segmented labial palps transformed into medially fused labella, one premental retractor, a dense vestiture of microtrichia on all exposed parts of the head except the labrum, and the presence of an epipharyngeal food channel. The systematic position of Axymyiidae is discussed. Our findings do not provide support for the phylogenetic position of Axymyiidae.

## Key words

Axymyiidae, Diptera, morphology, head, phylogeny, position.

## 1. Introduction

Axymyiidae are a poorly known group among the nematoceran lineages of Diptera. The family contains only eight described species, which are widely distributed throughout the Holarctic Region (WIHLM & COURTNEY 2011; SINCLAIR 2013). They are placed in three extant genera, *Axymyia* McAtee, *Mesaxymyia* Mamayev and *Protaxymyia* Mamayev & Krivosheina (MAMAYEV 1968). Additionally three fossil species are known from the Jurassic (ZHANG 2010).

The larvae live in small, lotic habitats, including seeps, springs and streams (WIHLM & COURTNEY 2011). The diets of larvae and adults are unknown (WIHLM 2009). Very little is known about the biology of the family. Adults are considered as short-lived based on the greatly reduced mouthparts (WOOD 1981). After WIHLM

& COURTNEY (2011) they have a life span of 2–7 days. The size of the adults (5–8 mm wing length) and pupae is highly variable within species, but the mechanism of this polymorphism is still unclear (WIHLM & COURTNEY 2011).

*Axymyia furcata* was first described by McATEE (1921) and placed in Bibionidae. SHANNON (1921) established a subfamily Axymyiinae and placed it in Anisopodidae, a concept which was later adopted by ALEXANDER (1942). In other contributions other species of *Axymyia* were placed in Pachyneuridae or Bibionidae, respectively (DUDA 1930; ISHIDA 1953). MAMAYEV & KRIVOSHEINA (1966) pointed out that the peculiar larval features suggest a more isolated lineage and consequently proposed a separate family Axymyiidae.

The phylogenetic relationships of Axymyiidae to the other nematoceran families is apparently one of the most persistent problems in “lower” dipteran systematics. ROHDENDORF (1974) (informal non-Hennigian interpretation of morphological characters) placed Axymyiidae in Tipulomorpha as a part of Perissommatidae. MAMAYEV & KRIVOSHEINA (1966) (morphological characters of the larvae) and WOOD & BORKENT (1989) (morphological characters) proposed a status as a separate infraorder Axymyiomorpha. HENNIG (1973) (based on morphological characters) suggested a placement in Bibionomorpha. This was also supported by OOSTERBROEK & COURTNEY (1995) (morphological characters, mainly of larvae and pupae), who placed the family as sistergroup of the remaining Bibionomorpha. And MICHELSEN (1996) suggested a closer relationship of Axymyiomorpha (containing only Axymyiidae) with Bibionomorpha and Psychodomorpha (Neodiptera) (morphological characters of the prothorax and neck). Analyses of a molecular data set (BERTONE et al. 2008) yielded a placement of Axymyiidae as sistergroup of Nymphomyiidae, and both groups together were placed as close relatives of Culicomorpha. However, it was pointed out by the authors that this is likely an artefact caused by long-branch attraction. Finally, in agreement with HENNIG (1973) and based on a comprehensive data set WIEGMANN et al. (2011) (combined analysis of 14 nuclear genes, complete mitochondrial genomes and morphological characters) suggested a placement within Bibionomorpha.

The situation is complicated by a number of hypotheses concerning the composition of Bibionomorpha: only Bibionidae, Pachyneuridae, Mycetophilidae, Sciaridae and Cecidomyiidae were included by WOOD & BORKENT (1989) and BLASCHKE-BERTHOLD (1994). OOSTERBROEK & COURTNEY (1995) added Axymyiidae as the sister group of a clade comprising these five families. Analyses of 28SrDNA suggested an expanded concept including also Anisopodidae and Scatopsidae (FRIEDRICH & TAUTZ 1997). Interestingly analyses of combined nuclear ribosomal (28S) and protein coding genes (CAD, PGD and TPI) supported the inclusion of Canthyloscelidae (BERTONE et al. 2008) but the exclusion of Axymyiidae. The most comprehensive data set to date, assembled by WIEGMANN et al. (2011; see above), again supported the inclusion of Axymyiidae in Bibionomorpha. The family was placed as the 3<sup>rd</sup> branch following Anisopodidae and a clade comprising Scatopsidae and Canthyloscelidae. However, only few superficial morphological data for Axymyiidae were available for these analyses.

Morphological treatments of an axymyiid fly were provided by MICHELSEN (1996) and WIHLM et al. (2012). MICHELSEN (1996) described the prothorax and neck region. WIHLM et al. (2012) documented the external morphology of all life stages of *Axymyia furcata*, with the main focus on the larvae. Internal morphological features of all life stages remained largely or completely unknown including the head of adults. Consequently the main aim of our study is to provide detailed data on this taxon. The phylogenetic implications we suggest here have to be

considered as preliminary. The comprehensive analysis of WIEGMANN et al. (2011) provides a solid phylogenetic framework for the discussion. A formal analysis of the characters treated here is presently not feasible due to a serious lack of detailed anatomical data for a number of relevant nematoceran taxa (see Discussion).

## 2. Materials and methods

### 2.1. Material examined

Axymyiidae (Diptera): *Axymyia furcata* McAtee, 1921 (95% Ethanol; North Carolina, USA), 2 females, 2 males.

Bibionidae (Diptera): *Bibio marci* Linnaeus, 1758 (70% Ethanol, Jena, Germany).

Anisopodidae (Diptera): *Sylvicola fenestralis* Scopoli, 1763 (70% Ethanol, Jena, Germany).

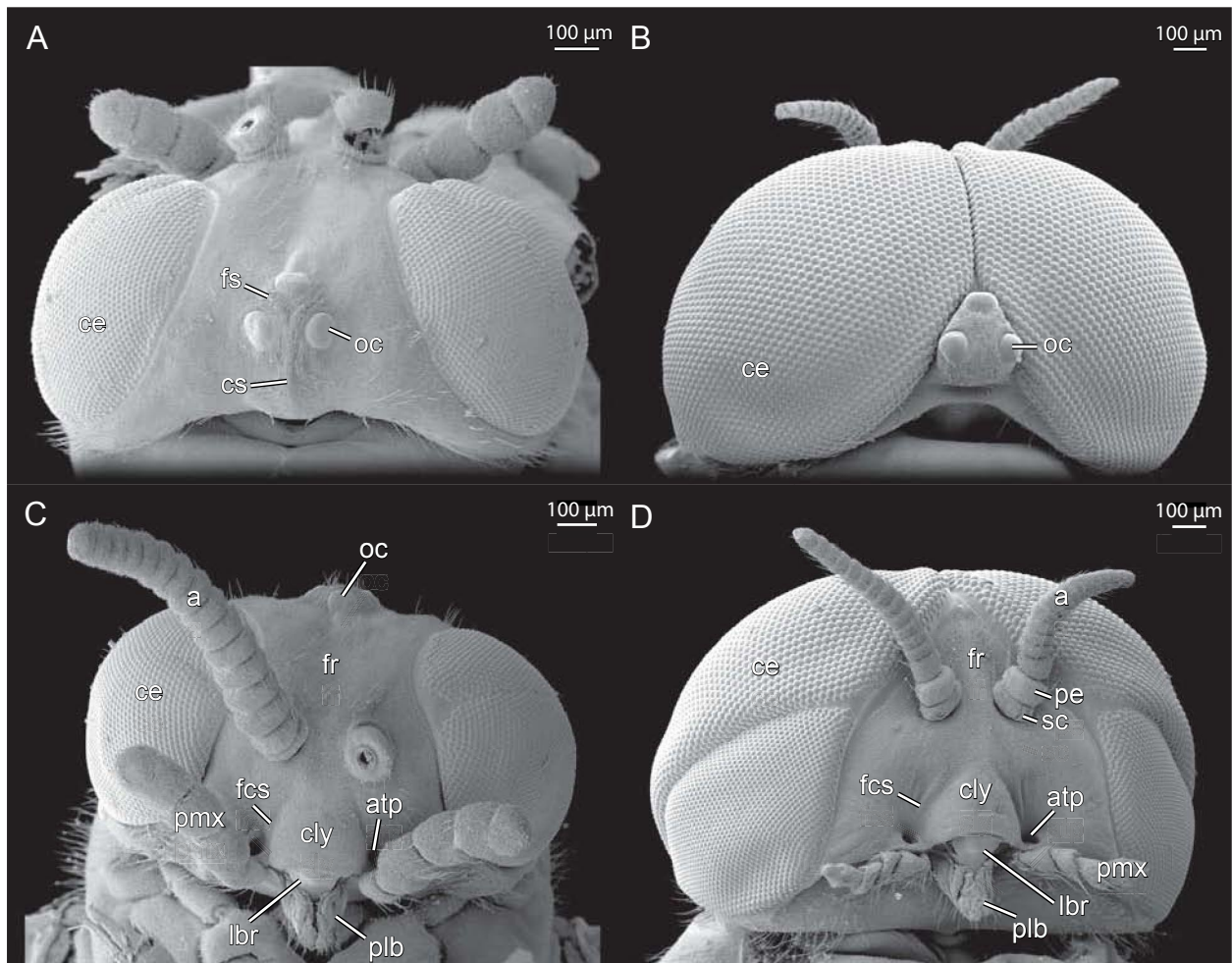
### 2.2. Methods

Drawings were made using a stereo microscope MZ 125 (Leica). Figures were processed with Adobe Photoshop<sup>®</sup> and Adobe Illustrator<sup>®</sup>. For scanning electron microscopy the specimens were dehydrated with ethanol (95%–100%) and acetone, critical point dried (EmiTech K500 Critical Point Dryer; Ashford, Kent, UK), glued on a fine pin and sputter coated. Images were taken with a Philips XL 30 ESEM using a specimen holder after POHL (2010).

Three-dimensional reconstructions were carried out using Imaris<sup>®</sup> 6.2.1 (Bitplane AG, Zürich, Suisse) and Maya 2012 (Autodesk) software based on the  $\mu$ CT-image stack. For the synchrotron radiation based micro-computed tomography the specimens were dehydrated with ethanol (95%–100%) and acetone, critical point dried (EmiTech K850 Critical Point Dryer; Ashford, Kent, UK) and mounted with superglue on a metal rod. The scans were performed at Beamline BW2 on the German Electron Synchrotron Facility (DESY, Hamburg) using a low energy beam (8 kV) and absorptions contrast (see FRIEDRICH et al. 2010).

### 2.3. Terminology

The regions and sclerites of the head are named following the nomenclature of DUPORTE (1946). Muscles are named following the nomenclature of v. KÉLER (1963).



**Fig. 1.** *Axymyia furcata*, female and male, entire head, SEM images. **A:** Female, dorsal. **B:** Male, dorsal. **C:** Female, frontal. **D:** Male, frontal. Abbreviations: a – antenna, atp – anterior tentorial pits, ce – compound eyes, cly – clypeus, cs – coronal suture, fcs – frontoclypeal suture, fr – frons, fs – frontal suture, lbr – labrum, oc – ocelli, pe – pedicellus, plb – labellum, pmx – maxillary palp, sc – scapus.

### 3. Results

#### 3.1. Head capsule

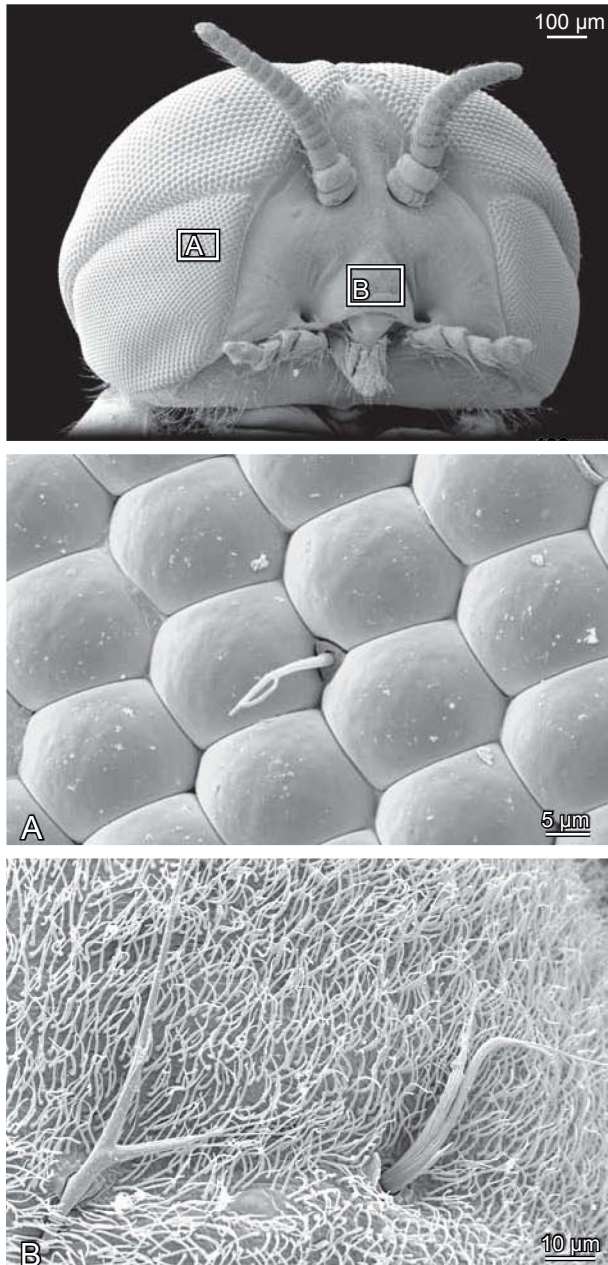
The orthognathous head is kidney-shaped in dorsal view and nearly oval in frontal view (Fig. 1). It is sclerotized and densely covered with microtrichia. The posterodorsal, lateroventral and posteroventral margins are covered with numerous bristles. A row of setae is present on the frons of the females, below the frontal ocellus and above the antennae (Fig. 1A). Their arrangement approximately agrees with the three sides of a triangle (pointing upward), with a horizontal row and two rows with an oblique longitudinal orientation. In males, the triangular area between the antennal insertions and the area of fusion of the compound eyes is densely covered with setae.

The frons extends between the compound eyes and completely separates them medially in females, whereas in males a part of the frons is reduced in size by expansion of the compound eyes (ce, Fig. 1B). It is approxi-

mately triangular and distinctly reduced in size in males. The frons is laterally separated from the clypeus by a faintly developed, oblique lateral part of the frontoclypeal ridge (= epistomal suture [SCHIEMENZ 1957]), which is interrupted medially (see below); the area of origin of *M. clypeobuccalis* (M 44) also defines the clypeal area. The triangular clypeus lies below the antenna (cly, Fig. 1C,D). It is distinctly convex, not subdivided into an anteclypeus and a postclypeus, and covered by a very dense vestiture of microtrichia. The labrum is separated from it by an inwardly directed membranous fold (lbr, Figs. 1C,D, 4). A pair of forked, antler-like setae is present on the central region of the clypeus of males (Fig. 2B).

The frontal sutures and the coronal suture are completely absent in males. Landmarks for the upper part of the frons are the origin of *M. frontobuccalis* posterior (M. 46) and the frontal ocellus (see v. KÉLER 1963: pp. 615, 732). The genae alongside the lower hemispheres of the compound eyes and the lower parts of the frons and clypeus form the ventrolateral and ventromedian marginal parts of the head capsule. Its ventral posterior margins are formed by the postgenae, which are adjacent below





**Fig. 2.** *Axymyia furcata*, male, SEM images. **A:** Higher magnification of compound eyes. **B:** Higher magnification of setae of the clypeus.

the compound eyes and fused medially, thus forming a postgenal or hypostomal bridge.

The vertex is separated from the occipital region by the transverse epicranial ridge. Its lateral parts extend along the circumocular suture reaching approximately the horizontal division of the eyes. In males the eyes are fused medially and the vertex lies directly above the dorsal area (Fig. 1B). It is prominent, cupola-shaped, and approximately triangular. Its entire surface is covered with setae. In females the vertex lies directly above the frons and is less convex (Fig. 1A). The Y-shaped line representing the parts of the frontal and coronal sutures around their junction in females (absent in males, see above) is flanked by setae on all sides. The median ocellus

is located on the upper part of the frons and the lateral ocelli are located on the vertex. The lateral ocelli of males are approximately round and smaller than the oval median ocellus (oc, Fig. 1B). All three ocelli are oval and of equal size in females (oc, Fig. 1A). The postoccipital region is almost vertical posterad the vertex. The postociput is a narrow bulge enclosing the foramen occipitale. It is separated from the occipital region by a faintly impressed postoccipital furrow. The foramen occipitale has the shape of a medially open 8, with the upper part almost 4 times as large as the ventral portion.

Cuticular strengthening ridges are distinctly developed. This includes the well-developed circumocular ridge, several internal folds laterad the clypeus and above the anterior tentorial pits, and a large, smooth extension above the posteroventral margin of the compound eyes. A frontal apodeme located between the antennal bases in many Diptera (and also Nannochoristidae) is lacking. A subgenal ridge and a prefrontal suture or ridge are also missing.

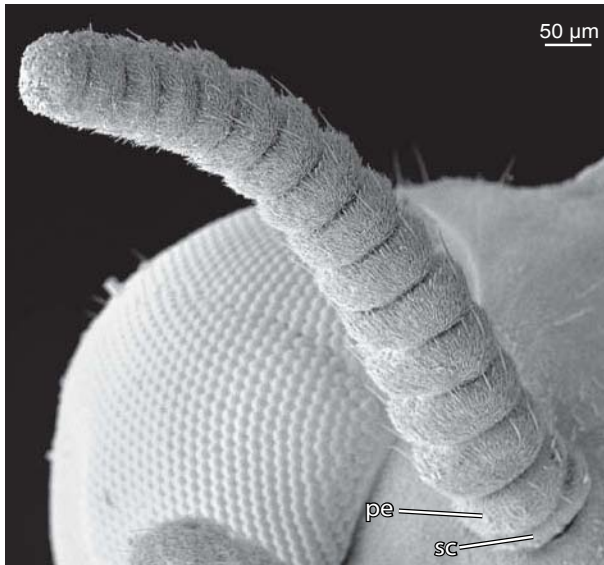
The compound eyes are sexually dimorphic. The outline of the dichoptic eyes of the females, which cover almost 1/3 of the surface of the head, is nearly circular (ce, Fig. 1A,C). The holoptic eyes of the males are oval and cover approximately 3/4 of the head surface (ce, Fig. 1B,D). The compound eyes are subdivided into a dorsal and a ventral part in both sexes by a flat horizontal chitinous stripe, which bears several terminally curved setae (Fig. 1C,D). The ventral part of females is slightly concave mesally and about twice as large as the dorsal portion. The dorsal hemisphere of males is about 2.5 times as large as the ventral one and concave posteroventrally.

### 3.2. Tentorium

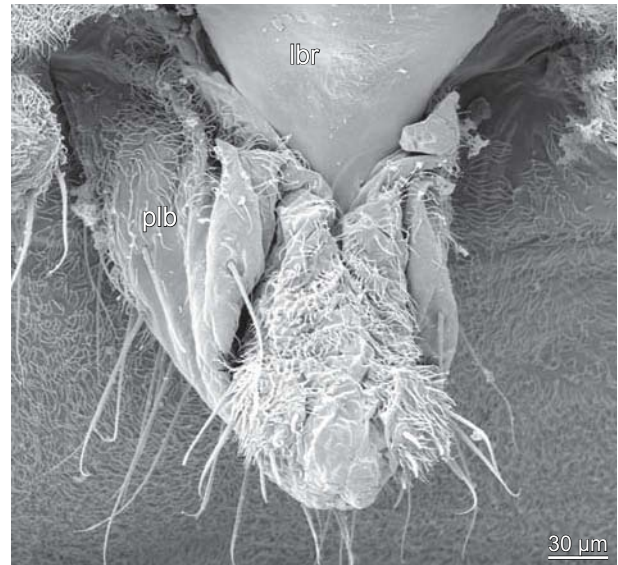
The anterior tentorial pits at the lateral ends of the frontoclypeal strengthening ridge are oval in outline and funnel-shaped (atp, Figs. 1C,D, 7A). The fissure-shaped posterior pits lie on the posterior side of the head beside the ventrolateral margins of the foramen occipitale (ptp, Fig. 7B). Anterior and posterior arms form a nearly straight, hollow tube-like structure on each side. The dorsal arms are absent. The posterior arms bear small thorn-shaped processes, they are directed mesally and are fused ventrally with the head capsule.

### 3.3. Antenna

The moniliform antennae insert on the frontal part of the head, approximately at the level of the division of the compound eyes (a, Fig. 1C,D). The distance between the antennal bases is approximately equal to their distance to the margin of the compound eyes. The antennae of



**Fig. 3.** *Axymyia furcata*, female, antenna, SEM image. Abbreviations: pe – pedicellus, sc – scapus.



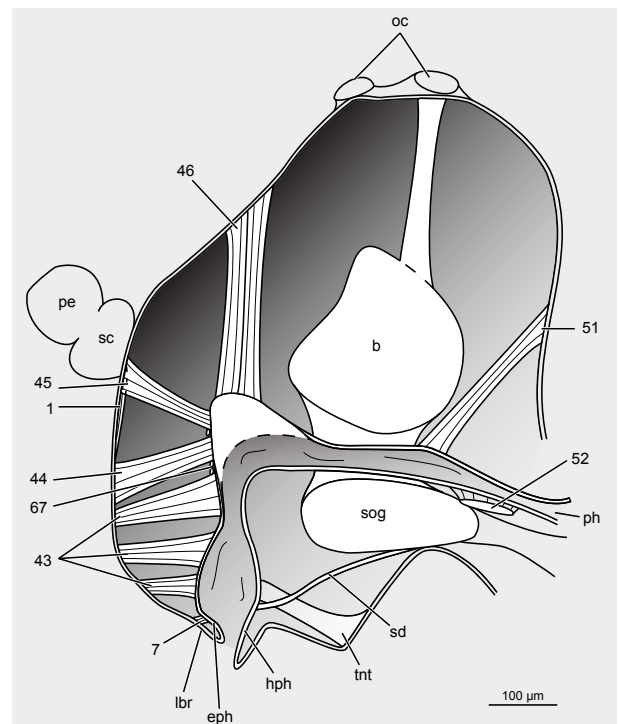
**Fig. 4.** *Axymyia furcata*, male, mouthparts, SEM image. Abbreviations: lbr – labrum, plb – labellum.

females are longer and thicker than those of the males. The 14 flagellomeres are rounded. All antennomeres are covered by a dense vestiture of microtrichia (Fig. 3). The scapus bears four longer setae medioventrally, three arranged in a line at the basal margin of the segment and the fourth placed above them. Additional lateroventral setae are present in males. The pedicellus is about twice as long as the scapus in both sexes. In males both basal antennomeres are longer and wider than the flagellomeres, and the basal flagellomere is approximately twice as long as the others. All flagellomeres bear a distal whorl of longer setae and a dense vestiture of sensilla trichodea. In females the whorl is placed at the distal margin on the pedicellus. In males the setae are not arranged in a regular row, but in alternating positions.

**Musculature:** (1, Fig. 5) M. 1: M. tentorioscapalis anterior: long and slender muscle; O (origin) – genae, laterally on the level of the most dorsal point of the frontoclypeal ridge; I (insertion) – anteroventral margin of the scapus; F (function) – depressor of the antenna. M. 2: M. tentorioscapalis posterior: long, slender muscle; O – genae, posterad M. tentorioscapalis anterior; I – posteriorly on the basal margin of the scapus; F – levator, antagonist of M. tentorioscapalis anterior. M. 3: M. tentorioscapalis lateralis: absent. M. 4: M. tentorioscapalis medialis: absent. Mm. 5/6: Mm. scapopedicellaris lateralis/medialis: not recognisable in the  $\mu$ CT data set.

### 3.4. Labrum

The small triangular labrum is largely exposed (lbr, Fig. 4). Its relatively broad basal margin is connected with the clypeus by an inwardly directed membranous fold. The surface is smooth and glabrous. The pointed

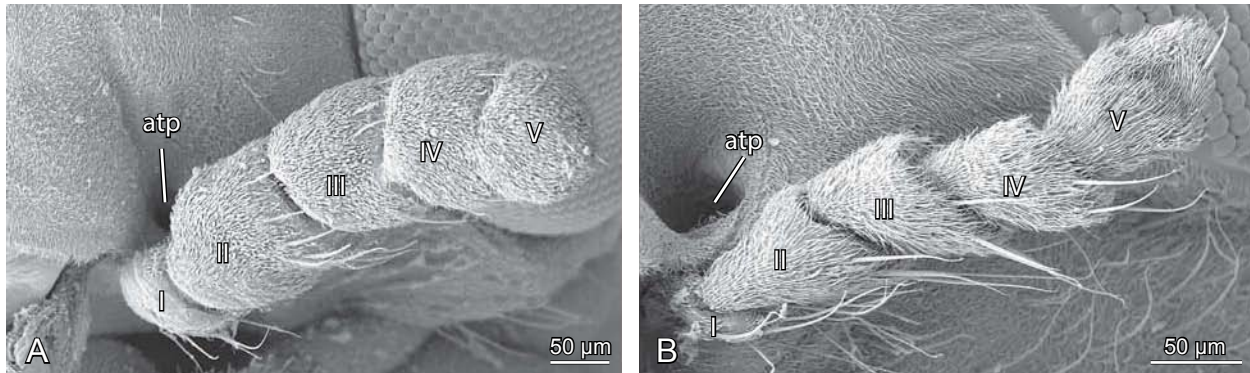


**Fig. 5.** *Axymyia furcata*, male, sagittal section of head. Abbreviations: b – brain, eph – epipharynx, hph – hypopharynx, lbr – labrum, oc – ocelli, pe – pedicellus, ph – pharynx, sc – scapus, sd – salivary duct, sog – sobesophageal ganglion, tnt – tentorium, 1 – M. tentorioscapalis anterior, 7 – M. labroepipharyngalis, 43 – M. clypeopalatalis, 44 – M. clypeobuccalis, 45 – M. frontobuccalis anterior, 46 – M. frontobuccalis posterior, 51 – M. verticopharyngalis, 52 – M. tentoriopharyngalis, 67 – M. transversalis buccae.

apex is embedded in the median fold between the labella. A paired bulge is present at the upper margin in males.

**Musculature:** (7, Figs. 5, 7A) M. 7: M. labroepipharyngalis: small paired muscle, diverging towards the in-





**Fig. 6.** *Axymyia furcata*, maxillary palp, SEM images. **A:** Female. **B:** Male. Abbreviations: atp – anterior tentorial pits; I–V – number of palpomeres.

sersion; O – dorsal labral wall; I – anteromesally on the epipharynx; F – levator of the anterior epipharynx. M. 8: M. clypeolabralis (by SCHIEMENZ 1957): not recognisable in the  $\mu$ CT-data set, apparently absent.

### 3.5. Mandible

Absent in both sexes.

### 3.6. Maxilla

The basal parts of the maxillae are fused with the head capsule. Externally only the moniliform palps are visible (pmx, Figs. 1C,D, 6). They insert below the anterior tentorial pits. The palps of the males are short and slender (Fig. 6B). Those of the females are longer and thicker (Fig. 6A). The 5 palpomeres are almost globular. The basal one bears a scattered vestiture of microtrichia in males and females. A denser ventral field of microtrichia is present on palpomere 1 of females. Several curved setae are present lateroventrally in both sexes. Palpomeres 2–5 bear a dense vestiture of microtrichia and sensilla on their entire surface. A sensorial pit, which is present in some dipteran groups, is absent in *Axymyia*. A distal row of setae is present on palpomeres 2–4. It is restricted to the ventral half in males but encloses the entire segment in females. Palpomere 5 bears setae on its apex. A circular concavity is present in males on this segment.

**Musculature:** absent.

### 3.7. Labium

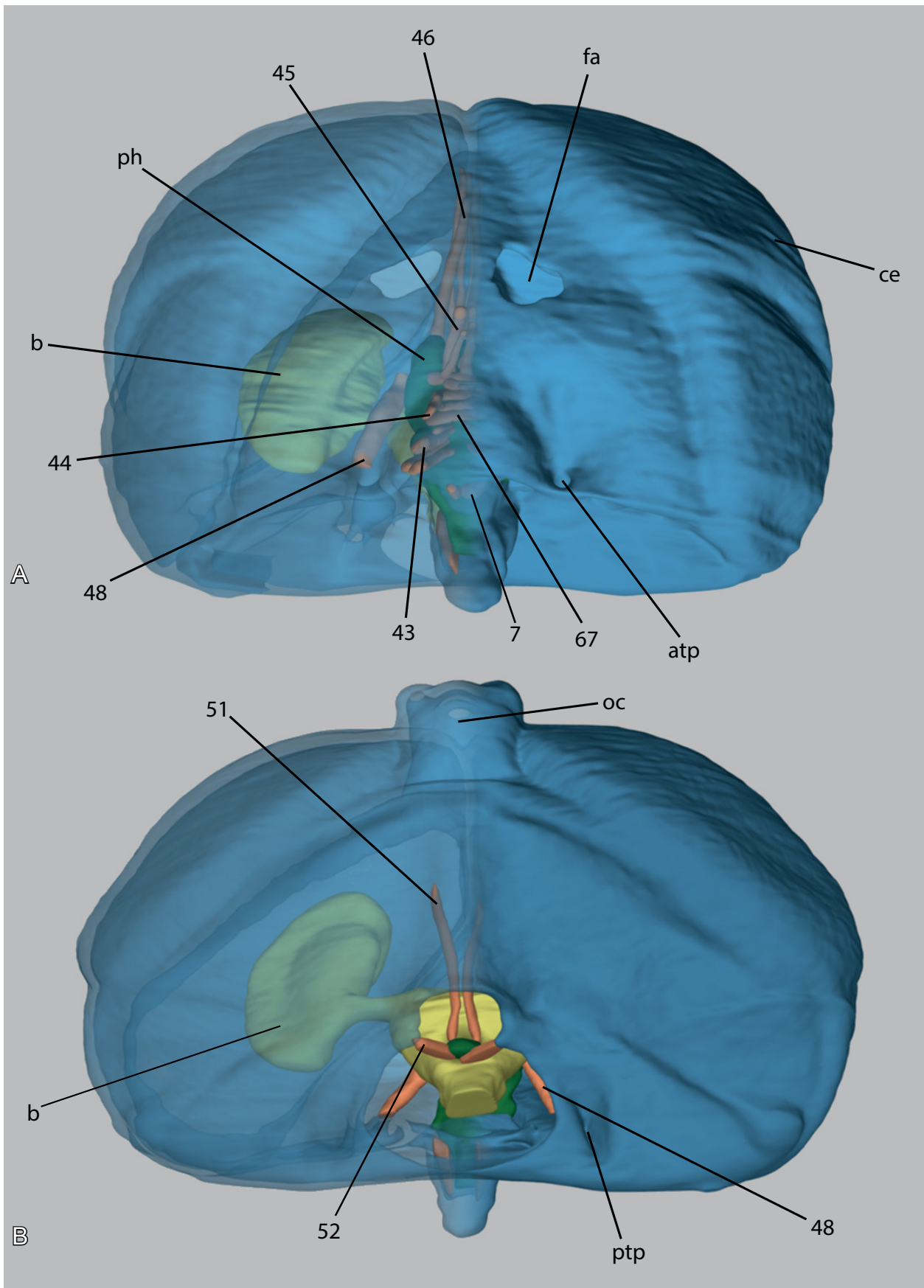
The labium is not distinctly delimited externally. The prementum forms a part of the ventral closure of the

head capsule and is partly fused with the head capsule. The postmentum is completely reduced or is a part of the ventral closure of the head. Glossae and paraglossae are absent. The labial palps are transformed into labella fused medially for most of their length (plb, Figs. 1C,D, 4). Their unsclerotized cuticle is folded, especially in the distal region. Pseudotracheae with internal strengthening ridges are missing. Basally and terminally numerous bristles are present, especially on the ventral surface. A small circle with dark setae is present on the apices of the labella.

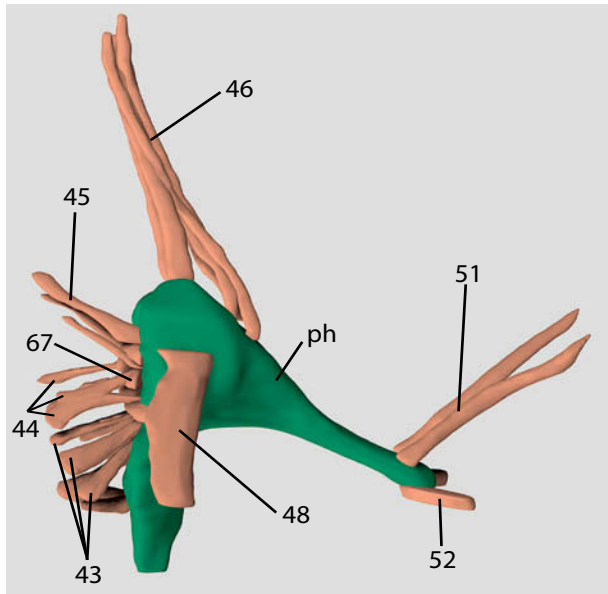
**Musculature:** M. 28: M. submentopraementalis: absent. M. 29: M. tentoriopraementalis inferior: the only premental retractor. O – mesoventral part of the tentorium; I – hind margin of the prementum; F – retractor of the prementum. M. 30: M. tentoriopraementalis superior: absent or completely merged with M. tentoriopraementalis inferior. M. 31: M. praemento-paraglossalis: absent. M. 32: M. praementoglossalis: absent. M. 33: M. praementopalpalis internus: absent. M. 34: M. praementopalpalis externus: apparently absent. M. 35: M. palpopalpalis labii primus: a slender, paired muscle of the labellum; O – laterobasally on the labellum (palpomere 1) I – lateral basal margin of palpomere 2; F – extensor of the labellum. M. 36: M. palpopalpalis labii secundus: absent.

### 3.8. Epi- and hypopharynx

The epipharynx forms the internal wall of the labrum and the roof of the preoral cavity (cibarium) (eph, Fig. 5). It is smooth and unsclerotized. The lateral walls of the epipharynx are lightly bent inwards and form a flat epipharyngeal food channel. The sclerotized hypopharynx is U-shaped in cross section (U dorsally open). Its anterior wall forms the floor of the preoral cavity. The distal hypopharynx is also U-shaped, but it becomes flatter in the posterior region of the preoral cavity. The lumen between the hypo- and epipharynx is rather narrow close to the anatomical mouth opening.



**Fig. 7.** *Axymyia furcata*, male, 3d-reconstruction of head. **A:** Frontal view. **B:** Caudal view. Abbreviations: atp – anterior tentorial pits, b – brain, ce – compound eye, fa – foramen antennale, oc – ocelli, ph – pharynx, ptp – posterior tentorial pits, 7 – *M. labroepipharyngalis*, 43 – *M. clypeopalatalis*, 44 – *M. clypeobuccalis*, 45 – *M. frontobuccalis anterior*, 46 – *M. frontobuccalis posterior*, 48 – *M. tentoriobuccalis anterior*, 51 – *M. verticopharyngalis*, 52 – *M. tentoriopharyngalis*, 67 – *M. transversalis buccae*.



**Fig. 8.** *Axymyia furcata*, male, 3d-reconstruction, part of digestive tract in head. Orientation: ← anterior, ↓ ventral. Abbreviations: ph – pharynx, 43 – M. clypeopalatalis, 44 – M. clypeobuccalis, 45 – M. frontobuccalis anterior, 46 – M. frontobuccalis posterior, 48 – M. tentoriobuccalis anterior, 51 – M. verticopharyngalis, 52 – M. tentoriopharyngalis, 67 – M. transversalis buccae.

**Musculature:** (43, 44, 48, Figs. 5, 7, 8) M. 41: M. frontohypopharyngalis: absent. M. 42: M. tentoriohypopharyngalis: absent. M. 43: M. clypeopalatalis: strongly developed, composed of several bundles arranged in a longitudinal row; O – clypeus; I – on the roof of the cibarium (palatum); F – cibarial dilator. M. 44: M. clypeobuccalis: strongly developed, oblique, directed posteriorly; O – clypeus, immediately posterad of M. clypeopalatalis; I – dorsal wall of the palatum, immediately beside anatomical mouth; F – dilator of the posterior cibarium and mouth opening.

### 3.9. Pharynx

The lateral walls of the pharynx are sclerotized. The broad precerebral pharynx has a wide lumen and appears U-shaped in cross section (U dorsally open). The anterior pharynx is enhanced bag-shaped dorsally (ph, Fig. 5). Laterodorsally, approximately at the level of the antennal bases, two massive apodemes are developed. These are the insertion points of the precerebral dilators, which form the precerebral pumping apparatus, together with M. tentoriobuccalis anterior (M. 48). After the foramen oesophageale of the massive complex formed by the brain and the suboesophageal ganglion, the lumen of the pharynx becomes narrow and it appears elliptic in cross section and continuous with the oesophagus. The dilators of the postcerebral pumping chamber insert at the edges of the foramen occipitale (51, 52, Figs. 5, 7B).

**Musculature:** (45, 46, 51, 52, 67, Figs. 5, 7, 8) M. 45: M. frontobuccalis anterior: O – frons, medially on the level of the lower margin of the antennal insertion areas; I – dorsally on the roof of the anterior pharynx, posterad the ganglion frontale; F – dilator of the anterior pharynx. M. 46: M. frontobuccalis posterior: strongly developed, long muscle; O – posterior frontal region; I – roof of the anterior pharynx, immediately before the brain; F – widens the lumen of the precerebral pharynx. M. 48: M. tentoriobuccalis anterior: strongly developed; O – dorsally on the anterior tentorial arms; I – laterally on the anterior pharynx (dorsolateral apophyses); F – dilator of the precerebral pharynx. M. 49: M. tentoriobuccalis lateralis: absent. M. 50: M. tentoriobuccalis posterior. M. 51: M. verticopharyngalis: long, slender muscle; O – dorsally on the postoccipital region, at the edge of the foramen occipitale; I – dorsal wall of the postcerebral pharynx, immediately posterad the brain. M. 52: M. tentoriopharyngalis: short muscle; O – on the lateral margin of the foramen occipitale; I – ventrolateral wall of the posterior pharynx; F – dilator of the posterior pharynx, together with M. verticopharyngalis. M. 67: M. transversalis buccae: five muscle bands extending over the dorsal wall of the anterior pharynx. The anteriormost band lies immediately posterad of anatomical mouth. M. 68: M. anularis stomodaei: Not recognisable in the  $\mu$ CT data set. M. 69: M. longitudinalis stomodaei: absent.

### 3.10. Salivary duct and glands

The salivary duct opens posteriorly on the hypopharynx, approximately at the level of the labral apex (sd, Fig. 5). It extends posteriorly below the suboesophageal ganglion and enters the thorax. It appears almost circular in cross section close to its anterior opening but its remaining part is transversely oval in cross section and narrowing posteriorly. The salivary duct is only sclerotized at its opening. The salivary glands lie in the prothorax.

**Musculature:** M. 37: M. hypopharyngosalivariialis: apparently absent. Mm. 38/39: Mm. praementosalivariialis anterior/posterior: absent. M. 40: M. anularis salivarii is not visible in the  $\mu$ CT-data set, but it cannot be excluded that the muscle is present.

### 3.11. Nervous system

The compact complex formed by the brain and suboesophageal ganglion lies in the medioventral area of the head and occupies a large proportion of the lumen (b, Fig. 7). The optic lobes are kidney-shaped and less strongly developed in the females. Each lobe is connected with the central part of the protocerebrum by a short, thick optic nerve. The protocerebrum is separated by a

shallow furrow from the deutocerebrum. The three ocelli are connected with the protocerebrum by a single ocellar ganglion and three ocellar nerves (Fig. 5). The deutocerebrum contains the hemispherical antennal lobes where the antennal nerve originates. It is round in cross section. The tritocerebrum is represented by the tritocerebral lobes below the antennal lobes. A tritocerebral commissure is not recognisable as a separate structure, brain and suboesophageal ganglion are largely fused and form a compact structure around the pharynx (b, sog, Fig. 5). The foramen oesophageale between the brain and the suboesophageal ganglion is elliptical. The frontal ganglion is connected with the tritocerebrum by the frontal connectives below the antennal lobes. The nervus frontalis is present, whereas the unpaired nervus connectivus is absent.

### 3.12. Tracheal system

A pair of dorsal tracheae enter the head. Several lateral tracheae originate from them and supply the brain, muscles and foregut with oxygen.

## 4. Discussion

The status as a separate family Axymyiidae was well established in more recent studies (MAMAYEV & KRIVOSHEINA 1966; OOSTERBROEK & COURTNEY 1995; WIEGMANN et al. 2011). Whereas this is widely accepted, the precise position of the group remains largely obscure (see below).

We identified an entire series of apomorphic cephalic characters. The complete subdivision of the compound eyes into a dorsal and ventral part in both sexes is apparently an autapomorphy in Axymyiidae. The dorsal portion is larger than the ventral one in males, and also the ommatidia are larger in the dorsal hemisphere. The compound eyes are undivided in all potentially related dipteran groups (e.g. SCHNEEBERG & BEUTEL 2011).

The absence (or extreme size reduction) of *M. clypeolabralis* is another potential autapomorphy of the family. Its presence is likely a derived groundplan apomorphy of Diptera (SCHNEEBERG & BEUTEL 2011). The muscle is probably homologous to *M. frontolabralis* in other insects (see *M. 8* in v. KELÉR 1963). *M. clypeolabralis* is also missing in adults of Deuterophlebiidae (SCHNEEBERG et al. 2011) and Nymphomyiidae (TOKUNAGA 1935). As the labrum is completely reduced in both groups and a close relationship with Axymyiidae is not supported by other features (and molecular data, e.g., WIEGMANN et al. 2011), we assume that the loss of the muscle occurred independently.

Another complex of potential autapomorphies of Axymyiidae is related to the greatly reduced condition of the maxillae. This includes the complete absence of the endite lobes and the reduction of *M. stipitopalpalis externus* and *M. palpopalpalis primus* and *secundus*. However, the great reduction of the maxillae is possibly a feature shared Axymyiidae, without the newly described *Plesioaxymyia vespertina* (SINCLAIR 2013), whereas they have well-developed mouthparts. Both maxillary endites are also missing in *Tipula* (Tipulidae) (SCHNEEBERG & BEUTEL 2011), *Erioptera* (Limoniidae), *Mycetophila* (Mycetophilidae), *Fucellia* (Anthomyiidae) (HOYT 1952) and *Mayetiola* (Cecidomyiidae) (SCHNEEBERG et al. 2013). The loss apparently occurred independently in different lineages of lower Diptera, as the absence does not correlate with recently suggested phylogenetic patterns (e.g., tree topology in WIEGMANN et al. 2011). This also applies to the missing *M. stipitopalpalis externus*, which is also absent in *Coboldia* (Scatopsidae), *Aedes* (Culicidae) and all examined members of Brachycera (BONHAG 1951; SCHIEMENZ 1957; CHRISTOPHERS 1960; SZUCSICH & KRENN 2000; SCHNEEBERG et al. 2013). The loss of *M. palpopalpalis maxillae primus* and *secundus* is widespread among nematoceran Diptera. Both muscles are absent in *Axymyia*, *Coboldia*, *Spathobdella* (Sciaridae) (SCHNEEBERG et al. 2013) and the brachyceran groups Bombyliidae (SZUCSICH & KRENN 2000) and Syrphidae (SCHIEMENZ 1957). *M. palpopalpalis maxillae secundus* is absent in *Sylvicola* (Anisopodidae), *Wilhelmia* (Simuliidae) (WENK 1962), *Edwardsina* (Blephariceridae) (SCHNEEBERG et al. 2011) and all representatives of Tipulomorpha examined (SCHNEEBERG & BEUTEL 2011), and also in *Tabanus* (Tabanidae) (BONHAG 1951). Apparently Bibionomorpha are characterized by a strong tendency to reduce the maxillary palp musculature (correlated with the reduction of the maxillary palpomere), but the muscles are present in the groundplan as they are preserved in *Bibio* (Bibionidae) and *Mayetiola* (SCHNEEBERG et al. 2013). To which extent this is correlated with a simplification of the palp is unclear. *Bibio* and *Sylvicola* have 5-segmented well developed maxillary palps, whereas they are 3-segmented in *Mayetiola* and *Spathobdella*, and only one palpomere is present in *Coboldia*.

Another potential autapomorphy of the head is the absence of the salivary pump musculature (*M. hypopharyngosalivarialis*). *M. hypopharyngosalivarialis* is present in the groundplan of Diptera and preserved in most taxa examined. However, it is missing in *Axymyia*, *Deuterophlebia* (Deuterophlebiidae) (SCHNEEBERG et al. 2011) and *Limonia* (Limoniidae) (SCHNEEBERG & BEUTEL 2011). Again, parallel loss is very likely. The muscle is present in other members of Tipulomorpha (SCHNEEBERG & BEUTEL 2011) and apparently in the groundplan of this lineage. An extremely reduced cephalic musculature characterizes adults of Deuterophlebiidae (SCHNEEBERG et al. 2011: 8 pairs of cephalic muscles versus more than 40 in adults of Neuropterida), which have a very short life span and are not feeding (COURTNEY 1991). They were



suggested as the sistergroup of all the remaining Diptera by WIEGMANN et al. (2011), which implies that different structures which occur in most dipteran lineages have been reduced independently in *Deuterophlebia*.

Another apparent apomorphy is the origin of *M. tentorioscapalis anterior* (M. 1) on the head capsule. This condition does also occur in *Mayetiola* (SCHNEEBERG et al. 2013), *Limonia*, *Tipula* (SCHNEEBERG & BEUTEL 2011) and *Nymphomyia* (Nymphomyiidae) (TOKUNAGA 1935). In the case of the last two genera this is obviously linked with the complete reduction of the tentorium. Aside from this, there is a general tendency in Diptera to shift the origin of the antennal muscles from the tentorium to the head capsule without a recognizable phylogenetic pattern. The second antennal muscle *M. tentorioscapalis posterior* also originates on the head in *Axymyia*, *Culicoides* (Ceratopogonidae) (GAD 1951), *Edwardsina*, *Deuterophlebia* (SCHNEEBERG et al. 2011), *Tipula* (SCHNEEBERG & BEUTEL 2011), and *Nymphomyia* (TOKUNAGA 1935). *M. tentorioscapalis medialis* also originates on the head capsule in several dipteran groups (*Trichocera*, *Bibio*, *Spathobdella*, *Mayetiola*, *Sylvicola*, *Tabanus*, *Tipula*, *Nymphomyia*; TOKUNAGA 1935; BONHAG 1951; SCHNEEBERG & BEUTEL 2011; SCHNEEBERG et al. 2013). This muscle is completely missing in *Axymyia*, but also in many other dipteran groups such as Deuterophlebiidae (SCHNEEBERG et al. 2011), Simuliidae (WENK 1962), and Culicidae (THOMPSON 1905; SCHIEMENZ 1957; CHRISTOPHERS 1960; OWEN 1985).

As mentioned above the shift of the antennal muscles to the head capsule is often correlated with a partial or complete reduction of the tentorium. In the dipteran groundplan a typical corpotentorium (establishing a left-right connection) is missing whereas anterior, posterior and dorsal arms are well developed (after PETERSON 1916; HENNIG 1973). This condition is described for Simuliidae (PETERSON 1916; WENK 1962) and the brachyceran Tabanidae (BONHAG 1951). In *Wilhelmia* all antennal muscles originate on the tentorium (WENK 1962), whereas *M. tentorioscapalis medialis* is shifted to the head capsule in *Tabanus* (BONHAG 1951). Short vestigial dorsal arms occur in several groups (*Mischoderus* [Tanyderidae], *Culiseta* [Culicidae], *Chironomus* [Chironomidae], *Eristalis* [Syrphidae], *Exoprosopa* [Bombyliidae]; PETERSON 1916; SCHIEMENZ 1957; OWEN 1985). In *Mischoderus* and *Eristalis* all antennal muscles still originate on the tentorium, whereas in *Culiseta* *M. tentorioscapalis medialis* is absent, like in all other members of Culicidae (SCHIEMENZ 1957; SCHNEEBERG & BEUTEL 2011). In most dipteran groups the dorsal arms are completely missing and the tentorium consists of a simple, more or less straight, wide and hollow tube on each side (e.g. *Axymyia*, Deuterophlebiidae, Trichoceridae, Bibionidae, Culicidae [with the exception of *Culiseta*]; SCHIEMENZ 1957; SCHNEEBERG & BEUTEL 2011; SCHNEEBERG et al. 2011). Tentorial structures are completely missing in Tipulidae and Nymphomyiidae and all preserved extrinsic antennal muscles consequently arise from the head capsule (see above; e.g., SCHNEEBERG & BEUTEL 2011; SCHNEEBERG et al. 2011).

Aside from the apomorphic features treated above, the head of *Axymyia* displays many plesiomorphic character states. As it can be assumed for the groundplan of Diptera the head is orthognathous. The presence of three ocelli on the vertex, 5-segmented maxillary palps, 2-segmented labial palps modified as labella, and only one premental retractor (see also SCHNEEBERG & BEUTEL 2011) are apparently also ancestral features.

All exposed parts of the head (except the labrum) are covered with short microtrichia in *Axymyia*. This is also the case in all other dipterans examined, including the two presumably basal families Deuterophlebiidae and Nymphomyiidae (TOKUNAGA 1935; SCHNEEBERG et al. 2011) (also in e.g. *Spathobdella*, *Mayetiola*, *Coboldia*, *Tipula*, *Limonia*, *Trichocera*, *Culex* [Culicidae], *Aedes*, *Edwardsina*; SCHNEEBERG & BEUTEL 2011; SCHNEEBERG et al. 2013).

The epipharyngeal food channel is apparently also a groundplan condition as this structural modification is generally found in dipterans and also in Nannochorista and Siphonaptera (e.g. VOGEL 1921; BONHAG 1951; GAD 1951; WENK 1953; SCHIEMENZ 1957; SNODGRASS 1959; WENK 1962; SUTCLIFFE 1985; SZUCSICH & KRENN 2000; BEUTEL & BAUM 2008; SCHNEEBERG & BEUTEL 2011).

Only few derived characters tentatively indicate systematic affinities between Axymyiidae and other nematoceran groups, notably with families of Bibionomorpha. A placement of Axymyiidae within this infraorder is presently best supported (WIEGMANN et al. 2011). However, there are still controversies on the composition of Bibionomorpha, especially with regard to the inclusion of Axymyiidae (see above).

The interpretation of cephalic features, especially a comparison with other groups assigned to Bibionomorpha, is impeded by a serious lack of detailed data. Information on adult internal head structures is only available for *Bibio*, *Mayetiola*, *Spathobdella*, *Coboldia*, *Sylvicola* and *Axymyia*. Derived cephalic characters shared by *Axymyia* and other bibionomorph taxa are the loss of *M. craniolacinalis* and the loss of the concavity on the dorsal surface of the anterior labium. *M. craniolacinalis* is clearly present in the groundplan of Diptera, but was also reduced in several groups not belonging to Bibionomorpha, such as for instance *Mischoderus*, *Edwardsina* (SCHNEEBERG et al. 2011) and Tipuloidea (*Pedicia*, *Limonia*, *Tipula*; SCHNEEBERG & BEUTEL 2011). The concavity on the dorsal surface of the labium is preserved in Scatopsidae, which suggests that the absence may be an apomorphy of a bibionomorph subgroup which includes Axymyiidae but not Scatopsidae. This would be in agreement with the concepts of Bibionomorpha of OOSTERBROEK & COURTNEY (1995) and WIEGMANN et al. (2011). The concavity is also absent in adults of *Tipula* (SCHNEEBERG & BEUTEL 2011) but present in other members of Tipulomorpha.

Apomorphic features occurring in bibionomorph groups but not in *Axymyia* are the presence of only one precerebral pharyngeal dilator and the origin of *M. tentorioscapalis medialis* on the head capsule. The homology of the single precerebral dilator of Scatopsidae, Bibio-

nidae, Sciaridae, Cecidomyiidae and examined members of Tipuloidea remains uncertain, whereas it is very likely *M. frontobuccalis* posterior in the case of *Deuterophlebia* (SCHNEEBERG et al. 2011). The origin of *M. tentorioscapalis* medialis on the frontal region of the head would be conform with an expanded concept of Bibionomorpha also including Anisopodidae (HENNIG 1973; FRIEDRICH & TAUTZ 1997; BERTONE et al. 2008; WIEGMANN et al. 2011), but an origin on the frons is also described for Trichoceridae and Tabanidae (BONHAG 1951; SCHNEEBERG & BEUTEL 2011). *M. tentorioscapalis* medialis is completely missing in *Axymyia* and it originates on the tentorium in Scatopsidae.

BERTONE et al. (2008) suggested a sistergroup relationship between Axymyiidae and Nymphomyiidae and between these two groups and the infraorder Culicomorpha. Nymphomyiidae is also a small and highly specialized group comprising only seven described species (COURTNEY 1994). This concept is in contrast with all previously suggested placements of Nymphomyiidae, for instance as the sistergroup of the remaining Diptera (HACKMAN & VÄISÄNEN 1982; BERTONE et al. 2008 [reduced data set]), as the second branch in the dipteran tree after Deuterophlebiidae (WIEGMANN et al. 2011), as a member of Blephariceromorpha (WOOD & BORKENT 1989; COURTNEY 1990, 1991; OOSTERBROEK & COURTNEY 1995), or as the sistergroup of Deuterophlebiidae (CUTTEN & KEVAN 1970; SCHNEEBERG et al. 2011, 2012).

The phylogenetic affinities between Axymyiidae and Nymphomyiidae are very likely an artifact of long branch attraction as discussed in BERTONE et al. (2008, p. 683), even though two derived cephalic features of adults are shared by the two groups (SCHNEEBERG et al. 2012), the fusion of the frons and clypeus at least near the midline and the loss of the mandibles incl. their musculature (TOGUNAKA 1935). But both features are reductions and common in the nematoceran dipteran lineages (see discussion in SCHNEEBERG et al. 2013).

It is apparent that most cephalic features of Axymyiidae are either plesiomorphic or autapomorphies of the family. This evidently impedes the systematic placement of the group. Convincing cephalic synapomorphies with other groups are apparently lacking. Taking everything into consideration, especially the comprehensive study of WIEGMANN et al. (2011), a placement in Bibionomorpha appears most likely at present. However, despite of strong efforts and an impressive progress in recent years, a sufficiently robust reconstruction of nematoceran relationships including the placement of Axymyiidae has not been achieved yet.

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### 3.4 Study IV

Neugart C, **Schneeberg K**, Beutel RG. 2009.

The morphology of the larval head of Tipulidae (Diptera, Insecta) – the dipteran groundplan and evolutionary trends. *Zoologischer Anzeiger* 248: 213-235.

#### **Abstract**

External and internal head structures of the larva of *Tipula montium* are described in detail. The results are compared to conditions found in other representatives of Tipuloidea and other dipteran and antliophoran lineages. Despite of the conceivably basal position of Tipulomorpha within Diptera, the larvae are mainly characterized by derived features. The partially retracted head, the specific hemicephalic condition and several other derived character states support the monophyly of Tipuloidea. A clade comprising Tipuloidea excluding Pediciidae is suggested by the strongly retracted head, by deep dorsolateral incisions of the head capsule, by a distinctly toothed anterior premental margin, by the loss of the second extrinsic maxillary muscle, and possibly by the loss of the pharyngeal filter. Eriopterinae and Hexatomiinae are characterized by a tendency towards an extreme reduction of the head capsule. Limoniinae, Cylindrotomidae, and Tipulidae form a clade supported by the presence of a premaxillary suture. This implies the non-monophyly of Limoniidae. A feature shared by Cylindrotomidae and Tipulidae is the presence of a movable lacinia mobilis. However, this is arguably a plesiomorphic feature, as it also occurs in Nannochoristidae. Features of the larval head of Trichoceridae, which were included in Tipulomorpha, do not show affinities with those of Tipuloidea. Trichocerid larvae share a specialized subdivided mandible with larvae of psychodomorph groups. Tipuloidea are a highly specialized group. The characters examined did not reveal plesiomorphic features supporting a basal position, and features suggesting closer affinities with Brachycera are vague. The evolution of dipteran larval head structures was apparently strongly affected by the loss of legs and the tendency to live in cryptic habitats. Diptera are the group of Endopterygota with the highest number of apomorphic features of the larval head. The appendages are generally simplified and the muscular apparatus is strongly reduced. Specialised features evolving within dipteran lineages include specifically arranged brushes of hairs on the labrum and epipharynx, movable messorae, subdivided mandibles, different mandibular brushes, and a far-reaching reduction of labial parts.

#### **Significance in the present thesis**

Tipulolidea is traditionally discussed as the most ‘primitive lineage’ of Diptera. In the study a list of phylogenetic relevant characters and data matrix on the larval head morphology is presented.

#### **Own contribution**

40%



# The morphology of the larval head of Tipulidae (Diptera, Insecta) – The dipteran groundplan and evolutionary trends

C. Neugart, K. Schneeberg, R.G. Beutel\*

*Institut für Spezielle Zoologie and Evolutionsbiologie, FSU Jena, 07743 Jena, Germany*

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## Abstract

External and internal head structures of the larva of *Tipula montium* are described in detail. The results are compared to conditions found in other representatives of Tipuloidea and other dipteran and antliophoran lineages. Despite of the conceivably basal position of Tipulomorpha within Diptera, the larvae are mainly characterised by derived features. The partially retracted head, the specific hemicephalic condition and several other derived character states support the monophyly of Tipuloidea. A clade comprising Tipuloidea excluding Pediciidae is suggested by the strongly retracted head, by deep dorsolateral incisions of the head capsule, by a distinctly toothed anterior premental margin, by the loss of the second extrinsic maxillary muscle, and possibly by the loss of the pharyngeal filter. Eriopterinae and Hexatomiinae are characterised by a tendency towards an extreme reduction of the head capsule. Limoniinae, Cylindrotomidae, and Tipulidae form a clade supported by the presence of a premaxillary suture. This implies the non-monophyly of Limoniidae. A feature shared by Cylindrotomidae and Tipulidae is the presence of a movable lacinia mobilis. However, this is arguably a plesiomorphic feature, as it also occurs in Nannochoristidae. Features of the larval head of Trichoceridae, which were included in Tipulomorpha, do not show affinities with those of Tipuloidea. Trichocerid larvae share a specialised subdivided mandible with larvae of psychodomorph groups. Tipuloidea are a highly specialised group. The characters examined did not reveal plesiomorphic features supporting a basal position, and features suggesting closer affinities with Brachycera are vague. The evolution of dipteran larval head structures was apparently strongly affected by the loss of legs and the tendency to live in cryptic habitats. Diptera are the group of Endopterygota with the highest number of apomorphic features of the larval head. The appendages are generally simplified and the muscular apparatus is strongly reduced. Specialised features evolving within dipteran lineages include specifically arranged brushes of hairs on the labrum and epipharynx, movable messoria, subdivided mandibles, different mandibular brushes, and a far-reaching reduction of labial parts.

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**Keywords:** Tipulidae; Larvae; Head; Anatomy; Phylogeny

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## 1. Introduction

With approximately 15.000 described species Tipulomorpha (sensu Wood and Borkent 1989 = Tipuloidea sensu Hennig 1973) is the most speciose lineage of the

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\*Corresponding author.

E-mail address: [Rolf.Beutel@uni-jena.de](mailto:Rolf.Beutel@uni-jena.de) (R.G. Beutel).

non-monophyletic nematoceran Diptera. Trichoceridae were included in a wider concept of Tipulomorpha by Hennig (1973) and Sary (1992, 2008), but excluded in other recent classifications (e.g., Wood and Borkent 1989; Oosterbroek and Theowald 1991). In Oosterbroek and Theowald (1991) Tipuloidea comprises the families Limoniidae, Cylindrotomidae and Tipulidae, and Limoniidae is divided into the subfamilies Eriopterinae, Hexatomiinae, Limoniinae and Pediciinae (see also Wood and Borkent 1989), but the last group was considered as a family in recent studies (see e.g., Yeates et al. 2007; Ribeiro 2008; World Crane fly Catalog: <http://nlbif.eti.uva.nl/ccw/>).

Tipulidae was considered as the "most primitive family of Diptera" (Foote 1991) for a long time and Tipulomorpha are represented in the fossil record since the Triassic (Blagoderov et al. 2007). The presence of an arolium and a larval lacinia mobilis and the absence of pulvilli (Beutel and Gorb 2001; Oosterbroek and Courtney 1995) were suggested as potential plesiomorphic features of the group, with the corresponding apomorphies in other dipteran lineages. Meanwhile, the hypothesized basal position of Tipulomorpha (e.g., Hennig 1973; Wood and Borkent 1989; Sinclair 1992; Michelsen 1996) has been challenged and a basal position of Culicomorpha + Ptychopteromorpha was suggested by Oosterbroek and Theowald (1991) and Oosterbroek and Courtney (1995) (see also Yeates and Wiegmann 1999 and Yeates et al. 2007) and a basal placement of the highly specialised Nymphomyiidae or Deuterophlebiidae by Hackman and Väisänen (1982) and Bertone et al. (2008), respectively. Recent analyses support either a closer relationship of Tipuloidea with Brachycera (e.g., Oosterbroek and Courtney 1995; Yeates et al. 2003) or re-confirm a basal position of the superfamily (Blagoderov et al. 2007). Considering the diverging branching patterns in recent studies it is apparent that a solid placement of Tipulomorpha and a solid phylogenetic arrangement of the basal grade dipteran lineages have not been achieved yet.

Nematoceran larvae are comparatively well known. They were extensively covered in Hennig's series on dipteran larvae (Hennig 1968a, b), in Stehr's Immature insects (e.g., Foote 1991), and a detailed evaluation of characters of tipuloid immature stages was presented by Oosterbroek and Theowald (1991). However, the knowledge of the anatomy is still quite limited. External and internal head structures of different groups were treated in detail in several studies by Kramer (1954), Anthon (1943a, b, 1988) and Anthon and Lyneborg (1968). However, the musculature and other soft parts are only covered in the first of these studies. The skeleton and musculature of the head of several nematoceran groups, among them representatives of Tipuloidea (*Holorusia rubiginosa*, *Hexatoma* sp. [as *Eriocera* sp.] [Tipulidae], *Dicranota* sp. [Pediciidae]),

were treated in several studies by Cook (1944a, b, 1949). However, the descriptive text is brief and the results were apparently exclusively based on simple dissection techniques. More detailed studies are available for *Bibio* (Perraudin 1961), *Dixa* (Felix 1962), *Phaenobremia* (Cecidomyiidae) (Solinas 1968), and Deuterophlebiidae (Courtney 1990).

The purpose of the present study is to provide detailed anatomical data for the head of the semiaquatic larva of *Tipula montium* and also to give an overview of the presently available morphological data. For this purpose characters of potential phylogenetic value are listed and discussed. Data on the external and internal structures of dipteran larvae were extensively collected from the literature, notably Cook (1944a, b, 1949), Kramer (1954), Anthon (1943a, b, 1988), Courtney (1990, 1994) and Oosterbroek and Theowald (1991). The comprehensive phylogenetic study of Oosterbroek and Courtney (1995) helped greatly to compile the data matrix which is presented as an Electronic Appendix in WinClada format. A cladistic analysis was mainly carried out for a more reliable determination of the character state polarity. We are well aware that the present set of characters of the larval head is insufficient for a clarification of the relationship of nematoceran lineages. The data matrix does also show the still very fragmentary state of the available anatomical data. Clearly, more detailed information and a numerical evaluation of a more comprehensive and complete morphological data set including features of adults is required for a reliable reconstruction of the early evolution of Diptera.

## 2. Materials and methods

### 2.1. Material examined

Diptera, Tipulidae: *Tipula (Yamatotipula) montium* Meigen, 1818 (70% ethanol; SEM, microtome sections; Jena, Germany, C. Neugart coll.)

*Tipula (Pterolachisus) irrorata* Macquart, 1826 (70% ethanol; dissections; Jena, Germany, C. Neugart coll.)

Culicidae: *Culex* spp., *Anopheles* spp. (FAE; microtome sections; Jena, R.G. Beutel coll.)

Bibionidae: *Bibio marci* Linnaeus, 1758 (70% ethanol; SEM, microtome sections; H. Pohl coll.)

Mecoptera, Nannochoristidae: *Nannochorista (Microchorista* auct., *Choristella* auct. nec Bush 1897) *philpotti* Tillyard, 1917 (Pampel's fluid, Bouin, 70% ethanol; SEM, microtome sections, whole mount preparations; Zoologisk Museum, Copenhagen, N. P. Kristensen)

Siphonaptera, Ceratophyllidae: *Ceratophyllus* Curtis, 1832 sp. (70% ethanol; SEM, microtome sections).

Pulicidae: *Synosternus cleopatrae* Rothschild, 1903 (70% ethanol; SEM, microtome sections)

Larvae of *Tipula montium* were collected between stones and leave litter in flat water at the edge of the Saale river at Maua (7 km south of Jena, Thuringia). The larvae were identified with Theowald (1967) and Brindle (1958). The *lateralis*-group (e.g., *Tipula lateralis* Meigen, 1804, *T. couckeii* Tonnoir, 1921, *T. montium* Egger, 1863) is characterised by the arrangement of microtrichia of the abdominal sternites and tergites, and *T. montium* by the color pattern of the tergites and a black pattern on the dorsal marginal lobes (see Brindle 1958).

## 2.2. Morphological methods

The deeply retracted head capsule was removed from the prothorax. Drawings were carried out using a Leica MZ 125 stereo microscope and a camera lucida. The drawings were scanned and processed with Adobe Photoshop 5.5<sup>®</sup> and Illustrator CS<sup>®</sup>. Specimens were embedded in Araldit CY 212<sup>®</sup> (Agar Scientific, Stansted/Essex, England) for sectioning. Cross section and longitudinal section (1.5 µm) series were carried out with a HM 360 (Microm, Walldorf, Deutschland) microtome. The sections were stained with Toluidin blue and Pyronin G (Waldeck GmbH and Co. KG/Division Chroma, Münster, Deutschland), documented with AnalySIS<sup>®</sup> software (Soft Imaging Systems, Münster, Deutschland) and examined with a light microscope Leica DME. For scanning electron microscopy specimens were cleaned with ultra sonic sound (2 × 5'), rinsed with distilled water, dehydrated with ethanol (20–100%) and acetone, and critical point dried and sputter coated with gold. Images were taken with a FEI (Philips) XL 30 ESEM. KOH was used for maceration.

The morphological terminology is based on v. Kéler (1963) (musculature) and Beutel et al. (2009).

## 2.3. Cladistic analysis

A character state matrix (see Electronic Appendix) with 67 external and internal features of the larval head was compiled using the results of the present investigation, characters from Oosterbroek and Courtney (1995), and from other literature sources (see above and list of characters). All characters were equally weighted and considered as non-additive. Several terminal taxa used in Oosterbroek and Courtney (1995) were excluded from the analysis as numerous entries were missing due to lack of information (e.g., Cecidomyiidae, Synneuridae, Corethrellidae). Panorpidae, Nannochoristidae and Pulicidae were used as outgroups.

For character coding and cladistic analyses the combination of WinClada and NONA 2.0 software was used (Nixon 1999–2002; Goloboff 1999). The

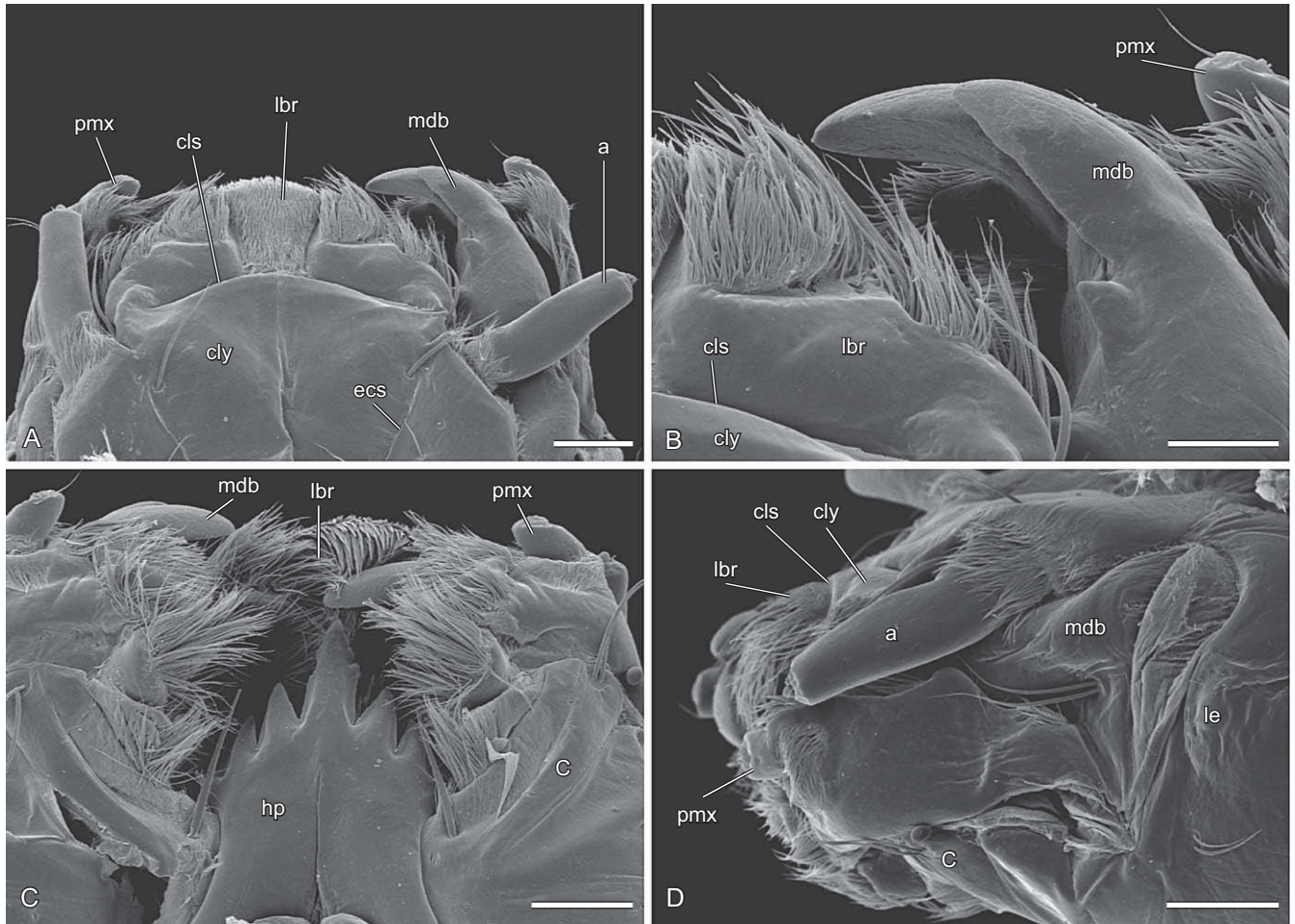
unconstrained analyses were performed using ratchet with the presetting of 1000 replications.

## 3. Results

### 3.1. External features of the head capsule

The prognathous and hemicephalic head capsule is deeply retracted into the prothorax and fixed by the cervical membrane at the articulatory fold close to its anterior margin. It is oval in dorsal view and distinctly compressed dorsoventrally (Fig. 2A). The coloration of most regions is dark brown or blackish. Some oval areas are less strongly pigmented. The exposed anterior parts of the lateral walls are robust and sclerotised. The extensive, apodeme-like internalised posterior parts (externolateral plates; Hennig 1968b) are less strongly sclerotised and characterised by concentric growth lines (gl, Fig. 2). Deep incisions measuring about 1/3 of the maximum length of the head capsule are present dorsolaterally. Anteriorly they are continuous with deep furrows ending at the level of the eyes (Fig. 2A). They separate the lateral genal regions from the dorsal fragment of the head capsule (externo- and internolateralia; Cook 1949; Oosterbroek and Theowald 1991) (ela, ily, Figs. 2 and 7E, F). The margin of the externo-lateralia is strengthened. The transverse strengthening line or frontoclypeal suture is absent. The clypeal region is represented by a narrow triangular plate-like area of the dorsal fragment of the cranium. Six setae and three or four pores are present on either side of it. Two anterior setae are long, two of medium length, and the other two pairs very short. The clypeolabral suture is distinct (cls, Figs. 1A, B, 2A). The dorsal eclypeal sutures (frontal sutures) are present but not meeting posteriorly to form a median coronal suture. They enclose the V-shaped frontoclypeus and reach the base of the labrum anteriorly (fs, Fig. 2A). A distinctly developed dorsal endocarina is present along the midline of the dorsal fragment of the head capsule, which is also reinforced by distinct, paired, paramedian external ridges (ec, Fig. 2A). On the dorsal side close to the mandibular articulation a line is recognisable which serves as attachment area of extrinsic head retractors. A distinct lateral premaxillary suture (Oosterbroek and Theowald 1991) is present. It separates the side plates (Peus 1952: Seitenplatten) from the remaining ventral and lateral sclerotised parts of the head capsule. The ventral wall of the head capsule is strongly reduced. The strongly sclerotised edges of the genal regions are widely separated and diverging posteriorly (Fig. 2). Thus the foramen occipitale appears strongly widened anteriorly. A separate plate-like structure, the hypostomium, is present anteromedially. The undivided larval eye spots are located anterodorsally immediately close to the mandibular articulation. The adjacent areas are more strongly pigmented.





**Fig. 1.** *Tipula montium*, head, SEM images: (A) dorsal view; (B) mandible; (C) ventral view; (D), lateral view. Abbreviations: a, antenna; c, cardo; cls, clypeolabral suture; cly, clypeus; ecs, epicranial suture; hp, hypostomium; lbr, labrum; le, larval eye; mdb, mandible; pmx, maxillary palp. Scale bar: A, C, D, 100  $\mu$ m; B, 50  $\mu$ m.

### 3.2. Endoskeleton

All elements of the tentorium are absent. The hemicephalic head capsule is mainly stabilised by the reinforced margins of the genal regions and the well sclerotised wall of the anterior parts.

### 3.3. Labrum

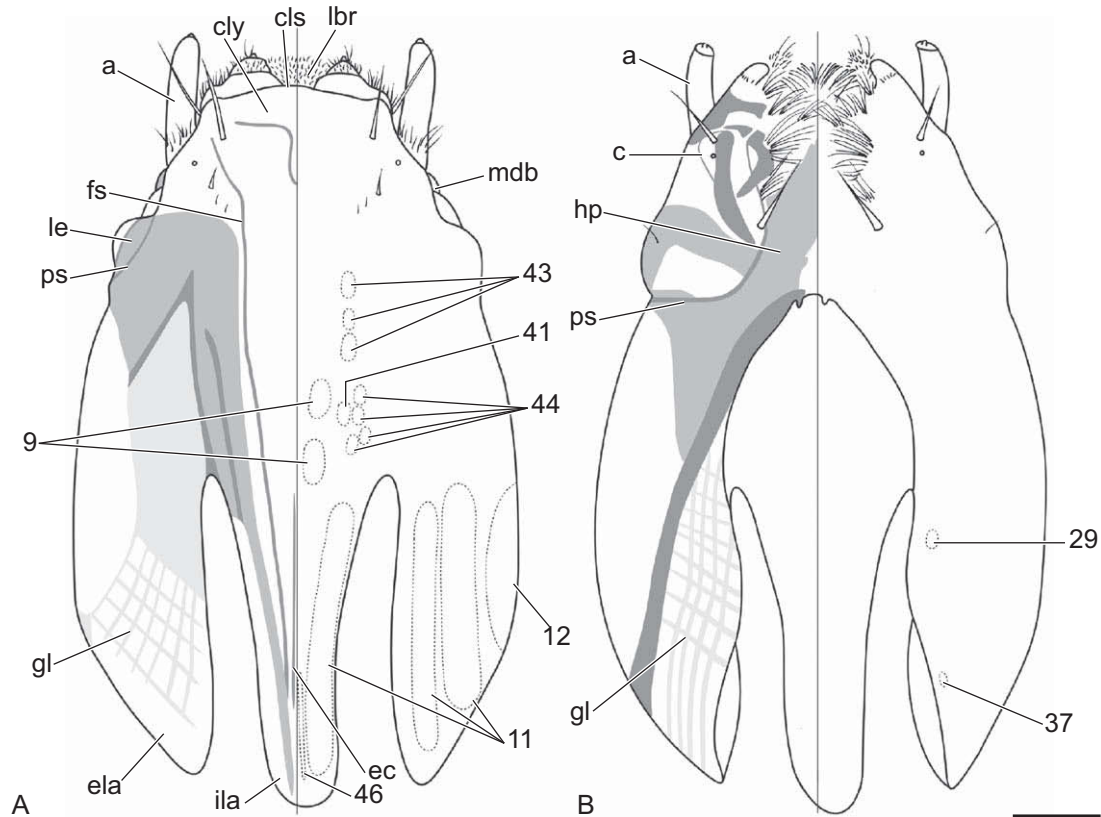
The highly modified tripartite labrum is separated from the anterior margin of the clypeal region by a deep fold (cls, Figs. 1A and 2A). It appears triangular in cross section, with a wedge-shaped, thick, unsclerotised ventral wall (anterior epipharynx, see below) (lbr, Fig. 7A). Dorsally, it is composed of two sclerotised lobes with dense brushes of numerous long and flexible microtrichia along the anterior and lateral margins, interspersed with some long setae. Two pairs of long setae and a short, stout process of the apical region are scarcely visible due to the densely arranged microtrichia

(Figs. 1 and 2). The convex median part of the labrum is enclosed between the mesal edges of the lateral lobes. It is unsclerotised, widening towards its convex anterior margin, and densely covered with anteriorly directed short microtrichia (Fig. 1A). A similar layer of posteriorly directed short microtrichia is present on the anterior epipharynx (Figs. 1C and 2B).

Musculature (nomenclature following v. K ler 1963): M. 7, M. labroepipharyngalis, absent; M. 8, M. frontolabralis, absent; M. 9, M. frontoepipharyngalis, bipartite, with a long tendon, O (= origin): posterior clypeofrontal region, I (= insertion): ventrally, on the epipharyngeal tormae; M. 10, M. epistomalabralis, absent.

### 3.4. Antenna

The antennae is distinctly developed but composed of only one segment. It articulates anteriorly of the mandibular articulation on a membranous field. Numerous microtrichiae of medium length are present at the



**Fig. 2.** *Tipula montium*, head: (A) dorsal view; (B) ventral view. Left: dark-grey for strongly sclerotised regions, light-grey for weakly sclerotised regions; right: muscle origins. Abbreviations: a, antenna; c, cardo; cls, clypeolabral suture; cly, clypeus; ec, endocarina; ela, externo-lateralia; fs, frontal suture; gl, growth lines; hp, hypostomium; ila, internolateralia; lbr, labrum; le, larval eye; mdb, mandible; ps, premaxillary suture 9, *M. frontoepipharyngalis*; 11, *M. craniomandibularis internus*; 12, *M. craniomandibularis externus*; 29, *M. tentoriopraementalis inferior*; 37, *M. hypopharyngosalivariialis*; 41, *M. frontohypopharyngalis*; 43, *M. clypeopalatalis*; 44, *M. clypeobuccalis*; 46, *M. frontobuccalis posterior*. Scale bar: 200  $\mu$ m.

antennal base. The single antennomere is cylindrical, slightly curved, and about 1/3 as long as the maximum head width (a, Figs. 1A, D, 2 and 3). The proximomesal region bears a specialised sensillum. The apex is only very slightly convex and bears a short seta, a moderately large globular sensillum and a group of smaller peg-like sensilla. The sensilla are surrounded by several small, shallow concavities (Fig. 3).

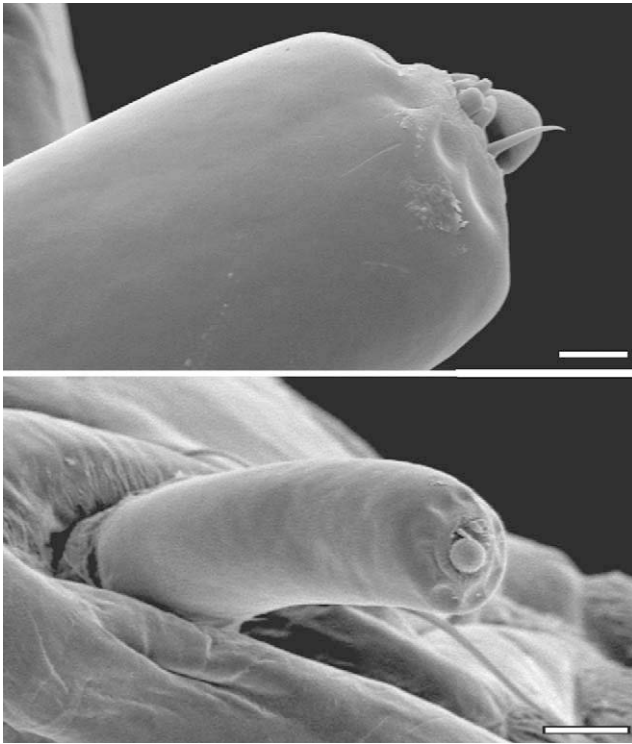
Musculature: *Mm.* 1/2/3/4, *M. tentorioscapalis anterior/posterior/lateralis/medialis*, absent; *Mm.* 5/6: *M. scapopedicellaris lateralis/medialis*, absent.

### 3.5. Mandible

The mandibles (mdb, Figs. 1, 4A, B, 6 and 7B) are one-segmented, strongly sclerotised, and distinctly curved inwards. They operate in a horizontal plane. The dorsal mandibular socket (secondary mandibular joint) articulates with the head capsule posteriorly of the antennal articulation area. The ventral condyle (primary mandibular joint) is part of a conspicuous triangular extension of the ventral mandibular base. The mesal

mandibular wall is distinctly shortened in relation to the lateral wall. Therefore the proximal part of the mandibles is open mesally and the strongly developed adductor tendon inserts far anteriorly of the slightly thinner abductor tendon. Six teeth are present on both mandibles. The largest apically pointed tooth is in an apical position and flanked by two well developed triangular teeth dorsally and ventrally. A truncate, blade-shaped tooth is present proximally of the ventral subapical tooth and two additional teeth close to the base of the mesal wall. The ventral proximal tooth is small whereas the dorsal one is strongly developed. A well developed articulated lacinia mobilis is present on the dorsal side of the mesal mandibular base, very close to the attachment of the adductor tendon (lm, Fig. 4A, B). It is sclerotised, distinctly widening distally, and set with a dense fringe of hairs along its nearly straight apical margin. Combs of hairs on the dorsal surface or on the apical region are absent. The lateral margin of the mandible is slightly bulging proximally and almost evenly curved inwards distally, with the curvature increasing towards the apex. A strong seta is present close to the base of the lateral mandibular wall.

Musculature: M. 11, *M. craniomandibularis internus*, largest muscle of the head, O: large areas on the dorsal and lateral wall of the head capsule; I: adductor tendon, closed to prosthema; M. 12, *M. craniomandibularis externus*, O: lateral wall of head capsule I: abductor tendon, Mm. 13/14, *M. hypopharyngo-mandibularis/zygomatiscus mandibulae*, absent.



**Fig. 3.** *Tipula montium*, head, antenna: (A) dorsal view; (B) lateral view. Scale bar: A, 10  $\mu$ m; B, 30  $\mu$ m.

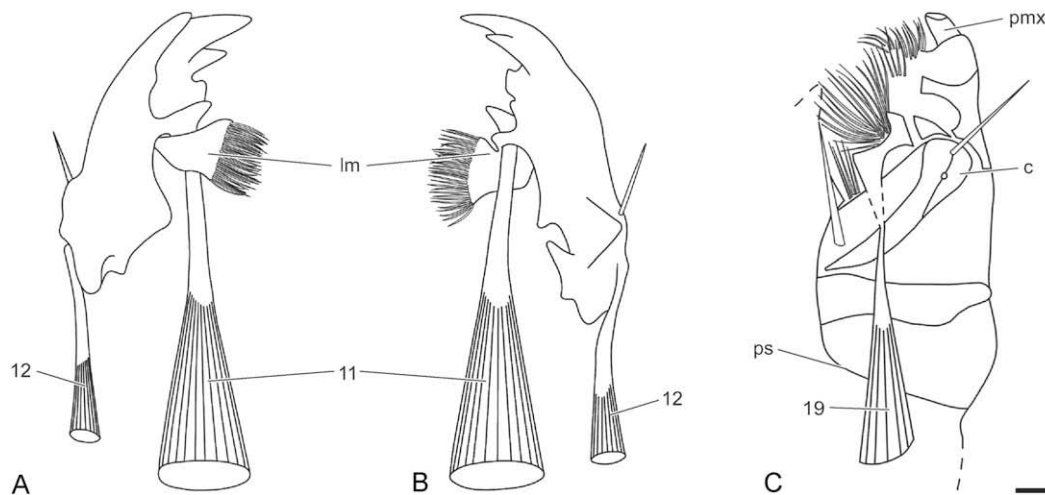
### 3.6. Maxilla

The cardo is visible as a distinctly developed triangular, oblique, slightly curved sclerite on the ventral side (c, Figs. 1C, D, 2B and 4C). A seta is present close to its base and two additional setae close to the anterior margin, which is separated from the distal part of the maxilla by a membranous zone. Stipes, galea and lacinia are not recognisable as separate structures. The mesal side of the distal maxillary element is equipped with a dense fringe of long microtrichiae and an additional brush of long hairs is present proximally (Figs. 1C, 2B, 4C). The short, 1-segmented, cylindrical palp is inserted laterally. It bears two sensilla ventrolaterally (pmx, Fig. 1B). A papilla is present on its membranous apex.

Musculature: Mm. 15/16, *M. craniocardinalis ext./int.*, absent; M. 17, *M. tentoriocardinalis*, absent; M. 18, *M. tentoriostipitalis*, absent; M. 19, *M. craniolacinalis*, well developed, bipartite, O: lateral cranial wall, dorsal part anteriorly of the insertion of M. 12, ventral part anteriorly of the insertion of M. 11, I: mesally on the galeolacinal sclerite; M. 20, *M. stipitlacinalis*, absent; M. 21, *M. stipitogalealis*, absent; Mm. 22/23, *M. stipitopalpalis ext./int.*, absent; intrinsic palp muscles (Mm. 24–27), absent.

### 3.7. Epipharynx

The anteriormost part of the epipharynx, i.e. the ventral wall of the labrum (see above), is densely covered with posteriorly directed microtrichiae (see above). Only few hairs are present on the surface of the middle epipharyngeal region, which is laterally reinforced by the sclerotised, plate-like tormae. Two lobes bearing few setae are present anteriorly of the



**Fig. 4.** *Tipula montium*, head structures: (A) mandible, dorsal view; (B) mandible, ventral view; (C) maxilla, ventral view. Abbreviations: c, cardo; lm, lacinia mobilis; pmx, maxillary palp; ps, premaxillary suture; 11, *M. craniomandibularis internus*; 12, *M. craniomandibularis externus*; 19, *M. craniolacinalis*. Scale bar: 100  $\mu$ m.



tormae, adjacent with a longitudinal row of four conical teeth posteriorly of them. Ventrolateral sclerites are present posterad the median part of the labrum. They are continuous with strongly sclerotised rods (Fig. 7B), which strengthen the lateral epipharyngeal wall and reach the anatomical mouth above the hind margin of the hypopharynx posteriorly.

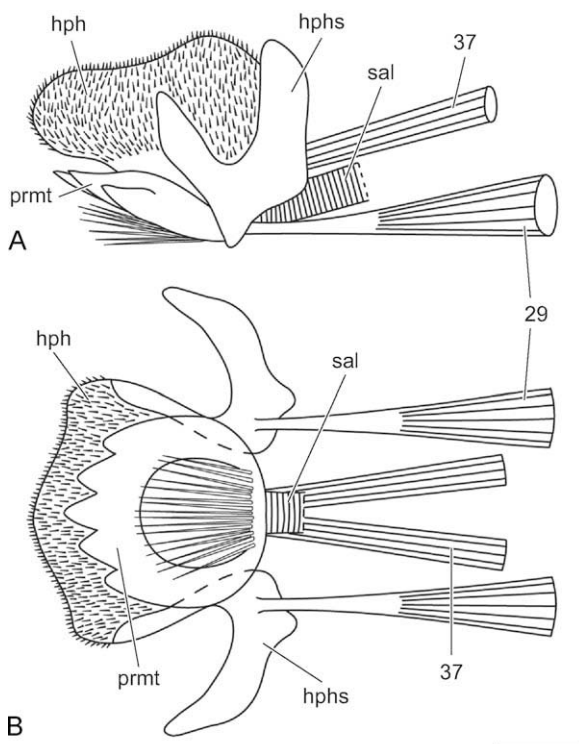
Musculature: M 43, M. clypeopalatalis, dorsal cibarial dilator, O: frontoclypeal region, I: roof of cibarium posteriorly of the epipharyngeal tormae.

### 3.8. Labium and hypopharynx

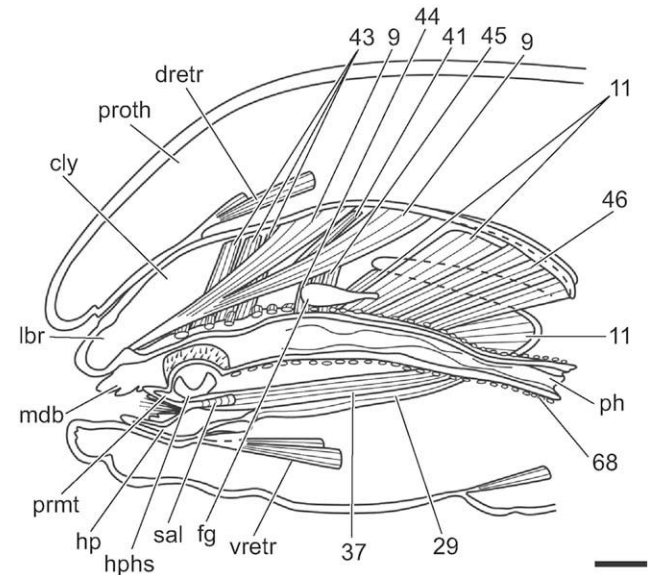
The hypostomium or hypostomal plate, which is at least partly formed by postlabial elements, is strongly sclerotised. It is widening towards its posterior margin and moderately narrowed proximad of its anterior part. The anterior margin is equipped with seven strongly developed triangular teeth. It is medially divided by a triangular membranous field which ends at the anterior third of the sclerite. Basally the hypostomium is fused with the ventral margins of the genae. Two small kidney-shaped sclerotisations are present at its posterior end (Fig. 2B). They serve as attachment devices of the external retractor muscles of the

head capsule. A prementum is present but not visible from below. The entire, nearly round structure is covered by the hypostomium. It bears five slightly rounded teeth at its anterior margin. It is strongly sclerotised except for an oval area on the ventral side, which is covered by a dense group of hairs originating from the posterior premental margin. Palps or other separate prelabial structures are absent. Dorsally the prementum is largely fused with the hypopharynx (prelabio-hypopharyngeal-complex). Both elements are separated medially by the opening of the salivary duct (Figs. 5 and 6). The hypopharynx is spoon-shaped in lateral view, membranous, and densely covered with very short microtrichiae. The lateral arms, the hypopharyngeal suspensoria, are strongly sclerotised (hphs, Figs. 5, 6, 7C). The rounded posterior edge of the U-shaped structure is the attachment area of the only labial retractor.

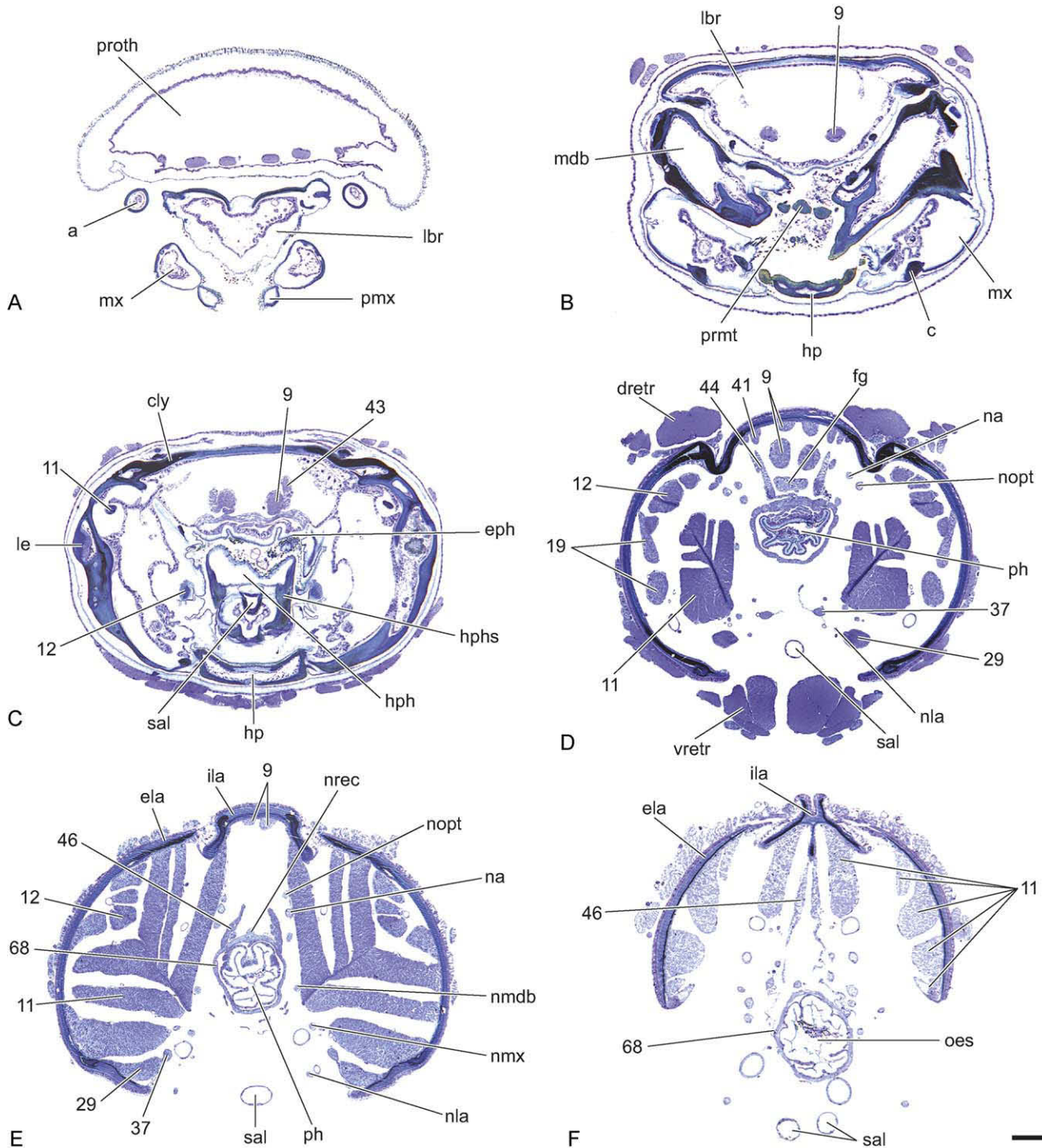
Musculature: M. 29, M. tentoriopraementalis inferior, the only labial muscle, O: ventrolaterally on the cranial wall, I: small process of the hypopharyngeal suspensorium laterad of salivary duct, with a short tendon; M. 41, M. frontohypopharyngalis, O: between M. 9 and M. 44, I: posterolateral edge of the cibarium, anterad the first pharyngeal ring muscle; M.42: M. tentoriohypopharyngalis, absent.



**Fig. 5.** *Tipula montium*, head, prelabio-hypopharyngeal-complex: (A) lateral view; (B) ventral view. Abbreviations: hph, hypopharynx; hphs, hypopharyngeal suspensoria; prmt, prementum; sal, salivary duct; 29, M. tentoriopraementalis inferior; 37, M. hypopharyngosalivarialis. Scale bar: 100  $\mu$ m.



**Fig. 6.** *Tipula montium*, head, sagittal section. Abbreviations: cly, clypeus; dretr, dorsal retractor muscle of the head; fg, frontal ganglion; hp, hypostomium; hphs, hypopharyngeal suspensoria; lbr, labrum; mdb, mandible; ph, pharynx; prmt, prementum; proth, prothorax; sal, salivary duct; vretr, ventral retractor muscle of the head; 9, M. frontoepipharyngalis; 11, M. craniomandibularis internus; 29, M. tentoriopraementalis inferior; 37, M. hypopharyngosalivarialis; 41, M. frontohypopharyngalis; 43, M. clypeopalatalis; 44, M. clypeobuccalis; 45, M. frontobuccalis anterior; 46, M. frontobuccalis posterior; 68, M. annularis stomodaei. Scale bar: 200  $\mu$ m.



**Fig. 7.** *Tipula montium*, head, cross sections: (A–C) cross sections of the anterior head region; (D–F) cross sections of the posterior head region. Abbreviations: a, antenna; c, cardo; cly, clypeus; dretr, dorsal retractor muscle of the head; ela, externo-lateralis; eph, epipharynx; fg, frontal ganglion; hp, hypostomium; hph, hypopharynx; hphs, hypopharyngeal suspensoria; ila, internolateralis; lbr, labrum; le, larval eye; mdb, mandible; mx, maxilla; na, nervus antennalis; nla, nervus labialis; nmdb, nervus mandibularis; nmx, nervus maxillaris; nopt, nervus opticus; nrec, nervus recurrens; oes, oesophagus; ph, pharynx; pmx, maxillary palp; prmt, prementum; proth, prothorax; sal, salivary duct; vretr, ventral retractor muscle of the head; 9, *M. frontoepipharyngalis*; 11, *M. craniomandibularis internus*; 12, *M. craniomandibularis externus*; 19, *M. craniolacinalis*; 29, *M. tentoriopraementalis inferior*; 37, *M. hypopharyngosalivariialis*; 41, *M. frontohypopharyngalis*; 43, *M. clypeopalatalis*; 44, *M. clypeobuccalis*; 46, *M. frontobuccalis posterior*; 68, *M. anularis stomodaei*. Scale bar: 200  $\mu$ m.

### 3.9. Pharynx

The pharynx is unsclerotised and does not form a functional complex with the cibarium. It is slightly wider anteriorly than in the posterior head region. The wall is thick and strongly folded longitudinally. The lumen is very narrow.

Musculature: M. 44, M. clypeobuccalis, two pairs of muscles, O: clypeofrons posteriorly of M. 43, I: anterad the frontal ganglion, dorsal wall of the buccal cavity; Mm. 45/46, Mm. frontobuccales anterior/posterior, slender muscles, posteriorly on the dorsomedian fragment of the head capsule, on a strongly developed septum, I: dorsolaterally on the pharyngeal wall; Mm. 47–50, Mm. tentoriobuccales anterior/lateralis/posterior, absent; M. 51, M. verticopharyngalis, absent; M. 52: M. tentoriopharyngalis, absent; M. 68, M. anularis stomodaei, well developed layer of ring muscles present throughout the entire length of the pharynx; M. 69, M. longitudinalis stomodaei, thin longitudinal muscle strands present on the dorsal side, below the ring muscles.

### 3.10. Salivarium

The salivarium is represented by the salivary duct, which opens between the anterior margins of the prementum and hypopharynx (sal, Fig. 5). The duct bifurcates posteriorly at the level of the insertion of the dorsal pharyngeal dilators. The elongate salivary glands in the prothorax are narrower than the pharynx.

Musculature: M. 37, M. hypopharyngosalivariialis, O: laterally on the cranial wall, posteriorly of M. 29, I: dorsally on a sclerotisation of the salivary duct; Mm. 38/39, Mm. praementosalivariialis anterior/posterior, absent; M. 40, M. anularis salivarii, absent.

### 3.11. Nervous system

The brain and suboesophageal complex are completely shifted to the thorax. They lie at approximately the same level. A subdivision of the brain into proto-, deuto- and tritocerebrum is not recognisable. The frontal ganglion (fg, Figs. 6 and 7D) is connected with the brain by elongate frontal connectives.

## 4. Phylogenetically relevant characters (character state matrix [WinClada format] see Appendix A)

The data presented in the following list of characters are based on our own observations made for ingroup and outgroup taxa, on the extensive data matrix in Oosterbroek and Courtney (1995), and on other

literature sources (e.g., Anthon 1943a, b, 1988; Cook 1944a, b, 1949; Anthon and Lyneborg 1968; Hennig 1968a, b, 1973; Oosterbroek and Theowald 1991; Courtney 1990, 1991, 1994). Following a convention the presumptive plesiomorphic character states (groundplan of Diptera) are coded as 0.

1. (*expo*) **Exposure of head: (0) fully exposed; (1) moderately retracted into prothorax, not fixed in this position; (2) strongly retracted, fixed in this position.** The head of Tipulidae (Fig. 6), Cylindrotomidae and Limoniidae is strongly retracted into the prothorax (Selke 1936; Cook 1949; Chiswell 1955; Hennig 1968b; Peus 1952; Podeniene and Gelhaus 2002; Oosterbroek and Theowald 1991). The head capsule is fixed by the cervical membrane at the articulatory fold close to its anterior margin (Hennig 1968b: Grenzlinie, Fig. 197: gr). In contrast to that, the head of Pediciidae (e.g., Cook 1949 [*Dicranota* Zetterstedt, 1838]; see also Oosterbroek and Theowald 1991: Figs. 93–94 [*Tricyphona* Zetterstedt, 1837]) is only moderately retracted and not fixed in this position (Hennig 1968b). This is likely a groundplan feature of the superfamily, whereas the more advanced condition is a potential synapomorphy of Tipuloidea excl. Pediciidae. Among the nematoceran lineages, a moderately retracted head is also found in Axymyiidae (Mamayev and Krivosheyina 1966; Foote 1991) and a strongly retracted head in Cecidomyiidae (Solinas 1968). The head is largely or fully exposed in most groups (e.g., Deuterophlebiidae, Nymphomyiidae, Trichoceridae, Tanyderidae, Ptychopteridae, Culicidae, Chironomidae, Ceratopogonidae, Blephariceridae [head strongly modified, with large apodemes reaching into the thorax], Bibionidae; Karandikar 1931; Anthon 1943a, 1988; Cook 1944a, b, 1949; Nielsen 1951; Anthon and Lyneborg 1968; Courtney 1990, 1994; Foote 1991). This is apparently the ancestral condition in Diptera, as an exposed head is also found in all other antliophoran larvae (e.g., Beutel et al. 2009). A more or less retracted head does also occur in brachyceran groups such as Tabanoidea or Asilidae (Foote 1991), but it is fully exposed in others (e.g., Xylophagidae, Therevidae; Foote 1991). It is apparent that different degrees of retractability have evolved in different lineages.
2. (*oriemp*) **Orientation of mouthparts: (0) prognathous or slightly inclined; (1) orthognathous.** A distinctly prognathous head as it is found in Tipuloidea is apparently a groundplan feature of Diptera. The same condition is found in the vast majority of nematoceran groups, in orthorrhaphan Brachycera (e.g., Anthon 1943a, b, 1988; Cook 1944a, b, 1949;



Courtney 1990, 1994; Foote 1991), and also in Nannochoristidae (Pilgrim 1972) and Siphonaptera (Sharif 1937). Secondarily orthognathous larval heads occur in Thaumaleidae and some terrestrial Ceratopogonidae (both Culicomorpha), possibly correlated with the formation of prothoracic pseudopods (Hennig 1973; Cook 1944a, 1949; Anthon 1988).

3. (*dlinc*) **Paired dorsolateral incisions: (0) absent; (1) present, short; (2) present, deep, at least reaching anterior half of head capsule.** The head capsule of larvae of Tipuloidea is usually referred to as hemicephalic, an unspecific description for partial reductions occurring in different dipteran lineages. A specific feature of tipuloid larvae is the presence of paired dorsolateral incisions, separating a dorsomedian fragment of the head capsule from the remaining parts (Selke 1936; Cook 1949; Oosterbroek and Theowald 1991: externo- and internolateralia). The incisions are short in larvae of *Dicranota*, *Ula* Haliday, 1837 (partim) and *Tricypbona* (Pediidae; Cook 1949; Oosterbroek and Theowald 1991: Figs. 93–96, 110–112), but at least 1/3 as long as the head capsule in almost all other tipuloid larvae (e.g., Cook 1949; Hennig 1968b; Foote 1991: unusually short in *Prionocera* Loew, 1844 [Fig. 37.89]; Oosterbroek and Theowald 1991: e.g., Figs. 14, 15 [Eriopterinae], 66 [Hexatominiae]; Podeniene and Gelhaus 2002). The presence of the incisions is a potential autapomorphy of Tipuloidea (Oosterbroek and Theowald 1991) and the advanced condition likely a synapomorphy of all subgroups except for Pedidae. An extremely hemicephalic condition is characteristic for Eriopterinae and Hexatominiae (Cook 1949; Oosterbroek and Theowald 1991: e.g., Figs. 33, 68). Incisions in a similar position but distinctly broader and rounded anteriorly are present in larvae of Axymyiidae (Mamayev and Krivosheyina 1966: Fig. 2). They have likely evolved independently. The incisions are generally absent in other nematoceran groups such as for instance Trichoceridae (Karandikar 1931; Anthon 1943a), Tanyderidae (Anthon 1988), *Bittacomorpha* Westwood, 1835 (Ptychopteridae) (Kramer 1954), Bibionidae, Culicidae, and Chironomidae (e.g., Cook 1944a, b, 1949; Foote 1991). The merging of the head with the thorax and the anterior abdominal segment in Blephariceridae (only partly in Edwardsininae) (Anthon and Lyneborg 1968) is probably correlated with tendency to form a hemicephalous condition somewhat similar to that of tipuloid larvae, and short and broad dorsolateral incisions are present in *Liponeura* Loew, 1844 (Anthon and Lyneborg 1968: Fig. 2). However, the head is eucephalic, without incisions in *Edwardsina* (Anthon and Lyneborg 1968: Figs. 3,

7). Different types of hemicephalic conditions (without dorsolateral incisions, scored as 0) have apparently evolved independently in larvae of orthorrhaphan groups such as Tabanidae (head capsule incised medially; Cook 1949: Fig. 24A), Asilidae and Stratiomyidae (Foote 1991) in correlation with a retracted or retractable head (see above). An acephalous condition is characteristic for Cyclorrhapha and has likely evolved independently in the nematoceran Synneuridae (Foote 1991) and in parasitic orthorrhaphan groups.

4. (*vminc*) **Ventromedian incision of head capsule: (0) absent; (1) present; (2) triangular median membranous sinus; (3) ventral head capsule entirely unsclerotised.** A very deep ventromedian incision of the head capsule (Fig. 2B) is present in all tipuloid larvae (Selke 1936; Cook 1949; Hennig 1968b; Peus 1952; Oosterbroek and Theowald 1991; Podeniene and Gelhaus 2002). This is a potential autapomorphy of the superfamily (Oosterbroek and Theowald 1991). The incision is absent in most other nematoceran groups such as Tanyderidae (Anthon 1988: Figs. 8, 25), Ptychopteridae (Kramer 1954), Bibionidae, Culicidae (Cook 1944a, b, 1949) and others, but the ventral head capsule is also reduced in some Blephariceridae (Anthon and Lyneborg 1968: Fig. 4, not in the groundplan: e.g., *Edwardsina* Alexander, 1920, Fig. 3). A triangular median membranous sinus is present in larvae of Simuliidae (Cook 1949: Fig. 7). Different degrees of reduction of the ventral side of the head occur in Brachycera. The ventral head capsule lacks sclerotised parts in *Tabanus*, in *Odontomyia* Meigen, 1803 and in a therevid larva examined by Cook (1949: Figs. 24, 39, 32).
5. (*extpl*) **Externolateral plates with growth lines (intermolt cuticle deposition): (0) absent; (1) present.** Extensive externolateral plates with a zonal structure or growth lines (gl, Fig. 2) are possibly generally present in tipuloid larvae (Hennig 1968b; Oosterbroek and Theowald 1991). They are also well developed in *Mischoderus* Handlirsch, 1909 (absent in other known tanyderid larvae; Anthon 1988 [metacephalic sclerotisation]; Oosterbroek and Courtney 1995) and probably also present in Axymyiidae (Mamayev and Krivosheyina 1966: “Lateral plates with large, elongate, non-sclerotised parts on the dorsal side”), and in Blephariceridae and Deuterophlebiidae (Oosterbroek and Courtney 1995: intermolt cuticle deposition). The phylogenetic interpretation is uncertain presently. It is conceivable that the formation of these structures is linked with the tendency to retract the head capsule and to form a hemicephalic condition. The externolateral plates are usually absent in nematoceran (e.g., Trichoceridae, Ptychopteridae, Culicidae, Chironomidae, Simuliidae, Ceratopogonidae,

- Bibionidae; e.g., Karandikar 1931; Anthon 1943a; Cook 1944a, b, 1949; Nielsen 1951; Foote 1991) and brachyceran larvae (Oosterbroek and Courtney 1995).
6. (*splset*) **Split cranial setae: (0) absent; (1) present.** Split cranial setae are absent in Tipuloidea (e.g., Hennig 1968b), but occur in Tanyderidae (Anthon 1988), Axymyiidae (Mamayev and Krivosheyna 1966), Deuterophlebiidae (Courtney 1990), Culicidae, Chaoboridae, Thaumaleidae, Ceratopogonidae, Simuliidae, Psychodidae (e.g., Hennig 1968a), and also in *Nannochorista*. It is conceivable that this is a groundplan feature of Diptera and a synapomorphy with Nannochoristidae (Beutel et al. 2009).
  7. (*fcslut*) **Transverse facial strengthening line (fronto-clypeal suture): (0) present; (1) absent.** The transverse facial strengthening line or frontoclypeal suture is absent in Tipuloidea and also generally lacking in other dipteran groups (e.g., Cook 1949; Hennig 1968a, 1973). A distinct, unsclerotised clypeal area is present in Pediciidae and other tipuloid larvae (Cook 1949; Oosterbroek and Theowald 1991: Fig. 93 [*Tricyphona*]), but this is not equivalent with the presence of a transverse strengthening line. Remarkably, a separate clypeus is explicitly described for larvae of Axymyiidae (Mamayev and Krivosheyna 1966: Fig. 3). The strengthening line is absent in Siphonaptera (Sharif 1937) but present in Mecoptera (Pilgrim 1972; Bierbrodt 1942; Byers 1987; Beutel et al. 2009).
  8. (*corsut*) **Coronal suture: (0) present at least 25% as long as dorsal wall of head capsule; (1) present, less than 25% of dorsal wall of head capsule; (2) absent.** A long coronal suture is absent in most tipuloid larvae (Hennig 1968b; Oosterbroek and Theowald 1991). However it is present and long in Pediciidae (Cook 1949: "...deep median phragma developed along the line of the coronal suture...", Fig. 18) and does also occur in Eriopterinae (Oosterbroek and Theowald 1991: Figs. 14, 21, 26). It is also well developed in larvae of Bibionidae, Tanyderidae (Anthon 1988) and Axymyiidae (Mamayev and Krivosheyna 1966), whereas it is very short or absent in larvae of Culicidae and Blephariceridae (Cook 1944a, 1949; Anthon and Lyneborg 1968), and probably generally lacking in Cecidomyiidae, Ceratopogonidae, Chironomidae, and Simuliidae (e.g., Cook 1944a, b, 1949; Nielsen 1951; Solinas 1968).
  9. (*shfron*) **Shape of frons: (0) V-shaped; (1) U-shaped** (char. 1 in Oosterbroek and Courtney 1995). The frons, if present as a recognisable delimited structure, is V-shaped in Tipuloidea and most other nematoceran families (e.g., Axymyiidae, Tanyderidae, Deuterophlebiidae, Nymphomyiidae, Trichoceridae; Oosterbroek and Theowald 1991; Oosterbroek and Courtney 1995). It is U-shaped in Culicidae (partim; e.g., Cook 1949), Simuliidae, Dixidae and few other groups (Oosterbroek and Courtney 1995). It is likely that this derived condition has evolved several times independently.
  10. (*prmxsut*) **Premaxillary suture and side plates: (0) absent; (1) present.** A premaxillary suture and side plates are present in Tipulidae (ps, Figs. 2 and 4C), Limoniinae and Cylindrotomidae (Cook 1949: Fig. 14; Oosterbroek and Theowald 1991), and apparently also in larvae of *Mischoderus* (Anthon 1988: Fig. 17). The interpretation as a synapomorphy of Tipulidae, Limoniinae and Cylindrotomidae (Oosterbroek and Theowald 1991) implies parallel evolution in Tanyderidae.
  11. (*stfmext*) **Strengthened margins of externo-lateralialia: (0) absent; (1) present.** The externo-lateralialia are strengthened in Tipulidae, Limoniinae and Cylindrotomidae (Oosterbroek and Theowald 1991). This is a potential synapomorphy of the three groups.
  12. (*endoc*) **Dorsal endocarina: (0) absent; (1) present.** A distinctly developed dorsal endocarina is present in *T. montium* and also in larvae of Pediciidae (Selke 1936; Cook 1949: Fig. 18; Oosterbroek and Theowald 1991: Fig. 93) and Eriopterinae (Oosterbroek and Theowald 1991: Fig. 21). It is missing in most other nematoceran groups (e.g., Ptychopteridae, Chironomidae, Culicidae; Cook 1944a, b, 1949; Kramer 1954), but does occur in larvae of Bibionidae (well developed but not visible externally; pers. obs. Beutel) and Tanyderidae (*Mischoderus*), where it is connected with a tube-like structure interpreted as a gland duct by Anthon (1988, Figs. 5–7). It is apparently also present in Axymyiidae (Mamayev and Krivosheyna 1966: Fig. 2) even though it is not explicitly mentioned by the authors.
  13. (*dta*) **Dorsal tentorial arm: (0) present; (1) absent.** The dorsal tentorial arm is absent in tipuloid larvae and in most other dipteran groups (Cook 1944a, b, 1949; Hennig 1973; Anthon 1943a, b, 1988). A short dorsal arm is present in *Trichocera* Meigen, 1803 (Trichoceridae), Anisopodidae, and possibly in Blephariceridae (Anthon 1943a, b; Denis and Bitsch 1973). It is conceivable that this is a plesiomorphic groundplan condition in Diptera, but reversal cannot be excluded considering the strongly reduced or absent dorsal arm in the other antliophoran groups. The dorsal arm is represented by a delicate ligament-like structure in *Panorpa* Linnaeus, 1758 (Bierbrodt 1942) and *Nannochorista* Tillyard, 1917 (Beutel et al. 2009), and is missing in *Boreus* Latreille, 1816 and *Apterobittacus* MacLachlan, 1893 (Applegarth 1939), and also in Siphonaptera (Sharif 1937; Widhalm-Finke 1974).
  14. (*tbr*) **Tentorial bridge: (0) well developed and sclerotised; (1) partly reduced with thin median connection; (2) absent.** The tentorial bridge is absent in

Tipuloidea, and also missing in Tanyderidae (Anthon 1988: tentorium vestigial, anterior arm not connected with posterior head capsule), Blephariceridae, Culicidae, Chironomidae, Dixidae, Simuliidae, and Brachycera (Felix 1962; Denis and Bitsch 1973; Cook 1944a, b, 1949). It is apparently present but scarcely fused medially in *Olbiogaster* (Anthon 1943b) and Bibionidae (narrow thread; Perraudin 1961), and does also occur in Mycetophilidae (connected by a thin, unscletrised thread in *Mycetophila fungorum* [De Geer, 1776]) and possibly in Sciaridae (Perraudin 1961; Denis and Bitsch 1973). The tentorial bridge is present in *Nannochorista* (Beutel et al. 2009) but is absent in Siphonaptera (Sharif 1937; Widhalm-Finke 1974).

15. **(ata)Anterior tentorial arms: (0) present; (1) vestigial or absent; (2) arising from paraclypeal phragma and strongly developed.** The anterior arms are largely reduced (e.g., Limoniinae; Lindner 1959) or completely absent in Tipuloidea (*T. montium*, *Holorusia* Loew, 1863, *Hexatoma* Latreille, 1809, *Dicranota*; Cook 1949). The tentorium including its anterior parts is also largely reduced or absent in Scatopsidae (Wood and Borkent 1989), *Chironomus* Meigen, 1803, Simuliidae, Sciaridae, and Mycetophilidae (Cook 1944a; Denis and Bitsch 1973). The anterior arms are present in Trichoceridae, Tanyderidae (Anthon 1988), Anisopodidae (*Olbiogaster* Osten-Sacken, 1886; Anthon 1943b), Bibionidae (Cook 1949), Culicidae (e.g., *Culiseta* Felt, 1904, *Anopheles* Meigen, 1816; Cook 1949), Chironomidae (partim; Cook 1944b, 1949), Dixidae, Blephariceridae, Cecidomyiidae (Denis and Bitsch 1973), and Ptychopteridae (Anthon 1943a). They are strongly developed and arise from the paraclypeal phragma in Tabanidae and Therevidae (Cook 1949).
16. **(cutlens)Cuticular lense: (0) present; (1) absent.** A cuticular lense is generally lacking in nematoceran larvae with developed larval eyes (e.g., Cook 1949; Hennig 1973; Wood and Borkent 1989). This is also the case in the aquatic larvae of Nannochoristidae (Melzer et al. 1994). The eyes are absent in many groups of Diptera (e.g., Hennig 1973) and also in Siphonaptera (e.g., Sharif 1937). Interestingly, a distinct lateral eye with a convex cuticular lense is present in *Odontomyia* (Stratiomyidae) (Cook 1949).
17. **(latey)Lateral eyes: (0) simplified compound eyes; (1) several stemmata; (2) eye spot; (3) absent.** The presumably ancestral condition for Diptera, the presence of a single or bipartite larval eye, is found in Tipulidae (e.g., Cook 1949), and eye spots also occur in Trichoceridae, Bibionidae, Nymphomyiidae, Deuterophlebiidae (widely separated), Ptychopteridae, Culicidae, Dixidae, Chironomidae, Simuliidae, Stratiomyidae and Rhagionidae (e.g., Constantineanu 1930; Anthon 1943a; Cook 1944a, b, 1949; Felix 1962; Hennig 1973; Courtney 1990, 1994; Foote 1991). Eyes are absent in *Hexatoma* (Hexatominae) and *Dicranota* (Pediidae), in Blephariceridae (Anthon and Lyneborg 1968: Figs. 2–9), *Rhyphus* Latreille, 1804 (Anisopodidae), *Philosepedon* Eaton, 1904 (Psychodidae) (Anthon 1943a), and also in the tabanid and therevid larva described by Cook (1949). Preformed adult compound eyes occur in Culicidae, Chaoboridae and Chironomidae (Cook 1944a, b, 1949; Hennig 1973). Simplified compound eyes without crystalline cone occur in Mecoptera (Melzer et al. 1994) and in symphytan larvae (e.g., Beutel et al. 2008). Several stemmata are present in larvae of Neuropterida (e.g., Beutel and Friedrich 2008) and larvae of other endopterygote lineages (e.g., Strepsiptera; Pohl 2000).
18. **(lbrfus)Articulation of labrum: (0) free; (1) partially fused with head capsule; (2) completely fused with head capsule.** A free labrum is generally present in most tipuloid larvae (e.g., *Dicranota*; Cook 1949), and in most other nematoceran lineages (e.g., Tanyderidae, Axymyiidae, Culicidae, Blephariceridae; Anthon 1943a, b, 1988; Cook 1944a, b, 1949; Kramer 1954; Mamayev and Krivosheyna 1966). It is also free in larvae of Mecoptera (Pilgrim 1972; Byers 1987) and Siphonaptera (Sharif 1937). The clypeolabral fold is partly reduced in Bibionidae (Cook 1949) and the labrum is completely fused with the head capsule in Trichoceridae, Sciaridae, Cecidomyiidae, and Simuliidae (Cook 1949; Anthon 1943a, 1988; Foote 1991), and apparently also in Nymphomyiidae (Courtney 1994: clypeolabrum, Fig. 51). It is also usually fused in brachyceran larvae (e.g., *Tabanus* Linnaeus, 1758, *Odontomyia*, Therevidae; Cook 1949).
19. **(lbrsh)Shape of labrum: (0) transverse; (1) narrow and conical** (char. 3 in Oosterbroek and Courtney 1995, modified). A transverse labrum is present in Tipulidae (lbr, Figs. 1A, 2A) and most other tipuloid groups (e.g., Pediidae; Selke 1936; Peus 1952; Oosterbroek and Courtney 1995), and also in other lineages of Diptera (e.g., Tanyderidae, Ptychopteridae; Anthon 1988; Kramer 1954). This is apparently a plesiomorphic feature. The labrum of larvae of Deuterophlebiidae is about as wide as long (Courtney 1990) and represents an intermediate condition (coded as 0 here). A narrow and conical labrum is present in Eriopterinae, Cylindrotomidae, Axymyiidae, Nymphomyiidae, Trichoceridae, Psychodidae, Anisopodidae, Scatopsidae, Blephariceridae, Thaumaleidae, and does also occur in Brachycera (e.g., *Tabanus*, *Odontomyia*, Therevidae) (Anthon 1943a; Cook 1949; Mamayev and Kri-



- vosheyina 1966; Courtney 1990, 1994; Oosterbroek and Courtney 1995). This feature was suggested as an autapomorphy of Psychodomorpha by Oosterbroek and Theowald (1991). However, the presumably plesiomorphic condition is present in some psychodids and in Synneuridae, and it is apparent that this feature has evolved several times independently in Diptera.
20. (*lbrpar*) **Subdivision of labrum: (0) absent; (1) present.** A distinctly tripartite labrum as it is present in Tipulidae (Figs. 1A; Selke 1936; Cook 1949: *Holorusia*; Chiswell 1955) is a potential autapomorphy of the family. The labrum is distinctly narrowed (see previous character) or transverse and undivided (e.g., Pediciidae) in the other tipuloid groups (e.g., Cook 1949; Peus 1952; Oosterbroek and Theowald 1991).
  21. (*lbrbru*) **Labral brush with dense field of hairs: (0) absent; (1) present, without specific arrangement; (2) specifically arranged labral brush; (3) complex arrangement of different types of hairs; (4) macrosetae** (char. 5 in Oosterbroek and Courtney 1995, modified). Dense fields or brushes of microtrichiae are present on the labrum of tipuloid larvae (Figs. 1A, 2A; e.g., *Gonompeda* Alexander, 1924, *Cylindrotoma* Macquart, 1834, *Holorusia*, less strongly developed *Dicranota*; Cook 1949; Peus 1952; Podeniene and Gelhaus 2002), and they also occur in other nematoceran lineages such as Tanyderidae, Trichoceridae, Anisopodidae, Ptychopteridae, Psychodidae, and Blephariceridae (Anthon 1943a, b, 1988; Anthon and Lyneborg 1968; Kramer 1954). Specifically arranged setal brushes are present in Culicidae, Simuliidae (Cook 1949: on messorial arms) and Dixidae (Cook 1944a, 1949; Felix 1962; Denis and Bitsch 1973), a complex arrangement of different types of hairs is present in *Chironomus* (Cook 1944b: Fig. 38), and specialised spatulate macrosetae in Nymphomyiidae and Deuterophlebiidae (Courtney 1990, 1994). It is conceivable that dense labral brushes are a derived groundplan feature of Diptera.
  22. (*lbrtth*) **Labral teeth: (0) very small or absent; (1) present.** Strongly sclerotised labral teeth are usually absent in Tipuloidea (e.g., Cook 1949; Podeniene and Gelhaus 2002), but occur in larvae of Cylindrotomidae (Peus 1952). Large teeth are also present in *Olbiogaster* (Anthon 1943b) and very small teeth in *Liponeura* (Blephariceridae; Anthon and Lyneborg 1968). Distinct teeth are absent in Axymyiidae (Mamayev and Krivosheyina 1966), Tanyderidae, Bibionidae, Trichoceridae, Deuterophlebiidae, Blephariceridae and other nematoceran lineages (e.g., Anthon 1943a, b, 1988; Cook 1944a, b, 1949; Nielsen 1951; Courtney 1990).
  23. (*tormcon*) **Connection of torma with labral sclerite: (0) tormae firmly connected with labral sclerite; (1) articulated** (char. 12 in Oosterbroek and Courtney 1995, modified). Tormae articulating with the labral sclerite occur in Tipuloidea (e.g., Pediciidae, Limoniinae), and also in Anisopodidae, Nymphomyiidae, Deuterophlebiidae (partim), Trichoceridae, Ptychopteridae, Blephariceridae, Ceratopogonidae, Chironomidae, Culicidae and some other groups (Oosterbroek and Theowald 1991; Courtney 1990, 1994; Oosterbroek and Courtney 1995). They are firmly connected with the labral base in Axymyiidae, Bibionidae, Cecidomyiidae, Mycetophilidae and Sciaridae (Oosterbroek and Theowald 1991; Oosterbroek and Courtney 1995). The tormae are absent in Siphonaptera (see M. 9; Sharif 1937).
  24. (*M7*) **M. labroepipharyngalis (M. 7): (0) present; (1) absent.** M. 7 is absent in *T. montium* but is present in *Hexatoma* (Cook 1949: labral compressor). The muscle is also present in *Mischoderus*, *Chironomus*, *Bittacomorpha* (Cook 1949; Kramer 1954) and *Tabanus* (Cook 1949), and in larvae of Mecoptera and Siphonaptera (Sharif 1937; Beutel et al. 2009). The unusual transverse intrinsic muscle described by Courtney (1990) for Deuterophlebiidae is almost certainly not homologous with M. 7 (coded as 1).
  25. (*M8*) **M. frontolabralis (M. 8): (0) present; (1) absent.** M. 8 is absent in *T. montium* and is probably generally lacking in dipteran larvae (e.g., Cook 1949). The frontal labral muscle (Cook 1944a, b, 1949; Kramer 1954; see also Hennig 1973) is never attached to the external basal margin of the labrum but to tormae or torma fragments or to the epipharyngeal roof. Therefore it is apparently homologous with M. frontoepipharyngalis (M. 9), even though the attachment of a subcomponent may be shifted mesally (e.g., Bibionidae; Perraudin 1961: rétracteur de l'épipharynx; Simuliidae, Culicidae; Cook 1949: median palatal muscle; see also Chaudonneret 1963 and Beutel et al. 2009).
  26. (*M9*) **M. frontoepipharyngalis (M. 9): (0) present; (1) absent.** M. frontoepipharyngalis is present in *T. montium*, like in other tipulid larvae and in larvae of *Bibio* Geoffroy, 1762, *Chironomus*, *Simulium* Latreille, 1902, *Dixa* Meigen, 1818, Culicidae, and *Bittacomorpha* (see previous character; Cook 1944a, b, 1949; Kramer 1954; Felix 1962). The muscle is extremely strong and medially intercrossing in *Simulium* (Cook 1949). This apparently correlated with the highly specialised messorial arms (see next character).
  27. (*messor*) **Movable premandible (messores): (0) absent; (1) present, separated from basal part by weakly sclerotised zone; (2) present as strongly developed movable messorial arms** (char. 7 in Oosterbroek and Courtney 1995, modified). A movable premandible is absent in Tipulidae and other tipuloid subgroups (Cook 1949), with some possible exceptions in Limoniidae (Hexatominae, Eriopterinae; Oosterbroek and Courtney 1995). It is also absent in

Nymphomyiidae, Deuterophlebiidae (Courtney 1990, 1994; Oosterbroek and Courtney 1995), Axymyiidae, Bibionidae, Blephariceridae and some other groups (e.g., Chaoboridae), and also missing in Brachycera (Oosterbroek and Courtney 1995). They are present in most nematoceran groups such as Anisopodidae, Ceratopogonidae, Culicidae, Psychodidae, Ptychopteridae, and Trichoceridae (Anthon 1943a, b; Oosterbroek and Courtney 1995). Strongly developed movable messorial arms form a sweeping device in Simuliidae (Cook 1949).

28. (*expepi*) **Exposure of anterior epipharynx (cibarial roof): (0) not or only slightly exposed; (1) largely exposed.** The anterior epipharynx is largely exposed in Tipulidae (e.g., *Tipula*, *Holorusia*), *Hexatoma* (labium completely reduced), and Cylindrotomidae (Selke 1936; Cook 1949; Peus 1952). It is also partly exposed but to a much lesser degree in Pediciidae (coded as 0; Cook 1949; Oosterbroek and Theowald 1991: Fig. 94). A largely exposed anterior epipharynx is also present in larvae of Nymphomyiidae, Deuterophlebiidae (Courtney 1990, 1994), Chironomidae, Culicidae (partim), Bibionidae, Simuliidae (Cook 1944a, b, 1949), Dixidae (Felix 1962), Tanyderidae, Trichoceridae, Psychodidae, Ptychopteridae (Anthon 1943a, b, 1988; Kramer 1954), Blephariceridae (partim: *Edwardsina*; Anthon and Lyneborg 1968: Fig. 4), and *Odontomyia* (Cook 1949), and also in nannochoristid larvae (Beutel et al. 2009). It is conceivable that this is a dipteran groundplan feature and a synapomorphy of Diptera and Nannochoristidae. The anterior epipharynx is concealed in larvae of *Tabanus* (Cook 1949), and in Mecoptera excl. Nannochoristidae (Bierbrodt 1942; Beutel et al. 2009) and the condition is unclear in Axymyiidae.
29. (*posant*) **Position of antennal insertion: (0) lateral; (1) close to midline.** The antenna is strongly shifted mesad in larvae of Cylindrotomidae, and a similar condition occurs in Eriopterinae (e.g., *Rhypholophus* Kolenati, 1860) and to a lesser degree in some Hexatomininae (e.g., *Conosia*) (Peus 1952; Oosterbroek and Theowald 1991) and Nymphomyiidae (Courtney 1994). It is likely that the apparently derived condition has evolved several times in correlation with the formation of a narrow, conical labrum.
30. (*antseg*) **Antennal segmentation: (0) with basal antennomere and one or several distinctly developed distal segments; (1) appearing 1-segmented, distal segment vestigial or absent; (2) antenna vestigial.** The antenna of *T. montium* appears 1-segmented like in other larvae of Tipulidae, Cylindrotomidae and Limoniinae (Selke 1936; Cook 1949; Peus 1952; Chiswell 1955) and in larvae of Nymphomyiidae (Courtney 1994 [in contrast to Foote 1991]), *Bittacomorpha*, Blephariceridae (elongated in *Liponeura*, short in *Edwardsina*; Denis and Bitsch 1973), Anisopodidae (distal segment disc-like, strongly reduced; Anthon 1943a, b), Trichoceridae, Dixidae, Chaoboridae, Culicidae (e.g., *Anopheles*, *Aedes* Meigen, 1818, *Culex* Linnaeus, 1758), *Chironomus*, *Tabanus* and *Odontomyia*. The antenna is very long, flattened, bifurcated and 2-segmented in Deuterophlebiidae (Courtney 1990), and also composed of two antennomeres in *Dicranota* (Pediciidae), Tanyderidae, Cecidomyiidae (Solinas 1968), and Anisopodidae (Anthon 1943a, 1988). It is 3-segmented in Nymphomyiidae (Courtney 1994) and Bolitophilidae, and thin and 4-segmented in Simuliidae (e.g., *Simulium*) (Denis and Bitsch 1973; Cook 1944a, b, 1949; Kramer 1954). The antenna is absent in *Pedicia* Latreille, 1809 (Pediciidae) and vestigial in Bibionidae (small elevation with sensorial papillae; e.g., Perraudin 1961) and Axymyiidae (Mamayev and Krivosheyna 1966). It is very small (Cook 1949) or only represented by a structure resembling a seta in Therevidae (Denis and Bitsch 1973). The larval antennae are 2-segmented in Siphonaptera (e.g., Sharif 1937) and Boreidae, and 3-segmented in other groups of Mecoptera (Pilgrim 1972; Byers 1987).
31. (*exantm*) **Extrinsic antennal muscles: (0) present; (1) absent.** Extrinsic antennal muscles are lacking in *T. montium* like in other tipuloid larvae examined (Cook 1949). They are also absent in *Biblio* (Cook 1949) and apparently also in Chironomidae and Simuliidae (Cook 1944b, 1949: Figs. 5–8), whereas a single muscle is present in Deuterophlebiidae (Courtney 1990), Dixidae (Denis and Bitsch 1973), *Bittacomorpha* (Kramer 1954), Culicidae (Cook 1944a, 1949; Schremmer 1949), and also in *Tabanus* (Cook 1949). Apparently they are almost generally absent in Brachycera (e.g., Therevidae, Stratiomyidae, all Cyclorrhapha; Cook 1949; Hennig 1973).
32. (*mdmxc*) **Mandibulo-maxillary complex: (0) absent; (1) present.** A mandibulo-maxillary complex is absent in all nematoceran lineages (e.g., Anthon 1943a, b, 1988; Cook 1944a, b, 1949; Kramer 1954). The presence (e.g., Cook 1949) is likely an autapomorphy of Brachycera (Anthon 1988).
33. (*mdplop*) **Plane of operation of mandibles: (0) horizontal or slightly oblique; (1) distinctly oblique or vertical; (2) horizontal in first larval stage and oblique or vertical in later instars** (char. 18 in Oosterbroek and Courtney 1995, modified). The plane of operation is horizontal in Tipulidae (e.g., *T. montium*, *Tanyptera* Latreille, 1804; Hennig 1973: Fig. 38) and in most other groups of Tipuloidea (e.g., Oosterbroek and Theowald 1991). It is also horizontal or slightly oblique in Pediciini (e.g., *Dicranota*; Cook 1949) but distinctly oblique in *Ula*, like in larvae of

- Eriopterinae and Hexatomiinae (Oosterbroek and Theowald 1991). In Cylindrotomidae they move horizontally in the first larval stage but vertically in later instars (Oosterbroek and Theowald 1991). Horizontally moving mandibles are present in the outgroup taxa and also in larvae of Tanyderidae (Anthon 1988), Axymyiidae (Mamyev and Krivosheyna 1966), Blephariceridae, *Trichomyiinae* (Psychodidae), and in some Chironomidae. The axis of movement is slightly oblique in *Biblio* (Cook 1949: 75°, coded as 0), but distinctly oblique or nearly vertical in Nymphomyiidae, Deuterophlebiidae, Ptychopteridae, Culicidae, Chironomidae (with few exceptions), Thaumaleidae, Simuliidae, Ceratopogonidae, Blephariceridae, Trichoceridae, Anisopodidae, Psychodidae (partim) and in the orthorrhaphan lineages (Cook 1944a, b, 1949; Kramer 1954; Anthon 1943a, b; Anthon and Lyneborg 1968; Oosterbroek and Courtney 1995). The mandibular mouth hooks of cyclorrhaphan larvae are always moved parallel to the body axis in vertical direction.
34. (*mdjpos*) **Position of the secondary mandibular joint: (0) not shifted posteriorly of the antennal foramen; (1) shifted posteriorly of the antennal foramen.** The unusual position of the secondary mandibular joint posteriorly of the antennal articulation area is a potential autapomorphy of Tipulidae. This condition is not found in the other groups of Tipuloidea (e.g., Cook 1949; Peus 1952). A slight posterior shift of the secondary joint was also observed in Simuliidae, Culicidae and Chironomidae by Cook (1944a, b, 1949), however to a much lesser degree than in Tipulidae (coded as 0). The dorsal mandibular articulation lies at a level with the antennal foramen.
35. (*mdsh*) **Shape of mandible: (0) without distinctly elongated distal part; (1) distal part elongated, sickle-shaped.** The distal part of the mandible is short and stout in most tipuloid larvae and larvae of other nematoceran groups (e.g., Axymyiidae, Tanyderidae, Trichoceridae, Simuliidae, Anisopodidae, Psychodidae, Ptychopteridae, Blephariceridae; Anthon 1943a, b, 1988; Anthon and Lyneborg 1968; Cook 1949; Mamyev and Krivosheyna 1966). It is sickle-shaped and elongated in some larvae of Hexatomiinae and in Pediciidae (Cook 1949; Oosterbroek and Theowald 1991).
36. (*lcmob*) **Movable lacinia mobilis: (0) present; (1) absent.** An articulated lacinia mobilis is present in *T. montium* (lm, Fig. 4A, B) and other Tipulidae, and also in larvae of Cylindrotomidae and Nannochoristidae (Wood and Borkent 1989; Oosterbroek and Theowald 1991; Pilgrim 1972). It is absent from other groups of Diptera and Mecoptera (Beutel et al. 2009). The presence is arguably a groundplan feature of Diptera and a synapomorphy with Nannochoristidae. However, considering the absence in almost all groups of Diptera including most tipuloid lineages (Pediciidae, Eriopterinae, Hexatomiinae, Limoiniinae) it appears more likely that it has independently evolved in Tipulidae and Cylindrotomidae. It is evidently a potential synapomorphy of both families.
37. (*mdhook*) **Anteriorly directed cone of mesal mandibular edge: (0) absent; (1) present** (char. 23 in Oosterbroek and Courtney 1995). A distinctly developed, anteriorly directed toothed cone is present on the mesal side of the mandible in Anisopodidae, Trichoceridae, Psychodidae (partim), and Scatopsidae (Anthon 1943a: “Haken”, Figs. 26, 29, 33, 35[kr]; Hennig 1968a: Fig. 22). Mesal processes occurring in some hexatomine larvae are clearly different structurally, even though they also likely interact with a movable apical part of the mandible (Oosterbroek and Theowald 1991: Fig. 61).
38. (*mdcomb*) **Mandibular comb on dorsal surface: (0) absent; (1) present** (char. 21 in Oosterbroek and Courtney 1995). A mandibular comb is absent in *T. montium* and in larvae of the other tipuloid groups (e.g., Peus 1952; Oosterbroek and Theowald 1991; Podeniene and Gelhaus 2002). It is present in Dixidae, Scatopsidae, Trichoceridae, Culicidae, Chironomidae (partim), Anisopodidae (e.g., *Mycetobia* Meigen, 1818) and Ptychopteridae (Anthon 1943a; Denis and Bitsch 1973; Hennig 1973; Wood and Borkent 1989; Oosterbroek and Courtney 1995). It is absent in *Olbiogaster*, in most groups of Culicomorpha, in Brachycera, and also in Mecoptera (incl. Nannochoristidae) and Siphonaptera (e.g., Byers 1987; Sharif 1937).
39. (*mdcoop*) **Apical multitoothed mandibular comb: (0) absent; (1) present (at least in instar 1)** (char. 22 in Oosterbroek and Courtney 1995). An apical multitoothed mandibular comb is absent in Tipuloidea and most other nematoceran groups. It is present in Deuterophlebiidae, Nymphomyiidae and some Blephariceridae (Courtney 1990, 1994; Oosterbroek and Courtney 1995).
40. (*mdsubd*) **Subdivision of mandible: (0) absent; (1) present, distal part separated by a furrow; (2) present, distal part separated from basal part by weakly sclerotised zone** (char. 20 in Oosterbroek and Courtney 1995). A movable distal part of the mandible is absent in Tipulidae and most other tipuloid subgroups but occurs in the hexatomine genus *Ulomorpha* Osten-Sacken, 1869 (Oosterbroek and Theowald 1991), and also in Anisopodidae, Trichoceridae, Psychodidae (partim), Deuterophlebiidae (Anthon 1943a, b; Oosterbroek and Courtney 1995), and generally in orthorrhaphous Brachycera



- (Anthon 1943a; Hennig 1973; Oosterbroek and Theowald 1991). The distal part is separated from the proximal mandible by a furrow in larvae of Ptychopteridae (Anthon 1943a; Kramer 1954). The mandible is compact and undivided in most nematoceran groups such as Tanyderidae, Axymyiidae, Bibionidae, and Culicidae (Oosterbroek and Courtney 1995).
41. **(insadtd) Insertion of adductor tendon: (0) nor or very slightly shifted anteriorly; (1) distinctly shifted anteriorly.** The mesal wall of the mandible is distinctly shortened in larvae of Tipulidae (Fig. 4A, B) and Cylindrotomidae (Peus 1952), and this is probably a general feature in tipuloid larvae (Anthon 1943a: Fig. 38; Oosterbroek and Theowald 1991: Figs. 61, 128). As a result, the adductor tendon is attached distinctly anteriorly of the tendon of the abductor. This condition is widespread in dipteran larvae. It does also occur in Tanyderidae, Deuterophlebiidae (Courtney 1990: Fig. 4), Trichoceridae, Psychodidae, Ptychopteridae, Anisopodidae (Anthon (1943a, b, 1988)), Dixidae (Felix 1962: Fig. 18), Ceratopogonidae (Nielsen 1951: Fig. 13C), Simuliidae (Cook 1949: Fig. 9d) and Blephariceridae (partim: *Liponeura*; Anthon and Lyneborg 1968), and also in larvae of Siphonaptera (Widhalm-Finke 1974: Fig. 6). The condition in Axymyiidae is somewhat unclear. The illustrations in Mamayev and Krivosheyna (1966: Figs. 2, 4, 5) suggest that the adductor tendon is moderately shifted anteriorly. Both tendons insert at approximately the same level in and *Chironomus* and *Culiseta* (Cook 1949). The adductor tendon is very slightly shifted anteriorly in *Nannochorista* (coded as 0), whereas both tendons are attached at the same level in the other groups of Mecoptera (Bierbrodt 1942; Beutel et al. 2009).
42. **(M13)M. tentoriomandibularis: (0) present; (1) absent.** M. tentoriomandibularis is absent in *T. montium* like in all other examined larvae of Diptera (e.g., Cook 1944a, b, 1949; Kramer 1954). The muscle is present in *Nannochorista*. It cannot be excluded that it was overlooked in some cases due to its extremely small size (e.g., Beutel et al. 2009).
43. **(galac) Endite lobes: (0) distinctly developed and separated; (1) partly fused, still recognisable as separate structures; (2) galea and stipes completely fused or absent.** Separate endite lobes were described for *Tipula* species by Selke (1936: Fig. 23) but are not distinctly recognisable in *T. montium* (Fig. 4C). Only one distinct endite lobe is present in other representatives of Tipuloidea as in almost all other dipteran larvae (e.g., Axymyiidae, Nymphomyiidae, Bibionidae, Ptychopteridae, Culicidae, Dixidae; Cook 1944a, b, 1949; Anthon 1943a, b, 1988; Mamayev and Krivosheyna 1966; Hennig 1973; Courtney 1994). A reduced but recognisable digitiform lacinia and a thorn-shaped galea are present in *Olbiogaster* (Anisopodidae) and the two endite lobes are also distinctly separated and different in Deuterophlebiidae (Courtney 1990) and *Mischoderus* (Tanyderidae) (Anthon 1943b, 1988).
44. **(nmxpl) Number of maxillary palp segments: (0) 3; (1) 2; (2) 1.** Only one palpomere is present in *T. montium* (Fig. 4C) like in larvae of other groups of Tipuloidea (e.g., Cook 1949; Peus 1952; Denis and Bitsch 1973), and in most other groups of Diptera (e.g., Tanyderidae, Dixidae, Bibionidae, Anisopodidae, Ptychopteridae, Psychodidae, Culicidae, Simuliidae, Tabanidae, Stratiomyidae; Cook 1944a, b, 1949; Anthon 1943a, b, 1988; Denis and Bitsch 1973). It is 2-segmented in Axymyiidae (Mamayev and Krivosheyna 1966), Chironomidae and Pachyneuridae (Foote 1991), in larvae of the orthorrhaphous Therevidae (Denis and Bitsch 1973; Cook 1944a, b, 1949), and in larvae of Siphonaptera (Sharif 1937) and Boreidae. Three are present in Nannochoristidae and Panorpidae (Bierbrodt 1942; Pilgrim 1972).
45. **(lmxpl) Length of single maxillary palpomere: (0) not elongated, about as long as wide; (1) distinctly elongated.** The single maxillary palpomere is usually short or more or less vestigial (e.g., Anthon 1943a, b, 1988; Oosterbroek and Theowald 1991) but distinctly elongated in Pediciidae and Hexatominiae. A correlation with secondary predaceous habits is likely.
46. **(M15)M. craniocardinalis (M. 15): (0) well developed; (1) absent.** M. 15 is generally absent in Diptera (e.g., Cook 1944a, b, 1949; Kramer 1954; Felix 1962) and is also missing in the other mecopterid orders (Hinton 1958; Bierbrodt 1942; Beutel et al. 2009).
47. **(M17/18)Mm. tentoriocardinalis/-stipitales (Mm. 17/18) with origin from the posterior tentorial arm: (0) present; (1) absent.** Cardinal and stipital muscles with an origin from the posterior tentorium are absent in larvae of Tipuloidea (Das 1937; Cook 1949). The typical extrinsic tentoriocardinal and -stipital muscles are almost generally missing in Diptera (Cook 1944a, b, 1949; Hinton 1958; Hennig 1973; Courtney 1990) and are also absent in Siphonaptera (Sharif 1937; Widhalm-Finke 1974). An extrinsic maxillary muscle originating from the posterior tentorium occurs in the culicid genera *Lutzia* and *Armigeres* (Cook 1944a), and a small subcomponent of the well developed maxillary flexor originates from the anterior tentorial arm in *Bittacomorpha* (coded as 1; Kramer 1954). It appears likely that these muscles are derived from M. 18.
48. **(stflex) Stipital flexor: (0) present; (1) absent.** The single extrinsic maxillary muscle of Tipulidae, *Hexatoma* and Brachycera is likely homologous with M. craniolacinalis (M. 19) (e.g., Cook 1949;

- Denis and Bitsch 1973). A second extrinsic muscle, a flexor of the stipes (see previous character), is present in *Dicranota* (Pediidae), *Bittacomorpha* (Kramer 1954), and other nematoceran groups (Cook 1949: retractor of maxilla and maxillary muscle).
49. (*M20/21*) **Muscles of the endite lobes: (0) two; (1) one; (1) absent.** Intrinsic maxillary muscles of the galea and lacinia are missing in dipteran larvae (e.g., Cook 1944a, b, 1949; Hennig 1973).
50. (*22/23*) **Mm. stiptopalpalis externus/internus (Mm. 22/23): (0) present; (1) absent.** Both muscles are generally absent in Diptera (Cook 1944a, b, 1949; Kramer 1954; Hennig 1973).
51. (*teethyp*) **Anterior teeth of hypostomium: (0) absent; (1) present.** A plate-like anteriorly toothed hypostomium, which is at least partly formed by postlabial elements (e.g., Cook 1949; Hennig 1973; but see Anthon 1943b and Oosterbroek and Theowald 1991) and basally fused with the genae, is usually present in Tipuloidea, but is apparently reduced at least in some members of Eriopterinae (Oosterbroek and Theowald 1991: Figs. 15, 26). It is well developed in Tanyderidae (Anthon 1988: Fig. 8) and does also occur in Nymphomyiidae (Courtney 1994: Fig. 55), Anisopodidae (partim; Oosterbroek and Courtney 1995), Ceratopogonidae, Chironomidae, Culicidae, Dixidae, Psychodidae, Ptychopteridae, Simuliidae and some other groups (Cook 1944a, b, 1949; Kramer 1954; Anthon 1943a, b). It is absent in Axymyiidae, Bibionidae, Cecidomyiidae, Blephariceridae, Mycetophilidae, Trichoceridae, Sciaridae and some other nematoceran groups, and is also missing in Brachycera (e.g., Cook 1949; Mamayev and Krivosheyna 1966; Solinas 1968; Oosterbroek and Courtney 1995). The absence in the outgroups shows that a toothed hypostomium is a derived feature, which possibly belongs to the groundplan of Diptera or a large subunit of the order. This would imply several reversals. The alternative interpretation is parallel evolution in several lineages.
52. (*hypdiv*) **Median division of hypostomium: (0) absent; (1) partly divided; (2) completely divided.** The hypostomium or hypostomal plate is medially partly divided by an incomplete triangular zone of weakness in *T. montium* (hp, Figs. 2B and 7C) and a similar condition is found in other tipuloid larvae (e.g., Cylindrotomidae; Peus 1952). It is completely divided (or reduced) in larvae of Eriopterinae, Hexatominiae and Pediciini (Oosterbroek and Theowald 1991: Figs. 13, 58, 94, 66).
53. (*sepsubm*) **Separate submentum: (0) present; (1) absent.** A separate submentum is absent in Tipuloidea and other groups of Diptera, but is present in *Olbiogaster* (Anisopodidae) (Anthon 1943a, b) and apparently in some representatives of Psychodidae (Oosterbroek and Courtney 1995).
54. (*hyspl*) **Hypostomal plate: (0) fused with elements of the head capsule; (1) present as a separate structure.** The hypostomal plate is fused with elements of the head capsule in Tipuloidea and this is also the case in most other nematoceran groups (e.g., Nymphomyiidae; Courtney 1994). A separate hypostomal plate is present in larvae of Ptychopteridae (Anthon 1943a; Kramer 1954).
55. (*prmttee*) **Premental teeth: (0) present; (1) indistinct or absent.** A characteristic plate-like prementum with a distinct anterior row of teeth similar to those of the hypostomium is present in Tipulidae, Cylindrotomidae and Limoniinae, and apparently also in the groundplan of Eriopterinae (*Molophilus*) and Hexatominiae (*Pseudolimmophila* Alexander, 1919) (Peus 1952; Oosterbroek and Theowald 1991). The teeth are very indistinct (*Tricyphona*) or absent in Pediciidae (*Ula*, *Dicranota*) (Cook 1949; Oosterbroek and Theowald 1991: Figs. 94, 111). We consider the former condition as derived and as a potential apomorphy of Tipuloidea excluding Pediciidae. Prementopharyngeal teeth have likely evolved independently in Nymphomyiidae (Courtney 1994: Fig. 55, lingua).
56. (*nlbpl*) **Labial palp segments: (0) more than one segment; (1) 1-segmented, more or less vestigial.** The labial palp is absent in most Tipuloidea (e.g., Cook 1949; Peus 1952), and this is also the case in Axymyiidae (Mamayev and Krivosheyna 1966), Dixidae, and Simuliidae. It is vestigial in *Metalimnobia* Matsumura, 1911 (Limoniinae), Bibionidae, Anisopodidae, Trichoceridae, Ptychopteridae, Culicidae, Chironomidae, Blephariceridae, and other groups (Cook 1944a, b, 1949; Anthon 1943a, b). A distinctly developed 1-segmented palp is present in *Tabanus*, whereas it is absent in *Ondontomyia* and the therevid larva described by Cook (1949). A 1-segmented palp is also present in Siphonaptera (Sharif 1937), whereas two segments are present in Mecoptera (e.g., Pilgrim 1972; Bierbrodt 1942).
57. (*M28*) **M. submentopraementalis (M. 28): (0) present; (1) absent.** *M. submentopraementalis* is missing in dipteran larvae (e.g., Hinton 1958; Cook 1944a, b, 1949; Kramer 1954; Denis and Bitsch 1973) and is probably also generally absent in the other groups of Mecoptera (Beutel et al. 2009).
58. (*M29/30*) **Tentorio-premental muscles (Mm. 29/30): (0) two; (1) one; (2) extrinsic labial muscles absent.** A single pair arising from the remnants of the ventral head capsule is present in Tipulidae and *Dicranota* (Pediidae) (Cook 1949), whereas labial muscles are completely missing in *Hexatoma* (Cook 1949: labium completely reduced). Two bundles arise near

the occipital condyle in Deuterophlebiidae (Courtney 1990). A single labial muscle with a cranial origin is present in *Biblio* (origin anteriorly of the posterior tentorial pits; Cook 1949), *Chironomus*, *Simulium* (Cook 1949) and *Dixa* (Felix 1962), whereas the single premental retractor originates from the posterior tentorial arms in Culicidae, *Bittacomorpha*, and *Odontomyia* (Stratiomyidae) (Cook 1949; Kramer 1954). This is likely the ancestral condition for Diptera. Extrinsic labial muscles are apparently absent in *Tabanus* and *Therevidae* (Cook 1949).

59. (M34) **M. praementopalpalis internus (M. 34): (0) present; (1) absent.** Labial palp muscles are apparently generally absent in Tipuloidea and the entire Diptera (e.g., Cook 1949; Kramer 1954; Denis and Bitsch 1973).
60. (M37) **M. hypopharyngosalivarialis (M. 37): (0) present; (1) absent.** The muscle is present in *Tipula* and *Holorusia*, but not described for other representatives of Tipuloidea such as *Dicranota* or *Hexatoma* (Cook 1949). It is also missing in some representatives of Culicomorpha such as *Culiseta incidens* (Thomson, 1869), *Simulium*, and *Chironomus*, and also in *Biblio* (Cook 1944a, b, 1949). It is described for *Dixa*, *Culex*, *Phaenobremia* (Cecidomyiidae) and *Bittacomorpha* (Cook 1944a, b, 1949; Kramer 1954; Gouin 1949; Denis and Bitsch 1973; Chaudonneret 1963). It is apparent that this muscle was reduced several times independently and it may have been overlooked in some cases.
61. (M42) **M. tentorihypopharyngalis (M. 42): (0) present; (1) absent.** The muscle is probably generally absent in Diptera (e.g., Cook 1949; Kramer 1954; Denis and Bitsch 1973).
62. (phfil) **Pharyngeal filter: (0) present; (1) absent** (char. 27 in Oosterbroek and Courtney 1995). The pharyngeal filter is absent in tipuloid larvae with the exception of the pediciid genus *Ula* (Oosterbroek and Theowald 1991). It is also missing in Deuterophlebiidae, Bibionidae, Ceratopogonidae, Simuliidae, Chironomidae, Psychodidae (excl. Psychodinae) and some other groups, but is present in Tanyderidae, Axymyiidae, Trichoceridae, Scatopsidae, Culicidae, Blephariceridae, Ptychopteridae, and Anisopodidae (Oosterbroek and Courtney 1995). As pointed out by Oosterbroek and Courtney (1995), considering the character state distribution it appears likely that the presence is a groundplan feature of Diptera, even though the filter is generally absent in other antliophoran groups (Sharif 1937; Bierbrodt 1942; Beutel et al. 2009).
63. (M48) **M. tentoriobuccalis anterior (M. 48): (0) present; (1) absent.** *M. tentoriobuccalis anterior*, a muscle which arises from the tentorial bridge, is absent in larvae of Tipuloidea and probably also missing in all other groups of Diptera (e.g., *Culiseta*, *Simulium*, *Dixa*, *Biblio*; Cook 1949; Felix 1962; Beutel, pers. obs.). The muscle is present in *Nannochorista* (Beutel et al. 2009).
64. (M51) **M. verticopharyngalis (M. 51): (0) present; (1) absent.** *M. verticopharyngalis* is probably generally missing in tipuloid larvae (Cook 1949). It is also missing in other dipteran groups but is present in Culicidae (Denis and Bitsch 1973), *Bittacomorpha* (Kramer 1954), *Biblio* (Beutel, pers. obs.) and probably also in Deuterophlebiidae (Courtney 1990: 86). Apparently the muscle is never subdivided and strongly developed as it is the case in Nannochoristidae (Beutel et al. 2009). The tendency to reduce *M. verticopharyngalis* is apparently linked with the trend to shift the brain to the thorax.
65. (posphd) **Posterior ventral pharyngeal dilators: (0) anterior and posterior subcomponent (M. 50 and M. 52); (1) one bundle; (2) absent.** Posterior ventral pharyngeal dilators (*Mm. tentoriobuccalis posterior* and *tentoriopharyngalis*) are probably generally absent in Tipuloidea (Cook 1949). This is possibly linked with the far-reaching reduction of the ventral wall of the head capsule and the loss of the tentorium. The muscles are present in *Dixa*, *Bittacomorpha* and *Biblio* (Felix 1962; Kramer 1954; Beutel, pers. obs.: origin from tentorium and from head capsule), and at least represented by a cranial component in *Simulium* (Cook 1949), *Chironomus* and some Culicidae (e.g., *Culiseta*, possibly absent in *Culex* and others) (Cook 1944a, 1949; Chaudonneret 1963; Denis and Bitsch 1973). It is absent in apparently missing in the brachyceran larvae described by Cook (1949).
66. (posbr) **Position of brain: (0) completely or largely within head capsule; (1) partly shifted to thorax; (1) completely shifted to thorax.** The entire brain is shifted to the prothorax in Tipuloidea (Cook 1949: Fig. 17). This is likely a general trend in Diptera even though the entire brain of *Dixa* (Felix 1962) and *Culex* (Chaudonneret 1963) lies within the head capsule, and at least the major part in *Bittacomorpha* (Kramer 1954). This is arguably a groundplan feature of Diptera. An intermediate condition is found in *Biblio* (Beutel, pers. obs.), where parts of the hemispheres are shifted to the anterior prothorax but a narrow median connection remains within the head capsule.
67. (cibph) **Cibariopharyngeal sclerotisation: (0) absent; (1) present.** A structural unit with a trough-like, sclerotised ventral wall is formed by the cibarium and pharynx in the brachyceran groups examined by Cook (1949). This condition is absent in Tipuloidea and larvae of other nematoceran groups examined (e.g., Cook 1944a, b, 1949; Kramer 1954; Denis and Bitsch 1973).



## 5. Results of the parsimony analysis (combined data set)

The cladistic analysis of the data set yielded 45 equally parsimonious trees (205 steps, CI=0.41). The strict consensus tree is poorly resolved, with only Diptera, Nymphomyiidae+Deuterophlebiidae, Brachycera and (Limoniinae+(Cylindrotomidae+Tipulidae)) supported as clades. The monophyly of Tipuloidea and a clade Tipuloidea excluding Pediciidae was confirmed in the majority of the trees (majority rule consensus tree). The preferred most parsimonious branching pattern for Tipuloidea is shown in Fig. 8 with apomorphies mapped on the branches.

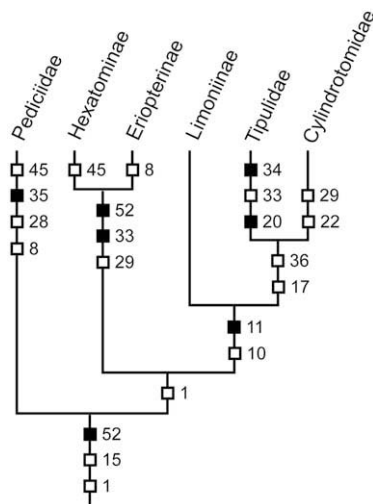
## 6. Discussion

The cladistic analyses carried out here was exclusively based on characters of the larval head, with emphasis on the hitherto strongly neglected internal structures including musculature. It contributed to the understanding of the phylogeny of Tipuloidea, which were in the focus of this study (Fig. 8). However, as was to be expected with the limited data set, the results did not clarify the interrelationships of the large nematoceran lineages, including the longstanding problem where Tipuloidea should be placed in the dipteran tree. As pointed out above, a basal placement of Tipuloidea (or Tipulomorpha) within Diptera is still a serious option (see e.g., Blagoderov et al. 2007). However, it is evident that the larvae are highly derived. A doubtlessly apomorphic complex of features supporting the monophyly of the superfamily is the retracted head capsule

and the specific hemicephalic condition with paired dorsolateral incisions and a deep median incision on the ventral side. The hemicephalic condition found in brachyceran lineages (e.g., Tabanoidea) is distinctly different from that of Tipuloidea, and a retracted partly dissolved head capsule is apparently not a brachyceran groundplan feature. An unusual feature possibly related with the retracted head is the presence of intermolt cuticular deposits, another potential autapomorphy of Tipuloidea. This condition is also present in Axymyiidae, which are also characterised by moderately hemicephalic condition, and in few other groups including Tanyderidae (Anthon 1988). Apparently this derived feature has evolved independently several times. Other potential autapomorphies of Tipuloidea are the loss of the tentorium (with the exception of vestiges of the anterior arm), the absence of antennal muscles, the partial division of the hypostomal plate, the complete loss of the labial palp, and the absence of M. tentoriopharyngalis posterior (M. 52). Derived features of Tipuloidea which also occur in several other groups including brachyceran taxa are the loss of the postcerebral dorsal pharyngeal dilator, and the posterior shift of the brain. Most or all of these derived characters are not found in larvae of Trichoceridae (internal features are largely unknown). The characters of the larval head analysed here do not support a clade Tipulomorpha (including Trichoceridae), which was suggested by earlier authors (e.g., Hennig 1973) and corroborated by adult features in the analyses carried out by Oosterbroek and Courtney (1995).

Within Tipuloidea our results are largely congruent with those obtained by Ribeiro (2008), who analysed 88 characters of the male imago. Features of the larval head also suggest a sistergroup relationship between Pediciidae and the remaining groups (Fig. 8). A moderately retracted head is present in Pediciidae, with a considerably movability of the head capsule preserved, whereas a clearly advanced condition is found all other tipuloid groups, with the head capsule strongly retracted and fixed in this position (Hennig 1968b; Oosterbroek and Theowald 1991). Other potential synapomorphies of Tipuloidea excluding Pediciidae is the elongation of the dorsolateral incisions, the shortening of the coronal suture, the loss of one of two antennomeres, the loss of one extrinsic maxillary muscle (confirmed for Tipulidae and *Hexatoma*; Cook 1949), and the presence of distinct premental teeth. The presence of a pharyngeal filter in larvae of the pediciid genus *Ula* is also arguably a plesiomorphic condition. It is absent in all other tipuloid larvae examined including other pediciid genera. Even though the basal placement of Pediciidae is not supported in all minimum length trees, this group has likely preserved the highest number of plesiomorphies in Tipuloidea.

Features shared by pediciid larvae and larvae of some or all groups of Eriopterinae and Hexatominae are the



**Fig. 8.** Tipuloid branch of one of 45 minimal length trees (tree length 205, CI: 0.1). All branches except for Diptera, Nymphomyiidae+Deuterophlebiidae, Brachycera and (Limoniinae+(Cylindrotomidae+Tipulidae)) collapse in the strict consensus tree. Full squares indicate unambiguous apomorphies.

elongate sickle-shaped apical part of the mandible, a characteristic likely related with secondarily predacious habits, and a completely divided hypostomal plate (undivided in *Ula*; Oosterbroek and Theowald 1991: Fig. 111), which may be largely reduced or absent in Eriopterinae and Hexatominae. A feature shared by Pediciidae and Hexatominae is the enlarged maxillary palp. A correlation with predacious habits is also conceivable in this case (see also Oosterbroek and Theowald 1991).

A trend shared by Cylindrotomidae, Eriopterinae and Hexatominae is to shift the antennae towards the median line. This condition has apparently evolved several times independently, in correlation with the formation of a narrow and conical labrum. A broad and undivided labrum is present in Pediciidae, and this is very likely a groundplan feature of Tipuloidea.

A clade comprising Tipulidae, Cylindrotomidae and Limoniinae (Fig. 8), implying the non-monophyly of Limoniidae (see Ribeiro 2008), was already suggested by Oosterbroek and Theowald (1991). This appears well supported by two likely synapomorphies, the presence of a premaxillary suture and strengthened margins of the externo-lateralia (Oosterbroek and Theowald 1991). A feature shared by Tipulidae and Cylindrotomidae is the presence of a movable lacinia mobilis on the mandible. However, this condition is also found in larvae of Nannochoristidae, and is arguably a groundplan feature of Tipuloidea and Diptera (see below). The monophyly of Tipulidae is supported by the tripartite labrum and by the posterior shift of the secondary mandibular joint. A plesiomorphy preserved in *Tipula* is the presence of muscles arising from the dorsal endocarina and inserting on the posterior pharynx (Mm. frontopharyngalis anterior and posterior). However, it cannot be excluded that the delicate muscles were overlooked by Cook (1949) in *Holorusia*, *Dicranota* and *Eriocrania*.

The features examined here do not contribute much to a clarification of the position of Tipuloidea. Characters supporting phylogenetic affinities with Brachycera as suggested by Yeates et al. (2003) and others are vague at best. It was pointed out above that the hemicephalic conditions of tipuloid larvae and larvae of Brachycera are distinctly different. A strongly retracted head is apparently not a groundplan feature of Tipuloidea, and a fully exposed head occurs in some brachyceran groups (e.g., Therevidae). A posterior shift of the brain is not only a feature shared by tipuloids and Brachycera, but this condition is rather common in other nematoceran groups (Cook 1949). Plesiomorphic characters preserved in Tipuloidea with possible apomorphic counterparts in the remaining groups, i.e. derived features supporting a basal tipuloid position, were not found during our study, with the possible exception of the lacinia mobilis. It is evident that Tipuloidea are remote from the ancestral condition of Diptera (see above), and a highly specialised group rather than “primitive” in its larval head structures.

Diptera as a whole are apparently the most highly specialised group of Endopterygota as far as the larval stages are concerned. Derived groundplan features of the order are the partly reduced tentorial bridge (absent in most groups, unknown e.g., in Axymyiidae), the reduced condition of the larval eyes, dense fields of hairs on the labrum and anterior epipharynx (with highly specialised arrangement in some aquatic groups), the loss of M. frontolabralis (M. 8), a distinctly reduced condition of the antenna (with reversal in few groups, especially with aquatic larvae), the presence of only one extrinsic antennal muscle, the absence of the tentorial mandibular muscle, the reduced number of maxillary palp segments (usually one, two in Axymyiidae and few other groups), the presence of only two extrinsic maxillary muscle, the complete absence of intrinsic muscles of the maxilla, the presence of only one extrinsic labial muscle, the 1-segmented labial palp (strongly reduced or absent in many groups), and the loss of M. tentoriohypopharyngalis (M. 42). Of 34 muscles of the larval head likely belonging to the groundplan of Endopterygota (e.g., Beutel and Friedrich 2008) 17 are absent in the groundplan of Diptera. Whether the toothed hypostomal plate and a pharyngeal filter apparatus are groundplan features of Diptera is uncertain. Both features are missing in several groups. Another derived feature considered as a dipteran autapomorphy (e.g., Hennig 1973) is the absence of the transverse facial strengthening line. However, the presence was recorded for Axymyiidae by Mamayev and Krivosheyina (1966). The endite lobes are almost always more or less completely fused, but distinctly separated in Tanyderidae. The latter condition may be ancestral for Diptera, with a strong tendency to merge both elements.

A prognathous head as it is found in almost all groups of Diptera is arguably a groundplan feature of Endopterygota as suggested by the presence of this condition in Neuropterida, Strepsiptera, Coleoptera (partim), Trichoptera (partim), Lepidoptera (basal groups), Nannochoristidae, and Siphonaptera (e.g., Beutel et al. 2009). Clearly, an orthognathous head is a secondarily derived condition within Diptera. Whether the presence of a separate submentum belongs to the groundplan is doubtful. It is only described for few groups (e.g., *Olbiogaster*; Anthon 1943b) and the presence may be due to reversal. Whether the separate hypostomal plate of Ptychopteridae and some Psychodidae (Anthon 1943a) is apomorphic or plesiomorphic is also unclear. The fusion with the head capsule may represent the ancestral condition for Diptera.

Features occurring in some groups of Diptera and in Nannochoristidae are the presence of a movable protheca (only Cylindrotomidae and Tipulidae, see above), the presence of split cranial setae (e.g., Axymyiidae, Tanyderidae), a largely exposed anterior epipharynx, and

the absence of a cuticular lense. It is presently uncertain whether these characters belong to the groundplan of Diptera and a dipteran-nannochoristid clade, or whether they have evolved independently. A derived feature which is also possibly present in the dipteran groundplan is shared with Siphonaptera, the anterior shift of the attachment of the adductor tendon.

Morphological modifications which have apparently played an important role in the evolution of diptera are those of the labrum, the mandibles and also the ventral mouthparts. Modifications of the labrum and epipharynx, especially in some aquatic groups (e.g., Simuliidae, Culicidae, Chironomidae) are apparently correlated with specialised feeding habits, such as filtering, scraping, gathering and collecting (Labandeira 2005). This development reaches a peak in Simuliidae with conspicuous movable messorial arms and very strongly developed, medially intercrossing messorial muscles. The ancestral feeding habits of Diptera are unclear. However, the presence of dense fields of hairs on the labrum and other appendages suggest a primary diet of more or less semiliquid or liquified substrates, possibly involving extraoral digestion in some groups. Extraoral digestion and uptake of liquified food takes also place in beetle larvae with dense preoral brushes (e.g., Carabidae, Histeridae, Cantharoida; Beutel 1993, 1995, 1999).

It is apparent that the loss of legs and the tendency to live in cryptic habitats such as crevices, decaying materials, leave litter or moist soil had a strong impact on the evolution of Diptera. Shortening or far-reaching or complete reduction of the antennae and palps can be seen in this functional context, with reversals occurring in predacious (e.g., Pediciidae: long maxillary palps) or aquatic groups (e.g., Simuliidae, Culicidae, Deuterophlebiidae: long antennae; Foote 1991).

A major trend in dipteran larvae likely linked with the loss of locomotory legs is the shift of the axis of movement of the mandibles. Apparently an oblique or even vertical articulation has evolved several times independently in Diptera including subgroups of Tipuloidea (e.g., *Ula*). This development reaches its peak in Cyclorrhapha, where the scythe-shaped mandibles operate in a vertical plane. Another unusual modification of the mandibles is the formation of a movable distal part, which interacts with an anteriorly directed, toothed, cone-shaped structure. This doubtlessly derived feature is found in several groups of Psychodomorpha (Anthon 1943a), suggesting close phylogenetic affinities of Trichoceridae, Psychodidae and Anisopodidae. A somewhat similar condition has independently evolved in *Ulomorpha* (Oosterbroek and Theowald 1991: Fig. 61) and also in Deuterophlebiidae. Further mandibular modifications are the acquisition of combs on the dorsal side (e.g., Culicidae, Dixidae) or apically (Nymphomyiidae, Deuterophlebiidae, Blephariceridae [partim]). This occurs in aquatic

groups, which are also characterised by specialised labral brushes, but also in different terrestrial lineages (dorsal combs; e.g., Trichoceridae, Scatopsidae, Anisopodidae).

The maxillae are generally highly modified, and the homologisation of the elements is often difficult. The labium is strongly simplified. The tendency to internalise the prementum and to reduce the palps is likely related with the absence of legs, and the resulting direct contact with the substrate. The formation of a strongly sclerotised toothed hypostomal plate is also likely related with an immediate surface contact. It does not only appear suitable as a mechanical protective device, but also for scraping material from surfaces. However, there is no direct evidence that it is involved in the process of gathering food. Generally, there is little known about feeding mechanisms in dipteran larvae (see e.g., Labandeira 2005), even though some aspects of the feeding process of *Tipula* larvae, especially mouthpart interactions and resorption, were described in detail by Selke (1936).

The question of which dipteran group has the most ancestral larva (i.e. with the maximum number of preserved plesiomorphies) is intriguing. Nymphomyiidae, which are possibly the most basal group of dipterans (e.g., Hackman and Väisänen 1982; Courtney 1994; R. Meier, pers. comm.), are apparently highly derived in their larval head structures (e.g., conical clypeolabrum, distinctly simplified maxilla). This is probably due to a high degree of miniaturisation and life in specialised habitats (Courtney 1994). Possible candidates are different groups such as Axymyiidae, Bibionidae, Tanyderidae, Ptychopteridae, and Anisopodidae (see Anthon 1943b: “...der primitivsten bisher gekannten Dipterenlarve”). Aside from the trivial fact that all groups display a combination of specialised and “primitive” features, a solid phylogenetic background would be required for a reliable assessment of the maximum number of preserved plesiomorphies. This is presently greatly impeded by the lack of detailed anatomical data for many groups (see Appendix A). The detailed anatomical study of apparent key taxa such as Axymyiidae or Tanyderidae is presently mainly impeded by the lack of suitably preserved material. The acquisition of well fixed specimens and well documented anatomical data should have high priority in future projects on the larval morphology, phylogeny and evolution of Diptera.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jcz.2009.10.001](https://doi.org/10.1016/j.jcz.2009.10.001).

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### 3.5 Study V

**Schneeberg K**, Friedrich F, Courtney GW, Wipfler B, Beutel RG. 2012.

The larvae of Nymphomyiidae (Diptera, Insecta) – ancestral und highly derived? *Arthropod Structure and Development* 41: 293-301.

#### **Abstract**

Larval head structures of *Nymphomyia dolichozeza* were examined and described in detail. The conditions are compared to those of other dipteran representatives. Our results support the monophyly of Nymphomyiidae. Potential apomorphies are dimorphic crochets on the abdominal prolegs and the complete loss of the tentorium. Possible synapomorphies of Nymphomyiidae and Deuterophlebiidae could be the rows of spatulate macrosetae covering the ventral surface of the labrum-epipharynx, the presence of distinct teeth along the anterior premento-hypopharyngeal margin, the absence of labral microtrichia and some other affinities concerning the life history of the two groups. A clade Blephariceromorpha is also supported by some larval features. Potential synapomorphies of Nymphomyiidae, Deuterophlebiidae and Blephariceridae are the vestigial *M. labroepipharyngalis*, the absence of a movable premandible, crochet-tipped prolegs, the complete loss of spiracles and non-retractable anal papillae. A clade Nymphomyiidae and Chironomidae is only weakly supported by characters of the larval head. The anteriorly serrate and posteriorly fused hypostoma is a potential apomorphic character. Our results support neither phylogenetic affinities between Nymphomyiidae and Axymyiidae nor a sistergroup relationship between Nymphomyiidae and the remaining Diptera. However, a comprehensive cladistic analysis is not presented in our study.

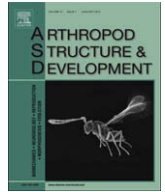
#### **Significance in the present thesis**

Nymphomyiidae is a very specialized group in terms of its lifestyle and morphology. Nymphomyiidae play a phylogenetic key role, because the group is suggested for different systematic placements in the last years. And the larval morphology, especially the inner morphology of the head is not known.

#### **Own contribution**

70%





## The larvae of Nymphomyiidae (Diptera, Insecta) – Ancestral and highly derived?

Katharina Schneeberg<sup>a,\*</sup>, Frank Friedrich<sup>b</sup>, Gregory W. Courtney<sup>c</sup>, Benjamin Wipfler<sup>a</sup>, Rolf G. Beutel<sup>a</sup>

<sup>a</sup> Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, 07743 Jena, Germany

<sup>b</sup> Biozentrum Grindel und Zoologisches Museum, Universität Hamburg, 20146 Hamburg, Germany

<sup>c</sup> Department of Entomology, 432 Science II, Iowa State University, Ames, IA 50011-3222, USA

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### ABSTRACT

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### 1. Introduction

Nymphomyiidae is a small nematoceran family comprising only seven described species (Courtney, 1994; Wagner et al., 2008). Like the potentially related Deuterophlebiidae it is a highly specialised group both in terms of morphology and lifestyle. Adults and larvae are exceptionally slender and very small, measuring approximately 1 mm in length. The immature stages are aquatic and usually associated with moss in small, cool mountain streams (Courtney, 1994). The non-feeding, short-lived adults die after copulation. Their simplified fringed wings can be shed at predetermined lines of fracture (Courtney, 1991).

A potential phylogenetic key role of Nymphomyiidae is suggested by numerous different systematic placements proposed by different authors since the family was introduced by Tokunaga (1932). Like Tokunaga (1932), Rohdendorf (1961, 1964) emphasized the isolated position of the group among extant Diptera. He suggested a suborder Archidiptera comprising the infraorder Nymphomyiomorpha (=Nymphomyiidae) and the Triassic groups †Dictyodipteromorpha and †Diplopolynneuromorpha (Rohdendorf, 1964). Crampton (1942)

placed the family in Culicoidea and a close relationship to Culicomorpha was also discussed later by Courtney (1991, 1994). Hennig (1950), Séguéy (1950) and Imms (1957) assumed a closer relationship to Psychodidae. Wood and Borkent (1989), Courtney (1990, 1991) and Oosterbroek and Courtney (1995) suggested a monophyletic group Blephariceromorpha comprising Nymphomyiidae, Deuterophlebiidae and Blephariceridae. Within this lineage, Nymphomyiidae were placed as the sistergroup of the other two families, i.e. Blephariceromorpha (Wood and Borkent, 1989; Courtney, 1990, 1991; Oosterbroek and Courtney, 1995). A clade comprising Nymphomyiidae and Deuterophlebiidae was suggested by Cutten and Kevan (1970) and this hypothesis was also supported by characters of the adult head (Schneeberg et al., 2011). Molecular data suggested that Nymphomyiidae are closely related to Axymyiidae, and this clade was placed as the sistergroup of Culicomorpha (Bertone et al., 2008). However, as discussed by Bertone et al. (2008) this is likely due to a long branch attraction artefact (see discussion in Bertone et al., 2008, p. 683). Analyses of a reduced data set of Bertone et al. (2008) suggested that Nymphomyiidae are the sistergroup of all remaining lineages of Diptera. However, a basal placement was also suggested for Deuterophlebiidae (Bertone et al., 2008; Wiegmann et al., 2011), Culicomorpha + Ptychopteromorpha (Oosterbroek and Courtney, 1995; Yeates and Wiegmann, 1999, 2005; Yeates et al., 2007), and

\* Corresponding author. Tel.: +49 3641 949181; fax: +49 3641 949142.

E-mail address: [Katharina.Schneeberg@gmx.de](mailto:Katharina.Schneeberg@gmx.de) (K. Schneeberg).

traditionally for Tipulomorpha (inclusive or exclusive Trichoceridae) (Hennig, 1973; Wood and Borkent, 1989; Beutel and Gorb, 2001; Blagoderov et al., 2007). In their comprehensive study of dipteran phylogeny, Wiegmann et al. (2011) propose that Nymphomyiidae is the sister to all remaining Diptera, except Deuterophlebiidae.

Considering the unusually problematic systematic positioning of Nymphomyiidae and the possible phylogenetic key position, the importance of detailed morphological data is apparent. External features of a nymphomyiid larva were first described by Cutten and Kevan (1970). A second, more comprehensive study was presented by Courtney (1994). However, a description of internal structures such as musculature, endoskeleton and digestive tract was still missing. Consequently, the main aim of the study is to provide detailed morphological data for the larval head of a nymphomyiid representative. External and internal head structures are described in detail and the results were compared to the conditions found in representatives of possibly related groups, like Deuterophlebiidae and Blephariceridae. A formal cladistic evaluation is not presented here, but will be carried out in a future study when more detailed anatomical data for larvae and adults of a broad spectrum of basal dipteran lineages are available.

## 2. Materials and methods

### 2.1. Material examined

Diptera, Nymphomyiidae: *Nymphomyia dolichozepe* Courtney, 1994 (95% ethanol; SEM, microtome section)

Culicidae: *Culex* sp. (FAE [=ethanol-formaldehyde-acetic acid]; microtome sections)

Bibionidae: *Bibio* sp. (SEM, microtome sections)

Tipulidae: *Tipula* sp. (dissections, microtome sections).

Blephariceridae: *Edwardsina williamsi* Zwick, 1977 (microtome sections)

Nannomecoptera, Nannochoristidae: *Nannochorista* (*Microchorista* auct., *Choristella* auct. nec Bush, 1897) *philpotti* Tillyard, 1917 (Pampel's fluid, Bouin, ethanol; SEM, microtome sections, whole mount preparations); *Nannochorista dipteroides* Tillyard, 1917 (ethanol; whole mount preparations)

Neomecoptera, Boreidae: *Boreus westwoodi* Hagen, 1866 (first instar, FAE; SEM, microtome sections)

Siphonaptera, Ceratophyllidae: *Ceratophyllus* sp. (SEM, microtome sections).

Pulicidae: *Synosternus cleopatrae* Rothschild, 1903 (70% ethanol; SEM, microtome sections)

### 2.2. Methods

Drawings were made using a stereo microscope MZ 125 (Leica) and camera lucida. Figures were processed with Adobe Photoshop®, Adobe Illustrator® and Image J 1.410 (freeware: <http://rsb.info.nih.gov/ij>). For sectioning, specimens were embedded in Araldit CY 212® (Agar Scientific, Stansted/Essex, UK). Longitudinal (1 µm) and cross sectioning (1 µm) were carried out with a HM 360 microtome (Microm, Walldorf, Germany). The sections were stained with Toluidin blue and Pyronin G (Waldeck GmbH & Co. KG/Division Chroma, Münster, Germany), examined with a light microscope Leica DME and documented with a PixelINK PL-A622C digital camera. The alignment of the image stack was calculated with Amira 5.2 software (Visage Imaging, Berlin, Germany). For scanning electron microscopy the specimens were dehydrated with ethanol (70%–100%) and acetone, critical point dried (EmiTech K850 Critical Point Dryer; Ashford, Kent, UK) glued on a fine pin and sputter coated. Images were taken with a Zeiss LEO 1525 using a specimen holder developed after Pohl (2010).

Muscles are named following the nomenclature of v. Kéler (1963).

## 3. Results

### 3.1. General appearance

The eucephalic larvae are approximately 1 mm long, slender and weakly sclerotised, with the exception of the head capsule (Fig. 1). The postcephalic body is unpigmented, with a smooth cuticle and very sparse setation. The thorax is legless, but paired ventral prolegs are present on abdominal segments I–VII and XI. Each proleg is equipped with apical crochets (Fig. 2).

### 3.2. Head capsule

The moderately elongate head is completely exposed, prognathous and usually slightly declined in fixed larvae (Fig. 3). It is well sclerotised and slightly longer than broad in dorsal view, oval in frontal view, and almost round in cross section (see Fig. 10). The surface of the head capsule is smooth and some widely spaced setae are present. On its dorsal side it bears an indistinct Y-shaped line, probably representing the frontal and coronal sutures (fs, Fig. 3B). The presumptive frontal region is V-shaped (fr, Fig. 3A). The antennal articulation fossae on this head region are distinctly separated. A distinct dorsal cavity with a wrinkled surface

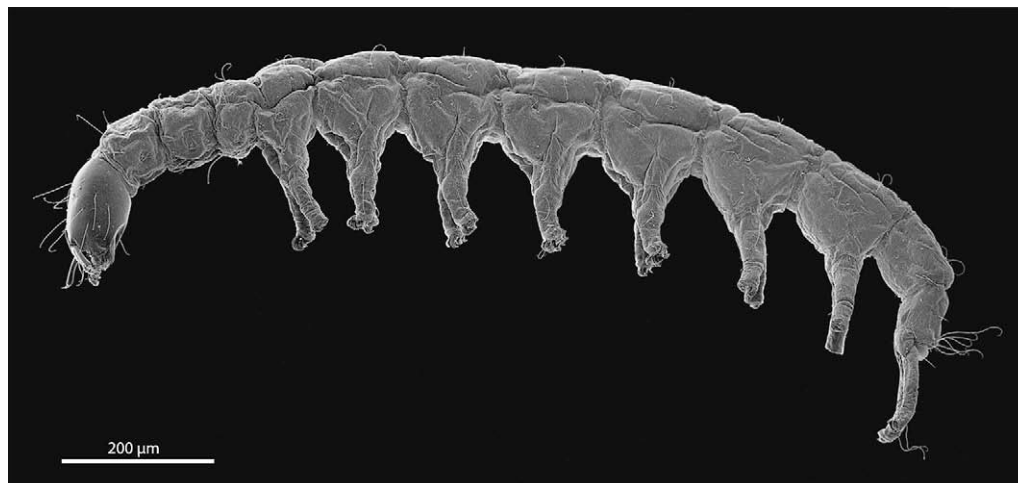


Fig. 1. *Nymphomyia dolichozepe*, larva, overview, SEM image.

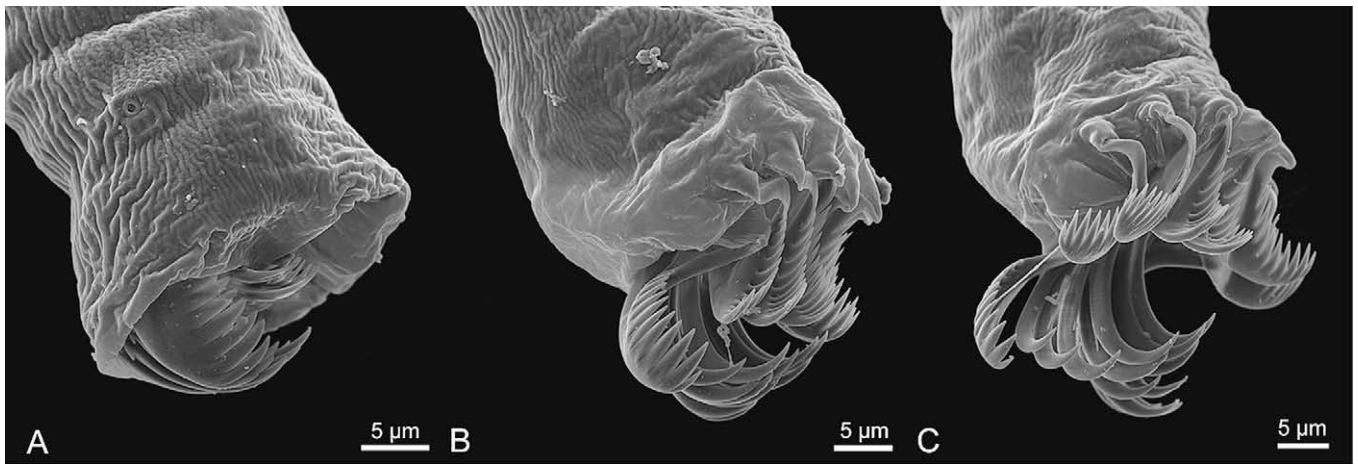


Fig. 2. *Nymphomyia dolichozepe*, larva, crochets, SEM images. (A) proleg 1; (B) proleg 3; (C) proleg 5.

structure is present between the antennal bases (Fig. 3B). The frontoclypeal suture and the clypeolabral suture are missing. The clypeolabrum is anteriorly elongated and the apex of the labral region and epipharynx bears a conspicuous fan-shaped structure (lf, Figs. 3A, C, D and 7). The ventral side of the head is completely sclerotised. The closure is formed by the postgenal regions, which are fused medially and form a postgenal bridge. Anteriorly the

labium is fused with the postgenal bridge (hypostoma). At the foramen occipitale the postgenae are separated from each other (Fig. 3D). The foramen is very large in relation to the size of the head. It is strongly widened anteriorly on the ventral side. Anterior and posterior tentorial grooves are not recognisable. Pigmented larval eyes are present on the posterolateral part of the head (le, Fig. 4).

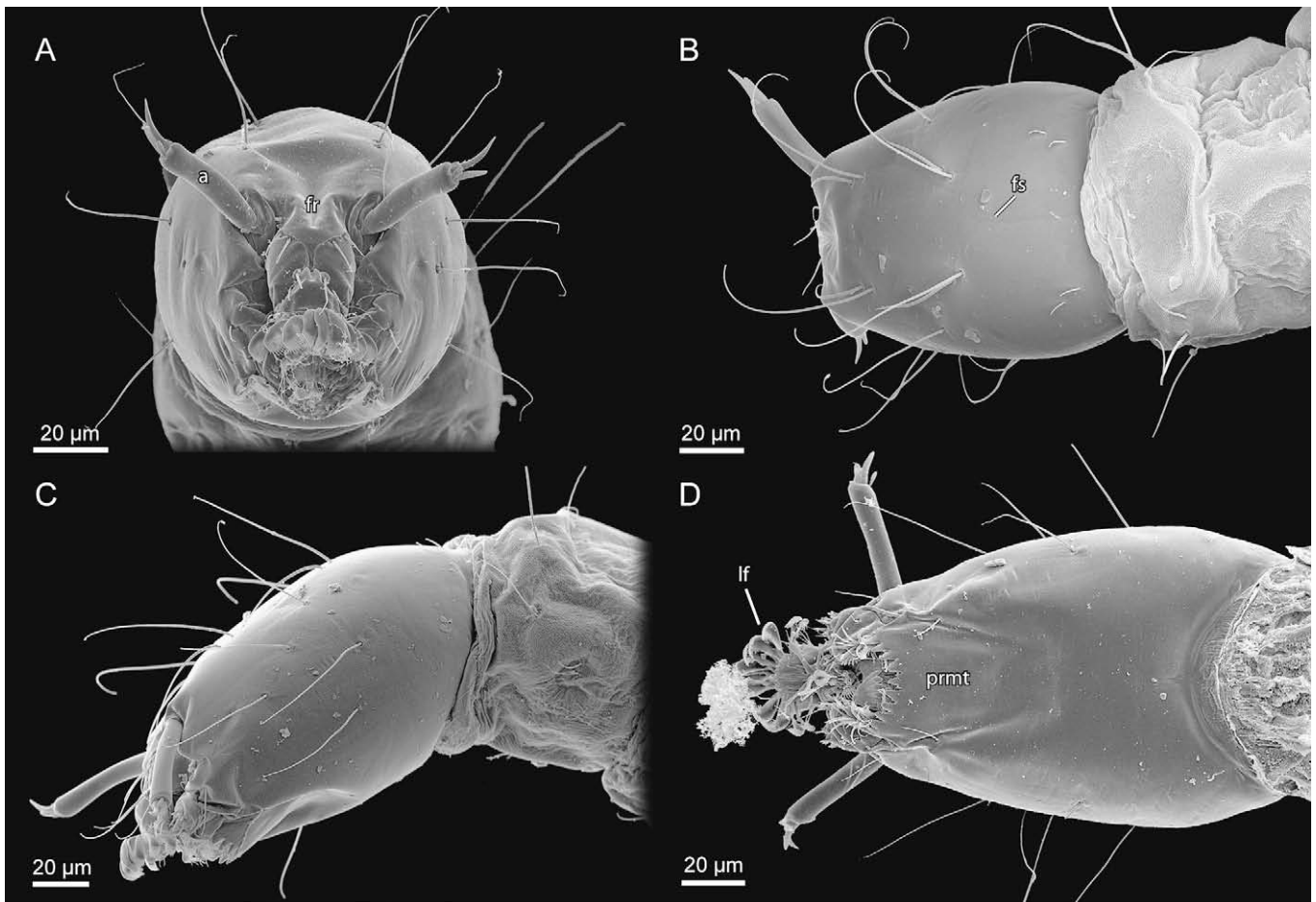


Fig. 3. *Nymphomyia dolichozepe*, larva, head, SEM images. (A) frontal view; (B) dorsal view; (C) lateral view; (D) ventral view. Abbreviations: a, antenna; fr, frons; fs, frontal and coronal sutures; lf, labral fan; prmt, prementum.





**Fig. 4.** *Nymphomyia dolichozepe*, larva, head, lateral view, CLSM image. Abbreviation: le, larval eye.

### 3.3. Tentorium

The tentorium is completely absent.

### 3.4. Antenna

The antennae are one-segmented but distinctly elongated, approximately five times longer than broad (a, Fig. 3). They articulate on a membranous field on the frontal region above a sclerotised lateral projection. The single antennomere is cylindrical, slightly curved laterally and very slightly narrowing towards the apex. On the apex the antenna bears four sensilla, two shorter dorsal ones and two longer ventral ones (Fig. 6). The ventral sensilla are conical and acuminate apically. The dorsal and ventrofrontal sensilla are short and stout; the ventrocaudal and dorsofrontal ones are conical. The length of the ventrocaudal is two times as long as the length of the short sensilla and the length of the dorsofrontal is four times as long as the short ones. The proximal part of the antennae is smooth and no sensilla are recognisable.

**Musculature:** Mm. 1/2/3/4: Mm. tentorioscapales anterior/posterior/lateralis/medialis: absent. Mm. 5/6: Mm. scapopedicellares lateralis/medialis: absent.

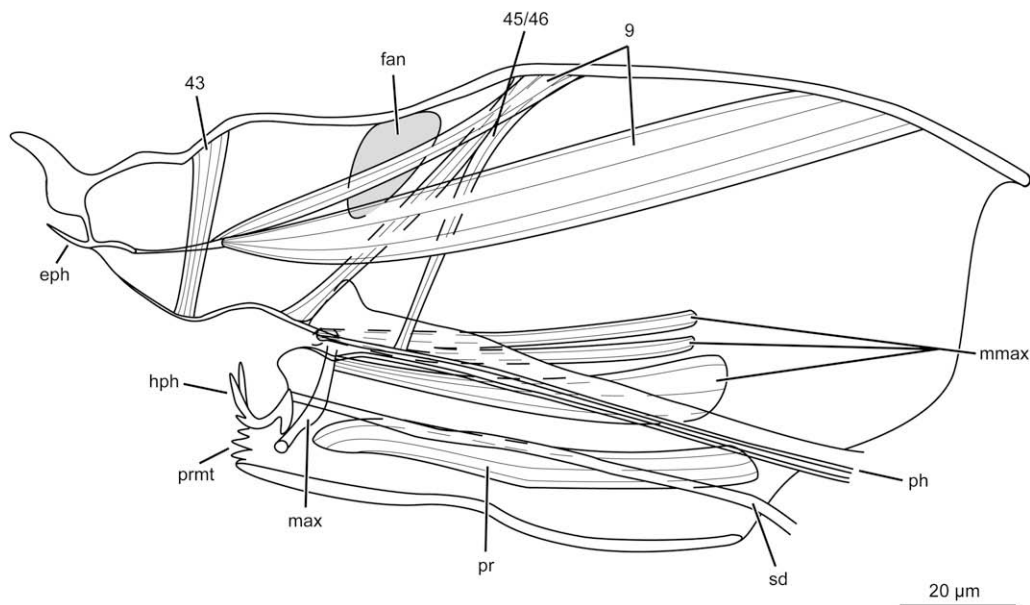
### 3.5. Labrum and epipharynx

The labrum is fused with the clypeus. Both elements together form a prominent structure between the antennae (Fig. 3). The clypeolabrum is conical and bears an anterior fan-shaped structure, which is composed of 14 apically spatulated macrosetae (lf, Fig. 7). The dorsal and lateral labral walls are well sclerotised and separated from each other by an unsclerotised area. The lateral strengthening sclerites likely represent the tormae (t, Fig. 10B). The epipharynx is largely membranous anteriorly but well sclerotised posteriorly. The posterior epipharyngeal bar is U-shaped in cross section, reinforces the epipharyngeal wall and serves as attachment area of M. frontoepipharyngalis (9, Fig. 5).

**Musculature:** (Figs. 5 and 10C–F) M. 7: M. labroepipharyngalis: absent. M. 8: M. frontolabralis: absent. M. 9: M. frontoepipharyngalis: strongly developed; O (=origin) – widely removed posteriorly, on the vertex; I (=insertion) – with a tendon on the ventral epipharyngeal bar; F (=function) – depressor of the labrum. M. 10: M. epistomalabralis: absent. M. 43: M. clypeopalatalis: long and slender muscle; O – clypeus; I – on the anterior part of the palatum; F – cibarial dilator. M. 44: M. clypeobuccalis: absent.

### 3.6. Mandible

The mandibles are strongly sclerotised, slightly curved inwards, longer than broad, with a flattened basal part (md, Fig. 7). The plane of operation is oblique. The intermediate section is distinctly constricted. The distal part is widening and bears a series of approximately triangular, acuminate teeth arranged in a comb-like row. The length of the teeth is about equal whereas the width increases towards the mesal margin of the spoon-shaped distal mandibular part, which has a distinctly concave inner surface. The basal mandibular lobe is broad at its base. It terminates in three blunt teeth, two of them distally oriented and one mesally. A prosthema is



**Fig. 5.** *Nymphomyia dolichozepe*, larva, head, sagittal section. Abbreviations: eph, epipharynx; fan, foramen antennale; hph, hypopharynx; max, maxillary plate; mmax, maxillary muscles; ph, pharynx; pr, premental retractor; prmt, prementum; sd, salivary duct; 9, M. frontoepipharyngalis; 43, M. clypeopalatalis; 45/46, Mm. frontobuccalis anterior/posterior.

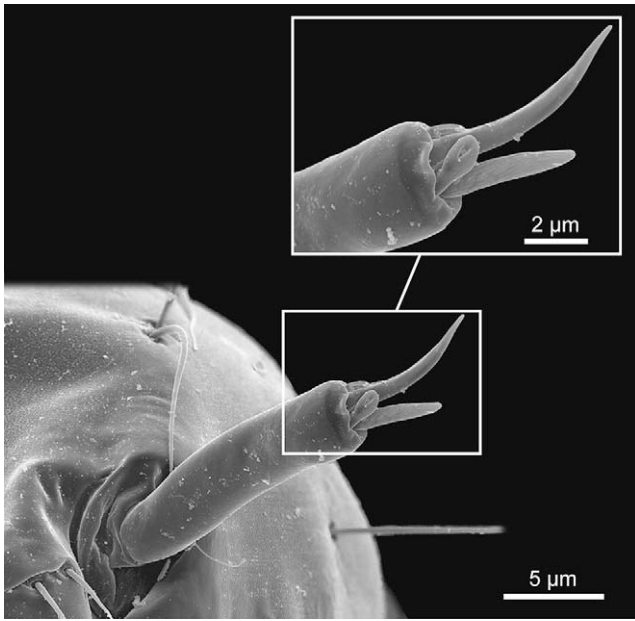


Fig. 6. *Nymphomyia dolichozepeza*, larva, antenna, SEM image.

present at the mesal margin beneath the basal lobe. It bears several clusters of compound, basally united macrotrichia, and a second group of macrotrichia laterad the basal lobe. Premandibles are completely absent.

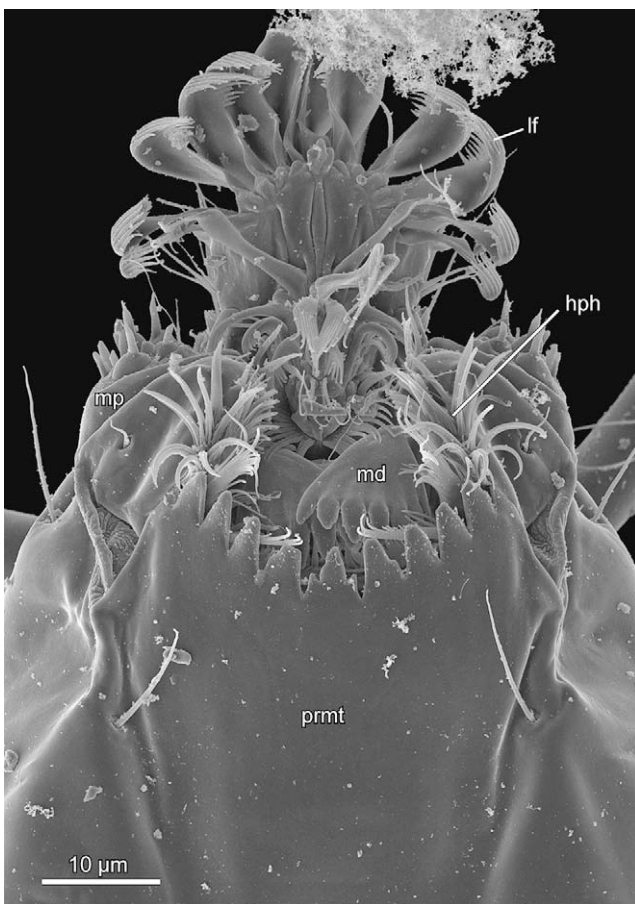


Fig. 7. *Nymphomyia dolichozepeza*, larva, head, mouthparts, ventral view, SEM image. Abbreviations: hph, hypopharynx; lf, labral fan; md, mandible; mp, maxillary palpus; prmt, prementum.

**Musculature:** (Figs. 8 and 10D–F) M. 11: M. craniomandibularis internus: the largest muscle of the head; O – with four subcomponents extensively on the dorsolateral part of the head capsule; I – with strongly developed adductor tendon (extended posteriorly fan-shaped) on the ventral margin of the mandible; F – adductor. M. 12: M. craniomandibularis externus: less strongly developed than M. 11; O – with four subcomponents dorsolaterally on the head capsule, anterolaterad M. 11; I – with long and slender tendon dorsolaterally on the mandibular base; F – abductor, antagonist of M. 11. M. 13: M. hypopharyngomandibularis; absent. M. 14: M. zygomaticus mandibulae: absent.

### 3.7. Maxilla

The maxilla is strongly simplified. The maxillary plate is longer than broad (max, Fig. 10D, E). The endites are fused with the palp and not recognisable as separate structures. The palpus is one-segmented and inserts on a small dorsal prominence likely representing the stipes (Fig. 9). On the apex it bears eight sensilla.

**Musculature:** (Fig. 5) Two antagonistic muscles are present, probably representing M. tentoriocardinalis (M. 17) and/or M. tentoriostipitalis (M. 18) and M. craniolacinalis (M. 19). Mm. 15/16: Mm. craniocardinalis externus/internus: absent. M. 20: M. stipitolacinalis: absent. M. 21: M. stipitogalealis: absent. Mm. 22/23: Mm. stipitopalpalis externus/internus: absent. Mm. 24–27: Mm. palpopalpalis maxillae primus/secundus/tertius/quartus: absent.

### 3.8. Labium and hypopharynx

The labium is composed of a broad, flat and well sclerotised hypostoma (postmentum *sensu* Courtney, 1994) and a complex, largely hidden prementum lacking distinct appendages (prmt, Fig. 7). Posteriorly the hypostoma is fused with the postgenal region. Its anterior margin bears one central tooth with two smaller accessory teeth on either side, and five additional lateral teeth. The simplified prelabium forms a complex with the hypopharynx. The anterior part of the hypopharynx, the lingua (see Cutten and Kevan, 1970), is plate like and bears eight teeth (hph, Fig. 5). Palpi are completely missing.

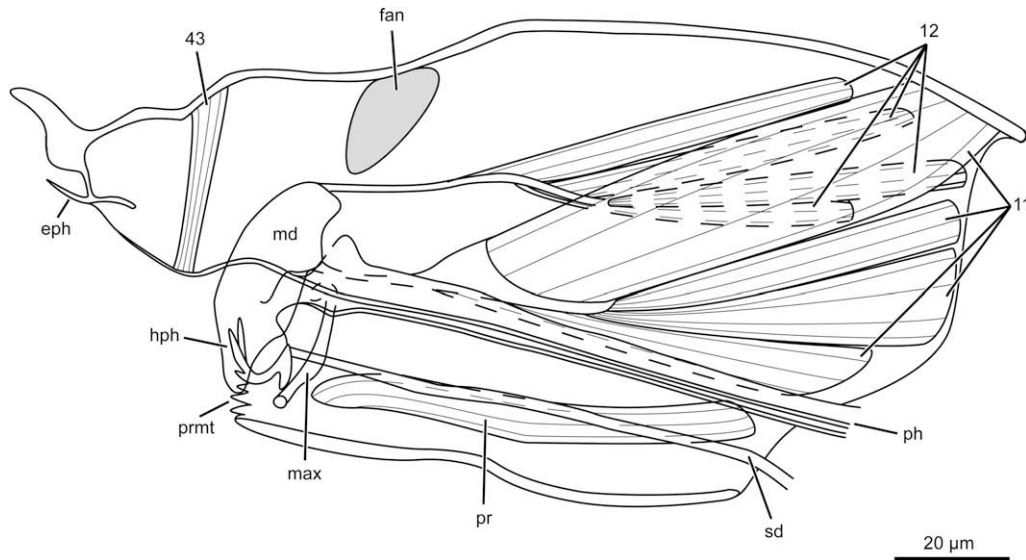
**Musculature:** (pr, Figs. 5, 8 and 10E, F) Mm. 28–30: M. submentopraementalis, Mm. tentoriopraementales inferior/superior: only one premental retractor is present. M. 31: M. praementoparaglossalis: absent. M. 32: M. praementoglossalis: absent. Mm. 33/34: Mm. praementopalpalis internus/externus: absent. Mm. 35/36: Mm. palpopalpalis labii primus/secundus: absent. M. 41: M. frontohypopharyngalis: absent. M. 42: M. tentoriohypopharyngalis: absent.

### 3.9. Pharynx

The pharynx is largely membranous and its opening lies approximately at the level of the antennal insertion. The lumen of the anterior part is very narrow and U-shaped in cross section (ph, Fig. 10D). The lateral edges are distinctly bent upwards. The middle section widens at the level of the insertion of the first bundle of M. 45/46. Approximately in the middle region of the head capsule the lateral pharyngeal folds split and there is no recognisable lumen (ph, Fig. 10E, F).

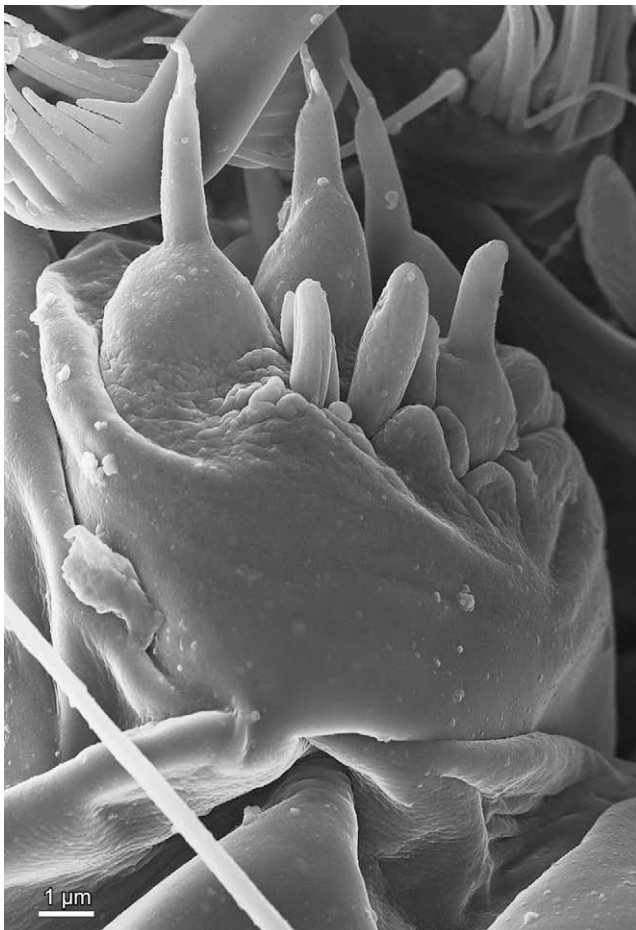
**Musculature:** (Fig. 6) Precerebral dorsal pharyngeal dilators: it cannot be assessed whether the two slender bundles represent M. frontobuccalis anterior (M. 45) or M. frontobuccalis posterior (M. 46) or both; O – postfrontal region; I – to separate successive insertion points dorsally on the precerebral pharynx, approximately at the level of opening of the salivary duct; F –





**Fig. 8.** *Nymphomyia dolichozepe*, larva, head, sagittal section. Abbreviations: eph, epipharynx; fan, foramen antennale; hph, hypopharynx; max, maxillary plate; ph, pharynx; pr, premental retractor; prmt, prementum; sd, salivary duct; 11, M. craniomandibularis internus; 12, M. craniomandibularis externus; 43, M. clypeolabralis.

prepharyngeal dilator. M. 47: M. frontobuccalis lateralis: absent. M. 48: M. tentoriobuccalis anterior: absent. Mm. 49/50: M. tentoriobuccales lateralis/posterior: absent. M. 51: M. verticopharyngalis: absent. M. 52: M. tentoriopharyngalis: absent.



**Fig. 9.** *Nymphomyia dolichozepe*, larva, head, maxillary palpus.

### 3.10. Salivarium

The salivary duct opens anteriorly on the posterior part of the hypopharyngeal plate, approximately at the level of the frontal apex (sd, Figs. 6 and 8). The external wall of the anterior part is sclerotised whereas the remaining duct is membranous. It is nearly round in cross section and slightly flattened posteriorly (sd, Fig. 10D–F). Approximately at the level of the insertion of M. 41 the duct is distinctly bent ventrad and runs closely adjacent to the ventral wall of the head capsule. It enters the prothorax on the ventral side.

**Musculature:** M. 37: M. hypopharyngosalivariialis: absent. Mm. 38/39: Mm. praementosalivariiales anterior/posterior: absent. M. 40: M. anularis salivarii: absent.

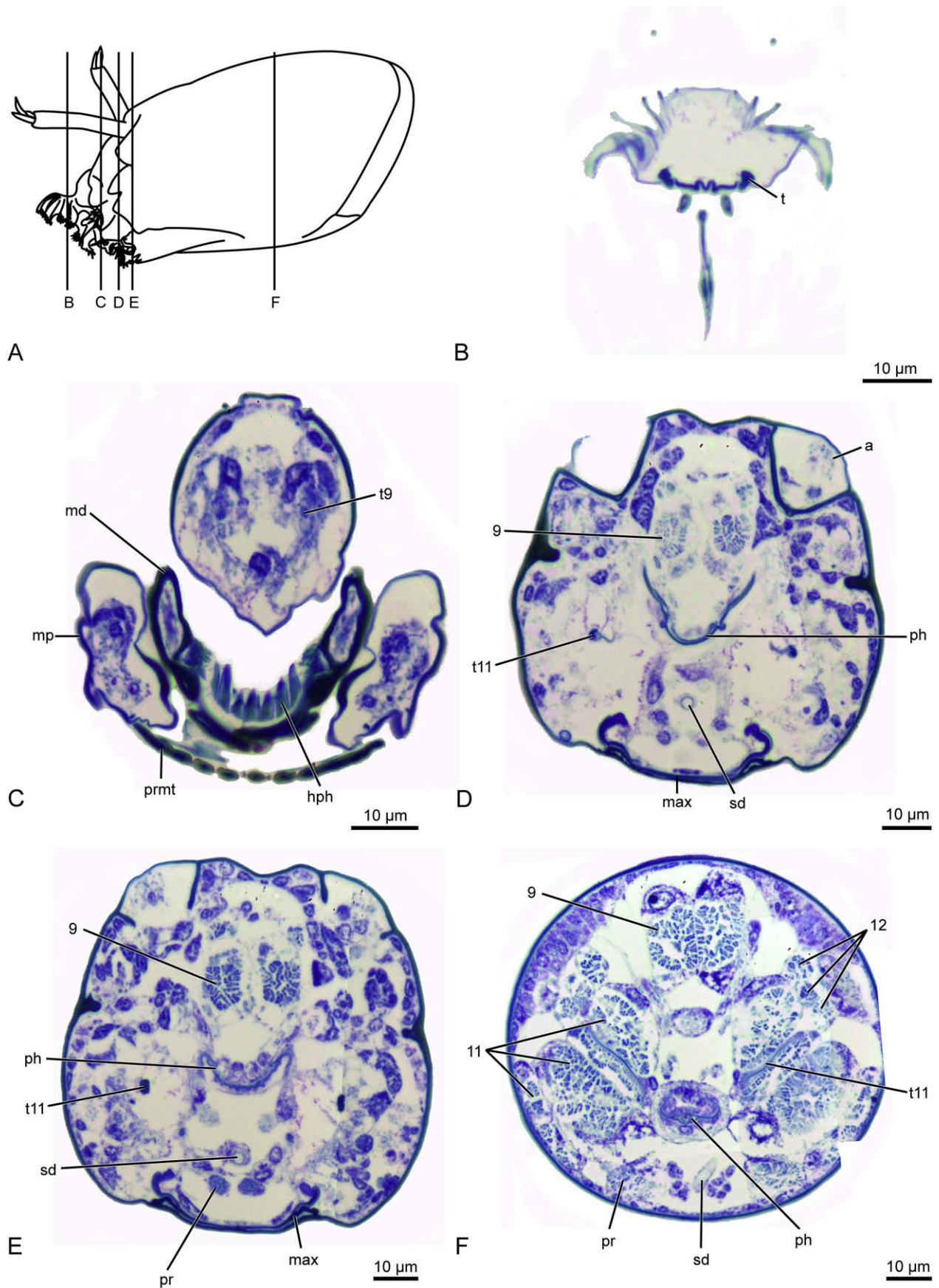
### 3.11. Nervous system

The brain and suboesophageal complex are completely shifted to the thorax.

## 4. Discussion

The monophyly of the highly specialised and uniform Nymphomyiidae is beyond serious doubt. It is strongly supported by autapomorphies of the larval, pupal and adult stages. Adult apomorphies include the ventrally contiguous compound eyes, the loss of the unpaired ocellus, the shift of the lateral ocelli posterior to the compound eyes and the complete reduction of the tentorium (Togunaka, 1935; Courtney, 1991; char. 20–32). A pupal apomorphy is the prognathous head (Courtney, 1991).

Previously suggested larval features supporting the monophyly are the dimorphic crochets on the abdominal prolegs and the presence of only two malpighian tubules borne on a common stalk originating on the ventral surface of the gut (Courtney, 1991). The complete loss of the tentorium is also a potential larval apomorphy of Nymphomyiidae (Courtney, 1991). However, the same condition is present in several other lineages such as Tipulidae (Cook, 1949; Neugart et al., 2009), Scatopsidae (Wood and Borkent, 1989), Simuliidae and in most Chironomidae (Cook, 1944), Psychodidae (Anthon, 1943) and Sciaroidea (Cook, 1944; Matile, 1990). In Mycetophilidae s. str. the tentorium is also mainly reduced (Matile,



**Fig. 10.** *Nymphomyia dolichozepe*, larvae, head, cross sections. (A) Overview of the sections. (B–F) Cross sections. Abbreviations: a, antenna; hph, hypopharynx; max, maxillary plate; md, mandible; mp, maxillary palpus; ph, pharynx; pr, premental retractor; prmt, prementum; sd, salivary duct; t, tormae; t9, tendon of *M. frontoepipharyngalis*; t11, tendon of *M. craniomandibularis internus*; 9, *M. frontoepipharyngalis*; 11, *M. craniomandibularis internus*; 12, *M. craniomandibularis externus*.



1967). Courtney (1991) discussed the anteriorly serrate and posteriorly fused hypostoma as a further autapomorphy. However, this condition occurs in many other groups of Diptera such as for instance in Chironomidae (Courtney, 1991) or Tipulidae (Neugart et al., 2009).

Cutten and Kevan (1970) and Schneeberg et al. (2011) discussed a close relationship between Nymphomyiidae and Deuterophlebiidae. As pointed out above, Deuterophlebiidae are also highly specialised and in a very similar way as Nymphomyiidae (Courtney, 1991). In larvae of Nymphomyiidae and in later-instars of Deuterophlebiidae the ventral surface of the labrum-epipharynx is equipped with rows of spatulate macrosetae (Courtney, 1991). However, similar structures occur on the epipharyngeal surface of larvae of some Chironomidae and Simuliidae (Oliver and Roussel, 1983; Craig, 1974; see also char. 18, Courtney, 1991). Some other affinities concerning the morphology and life history were pointed out by Courtney (1991). In both families females shed their wings after or during oviposition, the femur and tibia are subdivided by a membranous zone, all abdominal spiracles are absent, and the spermatheca is vestigial (see Courtney, 1991, chars. 29–32). These similarities were interpreted as convergences, related to an independent evolution of a short-lived adult stage (see discussion in Courtney, 1991, p. 167).

A sistergroup relationship between Nymphomyiidae and Deuterophlebiidae would be in contrast to a clade comprising Deuterophlebiidae and Blephariceridae (=Blephariceroida) as suggested by analyses of Courtney (1991) and Oosterbroek and Courtney (1995). Blephariceroida was supported by 13 or 17 synapomorphies, respectively (see Courtney, 1991, char. 33–45; Oosterbroek and Courtney, 1995). Potential larval apomorphies are an isolated first-instar frontal sclerite and a laterally shifted antennal socket (Courtney, 1991). Another potential apomorphy is the relatively broad and completely membranous postmentum (Courtney, 1991). However, this is not a very strictly defined character state and the condition in many nematoceran groups is unclear. The head and prothorax of the first two instars of Deuterophlebiidae and all instars of Blephariceridae are fused (Courtney, 1991). A similar fusion apparently also occurs in early instars of Tipulidae, Tanyderidae and Axymyiidae (Courtney, 1991, char. 37). Courtney (1991) interpreted also five Malpighian tubules and the posterodorsal margin of the cranium excised laterally or extended medially into the thorax, with a region of intermoult cuticle deposition, as synapomorphy of Blephariceroida.

A clade Blephariceromorpha (Nymphomyiidae, Deuterophlebiidae and Blephariceridae) was supported in several studies (Wood and Borkent, 1989; Courtney, 1990, 1991; Oosterbroek and Courtney, 1995). However, the potential larval synapomorphies are far from convincing. One shared derived feature is a vestigial *M. labroepipharyngalis* (an unusual transverse intrinsic muscle described by Courtney (1990) is almost certainly not homologous with *M. labroepipharyngalis* sensu Kéler (1963)), but this muscle is missing in Tipulidae (Neugart et al., 2009) and may be absent in other groups where anatomical data are still missing. Another potential apomorphy of Blephariceromorpha is the absence of a movable premandible (messores). However, messores are missing in many other nematoceran groups such as Axymyiidae, Bibionidae, Pachyneuridae, Chaoboridae, and Tipuloidea (with some possible exceptions in Limoniidae), and they are generally absent in Brachycera (Oosterbroek and Courtney, 1995). Crochet-tipped prolegs are present on the abdominal segments I–VII in *Nymphomyia* and Deuterophlebiidae (Courtney, 1990) and do also occur on segments II–VI of first instar larvae of Blephariceridae (Courtney, 1991). However, prolegs are absent in certain Blephariceridae, notably in Edwardsiniinae. Zwick (1977) pointed out that eversible prolegs are not part of the blepharicerid groundplan (see also Courtney, 1991). Other potential apomorphic features are obviously

linked with the life in aquatic habits, like the complete loss of spiracles and the non-retractile anal papillae (Courtney, 1991). These conditions have apparently evolved several times independently. They also occur in several other groups with aquatic larvae such as Tipulidae, Simuliidae, Ceratopogonidae (see also Courtney, 1991) and some Mycetophilidae (Hennig, 1973).

Courtney (1994) discussed a possible close relationship between Nymphomyiidae and Culicomorpha, based partly on the shared presence of an anteriorly serrate and posteriorly fused hypostoma. However, a similar condition occurs also in other dipteran larvae, including some Tipulidae and most Tanyderidae.

We did not find larval features suggesting phylogenetic affinities between Nymphomyiidae and Axymyiidae (Bertone et al., 2008). Likewise, an “isolated position” of Nymphomyiidae (Rohdendorf, 1964), a sistergroup relationship between Nymphomyiidae and all the remaining dipteran lineages (Bertone et al., 2008 [reduced data set]) or the Deuterophlebiidae followed by Nymphomyiidae are the two earliest branching lineages of Diptera (Wiegmann et al., 2011) is also not supported by the presently available larval features.

It is difficult to assess plesiomorphic characters in Nymphomyiidae, because of its extreme specialisation. A potential plesiomorphic feature could be the presence of a toothed hypostoma. Its occurrence in many dipteran groups suggest it as a groundplan feature of the order (e.g. some Tipulidae, Neugart et al., 2009; Anisopodidae, Oosterbroek and Courtney, 1995; Tanyderidae, Exner and Craig, 1976). However, it is missing in the possible sistergroup Nannochorista (Beutel et al., 2009). This interpretation implies that this structure was reduced in different lineages, very likely several times independently.

Considering conflicting results in hitherto published phylogenetic studies, it is evident that the systematic position of Nymphomyiidae can only be clarified with a formal analysis of a very broad spectrum of data including extensive and well documented morphological characters of all stages and an extensive molecular data set. However, in view of the results of this contribution and of Schneeberg et al. (2011), a nymphomyiid-deuterophlebiid lineage, which may or may not be the sistergroup of Blephariceridae, is possible. Although conceivable that a shared lifestyle may have led to parallel evolution of similar morphological adaptations in Nymphomyiidae and Deuterophlebiidae, it is also possible that a common ancestor adapted to fast flowing streams developed specific features such as small size, prolegs with crochets on specific abdominal segments, subdivided adult femora and tibiae, completely reduced abdominal spiracles, a vestigial spermatheca, short-lived adults, and wings of females shed at predefined zones of weakness.

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### 3.6 Study VI

Wipfler B, **Schneeberg K**, Löffler A, Hünefeld F, Meier R, Beutel RG. 2013.

The skeletomuscular system of the larva of *Drosophila melanogaster* (Drosophilidae, Diptera) – a contribution to the morphology of a model organism. *Arthropod Structure and Development* 42: 47-68.

#### **Abstract**

The morphological features of the third instar larva of the most important insect model, *Drosophila melanogaster*, are documented for the first time using a broad spectrum of modern morphological techniques. External structures of the body wall, the cephaloskeleton, and the musculature are described and illustrated. Additional information about other internal organs is provided. The systematic implications of the findings are discussed briefly. Internal apomorphic features of Brachycera and Cyclorrhapha are confirmed for *Drosophila*. Despite the intensive investigations of the phylogeny of the megadiverse Diptera, evolutionary reconstructions are still impeded by the scarcity of anatomical data for brachyceran larvae. The available morphological information for the life stages of three insect model organisms *D. melanogaster* (Diptera, Drosophilidae), *Manduca sexta* (Lepidoptera, Sphingidae) and *Tribolium castaneum* (Coleoptera, Tenebrionidae) - is addressed briefly. The usefulness of a combination of traditional and innovative techniques for an optimized acquisition of anatomical data for different life stages is highlighted.

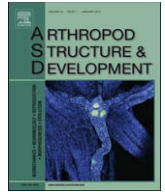
#### **Significance in the present thesis**

Surprisingly the morphology, especially the skeletomuscular system, of the larva of the model organism *Drosophila melanogaster* was not known in detail. The investigation completes our taxon sampling.

#### **Own contribution**

35%





## The skeletomuscular system of the larva of *Drosophila melanogaster* (Drosophilidae, Diptera) – A contribution to the morphology of a model organism

Benjamin Wipfler<sup>a</sup>, Katharina Schneeberg<sup>a,\*</sup>, Andreas Löffler<sup>a</sup>, Frank Hünefeld<sup>a</sup>, Rudolf Meier<sup>b</sup>, Rolf G. Beutel<sup>a</sup>

<sup>a</sup> Entomology Group, Institut für Spezielle Zoologie and Evolutionsbiologie, FSU Jena, Erbertstrasse 1, 07743 Jena, Germany

<sup>b</sup> Department of Biological Sciences and University Scholars Programme, National University of Singapore, 14 Science Dr 4, Block S2 #02-01, Singapore 117543, Singapore

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### ABSTRACT

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### 1. Introduction

The relative importance of morphological characters for the reconstruction of phylogenetic relationships is arguably decreasing in the era of phylogenomics, although the number of analyses based on morphological characters remains high (Meier and Lim, 2009). Very extensive molecular data sets with several tens of thousands of base pairs and improved analytical approaches lead to increasingly robust phylogenetic hypotheses (e.g., Regier et al., 2010; Meusemann, 2010) and dwarf even comparatively large morphological character sets. However, this does not affect the general importance of morphology. “Naked” trees without a biological context are of limited interest, whereas morphological structures are intrinsically interesting for scientists working in fields like evolutionary biology, developmental biology, genetics, applied entomology, or other disciplines of biology.

The famous American myrmecologist W.M. Wheeler (1865–1937) considered *Drosophila melanogaster* Meigen 1830 a “stupid little saprophyte” (Grimaldi and Engel, 2005). Nevertheless, there

are few organisms if any at all that have revolutionized biology to a similar degree as this unattractive and usually undesirable fruit fly. It is one of approximately 3,500 known species of the successful family Drosophilidae, which belongs to the Ephydroidea (ca. 5,400 spp.). Drosophilids are small or very small flies ranging between 1 and 7 mm in length. Many species are attracted to decaying plant materials such as leaves or fruit (fruit fly), where they deposit their eggs. The hatching maggots feed on yeast and bacteria, which are involved in the decomposition process. Other species are leaf miners, ectoparasites of cicada and land crabs, predators of scale insects, orinquilines of nests of solitary bees (Hennig, 1973; Ziegler, 2005).

The original distribution area of *D. melanogaster* is tropical Africa but it is cosmopolitan today. The females lay ca. 300 eggs of approximately 0.5 mm length. The 1st instar larvae hatch after 20–22 h at 25 °C (Sonnenblick, 1950). The first molt takes place after 25 h, the second after 48 h. After 96 h the puparium is formed (Bodenstein, 1950).

The high reproductive rate, short generation time, ease of maintenance in the lab, and the manageable genome size favored the small fruit fly as a model organism (see Brookes, 2002). The unparalleled career of *D. melanogaster* in genetics and developmental biology started in the first decade of the

\* Corresponding author. Tel.: +49 3641 49181; fax: +49 3641 49142.

E-mail address: [katharina.schneeberg@gmx.de](mailto:katharina.schneeberg@gmx.de) (K. Schneeberg).

20th century, when T.H. Morgan carried out cross-breeding experiment with mutants of the species. His investigations resulted in decisive breakthroughs in genetics (e.g., Morgan, 1912, 1914; Morgan and Bridges, 1916). Since the 1970s *D. melanogaster* is widely used as a model organism in developmental biology (Lewis, 1939–1985; Wieschaus and Szabad, 1979; Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard, 1990, 1994) and a wide variety of other biological investigations related to such diverse phenomena as insomnia, alcoholism, neurodegenerative diseases, or global warming. *D. melanogaster* was also the first complex eukaryote with a completely sequenced genome (Adams et al., 2000; see also Grimaldi and Engel, 2005).

It is surprising that despite its prominent role in developmental biology and its undisputed status as one of the most intensively investigated models, several aspects of the larval morphology remain only partly or insufficiently known. Hennig (1948–1952) studied the external features. Strasburger (1932) gave a detailed account of the cephaloskeleton (“cephalopharyngeal” skeleton) and the digestive system, and Hertweck (1931), Dambly-Chaudière and Ghysen (1986) and Melzer and Paulus (1989) studied the sensory organs and nervous system. Crossley (1978), Hooper (1986) and Bate (1990) provided information on the musculature of selected body regions. However, these descriptions were focussed on developmental aspects and only segmental muscles were considered. Despite these contributions, a complete and detailed description of the integument and the musculature of all body regions is missing. This motivated the present study, which is primarily aiming at a detailed documentation of the skeletomuscular system. In addition, some details on the fat body and imaginal anlagen, and the tracheal-, nervous- and digestive system are provided. Phylogenetically relevant characters are addressed briefly. A brief assessment of presently available morphological data for three important insect model species is presented (*D. melanogaster* [Diptera], *Tribolium castaneum* [Coleoptera], *Manduca sexta* [Lepidoptera]). Finally, the usefulness of traditional and innovative morphological techniques for an optimized acquisition of anatomical data for different life stages is evaluated.

## 2. Material and techniques

### 2.1. Material

A culture of *D. melanogaster* (wild type Canton S) was obtained from the Max-Planck-Institut für Chemische Ökologie (Jena, Germany). The maggots were kept in a glass container with growth medium. After 2–4 weeks adults were transferred to a new container. Third instar larvae (L3) were cleaned with water and detergent, numbed in mineral water containing CO<sub>2</sub> for relaxation of the body, and finally fixed in FAE (formaldehyde–ethanol–acetic acid 3:6:1). After sufficient fixation they were stored in 70% Ethanol.

### 2.2. Histological sectioning

Larvae were embedded in Araldit CY 212 (Agar Scientific, Stansted/Essex, UK). Sections of 1.5 µm thickness were made using a Microtome HM 360 (Microm, Walldorf, Germany). They were stained with Toluidine blue and Pyronin G (Waldeck GmbH and Co. KG/Division Chroma, Münster, Germany). The sections were digitized with an Axioskop microscope (Carl Zeiss AG, Jena, Germany) with an Pixelink PL-B686 camera (Pixelink, Ottawa, Canada) and the software Pixelink Capture OEM 7.12 (2008) (Pixelink, Ottawa, Canada).

### 2.3. Scanning electron microscopy (SEM)

Specimens were cleaned using ethanol (50%–10%), distilled water and detergent, and ultrasonic sound (Bandelin Elektronik, Berlin, FRG). After that the specimens were retransferred in ethanol (80%–100%) and from 100% ethanol to acetone (100%). Finally they were dried at the critical point (EmiTech K850 Critical Point Dryer), sputter coated (EmiTech K500 Sputter Coater [both Quorum Technologies Ltd., Ashford, UK]) and mounted on a special specimen holder (Pohl, 2010). SEM images were taken with an ESEM XL30 (Philips, Amsterdam, The Netherlands) and the software Scandium FIVE (Olympus, Münster, Germany). Figure plates with SEM images were processed with Adobe Photoshop and Illustrator CS2 (Adobe, San José, USA).

### 2.4. 3D-Reconstruction

Image stacks of the sections were aligned and processed using AMIRA 5.3 (Visage Imaging GmbH, Berlin, Germany). Surface polishing and rendering was done with MAYA 2011 (Autodesk, San Rafael, USA). Figure plates were completed with Adobe Photoshop and Illustrator CS2.

### 2.5. Maceration

Rehydrated larvae (70%–10% ethanol, aqua dest) were transferred in KOH (10%) and kept in an incubator for several hours (60 °C). After that they were gradually retransferred to ethanol (aqua dest- 10%–70%, 2 × 2 min each step).

Alternatively, for the examination of skeletal structures, larvae were dehydrated (80%, 90%, 96%, 2 × 2 min each step). After that the specimens were subsequently transferred in methyl salicylate and mounted on an object slide with a concavity. Photos were taken with a Nikon D90 (Nikon Corporation, Tokia, Japan) equipped with a Zeiss Lumina 16 mm objective (Carl Zeiss AG, Jena, Germany).

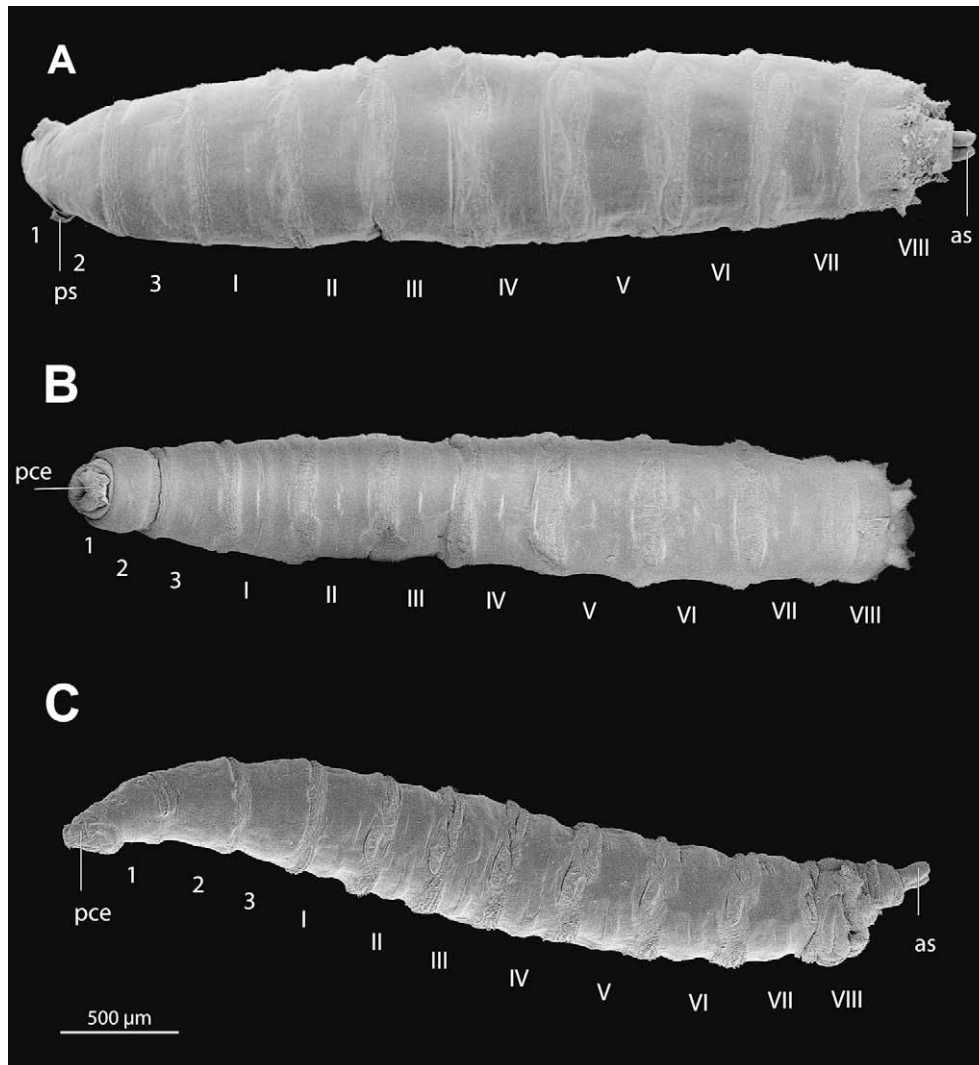
### 2.6. Terminology

The terminology used for skeletal structures follows Courtney et al. (2000) unless otherwise stated. Additionally, it is referred to the more complete nomenclature for the cephaloskeleton of Strasburger (1932). No previously introduced nomenclature is used for the muscles, which are simply numbered consecutively. In general, the origin of a muscle is defined as the attachment site on the immobile part, and the insertion on the movable element. For muscles of the body wall, which extend along the entire segment, this definition is not applicable. Consequently the anterior or ventral attachment areas are considered as the origin.

## 3. Results

### 3.1. Habitus and segmentation

The cylindrical 3rd instar larvae (maggots) are ca. 4–5 mm long and 1 mm wide. They reach the greatest width in the middle region of the body and taper cranially (Fig. 1). The cuticle is thin, very weakly sclerotized and transparent. The only strongly sclerotized parts are the mouth hooks and the cephaloskeleton (Fig. 4), which is visible through the external cuticle. The body is subdivided into the distinctly reduced pseudocephalon (often referred to as the “head” although it includes thoracic elements), three legless thoracic segments, and an elongate abdomen (Fig. 1). The pseudocephalon is distinctly retracted into the prothorax, which bears a pair of dorsolateral spiracles (pce, Figs. 1 and 2). The other thoracic segments and the abdominal segments I–VII lack spiracles and are



**Fig. 1.** *Drosophila melanogaster*, larva; habitus, SEM micrograph. (A) Dorsal; (B) ventral; (C) lateral. Abbreviations: 1–3, thoracic segments; I–VIII, abdominal segments (VIII = anal division); as, abdominal spiracles; pce, pseudocephalon; ps, prothoracic spiracles.

very similar in their general shape and composition. The last abdominal “segment” is more aptly named anal division given that it consists of several abdominal segments (Courtney et al., 2000). It bears a posterior pair of spiracles at its apex. Thoracic and abdominal legs or prolegs are missing. Several transverse rows of small cuticular teeth are present along the segmental borders (ct, Fig. 5). They are more distinct ventrally than dorsally.

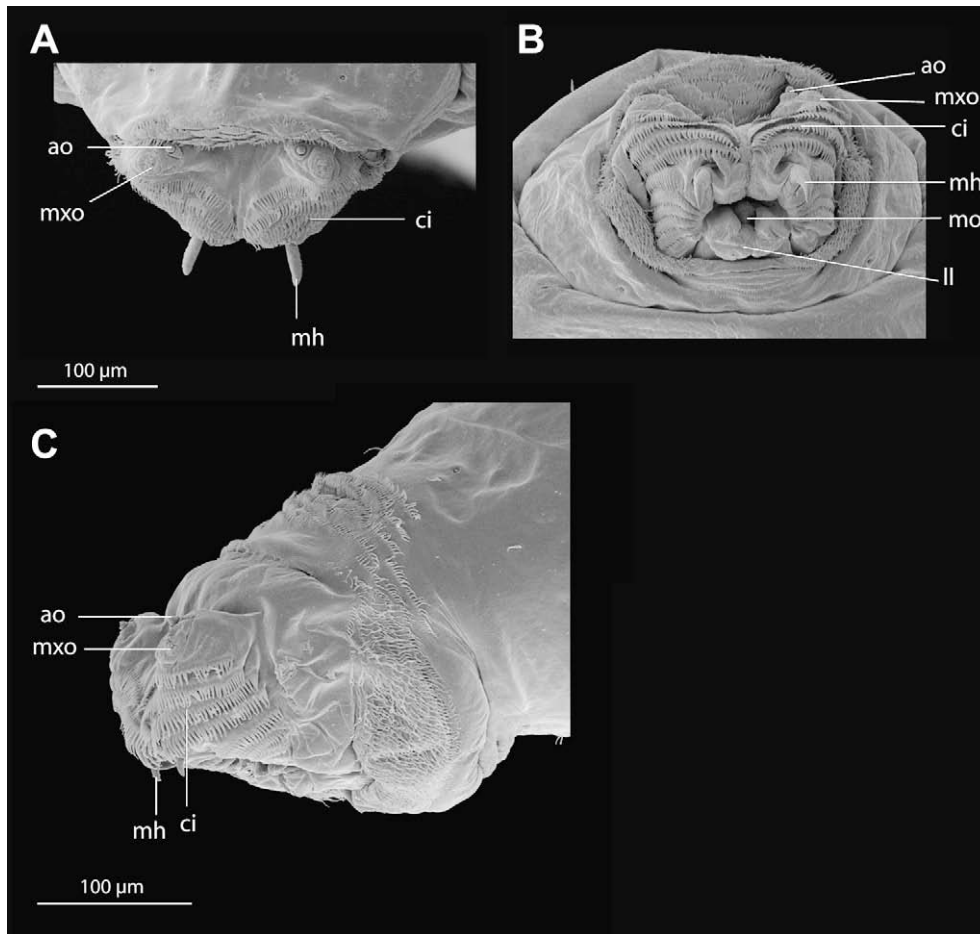
### 3.2. Pseudocephalon

The head capsule is strongly reduced and largely internalized. The orthognathous pseudocephalon (pce, Fig. 1) is unsclerotized, bilobate and twice as wide as long. Sutures or strengthening ridges are missing. Dorsally it is completely fused and partially retracted within the prothorax with the slightly sclerotized cephalic elements extending into the thorax on the ventral side. A well-defined foramen occipitale and the postoccipital ridge are absent. Dorsally a pair of antennae or antennal organs is present (‘Dorsalorgan’; Bolwig, 1946) (ao, Figs. 2 and 3) and laterad of them is a pair of maxillary sense organs (‘Terminalorgan’; Bolwig, 1946) (mxo, Figs. 2 and 3). The mouth hooks are inserted on the ventral side (mh, Figs. 2 and 3). Their orientation is parallel to the

body axis. Conspicuous structures are the cirri. Four parallel rows of larger cirri are arranged above the mouth hooks (ci, Figs. 2 and 3) and five additional rows of smaller cirri are present below them.

#### 3.2.1. External sense organs, pseudocephalic appendages and mouthparts

Compound eyes are absent. The Bolwig’s organs or stemmata are not visible externally. They are located laterad the cephaloskeleton on the level of the dorsal bridge (bo, Fig. 9E). The antennae or dorsal organs (ao, Figs. 2 and 3) are distinctly separated from the maxillary or terminal sense organs. They are about 12–15 µm wide and formed by a cupola-shaped sensory dome (7 µm) with pore channels in its cuticle. It is enclosed by a ring-shaped cuticular bulge (Singh and Singh, 1984: Fig. 1e). In its central area, the dome contains seven bundles of dendrite-triplets (rarely only two dendrites in one of them) arranged symmetrically in a space filled with sensillar fluid. Distally the dendrites divide into numerous branches (Singh and Singh, 1984: Fig. 1a–e). Around the dome six sensilla are located, five of them with two dendrites and one with a single dendrite (Singh and Singh, 1984: Fig. 1e). The maxillary sense organ (mxo, Figs. 2 and 3) is about 30 µm long and



**Fig. 2.** *Drosophila melanogaster*, larva; pseudocephalon, SEM micrograph. (A) Dorsal; (B) ventral; (C) lateral. Abbreviations: ao, antenna or antennal organ; ci, cirri; ll, labial lobes; mh, mouth hooks; mo, mouth opening; mxo, maxillary sense organ.

composed of two groups of sensilla, which are enclosed by a ring of lobe-like cuticular outgrowths. This includes two smaller knob-shaped sensilla (1.6  $\mu\text{m}$ ) and five larger papilla-shaped sensilla (4  $\mu\text{m}$ ) (Frederick and Denell, 1982) (a somewhat different configuration was described by Singh and Singh, 1984). The dendritic organization is illustrated in Singh and Singh (1984). Ventrad the maxillary sense organ of the ventral organs are located, each of them containing five sensilla. Four of them are innervated by one dendrite and one by four (Singh and Singh, 1984: Fig. 4c).

The only distinctly developed mouthparts are the mouth hooks (Figs. 2–4). They are black, strongly sclerotized, sickle-shaped and oriented parallel to the longitudinal body axis. Their exposed distal oral process bears seven teeth on its ventral side. A dorsal and a ventral process are present proximally. The mouth hooks contain a single dendrite close to their base (Singh and Singh, 1984). The labial lobe is placed further ventrad between the hooks (ll, Fig. 2B). It forms the posterior border of the mouth opening (mo, Fig. 2B). The labial organ on the ventral side of the lobe comprises three sensilla, one of them with three dendrites and two with one each (Singh and Singh, 1984: Fig. 4b).

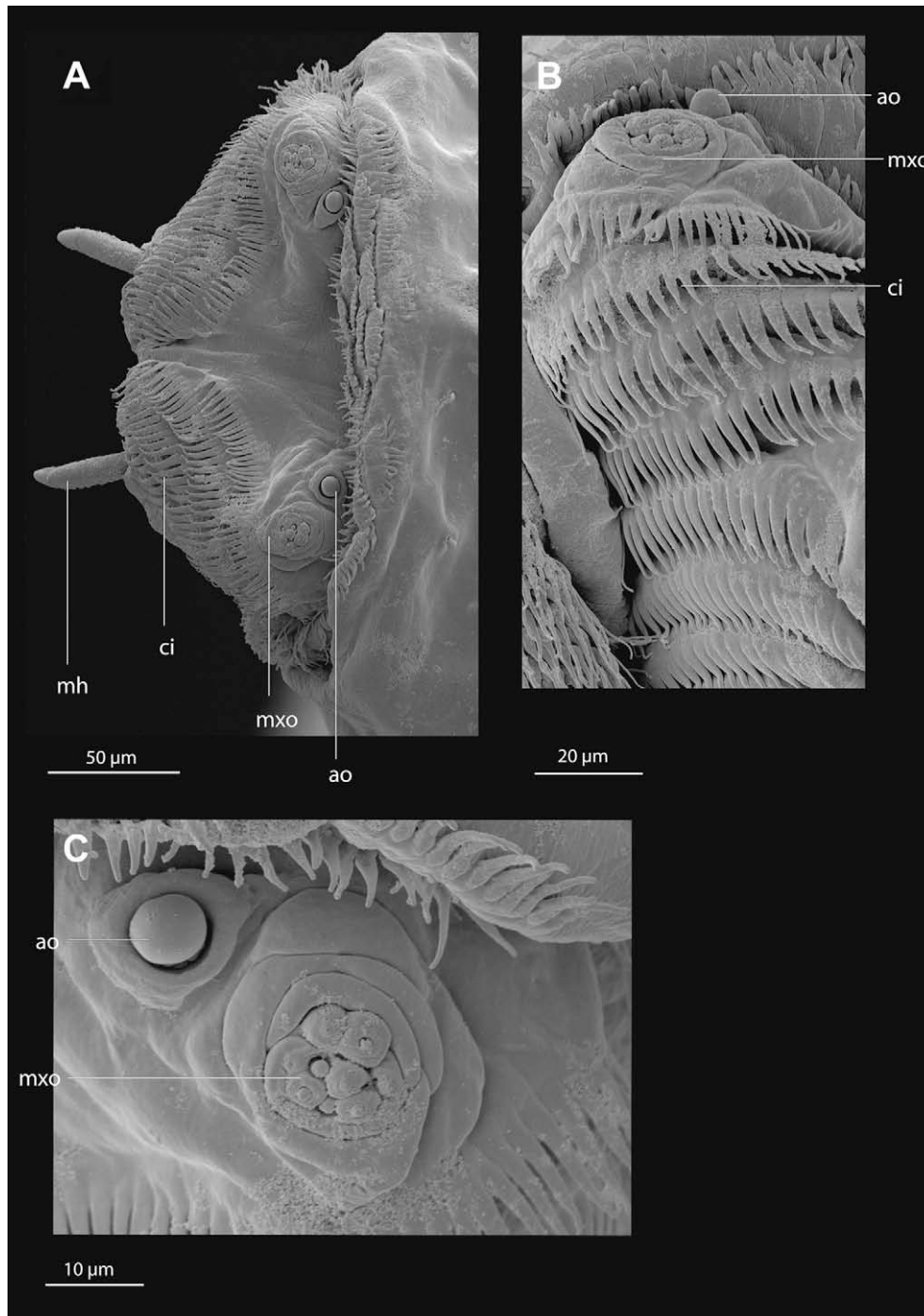
The paired dorsal pits on the dorsal side of the pseudocephalic region contain 5 + 2 cavities each. A paired knob in pit sensillum (KIP) is present on the dorsolateral surface (Singh and Singh, 1984).

### 3.2.2. Intermediate and basal sclerite

The cephaloskeleton consists from anterior to posterior of three main parts; i.e., the mouth hooks, intermediate sclerite and basal

sclerite. The intermediate sclerite is strongly sclerotized anteriorly, but the degree of sclerotization decreases towards its posterior region (Figs. 4 and 13). It lies within the pro- and mesothorax and is visible through the transparent cuticle (Fig. 12A). Two broad rods are continuous with the mouth hooks. The entire structure is sometimes also termed the H-piece (H, Figs. 4B and 9A–E). The dorsal bridge lies above this endoskeletal element and connects its caudal processes with each other (db, ppH, Figs. 4 and 9C, F). A sclerite posterior to the mouth hooks and below the H-piece is referred to as mouth angle piece (map, Figs. 4B and 9B). The largest part of the cephaloskeleton is the basal sclerite (“cephalopharyngeal plates” of Strasburger, 1932). It is continuous with the H-piece and the ventral anterior process (vap, Figs. 4B and 9D–F) is attached with a slightly oblique orientation directly posterad of the H-piece. Slightly posterodorsad, the dorsal anterior processes (dap, Figs. 4B and 9C–E) articulate and reach anteriorly towards the level of the posterior end of the mouth hooks. Posterad of these articulations, the basal sclerite forms two large and wing-shaped processes, the ventral (vpp, Figs. 4 and 9E–H) and dorsal posterior process or cornua (dpp, Figs. 4 and 9F–H). Both serve as muscle attachment areas. Their sclerotization decreases posteriorly. The ventral processes are slightly wider and longer than the dorsal ones. In the anterior third, the two processes are connected by a wide membrane and several cibarial dilators (see Muscle section). The ventral posterior process forms the roof of the cibarium, which displays nine T-shaped longitudinal ridges (T-ridges) in this area. The dorsal posterior process is distinctly bifurcated in its posterior region. The dorsal bridge articulates on the anterior margin of the basal





**Fig. 3.** *Drosophila melanogaster*, larva; pseudocephalon, details, SEM micrograph. (A) Dorsal; (B) ventral; (C) detail of A 2000 $\times$ . Abbreviations: ao, antenna or antennal organ; ci, cirri; mh, mouth hooks; mxo, maxillary sense organ.

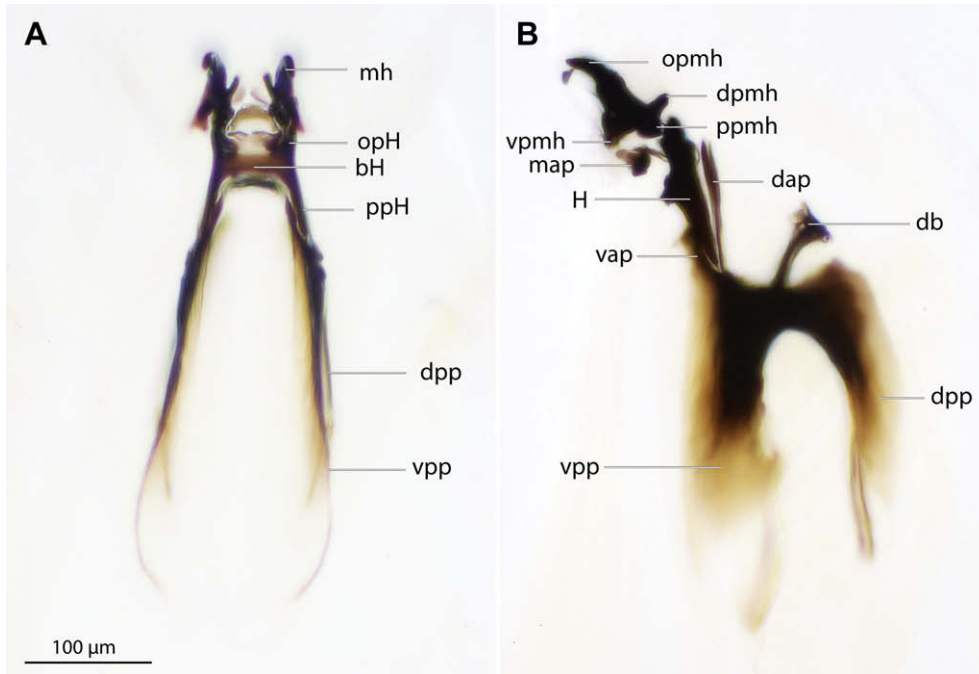
sclerite, dorsad the dorsal anterior process. It is composed of thin rods which run antiodorsad and form an arch mesally.

### 3.3. Thorax and anterior abdominal segments

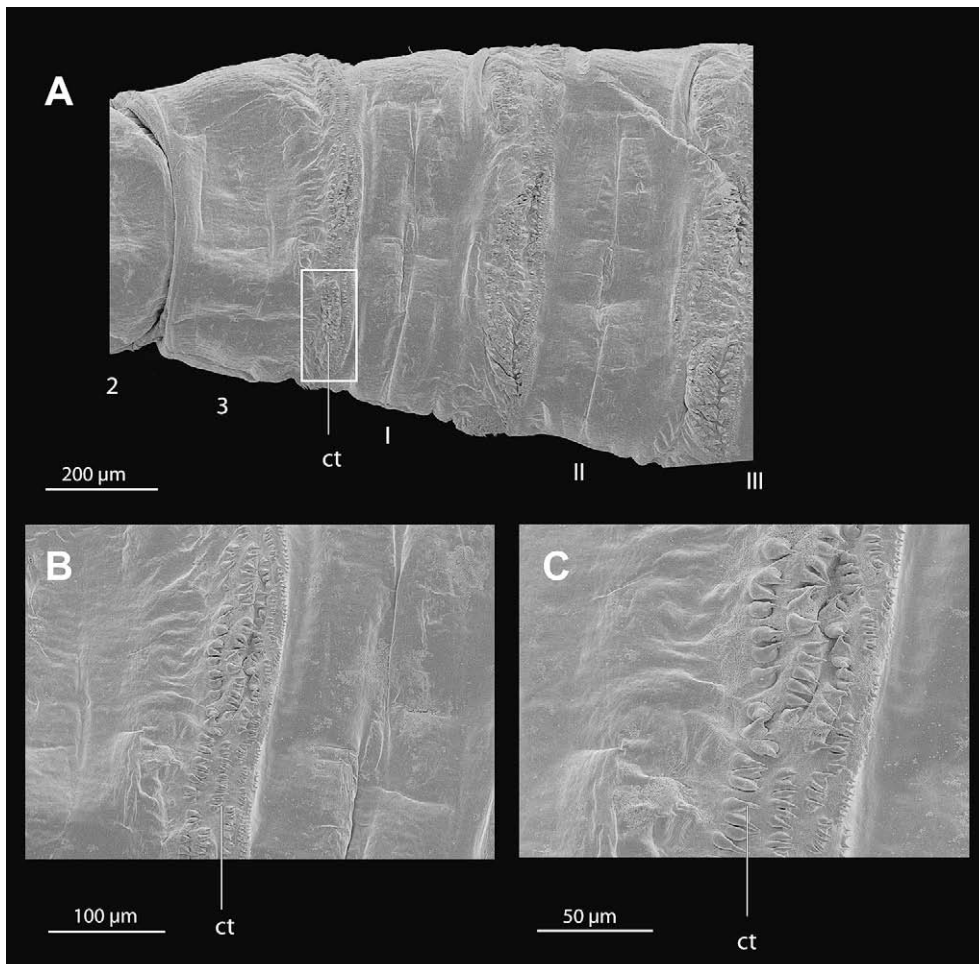
The meso- and metathorax and the abdominal segments I–VII are largely uniform. The prothorax contains the retraced pseudocephalon and additionally differs from the other segments by the presence of a pair of spiracles. These spiracles are hardly visible when retracted. Each of the three thoracic segments bears a pair of Keilin's organs (three sensorial sensilla together in a cavity) on

the ventral side. Several irregular rows of small cuticular denticles or spines (Lohs-Schardin et al., 1979; Szpila and Pape, 2008a; spines) are present along the anterior segmental borders (ct, Figs. 1 and 5). In the thoracic region these denticles are smaller than those on the abdominal segments and triangular rather than thorn-shaped. They are smaller laterally and dorsally while the ventral rows of segments I–VII form transverse creeping welts (ampullae). Lateral welts without denticles are present at the posterior end of the abdominal segments I–VII. A transverse furrow divides these segments in an equally sized anterior and posterior half.





**Fig. 4.** *Drosophila melanogaster*, larva; cephaloskeleton. (A) Ventral; (B) lateral. Abbreviations: bH, bridge of the H-piece; dap, dorsal anterior process; db, dorsal bridge; dpmh, dorsal process of the mouth hooks; dpp, dorsal posterior process; H, H-piece; mh, mouth hook; map, mouth angle piece; opH, oral process of the H-piece; opmh, oral process of the mouth hooks; ppH, posterior process of the H-piece; ppmh, posterior process of the mouth hooks; vpmh, ventral process of the mouth hooks; vap, ventral anterior process; vpp, ventral posterior process.



**Fig. 5.** *Drosophila melanogaster*, larva; (A) Ventral; (B) detail of A 300 $\times$ ; (C) same area 600 $\times$ . Abbreviations: 2–3, thoracic segments; I–III, abdominal segments; ct, cuticular tooth.

### 3.3.1. Prothoracic or anterior spiracles

The spiracles at the posterior margin of the prothorax bear an anteriorly directed tube of ca. 0.1 mm length (Fig. 6). Apically it bears several finger-shaped projections, processes or papilla (Bodenstein, 1950; see also Rühle, 1932). Only six of the eight papillae are recognizable externally (Fig. 6). They differ in length and can be retracted. A fissure-shaped spiracular opening lies at their distal end (so, Fig. 6C).

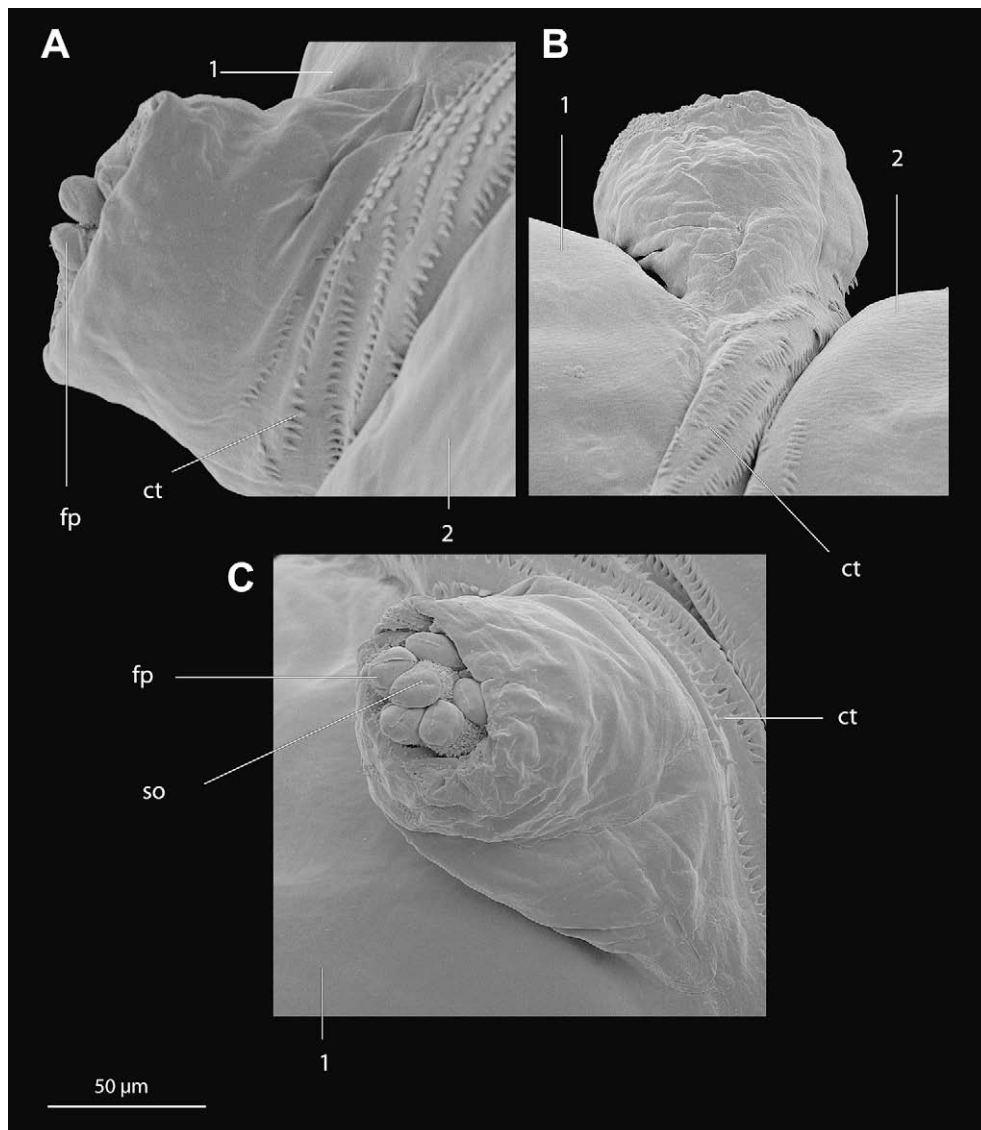
### 3.4. Anal division

The anal division (“8th abdominal segment”) is distinctly different from the other largely uniform postcephalic segments because it is the product of the fusion of several primary segments. Its entire surface (with exception of the anal organs and the spiracular tubes) is covered with microtrichia. The paired anal pad or anal organ lies on the ventral side (ano, Fig. 7B and C) together with the anus (an, Fig. 7B). It extends along the anus and reaches the lateral region of the segment. It has an oblong oval shape (width ca. 300  $\mu\text{m}$ , length 155–160  $\mu\text{m}$ ) and is almost completely divided by a transverse furrow (length 150  $\mu\text{m}$ ). The posterior pair of

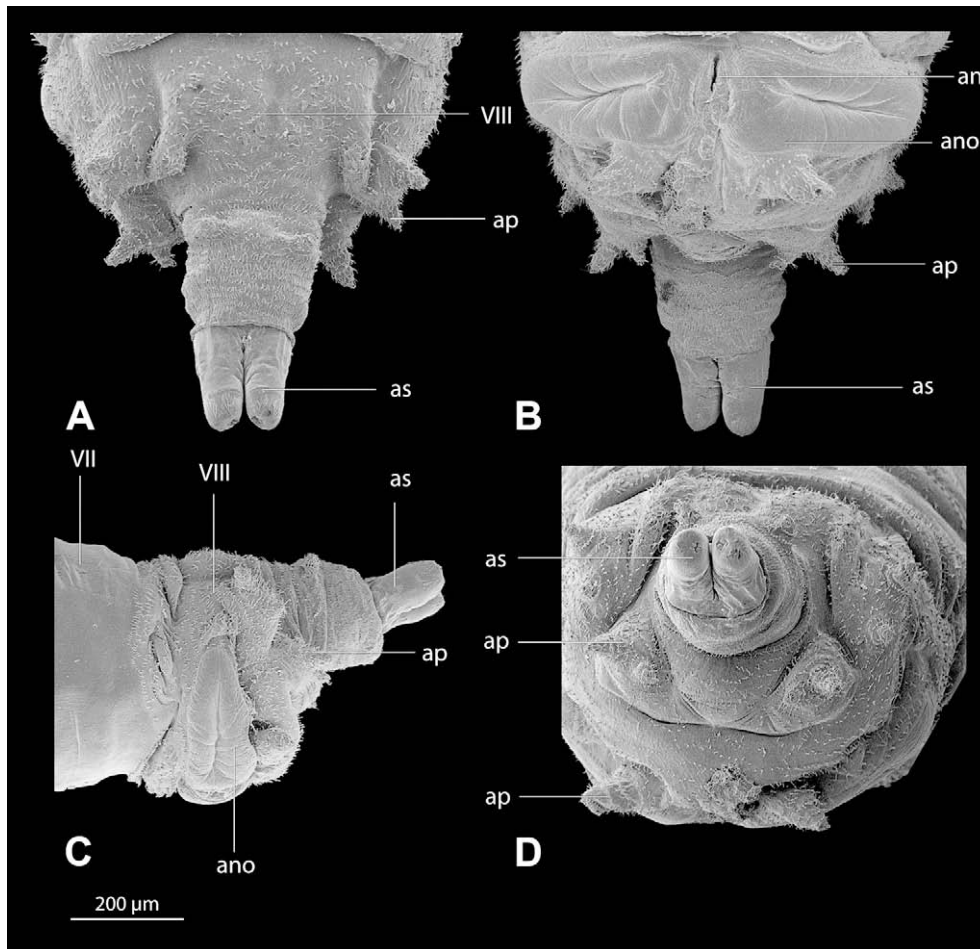
spiracles lies at the posterior end of the segment and also six pairs of cone-shaped anal papillae (ap, Fig. 7) (see also Hertweck, 1931 and Singh and Singh, 1984). A smaller pair is placed dorsad the spiracles and four larger ones laterally. The latter are arranged in groups of two. The sixth pair is placed ventrally close to the anus. The anal papillae bear setae with an annular arrangement.

### 3.4.1. Abdominal spiracles

The terminal spiracles (as, Figs. 1, 7 and 8) are connected with the adjacent parts of the anal division by the peritreme (p, Fig. 8). The paired, cylindrical spiracular tubes (st, Fig. 8) are about twice as long as wide (length ca. 140  $\mu\text{m}$ , width ca. 80  $\mu\text{m}$ ). The spiracular or ecdysial scar at their distal end is slightly shifted mesad (ss, Fig. 8A and D). It is a funnel-shaped concavity with folded walls (ca. 20  $\mu\text{m}$ ). Three oblong-oval spiracular openings arranged in a semi-circle are present laterad of the scar (so, Fig. 8A and D). Four gland openings are present between the spiracular openings and laterad the spiracular openings. Conspicuous peristigmatic tufts of spiracular hairs arise from them (sgh, Fig. 8) and cover the terminal fourth of the spiracular tubes.



**Fig. 6.** *Drosophila melanogaster*, larva; prothoracic spiracles, SEM micrograph. Abbreviations: 1, 2, pro- and mesothorax; ct, denticles; fp, finger-shaped processes; so, spiracular openings.



**Fig. 7.** *Drosophila melanogaster*, larva; posterior end of abdomen, SEM micrograph. (A) Dorsal; (B) ventral; (C) lateral; (D) caudal. Abbreviations: VII, abdominal segment VII; VIII, anal division; an, anus; ano, anal organ or pads; ap, anal papillae; as, abdominal spiracles.

### 3.5. Digestive tract

The anterior opening of the digestive tract lies on the ventral side. The position of the anatomical mouth cannot be defined.

Four small bent bristles (medial sensory organ) and two large cone-shaped structures on a structure referred to as 1st ventral throat bracelet were identified by Hertweck (1931). Three groups of paired sensory organs were described by Singh and Singh (1984). The anteroventral group comprises three sensilla. One of them ( $V_1$  in Singh and Singh, 1984) is a single pore compound sensillum with nine dendrites. The dorsal group is located posterior to the anteroventral group and contains six sensilla on both sides. They are innervated either by one, two or three dendrites. The posteroventral group consists of a single paired compound sensillum with six dendrites (Singh and Singh, 1984).

Eleven pairs of dilators originate on the cephaloskeleton. The anteriormost of the digestive tract is characterized by nine longitudinal ridges, which are T or Y-shaped in cross section (T, Fig. 9F, G and 10B). It is posteriorly continuous with the esophagus. In the posterior region of the foregut a proventriculus is developed and the cardia (anteriormost region of the midgut) with caeca (Fig. 11A). The main part of the midgut forms several loops while the hindgut is straight. It ends with the anal opening on the ventral side of the anal division.

The ventrally placed salivary glands are large and reach abdominal segment III posteriorly. Four Malpighian tubules are connected to the midgut-hindgut border with two common proximal portions in abdominal segment V. One pair extends posteriorly

and one anteriorly. The anterior pair ends in segment III, the posterior one in the anal division.

### 3.6. Other organ systems

The main elements of the tracheal system are two dorsolateral main branches between the prothoracic spiracles and the spiracles of the anal division (tb, Figs. 10D, E, 11D, E, 13 and 14).

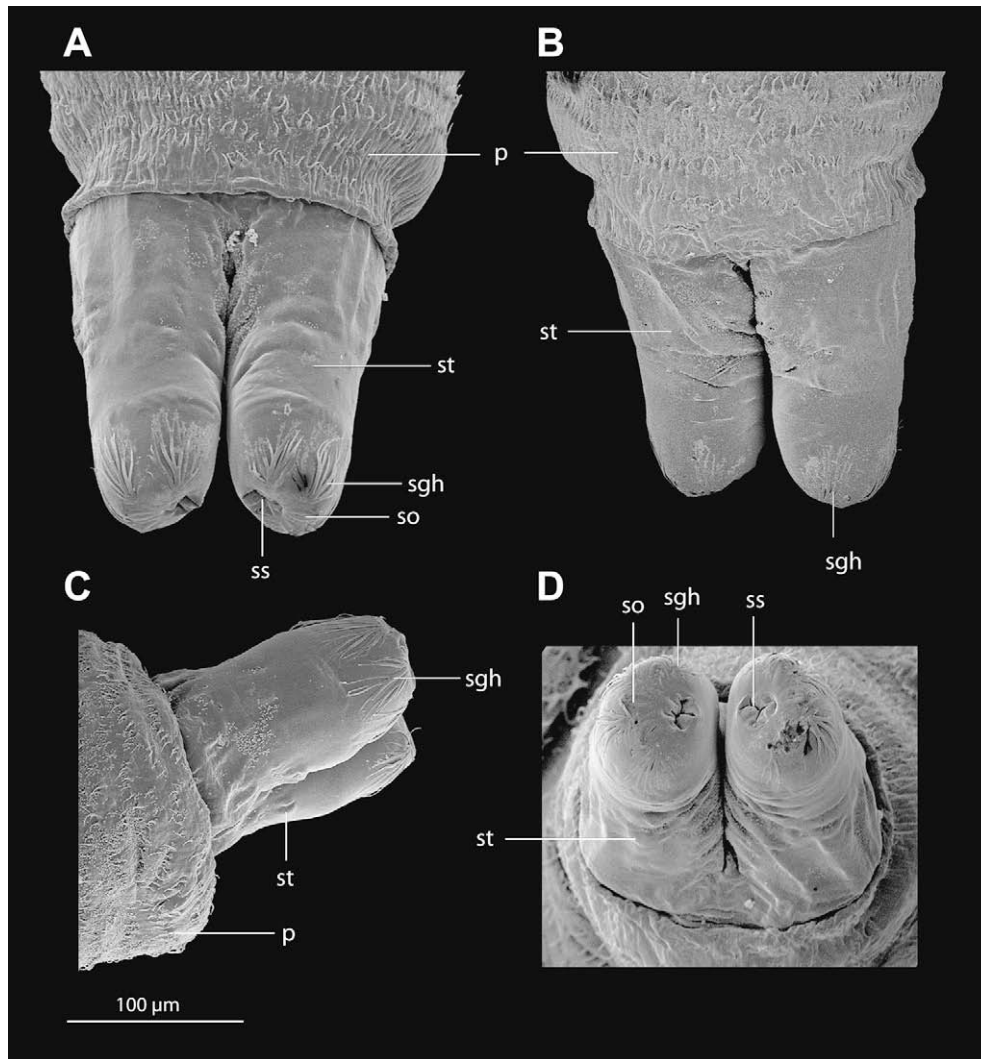
The central nervous system (CNS) is very compact and simplified (CNS, f Fig. 14). The main elements of the CNS are the brain with two large protocerebral hemispheres and the subesophageal ganglion. The latter is connected with the compact ventral nerve cord, which lacks defined segmental ganglia. The entire CNS is located in the metathorax and abdominal segment I.

The finger-shaped subunits of the fat body enclose the internal organs of the abdomen (fb, Figs. 10F and 11A–E). The imaginal anlagen are internally folded, sack-shaped structures (ia, Figs. 9B–D, 13 and 14). Those of the labium are placed ventrally in the pseudocephalon and laterad the mouth hooks. The posterior thorax contains the anlagen of the compound eyes, legs, wings and halteres. Those of the genital organs lie within the anal division anterior to the anus.

### 3.7. Musculature

The muscles form a dense 3-dimensional system below the body wall, superficially resembling the condition found in





**Fig. 8.** *Drosophila melanogaster*, larva; abdominal spiracles, SEM micrograph. (A) Dorsal; (B) ventral; (C) lateral; (D) caudal. Abbreviations: p, peritreme; sgh, spiracular gland hairs; so, spiracular opening; ss, spiracular scar; st, spiracular tube.

annelid worms. However, it comprises only longitudinal and dorsoventral muscles, whereas ring muscles of the body wall are lacking as in other adult or immature insects. Longitudinal muscles usually extend between two segmental borders (segmental muscles) (Fig. 16). Some of them extend over several segments and others insert within a segment (subsegmental muscles). Dorsoventral muscles are present in the lateral, ventrolateral and dorsolateral regions (Fig. 17). Accessory muscles extend between the cephaloskeleton, the mouth hooks, and the body wall. Muscles of head appendages, excluding the mouth hooks, are completely absent.

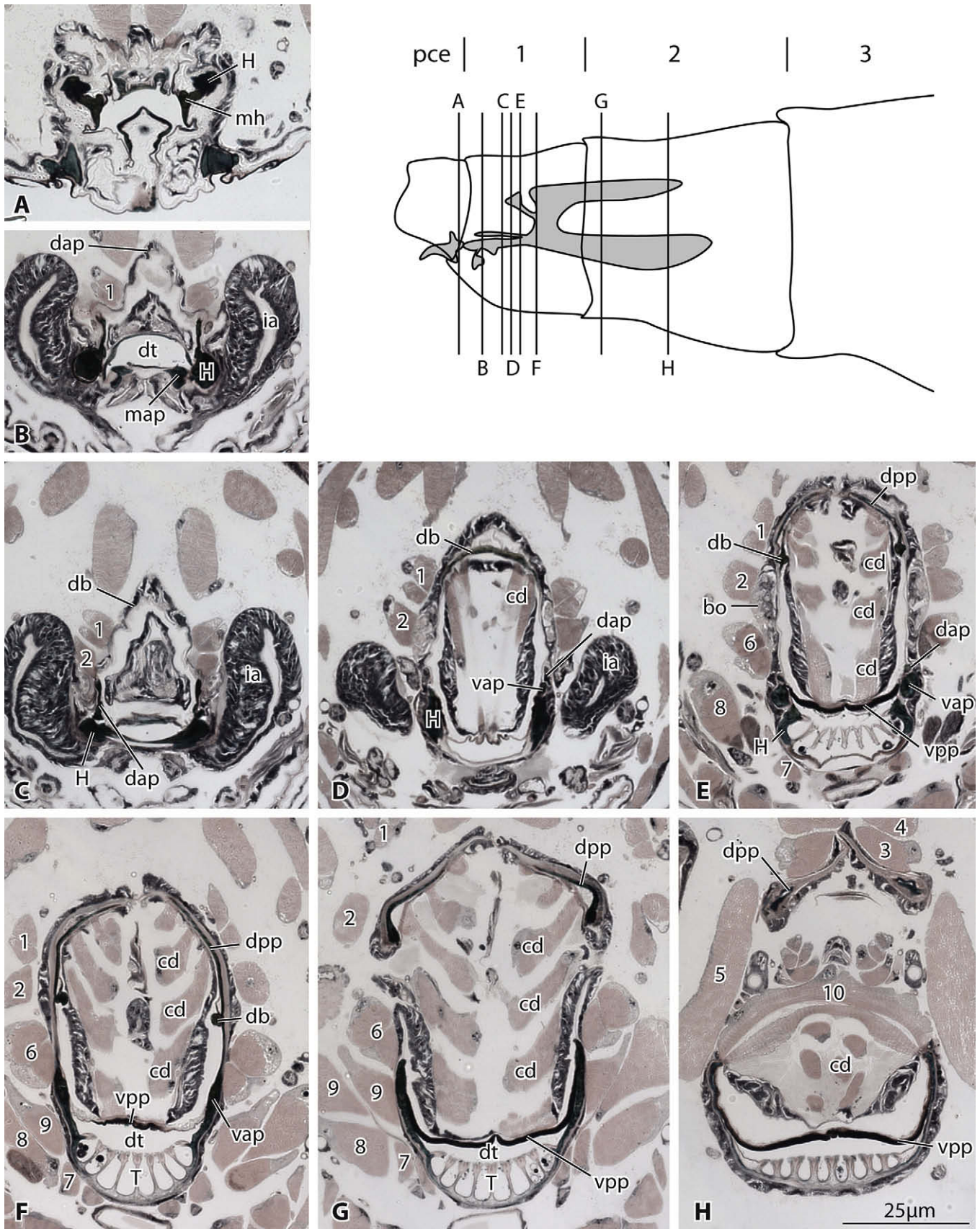
### 3.7.1. Cephaloskeleton (Figs. 9B–H, 12B–D and 15)

M1, origin (O): dorsolateral wall of prothorax, posterior segmental border; insertion (I): dorsal side of mouth hooks (Mh). M2, O: dorsolateral wall of mesothorax, middle region of segment; I: sclerite between mouth hooks and cephaloskeleton (CPS). M3, O: dorsal wall of prothorax, anterior region of segment; I: posterodorsal arms of CPS. M4, origin and, insertion of M4 above those of M3, O: orsal wall of prothorax, middle region of segment; I: posterodorsal arms of CPS. M5, broad muscle, O: ventrolateral wall of prothorax, posterior segmental border; I: posterodorsal arms of CPS. M6, O: posterior end of the ventral arms of the CPS; I:

posterodorsal process of Mh. M7, O: posterior end of the ventral arms of the CPS; I: posteroventral process of Mh. M8, origin and insertion below M7, O: posterior end of the ventral arms of the CPS; I: posteroventral process of Mh. M9, origin and insertion mesad of M8, covered by M8, O: posterior end of the ventral arms of the CPS; I: posteroventral process of Mh. M10, between ventral arms of CPS (posterior region); encloses the dorsal side of the gut. M11, O: dorsal wall of the salivary duct; I: lateral part of the ventral arms of the CPS, anterior of M7. Eleven pairs of cibarial dilators extend between the dorsal arms of the CPS and the dorsal side of the esophagus.

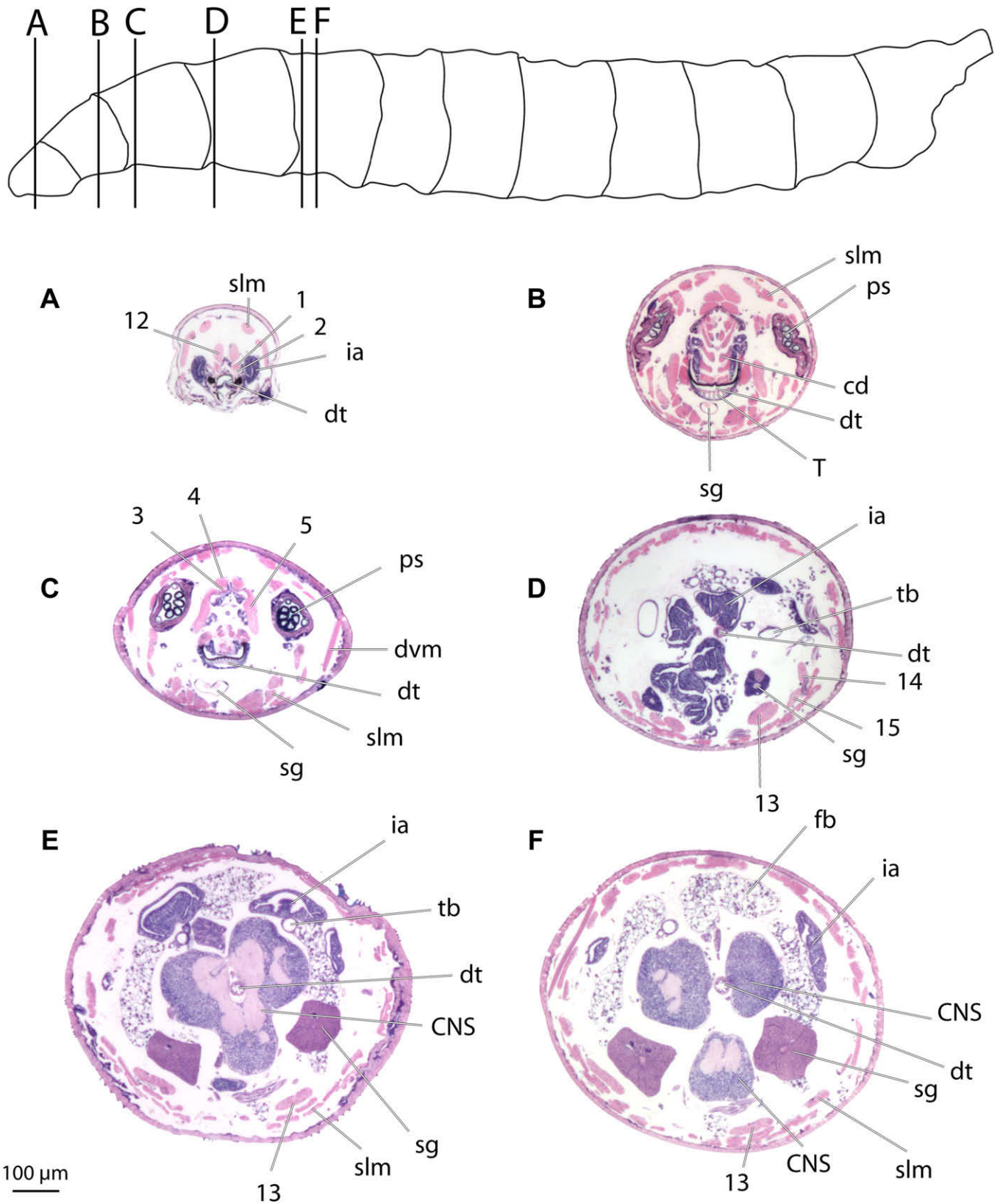
### 3.7.2. Pseudocephalon and prothorax (Figs. 12B–D and 15–17)

Three pairs of segmental longitudinal muscles are present in the prothorax dorsolaterally, laterally and ventrolaterally, and two pairs of dorsoventral muscles. M12, O: dorsal wall of prothorax, middle region of segment, laterad M3 and M4; I: ventral wall of pseudocephalon, mesad the Mh. M13, intersegmental muscle, O: ventral wall of anterior abdominal segments, posterior segmental border; I: ventral posterior edge of reduced head capsule. M14, intersegmental muscle, O and I dorsolaterad M13, O: ventral wall of prothorax, anterior segmental border, I: lateroventral wall of metathorax, posterior segmental border. M15, intersegmental

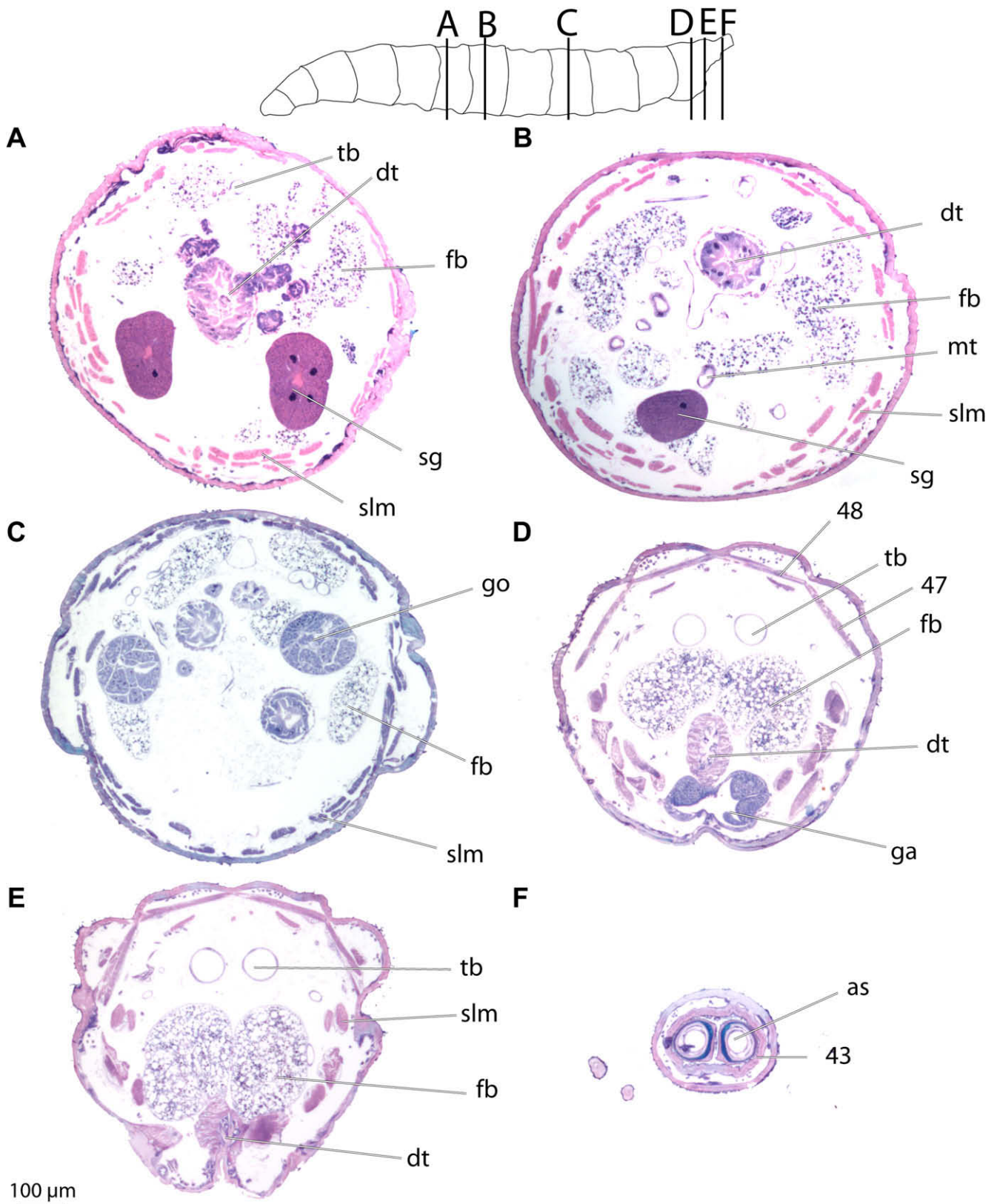


**Fig. 9.** *Drosophila melanogaster*, larva, cephaloskeleton; cross sections. Abbreviations: bo, Bolwig organ; cd, cibarial dilators; dap, dorsal anterior process; db, dorsal bridge; dpp, dorsal posterior process; dt, digestive tract; H, H-piece; ia, imago anlagen; map, mouth angle piece; mh, mouth hooks; T, T-ribs; vap, ventral anterior process; vpp, ventral posterior process, 1–11, muscles M1–M11.

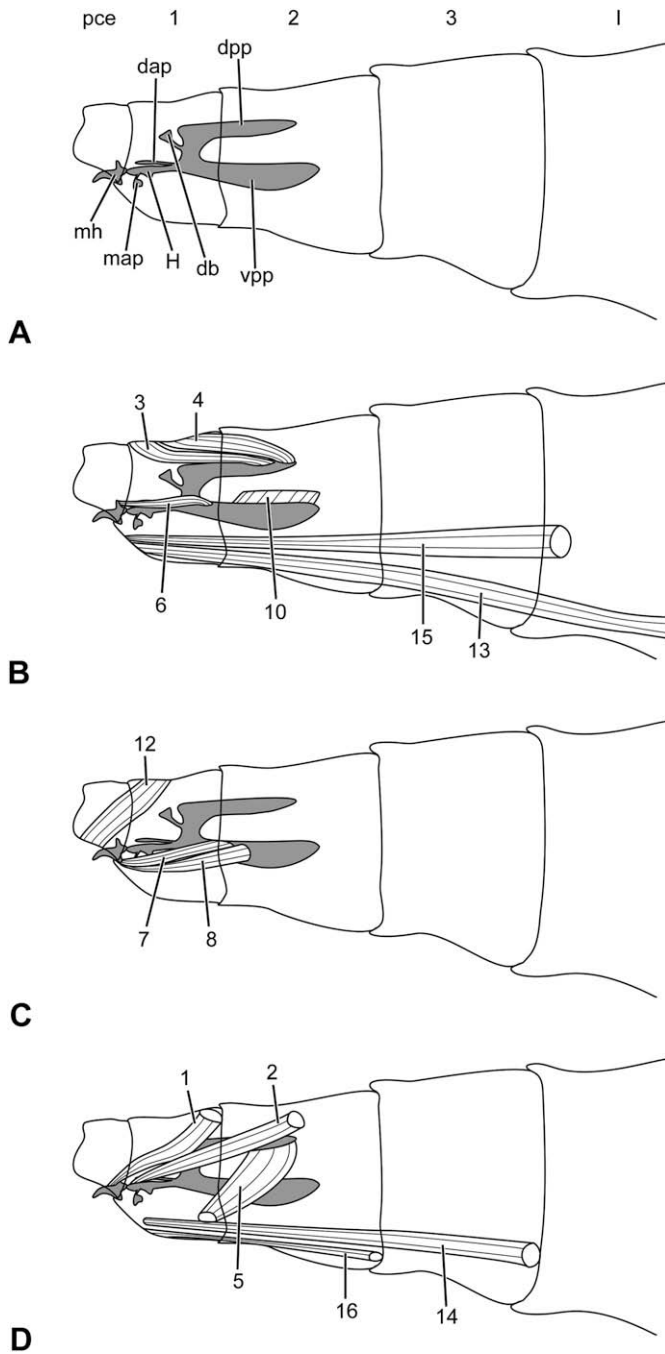




**Fig. 10.** *Drosophila melanogaster*, larva; cross sections. Abbreviations: cd, cibarial dilators; dt, digestive tract; dvm, dorsoventral muscle; fb, fat body; ia, imaginal anlage; 1–15, muscles M1–M15; ps, prothoracic spiracles; slm, segmental longitudinal muscles; sg, salivary glands; T, T-ribs; tb, main tracheal branches; CNS, central nervous system.



**Fig. 11.** *Drosophila melanogaster*, larva; cross sections. Abbreviations: as, abdominal spiracles; dt, digestive tract; fb, fat body; ga, genital anlagen; go, gonads; mt, malpighian tubules; slm, segmental longitudinal muscles; sg, salivary glands; tb, main tracheal branches; 43, 47, 48, muscles M43, M47, M48.



**Fig. 12.** *Drosophila melanogaster*, larva; line drawings, anterior body region, with view from outside to inside. (A) Cephaloskeleton; (B–D) musculature of the cephaloskeleton from the interior to the external layer. H-piece. Abbreviations: dap, dorsal anterior process; db, dorsal bridge; dpp, dorsal posterior process; H-piece map, mouth angle piece; mh, mouth hooks; pce, pseudocephalon; vpp, ventral posterior process.

muscle, O and I dorsolaterad M14, O: ventral wall of prothorax, anterior segmental border, I: lateroventral wall of metathorax, posterior segmental border. M16, intersegmental muscle, O and I ventrolaterad M13, O: ventral wall of prothorax, anterior segmental border, I: lateroventral wall of mesothorax, posterior segmental border. M17, intersegmental muscle, O: laterofrontal wall of pseudocephalon, I: dorsal wall of mesothorax, posterior segmental border. M18, intersegmental muscle, O: lateral wall of pseudocephalon, behind the Mh; I: mediadorsal wall of mesothorax, posterior segmental border.

### 3.7.3. Mesothorax (Figs. 16 and 17)

Contains 11 pairs of segmental longitudinal muscles. The muscle closest to the lateral body wall is very broad. Two pairs of dorsoventral muscles are present.

### 3.7.4. Metathorax (Figs. 15–17)

Contains also 11 pairs of segmental longitudinal muscles. Seven pairs of dorsoventral muscles are present. M19, intersegmental muscle, O: mediadorsal wall of mesothorax, anterior segmental border, I: mediadorsal wall of abdominal segment II, posterior segmental border. M20, Intersegmental muscle, O: ventral wall of metathorax, anterior segmental border, I: ventral wall of abdominal segment I, posterior segmental border. M21, intersegmental muscle, I and O ventrad M20, O: ventral wall of metathorax, anterior segmental border, I: ventral wall of abdominal segment I, posterior segmental border.

### 3.7.5. Abdominal segment I (Figs. 16 and 17)

Fourteen pairs of segmental longitudinal muscles are present in abdominal segment I, and six pairs of dorsoventral muscles.

### 3.7.6. Abdominal segments II and III (Figs. 15–17)

These two segments display a similar pattern of segmental longitudinal- and dorsoventral muscles. This comprises 16 pairs of longitudinal- and five pairs of dorsoventral muscles. Abdominal segments IV–VI are not treated here as they are very similar in their structural organization to segments II and III.

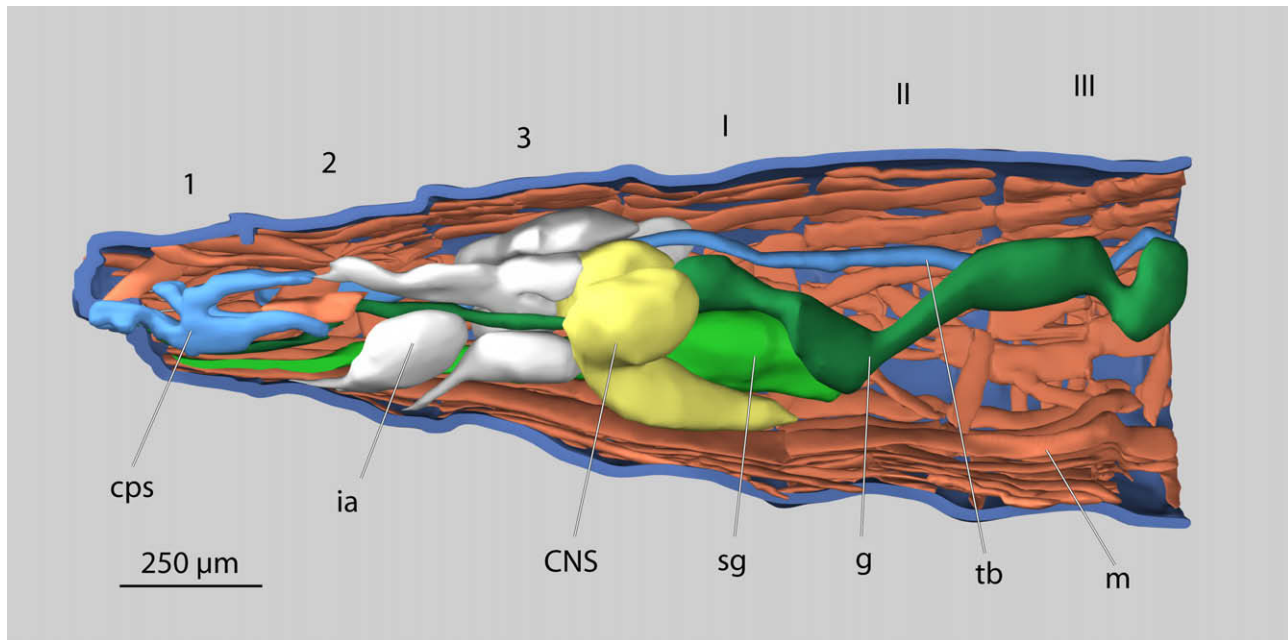
M22–25: intersegmental muscles. M22, O: ventral wall of abdominal segment II, anterior segmental border, I: lateroventral wall abdominal segment VII, posterior segmental border. M23, I and O dorsad M22, O: ventral wall of abdominal segment II, anterior segmental border, I: lateroventral wall of abdominal segment VII, posterior segmental border. M24, O: ventral wall of abdominal segment II, anterior segmental border, I: ventral wall abdominal segment III, middle region of segment. M25, ventrad M24, O: ventral wall of abdominal segment II, anterior segmental border, I: ventral wall of abdominal segment III, middle region of segment.

### 3.7.7. Abdominal segments VII and the anal division (Fig. 18)

The musculature of abdominal segment VII is similar to that of the preceding ones. It mainly consists of longitudinal and dorsoventral muscles. The only distinct difference is the increased number of bundles. In contrast, the anal division is characterized by a distinctly reduced muscle set. It contains only five bundles of both longitudinal and dorsoventral muscles and four muscles associated with the spiracle.

M26, 4 bundles, O: anterior margin of abdominal segment VII, paramedially on dorsal side, I: anterior margin of anal division, paramedially on dorsal side, F(=function): retractor of anal division. M27, 3 bundles, O: anterior margin of segment VII, laterad M26, I: anterior margin of anal division, ventrad M26. M28, 1 bundle, O: anterior part of segment VII, lateral region, I: anterior margin of anal division, ventrad M26. M29, 1 bundle, O: lateral wall of segment VII, anterior segmental border, ventrad M28, I: lateral wall of anal division, anterior segmental border. M30, 1 bundle, O: lateral wall of segment VII, anterior segmental border, ventrad M26, laterad M36 and M37, I: lateral wall of anal division, anterior segmental border. M31, 1 bundle, O: lateral wall of segment VII, anterior segmental border, ventrad M30, I: lateral wall of anal division, anterior segmental border, ventrad M29. M32, 5 bundles, O: ventral wall of segment VII, anterior segmental border, I: ventral wall of anal division, anterior segmental border. M33, 5 bundles, O: ventral wall of segment VII, anterior segmental border, laterad M32. I: ventrolateral wall of anal division, anterior segmental border, laterad M34. M34, 5 bundles, O: lateral wall of segment VII, anterior segmental border,





**Fig. 13.** *Drosophila melanogaster*, larva; 3D-reconstruction, anterior body region, sagittal section. Abbreviations: 1–3, thoracic segments; I–III, abdominal segments; cps, cephaloskeleton; g, gut; ia, imaginal anlagen; m, muscles; sg, salivary glands; tb, main tracheal trunk; CNS, central nervous system.

dorsad M31, I: lateral wall of anal division, anterior segmental border, mesad M33. M35, 1 bundle, O: lateral wall of segment VII, anterior segmental border, laterad M34, I: ventrolateral wall of anal division, anterior segmental border, laterad M33. M36, 1 bundle, dorsoventral muscle, O: laterally, anterior segmental border of segment VII, mesad M30. M37, 3 bundles, dorsoventral muscle, O: laterally, middle region of segment VII, posterad M36, laterad M28, mesad M30. M38, 1 bundle, dorsoventral muscle, O: laterally, hind margin of segment VII, posterad M37, laterad M27 and M28. M39, 1 bundle, ventral transverse muscle, close to hind margin of segment VII. M40, 1 bundle, O: dorsal wall of anal division, anterior segmental border, I: dorsally on terminal spiracles. M41, 1 bundle, O: ventral part of anal division, paralaterally, I: ventrally on terminal spiracles. M42, 1 bundle, O: ventral part of anal division, paramedially, I: ventrally on terminal spiracles. M43, ring-shaped, encloses terminal spiracles. M44, 1 bundle, O: dorsolateral body wall, I: dorsolateral body wall, posterad the origin. M45, 3 bundles, O: ventrolateral wall of anal division, anterior segmental border, ventrad M41, I: posteroventral wall of anal division, laterad M42. M46, 1 bundle, dorsoventral muscle, O: dorsum of anal division, close to anterior margin, I: ventrolateral wall of anal division, close to segmental border, F: depressor of anal division. M47, 1 dorsoventral bundle, O: dorso-laterally in anal division, posterad M46. I: ventrad the origin. M48, 1 bundle, O: dorsal transverse muscle, middle region of anal division, I: ventrad the origin. M49, 1 bundle, transverse muscle, obliquely extending through anal division, O: dorsolateral wall of anal division, posterior region, I: ventrolateral wall of anal division (opposite side). M50, 1 bundle, O: ventrolateral wall of anal division, I: rectum, laterad M41, I: lateral wall of anal division, close to the origin of M49. M51, 1 bundle, O: lateral wall of anal division, laterad M46, I: lateral wall of anal division, laterad M49.

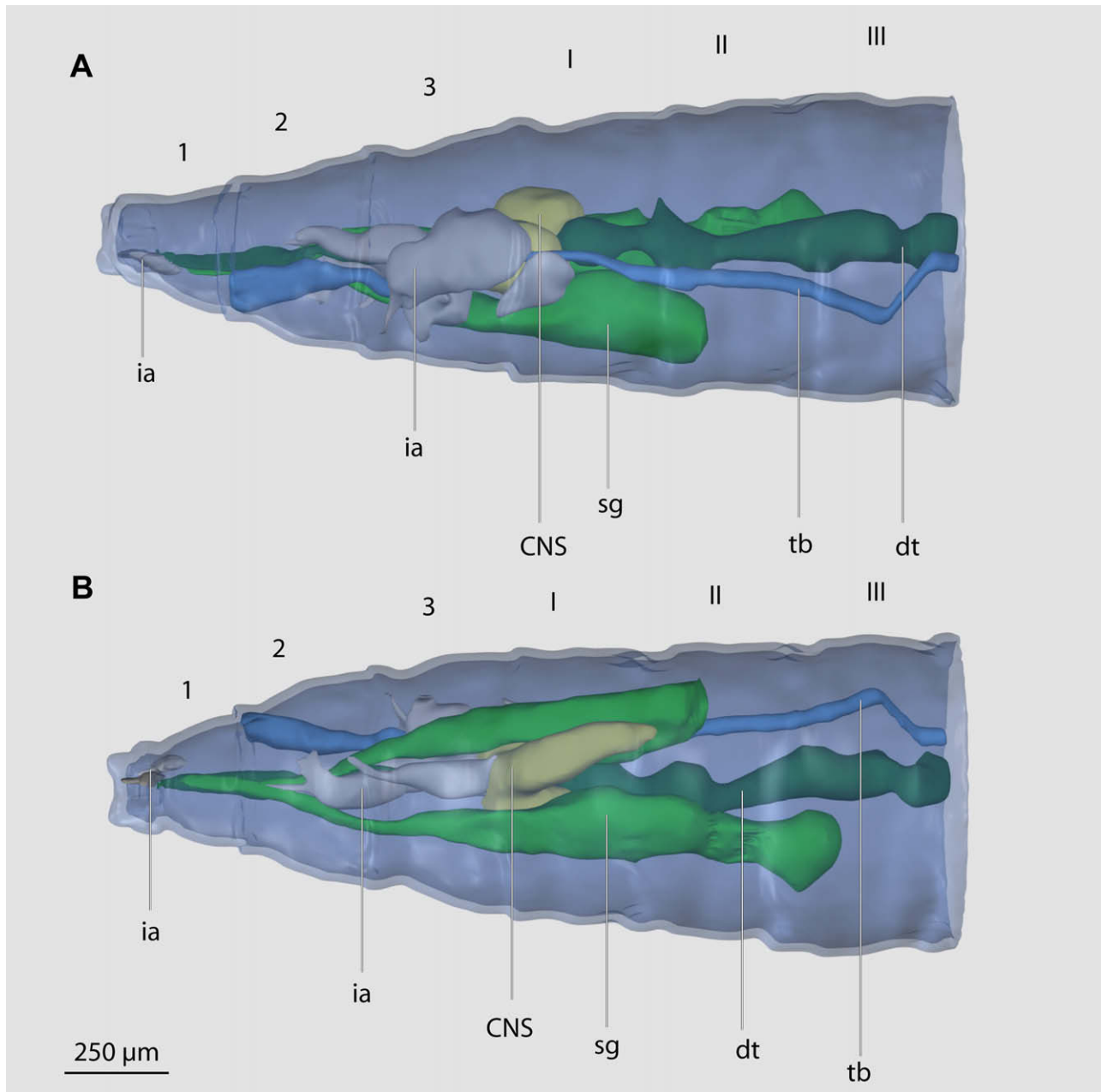
## 4. Discussion

### 4.1. Homology

Considering the role of *Drosophila* as a model organism in genetics and developmental biology, it is surprising that the

homology of important structures remains unclear. This applies to the antennal organ, which is apparently equivalent to the antennal organ of other schizophoran Diptera such as *Musca domestica* (Chu and Axtell, 1971) and Sepsidae (Meier, 1996). It was assigned to the antennal segment by Jürgens et al. (1986) but it was already pointed out by Frederick and Denell (1982) that there is no evidence supporting this. Likewise, the homology of the maxillary organ is problematic. Apparently it has a complex segmental origin. The two dorsal sensorial papillae are apparently not true maxillary elements (Frederick and Denell, 1982). Jürgens et al. (1986) assigned the sensilla to the segments of the antennae and mandibles, and the papillae as such to the maxillary segment. Due to the persistent homology problems, neutral terms should be used for these structures such as dorsal- and terminal organ, respectively (Bolwig, 1946; Chu and Axtell, 1971; Chu-Wang and Axtell, 1972). The homology of the mouth hooks is similarly controversial. It appears likely that they represent modified mandibles (Ludwig, 1949; Schremmer, 1956; Hartley, 1963; Sinclair, 1992) but alternative interpretations as maxillae (de Meijere, 1917; Snodgrass, 1953; Schoeller, 1962; Gouin, 1968; Jürgens et al., 1986) or as a product of fusion of both (Bischoff, 1922; Cook, 1949) were suggested. The muscular features we examined and comparisons with presumably basal lineages (e.g., Neugart et al., 2009; *Tipula*; Schneeberg et al., 2012; *Nymphomyia*; Wipfler et al., 2012; *Protanyderus*) did not provide unambiguous evidence allowing a reliable interpretation of these cephalic structures. One of the main obstacles is the lack of study of Empidoidea larvae. The Empidoidea and/or Apystomyiidae are sistergroup of Cyclorrhapha (Wiegmann et al., 2011; Lambkin et al., in press) and their larvae would be the logical point of comparison for understanding the highly apomorphic pseudocephalon of cyclorrhaphan flies.

Another body element with uncertain homology is the anal division. It differs distinctly from the anterior abdominal region and is apparently a product of fusion of several segments. It may include the original segments VIII, IX and X (Turner and Mahowald, 1979; Dambly-Chaudière and Ghysen, 1986; Szpila and Pape, 2008b; Szpila et al., 2008), but details remain uncertain and the configuration of muscles does not allow for the



**Fig. 14.** *Drosophila melanogaster*, larva; 3D-reconstruction, anterior body region. (A) Dorsal; (B) ventral. Abbreviations: 1–3, thoracic segments; I–III, abdominal segments; dt, digestive tract; ia, imaginal anlagen; sg, salivary gland; tb, main tracheal branches; CNS, central nervous system.

identification of the primary segments that form this terminal body part. The ventral intersegmental muscles M13, 14 and 15 are apparently homologous with those designated as 31, 34 and 35 by Hooper (1986) and Hanslik et al. (2010). The position of M16 is similar to that of 32 and 33 (Hooper, 1986). However, its origin lies at the posterior end of the metathorax and not on the mesothorax.

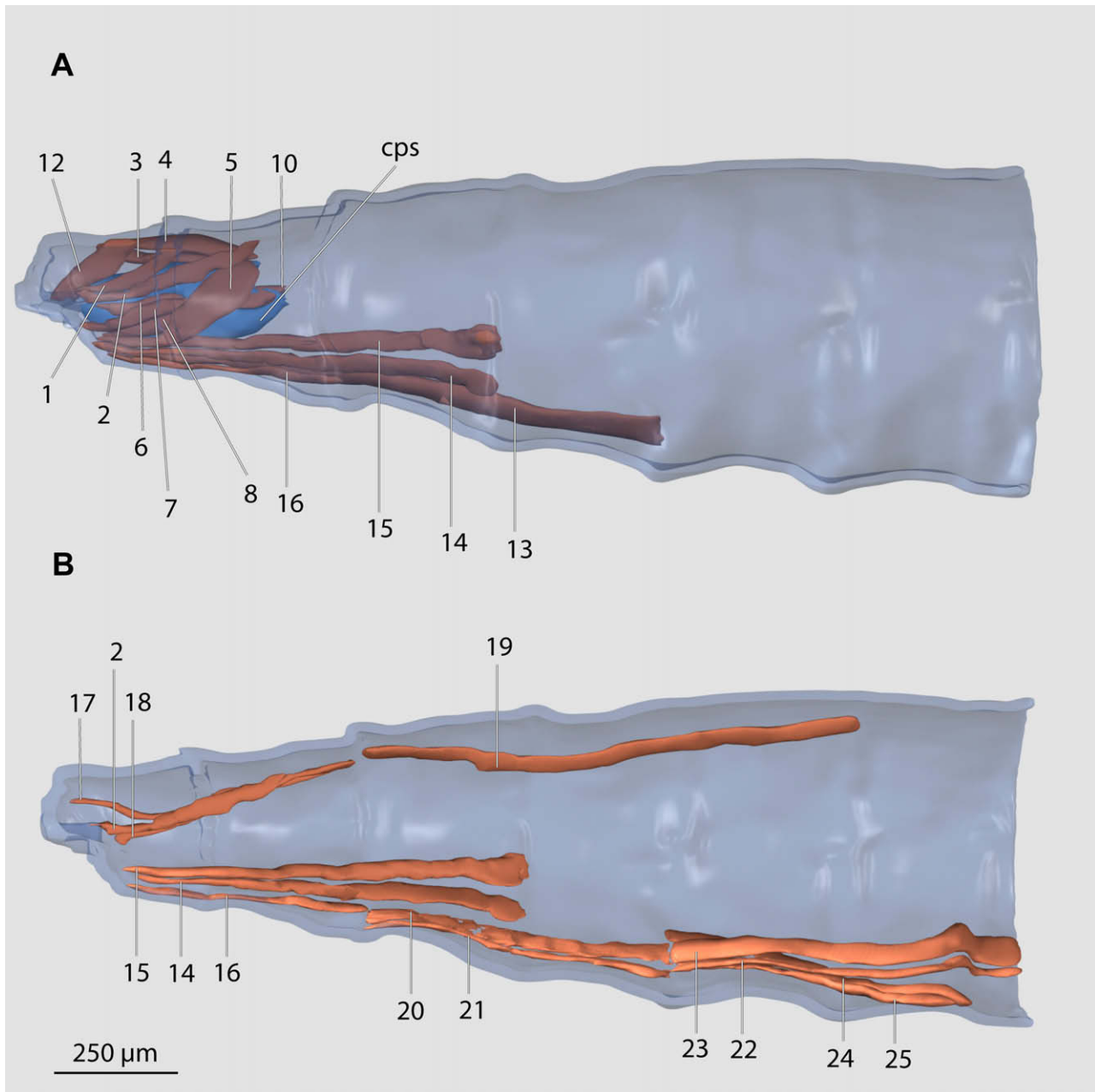
#### 4.2. Phylogenetic implications

The larva of *Drosophila* displays a series of potential autapomorphic groundplan features of Brachycera (e.g., Hennig, 1973; Ziegler, 2005). However, the phylogenetic interpretation is impeded by the surprising scarcity of well documented

morphological information for the immature stages of the Brachycera in general and the Eremoneura in particular.

The head region is externally not well separated from the prothorax, a derived feature which also evolved several times independently in nematoceran lineages (Hennig, 1973; Ceratopogonidae, Scatopsidae; Neugart et al., 2009; Tipuloidea). In *Drosophila* and other brachycerans the head is partially retracted and overgrown by the thoracic segments. A potential autapomorphy of Brachycera suggested in a little known study by Chaudonneret (1983) is the transformation of the dorsal cephalic levator into a protractor (Chaudonneret, 1983: Figs. 72–74; see also Roberts, 1970, 1971). Another potential brachyceran autapomorphy suggested in the same study is a strengthened dorsal thoracic collar region (Chaudonneret, 1983).





**Fig. 15.** *Drosophila melanogaster*, larva; 3D-reconstruction, anterior body region. (A) Musculature of the cephaloskeleton, lateral (M9, covered by M8); (B) intersegmental muscles, sagittal section (M12 not illustrated). Abbreviations: cps, cephaloskeleton; 1–8, 12–25, muscles M1–M8, M12–M25.

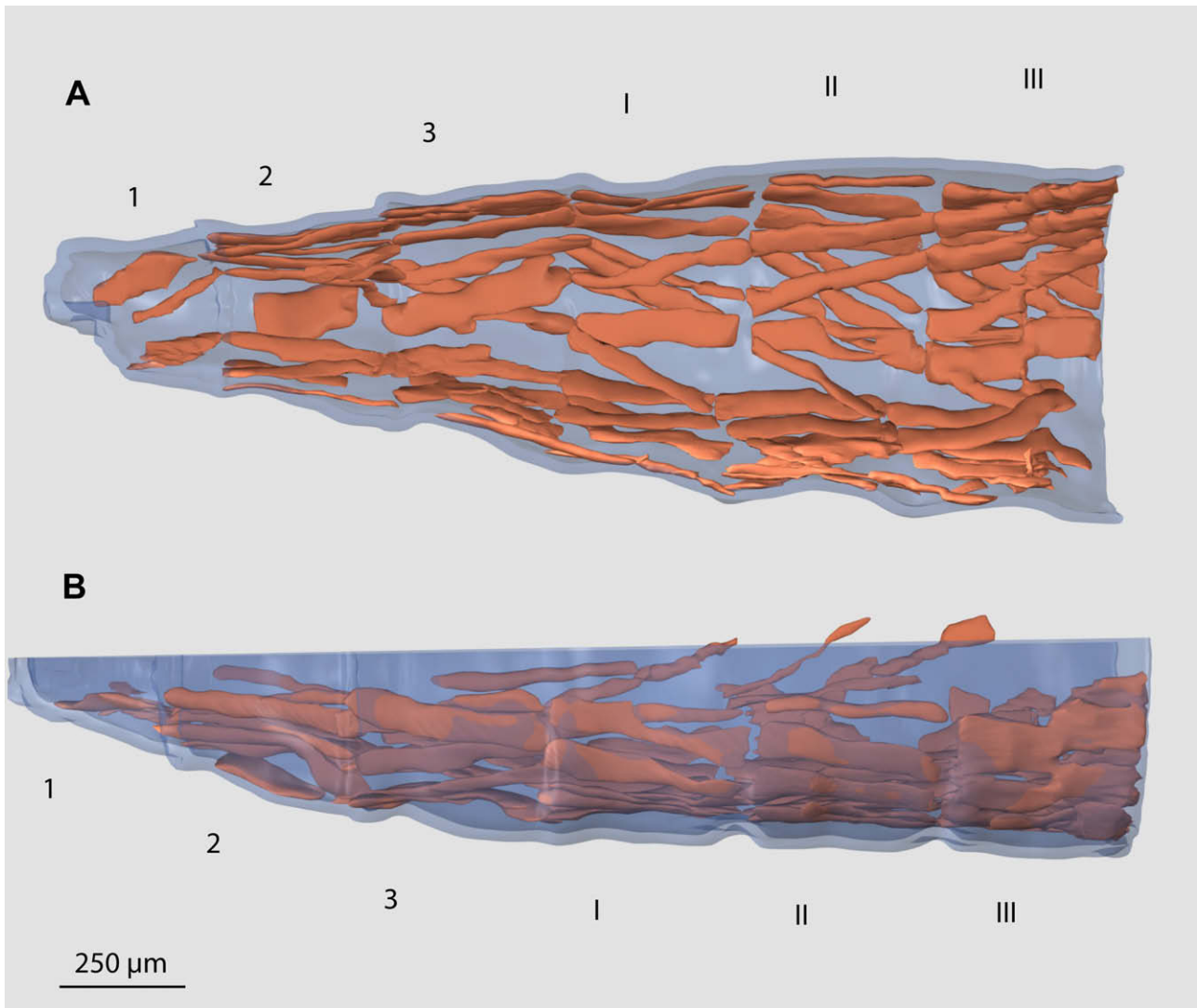
Within the Brachycera, the greatly reduced condition of the maxilla was interpreted as a synapomorphy of Empidoidea and Cyclorrhapha (Eremoneura) by Sinclair (1992). Another potential groundplan autapomorphy of Eremoneura is the presence of a V-shaped labial hypopharyngeal sclerite (Hennig, 1952; Sinclair, 1992). However, this structure is secondarily lost in most cyclorrhaphan lineages.

The head capsule is partly preserved in the brachyceran groundplan but already shows a distinct degree of reduction (e.g., Chaudonneret, 1983: “profonde échancrure”). Its complete reduction is a derived feature of Cyclorrhapha (pseudoccephalon; Chaudonneret, 1983; Stuke, 2000), but a similarly reduced condition of the head capsule evolved independently in the miniaturized Cecidomyiidae (Neugart et al., 2009). In Lonchopteridae a broad median projection of the cephaloskeleton is present; Ferrar (1987)

interpreted this as a vestige of the head capsule of larvae of lower Diptera. The ontogenetic process leading to the formation of a pseudoccephalon in *Calliphora* was described in detail by Schoeller (1962) and also discussed by Denis and Bitsch (1973).

The loss of all head sutures in *Drosophila* and all other groups of Cyclorrhapha is another presumptive autapomorphy of this lineage. Another groundplan feature of Cyclorrhapha is the amphipneustic 3rd instar larva (Hennig, 1948, 1973). The anterior spiracles are preserved in *Drosophila* but have no respiratory function as the larvae burrow in the food substrate. Functionally they are meta-pneustic as already pointed out by Rühle (1932).

Modifications of the mouthparts and associated elements were suggested as autapomorphies of Cyclorrhapha by Sinclair (1992), such as mouth hooks only composed of a single element with a single articulation, and the presence of ventral cibarial ridges



**Fig. 16.** *Drosophila melanogaster*, larva; 3D-reconstruction, anterior body region, segmental longitudinal muscles. (A) Sagittal section; (B) dorsal. Abbreviations: 1–3, thoracic segments; I–III, abdominal segments.

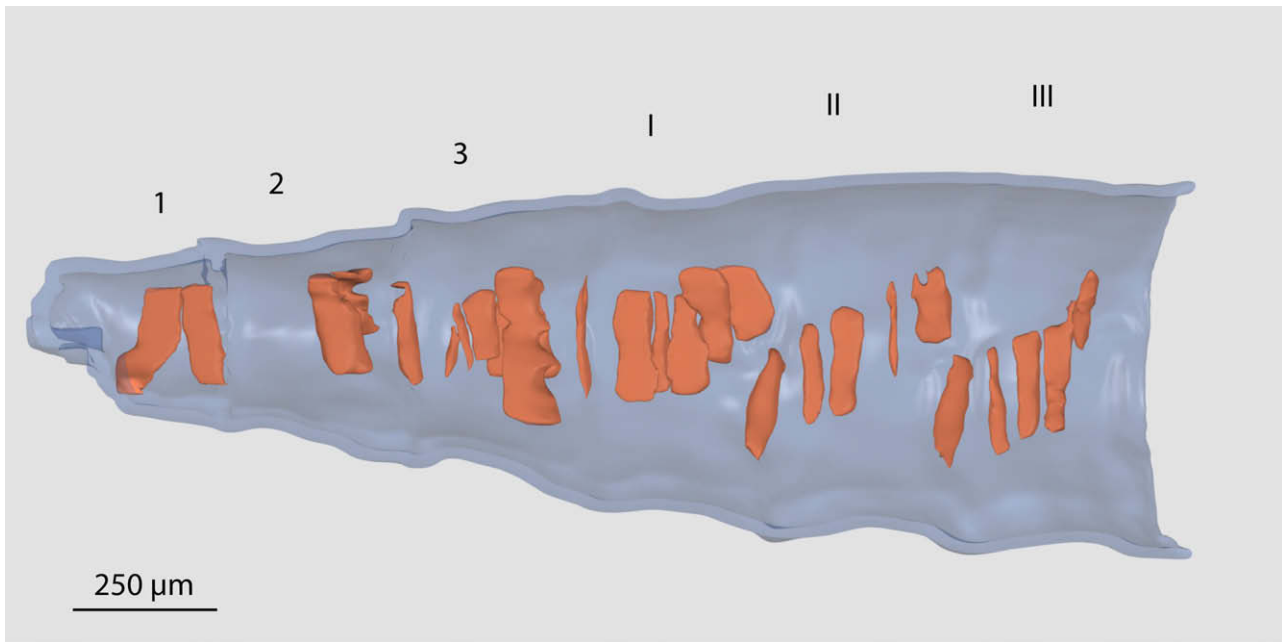
functioning as a filtering device (absent in the groundplan of Brachycera). The strongly reduced condition of the labium was pointed out as a derived feature characteristic of cyclorrhaphan larvae by Chaudonneret (1983).

The presence of a peculiar H-piece is another autapomorphy of Cyclorrhapha. It is absent in Empidoidea and other basal brachyceran lineages (e.g., Cook, 1949; McAlpine, 1989). Our observations of the cephaloskeleton (Hennig, 1973) largely conform with the description provided by Strasburger (1932). The formation of this complex structure from different cephalic elements was outlined by Denis and Bitsch (1973). Involved structures according to these authors are “phragmes paraclypeaux” (basal sclerite) and probably tentorial elements, fused with the lateral and ventral regions of the cibarial wall (“sclérifications hypopharygiennes”). Additional elements are small sclerites with uncertain affinity (Denis and Bitsch, 1973). In any case, the cephaloskeleton plays an important role in the food uptake and specifically in the movements of the mouth hooks and cibarial pump. Food is sucked into the foregut by contractions of cibarial dilators and the subsequent dilation of the cibarium. The mechanism is apparently very similar to that described for *Calliphora* (Schoofs et al., 2009; Hanslik et al., 2010)

and *Musca* (Hewitt, 1908), but the number of involved muscles varies considerably. Eleven are present in *Drosophila*, 13 in *Calliphora* (Schoofs et al., 2009; Hanslik et al., 2010) and only 8 in *Musca* (Hewitt, 1908). Aside from this, the muscle system of the cephaloskeleton of *Drosophila* is similar to that of *Calliphora*, *Musca* and *Rhagoletis* (Hewitt, 1908; Snodgrass, 1924; Hanslik et al., 2010). However, two additional pairs of dorsal retractors are present in *Calliphora* and *Musca*.

Another potential autapomorphy of Cyclorrhapha is the specific filter apparatus formed by T or Y-shaped longitudinal folds of the floor of the cibarium (Hennig, 1973; Chaudonneret, 1983). Note that the pharyngeal filter, which occurs in many nematoceran groups (e.g., Oosterbroek and Courtney, 1995), is apparently not homologous to the filter apparatus present in cyclorrhaphan larvae.

The absence of externally visible stemmata and the correlated formation of internal photoreceptors termed Bolwig’s organ (Steller et al., 1987; Melzer and Paulus, 1989; Sprecher et al., 2011) is also likely an apomorphic condition of Cyclorrhapha (Bolwig, 1946; Melzer and Paulus, 1989). Melzer and Paulus (1989, 1990) provide strong arguments for a homologization between Bolwig’s organ and the stemmata of nematoceran larvae. The internalization of the



**Fig. 17.** *Drosophila melanogaster*, larva; 3D-reconstruction, anterior body region, sagittal section, dorsoventral muscles. Abbreviations: 1–3, thoracic segments; I–III, abdominal segments.

larval eyes is likely a result of the internalization of the head capsule.

Another derived feature of Cyclorrhapha is the presence of only seven distinct abdominal segments followed by an undivided terminal anal division (see above), in contrast to nine distinct segments in the groundplan of Diptera (Hennig, 1948; Ziegler, 2005). The apparently derived condition is documented for many schizophoran larvae including those of *Ephydra hians* (Ephydriidae) (Cash-Clark and Bradley, 1994), *Cryptonevra diadema* (Chloropidae) (Grochowska, 2008), *Eutropha lindneri* (Chloropidae) (Kirk-Spriggs, 2007), *Curtonotum* sp. (Curtonotidae) (Kirk-Spriggs, 2008), Sepsidae (Meier, 1996), and *Sarconesia chlorogaster* (Calliphoridae) (Bonatto and de Carvalho, 1996). Abdominal prolegs are lacking in *Drosophila* and other brachyceran larvae (e.g., *Musca*, *Calliphora*). The presence of unpaired prolegs on abdominal segments I–VIII in *Ephydra hians* (Ephydriidae) (Cash-Clark and Bradley, 1994) or on segments I–VII in *Miltogramma przhewalkyi* (Calliphoridae) (Szpila and Pape, 2008b) are apomorphic conditions which have apparently evolved independently.

A derived groundplan feature of Schizophora is the branched condition of the tracheae originating on the prothoracic spiracles (Hennig, 1973). They show a bush-like pattern in *D. melanogaster*. The number of finger-like branches varies considerably. Ten processes are arranged in two groups of five in *Piophilidae casei* (Piophilidae), but six or 12 are present in other *Piophilidae* species (Sukontason et al., 2001). Between six and eight processes form a row in *Cryptonevra diadema* (Chloropidae) (Grochowska, 2008), whereas between nine and 12 are arranged in a fan-like manner in *Chrysomya rufifacies* (Calliphoridae). The number varies between eight and 12 in *Chrysomya megacephala*, between nine and 13 in *Chrysomya nigripes*, four and seven in *Lucilia cuprina* (Calliphoridae), and four and seven in *M. domestica* (Muscidae) (Sukontason et al., 2004). They are arranged in a fan-like or comb-like manner in these species. A similar range in number and a similar arrangement was described for Sepsidae (Meier, 1996).

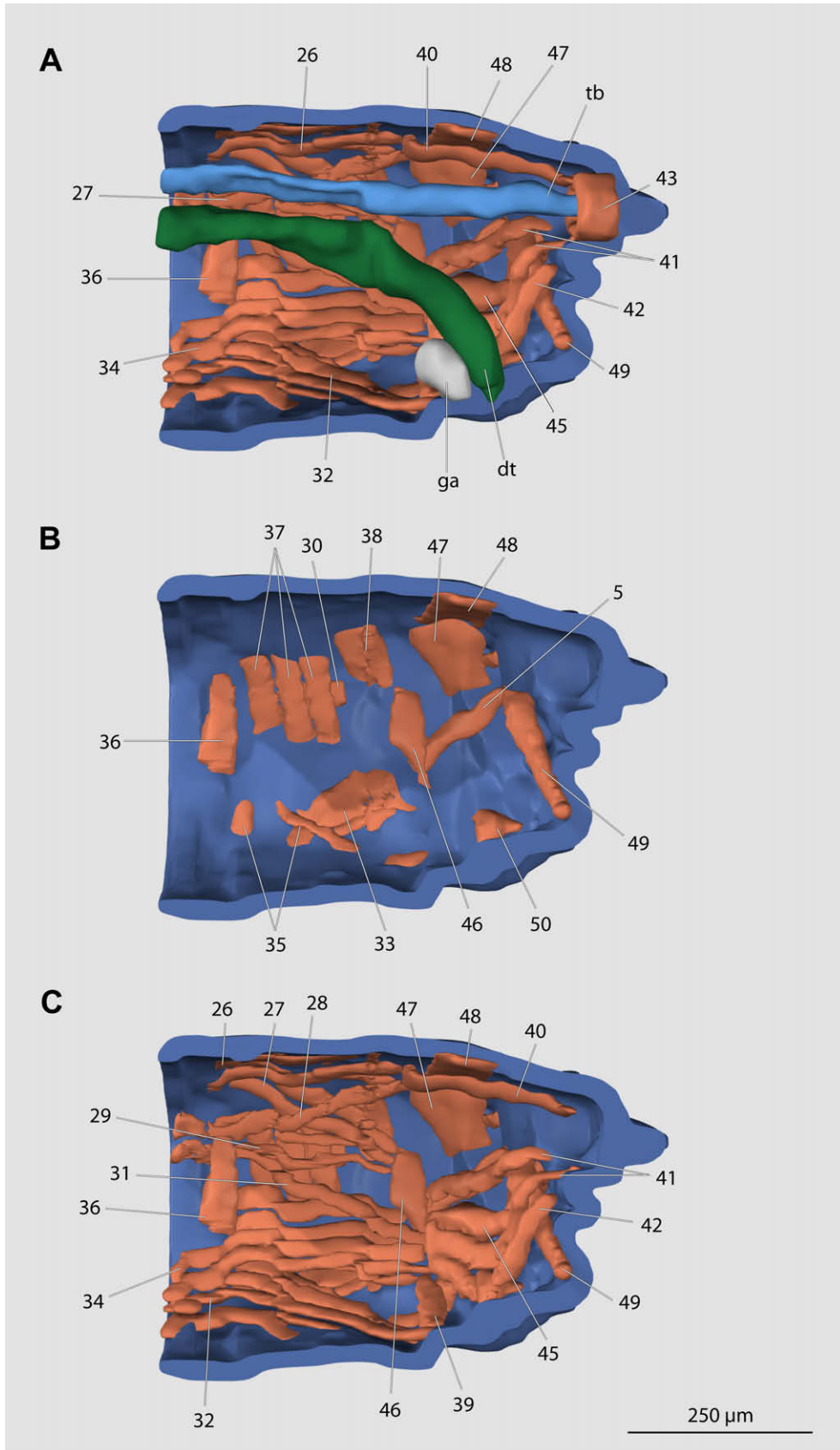
The hypodermal musculature in *Drosophila* is a network of muscles arranged in three layers, which is in congruence with observations made by Hooper (1986) and Bate (1990). However

these authors describe 24–30 muscle pairs per segment, Crossley (1978) 28, while the number observed by us ranges between 20 and 30. This discrepancy can be explained either by a varying muscle equipment between individuals or by different definitions of a muscle. A comparable pattern with varying numbers of muscles is described for other cyclorrhaphans such as *Calliphora* (Crossley, 1965: 29 pairs of muscles in each abdominal segment [except the anal division]; Hanslik et al., 2010: 33 in the mesothorax) or *Musca domestica* (Hewitt, 1908: 19–28 muscle pairs). In *Drosophila* (Crossley, 1978; Hooper, 1986; Bate, 1990) as well as in *Musca domestica* (Hewitt, 1908) and *Calliphora vicina* (Crossley, 1965; Hanslik et al., 2010), the three thoracic segments and the first and terminal abdominal segments show a varying muscular equipment, whereas the abdominal segments II–VII appear uniform. The musculature of the cephaloskeleton is discussed above.

For the anal division no comparative data for any cyclorrhaphan larva is available. Hewitt (1908) mentions that four groups of muscles are present in the anal division, but does not provide any further details. Thus a phylogenetic interpretation is not possible at present.

#### 4.3. The state of the morphological investigation of insect model organisms

The quality of the morphological reconstructions of insect models can potentially have a major impact on the genetic and developmental research that is conducted on these organisms (Blair Hedges, 2002). The most prominent insects in this context are *D. melanogaster*, *Tribolium castaneum* and *Manduca sexta*. The level of interest is high given the 32 000 hits in Google Scholar for *Tribolium*, 39 000 for *Manduca*, and 935 000 for *Drosophila*. Interestingly, despite this immense interest, the anatomy of these species is still rather poorly known, with considerable differences between the three species. Before this study, the information on the larval anatomy of *Drosophila* was quite fragmentary. External features and selected internal sclerotized parts were covered in Hennig (1948, 1950, 1952), the nervous system was treated by Hertweck (1931) and Dambly-Chaudière and Ghysen (1986), the tracheae by Rühle



**Fig. 18.** *Drosophila melanogaster*, larva; 3D-reconstruction, posterior body region, sagittal section. (A) General view; (B) inner layer of muscles; (C) outer layer of muscles. Abbreviations: dt, digestive tract; ga, genital anlagen; tb, main tracheal trunk, 5, 26–43, 45–50, muscles M5, M26–M43, M45–M50.



(1932) and Haskins and Enzman (1937), the digestive tract by Strasburger (1932). Bodenstein (1950) described the post-embryonic development and Crossley (1978), Hooper (1986) and Bate (1990) the musculature of selected body regions.

The adult anatomy of all body parts of *D. melanogaster* was treated by Ferris (1950) and Miller (1950), and additional fragmentary information was contributed by Bryant (1978). A comprehensive study of the thorax was presented by Zalokar (1947) and the adult nervous system was treated by Hertweck (1931).

Like in *Drosophila*, the adult anatomy of *Manduca* was treated in detail in several studies (Eaton, 1971, 1974, 1984, 1986) using traditional approaches. Eaton (1982) also described the thoracic anatomy of the larva. The larval hindgut was discussed by Reinecke et al. (1973). In a more recent study Davis and Hildebrand (2006) treated the sucking apparatus of the adults.

The anatomy of the 3rd insect model organism, *Tribolium castaneum*, is only poorly known. Studies of the adult morphology cover only the digestive tract (Sinha, 1958; Ameen and Rahman, 1973), external features of the male genital apparatus (Arnaud et al., 2001), and glands (Tschinkel, 1975). Of the larvae only the prothoracic glands (Srivastava, 1959), the gut (Ameen and Rahman, 1973), and sensory receptors (Ryan and Behan, 1973; Behan and Ryan, 1978) are described.

Whereas the adult morphology of two of three insect model organisms is comparatively well known, the anatomy of the immature stages lack far behind. As pointed out above, nearly complete treatments of the adults are available for *Manduca* and *Drosophila*. However, all model organisms have not benefited from the additional insights that can be obtained through the application of new innovative morphological methods. Detailed and well documented descriptions for all stages based on modern techniques would not only close gaps in a phylogenetic context (see above). They would also facilitate the interpretation of the effects of mutations on structures, functions and the development. Therefore, the detailed, modern and standardized investigation of the morphology of all life stages of insect model organisms should have high priority.

#### 4.4. The role and optimized application of innovative morphological techniques

The detailed documentation of the anatomy of all life stages of insects using traditional methods is very laborious and time consuming. New approaches developed and optimized in the last decade have changed the situation considerably. Using modern techniques, especially micro-computed tomography ( $\mu$ -Ct) and computer-based 3D-reconstruction, can accelerate the process very distinctly (see e.g., Friedrich and Beutel, 2008; Beutel et al., 2010). The best results can be obtained when modern and traditional methods are combined (Friedrich and Beutel, 2008). The use of 3D-reconstruction allows the efficient preparation of larger numbers of illustrations showing numerous details of an organism and has the additional option of three-dimensional animation. This allows for virtual dissections and viewing selected structures or the entire organism from all perspectives thus greatly increasing the information content of figures (e.g., Wipfler et al., 2012). In recent years, the workflow between the applications of different techniques has been optimized. Even with very little material, which is often a problem in phylogenetic investigations, excellent results can be obtained if a single specimen is examined using confocal laser scanning microscopy (CLSM) first, subsequently scanned with the  $\mu$ -CT (after drying at the critical point), then documented with a microscope-mounted camera, examined with the scanning electron microscope, and finally serially sectioned.

Lack of material is not a problem in the case of model organisms but the detailed documentation using a broad array of

morphological techniques is at least as important as for key taxa in phylogenetics. Aside from traditional and innovative illustrations, photographed serial sections and  $\mu$ -CT image stacks can be stored in suitable data banks (e.g., extended and modified Morph Dbase [DFG BE1789/9-1]), and thus made available to scientists working on different aspects of these organisms. This will create the foundation for critically evaluating published results and investigating structural details hitherto neglected. This will not only enrich the body of evidence for studies focused on phylogeny and evolution, but also create greatly improved background knowledge for research in genetics, developmental biology, and other disciplines. The present study is the prelude of a comprehensive atlas which will cover the morphology of all life stages of *D. melanogaster*.

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### 3.7 Study VII

Friedrich F, Hünefeld F, Pohl H, Beckmann F, **Schneeberg K**, Herzen J, Beutel RG. 2010.

Reconstructing the evolution of Holometabola (Hexapoda) using SR $\mu$ CT-based morphological data. HasyLab Annual Review 2009.

#### **Abstract**

The report presented the data acquisition at the Deutsches Elektronen Synchrotron (DESY) in Hamburg. The Synchrotron radiation-based Micro-Computer-Tomography (SR- $\mu$ CT) enables high quality morphological data within a short time. Beside the time efficiency the method is nondestructive and the image stacks are perfectly aligned.

#### **Significance in the present thesis**

The technique was used in some studies of the thesis and played an important role for data acquisition.

#### **Own contribution**

20%



# Reconstructing the evolution of Holometabola (Hexapoda) using SR $\mu$ CT-based morphological data

Frank Friedrich<sup>1</sup>, Frank Hünefeld<sup>2</sup>, Hans Pohl<sup>2</sup>, Felix Beckmann<sup>3</sup>,  
Katharina Schneeberg<sup>2</sup>, Julia Herzen<sup>3</sup> and Rolf G. Beutel<sup>2</sup>

<sup>1</sup>Universität Hamburg, Biozentrum Grindel und Zoologisches Museum, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany, <sup>2</sup>Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena, 07743 Jena, Germany, <sup>3</sup>GKSS-Research Centre, Max-Planck-Str. 1, 21502 Geesthacht, Germany

## Background

The primary aim of our DFG funded project (BE 1789/4-1) was the compilation of a very comprehensive morphological data set for a representative sample of the extremely species rich Holometabola (=Endopterygota). This group of insects can arguably be considered as the most successful group of organisms on this planet (ca. 800.000 *described* species). The acquisition of well-documented anatomical data in a conventional way is very time consuming and morphological characters used in recent phylogenetic studies were usually more or less uncritically extracted from the literature. Therefore, it was one of our goals to develop an optimised approach to combine traditional (e.g., histology) and innovative techniques (e.g.,  $\mu$ -CT, 3D reconstruction) in order to increase efficiency. The acquired data were used for the reconstruction of the phylogenetic relationships of holometabolan insects. The final aim was an evolutionary scenario for different body parts and life stages and a critical evaluation of the usefulness of morphological investigations in the “age of molecular systematics”.

## Material and specimen preparation

Larval and adult specimens (fixed in 70% ethanol, 100% ethanol or Formaldehyde-ethanol-acetic acid) of representatives of all orders of Holometabola (e.g., Trichoptera, Diptera, Mecoptera, Neuroptera) were dried at the critical point and mounted on cylindrical metal carriers with superglue.

## Results

The  $\mu$ -CT scans obtained were of exceptionally high quality. Especially the high density resolution of the image stacks produced at low photon energy (8 KeV) at the beamline BW2 was important for the examination of these biological specimens. Therefore, the anatomy of different body parts of larvae and adults of the taxa included in our study could be reconstructed very efficiently. Even with a single specimen available, an excellent anatomical documentation was possible with a successive application of SR $\mu$ CT, SEM and then histology (if necessary). The combined application of these techniques and the use of a combination of different 3D software (mainly Imaris and Maya) turned out as highly efficient. It was possible to create the most comprehensive morphological data set (356 characters) ever used in insect systematics within a time frame of only 3 years. Detailed anatomical information is now available for larvae and adults of 30 representatives of all holometabolan orders and several outgroup taxa. A large series of studies is published [e.g., 1, 2] and a final major work is submitted. A phylogenetic hypothesis and evolutionary scenarios for different body regions were developed. 3-dimensional reconstructions of copulating insects (Fig. 1) were obtained for the first time using  $\mu$ -CT scans. It could be demonstrated that studying insect anatomy with a modern approach is efficient and a highly useful and viable discipline.

## Outlook

The assessment of SR $\mu$ CT as a key innovation in insect morphology was fully confirmed. Follow up projects were successfully started, one of them on the evolution of one of the largest holometabolan orders, the Diptera (biting flies and flies) (DFG: BE 1789/6-1), and

another one on the evolution of the male genital apparatus of holometabolan insects (VolkswagenStiftung).

### Acknowledgements

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### References

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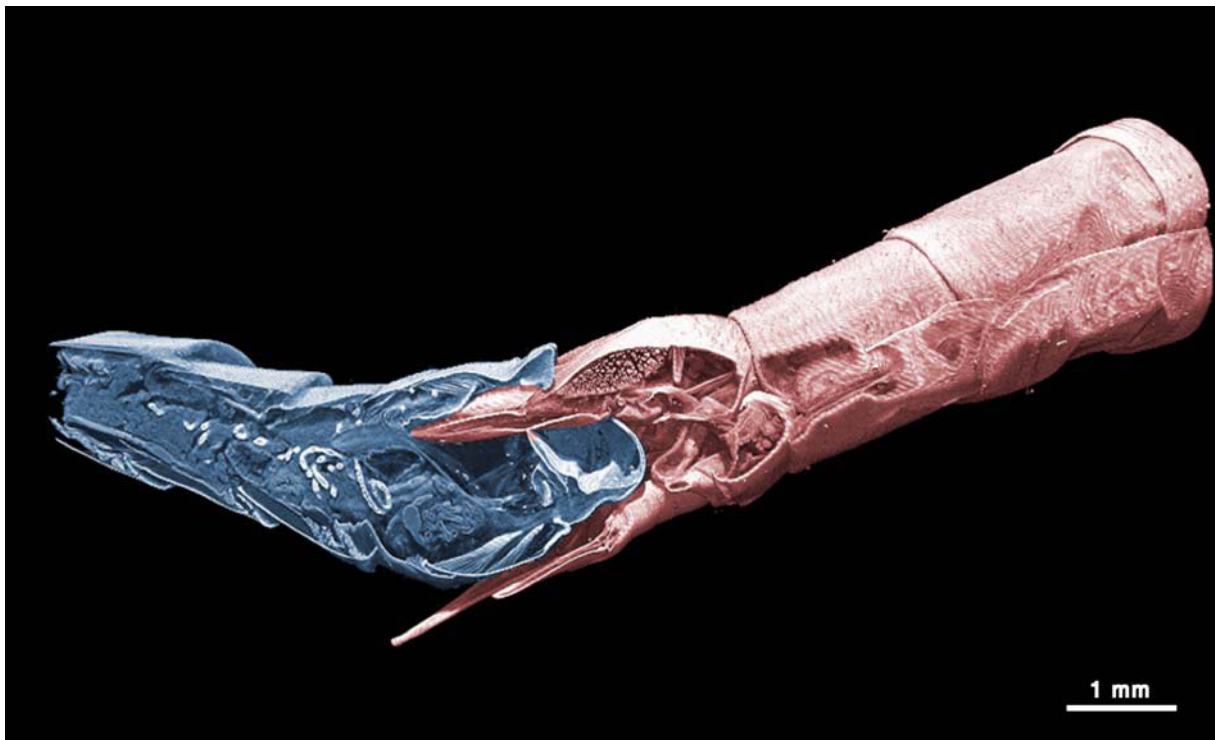


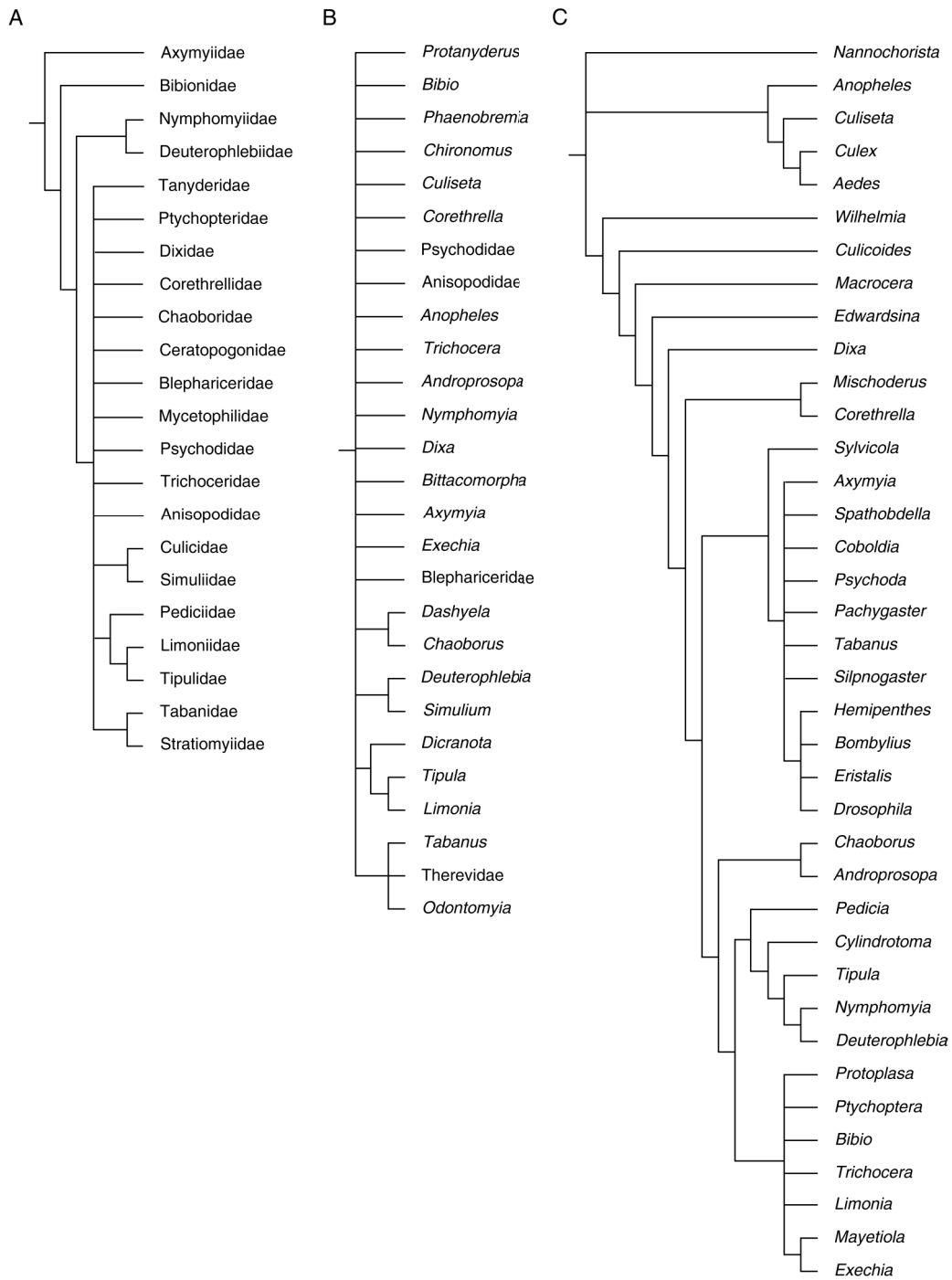
Figure 1: Abdomina of copulating crane flies (*Tipula* sp.; female [red] on right, male [blue] on left side), volume render of SR $\mu$ CT data obtained at DESY; virtually cut near the median line to show the interaction of the copulatory organs (resolution: 3,99  $\mu$ m; VG StudioMax).

### 3.8 Phylogenetic results

The Ratchet Island Hopper analyses resulted in 3 trees. The strict consensus tree is given in fig. 3A. Diptera and Brachycera are monophyletic. The strict consensus gives only poor information about the phylogenetic relationships among the lower Diptera. None of the eight subgroups is monophyletic in the analysis. Only Tipuloidea (Tipulomorpha, exclusive Trichoceridae) is monophyletic. Shared groups are Culicidae and Simuliidae, and Nymphomyiidae and Deuterophlebiidae. Axymyiidae form the sister to the remaining groups of Diptera.

The analyses of characters of the adult head resulted in 144 trees. In the strict consensus tree Diptera and also Brachycera are paraphyletic (Fig. 3C), as *Nannochorista* is nested within Diptera. Like in the analyses of the complete data set Nymphomyiidae and Deuterophlebiidae are close related.

Larval character analyses resulted in 90 trees. The strict consensus tree is given in fig. 3B. Diptera, Brachycera and Tipuloidea (excl. Trichoceridae) are monophyletic. Deuterophlebiidae shared with Simuliidae and Chaoboridae with Ceratopogonidae. Otherwise the tree is unresolved.



**Fig. 3:** Strict consensus trees based on: **A**, the complete data set (CI 0.33, RI 0.29). **B**, larval head structures (CI 0.26, RI 0.21). **C**, adult head structures (CI 0.25, RI 0.48).



## 4 Phylogenetically relevant characters

**1. Shape of the posterior region of the head capsule: (0) rounded; (1) flattened behind the compound eyes.** The posterior region of the head is rounded in *Culicoides* (Ceratopogonidae) (Szadziewski et al. 1997), *Chironomus* (Chironomidae) (Peterson 1916), *Chaoborus* (Chaoboridae), *Androprosopa* (Thaumaleidae), *Mischoderus* (Schneeberg & Beutel 2011), *Protoplasa* (Tanyderidae) (Williams 1933), *Biblio* (Bibionidae), *Exechia* (Mycetophilidae), *Coboldia* (Scatopsidae), *Psychoda* (Psychodidae), *Ptychoptera* (Ptychopteridae), *Deuterophlebia* (Deuterophlebiidae), *Edwardsina* (Blephariceridae) (Schneeberg et al. 2011), *Nymphomyia* (Nymphomyiidae) (Tokunaga 1935), and Tipulomorpha (Schneeberg & Beutel 2011), and this is also the case in *Nannochorista* (Nannochoristidae, Mecoptera) (Beutel & Baum 2008). It is flattened behind the large compound eyes in *Culex*, *Aedes*, *Anopheles*, *Culiseta* (Culicidae) (Schiemenz 1957), *Corethrella* (Corethrellidae), *Dixa* (Dixidae) (Peterson 1916), *Wilhelmia* (Simuliidae) (Wenk 1962), *Axymyia* (Axymyiidae) (Schneeberg et al. 2013b), *Macrocera* (Keroplastidae), *Mayetiola* (Cecidomyiidae) (Schneeberg et al. 2013a), *Spathobdella* (Sciaridae), *Sylvicola* (Anisopodidae), *Tabanus* (Tabanidae) (Bonhag 1951), *Pachygaster* (Stratiomyiidae), *Silpnogaster* (Asilidae), *Hemipenthes*, *Bombylius* (Bombyliidae) (Szucsich & Krenn 2000), *Drosophila* (Drosophilidae) (Ferris 1950), and *Eristalis* (Syrphidae) (Schiemenz 1957), and also in Mecoptera excl. Nannochoristidae (Beutel et al. 2008a; Heddergott 1938; Friedrich et al. 2013a).

**2. Vestiture of microtrichia: (0) present; (1) absent.** The head is densely covered with microtrichia in almost all dipterans examined (e.g., Tokunaga 1935; Ferris 1950; Schneeberg & Beutel 2011; Schneeberg et al. 2011; Schneeberg et al. 2013a, b). A similar vestiture is present on all cephalic regions of *Deuterophlebia* except for the ventral side (Schneeberg et al. 2011) and on all regions except for the vertex in *Ptychoptera*. The head of *Biblio* and *Pachygaster* is covered with longer setae and microtrichia are missing. The head of *Nannochorista* is densely covered with thin and very short setae (coded as 0). Large parts of the head of strepsipteran adults are densely covered with microtrichia (Beutel & Pohl 2006). They are absent in *Caurinus* (Beutel et al. 2008a), *Merope* (Friedrich et al. 2013a) and *Ctenocephalus* (Siphonaptera) (Wenk 1953).

**3. Orientation of the head: (0) orthognathous; (1) prognathous.** The head is orthognathous in *Wilhelmia* (Wenk 1962), *Culicoides* (Gad 1951), *Chironomus* (Chironomidae) (Peterson 1916), *Deuterophlebia*, (Schneeberg et al. 2011), *Edwardsina*, *Axymyia* (Schneeberg et al. 2013b), *Macrocera*, *Mayetiola* (Schneeberg et al. 2013a), *Coboldia*, *Spathobdella*, *Psychoda*, *Tabanus* (Bonhag 1951), *Silpnogaster*, *Hemipenthes* (Szucsich & Krenn 2000), *Drosophila* (Ferris 1950), *Eristalis* (Schiemenz 1957), *Caurinus* (Beutel et al. 2008a), *Nannochorista* (Beutel & Baum 2008), *Merope* (Friedrich et al. 2013a), *Ctenocephalus* (Wenk 1953). It is prognathous in *Culex*, *Aedes*, *Corethrella*, *Chaoborus*, *Androprosopa*, *Dixa* (Peterson 1916), *Mischoderus*, *Protoplasa* (Williams 1933), *Biblio*, *Sylvicola*, *Ptychoptera*, *Nymphomyia* (Tokunaga 1935), *Bombylius* (Szucsich & Krenn 2000), *Panorpa* (Heddergott 1938) and in Tipulomorpha (Schneeberg & Beutel 2011).

**4. Frontal apodeme: (0) present; (1) absent.** A frontal apodeme between the antennal bases is absent in *Deuterophlebia* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Chaoborus*, *Androprosopa*, *Tipula* (Tipulidae) (Schneeberg & Beutel 2011), *Pedicia*, *Biblio* (Bibionidae), *Axymyia* (Schneeberg et al. 2013b), *Coboldia*, *Sylvicola*, *Psychoda*, *Silpnogaster*, *Eristalis* (Syrphidae) (Schiemenz 1957), *Hemipenthes*, *Bombylius* (Bombyliidae) (Szucsich & Krenn 2000), and *Drosophila* (Ferris 1950), and it is also missing in *Ctenocephalus* (Wenk 1953) and in Mecoptera with the notable exception of Nannochoristidae (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008, fig. 5 [fap]). A small apodeme-like structure is present between the antennal bases in *Trichocera* (Trichoceridae) (Schneeberg & Beutel 2011), *Cylindrotoma* and *Pachygaster*. It is more distinctly developed in *Edwardsina*, *Limonia* (Tipulidae), *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Mischoderus* (Tanyderidae), *Ptychoptera*, *Culicoides* (Ceratopogonidae), (Gad 1951), *Corethrella*, in representatives of Culicidae (Schiemenz 1957; Christophers 1960; Owen 1985; Harbach & Kitching 1998), and in *Wilhelmia* (Simuliidae) (Wenk 1962). Bonhag (1951) described a median inflection between the antennae (m) in *Tabanus*, which is probably equivalent to the frontal apodeme (coded as 0).

**5. Frontoclypeal/epistomal border: (0) present as a strengthening ridge; (1) present as a joint; (2) absent.** Both regions are fused in *Deuterophlebia*, *Edwardsina* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Axymyia* (Schneeberg et al. 2013b), *Spathobdella*, *Ptychoptera*, *Culicoides* (Szadziewski et al. 1997), *Dixa* (Peterson 1916), *Simulium* (Wenk 1962, fig. 1) and *Ctenocephalus* (Wenk 1953). A ridge is present in *Corethrella*, *Chaoborus*, *Androprosopa*, *Limonia*, *Cylindrotoma*, *Pedicia*, *Trichocera*, *Biblio*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Coboldia*, *Sylvicola*, *Psychoda*, *Eristalis* (Schiemenz 1957), *Bombylius* (Szucsich & Krenn 2000), *Tabanus* (Bonhag 1951), *Mischoderus* and *Protoplasa* (Williams 1933). It is distinctly developed in *Tipula paludosa* (Schneeberg & Beutel 2011), whereas it appears to be absent in *Tipula reesi* (Rees & Ferris 1939) and a *Tipula* species examined by Bitsch et al. (1973). The ridge is also present in members of Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). A joint between the clypeus and frons in adults of Culicidae (e.g., Schiemenz 1957; Christophers 1960; Hennig 1973) is a potential autapomorphy of the family. In *Drosophila* it is replaced by a membrane (coded as 0) (Ferris 1950).

**6. Clypeus: (0) subdivided into ante- and postclypeus; (1) undivided.** An anterior anteclypeus is separated from the postclypeus in the groundplan of Diptera according to Crampton (1942). However, the division is generally missing in Diptera (e.g., Peterson 1916; Williams 1933; Tokunaga 1935; Ferris 1950; Bonhag 1951; Gad 1951; Hoyt 1952; Schiemenz 1957; Wenk 1962; Szucsich & Krenn 2000; Schneeberg et al. 2011). A transverse intraclypeal furrow is present in adults of Culicidae (e.g., *Culex*, *Culiseta*, *Aedes*; Schiemenz 1957; Christophers 1960; Owen 1985) and also in *Nannochorista* (Beutel & Baum 2008) and *Caurinus* (Beutel et al. 2008a). It is absent in other groups of Mecoptera (Heddergott 1938; Hepburn 1969; Friedrich et al. 2013a) and also in Siphonaptera (Wenk 1953) and some other groups of endopterygote insects (Hannemann 1956; Beutel & Pohl 2006; Beutel & Baum 2008).

**7. Rostrum: (0) absent or very short; (1) distinctly developed.**

A distinctly developed rostrum is present in adults of *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Cylindrotoma*, *Pedicia*, *Nymphomyia* (Tokunaga 1935), *Chaoborus*, *Dixa* (Peterson 1916), *Edwardsina*, *Sylvicola* (Hoyt 1952), *Mischoderus* and *Protoplasa* (Williams 1933). It is very short in *Corethrella*, *Androprosopa*, *Trichocera* (Schneeberg & Beutel 2011), *Biblio*, *Culicoides* (Gad 1951), *Ptychoptera* (Ptychopteridae) (Hoyt 1952), Culicidae (Schiemenz 1957; Christophers 1960; Owen 1985), and *Wilhelmia* (Wenk 1962). It is formed by a completely foldable rostral membrane in adults of most groups of Brachycera (Schiemenz 1957, fig. 34, *Eristalis* [Rstr]; Szucsich & Krenn 2000, fig 5, *Hemipenthes*, *Bombylius*; Ferris 1950, fig. 1, *Drosophila*; Hoyt 1952, figs 62-65, 69, 72, *Symphoromyia*, *Rhagio*, *Sepsis*, *Fucellia*) (see also next character). A rostrum is completely absent in most other examined members of Diptera (e.g., Peterson 1916; Bonhag 1951; Hoyt 1952; Schneeberg et al. 2011, 2013a, b), *Nannochorista* (Beutel & Baum 2008), *Caurinus* (Beutel et al. 2008a) and *Ctenocephalus* (Wenk 1953). A different type is present in adults of most groups Mecoptera (Otanen 1922; Heddergott 1938; Hepburn 1969; Beutel et al. 2008a; Friedrich et al. 2013a).

**8. Sclerotisation of the rostrum: (0) only sclerotized dorsally; (1) sclerotized on all sides; (2) completely membranous.** The rostrum of *Tipula*, *Cylindrotoma*, *Limnophila* (Hoyt 1952) and *Nymphomyia* (Tokunaga 1935) is entirely sclerotised. It is formed by the ventrally inflected lateral parts of the clypeus. Only the dorsal side is sclerotised in *Limonia*, *Erioptera*, *Dicranomyia* (Hoyt 1952), *Chaoborus*, *Dixa* (Peterson 1916), *Pedicia*, *Edwardsina*, *Mischoderus*, and *Protoplasa* (Williams 1933). It is completely membranous in *Sylvicola* (Hoyt 1952) and representatives of Brachycera (see char. 7; Ferris 1950, *Drosophila*; Hoyt 1952, *Symphoromyia*, *Rhagio*, *Sepsis*, *Fucellia*; Schiemenz 1957, *Eristalis*; Szucsich & Krenn 2000, *Hemipenthes*, *Bombylius*). In *Merope* the rostrum is covering the mouthparts and is not sclerotized on all sides (Friedrich et al. 2013a) (coded as 0).

**9. Reduced number of antennomeres: (0) not reduced; (1) reduced ( $\leq 6$ ).** The antennal flagellum composed of a relatively high number of flagellomeres in members of lower Diptera (e.g., Williams 1933; Wenk 1962; Lindegaard 1997; Szadziewski et al. 1997; Harbach & Kitching 1998; Schneeberg & Beutel 2011) and members of Mecoptera (Beutel & Baum 2008; Friedrich et al. 2013a). The antennae of *Tabanus* (Bonhag 1951) and *Pachygaster* comprise 7 segments. The antenna is 5-segmented in *Nymphomyia* (Tokunaga 1935) and 6-segmented in *Deuterophlebia* (Schneeberg et al. 2011). The number of antennomeres is also reduced in other members of Brachycera (Ferris 1950; Schiemenz 1957; Szucsich & Krenn 2000).

**10. Insertion of antennae: (0) frontally, not adjacent medially; (1) frontally, adjacent in midline; (2) dorsally, widely separated.** The antennae insert on the dorsal side of the head and are widely separated in *Deuterophlebia* (Schneeberg et al. 2011) and *Biblio*. They are also widely separated in *Caurinus* (Beutel et al. 2008a) and *Ctenocephalus* (Wenk 1953). They inserted frontally but are widely separated in *Psychoda* (coded as 0) and *Chaoborus*. The antennal insertions lie frontally between the compound eyes in other examined members of Diptera (Peterson 1916; Williams 1933; Tokunaga 1935;

Ferris 1950; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Harbach & Kitching 1998; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b) and also in Mecoptera (Heddergott 1938; Beutel & Baum 2008; Friedrich et al. 2013a).

**11. Shape of the antennae: (0) filiform; (1) moniliform; (2) club-shaped; (3) flabellate.** The antenna is filiform in most groups of lower Diptera (Williams 1933; Gad 1951; Schiemenz 1957; Snodgrass 1959; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011) and also in Mecoptera (Beutel & Baum 2008; Heddergott 1938; Beutel et al. 2008a). It is moniliform in *Wilhelmia* (Wenk 1962), *Axymyia* (Schneeberg et al. 2013b), *Coboldia* and *Bibio*, and club-shaped in *Nymphomyia*, *Tabanus* (Bonhag 1951), *Pachygaster*, *Drosophila* (Ferris 1950) and *Eristalis* (Schiemenz 1957). In *Mayetiola* the antennae are bead-like in males (coded as 0) (Schneeberg et al. 2013a). In *Ctenocephalus* the last 9 segments together form a club-shaped structure (Wenk 1953) (coded as 2).

**12. The first flagellomere of the antenna: (0) not enlarged; (1) enlarged.** The first flagellomere of the antenna is enlarged in *Nymphomyia* (Courtney 1994b), *Macrocera*, *Tabanus* (Bonhag 1951), *Bombylius* (Szucsich & Krenn 2000), *Drosophila* (Ferris 1950), *Eristalis* (Schiemenz 1957) and *Micropteryx* (Hannemann 1956). This is not the case in all other taxa examined (Peterson 1916; Williams 1933; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1953, 1962; Owen 1985; Szucsich & Krenn 2000; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b).

**13. Pedicellus with Johnston's organ: (0) absent; (1) present.** The Johnston's organ of the pedicellus was identified in members of Culicidae (Schiemenz 1957; Christophers 1960; Owen 1985), Corethrellidae, *Chaoborus*, *Androprosopa*, Ceratopogonidae, Chironomidae (Gad 1951), *Wilhelmia* (Wenk 1962), and *Drosophila* (Miller 1950), and also in *Caurinus* (Beutel et al. 2008a) and *Ctenocephalus* (Wenk 1953). This sense organ, which belongs to the groundplan of Insecta, is absent in other members of Diptera examined (Williams 1933; Tokunaga 1935; Schiemenz 1957; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b) and also in *Nannochorista* (Beutel & Baum 2008) and *Merope* (Friedrich et al. 2013a).

**14. Last segment of the antenna: (0) not elongated; (1) elongated.** The last antennal segment is extremely elongated in males of *Deuterophlebia* (Kennedy 1958, 1960; Schneeberg et al. 2011) and also elongated in *Nymphomyia* (Courtney 1994b, fig. 16, 26, 29). It is thick and elongated in *Eristalis* (Schiemenz 1957), *Drosophila* (Ferris 1950) and also enlarged in *Silpnogaster*, *Pachygaster*, and *Bombylius* (Szucsich & Krenn 2000). But the homology of the enlarged antennal segments is questionable. The segment is not elongated in members of most other groups of Diptera (Williams 1933; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b), and Mecoptera (Beutel et al. 2008a; Beutel & Baum 2008; Friedrich et al. 2013a).

**15. Ocelli: (0) present; (1) vestigial or absent.** Ocelli are absent in adults of *Deuterophlebia* (Schneeberg et al. 2011), *Tipula*, *Limonia*, *Cylindrotoma*, *Pedicia*, *Culex*, *Anopheles* (Schneeberg & Beutel 2011), *Corethrella*, *Chaoborus*, *Androprosopa*, *Dixa* (Peterson 1916), *Culiseta* (Schiemenz 1957; Owen 1985), *Wilhelmia* (Wenk 1962), *Mischoderus*, *Protoplasa* (Williams 1933), *Ptychoptera*, *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), and *Psychoda*, and they are also missing in *Caurinus* (Beutel et al. 2008a), *Merope* (Friedrich et al. 2013a), *Ctenocephalus* (Wenk 1953). Three ocelli are present on the vertex in *Edwardsina*, *Trichocera* (Schneeberg & Beutel 2011), *Bibio*, *Axymyia* (Schneeberg et al. 2013b), *Coboldia*, *Spathobdella*, *Sylvicola*, *Silpnogaster*, *Pachygaster*, *Eristalis* (Schiemenz 1957), *Drosophila* (Ferris 1950), *Exoprosopa* (Peterson 1916), *Nannochorista* (Beutel & Baum 2008) and *Panorpa* (Heddergott 1938). Christophers (1960) described a pair of degenerated ocelli on the frons of *Aedes*. A pair is present posterolaterad the large compound eyes of *Nymphomyia* (Tokunaga 1935; Courtney 1994b). Bonhag (1951) described three vestigial ocelli on the vertex of *Tabanus* (coded as 0). According to Szadziewski et al. (1997) ocelli are absent in Ceratopogonidae, but two are present on the frontal region in *Culicoides* according to Gad (1951).

**16. Subdivision of the compound eyes in dorsal and ventral part: (0) undivided; (1) subdivided.** The compound eyes are undivided in most dipteran groups (Williams 1933; Tokunaga 1935; Ferris 1950; Bonhag 1951; Gad 1951; Schiemenz 1957; Snodgrass 1959; Wenk 1962; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and also in Mecoptera (Heddergott 1938; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). They are subdivided in a dorsal and a ventral part in *Axymyia* (Schneeberg et al. 2013b).

**17. Coronal-/epicranial suture: (0) present; (1) absent.** The coronal suture is absent in most Diptera examined (Williams 1933; Bonhag 1951; Schiemenz 1957; Wenk 1962; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and also in *Ctenocephalus* (Wenk 1953), *Caurinus* (Beutel et al. 2008a) and many other representatives of Mecoptera (Hepburn 1969; Friedrich et al. 2013a). It is present in *Culex*, *Culiseta* (Schiemenz 1957; Owen 1985, fig. 1), *Aedes* (Christophers 1960, fig. 53/1), *Chironomus* (Peterson 1916, fig. 12 [e.s.]), *Nymphomyia* (Tokunaga 1935), *Drosophila* (Ferris 1950, fig. 1C [premandibular suture]), and *Nannochorista* (Beutel & Baum 2008). The presence is apparently a plesiomorphic condition preserved in Nymphomyiidae and Culicidae.

**18. Postgenal bridge: (0) present; (1) absent.** A postgenal bridge is present in *Deuterophlebia* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Tipula* (Schneeberg & Beutel 2011), *Limnophila* (Hoyt 1952), *Aedes* (Christophers 1960), *Bibio*, *Axymyia* (Schneeberg et al. 2013b), *Silpnogaster*, *Tabanus* (Bonhag 1951), *Eristalis* (Schiemenz 1957), *Exoprosopa* (Peterson 1916), and *Rhagio* (Hoyt 1952), and also in *Ctenocephalus* (Wenk 1953) and representatives of Mecoptera (e.g., Otanes 1922; Heddergott 1938; Hepburn 1969; Beutel et al. 2008a; Friedrich et al. 2013a) (with the possible exception of *Nannochorista*, Beutel & Baum 2008). The ventral closure of the head capsule is largely membranous in *Limonia*, but a narrow postgenal bridge is present anterior to the foramen occipital (coded as 0) (Schneeberg & Beutel 2011). The bridge is absent in *Edwardsina*, *Erioptera*, *Dicranomyia* (Hoyt 1952),



*Pedicia*, *Trichocera*, *Chironomus* (Peterson 1916), *Culicoides* (Gad 1951), *Corethrella*, *Chaoborus*, *Androprosopa*, *Dixa* (Peterson 1916), *Mischoderus* (Schneeberg & Beutel 2011), *Protoplasa* (Williams 1933), *Culiseta* (Schiemenz 1957, fig. 4 [gu]; Owen 1985), *Ptychoptera*, *Mycetophila*, *Mycomya* (Hoyt 1952), *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Coboldia*, *Sylvicola*, *Psychoda*, *Wilhelmia* (Wenk 1962), *Symphoromyia* (Hoyt 1952) and *Drosophila* (Ferris 1950). The head is largely membranous on its ventral side in adults of these taxa.

**19. Tentorium: (0) present; (1) absent.** The tentorium is present as a more or less straight tube-like rod in almost all examined groups of Diptera (e.g., Thompson 1905; Peterson 1916; Williams 1933, fig. 3; Gad 1951; Hoyt 1952; Christophers 1960; Wenk 1962; Schiemenz 1957; Owen 1985; Schneeberg et al. 2011). A similar condition is found in representatives of Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953). The tentorium is completely absent in *Nymphomyia* (Tokunaga 1935) and *Tipula* (Schneeberg & Beutel 2011). In *Mayetiola* (Schneeberg et al. 2013a), *Cylindrotoma* and *Pedicia* it is present as a short vestigial tube (coded as 0 as muscles are attached). Anterior and posterior tentorial grooves are present in *Exechia*, but the short anterior and posterior arms are not connected with each other.

**20. Dorsal tentorial arm: (0) present or present as a thin thread-like structure; (1) short vestigial structure; (2) absent.** The dorsal arm is completely missing in a number of Diptera (Thompson 1905; Peterson 1916; Ferris 1950; Gad 1951; Christophers 1960; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a) and in Siphonaptera (Wenk 1953). It is present as a short vestige in *Mischoderus*, *Culiseta* (Schiemenz 1957, fig. 5 [d.Ta]; Owen 1985, fig. 4), *Corethrella*, *Chaoborus*, *Chironomus* (Peterson 1916, fig. 152 [r.d.a.]), *Eristalis* (Schiemenz 1957, figs. 38, 39), *Exoprosopa* (Peterson 1916), *Pachygaster* and *Nannochorista* (Beutel & Baum 2008, fig. 2d [dta]) Wenk (1962) described a tentorial ridge for *Wilhelmia*, extending dorsad towards the antennal foramen (Wenk 1962, fig. 2). A typical, well developed dorsal arm is apparently almost generally missing in Antliophora (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008), but a thin, sclerotised structure is present and connected to the head capsule in *Caurinus* (Beutel et al. 2008a, figs. 5C, D) and *Merope* (Friedrich et al. 2013a). It is noteworthy that dorsal arms are also present in *Tabanus* (Bonhag 1951, fig. 5).

**21. Shape of the anterior tentorial arm: (0) thick, approximately round in cross section, hollow; (1) partly hollow; (2) massive.** The tentorium of almost all dipterans examined is a thick, hollow tube (Thompson 1905; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Schneeberg & Beutel 2011; Schneeberg et al. 2011), like in *Nannochorista* and *Bittacus* (Beutel & Baum 2008). A recognisable lumen is not present in *Eristalis* (Schiemenz 1957), *Drosophila*, *Exoprosopa* (Peterson 1916), *Mayetiola* (Schneeberg et al. 2013a), *Pedicia*, *Cylindrotoma*, *Boreus* (Beutel & Baum 2008) and *Caurinus* (Beutel et al. 2008a). The lumen of the anterior part of the tentorium is narrow in *Limonia*. It widens at the level of the brain and the posterior hollow part is approximately round in cross section (Schneeberg & Beutel 2011). In *Coboldia* the anterior part is also narrow and the tentorial rod is hollow in the following part (coded as 1). The vestigial anterior tentorial arm is round and hollow anteriorly

and narrow in its posterior region in *Exechia*. The anterior tentorial arms are missing in *Ctenocephalus* (Wenk 1953).

**22. Frontotentorial muscle band: (0) absent; (1) present.** A frontotentorial muscle band is absent in all dipterans examined (Bonhag 1951; Gad 1951; Schiemenz 1957; Snodgrass 1959; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) with the exception of *Drosophila* (Miller 1950 [19a]). It is also missing in Siphonaptera and Mecoptera (with the exception of *Nannochorista*) (Wenk 1953; Beutel & Baum 2008; Beutel et al. 2008a).

**23. Labrum: (0) present; (1) absent.** The labrum is almost generally present in Diptera (e.g., Thompson 1905; Williams 1933; Bonhag 1951; Hoyt 1952; Schiemenz 1957; Wenk 1962; Christophers 1960; Owen 1985; Schneeberg & Beutel 2011, 2013a, b) but is missing in *Deuterophlebia* (Schneeberg et al. 2011) and *Nymphomyia* (Togunaka 1935). It is also present in Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953).

**24. Clypeolabral connection: (0) separated; (1) fused.** The clypeus and labrum are fused in *Edwardsina* (Schneeberg et al. 2011), and also in *Ctenocephalus* (Wenk 1953) and most groups of Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008). They are recognisable as separate structures in other groups of Diptera (e.g., Thompson 1905; Williams 1933; Bonhag 1951; Gad 1951; Hoyt 1952; Schiemenz 1957; Wenk 1962; Christophers 1960; Owen 1985; Schneeberg & Beutel 2011, 2013a, b) and also in *Nannochorista* (Beutel & Baum 2008), *Caurinus* (Beutel et al. 2008a) and *Merope* (Friedrich et al. 2013a).

**25. Separation of clypeus and labrum: (0) transverse suture; (1) clypeus and labrum separated by a membrane.** Both elements are separated by an exposed membrane in *Tipula*, *Limonia*, *Trichocera*, *Mischoderus* (Schneeberg & Beutel 2011), *Protoplasia* (Williams 1933), *Ptychoptera*, *Biblio*, *Exechia*, and *Wilhelmia* (Wenk 1962), whereas a suture is present in Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985), in *Culicoides* (Gad 1951), *Dixa* (Peterson 1916), *Corethrella*, *Chaoborus*, *Androprosopa*, *Pedicia*, *Cylindrotoma*, *Coboldia*, *Sylvicola*, *Psychoda*, *Nannochorista* (Beutel & Baum 2008), and in *Caurinus* (Beutel et al. 2008a). Clypeus and labrum are separated by a rostral membrane in *Eristalis* (Schiemenz 1957), *Drosophila* (Ferris 1950) and *Toxophora* (Hoyt 1952), and by a small membranous area in *Tabanus* (Bonhag 1951). An indistinct clypeolabral suture is present in *Merope* (Friedrich et al. 2013a).

**26. Fulcrum: (0) absent; (1) present.** A fulcrum with lateral plates joining the external clypeal wall distally is generally present in Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985), and does also occur in *Protoplasia* (Williams 1933), Syrphidae (Schiemenz 1957), *Drosophila* (Ferris 1950), and Bombyliidae (Szucsich & Krenn 2000, *Hemipenthes*, *Bombylius*). It is absent in all other taxa examined (Tokunaga 1935; Heddergott 1938; Bonhag 1951; Gad 1951; Wenk 1953, 1962; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg & Beutel 2011; Friedrich et al. 2013a; Schneeberg et al. 2011, 2013a, b).

**27. Labro-epipharyngeal channel: (0) present; (1) absent.** The epipharynx forms a food channel in most adults of Diptera examined, and also in *Nannochorista* and Siphonaptera (e.g., Vogel 1921; Bonhag 1951; Gad 1951; Wenk 1953, 1962; Schiemenz 1957; Snodgrass 1959; Sutcliffe 1985; Szucsich & Krenn 2000; Beutel & Baum 2008; Schneeberg & Beutel 2011, 2013b). This structural modification is missing in Pistillifera and Boreidae (Heddergott 1938; Beutel & Baum 2008; Beutel et al. 2008a). In *Mayetiola* and *Macrocera* the anterior epipharynx is slightly bent upwards, but does not form a food channel (Schneeberg et al. 2013a). In *Exechia* the food channel is completely missing.

**28. Shape of the labro-epipharyngeal food channel: (0) ventrally open; (1) closed by the sides of the epipharynx; (2) ventrally closed by hypopharynx; (3) ventrally closed by the mandibles.** The food channel is open in most Diptera examined (Wenk 1962; Sutcliffe 1985; Schneeberg & Beutel 2011; Schneeberg et al. 2013b) and in *Nannochorista* (Beutel & Baum 2008) and *Ctenocephalus* (Wenk 1953). It forms a closed tube in representatives of Culicidae (Vogel 1921; Schiemenz 1957; Snodgrass 1959; Christophers 1960; Owen 1985) and in *Silpnogaster*. In *Eristalis* (Schiemenz 1957) and representatives of Bombyliidae (Szucsich & Krenn 2000, *Hemipenthes*, *Bombylius*) the channel is ventrally closed by the hypopharynx, whereas it is ventrally closed by the mandibles in *Tabanus* (Bonhag 1951) and *Culicoides* (Gad 1951).

**29. M. labroepipharyngalis (M. 7): (0) present; (1) absent.** A paired M. labroepipharyngalis connects the external and internal labral wall in a number of dipteran taxa examined (Ferris 1950; Bonhag 1951; Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). It is absent in representatives of Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985) and Simuliidae (Wenk 1962), *Coboldia* and *Pachygaster*, in *Ctenocephalus* (Wenk 1953), *Nannochorista* (Beutel & Baum 2008), and *Caurinus* (Beutel et al. 2008a), but is present in most groups of Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Friedrich et al. 2013a). Whether M. labroepipharyngalis is unpaired in the groundplan of Diptera as postulated by Gouin (1949) appears questionable. Radially arranged labroepipharyngeal muscles occur secondarily in Cyclorrhapha according to this author.

**30. M. frontolabralis (M. 8): (0) present; (1) absent.** Among all representatives of Diptera examined the muscle is only absent in *Deuteroephlebia* (Schneeberg et al. 2011), *Nymphomyia* (labrum reduced, Tokunaga 1935), *Pachygaster* and *Drosophila* (Miller 1950). In *Axymyia* (Schneeberg et al. 2013b) and *Cylindrotoma* it is not recognisable in the  $\mu$ CT data set. In all groups the muscle has an unusual origin on the clypeus (M. clypeolabralis). Schiemenz (1957) interpreted the muscle he found in *Culiseta* and *Eristalis* as M. epistomalabralis (M. 10). However, considering the function as labral levator it is much more plausible to assume that it is homologous with M. frontolabralis. M. 8 is generally absent in Mecoptera (e.g., Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a) and Siphonaptera (Wenk 1953, *Ctenocephalus*).

**31. Configuration of M. frontolabralis (M. 8): (0) separated; (1) fused.** M. clypeolabralis is distinctly paired in *Ptychoptera*, *Tipula*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Pedicia*, *Culicoides* (Gad 1951), *Bibio*, *Spathobdella* and *Micropteryx* (Hannemann 1956), whereas the two subcomponents are fused medially in *Mischoderus*, *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Psychoda*, *Corethrella*, *Androprosopa*, *Chaoborus*, Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985), *Tabanus* (Bonhag 1951) and *Eristalis* (Schiemenz 1957).

**32. M. frontoepipharyngalis (M. 9): (0) present; (1) absent.** The muscle is absent in all Diptera examined (e.g., Miller 1950; Bonhag 1951; Gad 1951; Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and also missing in Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953). It is present in *Bibio*, *Coboldia*, *Wilhelmia* (Wenk 1962) and Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985).

**33. Origin of M. tentorioscapalis anterior (M. 1): (0) tentorium; (1) head capsule.** In *Deuterophlebia* the muscles has three areas of origin on the head capsule and one on the dorsal side of the tentorium (Schneeberg et al. 2011). In *Exechia* it has an extensive area origin on the tentorium and another one the head capsule, and a similar condition with a bipartite muscle is found in *Psychoda*. It originates on the head capsule in *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Axyomyia* (Schneeberg et al. 2013b), *Mayetiola* (Schneeberg et al. 2013a), *Nymphomyia* (Tokunaga 1935) and *Pachygaster* and also in *Ctenocephalus* (Wenk 1953). The tentorial origin is preserved in most Diptera examined (Thompson 1905; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011) and in Mecoptera (Heddergott 1938; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a).

**34. Origin of M. tentorioscapalis posterior (M. 2): (0) tentorium; (1) head capsule.** M. 2 originates on the head capsule in *Deuterophlebia*, *Edwardsina* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Tipula* (Schneeberg & Beutel 2011, figs. 4, 11 [2]), *Pedicia*, *Axyomyia* (Schneeberg et al. 2013b), *Macrocera*, *Mayetiola* (Schneeberg et al. 2013a), *Hemipenthes* (Szucsich & Krenn 2000) and *Ctenocephalus* (Wenk 1953). In *Culicoides* M. tentorioscapalis posterior takes its origin on the apodeme of the frontal region (Gad 1951, fig. 21 [acc. adductor of the scape]), whereas the same condition occurs in *Corethrella* and *Androprosopa*. It is difficult to distinguish between M. 2 and M. 4 in *Limonia*. Both muscles lie very closely together and have a nearly identical point of insertion on the scapus and closely adjacent areas of origin on the tentorium (Schneeberg & Beutel 2011). The muscle originates on the tentorium in *Trichocera*, *Ptychoptera*, *Chaoborus*, *Aedes* (Christophers 1960), *Culiseta* (Schiemenz 1957; Owen 1985), *Wilhelmia* (Wenk 1962), *Bibio*, *Exechia*, *Coboldia*, *Spathobdella*, *Sylvicola*, *Tabanus* (Bonhag 1951) and *Silpnogaster* like in all adults of Mecoptera examined (e.g., Heddergott 1938; Beutel et al. 2008a; Beutel & Baum 2008; Friedrich et al. 2013a). The area of origin lies on the circumocular ridge in *Eristalis* (Schiemenz 1957) and a similar M. orbitoscapalis is also present in *Bombylius* (Szucsich & Krenn 2000). Schiemenz (1957) interpreted this muscle as M. tentorioscapalis posterior (M. 2). However, it cannot

be excluded that it is in fact *M. tentorioscapalis medialis* (M. 4). The muscle is bipartite in *Psychoda*. One subcomponent originates on the tentorium (lateral of M. 1) and the second one on the head capsule, ventrolateral of M. 1. *Cylindrotoma* have one muscle, which extends between the posteriomedial margin of the scapus and the dorsal wall of the tentorium. It is not clear if the muscle is homologous with *M. tentorioscapalis posterior* or *M. tentorioscapalis medialis*.

**35. Origin of *M. tentorioscapalis medialis* (M. 4): (0) tentorium; (1) frontal region of head capsule; (2) genal region of head capsule; (3) on the vertex.**

*M. tentorioscapalis medialis* originates on the head capsule in *Nymphomyia* (Tokunaga 1935), *Tipula* (Schneeberg & Beutel 2011, figs. 4, 11 [4]), *Trichocera*, *Ptychoptera*, *Bibio*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Sylvicola*, *Tabanus* (Bonhag 1951), *Pachygaster*, *Nannochorista* (Beutel & Baum 2008), *Panorpa* (Heddergott 1938) and *Ctenocephalus* (Wenk 1953). The muscle is absent in representatives of Culicidae (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985) and Simuliidae (Wenk 1962). It originates on the tentorium in *Mischoderus*, *Macrocera*, *Culicoides* (Gad 1951), *Corethrella*, *Chaoborus*, *Androprosopa*, *Hemipenthes* (Szucsich & Krenn 2000), *Merope* (Friedrich et al. 2013a) and *Caurinus* (Beutel et al. 2008a). As pointed out above, the homology of the muscle is not entirely clear in *Limonia*, *Eristalis* and *Bombylius* (see character 34). The muscle is bipartite in *Psychoda*. One subcomponent originates on the tentorium and the second one on the frontal region of the head capsule.

**36. Mandible: (0) present; (1) absent.** The mandibles are absent in most members of Diptera examined (Peterson 1916; Williams 1933; Tokunaga 1935; Ferris 1950; Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and also in *Ctenocephalus* (Wenk 1953). They are developed in females of *Edwardsina*, *Symphoromyia* (Rhagionidae) (Hoyt 1952) and Tabanidae (Bonhag 1951), whereas they are always present in adults of Ceratopogonidae (Blackwell 2004), Simuliidae (Wenk 1962), Mecoptera (e.g., Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a). In Culicidae mandibles are present in all females and the most males, except *Aedes* and *Ochlerotatus* (Schiemenz 1957; Christophers 1960; Owen 1985). The mandible in culicid males is much shorter than in females (Snodgrass 1959).

**37. Shape of the mandibles: (0) transformed into piercing stylets; (1) not transformed into piercing stylets.** The mandibles are transformed to piercing stylets in adults of Culicidae and Ceratopogonidae (Schiemenz 1957; Christophers 1960; Owen 1985; Blackwell 2004). The mandibles of *Wilhelmia* are spoon-shaped (Wenk 1962). According to Peterson (1916) the mandibles are also elongated in *Tabanus*, *Culicoides*, and females of *Bibiocephalia* and *Blepharocera* (Blephariceridae). Piercing stylets are absent in all adults of Mecoptera (e.g., Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a), but the mandibles are strongly modified, elongate and lamelliform in *Nannochorista* (Beutel & Baum 2008).



- 38. M. craniomandibularis internus (M. 11): (0) present; (1) absent.** The muscle is present in *Culiseta* (Schiemenz 1957; Owen 1985 [M. tentorio-mandibularis]), *Aedes* (Christophers 1960), *Culex*, *Anopheles* (Wenk 1961), *Culicoides* (Gad 1951), *Wilhelmia* (Wenk 1962), and also in adults of all mecopteran groups except for *Nannochorista* (Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a).
- 39. M. craniomandibularis externus (M. 12): (0) present; (1) absent.** The muscle is present in *Aedes* (Christophers 1960), *Culex*, *Anopheles* (Wenk 1961), *Culicoides* (Gad 1951), *Wilhelmia* (Wenk 1962), and females of *Tabanus* (Bonhag 1951), as in all mecopteran groups with the exception of *Nannochorista* (Beutel & Baum 2008; Friedrich et al. 2013a). Schiemenz (1957) described only one mandibular muscle for *Culiseta* (M. 11), but a second one is mentioned by Wenk (1961 [M. retractor mandibulae tentorialis]) and Owen (1985 [M. oculo-mandibularis, M. 12]).
- 40. M. hypopharyngomandibularis (M. 13): (0) present; (1) absent.** The muscle is usually absent in adults of Culicidae (Schiemenz 1957; Christophers 1960; Wenk 1961; Owen 1985) and in *Merope* (Friedrich et al. 2013a), but present in *Anopheles* according to Wenk (1961). It is also present in representatives of Simuliidae (Wenk 1962, 1admd), *Culicoides* (Gad 1951), females of *Tabanus* (Bonhag 1951), and in *Nannochorista*, *Boreus* and *Bittacus* (Beutel & Baum 2008). It is unusually large in *Caurinus* (Beutel et al. 2008a).
- 41. Maxilla: (0) present; (1) absent.** The maxilla is absent in *Deuterophlebia* (Schneeberg et al. 2011) and *Nymphomyia* (Togunaka 1935) and is also missing in some chironomids (Neumann 1976). It is present in all other members of Diptera (e.g., Thompson 1905; Williams 1933; Bonhag 1951; Hoyt 1952; Schiemenz 1957; Wenk 1962; Christophers 1960; Owen 1985; Blackwell 2004; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b), and also in the outgroup taxa (Heddergott 1938; Wenk 1953; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a).
- 42. Maxillary endite lobes: (0) lacinia; (1) lacinia and galea; (2) both absent.** Both endites are absent in *Tipula*, *Erioptera*, *Mycetophila* and *Fucellia* (Hoyt 1952), *Axymyia* (Schneeberg et al. 2013b) and *Mayetiola* (Schneeberg et al. 2013a). The lacinia is present in adults of most groups of Diptera (e.g., Williams 1933; Bonhag 1951; Hoyt 1952; Schiemenz 1957; Owen 1985; Szucsich & Krenn 2000; Blackwell 2004; Schneeberg & Beutel 2011), Siphonaptera (Wenk 1953; Michelsen 1996a) and *Nannochorista* (Beutel & Baum 2008). Both endite lobes are present in Boreidae and Pistillifera (Heddergott 1938; Hepburn 1969; Beutel et al. 2008a; Friedrich et al. 2013a).
- 43. Maxillary palp: (0) 5-segmented; (1) 4-segmented; (2) 3 palpomeres or less.** The maxillary palp is 5-segmented in almost all dipteran examined (Peterson 1916; Williams 1933; Hoyt 1952; Wenk 1962; Blackwell 2004; Schneeberg & Beutel 2011; Schneeberg et al. 2011) and also in Mecoptera (Heddergott 1938; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). In *Axymyia* the palps are also 5-segmented; however some individuals have four or five segments (Whilm 2009; Schneeberg et al. 2013b). The palp is 4-segmented in *Psychoda*, *Exechia* and representatives of Siphonaptera (Wenk 1953; Michelsen 1996a). In adults of Culicidae the palps can comprise 1-5 palpomeres (e.g., Christophers 1960,

*Aedes*, 5-segmented; Harbach & Kitching 1998, *Anopheles*, 4-segmented. In *Mayetiola* (Schneeberg et al. 2013a) and *Spathobdella* the palps are 3-segmented and in *Coboldia* and *Drosophila* (Ferris 1950) only one segment is present. The number is usually reduced in representatives of Brachycera (Bonhag 1951; Hoyt 1952; Schiemenz 1957).

**44. Last segment of the maxillary palp: (0) not elongated; (1) elongated.** The last segment of the maxillary palp is elongated in *Androprosopa*, *Tipula*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Chaoborus*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Ptychoptera* and *Protoplasa* (Williams 1933, fig. 9). It is not elongated in most other taxa examined (Peterson 1916; Heddergott 1938; Ferris 1950; Bonhag 1951; Gad 1951; Wenk 1953; Schiemenz 1957; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg et al. 2011, 2013b; Friedrich et al. 2013a).

**45. Sensorial field on maxillary palpomere 3: (0) present; (1) absent.** A sensorial field is present on the maxillary palpomere 3 of *Edwardsina* (Schneeberg et al. 2011), *Tipula*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Ptychoptera*, *Biblio*, *Sylvicola*, *Culicoides* (Blackwell 2004), *Corethrella*, *Wilhelmia* (Wenk 1962), *Mycetophila* (Hoyt 1952) and *Nannochorista* (Beutel & Baum 2008). It is absent in all other representatives of Mecoptera (e.g., Heddergott 1938; Beutel et al. 2008a; Friedrich et al. 2013a), in *Culiseta* (Schiemenz 1957), *Culex*, *Chaoborus*, *Androprosopa*, *Axymyia* (Schneeberg et al. 2013b), *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Psychoda* and also Siphonaptera (Wenk 1953; Michelsen 1996a). In *Exechia* a sensorial field is present on palpomere 2.

**46. Position of sensilla on sensorial field: (0) Sensilla placed in a groove; (1) each sensilla in a single groove; (2) Sensilla exposed on the surface.** The sensilla placed together in a large groove in *Edwardsina*, *Biblio*, *Culicoides* (Blackwell 2004), *Wilhelmia* (Wenk 1962, fig. 24), *Mycetophila* (Hoyt 1952), *Exechia* and *Nannochorista* (Beutel & Baum 2008). In *Tipula*, *Corethrella* and *Sylvicola* each sensillum is inserted in an individual groove and they are exposed on the surface of the palpomere in *Limonia*, *Trichocera* (Schneeberg & Beutel 2011) and *Ptychoptera*.

**47. Stipes: (0) exposed; (1) internalised (cryptostipes sensu Peterson 1916).** The stipites are exposed in females of *Tabanus*, whereas the stipites of males are largely reduced and internalised (Bonhag 1951). They are internalised in *Tipula*, *Limonia*, *Cylindrotoma*, *Culex*, *Anopheles* (Schneeberg & Beutel 2011), *Culiseta* (Schiemenz 1957; Owen 1985), *Aedes* (Christophers 1960), *Wilhelmia* (Wenk 1962), *Hemipenthes*, *Bombylius* (Szucsich & Krenn 2000), *Drosophila* (Ferris 1950), *Eristalis* (Schiemenz 1957) and *Toxophora* (Hoyt 1952).

**48. Stipites: (0) separated; (1) partly fused; (2) completely fused.** The stipites are fused in *Edwardsina*, *Tipula* (Schneeberg & Beutel 2011), *Cylindrotoma*, and some representatives of Brachycera (*Silpnogaster*, Hoyt 1952, figs. 66, 67, *Dioctria*, figs. 69, *Sepsis*, fig. 72, *Fucellia*, fig. 72). They are only fused posteriorly and thus form a Y-shaped rod-like structure in *Toxophora* (Hoyt 1952), *Limonia* (Schneeberg & Beutel 2011), *Pedicia* and some representatives of Tipulidae (Hoyt 1952, *Erioptera*, *Limnophila*, *Dicranomyia*). They are also partly fused in *Coboldia* and *Spathobdella*.

**49. Cardo and stipes: (0) not fused; (1) fused.** Both proximal maxillary elements are usually fused in adults of Diptera (e.g., Peterson 1916; Williams 1933; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011), and also in *Nannochorista* (Beutel & Baum 2008), *Caurinus* (Beutel et al. 2008a) and representatives of Siphonaptera (Michelsen 1996a).

**50. M. craniocardinalis (M. 15): (0) absent; (1) present.** The muscle is absent in all dipteran adults examined (e.g., Thompson 1905; Bonhag 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and is apparently generally missing in Antliophora (Beutel & Baum 2008).

**51. Origin of M. tentoriocardinalis (17): (0) tentorium; (1) head capsule; (2) fulcral plates.** M. 17 originates on the head capsule in *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Limnophila*, *Dicranomyia*, *Dioctria* (Hoyt 1952) and *Macrocera*, *Fucellia* (Hoyt 1952), *Tabanus* (Bonhag 1951) in representatives of Rhagionidae (Hoyt 1952, in *Symphoromyia*, *Rhagio*), and in *Panorpa* (Heddergott 1938), *Boreus* (Beutel & Baum 2008). The muscle is bipartite in *Wilhelmia*. One bundle originates lateroventrally on the tentorium, the other one on its ventral side (Wenk 1962, fig. 20 [1, 2adcd]). In *Eristalis* and *Bombyliidae* the muscle originates on the fulcral plates (Schiemenz 1957; Szucsich & Krenn 2000).

**52. Origin of M. tentoriostipitalis (M. 18): (0) tentorium; (1) head capsule.** M. tentoriostipitalis originates on the head capsule in *Mischoderus*, *Tipula*, *Limonia* (Schneeberg & Beutel 2011) and *Drosophila* (Ferris 1950 [maxillary muscle], homology not entirely clear).

**53. M. craniolacinalis (M. 19): (0) present; (1) absent.** M. 19 is missing in *Mischoderus*, *Ptychoptera*, *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Pedicia*, *Cylindrotoma*, *Corethrella*, *Androprosopa*, *Biblio*, *Macrocera*, *Axymyia* (Schneeberg et al. 2013b), *Mayetiola* (Schneeberg et al. 2013a), *Exechia*, *Coboldia*, *Spathobdella*, *Psychoda*, *Edwardsina* (Schneeberg et al. 2011), *Pachygaster*, *Eristalis* (Schiemenz 1957) and *Boreus* (Beutel & Baum 2008).

**54. M. stiptopalpalis externus (M. 22): (0) present; (1) absent.** The muscle is absent in *Aedes* (Christophers 1960), *Androprosopa*, *Axymyia* (Schneeberg et al. 2013b), *Coboldia*, and all examined members of Brachycera (Miller 1950; Bonhag 1951; Schiemenz 1957; Szucsich & Krenn 2000).

**55. M. stiptopalpalis internus (M. 23): (0) present; (1) absent.** The muscle is absent in *Mischoderus*, *Ptychoptera*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Culex* (Thompson 1905), *Culiseta* (Schiemenz 1957; Owen 1985), *Corethrella*, *Androprosopa*, *Wilhelmia* (Wenk 1962), *Axymyia* (Schneeberg et al. 2013b), *Exechia*, *Sylvicola*, *Spathobdella*, *Psychoda*, *Tabanus* (Bonhag 1951), *Pachygaster*, *Eristalis* (Schiemenz 1957), *Hemipenthes*, *Bombylius* (Szucsich & Krenn 2000), *Drosophila* (Miller 1950), *Nannochorista*, *Boreus*, *Bittacus* (Beutel & Baum 2008), *Caurinus* (Beutel et al. 2008a) and *Merope* (Friedrich et al. 2013a). Christophers (1960) described a muscle in *Aedes* extending from the anterior region of the stipes to the dorsal base of the palp. It functions as a levator of the entire palp and is only present in males (Christophers 1960 [13]).

**56. M. palpopalpalis maxillae primus (M. 24): (0) present; (1) absent.** The muscle is absent in *Ptychoptera*, *Axymyia* (Schneeberg et al. 2013b), *Coboldia*, *Spathobdella*, *Psychoda*, *Eristalis* (Schiemenz 1957), members of Bombyliidae (Szucsich & Krenn 2000), *Drosophila* (Miller 1950), and in *Caurinus* (Beutel et al. 2008a) and *Boreus* (Beutel & Baum 2008).

**57. M. palpopalpalis secundus (M. 25): (0) present; (1) absent.** This muscle is absent in *Chaoboridae*, *Androprosopa*, *Edwardsina*, *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Pedicia*, *Wilhelmia* (Wenk 1962), *Axymyia* (Schneeberg et al. 2013b), *Exechia*, *Coboldia*, *Spathobdella*, *Sylvicola*, *Psychoda*, *Tabanus* (Bonhag 1951), *Pachygaster*, *Eristalis* (Schiemenz 1957), representatives of Bombyliidae (Szucsich & Krenn 2000) and Mecoptera (Beutel & Baum 2008, *Nannochorista*, *Boreus*, *Bittacus*; Beutel et al. 2008a, *Caurinus*; Friedrich et al. 2013a, *Merope*) (with the exception of *Panorpa*, Heddergott 1938) and Siphonaptera (Wenk 1953). A muscle with an origin on the dorsolateral basal margin of palpomere 1 and an insertion on the lateral basal margin of palpomere 3 is present in *Mischoderus*, *Trichocera* (Schneeberg & Beutel 2011) and *Corethrella*. It is likely homologous with *M. stipitopalpalis secundus* (coded as 0).

**58. Labium: (0) present; (1) absent.** The labium is absent in *Deuterophlebia* (Schneeberg et al. 2011), *Nymphomyia* (Togunaka 1935) and some chironomids (Neumann 1976). It is present in all other members of Diptera (e.g., Thompson 1905; Williams 1933; Bonhag 1951; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Blackwell 2004; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b), Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953).

**59. Postmentum: (0) present; (1) absent.** The postmentum is reduced or completely fused with the prementum in members of Tipulomorpha (Schneeberg & Beutel 2011), *Mischoderus*, *Ptychoptera*, *Wilhelmia* (Wenk 1962), *Dixa* (Peterson 1916), *Chaoboridae*, *Androprosopa*, *Edwardsina*, *Biblio*, *Axymyia* (Schneeberg et al. 2013b), *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Coboldia*, *Spathobdella*, *Sylvicola*, *Psychoda*, *Silpnogaster*, *Pachygaster*, *Tabanus* (Bonhag 1951), *Hemipenthes*, *Bombylius* (Szucsich & Krenn 2000), *Drosophila* (Ferris 1950) and *Eristalis* (Schiemenz 1957). In *Nannochorista* the postmentum is indistinctly separated from the prementum (Beutel & Baum 2008).

**60. Dorsal surface of the anterior labium with distinct concavity: (0) present; (1) absent.** The concavity of the anterior labium is absent in *Tipula* (Schneeberg & Beutel 2011), *Biblio*, *Axymyia* (Schneeberg et al. 2013b), *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Pachygaster*, and *Mengenilla* (Beutel & Pohl 2006). The lateral premental walls are slightly bent upwards in *Edwardsina* (Schneeberg et al. 2011), *Mischoderus*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Pedicia*, *Cylindrotoma*, *Culicoides* (Gad 1951), *Androprosopa*, *Ptychoptera*, and distinctly in *Coboldia*, *Sylvicola*, *Psychoda*, *Corethrella*, *Chaoborus*, Simuliidae (Wenk 1962; Sutcliffe 1985), Bombyliidae (Szucsich & Krenn 2000), *Tabanus* (Bonhag 1951), *Eristalis* (Schiemenz 1957) and representatives of Culicidae (Thompson 1905; Vogel 1921; Schiemenz 1957; Christophers 1960; Owen 1985). In Culicidae the anterior labium forms a concavity for the reception of the piercing mouthparts in their resting position.

A concavity of the anterior labium is also present in adults of and Siphonaptera (Wenk 1953), and *Nannochorista* (Beutel & Baum 2008). It is absent in *Caurinus* and other mecopterans (Heddergott 1938; Hepburn 1969; Beutel et al. 2008a).

**61. Prementum: (0) without median ridge; (1) with median ridge.** A median ridge is present and the prementum is W-shaped in cross section in *Mischoderus*, *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Macrocera*, *Coboldia*, *Spathobdella*, *Sylvicola*, *Psychoda*, *Culicoides* (Gad 1951, fig. 27), *Corethrella*, *Chaoborus*, *Androprosopa*, and *Hemipenthes* (Szucsich & Krenn 2000, fig. 1c [pr]). It is T-shaped in cross section in representatives of Simuliidae (Wenk 1962; Sutcliffe 1985). A median ridge is also present in *Eristalis*, but small and inconspicuous (Schiemenz 1957, fig. 56).

**62. Number of labial palpomeres: (0) 2; (1) 3; (2) 5.** It is almost generally 2-segmented in adults of Diptera (e.g., Williams 1933; Bonhag 1951; Gad 1951; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Harbach & Kitching 1998; Schneeberg & Beutel 2011, 2013a, b) and Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). Schiemenz (1957) and Owen (1985) described 3-segmented palps for *Culiseta*, whereas they were interpreted as 2-segmented by Harbach & Kitching (1998). Only a single sclerite is present in *Anopheles*. It is divided dorsally, which suggests that it is a product of fusion and also 2-segmented (Harbach & Kitching 1998). Tokunaga (1935) described a small membranous appendage at the entrance of the mouth opening in *Nymphomyia*, which possibly represents vestigial labial palps. The palps are 5-segmented in representatives of Siphonaptera (Michelsen 1996a).

**63. Labialpalps modified as labellae: (0) present; (1) absent.** The 2-segmented labial palps (Crampton 1942) are modified as thickened labellae in all adults of Diptera with a preserved labium (e.g., Vogel 1921; Bonhag 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Sutcliffe 1985; Harbach & Kitching 1998; Szucsich & Krenn 2000; Blackwell 2004; Schneeberg & Beutel 2011; Schneeber et al. 2013a, b). This is not the case in the other antliophoran lineages (e.g., Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a).

**64. Pseudotracheae on the internal side of the labellae: (0) absent; (1) present.** The membranous mesal sides of the labellae are equipped with two rows of pseudotracheae in *Tipula* (Schneeberg & Beutel 2011). This is rarely the case in the nematoceran groups. They occur in Tipulini, and in some members of Mycetophilidae (some Sciophilinae and Mycetophilinae, e.g. *Exechia*) and Ptychopteridae (Hoyt 1952), but are usually missing. Within Brachycera pseudotracheae are more widespread and more complex. Two pseudotracheal collecting channels are present on the anterior edge of the labellae of *Eristalis* and about 40 pseudotracheae on the mesal wall (Schiemenz 1957, fig. 54). The number of pseudotracheae varies within Bombyliidae (Szucsich & Krenn 2000, fig. 4) and Tabanidae (Bonhag 1951). Within Brachycera they are lacking in representatives of Asilidae (Hoyt 1952). Pseudotracheae are present on the labellum of *Drosophila* (Ferris 1950).



**65. Simple furrows on the mesal sides of the labellae: (0) present; (1) absent.** Simple furrows are present on the mesal side of the labellae in *Androprosopa*, *Trichocera* (Schneeberg & Beutel 2011), *Pedicia*, *Cylindrotoma*, *Axymyia* (Schneeberg et al. 2013b), *Sylvicola*, *Psychoda* and some Culicids (*Culex*, *Anopheles*, *Culiseta*). They were referred to as pseudotracheae by Owen (1985) but it was already demonstrated by Schiemenz (1957) that their ultrastructure is distinctly different, i.e. that inner strengthening rings are absent and also secondary channels (e.g., Gilbert & Jervis 1998, figs. 3, 4; Ngernklun et al. 2007, fig. 2a, *Chrysomya*). The furrows are absent in *Corethrella*, *Chaoborus*, *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Silpnogaster* and *Nannochorista* (Beutel & Baum 2008). In *Culicoides* the wall of the second segment of the “labella are thin and much folded at the sides” (Gad 1951) (coded as 0).

**66. Scales on labial palps: (0) absent; (1) present.** Scales occur in some representatives of Culicidae on the external side of the labial palps (Harbach & Kitching 1998, figs. 9A, B, *Toxorhynchites*, *Tripteroidea*) and on the mesal side in *Nannochorista* (Beutel & Baum 2008).

**67. Glossa and paraglossa: (0) absent; (1) present.** Glossa and paraglossa are generally absent in Antliophora (e.g., Schiemenz 1957; Wenk 1962; Hepburn 1969; Owen 1985; Sutcliffe 1985; Szucsich & Krenn 2000; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg & Beutel 2011; Friedrich et al. 2013a).

**68. Retractor of the prementum (Mm. 28-30): (0) one muscle; (1) two muscles; (2) absent.** One large retractor is generally present in Diptera (Thompson 1905; Bonhag 1951; Gad 1951; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b) and Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). It is probably *M. tentoriopraementalis inferior* (M. 29) or a product of fusion of both tentoriopremental muscles (Beutel & Baum 2008). According to Miller (1950 [1, 2]) two retractors are present in *Drosophila*. Premental retractors are absent in Siphonaptera (Wenk 1953; Michelsen 1996a).

**69. M. palpopalpalis labii primus (M. 35): (0) present; (1) absent.** The muscle is absent in *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Pedicia*, *Coboldia*, *Spathobdella*, *Sylvicola*, *Psychoda*, *Corethrella* in representatives of Culicidae (Vogel 1921; Schiemenz 1957; Christophers 1960; Owen 1985), *Pachygaster*, *Eristalis* (Schiemenz 1957), *Hemipenthes*, *Bombylius* (Szucsich & Krenn 2000), *Drosophila* (Miller 1950), *Tabanus* (Bonhag 1951), *Fucellia* (Hoyt 1952), and in representatives of Mecoptera (Heddergott 1938; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953). A muscle originating on a median longitudinal premental ridge and inserting on the mesal basal margin of palpomere 2 occurs in Simuliidae (Wenk 1962; Imms 1944), and a similar muscle is also present in representatives of Bibionidae, Chironomidae, Rhagionidae, Asilidae and Sphaeroceridae (Hoyt 1952).

**70. M. frontohypopharyngalis (M. 41): (0) present; (1) absent.** The muscle is absent in *Deuterophlebia*, *Edwardsina* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Limonia*, *Trichocera* (Schneeberg & Beutel 2011), *Pedicia*, *Cylindrotoma*, *Bibio*, *Axymyia* (Schneeberg et al.

2013b), *Macrocera*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Spathobdella*, *Sylvicola*, *Psychoda*, *Wilhelmia* (Wenk 1962), *Culicoides* (Gad 1951), *Corethrella*, *Androprosopa*, *Pachygaster*, *Silpnogaster*, *Drosophila* (Ferris 1950), in representatives of Bombyliidae (Szucsich & Krenn 2000) and *Panorpa* (Heddergott 1938), but present in other mecopterans (Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a). A small bipartite muscle is present in *Tipula* and *Mischoderus*. It extends from the lateral clypeal wall to the lateral wall of the hypopharynx (Schneeberg & Beutel 2011). The homology with *M. frontohypopharyngalis* is questionable. A muscle connecting the postfrontal ridge and the dorsal fulcral apophyses is present in representatives of Culicidae (Thompson 1905; Schiemenz 1957; Owen 1985) and in *Eristalis* (Schiemenz 1957). It is likely that it mainly stabilises the cibarium and it is probably homologous with *M. frontohypopharyngalis*. However, Schiemenz (1957) assumed its homology with *M. frontobuccalis lateralis* (*M. 47*) (*Culiseta* and *Eristalis*) and a similar muscle is present in *Aedes*. It originates on the median frontal ridge and is inserted on the lateral horn of the cibarium (Christophers 1960, fig. 67/1 [21]). A muscle, which connects the clypeofrontal ridge and the anterior surface of the pharynx, is present in *Tabanus* (Bonhag 1951, fig. 10 [22]), but the homology with *M. 41* is also questionable. In *Coboldia* a muscle connecting the head capsule (ventral of the circumocular ridge) with the lateral wall of the hypopharynx, it is probably homologous with *M. 41*.

**71. Size of *M. clypeopalatalis* (*M. 43*): (0) long series of bundles; (1) bipartite, strongly developed; (2) not enlarged.** *M. clypeopalatalis* is a long series of bundles in all examined Diptera (Thompson 1905; Miller 1950; Bonhag 1951; Gad 1951; Schiemenz 1957; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). A similar condition is found in *Panorpa* (Heddergott 1938), *Boreus* (Beutel & Baum 2008), *Merope* (Friedrich et al. 2013a) and *Ctenocephalus* (Wenk 1953). Christophers (1960) described a bipartite muscle for *Aedes*, and a similar condition is present in *Wilhelmia* (Wenk 1962) and *Nannochorista* (Beutel & Baum 2008). It is composed of three subcomponents in *Caurinus* and is not enlarged (Beutel et al. 2008a). Tokunaga (1935) described a large muscle with three subcomponents for *Nymphomyia* (Tokunaga 1935, figs. 1, 7).

**72. *Mm. frontobuccalis anterior/posterior* (*Mm. 45/46*): (0) both present; (1) one muscle; (2) absent.** One muscle is present in *Deuterophlebia* (Schneeberg et al. 2011), *Tipula*, *Limonia* (Schneeberg & Beutel 2011), *Cylindrotoma*, *Androprosopa*, *Exechia*, *Mayetiola* (Schneeberg et al. 2013a), *Biblio*, *Coboldia*, *Spathobdella*, *Pedicia*, *Silpnogaster* and representatives of Bombyliidae (Szucsich & Krenn 2000). Both muscles are absent in *Eristalis* (Schiemenz 1957), *Nannochorista*, *Boreus*, *Bittacus* (Beutel & Baum 2008), and *Panorpa* (Heddergott 1938). A series of bundles is present between the frontal ganglion and the brain in *Nymphomyia* (Tokunaga 1935) and *Culicoides* (Gad 1951). It probably comprises both muscles.

**73. *M. tentoriobuccalis anterior* (*M. 48*): (0) present; (1) absent.** The muscle is absent in *Deuterophlebia* (Schneeberg et al. 2011), *Nymphomyia* (Tokunaga 1935), *Eristalis* (Schiemenz 1957), *Nannochorista*, *Bittacus* (Beutel & Baum 2008), *Panorpa* (Heddergott 1938), *Caurinus* (Beutel et al. 2008a), *Merope* (Friedrich et al. 2013a), *Ctenocephalus* (Wenk 1953) and *Mengenilla* (Beutel & Pohl

2006). Szucsich & Krenn (2000) described a protractor of the fulcrum in *Hemipenthes* and *Bombylius*, which is possibly homologous with M. 48 (mgc). A muscle which extends from the ventrolateral wall of the anterior pharynx, below the frontal ganglion, to the circum ocular ridge is present in *Edwardsina*. This muscle is probably homologous with M. tentoriobuccalis anterior.

**74. Postcerebral pharyngeal pump (Mm. 51/52): (0) present; (1) absent.** The postcerebral pharyngeal pump is absent in *Eristalis* (Schiemenz 1957), *Drosophila* (Ferris 1950) and representatives of Bombyliidae (Szucsich & Krenn 2000). It is functionally replaced by the labro-epipharyngeal pump in Cyclorrhapha according to Gouin (1949). It is strongly developed in all other taxa examined (e.g., Thompson 1905; Tokunaga 1935; Heddergott 1938; Bonhag 1951; Gad 1951; Wenk 1953; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Beutel & Baum 2008; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b; Friedrich et al. 2013a).

**75. M. anularis stomodaei (M. 68): (0) enclosing the lateral and ventral wall of the posterior pharynx; (1) ring muscle.** The bundles enclose only the ventral and lateral walls of the posterior pharynx and of the anterior pharynx in *Edwardsina*, *Trichocera* (Schneeberg & Beutel 2011) and *Culiseta* (Schiemenz 1957).

**76. M. hypopharyngosalivarialis (M. 37): (0) present; (1) absent.** A salivary pump is absent in *Deuterophlebia* (Schneeberg et al. 2011), *Axymyia* (Schneeberg et al. 2013b) and *Limonia* (Schneeberg & Beutel 2011). A typical M. hypopharyngosalivarialis is absent in Mecoptera (excluding Nannochoristidae, e.g. Beutel & Baum 2008), but a strong intrinsic muscle of the wall of the salivary duct (Heddergott 1938; Beutel et al. 2008a) may be a derivative of this muscle. M. hypopharyngosalivarialis is bipartite in *Nymphomyia*. It originates on the ventral membrane of the basipharynx and inserts on the salivarium (Tokunaga 1935, fig. 1).

**77. Exposure of the head: (0) fully exposed; (1) moderately retracted into prothorax, not fixed in this position; (2) strongly retracted, fixed in this position.** The head capsule of *Tipula* (Neugart et al. 2009), *Limonia* (Oosterbroek & Theowald 1991) and *Tabanus* (Cook 1949) is strongly retracted and fixed in this position. In *Axymyia* (Dvash 2013), *Dicranota* (Cook 1949) and *Odontomyia* (Cook 1949) the head is moderately retracted into the prothorax and not fixed in this position.

**78. Orientation of the mouthparts: (0) prognathous or slightly inclined; (1) orthognathous.** The orientation of the head is prognathous in most dipteran larvae examined (Anthon 1943a; Cook 1944a, b, 1949; Gouin 1949; Schremmer 1949, 1950a; Kramer 1954; Anthon & Lyneborg 1968; Solinas 1968; Courtney 1990b; Oosterbroek & Theowald 1991; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013) and also in *Nannochorista* (Beutel et al. 2009) and *Nosopsyllus* (Sharif 1937) is prognathous. The head is orthognath in *Panorpa* (Bierbrodt 1942).

**79. Paired dorsolateral incisions: (0) absent; (1) present, short; (2) present, deep, at least reaching anterior half of head capsule.** In tipulid larval head have paired dorsolateral incisions, separating a dorsomedian fragment of the head capsule from the remaining parts, this condition is also present in *Limonia* (Selke 1936; Cook 1949; Oosterbroek & Theowald 1991; Neugart et al. 2009). They

are present and short in *Dicranota* (Cook 1949). The incisions are absent in larvae of *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Protanyderus* (Wipfler et al. 2012a), *Trichocera* (Anthon 1943a), *Bibio* (Cook 1949), *Axymyia* (Dvash 2013), *Phaenobremia* (Solinas 1968), *Bittacomorpha* (Kramer 1954), *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Dasyhelea* (v. Lieven 1998), *Anopheles* (Cook 1944a; Schremmer 1949), *Culiseta* (Cook 1944a), *Androprosopa*, *Chaoborus* (Schremmer 1950a; Förster 2013), *Corethrella*, Chironomidae (Cook 1944b; Gouin 1949), Anisopodidae (Anthon 1943a), *Exechia*, Psychodidae (Anthon 1943a), Therevidae (Cook 1949), *Tabanus* (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). The merging of the head with the thorax and the anterior abdominal segment in Blephariceridae (only partly in Edwardsiinae) (Anthon and Lyneborg 1968) is probably correlated with tendency to form a hemicephalous condition somewhat similar to that of tipuloid larvae, and short and broad dorsolateral incisions are present in *Liponeura* (Anthon & Lyneborg 1968, fig. 2). However, the head is eucephalic, without incisions in *Edwardsina* and *Anisopous* (Anthon & Lyneborg 1968, figs. 3, 7).

**80. Ventromedian incision of head capsule: (0) absent; (1) present; (2) triangular median membranous sinus; (3) ventral head capsule entirely sclerotised.** A ventromedian incision is present in larvae of *Tipula*, *Limonia*, *Dicranota* and *Trichocera* (Selke 1936; Anthon 1943a; Cook 1949; Hennig 1968b; Peus 1952; Oosterbroek & Theowald 1991; Neugart et al. 2009). A triangular median membranous sinus is present in *Simulium* (Cook 1949). The ventral head capsule is entirely sclerotized in Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). The ventral head capsule is also reduced in some Blephariceridae (Anthon & Lyneborg 1968; but not in the groundplan, see *Edwardsina*, fig. 3).

**81. Externolateral plates with growth lines (intermolt cuticle deposition): (0) absent; (1) present.** Extensive externolateral plates with a zonal structure or growth lines are probably generally present in larvae of Tipuloidea (Cook 1949, Hennig 1968b; Oosterbroek & Theowald 1991; Neugart et al. 2009), Deuterophlebiidae (Courtney 1990b; Oosterbroek & Courtney 1995), Blephariceridae (Oosterbroek & Courtney 1995) and in *Protanyderus* (Wipfler et al. 2012a).

**82. Split cranial setae: (0) absent; (1) present.** Split cranial setae are present in Deuterophlebiidae (Courtney 1990b), *Axymyia* (Dvash 2013), Culicidae (see Neugart et al. 2009), *Androprosopa*, Chaoboridae (Förster 2013), *Simulium* (Cook 1949), Ceratopogonidae (Hennig 1968a, b), Psychodidae (Hennig 1968a, b) and *Nannochorista* (Beutel et al. 2009).

**83. Frontoclypeal suture: (0) present; (1) absent.** A frontoclypeal suture is generally lacking in dipteran larvae (e.g., Anthon 1943a; Cook 1944a, b, 1949; Gouin 1959; Schremmer 1949, 1950a, b; Kramer 1954; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; Hennig 1950, 1968a, b, 1973; v. Lieven 1998; Courtney 1990b; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a) and also in Siphonaptera (Sharif 1937). A distinct, unsclerotised clypeal area is present in Pediciidae and other larvae of Tipuloidea (Cook 1949; Oosterbroek & Theowald 1991, fig. 93, *Tricyphona*), but this is not equivalent to the frontoclypeal suture (Neugart et al. 2009). A frontoclypeal suture is present in *Axymyia* (Dvash 2013,

fig. 3A [ts]) and *Panorpa* (Bierbrodt 1942, fig. 1 [Epi.N.]). An internal ridge is present in *Nannochorista*, but it is morphological rather a transclypeal sulcus than a true frontoclypeal suture (Beutel et al. 2009, fig. 2 [tcs]).

**84. Coronal suture: (0) present at last 25% as long as the wall of head capsule; (1) present, less than 25% of dorsal wall of head capsule; (2) absent.** A long coronal suture is absent in most larvae of Tipulomorpha (Anthon 1943a; Hennig 1968b; Oosterbroek & Theowald 1991), in Chironomidae (Cook 1944b; Gouin 1959), *Anopheles* (Cook 1944a; Schremmer 1949), *Culiseta* (Cook 1944a), *Dixa* (Felix 1962), *Corethrella*, *Simulium* (Cook 1949), *Dasyhelea* (v. Lieven 1998), *Phaenobremia* (Solinas 1968), *Exechia*, Psychodidae (Anthon 1943a), *Tabanus* (Cook 1949) and *Nosopsyllus* (Sharif 1937). However it is present and long in Pediciidae (Cook 1949: "...deep median phragma developed along the line of the coronal suture...", fig. 18) and does also occur in Eriopterinae (Oosterbroek & Theowald 1991, figs. 14, 21, 26) (see Neugart et al. 2009). In *Protanyderus* (Wipfler et al. 2012a), *Androprosopa*, *Biblio* (Cook 1949; Perraudin 1961), *Axymyia* (Dvash 2013, fig. 3A [cs]), Anisopodidae (Anthon 1943a, fig. 2 [c.s.]), Blephariceridae (Anthon & Lyneborg 1968 [co.ec.li.]), *Nannochorista* (Beutel et al. 2009, fig. 2 [ecl]) and *Panorpa* (Bierbrodt 1942, fig. 1 [Cor.N.]) the coronal suture is present at last 25% long as the head capsule. In *Chaoborus* the coronal suture is present following Förster (2013, fig. 2B [cs]), but absent according to Schremmer (1950a). In *Bittacomorpha* (Kramer 1954) the coronal suture is a remnant line and in *Nymphomyia* it is present, but short (Schneeberg et al. 2012, fig. 3B). It is absent in *Deuterophlebia* (Courtney 1990b) and *Odontomyia* (Cook 1949).

**85. Shape of frons: (0) V-shaped; (1) U-shaped.** The frons is U-shaped in *Chaoborus* (Förster 2013) and *Dasyhelea* (v. Lieven 1998). In *Deuterophlebia* (Courtney 1990b) and *Bittacomorpha* (Kramer 1954) it is completely fused with the clypeus and not recognizable as a separate sclerite. It is V-shaped in all other taxa examined (Bierbrodt 1942; Anthon 1943a; Cook 1944b, 1949; Gouin 1959; Perraudin 1961; Felix 1962; Anthon & Lyneborg 1968; Oosterbroek & Theowald 1991; Oosterbroek & Courtney 1995; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Dvash 2013).

**86. Clypeus divided in ante- and postclypeus: (0) divided; (1) undivided.** The clypeus is divided in two areas in *Limonia* (Kramer 1959, fig. 9), *Axymyia* (Dvash 2013), *Nannochorista* (Beutel et al. 2009, figs. 1A, 2 [acl, fcl]) and *Panorpa* (Bierbrodt 1942, fig. 1 [A.Cly., P.Cly.]).

**87. Premaxillary suture and side plates: (0) absent; (1) present.** A premaxillary suture and side plates are present in Tipulidae, Limoniinae, Cylindrotomidae (Cook 1949, fig. 14; Oosterbroek & Theowald 1991; Neugart et al. 2009) and in *Protanyderus* (Wipfler et al. 2012a).

**88. Strengthened margins of externo-lateralia: (0) absent; (1) present.** The externo-lateralia are strengthened in Tipulidae, Limoniinae and Cylindrotomidae (Oosterbroek & Theowald 1991; Neugart et al. 2009), but not in other larvae of Diptera (e.g., Cook 1944a, b, 1949; Schremmer 1949, 1950; Courtney 1990b; Schneeberg et al. 2012; Wipfler et al. 2012a).



- 89. Dorsal endocarina: (0) absent; (1) present.** A distinctly developed dorsal endocarina is present in *Tipula* (Neugart et al. 2009, fig. 2A [ec]), *Limonia* (Oosterbroek & Theowald 1991) and also in larvae of Pediciidae (Selke 1936; Cook 1949, fig. 18; Oosterbroek & Theowald 1991, fig. 93), Eriopterinae (Oosterbroek & Theowald 1991, fig. 21 [93]), *Protanyderus* (Wipfler et al. 2012a), *Biblio* (see char. 12 in Neugart et al. 2009) and *Axymyia* (Mamayev & Krivosheyina 1966, fig. 2).
- 90. Tentorium: (0) present; (1) absent.** The tentorium is completely absent in larvae of *Tipula* (Neugart et al. 2009) and *Nymphomyia* (Schneeberg et al. 2012), *Dicranota* (Cook 1949), *Androprosopa*, *Exechia* and *Axymyia* (Dvash 2013). In Psychodidae the tentorium mainly vestigial (coded as 0) (Anthon 1943a).
- 91. Dorsal tentorial arm: (0) present; (1) absent.** Dorsal arms are present in *Trichocera* (Anthon 1943a [te.d.]), *Dasyhelea* (v. Lieven 1998, fig. 3 [ttd]), Anisopodidae (Anthon 1943a, fig. 2 [te.d.]) and *Panorpa* (Bierbrodt 1942). They are absent in all other taxa examined (Sharif 1937; Anthon 1943a; Cook 1944a, 1949; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Courtney 1990b; Wipfler et al. 2012a; Förster 2013). Dorsal arms are also absent in *Nannochorista*, but a vestige of the dorsal arm is present in form of a slender tissue strand (Beutel et al. 2009, fig. 5 [dta]).
- 92. Tentorial bridge: (0) well developed and sclerotised; (1) partly reduced with thin median connection; (2) absent.** The tentorial bridge is apparently present in *Deuterophlebia* (Courtney 1990b), *Biblio* (Perraudin 1961), *Nannochorista* (Beutel et al. 2009, fig. 5 [tb]), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937, fig. 1 [t.b.]). The tentorial arms are connected by a thin median connection in Anisopodidae (Anthon 1943a) and *Paenobremia* (Solinas 1968). The tentorial bridge is absent in all other dipterans examined (Anthon 1943a; Cook 1944a, 1949; Schremmer 1949; Kramer 1954; Gouin 1959; Felix 1962; Anthon & Lyneborg 1968; v. Lieven 1998; Wipfler et al. 2012a).
- 93. Anterior tentorial arms: (0) present; (1) vestigial or absent; (2) arising from paraclypeal phragma and strongly developed.** Anterior tentorial arms are absent in *Biblio* (Perraudin 1961), *Simulium* (Cook 1949) and partly in Chironomidae (Cook 1944b; Gouin 1959). The anterior tentorial arms are strongly developed and arising from the paraclypeal phragma in Therevidae (Cook 1949 [fig. 31B]) and *Tabanus* (Cook 1949).
- 94. Cuticular lense: (0) present; (1) absent.** A cuticular lense is generally lacking in nematoceran larvae with developed larval eyes (e.g., Cook 1944b, 1949; Schremmer 1950a; Kramer 1954; Gouin 1959; Solinas 1968; Hennig 1973; Wood & Borkent 1989; v. Lieven 1998; Schneeberg et al. 2012; Förster 2013) and also in *Androprosopa*, *Axymyia* (Dvash 2013), *Exechia*, *Protanyderus* (Wipfler et al. 2012a) and *Nannochorista* (Beutel et al. 2009). A convex cuticular lens is present in *Limonia* (Kramer 1959, fig. 9 [“Cornealinse”]), *Odontomyia* (Cook 1949) and *Panorpa* (Bierbrodt 1942).
- 95. Lateral eyes: (0) simplified compound eyes; (1) several stemmata; (2) eyes spot; (3) absent.** A single or bipartite larval eye is found in Tipulidae (e.g., Cook 1949). Eye spots are present in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Trichocera* (Anthon 1943a), *Simulium* (Cook 1949), Bibionidae (Cook 1949; Perraudin 1961), *Paenobremia* (Solinas 1968),

Ptychopteridae (Kramer 1954), Dixidae (Felix 1962), *Dasyhelea* (v. Lieven 1998), Culicidae (see Neugart et al. 2009), Chironomidae (Cook 1944b; Gouin 1959), *Odontomyia* (Cook 1949) and *Nannochorista* (Beutel et al. 2009). Simplified compound eyes are present in Chaoboridae (Schremmer 1950a; Förster 2013) and *Panorpa* (Bierbrodt 1942). Larval eyes are completely absent in *Dicranota* (Cook 1949), *Limonia* (Lindner 1959), *Axymyia* (Dvash 2013), *Protanyderus* (Wipfler et al. 2012a), Anisopodidae (Anthon 1943a), Psychodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968), Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Nosopsyllus* (Sharif 1937).

**96. Articulation of labrum: (0) free; (1) partially fused with head capsule; (2) completely fused with head capsule.** The labrum in Bibionidae (Cook 1949; Perraudin 1961) and *Axymyia* (Dvash 2013) is partly fused with the head capsule, whereas it is completely fused in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Trichocera* (Anthon 1943a), *Paenobremia* (Solinas 1968), *Simulium* (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949).

**97. Shape of labrum: (0) transverse; (1) narrow and conical.** The labrum is narrow in *Trichocera* (Anthon 1943a), *Dicranota* (Cook 1949) and Blephariceridae (Anthon & Lyneborg 1968). It is conical in *Nymphomyia* (Schneeberg et al. 2012), *Androprosopa*, *Axymyia* (Dvash 2013), Therevidae (Cook 1949), Anisopodidae (Anthon 1943a, fig. 18 [lb.]), Psychodidae (Anthon 1943a, fig. 9 [lb]), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). A transverse labrum is present in all other taxa examined (Sharif 1937; Bierbrodt 1942; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Courtney 1990b; Oosterbroek & Courtney 1995; v. Lieven 1998; Beutel et al. 2009; Neugart et al. 2009; Wipfler et al. 2012a; Förster 2013).

**98. Subdivision of labrum: (0) absent; (1) present.** A distinctly tripartite labrum as it is present in Tipulidae (Selke 1936; Cook 1949, *Holorusia*; Chiswell 1955) is a potential autapomorphy of the family.

**99. Labral brush with dense field of hairs: (0) absent; (1) present, without specific arrangement; (2) specifically arranged labral brush; (3) complex arrangement of different types of hairs; (4) macrosetae.** Dense fields or brushes of microtrichiae are present on the labrum of larvae of Tipuloidea (e.g., *Gonompoda*, *Cylindrotoma*, *Holorusia*, less strongly developed *Dicranota*; Cook 1949; Peus 1952; Podeniene & Gelhaus 2002; *Tipula*, Neugart et al. 2009), *Trichocera* (Anthon 1943a), *Dasyhelea* (v. Lieven 1998), *Corethrella*, Anisopodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968) and Psychodidae (Anthon 1943a). The labral brushes consist of a specific arrangement of different types of hairs in *Chironomus* (Cook 1944b; Gouin 1959), *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Culiseta* (Cook 1944a, fig. 19), *Androprosopa*, *Chaoborus* (Förster 2013), *Anopheles* (Cook 1944a, fig. 25; Schremmer 1949, fig. 2a [LPi]), *Protanyderus* (Wipfler et al. 2012a, fig. 2 [pb]) and *Axymyia* (Dvash 2013, fig. 5B). The labrum of *Bittacomorpha* (Kramer 1954, fig. 5 [lm]) is bearing two tufts of longer setae on the apex. The labral brush consists of macrotrichia in *Deuterophlebia* (Courtney 1990b) and *Nymphomyia* (Schneeberg et al. 2012). Labral brushes are absent in Bibionidae (Cook 1949; Perraudin 1961), *Exechia*, Therevidae (Cook 1949), *Tabanus* (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937).

**100. Labral teeth: (0) very small or absent; (1) present.** Labral teeth are absent in *Nymphomyia* (Schneeberg et al. 2012), Deuterophlebiidae (Courtney 1990b), *Protanyderus* (Wipfler et al. 2012a), Tipuloidea (major part; e.g., Cook 1949; Podeniene & Gelhaus 2002; Neugart et al. 2009), *Trichocera* (Anthon 1943a), *Androprosopa*, *Exechia*, *Axymyia* (Dvash 2013), Bibionidae (Cook 1949; Perraudin 1961), Ptychopteridae (Kramer 1954), Psychodidae (Anthon 1943a), *Dasyhelea* (v. Lieven 1998), Chironomidae (Cook 1944b; Gouin 1959), Culicidae (Cook 1944a; Schremmer 1949), *Chaoborus* (Schremmer 1950; Förster 2013), *Dixa* (Felix 1962), *Simulium* (Cook 1949), Blephariceridae (Anthon & Lyneborg 1968), *Tabanus* (Cook 1949), Therevidae (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942), *Nosopsyllus* (Sharif 1937) and *Leptopsylla* (Perfiljew 1927), but occur in larvae of Cylindrotomidae (Peus 1952) and Anisopodidae (Anthon 1943a).

**101. Connection of tormae with labral sclerite: (0) firmly connected with labral sclerite; (1) articulated.** Tormae articulating with the labral sclerite occur in Deuterophlebiidae (partim) (Courtney 1990b), Tipulomorpha (e.g., Pediciidae, Limoniinae) (Anthon 1943a; Oosterbroek & Theowald 1991; Oosterbroek & Courtney 1995; Neugart et al. 2009), Culicidae (Cook 1944a, 1949), Chironomidae (Cook 1944b; Gouin 1959), *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Androprosopa*, Ceratopogonidae (Oosterbroek & Courtney 1995), *Axymyia* (Mamayev & Krivosheyina 1966), Psychodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968), *Nymphomyia* (Schneeberg et al. 2012) and *Bittacomorpha* (Kramer 1954). The tormae are firmly connected with the labral sclerite in *Bibio* (Cook 1949; Perraudin 1961), *Paenobremia* (Solinas 1968), Anisopodidae (Anthon 1943a), *Nannochorista* (Beutel et al. 2009) and *Panorpa* (Bierbrodt 1942).

**102. M. labroepipharyngalis (M. 7): (0) present; (1) absent.** M. 7 is absent in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949), *Bibio* (Perraudin 1961), *Exechia*, *Paenobremia* (Solinas 1968), *Axymyia* (Dvash 2013), *Dasyhelea* (v. Lieven 1998), *Anopheles* (Schremmer 1949), *Culiseta* (Cook 1944a), *Androprosopa*, *Chaoborus* (Schremmer 1950a; Förster 2013), *Corethrella*, *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Odontomyia* (Cook 1949) and Therevidae (Cook 1949).

**103. M. frontolabralis (M. 8): (0) present; (1) absent.** M. 8 is absent in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Protanyderus* (Wipfler et al. 2012a), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949), *Bibio* (Perraudin 1961), *Exechia*, *Paenobremia* (Solinas 1968), *Axymyia* (Dvash 2013), *Bittacomorpha* (Kramer 1954), *Dasyhelea* (v. Lieven 1998), *Anopheles* (Schremmer 1949), *Culiseta* (Cook 1944a), *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Androprosopa*, Chironomidae (Cook 1944b; Gouin 1959), Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949).

**104. M. frontoepipharyngalis (M. 9): (0) present; (1) absent.** M. 9 is absent in *Bibio* (Perraudin 1961), *Paenobremia* (Solinas 1968), Therevidae (Cook 1949), *Odontomyia* (Cook 1949) and *Panorpa* (Bierbrodt 1942).

**105. Movable premandibles (messores): (0) absent; (1) present, separated by a weakly sclerotised zone; (2) present as strongly developed movable messorial arms.** A premandible separated by a weakly sclerotized zone is present in Limoniidae (Hexatominae, Eriopterinae; Oosterbroek & Courtney 1995), Chironomidae (Gouin 1959), *Androprosopa*, *Dasyhelea* (v. Lieven 1998, fig. 11 [mess]), *Dixa* (Felix 1962), Anisopodidae (Anthon 1943a, fig. 19 [pr.md.]), Psychodidae (Anthon 1943a, fig. 48 [pr.md.]), *Trichocera* (Anthon 1943a) and *Bittacomorpha* (Kramer 1954, fig. 9 [dmd]) and Culicidae (Cook 1944a, 1949; Schremmer 1949). They are modified as strongly developed messorial arms in *Simulium* (Cook 1949).

**106. Exposure of anterior epipharynx (cibarial roof): (0) not or only slightly exposed; (1) largely exposed.** The anterior epipharynx is largely exposed in Deuterophlebiidae (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Protanyderus* (Wipfler et al. 2012a), Tipulidae (Neugart et al. 2009), *Trichocera* (Anthon 1943a, fig. 7 [ep.]), Bibionidae (Cook 1949; Perraudin 1961), Anisopodidae (Anthon 1943a), Psychodidae (Anthon 1943a), some Blephariceridae (Anthon & Lyneborg 1968, fig. 4, *Edwardsina*), *Axymyia* (Dvash 2013), Ptychopteridae (Kramer 1954), Chironomidae (Cook 1944b; Gouin 1959), *Corethrella*, *Anopheles* (Schremmer 1949), *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Odontomyia* (Cook 1949), Therevidae (Cook 1949) and *Nannochorista* (Beutel et al. 2009). It is slightly exposed in *Androprosopa* and exposed at all in *Chaoborus* (Schremmer 1950a; Förster 2013), *Dasyhelea* (v. Lieven 1998), *Dicranota* (Cook 1949), *Tabanus* (Cook 1949), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937).

**107. Position of antennal insertion: (0) lateral; (1) close to midline.** The insertion is slightly shifted towards the midline in *Androprosopa* still closer to the lateral margin of the head. The antennae insert close to the midline in *Chaoborus* (Schremmer 1950; Förster 2013), *Corethrella* and *Dasyhelea* (v. Lieven 1998, fig. 6 [ant]). The antenna inserted laterally in all other taxa examined (Sharif 1937; Bierbrodt 1942; Anthon 1943a; Cook 1944a, b, 1949; Kramer 1954; Gouin 1959; Lindner 1959; Perraudin 1961; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; Courtney 1990b; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Dvash 2013).

**108. Antennal segmentation: (0) with basal antennomere and one or several distinctly developed distal segments; (1) appearing 1-segmented, distal segment vestigial or absent; (2) antenna vestigial.** The antenna of *Tipula* appears 1-segmented like in other larvae of Tipulidae, and a similar condition is found in Cylindrotomidae, Limoniinae (Selke 1936; Cook 1949; Peus 1952; Chiswell 1955; Neugart et al. 2009), Trichoceridae (Anthon 1943a), *Nymphomyia* (Schneeberg et al. 2012), Chironomidae (Cook 1944b; Gouin 1959), Culicidae (Cook 1944a; Schremmer 1949), *Androprosopa*, Chaoboridae (Schremmer 1950a; Förster 2013), *Corethrella*, *Dixa* (Felix 1962), *Dasyhelea* (v. Lieven 1998), *Paenobremia* (Solinas 1968), *Bittacomorpha* (Kramer 1954), Psychodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968), Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). The antenna of *Deuterophlebia* consists of a basal antennomere with a large bifurcated distal article (Courtney 1990b). It is composed of four segments in *Simulium* (Cook 1949) and

two-segmented in Anisopodidae (Anthon 1943a), *Dicranota* (Cook 1949), *Protanyderus* (Wipfler et al. 2012a), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). A three-segmented antenna is present in *Nannochorista* (Beutel et al. 2009). It is completely missing in Bibionidae (Cook 1949; Perraudin 1961), *Exechia* and *Axymyia* (Dvash 2013).

**109. Distal antennal segment: (0) simple; (1) bifurcate.** In *Anopheles* (Schremmer 1949, fig. 1) and *Corethrella* the antenna is simple with two thorns on the apex (coded as 0). The distal antennomere is bifurcate in *Deuterophlebia* (Courtney 1990b).

**110. Extrinsic antennal muscles: (0) present; (1) absent.** Extrinsic antennal muscles are lacking in Tipulomorpha (Cook 1949; Neugart et al. 2009), *Nymphomyia* (Schneeberg et al. 2012), Chironomidae (Cook 1944b), *Androprosopa*, *Paenobremia* (Solinas 1968), Therevidae (Cook 1949) and *Odontomyia* (Cook 1949). One extrinsic antennal muscle is present in other dipterans examined (Cook 1944a, 1949 [Antennal muscle]; Schremmer 1949; Schremmer 1950a [M. retractor antennae]; Kramer 1954 [antm]; Felix 1962 [mant]; Courtney 1990b [Mant]; Wipfler et al. 2012a; Förster 2013 [M5, M. tentorioscapalis posterior]) and also in Mecoptera (Beutel et al. 2009 [M1/2/3/4]; Bierbrodt 1942 [M.d.sc.]). Three extrinsic antennal muscles are present in *Nosopsyllus* (Sharif 1937 [d.a., l.a., f.a.]).

**111. Mandibulo-maxillary complex: (0) absent; (1) present.** A mandibulo-maxillary complex is absent in all members of lower Diptera (e.g., Anthon 1943a, b, 1988; Cook 1944a, 1949; Schremmer 1949; Schremmer 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Courtney 1990b; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013), in *Nannochorista* (Beutel et al. 2009), and *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). The presence (e.g., Cook 1949) is likely an autapomorphy of Brachycera (Anthon 1988). Among the taxa examined it was found in Therevidae (Cook 1949, fig. 31C), *Tabanus* (Cook 1949, fig. 27) and *Odontomyia* (Cook 1949, fig. 35A).

**112. Plane of operation of mandibles: (0) horizontal or slightly oblique; (1) distinctly oblique or vertical; (2) horizontal in first larval stage and oblique or vertical in later instars.** The plane of operation is horizontal in Tipulidae (e.g., *Tipula*, *Tanyptera*; Hennig 1973; Neugart et al. 2009), *Dicranota* (Cook 1949), *Trichocera* (Anthon 1943a), *Protanyderus* (Wipfler et al. 2012a), *Exechia*, *Axymyia* (Dvash 2013), *Paenobremia* (Solinas 1968), *Nannochorista* (Beutel et al. 2009) and *Panorpa* (Bierbrodt 1942). The axis of movement is slightly oblique in *Biblio* (Cook 1949; coded as 0), *Corethrella* and *Nosopsyllus* (Sharif 1937). The plane is distinctly oblique in *Dixa* (Felix 1962), *Simulium* (Cook 1949), *Anopheles* (Cook 1944a; Schremmer 1949), *Chaoborus* (Schremmer 1950a; Förster 2013), *Culiseta* (Cook 1944a) and *Tabanus* (Cook 1949). It is horizontal in some Chironomidae, whereas the plane is oblique or nearly vertical in other chironomid larvae (Gouin 1959), like in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Bittacomorpha* (Kramer 1954), *Dasyhelea* (v. Lieven 1998), *Androprosopa*, Anisopodidae (Anthon 1943a), Psychodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968), Therevidae (Cook 1949) and *Odontomyia* (Cook 1949).



**113. Position of secondary mandibular joint: (0) not shifted posterad the antennal foramen; (1) shifted posterad the antennal foramen.** The unusual position of the secondary mandibular joint is shifted posterad the antennal articulation in Tipulidae (Neugart et al. 2009). This condition is not found in other groups of Tipulomorpha (e.g., Cook 1949; Peus 1952). The mandibular joint is not shifted or only slightly shifted posteriorly in all other taxa examined (Sharif 1937; Bierbrodt 1942; Anthon 1943a; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; Courtney 1990b; Beutel et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**114. Shape of mandible: (0) without distinctly elongated distal part; (1) distal part elongated, sickle-shaped.** The mandible is sickle-shaped and elongated in *Dicranota* (Cook 1949). It is slender and moderately elongated in *Nannochorista* (Beutel et al. 2009, figs. 8A, B).

**115. Movable lacinia mobilis: (0) absent; (1) present.** An articulated lacinia mobilis is present in *Tipula* (Neugart et al. 2009), *Chaoborus* (Schremmer 1950a; Förster 2013, fig. 5C [lm]) and *Nannochorista* (Beutel et al. 2009, fig. 8A [lcm]).

**116. Anteriorly directed cone of mesal mandibular edge: (0) absent; (1) present.** The anteriorly directed cone is present in Anisopodidae (Anthon 1943a, figs. 26, 29 [kr.]), *Trichocera* (Hennig 1968a) and some Psychodidae (Hennig 1968a).

**117. Mandibular comb on dorsal surface: (0) absent; (1) present.** A mandibular comb is present in *Deuterophlebia* (Courtney 1990b), *Bittacomorpha* (Kramer 1954 [mdbl]), Chironomidae (Cook 1944b; Gouin 1959), Culicidae (Cook 1944a; Schremmer 1949), *Chaoborus* (Schremmer 1950a; Förster 2013), *Dixa* (Felix 1962), *Simulium* (Cook 1949) and Anisopodidae (Anthon 1943a).

**118. Apical multitoothed mandibular comb: (0) absent; (1) present (at least in instar 1).** An apical multitoothed mandibular comb is absent in most groups of lower Diptera (Anthon 1943a; Cook 1944a, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; v. Lieven 1998; Neugart et al. 2009; Wipfler et al. 2012a; Dvash 2013) and also in *Tabanus* (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). It is present in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Androprosopa* and some members of Blephariceridae (Oosterbroek & Courtney 1995).

**119. Subdivision of mandible: (0) absent; (1) present, distal part separated from basal part by a furrow; (2) present, distal part separated from basal part by weakly sclerotised zone.** A movable distal part of the mandible is present in *Bittacomorpha* (Kramer 1954) and Anisopodidae (Anthon 1943a). The mandible of *Tabanus* (Cook 1949), *Odontomyia* (Cook 1949) and Therevidae (Cook 1949) is subdivided into a distal and a basal part by a weakly sclerotised zone.

**120. Insertion of adductor tendon: (0) not or very slightly shifted anteriorly; (1) distinctly shifted anteriorly.** The mesal wall of the mandible is distinctly shortened in larvae of Tipulidae and Cylindrotomidae (Peus 1952), and this is probably a general feature in larvae of Tipulomorpha (Anthon 1943a, fig. 38; Oosterbroek & Theowald 1991, figs. 61, 128; Neugart et al. 2009, figs. 4A, B). It is also

distinctly shifted anteriorly in *Deuterophlebia* (Courtney 1990b, fig. 4 [M21]), *Bittacomorpha* (Kramer 1954, fig. 9 [mdf]), *Dixa* (Felix 1962, fig. 18 [tamdb]), *Simulium* (Cook 1949 [mandibular adductor muscle]), *Dasyhelea* (v. Lieven 1998), Anisopodidae (Anthon 1943a, figs. 26-31 [ap.ad.]), Psychodidae (Anthon 1943a), some Blephariceridae (Anthon & Lyneborg 1968), Therevidae (Cook 1949) and *Odontomyia* (Cook 1949 [mandibular adductor apodeme]) and in *Nosopsyllus* (Sharif 1937, fig. 6 [ad.a.]).

**121. M. tentoriomandibularis (M. 13): (0) present; (1) absent.** M. 13 is present even though extremely thin in *Nannochorista* (Beutel et al. 2009). It is absent in all other taxa examined (e.g., Sharif 1937; Bierbrodt 1942; Cook 1944a, 1949; Schremmer 1949, 1950; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Courtney 1990b; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**122. Cardo: (0) mainly membranous; (1) with a clearly delimited sclerotized area; (2) absent.** The cardo is absent or completely fused with the head capsule in *Nymphomyia* (Schneeberg et al. 2012), *Chironomus* (Cook 1944b), *Anopheles* (Cook 1944a), *Culiseta* (Cook 1944a), Psychodidae (Anthon 1943a, fig. 17, [car.] fused), and *Axymyia* (Dvash 2013). It is largely membranous in *Deuterophlebia* (Courtney 1990b).

**123. Endite lobes: (0) distinctly developed and separated; (1) partly fused, still recognisable as separate structures; (2) galea and stipes completely fused or absent.** Separate endite lobes are present in *Protanyderus* (Wipfler et al. 2012a), Anisopodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968, fig. 25 [lac, gal]), *Nannochorista* (Beutel et al. 2009) and they were described for *Tipula* species by Selke (1936, fig. 23) but are not recognisable in *Tipula montium* (Neugart et al. 2009, Fig. 4C). The endites are partly fused, but recognizable as separate structures in *Axymyia* (Dvash 2013) and *Panorpa* (Bierbrodt 1942). One maxillary endite is present in *Dasyhelea* (v. Lieven 1998 [lob]), *Deuterophlebia* (Courtney 1990b), *Tabanus* (Cook 1949) and *Nosopsyllus* (Sharif 1937). Both endites are absent or completely fused in a number of dipteran taxa examined (Karandikar 1931; Anthon 1943a; Cook 1944a, 1949; Schremmer 1949, 1950a; Gouin 1959; Lindner 1959; Perraudin 1961; Felix 1962; Solinas 1968; Schneeberg et al. 2012; Förster 2013).

**124. Number of maxillary palp segments: (0) 3 or more; (1) 2; (2) 1.** The maxillary palp is two-segmented in larvae of Bibionidae (Cook 1949; Perraudin 1961), *Chironomus* (Foote 1991), *Deuterophlebia* (Courtney 1990b), *Axymyia* (Dvash 2013), Therevidae (Cook 1949) and *Nosopsyllus* (Sharif 1937). It is three-segmented in *Nannochorista* (Beutel et al. 2009) and 5-segmented in *Panorpa* (Bierbrodt 1942). Palps are completely absent in *Exechia*. The palp is one-segmented in other dipterans examined (Anthon 1943a; Cook 1944a, 1949; Schremmer 1949, 1950a; Kramer 1954; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013).

**125. Length of single maxillary palpomere: (0) not elongated, about as long as wide; (1) distinctly elongated.** The maxillary palpomere is distinctly elongated in *Dicranota* (Cook 1949).

**126. M. craniocardinalis (M. 15): (0) well developed; (1) absent.** The muscle is present in *Nosopsyllus* (Sharif 1937 [ab.m.]). One maxillary muscle is present in *Exechia*, but it is not clear if it is homologous with *M. craniocardinalis*. Two maxillary muscles with unknown homology are present in *Androprosopa*. *M. 15* is absent in all other taxa examined (Bierbrodt 1942; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Courtney 1990b; v. Lieven 1998; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**127. M. tentoriocardinalis (M. 17) with origin from posterior tentorial arm: (0) present; (1) absent.** It originates on the ventrolateral cranium in *Deuterophlebia* (Courtney 1990b) and *Nymphomyia* (Schneeberg et al. 2012), and on the ventral wall of the head capsule in *Axymyia* (Dvash 2013). The origin lies on the anterior tentorial arm in *Nannochorista* (Beutel et al. 2009).

**128. M. tentoriostipitalis (M. 18) with origin from posterior tentorial arm: (0) present; (1) absent.** It originates on the ventral head capsule in *Axymyia* (Dvash 2013). The muscle is present with two subcomponents in *Panorpa* (Bierbrodt 1942). One component originates on the tentorium (M.add.st.1) and the other on the head capsule (M.add.st.2). In contrast, the entire muscle originates on the anterior tentorial arm in *Nannochorista* (Beutel et al. 2009). A maxillary muscle with unclear homology originates on the head capsule in *Nymphomyia* (Schneeberg et al. 2012). The maxillary endites are not recognizable as separate structures.

**129. Muscles of the endite lobes (Mm. 20/21): (0) 2; (1) 1; (2) absent.** Intrinsic maxillary muscles of the galea and lacinia (Mm. 20/21) are missing in dipteran larvae (e.g., Cook 1944a, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Hennig 1973; Courtney 1990b; v. Lieven 1998; Schneeberg et al. 2012; Wipfler et al. 2012a; Dvash 2013) and also in *Nannochorista* (Beutel et al. 2009). One muscle is present in *Panorpa* (Bierbrodt 1942 [M.prom.lac.]) and *Nosopsyllus* (Sharif 1937 [l.s.]).

**130. Anterior teeth of hypostomium: (0) absent; (1) present.** The hypostomium is equipped with triangular teeth on its anterior margin in *Nymphomyia* (Schneeberg et al. 2012), *Protanyderus* (Wipfler et al. 2012a [hp, fig. 1B]), *Tipula* (Neugart et al. 2009), *Limonia* (Oosterbroek & Theowald 1991), *Dicranota* (Cook 1949), *Anopheles* (Schremmer 1949), *Culiseta* (Cook 1944a), *Corethrella*, *Chironomus* (Cook 1944b), *Simulium* (Cook 1949) and Ptychopteridae (Neugart et al. 2009). The teeth are absent in *Deuterophlebia* (Courtney 1990b), *Trichocera* (Anthon 1943a), Bibionidae (Cook 1949; Perraudin 1961), *Exechia*, *Axymyia* (Dvash 2013), *Dasyhelea* (v. Lieven 1998), *Chaoborus* (Schremmer 1950; Förster 2013), *Androprosopa*, Therevidae (Cook 1949), *Tabanus* (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009) and *Panorpa* (Bierbrodt 1942).

**131. Median division of hypostomium: (0) absent; (1) partly divided; (2) completely divided.** The hypostomium or hypostomal plate is medially partly divided by an incomplete triangular zone of weakness in *Tipula* (Neugart et al. 2009) and *Limonia* (Lindner 1959, fig. 10 ["Hypostomium"]), and it is also partly divided in *Dixa* (Felix 1962, fig. 24, 25). It is completely divided in *Dicranota* (Cook 1949). An undivided

hypostomium is present in all other taxa examined (Anthon 1943a; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Courtney 1990b; Beutel et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013)

**132. Separate submentum: (0) present; (1) absent.** A separate submentum is present in *Chironomus* (Cook 1944b), *Simulium* (Cook 1949, fig. 9B), Therevidae (Cook 1949, fig. 29C), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949, fig. 32B). The submentum is present, but fused with the head capsule in *Panorpa* (Bierbrodt 1942, fig. 9 [Subm.]). It is present in *Olbiogaster* (Anisopodidae) (Anthon 1943b) and some Psychodidae (Oosterbroek & Courtney 1995). It is absent in all other taxa examined (Sharif 1937; Anthon 1943a; Cook 1944a, 1949; Schremmer 1949, 1950a; Kramer 1954; Perraudin 1961; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; Courtney 1990b; v. Lieven 1998; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**133. Hypostomal plate: (0) fused with elements of the head capsule; (1) present as a separate structure.** It is present as a separate structure in *Protanyderus* (Wipfler et al. 2012a), *Biblio* (Cook 1949; Perraudin 1961) and *Bittacomorpha* (Kramer 1954, fig. 6 [hst]).

**134. Premental teeth: (0) present; (1) indistinct or absent.** The prementum bears five teeth at its anterior margin in *Tipula* (Neugart et al. 2009), *Anopheles* (Schremmer 1949, fig. 9b), *Culiseta* (Cook 1944a), *Nymphomyia* (Schneeberg et al. 2012), Chironomidae (Cook 1944b; Gouin 1959), *Androprosopa*, *Trichocera* (Anthon 1943a) and Therevidae (Cook 1949). Two teeth are present in *Tabanus* (Cook 1949). Premental teeth are absent in *Deuterophlebia* (Courtney 1990b), *Protanyderus* (Wipfler et al. 2012a), *Biblio* (Cook 1949; Perraudin 1961), *Axymyia* (Dvash 2013), Anisopodidae (Anthon 1943a, b), Psychodidae (Anthon 1943a), Blephariceridae (Anthon & Lyneborg 1968), *Dicranota* (Cook 1949), *Chaoborus* (Schremmer 1950; Förster 2013), *Corethrella*, *Dixa* (Felix 1962), *Dasyhelea* (v. Lieven 1998), *Simulium* (Cook 1949), *Odontomyia* (Cook 1949), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937).

**135. Labial palp segments: (0) more than one; (1) one, palp more or less vestigial.**

Labial palps are completely absent in larvae of *Nymphomyia* (Schneeberg et al. 2012), Tipulomorpha (Cook 1949; Neugart et al. 2009), Trichoceridae (Anthon 1943a), Chironomidae (Cook 1944b; Gouin 1959), Culicidae (Cook 1944a; Schremmer 1949), *Androprosopa*, *Dasyhelea* (v. Lieven 1998), *Deuterophlebia* (Courtney 1990b), *Protanyderus* (Wipfler et al. 2012a), *Biblio* (Cook 1949; Perraudin 1961), *Exechia*, *Axymyia* (Dvash 2013), *Chaoborus* (Schremmer 1950; Förster 2013), *Corethrella*, *Dixa* (Felix 1962), Therevidae (Cook 1949) and *Odontomyia* (Cook 1949). They are more or less vestigial in Psychodidae (Anthon 1943a) and *Paenobremia* (Solinas 1968). According to Kramer (1954) the labial palps in *Bittacomorpha* are greatly reduced and possibly represented by paired apical projections (lbp, Kramer 1954). The prementum bears two small lobes in *Simulium* (Cook 1949), which are vestiges of palps. The palps are one-segmented in Anisopodidae (Anthon 1943a, fig. 19 [la.p.]; Anthon 1943b, fig. 7 [la.p.]), Blephariceridae (Anthon & Lyneborg 1968, fig. 26 [lb.p.]), *Tabanus* (Cook 1949), *Nannochorista*

(Beutel et al. 2009) and *Nosopsyllus* (Sharif 1937, fig. 8 [l.b.]). Three-segmented palps are present in *Panorpa* (Bierbrodt 1942, fig. 9 [P.l.b.]).

**136. M. submentopraementalis (M. 28): (0) present; (1) absent.** M. 28 is absent in all examined antliophoran larvae (Sharif 1937; Bierbrodt 1942; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Courtney 1990b; v. Lieven 1998; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**137. Tentorio-premental muscles (Mm. 29/30): (0) 2; (1) 1; (2) extrinsic labial muscles are absent.** One extrinsic labial muscle (M. 29) is present in *Deuterophlebia* (Courtney 1990b [Mlm]), *Nymphomyia* (Schneeberg et al. 2012 [pr]), *Protanyderus* (Wipfler et al. 2012a), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949 [labial muscle]), *Biblio* (Cook 1949; Perraudin 1961 [rhy]), *Paenobremia* (Solinas 1968 [MML]), *Bittacomorpha* (Kramer 1954 [lbn]), *Dixa* (Felix 1962 [mrtlb]), *Dasyhelea* (v. Lieven 1998 [78]), *Simulium* (Cook 1949 [labial adductor muscle]), *Anopheles* (Schremmer 1949 [Fl.hph]), *Culiseta* (Cook 1944a [labial adductor muscle]), *Chaoborus* (Schremmer 1950a [M. adductorlabii]; Förster 2013 [M13, M. tentoriopraementalis inferior/superior]), *Chironomus* (Cook 1944b), *Odontomyia* (Cook 1949 [labial muscle]) and *Nosopsyllus* (Sharif 1937 [p.l.a.]). Extrinsic labial muscles are completely absent in *Exechia*, *Axymyia* (Dvash 2013), *Androprosopa*, *Corethrella*, Therevidae (Cook 1949), *Tabanus* (Cook 1949), *Nannochorista* (Beutel et al. 2009) and *Panorpa* (Bierbrodt 1942).

**138. M. praementopalpalis internus (M. 34): (0) present; (1) absent.** M. 34 is absent in all taxa examined (Sharif 1937; Bierbrodt 1942; Cook 1944a, b, 1949; Schremmer 1949, 1950a; Kramer 1954; Gouin 1959; Perraudin 1961; Felix 1962; Solinas 1968; Courtney 1990b; v. Lieven 1998; Beutel et al. 2009; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013).

**139. M. hypopharyngosalivariialis (M. 37): (0) present; (1) absent.** M. 37 is absent in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Biblio* (Cook 1949; Perraudin 1961), *Paenobremia* (Solinas 1968), *Dicranota* (Cook 1949), *Anopheles* (Schremmer 1949), *Culiseta* (Cook 1944a), *Chaoborus* (Schremmer 1950a; Förster 2013), *Dixa* (Felix 1962), *Dasyhelea* (v. Lieven 1998) and *Simulium* (Cook 1949).

**140. M. tentoriohypopharyngalis (M. 42): (0) present; (1) absent.** The muscle is generally absent in Diptera (e.g., Cook 1949; Schremmer 1949, 1950a; Kramer 1954; Perraudin 1961; Felix 1962; Solinas 1968; Denis & Bitsch 1973; Courtney 1990b; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013) and also missing in *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). It is present in *Nannochorista* (Beutel et al. 2009, fig. 11 [42]).

**141. Pharyngeal filter: (0) present; (1) absent.** A pharyngeal filter is absent in larvae of *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Tipula* (Neugart et al. 2009), *Limonia* (Oosterbroek & Courtney 1995), *Dicranota* (Cook 1949), *Biblio* (Perraudin 1961), *Paenobremia* (Solinas 1968), *Dasyhelea* (v. Lieven 1998), *Simulium* (Cook 1949), Chironomidae (Cook 1944b; Gouin 1959), *Androprosopa*, Anisopodidae (Anthon 1943a), *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937). It is present in Culicidae (Cook 1944a; Schremmer 1949),



Chaoboridae (Förster 2013), *Bittacomorpha* (Kramer 1954), *Trichocera* (Oosterbroek & Courtney 1995), *Protanyderus* (Wipfler et al. 2012a), Blephariceridae (Anthon & Lyneborg 1968), and also in Psychodinae (absent in other Psychodidae; Oosterbroek & Courtney 1995). It is also present and very strongly developed in *Axymyia* (Dvash 2013).

**142. M. tentoriobuccalis anterior (M. 48): (0) present; (1) absent.** M. 48 is absent in *Deuterophlebia* (Courtney 1990b), *Nymphomyia* (Schneeberg et al. 2012), *Protanyderus* (Wipfler et al. 2012a), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949), *Anopheles* (Schremmer 1949), *Culiseta* (Cook 1944), *Chaoborus* (Schremmer 1950a; Förster 2013), *Dixa* (Felix 1962), *Dasyhelea* (v. Lieven 1998), *Simulium* (Cook 1949), *Exechia*, Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949).

**143. M. verticopharyngalis (M. 51): (0) present; (1) absent.** M. 51 is absent in *Nymphomyia* (Schneeberg et al. 2012), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949), *Simulium* (Cook 1949), *Culiseta* (Cook 1944a), *Corethrella*, *Androprosopa*, *Exechia*, Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949).

**144. Posterior ventral pharyngeal dilators: (0) anterior and posterior subcomponent (M. 50 and M. 52): (1) one bundle; (2) absent.** A posterior pharyngeal pump is absent in *Nymphomyia* (Schneeberg et al. 2012), *Tipula* (Neugart et al. 2009), *Dasyhelea* (v. Lieven 1998), *Dicranota* (Cook 1949), *Exechia*, Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). One muscle bundle is present in *Deuterophlebia* (Courtney 1990b [M87-90]), *Biblio* (Perraudin 1961 [dvphy]), *Simulium* (Cook 1949 [ventral pharyngeal muscle]), *Androprosopa*, *Corethrella*, *Culiseta* (Cook 1944a [ventral pharyngeal muscle]) and *Axymyia* (Dvash 2013). Both muscles are present in *Bittacomorpha* (Kramer 1954 [phd3, vphm3]), *Paenobremia* (Solinas 1968 [MDP]), *Protanyderus* (Wipfler et al. 2012a), *Dixa* (Felix 1962 [dvphy2]), *Anopheles* (Schremmer 1949 [v.Dil. and M.51 not mentioned in text but shown in illustrations]), *Chaoborus* (Schremmer 1950; Förster 2013), *Nannochorista* (Beutel et al. 2009, fig. 11 [50, 52]), *Panorpa* (Bierbrodt 1942 [M.tent.phar.sup. und inf., M.gul.phar.]) and *Nosopsyllus* (Sharif 1937 [v.d.a., v.d.p.]).

**145. Position of brain: (0) completely or largely within head capsule; (1) partly shifted to thorax; (2) completely shifted to thorax.** The brain is completely shifted into the prothorax in *Nymphomyia* (Schneeberg et al. 2012), *Tipula* (Neugart et al. 2009), *Dicranota* (Cook 1949), *Axymyia* (Dvash 2013), *Dixa* (Felix 1962), *Androprosopa*, Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). It is partly located within the prothorax in Chironomidae (Cook 1949; Gouin 1959), *Simulium* (Neugart et al. 2009), *Biblio* (Neugart et al. 2009), *Exechia* and *Protanyderus* (Wipfler et al. 2012a). The plesiomorphic position within the head capsule is preserved in *Deuterophlebia* (Courtney 1990b), *Bittacomorpha* (Kramer 1954), Culicidae (Cook 1944a), *Chaoborus* (Förster 2013 [b, 6B]), *Corethrella*, *Nannochorista* (Beutel et al. 2009, fig. 11A [br, sog]), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937, fig. 9 [br., s.g.]).

**146. Cibariopharyngeal sclerotisation: (0) absent; (1) present.** A structural unit with a trough-like sclerotised ventral wall is formed by the cibarium and pharynx in the brachyceran groups examined by Cook (1949), i.e. in Therevidae (Cook 1949), *Tabanus* (Cook 1949) and *Odontomyia* (Cook 1949). This

condition is generally absent in larvae of lower Diptera (e.g., Cook 1944a, 1949; Kramer 1954; Perraudin 1961; Denis & Bitsch 1973; Courtney 1990b; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a; Förster 2013; Dvash 2013) and also in *Nannochorista* (Beutel et al. 2009), *Panorpa* (Bierbrodt 1942) and *Nosopsyllus* (Sharif 1937).

## 5 Discussion

### 5.1 Phylogeny

#### 5.1.1 The phylogenetic potential of characters of the head of larvae and adults

The analysis of adult and larval head structures revealed a considerable number of potential autapomorphies of Diptera, but the relationships of the basal lineages, which were the target of the cladistic character evaluations, were not well resolved, especially using only the characters of the adult head (Fig. 3C). The consistency index of trees based on the separated character set is 0.25 and the retention index is 0.48; in the complete analyses the consistency index is 0.33 and the retention index 0.29 (Fig. 3A). In the trees based on the larval head structures the consistency index is 0.26 and the retention index 0.21 (Fig. 3B). Commonly accepted clades were not recovered in the analyses of adult head structures and Diptera was rendered paraphyletic in this analysis, as Nannochoristidae was nested within the order (Fig. 3C). In the analyses of larval head structures Diptera is monophyletic and also Brachycera and Tipuloidea. The same clades are confirmed in the combined analyses.

Characters of the adult head are not only relatively conservative within the order, but also greatly affected by homoplasy, especially parallel losses (e.g., antennal segments, mandibles, muscles). This may be due to the relatively similar life style of adult dipterans belonging to different lineages. Apparently adult head structures alone are unsuitable for reconstructing the intraordinal relationships of Diptera, even though dramatic character transformation must have taken place in the dipteran stem group (e.g., modifications of the mouthparts), and some very characteristic features evolved within the group (e.g., pseudotracheae, aristate antennae in Brachycera). The larvae differ much more strongly in their habitats, general life style and feedings habits, and not surprisingly also in their head structures. The absence of the thoracic legs is arguably a key feature of dipteran larvae and no variation occurs within the group in this feature. In contrast to that, cephalic structures can vary dramatically, reaching from well-developed heads with functioning complex mouthparts, antennae composed of several segments, distinct light sense organs, endoskeletal structures and a complex muscle system, to strongly retracted simplified heads (e.g., Tipulomorpha; Neugart et al. 2009) or largely reduced and highly specialized head structures as they are characteristic for most groups of Brachycera (Cyclorrhapha; e.g., Wipfler et al. 2013). Despite of a strong variation within the group, characters of the larval head, like those of the adults, are not sufficient for resolving basal dipteran relationships. Analyses of larval feature are also strongly affected by homoplasy, especially parallel reductions, which affected sense organs, mouthparts, the external head structures, the endoskeleton, and also the muscle system.

Resolving the relationships of the dipteran lineages using morphology is likely impeded by episodic bursts of rapid radiation in the early Mesozoic (Rabosky 2010; Wiegmann et al. 2011), lower Diptera in the Triassic (220 Ma), lower Brachycera in the early Jurassic (180 Ma) and Schizophora in the early Paleocene (65 Ma).

### 5.1.2 Phylogenetic implications of the examined characters

#### Diptera

Diptera is monophyletic in the analyses of the complete data set and also in the analyses of characters of the larval head (Figs. 3A, B), but not in the analyses of adult head features (Fig. 3C). Potential autapomorphies are the dense vestiture of microtrichia on the head, the reduction of the postmentum, the two-segmented labial palps transformed into labellae (equipped with simple furrows), and the presence of a clypeolabral muscle.

A groundplan apomorphy of the larval head is a labrum equipped with a dense field of hairs. The presence of a larval hypostoma (= hypostomal bridge) with triangular teeth is most likely a groundplan apomorphy of Diptera, a condition found in most groups of lower Diptera.

#### Antliophora

Although the name of this clade refers to the presence of a sperm pump (“pump bearers”), this feature is not an autapomorphy of Antliophora (Hünefeld & Beutel 2005; Mickoleit 2008). Nevertheless, the monophyly is well supported by morphological and molecular data (Beutel & Baum 2008; Beutel et al. 2008a; Wiegmann et al. 2009; Friedrich & Beutel 2010a; McKenna & Farrell 2010; Ishiwata et al. 2011). A potential apomorphy is the distinct reduction of the dorsal tentorial arm in the adults (see below). A thin sclerotized structure is present in *Caurinus* (Boreidae; Beutel et al. 2008a), *Merope* (Meropidae; Friedrich et al. 2013a) and *Nannochorista* (Nannochoristidae; Beutel & Baum 2008), but these are apparently vestiges compared to the dorsal arms in other groups (e.g., Neuroptera; Zimmermann et al. 2011). The dorsal arms are completely absent in *Ctenocephalus* (Siphonaptera; Wenk 1953). A second potential antliophoran apomorphy is the postgenal bridge. A completely sclerotized postgenal region of the head is present in mecopterans (Otanés 1922; Heddergott 1938; Hepburn 1969; Beutel et al. 2008a; Friedrich et al. 2013a) with the exception of *Nannochorista* (Beutel & Baum 2008), and also in *Ctenocephalus* (Wenk 1953). It is conceivable that the bridge is also present in the groundplan of Diptera (see below). A postgenal bridge is also present in few groups of Neuroptera (e.g., Ferris 1940) and Hymenoptera (Siricidae, Orussidae, Apocrita; Beutel & Vilhelmsen 2007), but since it is absent in most groups of the former group and in the sister group of all the remaining hymenopteran lineages (Xyelidae; Beutel & Vilhelmsen 2007), it is very likely absent in the groundplan of both orders. Other apomorphic characters of Antliophora are the reduction of *M. craniocardinalis* (M. 15) and the presence of only one premental retractor. *M. craniocardinalis* is absent in *Ctenocephalus* and all dipterans and mecopterans examined (Thompson 1905; Heddergott 1938; Bonhag 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg & Beutel 2011; Schneeberg et al. 2011; Friedrich et al. 2013a) and most likely missing in the antliophoran groundplan. Only one premental retractor is preserved in the groundplan of Diptera (see below) and also

in Mecoptera (Heddergott 1938; Hepburn 1969; Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a), whereas none is preserved in Siphonaptera (Wenk 1953; Michelsen 1996a).

The presence of only one premental retractor is also a larval groundplan feature of Antliophora (e.g., Sharif 1937; Cook 1949; Solinas 1968; Courtney 1990b; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a). As in adults it is either *M. tentoriopraementalis* inferior, *M. tentoriopraementalis* superior (Mm. 29/30), or a product of fusion of both. *M. submentopraementalis* (M. 28) is completely absent. Premental retractors are completely missing in mecopteran larvae (*Panorpa*, *Nannochorista*; Bierbrodt 1942; Beutel et al. 2009). In Amphiesmenoptera two premental retractor muscles are present (see Hasenfuss & Kristensen 2003) and three in larvae of most groups of Coleoptera (e.g., Beutel 1993). *M. praementopalpalis* (M. 34) is absent in larvae in the groundplan of Antliophora as it is also the case in strepsipteran larvae which lack labial palps (Pohl 2000), whereas the muscle occurs in other groups of Holometabola (e.g., Coleoptera, Neuroptera, Megaloptera; Das 1937; Röber 1942; Wundt 1961; Beutel 1993, 1995; Beutel et al. 2010a).

### **Diptera + Nannochoristidae**

The analysis of cephalic features (larval head and complete data set) suggests a sistergroup relationship between Diptera and Nannochoristidae as it was suggested earlier (e.g., Beutel & Baum 2008; Beutel et al. 2009) (see fig. 1H). Characters of the adult head supporting this hypothesis are the frontal apodeme, the presence of a sensorial field on the third maxillary palpomere and scales on the labial palps. However, the presence of a frontal apodeme in the groundplan of Diptera is questionable (see below). A sensorial field is present in most lineages of lower Diptera, but the pattern differs distinctly in these groups. In some cases the sensilla are placed together in a large groove (e.g., Bibionidae, Ceratopogonidae, Blephariceridae; Blackwell 2004), like in Nannochoristidae (Beutel & Baum 2008). In other groups each sensillum is located in an individual groove (e.g., Tipulidae, Corethrellidae; Schneeberg & Beutel 2011) or they are exposed on the surface of the palpomere (Limoniidae, Trichoceridae, Ptychopteridae; Schneeberg & Beutel 2011). The presence of scales on the labial palps is hardly convincing as a synapomorphy as these surface structures are almost generally missing in Diptera, with the exception of some Culicidae (e.g., Williams 1933; Bonhag 1951; Schiemenz 1957; Wenk 1962; Sutcliffe 1985; Harbach & Kitching 1998; Szucsich & Krenn 2000; Blackwell 2004; Schneeberg & Beutel 2011). Moreover, the scales are present on the mesal side of the labial palps in *Nannochorista* (Beutel & Baum 2008), but on the external surface in Culicidae (Harbach & Kitching 1998).

In the dipteran groundplan intrinsic muscles of the larval maxillae (Mm. 20/21) are missing (see below). This is also the case in Nannochoristidae (Beutel et al. 2009), whereas one muscle is present in Panorpidae and Siphonaptera (Sharif 1937; Bierbrodt 1942). In the groundplan of dipteran larvae the hypostomal plate is fused with elements of the head capsule. This is also the case in *Nannochorista* and arguably synapomorphic. One-segmented labial palps (e.g., Anthon 1943a; Cook 1944a, 1949; Beutel et al. 2009; Schneeberg et al. 2012) are also a potential synapomorphy.



### Deuterophlebiidae + Nymphomyiidae

Deuterophlebiidae and Nymphomyiidae are sistergroups according to Cutten & Kevan (1970) and this is also suggested by the analyses of the data presented here (adult head and complete data set) (Figs. 3A, C). Cutten & Kevan (1970) suggested the presence of lateral eyespots of older larvae, the anteriorly projecting larval rostrum, the presence of abdominal prolegs, and a pair of appendages on the larval abdomen as potential synapomorphies. The studies of Schneeberg et al. (2011) and Schneeberg et al. (2012) also suggested phylogenetic affinities between the two groups.

Both families share a large number of derived features of the adult head. In both groups the labrum, maxillae, and labium are completely missing including their extrinsic and intrinsic muscles. *M. tentoriobuccalis anterior* (M. 48) is completely reduced and the terminal antennal segment is distinctly elongated (Schneeberg et al. 2011).

Larval features supporting this hypothesis are the presence of rows of spatulate macrosetae on the ventral surface of the labrum-epipharynx (only in later-instar larvae in Deuterophlebiidae) (Courtney 1991a; Schneeberg et al. 2012). The labrum is completely fused with the head capsule in the larvae, accompanied by the loss of *M. labroepipharyngalis* (M. 7) and *M. frontolabralis* (M. 8), a condition also occurring in some groups of Brachycera, and in Cecidomyiidae and Simuliidae (Anthon 1943a; Cook 1949; Solinas 1968; Courtney 1990b). Another potential synapomorphy is the apical multitoothed mandibular comb. The comb is also present in larvae of Thaumaleidae and some Blephariceridae (Oosterbroek & Courtney 1995), which is likely the result of parallel evolution.

Other similarities concerning the morphology and life history were pointed out by Courtney (1991a). The females shed their wings after or during oviposition, the femora and tibiae are subdivided by a membranous zone, and all abdominal spiracles and the spermatheca are reduced. Courtney (1991a) interpreted all shared similarities as convergences, related to the short live span of adults. This interpretation is supported by the comprehensive molecular study of Wiegmann et al. (2011), where a clade Nymphomyiidae + Deuterophlebiidae was not confirmed (see below) (Fig. 2O).

Deuterophlebiidae and Nymphomyiidae are both highly specialized in their morphology and lifestyle and doubtlessly monophyletic (Courtney 1991a; Schneeberg et al. 2011). Autapomorphic features of Deuterophlebiidae are prolegs with curved crochets with five teeth, five anal papillae, malpighian tubules terminating in anal papillae, a pre-hardened pupal cuticle, an ultimate antennal flagellum extremely elongated in adult males, a male pretarsus densely set with capitate microtrichia, and reduced tarsal claws (Courtney 1990b, 1991b). Apomorphies of the head are the widely separated antennal insertions, an *M. tentorioscapalis anterior* (M. 1) with multiple areas of origin, and the absence of *M. hypopharyngosalivarialis* (M. 37) (Schneeberg et al. 2011). Possible larval apomorphies are the growth lines on the externolateral plates (see also Courtney 1991b), the fusion of clypeus and frons, the bifurcate distal antennal segment, and the largely membranous cardo.

Autapomorphies of Nymphomyiidae are the dimorphic larval crochets, malpighian tubules originating on a common stalk on the ventral gut surface, and the prognathous head of the pupa. The narrow wings

are highly derived and shed after flight or during oviposition. They are characterized by a marginal fringe of elongate macrotrichia, a poorly developed anal lobe, reduced posterior veins, and anterior veins concentrated along the costal margin. Other potential autapomorphies suggested by Courtney (1991b) are the neotenic adults, an anterolateral angle of abdominal tergite VIII equipped with prominent tubercles, subdivided femora and tibiae, missing abdominal spiracles and the reduced spermatheca.

Characters of the adult head are the ventrally contiguous compound eyes, the loss of the unpaired ocellus, the shift of the paired ocelli posterior to the compound eyes, the completely sclerotized rostrum, the club-shaped antenna and the strongly enlarged first antennal flagellomere (Tokunaga 1935).

### Tipuloidea

Tipuloidea (= Tipulomorpha, excl. Trichoceridae) was consistently confirmed as monophyletic in previous studies, but a close relationship with Trichoceridae was discussed controversially. Wood & Borkent (1989) suggested an inclusion of Trichoceridae in Psychodomorpha mainly based on larval characters (Fig. 2E). Tipulomorpha (including Trichoceridae) was also rejected as a clade in some morphological studies (e.g., Sæther 2000; Sinclair et al. 2007; Lambkin et al. 2013) (Figs. 2H, L). In contrast, it was supported in a considerable number of older and more recent studies, based on morphological characters of larvae and adults (including or excluding extinct taxa) (Hennig 1973, 1981; Dahl 1980; Hackmann & Väisänen 1982; Oosterbroek & Courtney 1995; Shcherbakov et al. 1995; Blagoderov et al. 2007; Sinclair et al. 2013) (Figs. 2B, D, G, J, K) or molecular data (Bertone et al. 2008; Wiegmann et al. 2011) (Figs. 2N, O). This concept was also supported in a recent study focused on dipteran attachment structures (Friedemann et al. 2014). The analyses suggest that an arolium was secondarily acquired in Tipulomorpha inclusive Trichoceridae. It is missing in all other groups of Diptera.

Tipuloidea is supported in the complete analyses and in the analyses of larval head structures (Figs. 3A, B), and Trichoceridae is not the sister group of this clade. An apomorphic character of Tipuloidea is the partial fusion of the stipites in adults. This groundplan feature is found in *Pedicia* and *Limonia*, whereas the stipites are completely fused in Tipulidae (Schneeberg & Beutel 2011). A partial fusion also occurs in Sciaridae and Scatopsidae, apparently the result of parallel evolution. Other shared derived characters of Tipuloidea are the absence of M. craniolacinalis (M. 19) and the presence of only one muscle of the precerebral pharyngeal pump, but both characters also occur in several other dipteran groups (Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011). The anterior tentorial arm is a thick hollow tube in the dipteran groundplan, as it is the case in most species examined. The anterior arm is massive in most groups of Tipuloidea (entire anterior arm in *Pedicia* and *Cylindrotoma*, anterior part in *Limonia*; see Schneeberg & Beutel 2011). Similar conditions have evolved independently in Syrphidae and some members of Bibionomorpha.

The head capsule of tipuloid larvae is separated from the dorsomedian fragment by paired dorsolateral incisions (Selke 1936; Cook 1949; Oosterbroek & Theowald 1991; Neugart et al. 2009). This is another potential autapomorphy of the group. A similar condition is present in *Liponeura*, but is absent

in other members of Blephariceridae (Anthon & Lyneborg 1968). Another apomorphic character is the presence of growth lines (zonal structures as intermold cuticle depositions) on the extensive externolateral plates (Hennig 1968b; Oosterbroek & Theowald 1991; Neugart et al. 2009). A similar condition has probably evolved independently in larvae of Deuterophlebiidae, Blephariceridae and Tanyderidae (Courtney 1990b; Oosterbroek & Courtney 1995; Wipfler et al. 2012a). The hypostoma of tipuloid larvae is partly (Pediciidae; Cook 1949) or completely divided (Limoniidae, Tipulidae; Lindner 1959; Neugart et al. 2009). It is undivided in the dipteran groundplan, as it is the case in almost all larvae of Diptera examined (with the exception of Dixidae; Felix 1962). The partial division is likely a transition state between the undivided hypostoma in the groundplan of the order and the complete division in Tipulidae and Limoniidae.

### **Brachycera**

Brachycera turn out as monophyletic in the analyses of the complete data set (Fig. 3A), and also using only larval features (Fig. 3B). Surprisingly it is not supported in analyses of characters of the adult head (Fig. 3C). The monophyly of Brachycera has never been questioned. They are supported by a broad spectrum of morphological characters of adults and larvae (e.g., thoracic or genital morphology) (e.g., Hennig 1973; Oosterbroek & Courtney 1995; Krzemiński & Krzemińska 2003; Blagoderov et al. 2007; Lambkin et al. 2013) and also by molecular data (Friedrich & Tautz 1997; Bertone et al. 2008; Wiegmann et al. 2011).

The presence of only 7 antennomeres is likely a derived groundplan feature of Brachycera. The number of antennomeres is also reduced in *Deuterophlebia* (Schneeberg et al. 2011) and *Nymphomyia* (Tokunaga 1935), but in both cases linked with a strong elongation of the distal segment. The reduction of the number of the maxillary palpomeres is also an autapomorphy of Brachycera. The palps are 2- or 1-segmented in the examined species. Hesse (1938, 1956) described 3-segmented palps for Bombyliidae, but this is probably due to reversal. A reduction of palpomeres is rare in the lower dipteran groups. It occurs in some Culicidae, Cecidomyiidae and Sciaridae (Harbach & Kitching 1998; Schneeberg et al. 2013a), very likely as a result of parallel evolution. Another apomorphic condition is the presence of pseudotracheae on the inner surface of the labellae of Brachycera, but these structural modifications also occur in some groups of lower Diptera (Tipulidae, Ptychopteridae, some Mycetophilidae; Hoyt 1952; Schneeberg & Beutel 2011).

An apomorphic character of brachyceran larvae is the mandibulo-maxillary complex, which is absent in all members of lower Diptera examined (e.g., Cook 1949; Anthon 1988). The mandible of the larvae is subdivided into a distal and a basal part by a weakly sclerotized zone in all brachyceran larvae examined. This is a potential autapomorphy, even though a subdivision with a membranous zone is also present in larvae of Anisopodidae and Ptychopteridae, and both parts are separated by a furrow in ptychopteran larvae (Anthon 1943a; Kramer 1954). Another larval autapomorphy is the presence of a complex structural unit with a trough-like, sclerotized ventral wall formed by the cibarium and pharynx (Cook 1949). This

condition does not occur in lower Diptera (Cook 1944a, b, 1949; Kramer 1954; Perraudin 1961; Denis & Bitsch 1973; Courtney 1990a; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a).

## 5.2 Character transformations

Wiegmann et al. (2011) presented a robust phylogenetic tree based on molecular data from species of 149 families, including 30 kb from 14 nuclear loci and the complete mitochondrial genomes. This tree was used to develop an evolutionary scenario for adult and larval head structures.

### 5.2.1 Adult head

#### Head capsule

The head capsule is completely covered with microtrichia (apomorphy) in the dipteran groundplan. This condition is found in almost all dipteran taxa examined (e.g., Tokunaga 1935; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b), with the exception of *Biblio* (Bibionidae) and *Pachygaster* (Stratiomyiidae). The characteristic vestiture is also missing in other groups of Bibionidae (Duda 1930), apparently due to secondary loss. In both families the microtrichia are replaced by longer setae. Adults of species of both groups visit flowers and a possible function of the longer setae on the head and other body parts is to keep the pollen away from the cuticular surface (Hardy 1981; James 1981). In *Nannochorista* the head capsule is also covered with very short and fine hairs (Beutel & Baum 2008), but this condition differs strongly from what is usually found in Dipterans. Microtrichia are absent in other groups of Mecoptera (Beutel et al. 2008a; Friedrich et al. 2013a) and also in Siphonaptera (Wenk 1953). A dense vestiture of microtrichia on the head capsule has evolved independently in Strepsiptera (Beutel & Pohl 2006) and some members of Heteroptera (e.g., Swart & Felgenhauer 2003; Weirauch 2012). The microtrichia are the only cephalic character shared by Diptera and Strepsiptera identified in the present study. This is certainly the result of convergency, as both groups are not closely related. In contrast to studies based on ribosomal genes (e.g., Wheeler et al. 2001: Halteria-hypothesis, see fig. 1D) Strepsiptera are the sistergroup of Coleoptera and both are nested within a more inclusive group Neuropteroidea (Wiegmann et al. 2009; Beutel et al. 2011; Niehuis et al. 2012; Peters et al. 2014). It is likely that the resulting surface properties improve the flight performance as it is the case in Strepsiptera (H. Pohl, pers. comm.).

The adult head is orthognathous in the dipteran groundplan and in most taxa examined, whereas it is prognathous in Tipulomorpha (Schneeberg & Beutel 2011), Nymphomyiomorpha (Tokunaga 1935), Ptychopteromorpha, and most members of Culicomorpha (partim, not in Simuliidae and Ceratopogonidae) (Peterson 1916). The head of Mecoptera (with the exception of *Nannochorista*) (Beutel & Baum 2008; Beutel et al. 2008a; Friedrich et al. 2013a) and Siphonaptera (Wenk 1953) is orthognathous, indicating that this is a groundplan feature of Antliophora. Prognathism is possibly partly linked with the development

of a rostrum. It is present in members of Tipulomorpha, Nymphomyiomorpha and some Culicomorpha, but not in Ptychopteromorpha.

Whether a rostrum is present in the groundplan of Diptera is ambiguous as it is missing in Deuterophlebiidae and many other groups of lower Diptera. If present it is usually only sclerotized on the dorsal side in the lower dipteran families, arguably a plesiomorphic condition. A completely sclerotized rostrum has evolved in Nymphomyiidae and in the tipulomorph clade *Cylindrotomidae* + *Tipulidae*, and in contrast a completely membranous rostrum in *Anisopodidae* and independently in *Brachycera*. The membranous condition is a characteristic and probably autapomorphic feature of the latter groups. The membranous rostrum is probably an adaptation to feeding on nectar and other liquid substances (e.g., Malloch 1917; Krenn et al. 2005).

In the groundplan and all examined species of *Brachycera* the clypeus and labrum are separated by the rostral membrane (haustellum). It ensures a high degree of movability, which facilitates flower-probing movements of the rostrum and its retraction in a resting position (Krenn et al. 2005). A separation of the sclerites by a membrane also occurs in some groups of lower Diptera, i.e. in *Tipulomorpha*, *Tanyderidae*, *Ptychopteridae*, *Bibionidae*, *Mycetophilidae* and *Simuliidae* (Williams 1933; Wenk 1962; Schneeberg & Beutel 2011), arguably a result of parallel evolution. Some of the species in question also feed on plant sap (e.g., Oosterbroek 2006) but the available data are insufficient for a reliable interpretation.

A frontoclypeal suture is part of the dipteran groundplan. It is usually present in the lower dipteran lineages and in *Brachycera* (Ferris 1950; Bonhag 1951; Schiemenz 1957; Szucsich & Krenn 2000) but was apparently reduced independently in several groups (e.g., *Deuterophlebiidae*, *Nymphomyiidae*, *Ptychopteridae*, *Blephariceridae*; Tokunaga 1935; Schneeberg et al. 2011). A unique apomorphic condition is found in *Culicidae*, a joint between the clypeus and frons (Schiemenz 1957; Christophers 1960; Hennig 1973). This probably facilitates the insertion of the mouthparts prior to the blood sucking process.

The presence of a coronal suture is plesiomorphic for *Diptera* and *Brachycera*, but it is absent in most groups (preserved in *Culicidae*, *Chironomidae*, *Nymphomyiidae*, *Drosophilidae*; Peterson 1916; Tokunaga 1935; Ferris 1950; Schiemenz 1957; Christophers 1960; Owen 1985). Whether the frontal apodeme is part of the dipteran groundplan is questionable, as it is missing in most taxa of lower *Diptera* (e.g., *Deuterophlebiidae*, *Nymphomyiidae*, *Axymyiidae*, *Brachycera*; Tokunaga 1935; Ferris 1950; Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg et al. 2011; Schneeberg et al. 2013b). This structure is also present in *Nannochorista* (Beutel & Baum 2008) (see above) but absent in other mecopteran groups (Friedrich et al. 2013a) and in *Siphonaptera* (Wenk 1953).

Three ocelli are present on the vertex in the groundplan, as it is also the case in most groups of insects (e.g., Snodgrass 1994; Beutel & Vilhelmsen 2007; Beutel et al. 2010b; Wipfler et al. 2012b). They were reduced several times independently (e.g., most *Culicomorpha*, *Tipuloidea*, *Keroplastidae*, *Mycetophilidae*, *Cecidomyiidae*; Schiemenz 1957; Schneeberg & Beutel 2011; Schneeberg et al. 2013a)



without a recognizable phylogenetic pattern. Within Brachycera they are only preserved in *Tabanus* (Bonhag 1951; Ferris 1950; Schiemenz 1957).

The compound eyes are unusually large and undivided and this is certainly a groundplan feature of the order. The subdivision into a dorsal and a ventral part is an autapomorphy of the controversial family Axymyiidae (Schneeberg et al. 2013b), which is placed in Bibionomorpha in Wiegmann et al. (2011).

An unusual structure which has evolved in several groups is the fulcrum which is composed of lateral plates which join the external clypeal wall. The presence is probably an autapomorphy of Culicidae and Tanyderidae, respectively, and it is also common in brachyceran groups (Williams 1933; Ferris 1950; Schiemenz 1957; Szucsich & Krenn 2000).

Peterson (1916) postulated the presence of a postgenal bridge in the groundplan of Brachycera, but not as an ancestral feature of the entire order. Its presence in several families of lower Diptera (e.g., Deuterophlebiidae, Nymphomyiidae, Tipulidae, Axymyiidae, Bibionidae, Sciaridae; Tokunaga 1935; Hoyt 1952; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a) rather suggests that it may be a dipteran groundplan feature. Hennig (1973) discussed a possible correlation between the presence of a hypostomal bridge and the reduction of the tentorium.

### **Tentorium**

A nearly complete tentorium is present in the groundplan but a massive tentorial bridge and strongly developed dorsal arms are never present (Petersen 1916). A complete tentorium was described for *Simulium* by Peterson (1916) but was not found in any of the species considered in this study (Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). More or less far-reaching reductions occur in several lineages. The complete loss of the tentorium is autapomorphic for Nymphomyiidae and Tipulidae, respectively. In Nymphomyiidae this is probably a result of the extreme size reduction. The tentorium is a thick, hollow and simple tube in most dipteran groups (e.g., Culicomorpha, Psychodomorpha, Ptychopteridae, Deuterophlebiidae; Schiemenz 1957; Wenk 1962; Schneeberg & Beutel 2011; Schneeberg et al. 2011). Dorsal arms occur as short vestiges in some families but are completely reduced in many groups. Surprisingly distinct dorsal arms are present in *Tabanus* (Bonhag 1951) and *Pipunculus* (Pipunculidae). As both groups are nested within Brachycera this is likely a secondary condition, and possibly autapomorphic in both cases.

It is possible, that the far-reaching reduction of the tentorium is correlated with the diet of the adults. Many of them feed on liquid substances such as nectar, some prefer pollen grains and dried honeydew, and some are sucking blood or are predators (McAlpine 1981; Oosterbroek 2006). In all cases biting, which create strong mechanical forces, is not involved. For feeding on liquid substrates a stabilization of the head capsule is not necessary, as it is the case in insects with typical orthopteroid mouthparts (see e.g., Wipfler et al. 2012b) where the head capsule is strengthened by a well-developed and strongly sclerotized tentorium and a more or less continuous system of internal ridges. A clear tendency to reduce the tentorium is apparent in Diptera, especially in groups feeding on liquid substances or pollen. However,

a reliable interpretation of the functional and phylogenetic background would require more specific information on the adult feeding habits in different groups.

### **Labrum**

A labro-epipharyngeal food-channel closed by the hypopharynx is part of the groundplan according to Hennig (1973). The food-channel is indeed present in most dipteran groups, but it is ventrally open in most cases (e.g., Tipulomorpha, Psychodomorpha, Bibionomorpha; Schneeberg & Beutel 2011). Therefore it appears more likely that a ventrally open channel is ancestral for the order. Its specific condition differs within the group. In Culicidae it is completely enclosed by the epipharyngeal wall (Vogel 1921; Schiemenz 1957; Snodgrass 1959; Christophers 1960; Owen 1985) and used for sucking blood. Correlated with the closure *M. labroepipharyngalis* (M. 7) is missing in this family (Thompson 1905; Schiemenz 1957; Christophers 1960; Owen 1985). Females of Simuliidae are also hematophagous but in contrast to Culicidae pool feeders. They generate wounds with their mandibles and ingest the collected blood (Reid 2008). Like in Culicidae *M. labroepipharyngalis* (M. 7) is missing.

A clypeolabral muscle is present in most species of Diptera examined (autapomorphy) (e.g., Thompson 1905; Bonhag 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b), but is missing in *Pachygaster* and *Drosophila* (Miller 1950), and also in Deuterophlebiidae, and Nymphomyiidae, which lack a labrum and are characterized by an extremely reduced cephalic musculature (Tokunaga 1935; Schneeberg et al. 2011). According to Matsuda (1965) the primarily paired muscle is fused and unpaired in the groundplan of the order (M. 62 in Matsuda 1965). However, the muscle is paired in most groups of lower Diptera (e.g., Tipulomorpha, Ptychopteromorpha, Ceratopogonidae, Bibionidae, Sciaridae; Gad 1951; Schneeberg & Beutel 2011) which is most likely the ancestral condition. The muscle is probably homologous with *M. frontolabralis* (M.8 of v. Kéler 1963) of other insects, but originates consistently on the clypeus in Diptera. Hennig (1973) discussed the possibility, that the dipteran clypeus may contain parts of the frons, as precerebral pharyngeal dilators originate on this area in *Drosophila* (Ferris 1950; Miller 1950). However, such a condition was not observed in other dipterans examined. *M. frontoepipharyngalis* (M. 9), which is present in the dipteran groundplan is generally absent in Brachycera.

### **Antenna**

According to Hennig (1973) the antenna is composed of a scape, pedicel and 14 flagellomeres in the groundplan of the order. However, the number of antennomeres varies strongly among the groups of lower Diptera, from 5 segments in Nymphomyiidae (Tokunaga 1935) to 113 in some Psychodidae (Hennig 1973), and it is strongly reduced in the groundplan of Brachycera (Ferris 1950; Bonhag 1951; Schiemenz 1957; Szucsich & Krenn 2000). Aside from the number of segments, the antenna is filiform and inserted frontally between the compound eyes in the dipteran groundplan (e.g., Peterson 1916; Tokunaga 1935; Harbach & Kitching 1998; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b). Closely adjacent

antennal insertions on the frontal region are also found in Mecoptera (with the exception of *Caurinus*; Beutel et al. 2008a) (Heddergott 1938; Beutel & Baum 2008; Friedrich et al. 2013a). Widely separated antennal bases with a dorsal insertion have apparently evolved independently in Deuterophlebiidae and Bibionidae (Schneeberg et al. 2011). Interestingly the antennae are distinctly different in these two groups, as they are elongated and slender in Deuterophlebiidae and moniliform and robust in Bibionidae.

Moniliform antennae have evolved independently in Simuliidae and within Bibionomorpha in Bibionidae, Axymyiidae and Scatopsidae (Schneeberg et al. 2013b). Whether this is an apomorphy of Bibionomorpha, excluding Anisopodidae with reversal in a subgroup comprising Sciaridae, Cecidomyiidae, Keroplatidae and Mycetophilidae is presently unclear. It is also possible that a moniliform antenna evolved three times independently in Bibionomorpha. A club-shaped antenna evolved independently in Nymphomyiidae and within Brachycera. This is clearly autapomorphic in the case of Nymphomyiidae but the interpretation in Brachycera is ambiguous. Club-shaped antennae occur in Drosophilidae, Syrphidae, Tabanidae and Stratiomyiidae (Ferris 1950; Bonhag 1951; Schiemenz 1957), linked with the presence of an arista in Cyclorrhapha (Hennig 1973). The club-shaped condition is due to the enlargement of the first flagellomere in Nymphomyiidae and in the brachyceran Drosophilidae, Tabanidae and Bombyliidae (where the antenna is filiform; but also shortened; Szucsich & Krenn 2000). A characteristic apomorphic condition occurring in Brachycera is the reduction of antennal segments (see above). The presence of a Johnston's organ in Culicomorpha and *Drosophila* (Miller 1950) is arguably due to reversal. It is conceivable that it also occurs in other groups but the presently available data are too fragmentary for a reliable interpretation.

Adults of several groups of lower Diptera are short-lived (e.g., Deuterophlebiidae, Nymphomyiidae, Cecidomyiidae) and have to find their mating partner in a short time span. Therefore the antenna is well developed and bears numerous olfactory sensilla (see Schneeberg et al. 2013a). Interestingly the antennae of adult Brachycera are greatly shortened, compared to the presumptive dipteran groundplan, but apparently still equipped with a highly efficient set of sensilla (e.g., *Drosophila*; Stocker 1994). It was shown in recent studies that the olfactory system performs well and that the short antennae play an important role in finding food sources and suitable oviposition sites (e.g., Hanssen & Stensmyr 2011; Date et al. 2013; Dweck et al. 2013).

The shift of the antennal musculature from the tentorium to the head capsule is very likely correlated with the more or less far-reaching reduction of the tentorium, but a phylogenetic pattern is not recognizable. In the groundplan of the order Mm. tentorioscapalis anterior, posterior and medialis (Mm.1/2/4) still originate on the tentorium. All muscles originate on the head capsule in Tipulidae and Nymphomyiidae, as in both cases the tentorium is completely reduced (Tokunaga 1935; Schneeberg & Beutel 2011). In groups with a short vestige of the dorsal arm all or at least two antennal muscles originate on the tentorium (e.g., Trichoceridae, Tanyderidae, Chaoboridae, Syrphidae, Bombyliidae; Schiemenz 1957; Szucsich & Krenn 2000; Schneeberg & Beutel 2011). In Psychodidae M. tentorioscapalis anterior (M. 1) is bipartite and one subcomponent originates on the tentorium, whereas the second one has its

origin on the head capsule. The muscle is also subdivided in Deuterophlebiidae (Schneeberg et al. 2011), but this is most likely a result of parallel evolution.

### **Mandibles**

Mandibles are present in the groundplan of Diptera like in Mecoptera and other holometabolan groups (e.g., Heddergott 1938; Hepburn 1969; Hennig 1973; Beutel & Baum 2008; Beutel et al. 2008a). However, they are never robust structures functioning as biting devices but more or less narrow stylet-like structures if present. In most groups they are completely absent (e.g., Tipulomorpha, Deuterophlebiidae, Nymphomyiidae, Bibionomorpha, Psychodomorpha; Tokunaga 1935; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). The reduction is apparently mainly correlated with the feeding habits. In blood-sucking dipterans mandibles are present and usually elongated and transformed into piercing stylets (e.g., Culicidae, Ceratopogonidae, Tabanidae; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Owen 1985). In Culicidae they are elongated in females, as only they need a blood meal for egg development. They are much shorter in the males which feed on plant sap (Snodgrass 1959). In Tabanidae mandibles are only present in the blood-sucking females, whereas they are absent in the flower-visiting males (Bonhag 1951). Similarly, in Blephariceridae they are present in predaceous females but also missing in males (Bellmann & Honomichl 2007).

### **Maxilla**

The stipites are internalized in Culicidae (cryptostipes of Peterson 1916) and also in some groups of Tipuloidea, in Simuliidae and in Brachycera (e.g., Ferris 1950; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011). An apomorphic feature of Tipuloidea is the partial fusion of the stipites and the complete fusion is an apomorphy of Tipulidae (Schneeberg & Beutel 2011) (see above). The stipites are also completely fused in Blephariceridae and some brachyceran groups (Hoyt 1952; Schneeberg & Beutel 2011), apparently as a result of parallel evolution.

Cardo and stipes are delimited from each other in the groundplan of Diptera and Brachycera according to Hennig (1973). However, a recognizable border is absent in most dipteran species examined (e.g., Peterson 1916; Williams 1933; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b) and is also missing in Siphonaptera (Wenk 1953), Nannochoristidae (Beutel & Baum 2008) and Boreidae (Beutel et al. 2008a). It was assumed by earlier authors that the cardines are reduced (e.g., Rees & Ferris 1939). However, the condition of the musculature suggests that the sclerite in question is a product of fusion (e.g., Imms 1944; Gouin 1949; Schiemenz 1957; Snodgrass 1959). Among the dipterans examined, cardo and stipes are only separated in Trichoceridae, Dixidae and Tabanidae (Bonhag 1951; Schneeberg & Beutel 2011). It is not entirely clear whether this separation is a secondary condition or ancestral for Diptera, implying that cardo and stipes fused several times independently within the group.

In the groundplan of the order and in most dipteran taxa only a single maxillary endite lobe is present (e.g., Williams 1933; Bonhag 1951; Hoyt 1952; Schiemenz 1957; Owen 1985; Szucsich & Krenn 2000; Blackwell 2004; Schneeberg & Beutel 2011). This was referred to as galea in older contributions (Peterson 1916; Williams 1933; Crampton 1942), but its homology with the lacinia has been established in more recent studies (e.g., Rees & Ferris 1939; Imms 1944; Schiemenz 1957; Snodgrass 1959; Wenk 1962; Krenn et al. 2005). Hoyt (1952) interpreted the structure as a product of fusion of both elements. However, this interpretation appears questionable, as *M. craniolacinalis* (*M.* 19) is preserved in some dipteran lineages (e.g., Culicidae, Simuliidae, Bombyliidae; Schiemenz 1957; Wenk 1962; Szucsich & Krenn 2000) as in Mecoptera (*Nannochorista*, *Caurinus*, *Bittacus*, *Merope*; Beutel & Baum 2008; Friedrich et al. 2013a), whereas a muscle of the galea is consistently absent. The complete reduction of the maxillary endite lobes is an apomorphic feature occurring in Tipulidae, Limoniidae, Cecidomyiidae, some Mycetophilidae, Axymyiidae and Anthomyiidae (see Hoyt 1952; Schneeberg et al. 2013a, b). It is apparent that this condition evolved several times independently.

The maxillary palp is 5-segmented in the dipteran groundplan and this condition was found in most species examined. Four segmented palps evolved in Psychodidae, Mycetophilidae and Siphonaptera (Wenk 1953; Michelsen 1996a). Palp segments are also reduced in some members of Culicidae (Harbach & Kitching 1998) and generally in Brachycera (see above). The last palp segment is distinctly elongated in several groups of lower Diptera (and slightly in Syrphidae; Schiemenz 1957) without a recognizable phylogenetic pattern. A sensorial field on the third maxillary palp segment is present in most species of lower Diptera and in Nannochoristidae (Beutel & Baum 2008), but the specific condition varies distinctly between the taxa. An apomorphic condition that evolved in Tipulomorpha is the exposed position of the sensilla of the sensorial field, and as a secondarily modified version of Tipulidae the sensilla are placed in individual, separated grooves (Schneeberg & Beutel 2011). A sensorium with a single groove belongs to the groundplan of Diptera and is also present in Nannochoristidae. A derived condition similar to that found in Tipulidae has evolved in Anisopodidae and Corethrellidae.

The origin of *M. tentoriostipitalis* (*M.* 18) is shifted to the head capsule in Tanyderidae and Limoniidae, and apparently also in Drosophilidae (Ferris 1950; Schneeberg & Beutel 2011) even though the homology of the muscle is not entirely clear in this case.

*M. palpopalpalis externus* (*M.* 22) is present in the dipteran groundplan, but absent in the groundplan of Brachycera. The muscle is also missing in Thaumaleidae, Axymyiidae, Scatopsidae and *Aedes*, whereas it is present in *Culex* (Christophers 1960).

## Labium

The dorsal surface of the anterior labium forms a distinct concavity in the groundplan of Diptera, a condition also found in Nannochoristidae and Siphonaptera (Beutel & Baum 2008). In Culicidae it forms a groove for the piercing mouthparts in their resting position (Thompson 1905; Vogel 1921; Schiemenz 1957; Christophers 1960; Owen 1985), and a similar condition is found in Simuliidae (Wenk 1962; Sutcliffe



1985), Tabanidae (Bonhag 1951) and Siphonaptera (Wenk 1953). The concavity is reduced in Bibionomorpha, excluding Anisopodidae and Scatopsidae. It is also absent in Tipulidae and Asilidae, but present in the groundplan of Tipulomorpha and Brachycera. The loss apparently occurred three times independently (Schneeberg & Beutel 2011).

Hennig (1973) considered the postmentum (submentum after Gouin 1950; mentum after Peterson 1916; Crampton 1942) as part of the dipteran groundplan. However, it is almost generally absent (Peterson 1916; Ferris 1950; Bonhag 1951; Schiemenz 1957; Wenk 1962; Szucsich & Krenn 2000; Schneeberg & Beutel 2011), with the only exception of Ceratopogonidae (Gad 1951). It appears likely that the postmentum is fused with other cephalic elements or completely reduced in the dipteran groundplan (potential autapomorphy of the group), implying that the structure occurring in Ceratopogonidae is not homologous with the postmentum of other insects. A correlation between the reduction of the postmentum and the presence of a postgenal bridge (Hennig 1973) is possible but not supported by specific evidence.

Two-segmented labial palps as thickened labellae are an autapomorphy of Diptera (e.g., Vogel 1921; Bonhag 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Sutcliffe 1985; Harbach & Kitching 1998; Szucsich & Krenn 2000; Blackwell 2004; Schneeberg & Beutel 2011; Schneeberg et al. 2013a, b). They are present in all dipterans with developed mouthparts. Pseudotracheae are a specialized modification of the labellae. This condition evolved independently in some groups of lower Diptera such as Ptychopteridae, Tipulidae and Mycetophilidae (partim) and it is typical for Brachycera. The pseudotracheae of brachycerans are more complex and cover a larger area. In Syrphidae the labellae consist of two rows of collecting channels on the anterior edge and about 40 on the mesal wall (Schiemenz 1957), whereas in *Tipula* two rows of collecting channels enclose the functional mouth opening (Schneeberg & Beutel 2011). The brachyceran pseudotracheae consist of inner strengthening rings and a number of secondary channels (e.g., Frey 1921; Gilbert & Jervis 1998; Ngeru-Klun et al. 2007). The function is the uptake of liquid food by capillary forces and the distribution of saliva (Elzinga & Bronce 1986). Simple furrows on the inner wall of the labellae are obviously present in the groundplan of Diptera. Owen (1985) considered them as pseudotracheae, but Schiemenz (1957) demonstrated, that the ultrastructure is distinctly different, which implies that they are precursors or were formed independently. The furrows are absent in Blephariceridae and Limoniidae, and in subgroups of Culicomorpha and Bibionomorpha. The absence is possibly linked with the feeding habit in these groups, but the available information is insufficient for a reliable interpretation.

Glossa and Paraglossa are absent in the groundplan of Diptera, as in all other antliophoran groups (e.g., Schiemenz 1957; Wenk 1962; Hepburn 1969; Owen 1985; Sutcliffe 1985; Szucsich & Krenn 2000; Beutel & Baum 2008; Beutel et al. 2008a; Schneeberg & Beutel 2011; Friedrich et al. 2013a).

The presence of only one premental retractor muscle is ancestral for Diptera. This condition is found in all examined taxa (e.g., Thompson 1905; Bonhag 1951; Gad 1951; Hoyt 1952; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011) with the exception of *Drosophila* (Miller 1950). As *Drosophila* is deeply nested within Brachycera the second

bundle is very likely the result of a subdivision of a primarily undivided retractor. An investigation of more brachyceran representatives is necessary for a reliable evaluation of this character. *M. palpopalpalis labii primus* (M. 35) is absent in the groundplan of Brachycera.

### **Pharynx and its musculature**

*M. clypeopalatalis* (M. 43) is distinctly enlarged in the groundplan of Diptera and forms a long series of bundles in most taxa (Thompson 1905; Miller 1950; Bonhag 1951; Gad 1951; Schiemenz 1957; Owen 1985; Szucsich & Krenn 2000; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). Likewise, a postcerebral pharyngeal pump (Mm. 51/52) is ancestral for the group. It is strongly developed in all groups examined, with the noteworthy exception of Cyclorrhapha (Thompson 1905; Tokunaga 1935; Bonhag 1951; Gad 1951; Schiemenz 1957; Christophers 1960; Wenk 1962; Owen 1985; Schneeberg & Beutel 2011; Schneeberg et al. 2011, 2013a, b). The well-developed sucking pump of dipteran adults is apparently correlated with the uptake of liquid food. It is also well developed in the blood-sucking fleas (Wenk 1953) and also in Nannochoristidae, which very likely also feed on liquid substrates (Beutel & Baum 2008).

### **Salivary pump**

A salivary pump with a well-developed muscle is present in the groundplan of Diptera and this condition is found in almost all groups examined. The single muscle (*M. hypopharyngosalivarialis*, M. 37) is reduced in Axymyiidae, Deuterophlebiidae and Limoniidae (Schneeberg & Beutel 2011; Schneeberg et al. 2011). Deuterophlebiid adults do not consume food, which makes a salivary pumping apparatus superfluous.

## **5.2.2 Larval head**

### **Head capsule**

The larval head is prognathous and fully exposed in the groundplan and this condition is present in most groups of lower Diptera (Anthon 1943a; Cook 1944a, b, 1949; Gouin 1950; Schremmer 1950a; Kramer 1954; Perraudin 1961; Felix 1962; Anthon & Lyneborg 1968; Solinas 1968; Courtney 1990b; v. Lieven 1998; Schneeberg et al. 2012; Wipfler et al. 2012a). This is also the case in Siphonaptera and Nannochoristidae, and therefore arguably part of the antliophoran groundplan. According to Hennig (1973) the prognathous head is a derived groundplan feature of Diptera and correlated with the loss of larval thoracic legs. This interpretation appears uncertain as the head is also distinctly prognathous in larvae of *Nannochorista* (Beutel et al. 2009), which possess well developed legs. A similar combination of prognathism and normally developed legs occurs also in other groups of holometabolan insects such as Coleoptera (Adephaga, Histeroidea, Staphylinidae partim), Neuropterida and Trichoptera (Rhyacophilidae) (e.g., Beutel et al. 2011).

Tipuloidea are characterized by a tendency to retract the larval head capsule into the prothorax. The head is strongly retracted in members of Limoniidae, Cylindrotomidae and Tipulidae (see Neugart et al. 2009). In the presumably basal Pediciidae the head is only moderately retracted (see Cook 1949) and this probably represents a transition state between the fully exposed head in the dipteran groundplan and the completely retracted one in Tipuloidea excl. Pediciidae. The head is also moderately retracted into the prothorax and not fixed in this position in larvae of Axymyiidae and Stratiomyiidae (Cook 1949), apparently as a result of parallel evolution. A partly reduced head capsule is usually found in dipteran larvae living in soft substrate, whereas it is well developed in larvae exposed in their environment, such as for instance in Culicomorpha or Bibionidae. However, the immature stages of Tipuloidea occupy a wide range of different habitats (Alexander & Byers 1981). The investigated larvae live mostly in semiaquatic or aquatic environments (*Limonia*, *Tipula*, many Pediciidae) or alternatively in moss (Cylindrotomidae). In Brachycera the tendency to retract and to reduce the head capsule is apparent. In Cyclorrhapha the larval head is strongly retracted and the head capsule completely reduced (Chaudonneret 1983; Stuke 2000; Wipfler et al. 2013).

A larval apomorphy of Tipulomorpha is the ventromedian incision of the head capsule. This condition is only found in members of Tipuloidea and Trichoceridae. A triangular median sinus on the ventral side of the head occurs in Simuliidae (Cook 1949), but this is clearly a different condition, which is unique in simuliid larvae. A further autapomorphy of tipuloid larvae is the separation of the dorsomedian fragment from the remaining head capsule by paired dorsolateral incisions. This is related with the general trend to reduce the sclerotized cephalic elements in Tipuloidea. Another derived character of this group is the presence of growth lines on the externolateral plates, as it has also evolved in Deuterophlebiidae, Blephariceridae and Tanyderidae. In Tanyderidae a premaxillary suture is present and a possible apomorphy of the group (Wipfler et al. 2012a). The suture has also evolved in subgroups of Tipuloidea (Tipulidae, Cylindrotomidae, Limoniinae: Cook 1949; Oosterbroek & Theowald 1991; Neugart et al. 2009).

A long coronal suture is present in the larval groundplan of Diptera, although it is missing or shortened in most groups. The frontoclypeal transverse ridge is missing in all larvae examined, with the exception of Axymyiidae. Based on the absence of the suture in all other dipteran groups and also in *Nannochorista* (Beutel et al. 2009) and Siphonaptera (Sharif 1937) it is plausible to assume that the presence is a secondary feature. The clypeus is subdivided in larvae of Axymyiidae. The same condition is only found in larvae of Limoniidae (Kramer 1959) and Mecoptera (Bierbrodt 1942; Beutel et al. 2009). As Axymyiidae is nested within Bibionomorpha and Limoniidae in Tipulomorpha it is most likely autapomorphic for both groups. The presence of a frontoclypeal suture and the divided clypeus in larvae of Axymyiidae possibly increases the flexibility of the anterior head region. The frons is primarily V-shaped in dipteran larvae (Oosterbroek & Courtney 1995) but a U-shaped frons has evolved in Chaoboridae and independently in Ceratopogonidae (v. Lieven 1998).

Larvae of Chaoboridae have well-developed imaginal compound eyes (Melzer & Paulus 1990). This is likely correlated with the predaceous lifestyle, as they detect their prey visually. Compound eyes also

found in larvae of *Panorpa* (Bierbrodt 1942). However, the compound eyes of *Panorpa* are larval structures and the larvae live in soil and feed on dead insects and carrion of other small animals (Sauer & Hensle 1977).

The hypostomal plate (hypostomium) fused with elements of the head capsule is a groundplan feature of the order and a common feature in lower Diptera. A partly divided hypostomium is present in larvae of Dixidae. A similar condition evolved independently in Tipulidae and Limoniidae (Neugart et al. 2009). In Pediciidae it is completely divided (Cook 1949) which is a potential autapomorphy of the family. It is separated from the head capsule by a distinct suture in Ptychopteridae, Bibionidae and Tanyderidae (Cook 1949; Kramer 1954; Perraudin 1961; Wipfler et al. 2012a), likely an apomorphic condition which evolved independently in these three families. As discussed by Solinas (2011) the condition of the hypostomal plate depends mainly on the diet of the larvae of Mycetophilidae. He assumed that it is strongly developed in species feeding on soft gall issues, moderately in larvae feeding on fibrous plant tissue, and poorly in species lacking sap from the gall walls (Solinas 2011). In the groundplan of Brachycera a hypostomal bridge is missing, which is arguably correlated with the retraction of the head (see above and Hennig 1973). However, this is not the case or only to a lesser degree in tipuloid larvae (Neugart et al. 2009). The hypostomium is equipped with triangular teeth in the groundplan, arguably an autapomorphy of the order. Ancestral dipteran larvae probably lived in a moist or wet environment (e.g., Deuterophlebiidae, Nymphomyiidae, Tipuloidea; Courtney 1990b, 1994b; de Jong et al. 2008) where they feed on algae, decaying plant material or moss. The toothed hypostoma probably helps to remove algae or microorganic material from rocks or other surfaces.

### **Tentorium**

A tentorium with anterior, posterior and dorsal arms and a tentorial bridge is present in the groundplan. However, dorsal arms and the bridge are missing in most groups and in the groundplan of Brachycera, as it is also the case in adults (see above).

The tentorium is completely reduced in the larvae of Mycetophilidae. Their head forms a “cutterhead” (pers. observation R. Bauernfeind) and they live in and feed on fungus (Madwar 1937). The same occurs in larvae of Axymyiidae which live in chambers they produce in wood saturated with water. Their diet is probably also soft material and the tentorium is also reduced (Krogstad 1959). A tendency to reduce the larval tentorium is also present in Bibionomorpha, as the anterior tentorial arms are reduced in most groups (Bibionomorpha excl. Anisopodidae and Scatopsidae). As in the adults, reductions of tentorial elements are common. Anterior arms are also missing in Simuliidae, Tipulidae, Pediciidae and Nymphomyiidae (Cook 1949; Neugart et al. 2009; Schneeberg et al. 2012).

### **Labrum**

The labrum is partly fused with the head capsule in larvae of Axymyiidae, and also in Bibionidae and Mycetophilidae. This condition could be a transition state between the free labrum of Anisopodidae and

the completely fused labrum in Cecidomyiidae. The labrum is also fused with the head capsule in several other dipteran groups, but without a recognizable phylogenetic pattern. A subdivided labrum is a characteristic feature of Tipulidae and an autapomorphy of the family.

A broad larval labrum is probably a dipteran groundplan feature. A conical labrum is ancestral for brachyceran larvae. However, the same condition has evolved in several lower dipteran families such as for instance Nymphomyiidae, Thaumaleidae, Axymyiidae, Anisopodidae, and Psychodidae (Anthon 1943a; Schneeberg et al. 2012).

A labrum equipped with a dense field of hairs is a groundplan apomorphy of Diptera. However, the specific condition in different groups varies strongly, and in some families a specific arrangement of the labral brush is missing. It is likely used to remove or sweep algae or other small food particles from surfaces. An unusual feature occurring in basal dipteran lineages is the formation of movable premandibles or messoroes. These structures, which do not occur in other holometabolan groups, form a conspicuous and efficient collecting apparatus in the aquatic larvae of Simuliidae (Craig & Chance 1982; Lacoursière & Craig 1993).

Strongly developed labral teeth are present in larvae of Anisopodidae. This apomorphic character has independently evolved in Cylindrotomidae (Peus 1952), as this structural modification is absent in *Trichocera* and other members of Tipuloidea (Anthon 1943a; Cook 1949; Podenine & Gelhaus 2002; Neugart et al. 2009) and consequently in the groundplan of Tipulomorpha.

M. frontoepipharyngalis (M. 9) is absent in larvae of Bibionidae. It is also missing in Cecidomyiidae and some Brachycera (Therevidae, Stratiomyiidae; Cook 1949; Solinas 1968), but present in the groundplan of the order and in most dipteran larvae examined (e.g., Cook 1944a, b, 1949; Schremmer 1949; Kramer 1954; Gouin 1959; Felix 1962; Courtney 1990b; v. Lieven 1998; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a). The loss obviously occurred several times independently within Bibionomorpha and Brachycera.

## Antenna

The larval antennae consist of a basal antennomere and several distinctly developed segments in the groundplan. However, it is 1-segmented in most groups and in the groundplan of Brachycera. The presence of only one extrinsic muscle is ancestral and this condition is found in most groups of lower Diptera. One muscle is also present in Nannochoristidae and Panorpidae, but two in Boreidae (Röber 1942; Beutel et al. 2009). The loss of the extrinsic antennal muscles occurred several times in Diptera. A phylogenetic pattern is not recognizable.

The antennae are vestigial in Axymyiidae, Bibionidae and Mycetophilidae, but present in larvae of Cecidomyiidae (Cook 1949; Perraudin 1961; Solinas 1968). The antenna is distinctly developed in the groundplan of Bibionomorpha but the group shows a clear tendency towards reduction.

In Chaoboridae, Corethrellidae and Ceratopogonidae (v. Lieven 1998) the antennal insertion is shifted towards the midline, whereas a lateral insertion belongs to the dipteran groundplan (e.g., Anthon 1943a;

Cook 1949; Kramer 1954; Lindner 1959; Perraudin 1961; Solinas 1968; Courtney 1990b; Neugart et al. 2009; Schneeberg et al. 2012; Wipfler et al. 2012a). The antenna insert on the anterior margin of the head capsule in *Chaoborus*, in front of the compound eyes. It is likely that this improves the coordination of prey catching in the specialized predators. Larvae of *Chaoborus* capture copepods and water fleas with this unusual apparatus (Sæther 1967). The raptorial antennae are an autapomorphy of Chaoboridae. In Corethrellidae the antenna is simple and bears two horns on the apex, and the same condition is found in *Anopheles* (Schremmer 1949). A bifurcate antenna is also present in larvae of Deuterophlebiidae, but this is likely due to parallel evolution considering the basal position of Deuterophlebiidae.

### **Mandible**

Well-developed, undivided and relatively stout mandibles moving in a horizontal plane are probably part of the larval groundplan of Diptera. A major evolutionary trend in Diptera is the shift of the axis of movement from vertical to oblique to horizontal (e.g., Oosterbroek & Theowald 1991; Neugart et al. 2009). The mandibles of several groups of lower Diptera move in a vertical plane and this applies generally to the hook-shaped mandibles or mouthhooks in Brachycera. It is conceivable that this is linked with the lack of thoracic legs. The more or less hook-shaped mandibles are moved as accessory locomotor organs in several groups of Diptera.

The mandible is sickle-shaped and elongated in Pediciidae (Cook 1949), apparently an autapomorphy of this family. In Tipulidae the secondary mandibular joint is shifted posterior to the antennal foramen (Selke 1936; Cook 1949; Chiswell 1955; Neugart et al. 2009), a unique feature and very likely an autapomorphy of this family. A movable lacinia mobilis is probably a synapomorphy of Tipulidae and Cylindrotomidae, but it also occurs in Chaoboridae and Nannochoristidae (Oosterbroek & Theowald 1991; Beutel et al. 2009), obviously as a result of parallel evolution. In Chaoboridae a small intrinsic extensor muscle is present. That this muscle was not found in any other group of Dipteran suggests that this is an autapomorphic feature which is possibly linked with the predaceous life style.

An anteriorly directed cone on the mesal edge of the mandible is a unique feature of Anisopodidae and an autapomorphy of this family (Anthon 1943a). The mandible is subdivided in larvae of this family as it is also the case in Ptychopteridae and Brachycera (Anthon 1943a; Cook 1949; Kramer 1954). In Ptychopteridae the mandible is divided by a furrow, whereas the distal and the proximal parts are separated by a weakly sclerotized zone in Anisopodidae and Brachycera. It cannot be fully excluded that the subdivided mandible is a synapomorphy of Brachycera and Bibionomorpha. But this interpretation would imply that an undivided mandible evolved secondarily in Bibionomorpha, excluding Anisopodidae.

A multitoothed comb is present on the apical part of the mandible in larvae of Thaumaleidae. This is also the case in larvae of Deuterophlebiidae, Nymphomyiidae and some Blephariceridae (Courtney 1990b; Oosterbroek & Courtney 1995; Schneeberg et al. 2012). This is probably linked with the feeding habits of the larvae. All of them live in cold streams and scrape diatoms, microscopic plant material and algae from rocks (Hogue 1981; Kevan & Cutten 1981; Stone & Peterson 1981).



*M. tentoriomandibularis* (M. 13) is missing in all dipteran larvae examined, and also in Siphonaptera and Mecoptera excl. Nannochoristidae (Beutel et al. 2009). It is conceivable that the delicate muscle was overlooked in some cases.

### Maxilla

A more or less complete and free larval maxilla belongs to the groundplan of Diptera. A maxilla with a cardo which is completely fused with the head capsule or reduced has evolved independently in Axymyiidae, Nymphomyiidae, Psychodidae, and some members of Culicomorpha. The cardo is present as a clearly defined sclerite in the groundplan of the order. In Axymyiidae it is apparently preserved even though indistinguishably fused with the stipes. *M. tentoriocardinalis* (M. 17) is distinctly developed in larvae of *Axymyia*.

Different degrees of fusion of the maxillary endite lobes occur in lower dipteran larvae. They are partly fused in Axymyiidae but still recognizable as separate structures, whereas the fusion is complete in Bibionidae and Cecidomyiidae (Cook 1949; Perraudin 1961; Solinas 1968). The homology of the maxillary elements is still not entirely clear in larvae of some groups such as for instance in Mycetophilidae, Corethrellidae, and Thaumaleidae.

A dipteran groundplan feature preserved in Deuterophlebiidae is the presence of 2-segmented maxillary palps (Courtney 1990b). This condition is also maintained in few other groups of lower Diptera (Bibionidae, Chironomidae, Axymyiidae; Cook 1949; Perraudin 1961; Foote 1991) and also in Therevidae (Brachycera) (Cook 1949). The reduction of one palpomere has taken place several times independently and it is 1-segmented in most groups.

The absence of *M. craniocardinalis* (M. 15) is probably ancestral for Diptera, even though one extrinsic maxillary muscle with a cranial origin is present in *Exechia* and even two in *Androprosopa*. The homology of these muscles is presently completely unclear, mainly due to the unclear homology of the corresponding maxillary elements. *M. craniocardinalis* is also missing in larvae of other groups of Mecoptera (Hinton 1958) and this is possibly an autapomorphy of this lineage (Beutel et al. 2009). An extrinsic maxillary muscle occurring in Siphonaptera is probably not homologous with *M. craniocardinalis* (Beutel et al. 2009). Intrinsic maxillary muscles (Mm. 20/21) are also missing in the groundplan of Diptera.

*M. tentoriostipitalis* (M. 18) is arguably present in the groundplan of Diptera as it is preserved in larvae of Nymphomyiidae. However, it originates on the head capsule due to the loss of the tentorium, and it cannot be fully excluded that it is in fact *M. craniolacinalis* (M. 19). The maxillary endites are not recognizable as separate structures in larvae of Nymphomyiidae and likely fused with the stipites (see Schneeberg et al. 2012).

### Labium

The labium is distinctly simplified in the groundplan of Diptera. A separate submentum (as a part of the postmentum) is present in the dipteran groundplan and also in the groundplan of Brachycera. The

ancestral condition occurs in *Olbiogaster* (Anisopodidae) (Anthon 1943b) and Psychodidae (Oosterbroek & Courtney 1995), but the basal labial element is absent in most groups of lower Diptera (e.g., Anthon 1943a; Cook 1944a, b, 1949; Neugart et al. 2009; Schneeberg et al. 2012).

Labial palps are greatly reduced and appear 1-segmented in the groundplan. They are present in Anisopodidae, Blephariceridae, and Tabanidae (Anthon 1943a; Cook 1949; Anthon & Lyneborg 1968). Even though they are completely reduced in almost all groups of lower Diptera, they are preserved in the groundplan of Brachycera according to Hennig (1973) (Tabanidae; Cook 1949) and also in the groundplan of Cyclorrhapha (Keilin 1916; de Meijere 1916).

Only one extrinsic labial muscle, a premental retractor, occurs in larvae of Diptera, as it is also the case in adults. *M. praementopalpalis* (M. 34) is probably absent in the groundplan.

### Pharynx

A pharyngeal filter is present in a number of families of lower Diptera. It is absent in Mecoptera and Siphonaptera (Sharif 1937; Bierbrodt 1942; Beutel et al. 2009) and also in Deuterophlebiidae and Nymphomyiidae, and is therefore probably not a part of the dipteran groundplan. Its presence is arguably a groundplan apomorphy of Diptera excluding Deuterophlebiidae and Nymphomyiidae, with parallel secondary loss in different groups such as Tipuloidea, Bibionomorpha (partim), Simuliidae, Thaumaleidae, and the entire Brachycera (Anthon 1943a; Cook 1944a, 1949; Gouin 1959; Perraudin 1961; Solinas 1968; Oosterbroek & Courtney 1995; v. Lieven 1998; Neugart et al. 2009). The homology of the filter in different groups is also not entirely clear as it differs considerably among the lower dipteran lineages. It is for instance highly specialized and complex in Axymyiidae, whereas it consists of simple combs in *Anopheles* (see Schremmer 1949, fig. 14).

*M. tentoriohypopharyngalis* (M. 42) is probably absent in the groundplan. The homology of a tentoriohypopharyngeal muscle described for *Trichocera* (Winkler 1959; Fotius-Jaboulet 1961) is questionable. The character polarity of the presence or absence of this muscle is also unclear. Hinton (1958) interpreted the loss as a possible synapomorphy of Diptera, Siphonaptera and Pistillifera, but this interpretation is not convincing according to Beutel et al. (2009), who assumed that the muscle is present in *Nannochorista* and *Panorpa* (Bierbrodt 1942) and considered a 'Musculus palpi labialis' as possible homologue of *M. tentoriohypopharyngalis* (M. 42).

*M. tentoriobuccalis anterior* (M. 48) and the muscles forming the postcerebral pharyngeal pump (Mm. 50-52) are completely missing in brachyceran larvae. The absence of *M. tentoriobuccalis anterior* is a common feature in Diptera, but as it occurs in several families and also in Mecoptera (Bierbrodt 1942; Beutel et al. 2009) and Siphonaptera (Sharif 1937); it belongs apparently to the groundplan of the order. The same applies to the muscles of the postcerebral pharyngeal pump.

### 5.3 Conclusion

Adults of Diptera are usually short lived, with a life span hardly extending beyond copulating and laying eggs. Feeding is not necessary in many cases and the mouthparts are largely or completely reduced in different groups. Adults of most groups are quite similar in their life habits and the head is characterized by similar reductions in many groups. One character complex which shows already far-reaching modifications in the groundplan is the food uptake apparatus. The paired mouthparts are strongly modified with a distinct tendency towards reduction, and the labium is transformed into a device for the uptake of liquid food. Strongly developed cibarial and postcerebral pumping apparatuses, which belong to the groundplan of the order, are also relevant in this functional context. The optimization of the uptake of more or less liquefied food is one major evolutionary trend in Diptera, with several cases of parallel evolution. Mandibles are already strongly modified in the groundplan and completely reduced along with their muscles in most lineages, a loss which obviously occurred several times independently. The mandibles are usually preserved in blood-sucking species, especially in females which need a blood meal for producing fertile eggs. They still occur in very few groups of Brachycera (e.g., Tabanidae, Dolichopodidae; Langhoffer 1901; Bonhag 1951), but are absent in the groundplan of Cyclorrhapha (Hennig 1973). The maxillae, which are already strongly modified in the groundplan, are further simplified within the group. They play only a minor role in the food uptake if at all. Transformations of the labium play a major role. The labellae are one of the conspicuous autapomorphies of the order. Pseudotracheae evolved several times within the group. These specialized surface structures create capillary forces and thus facilitate the uptake of liquid food.

Conspicuous transformations of the antennae take place within the group, with largely unmodified filiform antennae in the groundplan (e.g., Culicidae), moniliform antennae in some lower dipteran families (e.g., Bibionidae), and strongly shortened and transformed antennae (7 or less antennomeres) in Brachycera, with a characteristic whip-like arista as a common feature in Cyclorrhapha. The functional interpretation is difficult in this case. The antennae and their sensilla play an important role in the identification of suitable food substrates and oviposition sites. (e.g., Hanssen & Stensmyr 2011; Date et al. 2013; Dwecke et al. 2013).

A characteristic found in many groups of Diptera is the very large size of the compound eyes. This is probably related with the excellent flying abilities and probably also with the necessity to find a mating partner within a relatively short time span, as it is for instance the case in the short-lived strepsipterans (e.g., Pohl & Beutel 2008). As adult dipterans take up liquid food and strong mechanical forces are not created during this process, they do not need a strong reinforcement of the head capsule. This was likely a precondition for the formation of very large compound eyes, which results in extensive zones of mechanical weakness.

Another characteristic likely related with the exceptional flight capacity is the cuticular surface covered with a dense vestiture of microtrichiae, not only on the head but also on other body parts. As in

strepsipterans (e.g., Pohl & Beutel 2008) this specific surface sculpture probably improves the flight performance. Another possible function could be water repellence.

Dipteran larvae live mostly in wet environment and are frequently aquatic. Most of them, especially brachyceran larvae, live in the substrate they feed on. In aquatic and free living larvae the head is fully exposed, whereas larvae living in their substrate show a tendency to retract it into the anterior thorax. This process is accompanied by a more or less far-reaching reduction of the sclerotized parts of the head capsule.

The movability of the larvae is strongly constrained as thoracic legs are completely missing in all groups. The mouthparts of several larvae are involved in the locomotion, especially in brachyceran groups, and the oblique or vertical plane of movement of the mandibles (mouthhooks in Cyclorrhapha) can be seen in this context. The feeding habits and mouthparts of dipteran larvae are much more diverse than in the adults. The larvae can be grazers, filter feeders, predators or simply feed on the substrate they live in. They evolved specific adaptations to their feeding habit, such as a toothed hypostoma, mandibular combs, a pharyngeal filter apparatus, or a more or less extensive labral fan.

Larval antennae play a very minor role compared to those of the adults. They are greatly or completely reduced in many groups. This is possibly related to the habit of females to deposit the eggs at oviposition sites where suitable food for the larvae is readily available.

## 6 Summary

The aims of the present study were a documentation of morphological characters using innovative techniques (1), the compilation of an extensive morphological data set (2), the phylogenetic evaluation of the characters, (3) and the reconstruction of an evolutionary scenario for adult and larval head structures in Diptera.

Detailed morphological studies covering adults of 28 species and larvae of 10 species were carried out, including potential dipteran key taxa such as for instance Deuterophlebiidae (Study I), Axymyiidae (Study III), Tipulomorpha (Study IV) and Nymphomyiidae (Study V).

Characters of the adult and larval head were defined, coded and entered in a data matrix (see Appendix 1, 2). The character sets were analyzed separately and combined (adult and larval data). The characters of the larval head and the combined analyses support the monophyly of Diptera, Tipuloidea and Brachycera. Analyses of characters of the adult head do not recover commonly accepted clades. Cephalic features are greatly affected by homoplasy and therefore of limited value for phylogenetic reconstruction. The larvae differ more strongly in their head structures than the adults but are also affected by convergences and not sufficient for resolving basal dipteran relationships. Consequently the morphological data were mapped on a recently published phylogenetic tree based on the most comprehensive molecular data set analyzed so far (Wiegmann et al. 2011). With this approach an evolutionary scenario for adult and larval head structures was developed. Important potential apomorphies of the head are summarized in the following (see fig. 4):

**1 Diptera:** Adult head: dense vestiture of microtrichia, presence of a clypeolabral muscle, reduction of the postmentum, transformation of the labial palps into labellae (equipped with simple furrows). Larval head: labrum equipped with a dense field of hairs, presence of a hypostoma (hypostomal bridge) with triangular teeth.

**2 Deuterophlebiidae:** Adult head: antenna inserted laterally, widely separated, last antennal segment extremely elongated, labrum, maxilla and labium reduced (inclusive associated muscles), reduction of *M. tentoriobuccalis* anterior and *M. hypopharyngosalivarialis*. Larval head: presence of externolateral plates with growth lines (homoplasious character), labral brush consists of macrosetae, reduction of *M. labroepipharyngalis*, bifurcate distal antennal segment, presence of apical multitoothed mandibular comb (homoplasious character), largely membranous cardo.

**3 Nymphomyiidae:** Adult head: completely sclerotized rostrum, club-shaped antenna with enlarged first and elongated terminal segment, completely reduced tentorium, reduction of labrum, maxilla and labium (inclusive associated muscles), loss of *M. tentoriobuccalis* (homoplasious character). Larval head: completely reduced tentorium, labral brush consisting of macrosetae, reduction of *M. labroepipharyngalis*, bifurcate distal antennal segment, presence of an apical multitoothed mandibular comb.

**4 Diptera, exclusive Deuterophlebiidae and Nymphomyiidae:** Larval head: loss of *M. tentoriostipitalis*, presence of a pharyngeal filter.

**5 Tipulomorpha:** Adult head: sensilla on sensorial field of third maxillary palpomere exposed on surface (homoplasious character). Larval head: deep ventromedian incisions of the head capsule.

**6 Tipuloidea:** Adult head: stipites partly fused. Larval head: presence of paired dorsolateral incisions, presence of growth lines on the extensive externolateral plates (homoplasious character).

**7 Pediciidae:** Larval head: mandible elongated and sickle-shaped, distinctly elongated maxillary palpomere.

**8 Tipuloidea, exclusive Pediciidae:** Larval head: head strongly retracted and fixed in this position, presence of premaxillary suture and side plates (homoplasious character).

**9 Tipulidae + Cylindrotomidae:** Adult head: entirely sclerotized rostrum (homoplasious character). Larval head: movable lacinia mobilis (homoplasious character).

**10 Tipulidae:** Adult head: completely reduced tentorium and maxillary endites, sensilla on sensorial field of third maxillary palpomere inserted in individual grooves (homoplasious character), absence of the dorsal labial concavity (homoplasious character), presence of pseudotracheae (homoplasious character). Larval head: subdivided labrum, secondary mandibular joint shifted posterior to antennal foramen.

**11 Ptychopteromorpha:** Adult head: sensilla on sensorial field of third maxillary palpomere exposed on surface (homoplasious character), presence of pseudotracheae (homoplasious character). Larval head: subdivided mandible (distal part separated by a furrow), hypostomium present as separate structure (homoplasious character).

**12 Blephariceridae:** Adult head: completely fused stipites, absence of furrows on internal side of labellae (homoplasious character). Larval head: presence of externolateral plates with growth lines (homoplasious character).

**13 Psychodidae:** Adult head: *M. tentorioscapalis* anterior bipartite with one subcomponent originating on the tentorium and one on the head capsule, 4-segmented maxillary palp (homoplasious character), loss of *M. palpopalpalis maxillae primus*.

**14 Tanyderidae:** Adult head: *M. tentoriostipitalis* originates on head capsule. Larval head: presence of premaxillary suture and side plates (homoplasious character), specific arrangement of labral brush, hypostomium present as separate structure (homoplasious character).

**15 Chaoboridae + Corethrellidae:** Adult head: absence of furrows on internal side of labellae (homoplasious character). Larval head: antennal insertion shifted close to the midline.

**16 Chaoboridae:** Larval head: U-shaped frons (homoplasious character), presence of simplified compound eyes, presence of movable lacinia mobilis (homoplasious character).

**17 Corethrellidae:** Adult head: sensilla on the sensorial field of third maxillary palpomere inserted in individual grooves (homoplasious character). Larval head: antenna with two apical horns.



**18 Culicidae:** Adult head: joint between clypeus and frons, subdivision of clypeus, fulcrum, labroepipharyngeal food channel closed by sides of epipharynx, loss of *M. labroepipharyngalis* (homoplasious character), internalized stipites.

**19 Dixidae:** Larval head: median division of hypostomium.

**20 Ceratopogonidae:** Larval head: U-shaped frons (homoplasious character).

**21 Simuliidae:** Adult head: loss of *M. labroepipharyngalis* (homoplasious character), internalized stipites (homoplasious character). Larval head: triangular median membranous sinus of head capsule, absence of anterior tentorial arms (homoplasious character).

**22 Thaumaleidae:** Adult head: loss of *M. stipitopalpalis externus*. Larval head: apical multitoothed mandibular comb (homoplasious character).

**23 Anisopodidae:** Adult head: sensilla of the sensorial field of the third maxillary palpomere inserted in individual grooves (homoplasious character). Larval head: presence of strongly sclerotized labral teeth, presence of an anterior directed cone of the mesal mandibular edge, subdivided mandible (distal part separated by a furrow).

**24 Bibionomorpha, excluding Anisopodidae and Scatopsidae:** Adult head: absence of the concavity on the dorsal surface of the anterior labium (homoplasious character). Larval head: absence of anterior tentorial arms (homoplasious character).

**25 Axymyiidae:** Adult head: subdivided compound eyes, loss of *M. hypopharyngosalivarialis*. Larval head: head moderately retracted (homoplasious character), presence of a secondary frontoclypeal ridge (probably not homologous with that of other insects), subdivided clypeus.

**26 Bibionomorpha, exclusive Anisopodidae, Scatopsidae and Axymyiidae:** Larval head: reduction or complete fusion of maxillary endite lobes.

**27 Bibionidae:** Adult head: absence of vestiture of microtrichia (head covered with long setae), antennae inserted laterally. Larval head: loss of *M. frontoepipharyngalis*, hypostomium present as separate structure (homoplasious character).

**28 Mycetophilidae:** Adult head: partly hollow anterior tentorial arm, 4-segmented maxillary palp (homoplasious character).

**29 Brachycera:** Adult head: reduced number of antennomeres and maxillary palp segments, presence of pseudotracheae on the internal side of the labellae. Larval head: loss of extrinsic antennal muscles, cibariopharyngeal sclerotization.

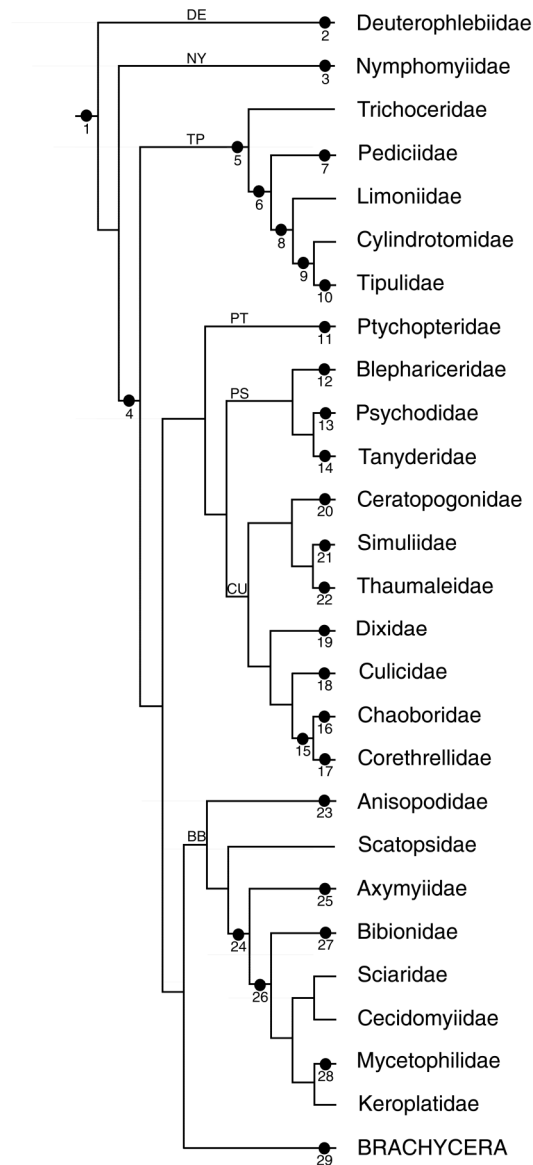
The head of adult dipterans is mainly characterized by reduction features of the mouthparts (e.g., mandibles, maxillae), linked with the minor role of feeding and the specialization on liquid substrates. In contrast, the compound eyes and the antennae, sense organs used for orientation and for finding a suitable mating partner and oviposition site are well developed. The dense vestiture of microtrichia likely creates surface properties facilitating flight. Some evolutionary novelties are apparently adaptations to feeding on more or less liquefied food, such as labellae with furrows or pseudotracheae on their surface

and the strongly developed pre- and postcerebral pumping apparatuses. In some dipteran groups specialized as blood feeders the mandibles are still present as piercing stylets. They are completely reduced in the vast majority of dipteran groups. Within the group far-reaching modifications of the antennae take place, with a strongly reduced number of segments and a specific configuration in Brachycera.

The legless dipteran larvae or maggots usually live in moist environments and several groups invaded aquatic habitats independently. Larvae of most groups, especially in Brachycera, live in the substrate they are feeding on. The feeding habits and mouthparts are much more diverse than in the adults. The larval head is prognathous and fully exposed in the dipteran groundplan and most groups of lower Diptera. The mouthparts are only slightly modified compared to the holometabolous groundplan, whereas the larval eyes show a clear tendency towards reduction. In Tipuloidea and Brachycera the head is partly or largely retracted and the sclerotized elements of the external head capsule are partly or fully reduced. Larvae of the potentially basal families Deuterophlebiidae and Nymphomyiidae and of some other groups live in streams and feed on algae and microorganisms. Adaptations to this feeding habit are the toothed hypostoma (derived groundplan feature of Diptera), the presence of a mandibular comb, and the presence of a specifically developed labral fan, which is composed of macrosetae in Deuterophlebiidae and Nymphomyiidae. The latter feature is likely a groundplan apomorphy of Diptera but the specific condition of this structure is highly variable. The larval antennae are distinctly reduced, usually one-segmented, and completely reduced in some bibionomorph groups. As the eggs are usually deposited on or in the substrate preferred by the larvae they only have to find a suitable place for pupation. The mandibles and maxillae can be modified in different ways in larvae of lower Diptera. The mandible can be subdivided or equipped with different appendages and its axis of movements can be shifted from horizontal to vertical or oblique. Elements of the maxillae can be more or less strongly reduced or fused. The labium is strongly reduced in the groundplan and can be more or less completely reduced in some groups. The head of Cyclorrhapha is largely reduced and a complex and unique feature of these groups is the cephaloskeleton. The movability of the larvae is limited due to the lack of thoracic legs. This can be partly compensated by the mouthparts which are involved in the locomotion in different groups. The mouthhooks associated with the cyclorrhaphan cephaloskeleton serve as anchorage in the substrate.

Recent investigations suggest that the dipteran radiation is characterized by three episodic bursts: lower Diptera, Brachycera and Schizophora. Profound evolutionary changes in larvae and adults took place in the dipteran stemlineage, resulting in many autapomorphies of the group as a whole. Similarly Brachycera are well characterized, mainly by larval features. The adult head structures are relatively constant within Diptera, with a high degree of homoplasy in the characters showing variation. In the radiation of lower Diptera an impressive diversity of larval forms evolved, even though the diversity in terms of species numbers remained relatively low. Like in the adults, larval characters show a high degree of homoplasy. Therefore it is not surprising that the relationships of the lower dipteran lineages could not be well resolved with the set of cephalic characters presented here. Molecular markers are apparently

more suitable to reconstruct the phylogeny of the order. However, the morphological features are essential for understanding the evolution of the group on the phenotypic level. Consequently, an evolutionary scenario for cephalic features was developed based on a phylogenetic hypothesis on the basis of an extensive molecular data set.



**Fig. 4:** Characters mapped on the cladogram of Wiegmann et al. (2011, fig. 1). Potential apomorphies indicate by black points. Numbers refers to characters in the text.

## 7 Zusammenfassung

Die Ziele der vorgelegten Arbeit waren die Dokumentation morphologischer Daten mittels innovativer Techniken (1), die Zusammenstellung eines umfangreichen morphologischen Datensatzes (2), die phylogenetische Auswertung der Merkmale und die Entwicklung eines evolutiven Szenarios für adulte und larvale Kopfstrukturen bei Dipteren.

Es wurden 28 adulte und 10 larvale Vertreter detailliert morphologisch untersucht, inklusive potentieller Schlüsseltaxa, wie beispielsweise Deuterophlebiidae (Study I), Axymyiidae (Study III), Tipulidae (Study IV) und Nymphomyiidae (Study V).

Die untersuchten Kopfmerkmale wurden definiert und in einer Datenmatrix kodiert (siehe Appendix 1, 2). Die Datensätze wurden kombiniert, sowie separat, analysiert (adulte und larvale Kopfmerkmale). Die Ergebnisse der kombinierten Analyse stützen die Monophylie der Diptera, Brachycera und Tipuloidea. Die gleichen monophyletischen Gruppen werden in der Analyse der larvalen Merkmale gestützt, nicht aber durch die Analyse adulter Kopfmerkmale. Die Analyse der adulten Kopfmerkmale unterstützt keine der allgemein anerkannten Gruppen. Die Kopfmerkmale sind größtenteils durch Homoplasien beeinflusst und dadurch eher ungeeignet für phylogenetische Untersuchungen. Deshalb wurden die morphologischen Daten auf ein aktuelles stabiles Kladogramm (Wiegmann et al. 2011), basierend auf einem umfangreichen molekularen Datensatz, aufgetragen. Auf dieser Grundlage wurde ein evolutives Szenario für adulte und larvale Kopfstrukturen entwickelt. Wichtige potentielle Autapomorphien sind im Folgenden zusammengefasst (siehe Abb. 4):

**1 Diptera:** Adulter Kopf: Kopf dicht mit Microtrichia besetzt, Clypeolabralmuskel vorhanden, Postmentum reduziert, Labialpalpen zu Labellen umgewandelt (mit einfachen Furchen besetzt). Larvaler Kopf: Labrum mit dichten Borstenfeldern, gezähntes Hypostoma (Hypostomale Platte).

**2 Deuterophlebiidae:** Adulter Kopf: Antennen inserieren lateral, weit voneinander entfernt, letztes Antennensegment extrem verlängert, Labrum, Maxille und Labium komplett reduziert (inklusive deren Muskulatur), *M. tentoriobuccalis anterior* und *M. hypopharyngosalivialis* reduziert (Homoplasie). Larvaler Kopf: externolaterale Platten mit Wachstumsstreifen vorhanden (Homoplasie), labraler Fächer besteht aus Macrosetae, *M. labroepipharyngalis* reduziert, letztes Antennensegment gegabelt, apikaler mandibulärer Kamm vorhanden (bestehend aus einer Reihe von Zähnen) (Homoplasie), überwiegend membranöser Cardo.

**3 Nymphomyiidae:** Adulter Kopf: komplett sklerotisiertes Rostrum (Homoplasie), keulenförmige Antenne mit vergrößertem ersten und verlängertem letzten Segment, Tentorium komplett reduziert, Labrum, Maxille und Labium komplett reduziert (inklusive deren Muskulatur), *M. tentoriobuccalis* reduziert (Homoplasie). Larvaler Kopf: Tentorium komplett reduziert, labraler Fächer besteht aus Macrosetae, *M. labroepipharyngalis* reduziert (Homoplasie), letztes Antennensegment gegabelt, apikaler mandibulärer Kamm vorhanden (bestehend aus einer Reihe von Zähnen) (Homoplasie).

**4 Diptera, exklusive Deuterophlebiidae und Nymphomyiidae:** Larvaler Kopf: *M. tentoriostipitalis* reduziert, pharyngealer Filterapparat vorhanden.

**5 Tipulomorpha:** Adulter Kopf: Sensillen des Sinnesfeldes auf dem dritten Segment des Maxillarpalpus befinden sich freiliegend auf der Oberfläche (Homoplasie). Larvaler Kopf: tiefe ventromediane Einschnitte der Kopfkapsel.

**6 Tipuloidea:** Adulter Kopf: Stipites teilweise miteinander verschmolzen. Larvaler Kopf: paarige dorsolaterale Einschnitte der Kopfkapsel, externolaterale Platten mit Wachstumsstreifen (Homoplasie).

**7 Pediciidae:** Larvaler Kopf: Mandibeln verlängert und sichelförmig, Maxillarpalpus deutlich verlängert.

**8 Tipuloidea, exklusive Pediciidae:** Larvaler Kopf: Kopf ist tief in den Thorax eingezogen und unbeweglich in dieser Position, prämaxilläre Naht und Seitenplatten vorhanden (Homoplasie).

**9 Tipulidae + Cylindrotomidae:** Adulter Kopf: komplett sklerotisiertes Rostrum (Homoplasie). Larvaler Kopf: bewegliche *Lacinia mobilis* (Homoplasie).

**10 Tipulidae:** Adulter Kopf: Tentorium, *Lacinia* und *Galea* komplett reduziert, Sensillen des Sinnesfeldes auf dem dritten Segment des Maxillarpalpus befinden sich jeweils in einzelnen Gruben (Homoplasie), Vertiefung auf der dosalen Seite des Labium reduziert (Homoplasie), Pseudotracheen vorhanden (Homoplasie). Larvaler Kopf: geteiltes Labrum, das sekundäre Mandibelgelenk hinter das Foramen antenale verschoben.

**11 Ptychopteromorpha:** Adulter Kopf: Sensillen des Sinnesfeldes auf dem dritten Segment des Maxillarpalpus befinden sich freiliegend auf der Oberfläche (Homoplasie), Pseudotracheen vorhanden (Homoplasie). Larvaler Kopf: Mandibel geteilt (distaler Teil durch Furche abgetrennt), Hypostomium als separate Struktur erkennbar (Homoplasie).

**12 Blephariceridae:** Adulter Kopf: Stipites komplett miteinander verschmolzen, Furchen auf der Innenseite der Labellen fehlen (Homoplasie). Larvaler Kopf: externolaterale Platten mit Wachstumsstreifen (Homoplasie).

**13 Psychodidae:** Adulter Kopf: *M. tentorioscapalis anterior* zweiteilig, ein Bündel entspringt am Tentorium, das Andere an der Kopfkapsel, 4-segmentige Maxillarpalpen (Homoplasie), *M. palpopalpalis maxillae primus* reduziert.

**14 Tanyderidae:** Adulter Kopf: *M. tentoriostipitalis* entspringt an Kopfkapsel. Larvaler Kopf: prämaxilläre Naht und Seitenplatten (Homoplasie), speziell angeordneter labraler Fächer, Hypostomium als separate Struktur erkennbar (Homoplasie).

**15 Chaoboridae + Corethrellidae:** Adulter Kopf: Furchen auf der Innenseite der Labellen fehlen (Homoplasie). Larvaler Kopf: Antenne inseriert an Vorderseite des Kopfes.

**16 Chaoboridae:** Larvaler Kopf: U-förmige Frons (Homoplasie), einfache Komplexaugen, bewegliche *Lacinia mobilis* (Homoplasie).

**17 Corethrellidae:** Adulter Kopf: Sensillen des Sinnesfeldes auf dem dritten Segment des Maxillarpalpus befinden sich jeweils in einzelnen Gruben (Homoplasie). Larvaler Kopf: Antenne mit zwei apikalen Hörnern.

**18 Culicidae:** Adulter Kopf: Gelenk zwischen Clypeus und Frons, geteilter Clypeus, Fulcrum vorhanden, Labro-epipharyngealer Nahrungskanal durch Seitenränder des Epipharynx geschlossen, M. labroepipharyngalis reduziert (Homoplasie), Stipites sind nach Innen verlagert (Homoplasie).

**19 Dixidae:** Larvaler Kopf: Median geteiltes Hypostomium.

**20 Ceratopogonidae:** Larvaler Kopf: U-förmige Frons (Homoplasie).

**21 Simuliidae:** Adulter Kopf: M. labroepipharyngalis reduziert (Homoplasie), Stipites nach Innen verlagert (Homoplasie). Larvaler Kopf: dreieckiger medianer membranöser Sinus auf der Ventralseite der Kopfkapsel vorhanden, anteriore Tentorialarme komplett reduziert (Homoplasie).

**22 Thaumaleidae:** Adulter Kopf: M. stipitopalpalis externus reduziert. Larvaler Kopf: apikaler mandibulärer Kamm vorhanden (bestehend aus einer Reihe von Zähnchen) (Homoplasie).

**23 Anisopodidae:** Adulter Kopf: Sensillen des Sinnesfeldes auf dem dritten Segment des Maxillarpalpus befinden sich jeweils in einzelnen Gruben (Homoplasie). Larvaler Kopf: stark sklerotisierte labrale Zähne vorhanden, nach anterior gerichteter Zapfen am mesalen Mandibelrand vorhanden, geteilte Mandibel (distaler Teil durch Furche abgetrennt).

**24 Bibionomorpha, exklusive Anisopodidae und Scatopsidae:** Adulter Kopf: Vertiefung auf der dosalen Seite des Labium reduziert (Homoplasie). Larvaler Kopf: anteriore Tentorialarme komplett reduziert (Homoplasie).

**25 Axymiidae:** Adulter Kopf: geteilte Komplexaugen, M. hypopharyngosalivariialis reduziert (Homoplasie). Larvaler Kopf: Kopf mäßig in den Thorax eingezogen (Homoplasie), Frontoclypealnaht vorhanden (wahrscheinlich nicht homolog mit der Naht anderer Insekten), Clypeus geteilt.

**26 Bibionomorpha, exklusive Anisopodidae, Scatopsidae und Axymiidae:** Larvaler Kopf: Lacinia und Galea reduziert oder komplett verschmolzen.

**27 Bibionidae:** Adulter Kopf: Mikrotrichia auf der Kopfkapsel reduziert (Kopf mit langen Setae besetzt), Antenne inseriert lateral. Larvaler Kopf: M. frontoepipharyngalis reduziert, Hypostomium als separate Struktur erkennbar (Homoplasie).

**28 Mycetophilidae:** Adulter Kopf: Anteriorer Tentorialarm nur teilweise hohl, Maxillarpalpus 4-segmentig (Homoplasie).

**29 Brachycera:** Adulter Kopf: Zahl der Antennenglieder und der Segmente des Maxillarpalpus reduziert, komplexe Pseudotracheen auf der Innenseite der Labellen vorhanden. Larvaler Kopf: Äußere Antennenmuskulatur komplett reduziert, cibariopharyngeale Verfestigung vorhanden.

Der Kopf adulter Dipteren ist durch zahlreiche Reduktionen der Mundwerkzeuge (z.B. Mandibeln, Maxillen) charakterisiert, da die Nahrungsaufnahme meist nur eine untergeordnete Rolle spielt. Nehmen die adulten Tiere Nahrung auf, dann ernähren sie sich meist von flüssigen Substanzen. Im Gegensatz



dazu sind Komplexaugen und Antennen, die Sinnesorgane, welche dem Auffinden eines geeigneten Sexualpartners oder einer geeigneten Stelle zur Eiablage dienen, sehr gut entwickelt. Der dichte Besatz mit Mikrotrichia dient wahrscheinlich der Verbesserung der Flugeigenschaften. Einige evolutionäre Neuerungen der Diptera sind augenscheinlich Anpassungen an die Ernährung von mehr oder weniger flüssigen Substanzen, wie die Labellen mit Furchen oder Pseudotracheen auf der Oberfläche und die kräftig ausgebildeten prä- und postcerebralen Pharynxpumpen. In einigen spezialisierten blutsaugenden Dipteregruppen sind die Mandibeln als Stechborsten ausgebildet. Allerdings sind Mandibeln bei den meisten Dipteren komplett reduziert. Innerhalb der Gruppe finden umfangreiche Änderungen der Antenne statt, wie die stark reduzierte Segmentzahl und die spezielle Ausprägung bei den Brachyceren.

Die beinlosen Larven oder Maden leben meist in feuchter Umgebung und haben mehrfach unabhängig voneinander aquatische Habitate besiedelt. Zu einem großen Teil leben sie minierend (vor allem Brachycerenlarven). In ihrer Ernährungsweise und der Struktur der Mundwerkzeuge unterscheiden sie sich deutlich mehr voneinander als die Adulten. Im Grundplan und bei den meisten Vertretern der niederen Dipteren (lower Diptera) ist der Kopf prognath und komplett freiliegend. Die Mundwerkzeuge sind bei Dipterenlarven, im Vergleich zum Grundplan der Holometabola, nur wenig modifiziert, während hingegen die Larvalaugen eine klare Tendenz zur Reduktion zeigen. Bei Larven der Tipuloidea und Brachycera ist der Kopf teilweise oder komplett in den Thorax eingezogen und die sklerotisierten Teile der Kopfkapsel sind teilweise oder komplett reduziert. Die Larven der potentiell basalsten Dipterenfamilien Deuterophlebiidae und Nymphomyiidae, sowie einiger anderer Gruppen, leben in Bächen und ernähren sich von Algen und Mikroorganismen. Anpassungen an diese Ernährungsweise sind das gezähnte Hypostoma (Autapomorphie der Diptera), ein apikaler mandibulärer Kamm und ein spezifisch ausgebildeter labraler Fächer, welcher bei den Deuterophlebiidae und Nymphomyiidae aus Macrosetae besteht. Letzteres ist möglicherweise ein Grundplanmerkmal der Dipteren, wobei sich die spezifische Ausprägung in den einzelnen Gruppen deutlich unterscheidet. Die Antenne der Larven ist weitestgehend reduziert, meist ein-segmentig und komplett reduziert bei den Bibionomorpha. Da die Eier oft direkt auf geeignetem Nahrungssubstrat abgelegt werden, müssen die Larven meist auch keine geeignete Futterquelle suchen, nur einen geeigneten Platz zur Verpuppung. Mandibeln und Maxillen können bei Dipterenlarven auf verschiedene Weise modifiziert sein. Die Mandibeln können geteilt sein oder verschiedene Anhänge haben, ihre Bewegungsachse kann von horizontal zu schräg bis hin zu vertikal verschoben sein. Die Maxillen können mehr oder weniger reduziert oder komplett verschmolzen sein. Der Kopf der Cyclorrhaphenlarven ist weitgehend reduziert und ein spezifisches Merkmal der Gruppe ist das Cephalopharyngealskelett. Durch das fehlen der Thorakalbeine ist die Beweglichkeit der Dipterenlarven stark eingeschränkt. Das wird in vielen Gruppen durch die Mundwerkzeuge kompensiert, welche dann lokomotorische Funktionen übernehmen, wie beispielsweise die Mundhaken der Cyclorrhapha, welche der Verankerung im Substrat dienen.

Aktuelle Untersuchungen deuten darauf hin, dass die Radiation der Dipteren in drei explosionsartigen Aufspaltungsphasen erfolgte: niedere Dipteren, Brachycera und Schizophora. Umfangreiche evolutionäre

Modifikationen adulter und larvaler Dipteren haben in der Stammlinie der Gruppe statt gefunden. Daraus resultierend gibt es eine Reihe apomorpher Merkmale für die gesamten Diptera. Die Brachycera sind ebenfalls klar monophyletisch und durch eine Reihe, vor allem larvaler Merkmale, charakterisiert. Adulte Kopfmerkmale sind relativ konstant innerhalb der Dipteren, wobei variierende Merkmale ein hohes Maß an Homoplasie aufweisen. Während der Ausbreitung der niederen Dipteren entstand eine hohe Diversität verschiedener Larvenformen, obwohl die Diversität bezüglich der Artenzahl relativ gering ist. Wie bei den Adulten zeigen die larvalen Merkmale ein hohes Maß an Homoplasie. Demnach ist es nicht überraschend, dass die Verwandtschaftsverhältnisse der niederen Dipteren anhand von Kopfmerkmalen allein nicht aufgeklärt werden können. Molekulare Daten sind offensichtlich geeigneter für die Aufklärung der Verwandtschaftsverhältnisse innerhalb der Gruppe. Dennoch sind die morphologischen Merkmale enorm wichtig um die Evolution der Diptera zu verstehen. Konsequenterweise wurde ein evolutives Szenario anhand der in der vorgelegten Arbeit erhobenen morphologischen Daten, basierend auf der stabilen molekularen Phylogenie, entwickelt.

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## 9 Appendix

Appendix 1. Character matrix (\$, 0/1; \$, 1/2), Adult head

	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	1	1	
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Deuterophlebiidae	0	0	0	1	2	1	0	-	1	2	0	0	0	1	1	0	1	0	0	0
Nymphomyiidae	0	0	1	1	2	1	1	1	1	0	2	1	0	1	0	0	0	0	0	1
Trichoceridae	0	0	1	0	0	1	0	-	0	1	0	0	0	0	0	0	1	1	0	0
Pediciidae	0	?	1	1	0	1	1	0	0	0	0	0	0	?	1	0	1	1	0	0
Limoniidae	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0
Cylindrotomidae	0	0	1	0	0	1	1	1	0	1	0	0	?	?	1	0	1	?	0	0
Tipulidae	0	0	1	1	\$	1	1	1	0	0	0	0	0	0	1	0	1	0	1	0
Ptychopteridae	0	0	1	0	2	1	0	-	0	0	0	0	0	0	1	0	1	1	0	0
Blephariceridae	0	0	0	0	2	1	1	0	?	0	0	?	0	0	0	0	1	1	0	0
Tanyderidae ( <i>Mischoderus</i> )	0	?	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	0
Tanyderidae ( <i>Protoplasa</i> )	0	?	1	?	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	0
Psychodidae	0	0	0	1	0	1	0	-	0	0	0	0	?	0	1	0	1	1	0	0
Ceratopogonidae	0	?	0	0	2	1	0	-	0	0	0	0	1	0	\$	0	?	1	0	0
Thaumaleidae	0	0	1	1	0	1	0	-	0	1	0	0	1	0	1	0	1	1	0	0
Simuliidae	1	0	0	0	2	1	0	-	0	0	1	0	1	0	1	0	1	1	0	0
Culicidae ( <i>Aedes</i> )	1	0	1	0	1	0	0	-	0	0	0	0	1	0	1	0	0	0	0	0
Culicidae ( <i>Anopheles</i> )	1	?	1	0	1	?	0	-	0	0	0	0	1	0	1	0	?	?	0	0
Culicidae ( <i>Culex</i> )	1	0	1	0	1	0	0	-	0	0	0	0	1	0	1	0	0	?	0	0
Culicidae ( <i>Culiseta</i> )	1	?	1	0	1	0	0	-	0	0	0	0	1	0	1	0	0	1	0	0
Corethrellidae	1	0	1	0	0	1	0	-	0	0	0	0	1	0	1	0	1	1	0	0
Chaoboridae	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	1	0	0
Anisopodidae	1	0	1	1	0	1	1	2	0	0	0	0	0	0	0	0	1	1	0	0
Scatopsidae	0	0	0	1	0	1	0	-	0	0	1	0	0	0	0	0	1	1	0	0
Axymyiidae	1	0	0	1	2	1	0	-	0	0	1	0	0	0	0	1	1	0	0	0
Bibionidae	0	1	1	1	0	1	0	-	0	2	1	0	0	0	0	0	1	0	0	0
Sciaridae	1	0	0	-	2	1	0	-	0	0	0	0	?	0	0	0	?	?	0	0
Cecidomyiidae	1	0	0	0	0	?	0	-	0	0	0	0	0	0	1	0	1	1	0	0
Mycetophilidae	0	0	?	0	0	1	0	-	0	0	0	0	?	0	1	0	1	1	0	0
Keroplastidae	1	?	0	0	?	?	0	-	?	0	?	1	?	?	1	0	?	1	0	0
Syrphidae	1	?	0	1	0	1	1	2	1	1	2	1	0	1	0	0	1	0	0	0
Tabanidae	1	?	0	0	0	1	0	-	0	0	2	1	?	0	0	0	1	0	0	0
Stratiomyiidae	1	1	?	0	0	1	0	-	0	0	2	0	0	1	0	0	1	?	0	0
Asilidae	1	0	0	1	?	1	0	-	1	0	0	0	0	1	0	0	?	0	0	0
Bombyliidae ( <i>Bombylius</i> )	1	?	1	1	0	1	1	2	1	0	0	1	?	1	?	0	1	?	0	0
Bombyliidae ( <i>Hemipenthes</i> )	0	?	0	1	0	1	1	2	1	0	0	0	?	0	?	0	1	?	0	0
Drosophilidae	1	0	0	1	0	1	1	2	1	0	2	1	1	1	0	0	0	1	0	0
Nannochoristidae	0	0	0	0	0	0	0	-	0	0	0	0	0	?	0	0	0	1	0	0
Meropidae	1	1	0	?	0	1	1	0	0	0	?	?	0	0	1	0	1	0	0	0
Boreidae ( <i>Caurinus</i> )	1	1	0	1	0	0	0	-	0	2	0	0	1	?	1	0	1	0	0	0
Panorpidae	1	?	1	1	0	1	1	-	?	0	0	0	?	?	0	0	0	0	0	0
Siphonaptera ( <i>Ctenocephalus</i> )	0	1	0	1	2	1	0	-	0	2	2	0	1	0	1	-	1	0	0	0

	2 0	2 1	2 2	2 3	2 4	2 5	2 6	2 7	2 8	2 9	3 0	3 1	3 2	3 3	3 4	3 5	3 6	3 7	3 8	
Deuterophlebiidae	2	0	0	1	-	-	0	-	-	-	-	-	-	-	§	1	-	1	-	-
Nymphomyiidae	-	-	-	1	-	-	0	-	-	-	-	-	-	-	1	1	2	1	-	-
Trichoceridae	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1	-	-	
Pediciidae	2	2	0	0	0	0	0	0	0	0	0	0	1	0	1	?	1	-	-	
Limoniidae	2	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	-	-	
Cylindrotomidae	2	2	0	0	0	0	0	0	0	0	1	-	1	0	-	0	1	-	-	
Tipulidae	-	-	-	0	0	1	0	0	0	0	0	0	0	1	1	1	2	1	-	-
Ptychopteridae	2	0	?	0	0	1	0	0	0	0	0	0	1	0	0	1	1	-	-	
Blephariceridae	2	0	0	0	1	-	0	?	?	0	?	?	1	0	1	?	§	-	-	
Tanyderidae ( <i>Mischoderus</i> )	1	0	0	0	0	1	0	0	0	?	0	1	1	0	?	0	1	-	-	
Tanyderidae ( <i>Protoplasa</i> )	?	?	?	0	0	1	1	?	?	?	?	?	?	?	?	?	1	-	-	
Psychodidae	2	0	0	0	0	0	0	0	0	0	1	1	§	§	§	§	1	-	-	
Ceratopogonidae	2	0	0	0	0	0	0	0	3	0	0	0	1	0	1	0	0	0	0	
Thaumaleidae	2	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	-	-		
Simuliidae	1	0	?	0	0	1	0	0	0	1	0	?	0	0	0	-	0	1	0	
Culicidae ( <i>Aedes</i> )	2	0	0	0	0	0	1	0	1	1	0	1	0	0	0	-	0	0	0	
Culicidae ( <i>Anopheles</i> )	?	0	?	0	0	0	?	0	1	1	0	?	0	0	0	-	0	0	0	
Culicidae ( <i>Culex</i> )	2	0	0	0	0	0	1	0	1	1	0	1	0	0	0	-	0	0	0	
Culicidae ( <i>Culiseta</i> )	1	0	0	0	0	0	1	0	1	1	0	1	0	0	0	-	0	0	0	
Corethrellidae	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	-	-	
Chaoboridae	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	-	-	
Anisopodidae	2	0	0	0	0	0	0	0	0	0	?	?	?	0	0	1	1	-	-	
Scatopsidae	2	1	0	0	0	0	0	0	0	1	?	?	0	0	0	?	1	-	-	
Axymyiidae	2	0	0	0	0	0	0	0	0	0	1	-	1	1	1	-	1	-	-	
Bibionidae	2	0	?	0	0	1	0	0	0	0	0	0	0	0	1	1	-	-		
Sciaridae	2	0	0	0	0	?	?	0	0	0	0	1	0	0	1	1	-	-		
Cecidomyiidae	2	2	0	0	?	?	0	1	-	0	0	?	1	1	1	1	1	-	-	
Mycetophilidae	2	1	0	0	0	1	0	1	-	0	0	1	1	§	0	1	1	-	-	
Keroplastidae	2	0	0	0	?	?	0	1	-	0	0	1	1	0	1	0	1	-	-	
Syrphidae	1	2	0	0	0	1	1	0	2	0	0	1	1	0	1	0	1	-	-	
Tabanidae	0	0	0	0	0	1	0	0	3	0	0	1	1	0	0	1	0	0	§	
Stratiomyiidae	1	0	0	0	?	1	0	0	0	1	1	-	1	1	-	1	1	-	-	
Asilidae	2	0	?	0	0	?	0	0	1	?	?	?	1	0	0	-	1	-	-	
Bombyliidae ( <i>Bombylius</i> )	?	?	?	0	0	1	1	0	2	0	0	?	1	0	1	0	1	-	-	
Bombyliidae ( <i>Hemipenthes</i> )	?	?	?	0	0	1	1	0	2	0	0	?	1	0	1	0	1	-	-	
Drosophilidae	2	2	1	0	0	1	1	?	?	0	1	-	1	?	?	?	1	-	-	
Nannochoristidae	1	0	1	0	0	0	0	0	0	1	1	-	1	0	0	1	0	1	1	
Meropidae	0	?	0	0	0	0	0	?	?	0	?	?	1	0	0	0	0	1	0	
Boreidae ( <i>Caurinus</i> )	0	2	0	0	0	0	0	1	-	1	1	-	1	0	0	0	0	1	0	
Panorpidae	2	?	0	0	1	-	0	1	-	0	1	-	1	0	0	3	0	1	0	
Siphonaptera ( <i>Ctenocephalus</i> )	2	-	0	0	1	-	0	0	0	1	1	-	1	1	1	-	1	-	-	

	3	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	
	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7
Deuterophlebiidae	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nymphomyiidae	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoceridae	-	-	0	0	0	1	0	2	0	0	0	0	0	0	?	0	1	0	0
Pediciidae	-	-	0	?	?	?	?	?	0	1	1	0	0	0	1	0	0	0	1
Limoniidae	-	-	0	0	0	1	0	2	1	1	1	0	1	1	1	0	1	0	1
Cylindrotomidae	-	-	0	0	?	?	?	?	1	2	1	0	-	0	1	0	0	0	0
Tipulidae	-	-	0	2	0	1	0	1	1	2	1	0	1	1	1	0	0	0	1
Ptychopteridae	-	-	0	?	0	1	0	2	0	0	1	0	0	0	1	0	1	1	0
Blephariceridae	-	-	0	0	0	0	0	0	0	2	1	0	0	0	1	0	0	0	1
Tanyderidae ( <i>Mischoderus</i> )	-	-	0	0	0	0	?	?	0	0	1	0	0	1	1	0	1	0	0
Tanyderidae ( <i>Protoplasa</i> )	-	-	0	0	0	1	?	?	0	0	1	?	?	?	?	?	?	?	?
Psychodidae	-	-	0	0	1	0	1	-	0	0	1	0	0	0	1	0	1	1	1
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	?
Thaumaleidae	-	-	0	0	0	1	1	-	0	0	1	0	0	-	1	1	1	0	1
Simuliidae	0	0	0	0	0	?	0	0	1	0	1	0	0	0	0	0	1	0	1
Culicidae ( <i>Aedes</i> )	0	1	0	0	0	0	?	?	1	0	1	0	0	0	0	1	0	0	?
Culicidae ( <i>Anopheles</i> )	0	0	0	0	0	0	?	?	1	?	?	0	?	?	?	?	?	?	?
Culicidae ( <i>Culex</i> )	0	1	0	0	0	1	-	1	0	?	0	0	0	0	?	1	?	?	?
Culicidae ( <i>Culiseta</i> )	§	1	0	0	1	0	1	-	1	0	1	0	0	0	0	0	1	0	0
Corethrellidae	-	-	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0
Chaoboridae	-	-	0	0	0	1	1	-	0	0	1	?	?	?	?	0	?	0	1
Anisopodidae	-	-	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	1
Scatopsidae	-	-	0	0	2	1	-	-	0	1	1	0	-	0	1	1	0	1	1
Axymyiidae	-	-	0	2	0	0	1	-	?	?	?	0	-	-	1	1	1	1	1
Bibionidae	-	-	0	0	0	0	0	0	0	0	1	0	-	0	1	0	§	?	?
Sciaridae	-	-	0	0	2	1	1	-	0	1	1	0	0	0	1	0	1	1	1
Cecidomyiidae	-	-	0	2	2	1	1	-	0	0	1	0	0	0	1	?	?	?	?
Mycetophilidae	-	-	0	0	1	1	1	-	0	0	1	0	0	0	1	0	1	0	1
Keroplastidae	-	-	0	0	0	0	?	?	0	0	1	0	1	0	1	0	0	0	0
Syrphidae	-	-	0	0	2	1	-	-	1	0	1	0	2	0	1	1	1	1	1
Tabanidae	§	§	0	0	2	0	-	-	§	0	0	0	1	0	§	1	1	0	1
Stratiomyiidae	-	-	0	0	2	0	-	-	0	0	1	0	0	0	1	1	1	1	1
Asilidae	-	-	0	0	?	?	?	?	?	2	1	?	?	?	?	?	?	?	?
Bombyliidae ( <i>Bombylius</i> )	-	-	0	0	?	?	?	?	?	1	?	1	0	2	0	0	1	1	1
Bombyliidae ( <i>Hemipenthes</i> )	-	-	0	0	?	?	?	?	?	1	?	1	0	2	0	0	1	1	1
Drosophilidae	-	-	0	0	2	0	-	-	1	0	1	0	-	1	0	1	1	1	-
Nannochoristidae	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1
Meropidae	0	1	0	1	0	0	1	-	0	0	0	0	0	0	0	0	1	0	1
Boreidae ( <i>Caurinus</i> )	0	0	0	1	0	0	1	-	0	1	1	0	0	0	0	0	1	1	1
Panorpidae	0	0	0	1	0	0	1	-	0	0	0	0	1	0	0	0	?	0	0
Siphonaptera ( <i>Ctenocephalus</i> )	-	-	0	0	1	0	1	-	0	0	1	0	-	-	0	0	?	0	1



	5 8	5 9	6 0	6 1	6 2	6 3	6 4	6 5	6 6	6 7	6 8	6 9	7 0	7 1	7 2	7 3	7 4	7 5	7 6
Deuterophlebiidae	1	-	-	-	-	-	-	-	-	-	-	-	1	2	1	1	0	1	1
Nymphomyiidae	1	-	-	-	-	-	-	-	-	-	-	-	1	0	\$	1	0	1	0
Trichoceridae	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Pediciidae	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0
Limoniidae	0	1	0	1	0	0	0	1	0	0	0	1	1	0	1	0	0	1	1
Cylindrotomidae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
Tipulidae	0	1	1	0	0	0	1	-	0	0	0	0	0	0	1	0	0	1	0
Ptychopteridae	0	1	0	0	0	0	1	-	0	0	0	0	1	0	\$	0	0	?	0
Blephariceridae	0	1	0	?	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Tanyderidae ( <i>Mischoderus</i> )	0	1	0	1	0	0	0	?	?	0	0	0	0	0	0	0	0	1	0
Tanyderidae ( <i>Protoplasa</i> )	0	\$	?	?	0	0	?	?	0	0	?	?	?	?	?	?	?	?	?
Psychodidae	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0
Ceratopogonidae	0	0	0	1	0	0	0	0	0	0	0	0	1	0	\$	0	0	?	0
Thaumaleidae	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	?	0
Simuliidae	0	1	0	1	0	0	0	?	0	0	0	0	1	1	0	0	0	1	0
Culicidae ( <i>Aedes</i> )	0	?	0	0	0	0	0	?	1	0	0	1	0	1	0	0	0	1	0
Culicidae ( <i>Anopheles</i> )	0	?	0	0	0	0	0	0	1	0	0	?	0	0	0	0	0	?	0
Culicidae ( <i>Culex</i> )	0	?	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	?	0
Culicidae ( <i>Culiseta</i> )	0	0	?	0	0	\$	0	0	1	0	0	1	0	0	0	0	0	0	0
Corethrellidae	0	1	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0
Chaoboridae	0	1	0	1	0	0	0	1	0	0	?	?	?	?	?	0	0	?	0
Anisopodidae	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0
Scatopsidae	0	1	0	1	?	0	0	1	0	0	0	1	0	0	1	0	0	1	0
Axymyiidae	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	?	1
Bibionidae	0	1	1	0	0	0	0	0	0	0	\$	0	1	0	1	0	0	?	0
Sciaridae	0	1	1	1	0	0	0	1	0	0	0	1	1	0	1	0	0	1	?
Cecidomyiidae	0	1	1	0	0	0	0	1	0	0	0	?	1	0	1	0	0	1	0
Mycetophilidae	0	1	1	0	0	0	1	-	0	0	0	0	1	0	1	0	0	1	0
Keroplastidae	0	1	1	1	0	0	0	?	?	0	0	0	1	0	0	0	0	1	0
Syrphidae	0	1	0	1	0	0	1	-	0	0	0	1	0	0	2	1	1	?	0
Tabanidae	0	1	0	0	0	0	1	-	0	0	0	1	0	0	0	0	0	1	0
Stratiomyiidae	0	1	1	0	0	0	1	-	0	0	0	1	1	0	0	0	0	?	?
Asilidae	0	1	?	?	0	0	0	1	0	0	0	?	1	0	1	0	0	1	0
Bombyliidae ( <i>Bombylius</i> )	0	1	0	0	0	0	1	-	0	0	0	1	1	0	1	0	1	?	0
Bombyliidae ( <i>Hemipenthes</i> )	0	1	0	1	0	0	1	-	0	0	0	1	1	0	1	0	1	?	0
Drosophilidae	0	1	?	?	0	0	1	-	0	0	1	1	1	0	0	0	1	?	0
Nannochoristidae	0	1	0	0	0	1	-	1	1	0	0	1	0	1	2	1	0	1	0
Meropidae	0	0	?	?	0	1	-	-	0	0	0	1	0	0	0	1	0	?	?
Boreidae ( <i>Caurinus</i> )	0	0	1	1	0	1	-	?	0	0	0	1	0	2	0	1	0	?	1
Panorpidae	0	0	1	0	0	1	-	?	0	0	0	1	1	0	2	1	0	1	1
Siphonaptera ( <i>Ctenocephalus</i> )	0	0	0	0	2	1	-	?	?	0	-	1	0	0	0	1	0	?	0

Appendix 2. Character matrix (\$, 0/1; \$, 1/2; \*, 0/2), larval head.

	7	7	7	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9
	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5
Deuterophlebiidae	0	0	0	0	1	1	1	2	-	1	0	-	0	0	1	0	0	1	2
Nymphomyiidae	0	0	0	0	0	0	1	1	0	1	0	-	0	1	-	-	-	1	2
Trichoceridae	0	0	0	1	0	0	1	2	0	1	?	0	0	0	0	2	0	1	2
Pediciidae	1	0	1	1	1	0	1	0	?	1	0	0	1	1	-	-	-	?	3
Limoniidae	2	0	2	1	1	0	1	2	0	0	1	1	1	0	1	2	0	0	3
Tipulidae	2	0	2	1	1	0	1	2	0	1	1	1	1	1	-	-	-	1	2
Ptychopteridae	0	0	0	0	0	0	1	1	-	1	0	-	0	0	1	2	0	1	2
Blephariceridae	0	0	\$	\$	1	0	1	0	0	1	?	0	0	0	?	2	0	?	3
Tanyderidae	0	0	0	0	1	0	1	0	?	1	1	?	1	0	1	2	0	1	3
Psychodidae	0	0	0	0	0	1	1	2	0	1	0	?	0	0	1	2	0	?	3
Dixidae	0	0	0	0	0	0	1	2	0	1	0	?	0	0	1	2	0	?	2
Chironomidae	0	0	0	0	0	0	1	2	0	1	0	?	0	0	1	2	\$	1	2
Ceratopogonidae	0	0	0	0	0	1	1	2	1	1	0	?	0	0	0	2	0	1	2
Thaumaleidae	0	0	0	0	0	1	1	0	-	1	0	-	0	1	-	-	-	1	?
Simuliidae	0	0	0	2	0	1	1	2	-	1	0	?	0	0	1	2	1	1	2
Culicidae ( <i>Anopheles</i> )	0	0	0	0	0	1	1	2	?	1	0	-	0	0	1	2	0	1	2
Culicidae ( <i>Culiseta</i> )	0	0	0	0	0	1	1	2	?	1	0	-	0	0	1	2	0	1	2
Corethrellidae	0	0	0	0	0	0	?	2	?	1	0	?	0	0	1	2	0	1	?
Chaoboridae	0	0	0	0	0	1	1	\$	1	1	0	-	0	0	1	2	0	1	0
Anisopodidae	0	0	0	0	0	0	1	0	0	1	0	?	?	0	0	1	0	?	3
Axymyiidae	1	0	0	0	0	1	0	0	0	0	0	0	1	1	-	-	-	1	3
Bibionidae	0	0	0	0	0	0	1	0	0	1	?	0	1	0	1	0	1	1	2
Cecidomyiidae	0	0	0	0	0	0	1	2	?	1	0	?	0	0	?	1	0	1	2
Mycetophilidae	0	0	0	0	0	0	1	2	0	1	0	-	0	1	-	-	-	1	?
Tabanidae	2	0	0	3	0	0	1	2	-	?	0	?	0	0	1	2	2	?	3
Stratiomyiidae	1	0	0	3	0	0	1	2	-	1	0	?	0	0	1	2	0	0	2
Therevidae	0	0	0	3	?	0	1	?	-	1	0	?	0	0	1	2	2	?	3
Nannochoristidae	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	2
Panorpidae	0	1	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0
Siphonaptera ( <i>Nosopsyllus</i> )	0	0	0	0	0	0	1	2	?	?	0	?	0	0	1	0	0	?	3

	9 6	9 7	9 8	9 9	1 0 0	1 0 1	1 0 2	1 0 3	1 0 4	1 0 5	1 0 6	1 0 7	1 0 8	1 0 9	1 1 0	1 1 1	1 1 2	1 1 3	1 1 4
Deuterophlebiidae	2	0	0	4	0	§	1	1	0	0	1	0	0	1	0	0	1	0	0
Nymphomyiidae	2	1	0	4	0	1	1	1	0	0	1	0	1	0	1	0	1	0	0
Trichoceridae	2	1	0	1	0	1	?	?	?	1	1	0	1	0	?	0	0	0	0
Pediciidae	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1
Limoniidae	0	0	0	1	0	1	?	?	?	§	?	0	1	0	?	0	?	0	0
Tipulidae	0	0	1	1	0	1	1	1	0	0	1	0	1	0	1	0	0	1	0
Ptychopteridae	0	0	0	1	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0
Blephariceridae	?	1	0	1	0	1	?	?	?	0	§	0	1	0	?	0	1	0	0
Tanyderidae	0	0	0	2	0	?	0	1	0	0	1	0	0	0	0	0	0	0	0
Psychodidae	0	1	0	1	0	1	?	?	?	1	1	0	1	0	?	0	1	0	0
Dixidae	0	0	0	2	0	1	1	1	0	1	1	0	1	0	0	0	1	0	0
Chironomidae	0	0	0	3	0	1	0	1	0	1	1	0	1	0	1	0	*	0	0
Ceratopogonidae	0	0	0	1	0	1	1	1	0	1	0	1	1	0	?	0	1	0	0
Thaumaleidae	0	1	0	2	0	1	1	1	0	1	0	0	1	0	1	0	1	0	0
Simuliidae	2	0	0	2	0	1	1	1	0	2	1	0	0	0	?	0	1	0	0
Culicidae ( <i>Anopheles</i> )	0	0	0	2	0	1	1	1	0	1	1	0	1	0	0	0	1	0	0
Culicidae ( <i>Culiseta</i> )	0	0	0	2	0	1	1	1	0	1	?	0	1	0	0	0	1	0	0
Corethrellidae	?	?	?	1	?	?	1	0	0	0	1	1	1	0	0	0	0	0	0
Chaoboridae	0	0	0	2	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0
Anisopodidae	0	1	0	1	1	0	?	?	?	1	1	0	0	0	?	0	1	0	0
Axymyiidae	1	1	0	2	0	1	1	1	0	0	1	0	2	-	-	0	0	0	0
Bibionidae	1	0	0	0	0	0	1	1	1	0	1	0	2	-	-	0	0	0	0
Cecidomyiidae	2	0	0	?	?	0	1	1	1	?	?	0	1	0	1	0	0	0	0
Mycetophilidae	0	0	0	0	0	?	1	1	0	0	?	0	2	-	-	0	0	0	0
Tabanidae	2	1	0	0	0	?	0	1	0	0	0	0	1	0	0	1	1	0	0
Stratiomyiidae	2	1	0	0	0	?	1	1	1	0	1	9	1	0	1	1	1	0	0
Therevidae	0	1	0	0	0	?	1	1	1	0	1	0	1	0	1	1	1	0	0
Nannochoristidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Panorpidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Siphonaptera ( <i>Nosopsyllus</i> )	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0

	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3	3	3	3
	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3
Deuterophlebiidae	0	0	1	1	0	1	1	0	2	1	0	1	1	1	2	0	0	1	?
Nymphomyiidae	0	0	0	1	0	0	1	2	2	2	0	1	1	1	2	1	0	1	0
Trichoceridae	0	1	0	0	0	1	?	1	2	2	0	?	?	?	?	0	0	1	?
Pediciidae	0	0	0	0	0	1	1	1	2	2	1	1	1	1	2	1	2	1	0
Limoniidae	0	0	0	0	0	1	?	1	2	2	0	?	?	?	?	1	1	1	0
Tipulidae	1	0	0	0	0	1	1	1	*	2	0	1	1	1	2	1	1	1	0
Ptychopteridae	0	0	1	0	1	1	1	1	2	2	0	1	1	1	2	1	0	1	1
Blephariceridae	0	0	0	§	0	§	?	1	0	2	0	?	?	?	?	0	0	1	0
Tanyderidae	0	0	0	0	0	0	1	1	0	2	0	1	1	1	2	1	0	1	1
Psychodidae	0	§	§	0	0	1	?	2	2	2	0	?	?	?	?	?	0	§	0
Dixidae	0	0	1	0	0	1	1	1	2	2	0	1	1	1	2	1	1	1	0
Chironomidae	0	0	§	0	?	0	1	2	2	1	0	1	1	1	2	1	0	0	0
Ceratopogonidae	0	0	0	0	0	1	1	?	2	2	0	1	1	1	2	0	0	1	0
Thaumaleidae	?	0	0	1	0	0	1	?	?	?	?	?	?	?	?	0	0	1	0
Simuliidae	0	0	1	0	0	1	1	-	2	2	0	1	1	1	2	1	0	0	0
Culicidae ( <i>Anopheles</i> )	0	0	1	0	0	0	1	2	2	2	0	1	1	1	2	1	0	1	0
Culicidae ( <i>Culiseta</i> )	0	0	1	0	0	0	1	2	2	2	0	1	1	1	2	1	0	1	0
Corethrellidae	0	0	0	?	0	?	1	?	?	2	0	?	?	?	2	1	0	1	0
Chaoboridae	1	0	1	0	0	0	1	-	2	2	0	1	1	1	2	0	0	1	0
Anisopodidae	0	1	1	0	1	1	?	1	0	2	0	?	?	?	?	?	0	0	0
Axymyiidae	0	0	0	0	0	0	1	2	1	1	0	1	1	1	2	0	0	1	0
Bibionidae	0	0	0	0	0	?	1	1	2	1	0	1	1	1	2	0	0	1	1
Cecidomyiidae	0	0	0	0	0	0	1	?	2	2	0	1	1	1	2	?	?	1	?
Mycetophilidae	0	0	0	0	0	?	1	?	?	-	-	?	?	?	2	0	0	1	?
Tabanidae	0	0	0	0	2	0	1	1	2	2	0	1	1	1	2	0	0	0	?
Stratiomyiidae	0	0	0	0	2	1	1	?	2	2	0	1	1	1	2	0	0	0	?
Therevidae	0	0	0	0	2	1	1	1	2	1	0	1	1	1	2	0	0	0	?
Nannochoristidae	1	0	0	0	0	0	0	1	0	0	0	1	1	1	2	0	0	1	0
Panorpidae	0	0	0	0	0	0	1	1	1	0	0	1	1	§	1	0	?	0	?
Siphonaptera ( <i>Nosopsyllus</i> )	0	0	0	0	0	1	1	1	2	1	0	0	1	1	1	?	?	1	?

	1	1	1	1	1	1	1	1	1	1	1	1	
	3	3	3	3	3	3	4	4	4	4	4	4	
	4	5	6	7	8	9	0	1	2	3	4	5	6
Deuterophlebiidae	1	1	1	1	1	1	1	1	1	0	1	0	0
Nymphomyiidae	0	1	1	1	1	1	1	1	1	1	2	2	0
Trichoceridae	0	1	?	?	?	?	?	0	?	?	?	?	0
Pediciidae	1	1	1	1	1	1	1	1	1	1	2	2	0
Limoniidae	1	1	?	?	?	?	?	1	?	?	?	?	0
Tipulidae	0	1	1	1	1	0	1	1	1	1	2	2	0
Ptychopteridae	?	1	1	1	1	0	1	0	0	0	0	0	0
Blephariceridae	1	1	?	?	?	?	?	0	?	?	?	?	0
Tanyderidae	1	1	1	1	1	0	1	0	1	0	0	1	0
Psychodidae	1	1	?	?	?	?	?	?	?	?	?	?	0
Dixidae	1	1	1	1	1	1	1	?	1	0	0	0	0
Chironomidae	0	1	1	1	1	?	?	1	?	?	?	1	0
Ceratopogonidae	1	1	1	1	1	1	1	1	1	0	2	?	0
Thaumaleidae	0	1	1	2	1	0	1	1	0	1	1	2	0
Simuliidae	1	1	1	1	1	1	1	1	1	1	1	1	0
Culicidae ( <i>Anopheles</i> )	0	1	1	1	1	1	1	0	1	0	0	0	0
Culicidae ( <i>Culiseta</i> )	0	1	1	1	1	1	1	0	1	1	1	0	0
Corethrellidae	1	1	1	2	1	0	1	?	0	1	1	0	0
Chaoboridae	1	1	1	1	1	1	1	0	1	0	0	0	0
Anisopodidae	1	1	?	?	?	?	?	1	?	?	?	?	0
Axymyiidae	1	1	1	2	1	0	1	0	0	0	1	2	0
Bibionidae	1	1	1	1	1	1	1	1	0	0	1	1	0
Cecidomyiidae	?	1	1	1	1	1	1	1	?	0	0	?	0
Mycetophilidae	?	1	1	2	1	0	1	0	1	1	2	1	0
Tabanidae	0	1	1	2	1	?	1	?	1	1	2	2	1
Stratiomyiidae	1	1	1	1	1	?	1	?	1	1	2	2	1
Therevidae	0	1	1	2	1	?	1	?	1	1	2	2	1
Nannochoristidae	1	1	1	2	1	0	0	1	0	0	0	0	0
Panorpidae	1	0	1	2	1	0	1	1	0	0	0	0	0
Siphonaptera ( <i>Nosopsyllus</i> )	1	1	1	1	1	0	?	1	0	0	0	0	0

Appendix 3. Adult head muscle homology with the muscular abbreviations. Abbreviations: +, present; ++, unusually large; -, absent; ?: unclear homology.

		<b>Deuterophlebiidae</b>	<b>Nymphomyiidae</b>	<b>Trichoceridae</b>	<b>Pediciidae</b>	<b>Limoniidae</b>	<b>Cylindrotomidae</b>	<b>Tipulidae</b>	<b>Ptychopteridae</b>	<b>Blephariceridae</b>	<b>Tanyderidae</b>	<b>Psychodidae</b>	<b>Ceratopogonidae</b>	<b>Thaumaleidae</b>
		<i>Deuterophlebia</i> Schneeberg et al. 2011	<i>Nymphomyia</i> Tokunaga 1935	<i>Trichocera</i> Schneeberg & Beutel 2011	<i>Pedicia</i> present study	<i>Limonia</i> Schneeberg & Beutel 2011	<i>Cylindrotoma</i> present study	<i>Tipula</i> Neugart et al. 2009	<i>Ptychoptera</i> present study	<i>Edwardsina</i> Schneeberg et al. 2011	<i>Mischoderus</i> Schneeberg & Beutel 2011	<i>Psychoda</i> present study	<i>Culicoides</i> Gad 1951	<i>Andrososopa</i> present study
M.1	0an1	+	6	+	+	+	+	+	+	+	+	+	a1	+
M.2	0an2	+	5	+	+	+	-	+	+	+	?	+	a3	+
M.3	0an3	-	-	-	-	-	-	-	-	-	?	-	-	-
M.4	0an4	-	4	+	+	?	+	+	+	-	?	+	a2	+
M.5	0an6	+	3	+	+	+	+	+	+	+	?	+	a4	+
M.6	0an7	+	1,2	+	+	+	+	+	+	+	?	+	a5	+
M.7	0lb5	-	-	+	+	+	+	+	+	+	?	++	b2	+
M.8	0lb1	-	-	+	+	+	?	+	+	?	+	+	b1	+
M.9	0lb2	-	-	-	-	-	-	-	-	-	-	-	-	-
M.10	0lb3	-	-	-	-	-	-	-	-	-	-	-	-	-
M.11	0md1	-	-	-	-	-	-	-	-	-	-	-	c1	-
M.12	0md3	-	-	-	-	-	-	-	-	-	-	-	c3	-
M.13	0md4	-	-	-	-	-	-	-	-	-	-	-	c2	-
M.14	0md6, 0md8 ?	-	-	-	-	-	-	-	-	-	-	-	-	-
M.15	0mx1	-	-	-	-	-	-	-	-	-	-	-	-	-
M.16	0mx1	-	-	-	-	-	-	-	-	-	-	-	-	-
M.17	0mx3	-	-	+	+	+	-	+	-	+	+	+	d1	+
M.18	0mx4, 0mx5	-	-	+	+	+	+	+	+	+	+	+	d2	-
M.19	0mx2	-	-	?	-	-	-	-	-	-	-	-	d3	-
M.20	0mx6	-	-	-	-	-	-	-	-	-	-	-	d4?	+
M.21	0mx7	-	-	-	-	-	-	-	-	-	-	-	-	-



M.22	0mx8	-	-	+	++	+	+	++	++	+	+	+	d5	-
M.23	0mx10	-	-	-	+	-	+	+	-	+	-	-	d6	+
M.24	0mx12	-	-	+	+	+	+	+	-	+	+	-	-	+
M.25	0mx13	-	-	+	-	-	+	-	+	-	+	-	-	-
M.26	0mx14	-	-	-	-	?	-	-	?	-	-	-	-	+
M.27	0mx15	-	-	-	-	?	-	-	?	-	-	-	-	-
M.28	0la8	-	-											
M.29	0la4, 0la5	-	-	+	+	+	+	+	+	+	+	+	e1	+
M.30	0la6	-	-											
M.31	0la11	-	-	-	-	-	-	-	-	-	-	-	-	-
M.32	0la12	-	-	-	-	-	-	-	-	-	-	-	-	-
M.33	0la13	-	-	+	+	+	+	+	+	+	+	+	e2	
M.34	0la14	-	-	+	+	+	+	+	+	+	+	+	e3	+
M.35	0la16	-	-	-	-	-?	+	+	-	+	+	-	e4	+
M.36	0la17	-	-	-	-	-	-	-	-	-	-	-	-	-
M.37	0hy12	-	8	+	+	-	+	+	?	+	+	+	h	+
M.38	0hy7	-	-	-	-	-	-	-	?	-	-	-	-	-
M.39	0hy8	-	-	-	-	-	-	-	?	-	-	-	-	-
M.40	0hy13	-	-	-	-	-	-	-	?	-	-	+	-	?
M.41	0hy1	-	-	-	-	-	-	+	-	+	+	-	-	-
M.42	0hy3	-	-	-?	-	-	-	-	-	-	-	-	-	-
M.43	0ci1	+	+	++	++	++	++	++	++	++	++	++	f1	++
M.44	0bu1	-	-	+	+	+	+	+	+	+	+	-	f2	?
M.45	0bu2	-	9	+		-	-	-	-	++	+	+		
M.46	0bu3	-	-	+	+	+	+	+	+	+	+	+	g1	+
M.47	0hy2	-	-	-	-	-	-	-	-	-	-	+	-	-
M.48	0hy5?, 0bu5	-	-	+	+	+	+	+	-	+	+	+	f3	+
M.49	0bu4	-	-	-	-	-	-	-	-	-	-	-	-	-
M.50	0bu6	-	-	-	-	-	-	-	-	-	-	-	-	-
M.51	0ph1	+	10	++	++	++	++	++	++	++	++	++	g2	+
M.52	0ph2	+	11	++	++	++	++	++	++	++	++	++	g3	+
M.67	0hy9	+	-	+	?	+	?	+	?	+	+	+	?	+
M.68	0st1	+	12	+	+	+	+	+	?	+	+	+	?	+
M.69	0st2	-	-	+	?	-	?	-	?	-	?	-	?	-

		<b>Simuliidae</b>	<b>Culicidae</b>	<b>Culicidae</b>	<b>Corethrellidae</b>	<b>Anisopodidae</b>	<b>Scatopsidae</b>	<b>Axymyiidae</b>	<b>Bibionidae</b>	<b>Sciaridae</b>	<b>Cecidomyiidae</b>	<b>Mycetophilidae</b>	<b>Keroplatidae</b>	<b>Syrphidae</b>	<b>Tabanidae</b>
	v. Kéler 1963	<i>Wilhelmia</i> Wenk 1962	<i>Aedes</i> Christophers 1960	<i>Culiseta</i> Schliemanz 1957; Owen 1985	<i>Corethrella</i> present study	<i>Sylvicola</i> present study	<i>Coboldia</i> present study	<i>Axymyia</i> Schneeberg et al. 2013b	<i>Biblio</i> Hoyt 1952; present study	<i>Spathobdella</i> present study	<i>Mayetiola</i> Schneeberg et al. 2013a	<i>Exechia</i> present study	<i>Macrocera</i> present study	<i>Eristalis</i> Schliemanz 1957	<i>Tabanus</i> Bonhag 1951
M.1	abant	+	+	+	+	+	+	+	+	+	+	++	+	+	3
M.2	adant	+	+	+	+	+	+	+	+	+	+	+	+	+	5
M.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.4	-	-	-	+	+	?	-	+	+	+	+	+	+	-?	4
M.5	dpdc	3c	+	+	+	+	?	+	+	+	+	+	++	+	7
M.6	lpdc	3a, 3b	+	+	+	+	?	+	+	+	+	+	++	+	6
M.7	-	-	-	+	+	-	+	+	+	+	+	+	+	+	1
M.8	mira	4	+	+	?	?	?	+	+	+	+	+	+	+	2
M.9	cplr	5	+	-	-	+	-	+	-	-	-	-	-	-	-
M.10	-	-	-?	-	-	-	-	-	-?	-	-	-	-	-?	-
M.11	2admd	8	+	-	-	-	-	-	-	-	-	-	-	-	9, nur ♀
M.12	abmd	9	- / +	-	-	-	-	-	-	-	-	-	-	-	8, nur ♀
M.13	1admd	-	-	-	-	-	-	-	-	-	-	-	-	-	10, nur ♀
M.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.17	1, 2adcd	12	+	+	+	-	-	-	-	+	+	+	+	+	11
M.18	adst	11	+	+	+	+	-	+	+	+	+	+	+	+	12
M.19	lplp	10	+	-	+	-	-	-	-	-	-	-	-	-	19, nur ♀
M.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.22	2 lplp	-	+	+	+	-	?	+	+	+	-	++	+	-	?
M.23	-	13 only ♂	-	-	-	+	?	+	+ / -	+	?	-	+	-	-

M.24	lv, dp	14	+	+	+	-	?	?	-	?	+	+	-	14?
M.25	-	?	+	+	-	-	?	?	-	?	-	+	-	-
M.26	-	?	+	-	-	-	?	?	-	?	+	-	-	-
M.27	fl	?	-	+	-	-	?	?	-	?	?	+	-	-
M.28	2adlb													
M.29		15	+	+	+	+	+	+	+	+	+	+	+	15
M.30														
M.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.32	-	-	-	-	-	-	-	-	-	-	-	-	? M.ph.	18?
M.33	1dplp	17	+				-	+		+		+	+	17
M.34	1lplp	16	+				-	+		+		+	+	16
M.35	2dplp	-	-	-	-	-	+	+	-?	-	+	+	-	-
M.36	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.37	dlsyr	18	+	+	+	+	-	+	+	+	+	+	+	29
M.38	-	-	-	-	?	-	-	-	-	-	-	-	-	-
M.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.40	-	-	-	-	-	-	-	-	-	-	-	?	-	-
M.41	?	21?	+?	-	-	+	-	-	-	-	-	-	+?	22?
M.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.43	1, 2dlcp	6,7	++	++	++	++	++	++	++	+	++	+	++	19++
M.44	?	?	?	+	+	?	+	+	?	+	+	+	?	-
M.45	rao	19	+	+	+		+	+		+	+	+	-	20
M.46	1, 2dlphy	22	+	+	+		+	-		+	+	+	-	23
M.47	-	-	-?	-	-	-	-	-	-	-	-	-	-	-
M.48	pan	20	+	+	+	+	+	+	+	+	++	++	-	21
M.49	-	-	-	+	-	-	-	-	-	-	-	-	-	-
M.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.51	1dlppy	23	++	+	++	+	+	++	+	+	++	+	-	27
M.52	2dlppy	24	++	+	++	++	+	++	+	+	++	+	-	28
M.67	csphy	?	?	+	+	+	+	+?	+	+	+	+	? M.flc.- flcr.	24
M.68	csoe	c	?	+	+	+	-	?	+	+	+	+	?	26
M.69	dphy	?	?	-	+	-	-	?	?	?	?	?	?	25

	<b>Stratiomyidae</b>	<b>Asilidae</b>	<b>Bombyliidae</b>	<b>Bombyliidae</b>	<b>Drosophilidae</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Siphonaptera</b>	<b>Lepidoptera</b>	
	v. Kéler 1963	<i>Pachygaster</i> present study	<i>Silpnogaster</i> present study	<i>Hemipenthes</i> Szucsich & Krenn 2000	<i>Bombylius</i> Szucsich & Krenn 2000	<i>Drosophila</i> Ferris 1950; Miller 1950	<i>Nannochorista</i> Beutel & Baum 2008	<i>Merope</i> Friedrich et al. 2013a	<i>Caurinus</i> Beutel et al. 2008a	<i>Boreus</i> Beutel & Baum 2008	<i>Bittacus</i> Beutel & Baum 2008	<i>Panorpa</i> Heddergott 1938	<i>Ctenocephalus</i> Wenk 1953	<i>Micropteryx</i> Hannemann 1956
M.1	+	+	mfsdm	mfsdm	?	+	+	+	+	+	+	172	1,2	
M.2	-	+	mfsv	mos, ?mfsv	?	+	+	+	+	+	+	162	4	
M.3	-	-	-	-	?	-	-	-	-	-	-	-	-	
M.4	+	+	mta	?	?	+	+	+	+	+	+	18	3	
M.5	+	+	mspd	mspd	14	+	+	+	+	+	+	19	7,8	
M.6	+	+	mospv	mospv	15	+	+	+	+	+	+	20	5,6	
M.7	-	?	mle	mle	5	-	+	-	+	+	+	-	21	
M.8	-	?	mcl	mcl	-	-	-	-	-	-	-	-	19	
M.9	-	-	-	-	-	-	-	-	-	-	-	-	20	
M.10	-	-	-?	-?	-	-	-	-	-	-	-	-	-	
M.11	-	-	-	-	-	-	++	++	+	+	+	-	22	
M.12	-	-	-	-	-	-	+	+	+	+	+	-	23	
M.13	-	-	-	-	-	+	-	+	+	+	+	-	-	
M.14	-	-	-	-	-	-	+	-	-	-	-	-	-	
M.15	-	-	-	-	-	-	-	-	-	-	-	-	24,25	
M.16	-	-	-	-	-	-	-	-	-	-	-	-	-	
M.17	-	-	mfsl	mfsl	4? (maxillary retractor muscle)	+	+	+	+	+	+	-	26	
M.18	+	+	mfsm	mfsm	maxillary muscle	+	+	+	?	+	+	-	27	
M.19	-	+	mtl	mtl	-	+	+	+	-	+	+	8, 10?- 12	29	
M.20	-	-	-	-	-	-	-	-	+	-	-	9	28	
M.21	-	-	-	-	-	-	-	-	-	-	-	-	30	
M.22	-	-	-	-	-	++	+	+	++	++	+	13a b	32	

M.23	-	-	-	-	-	-	-	-	-	-	-	?	?	31
M.24	?	-	-	-	-	+	+	-	-	+	+	14	33	
M.25	-	-	-	-	-	-	-	-	-	-?	+	-	34	
M.26	-	-	-	-	-	+	+	+	-	+	+	-	35	
M.27	-	-	-	-	-	-	+	-	-	?	-	-	36	
M.28													-	41
M.29	+	+	mtlb	mtlb	1/2	+	+	+	+	+	+	-	40	
M.30													-	39
M.31	-	-	-	-	-	-	-	-	-	-	-	-	-	37
M.32	-	-	?mpp	?mpp	-	-	-	-	-	-	-	-	-	38
M.33	+	+	mpe	mpe	6		-						-	42
M.34	+	+	mpk	mpk	7	++	-	+	+	++	+		15	43
M.35	?	?	-	-	-	-	-	-	-	?	-	-	-	44
M.36	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.37	++	++	mcs	mcs	13	+	+	-	-	-	+	+	56, 57	
M.38	?	?	-	-	-	-	-	-	-	-	-	-	-	-
M.39	-	-	-	-	-	-	-	-	-	-	-	-	-	58
M.40	-	-	-	-	-	-	?	-	-	-	-	?	?	
M.41	-	-	-	-	-	+	+	+	+	+	-	6	-	
M.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.43	++	++	mcc++	mcc++	12	++	++	+	+	+	++	1	45	
M.44	?	+	-	-	11	-	+	-	-	-	+	1	-	
M.45	+		-	-		-	+	+			-	2	48	
M.46	+	+	mfc	mfc	10, 17?	-	+	++		+	+	-	3	50
M.47	-	-	-	-	-	-	+	?	-	-	-	4	49	
M.48	+	+	?mgc	?mgc	9	-	-	-	+	-	-	-	46	
M.49	-	+	-	-	-	-	-	-	-	-	-	-	-	
M.50	-	-	-	-	-	-	-	-	-	-	-	-	54	
M.51	+	+	-	-	-	++	+	+	+	+	++	5	51	
M.52	+	+	-	-	-	++	+	+	+	++	++	5	55	
M.67	?	+	mcca, mccp	mcca, mccp	?	+	?	+	+	+	+	-	47	
M.68	+	+	mph	mph	?	+	?	+	+	+	+	?	?	
M.69	-	?	?mph	?mph	?	+	?	+	+	+	+	?	?	

Appendix 4. Larval head muscle homologisation with the muscular abbreviations. Abbreviations: +, present; ++, unusually large; -, absent; /, not included in the work; ?: unclear homologisation.

					Deuterophlebiidae	Nymphomyiidae	Tipulidae	Ptychopteridae	Ptychopteridae	Tanyderidae	Dixidae	Dixidae	Chironomidae	Ceratopogonidae	Thaumaleidae
	v. Kéler 1963	Wipfler et al. 2011	Matsuda 1956	Das 1937	<i>Deuterophlebia</i> Courtney 1990b	<i>Nymphomyia</i> Schneeberg et al. 2012	<i>Tipula</i> Neugart et al. 2009	<i>Bittacomorpha</i> Kramer 1954	<i>Ptychoptera</i> Wipfler et al. 2012a	<i>Protanyderus</i> Wipfler et al. 2012a	<i>Dixa</i> Felix 1962	<i>Dixa</i> Schremmer 1950b	<i>Chironomus</i> Cook 1944b	<i>Dasyhelea</i> v. Lieven 1998	<i>Androsocopa</i> present study
M.1	0an1	52	/		-	-							-		-
M.2	0an2	54	/		-	-							-		-
M.3	0an3	51	/		Mant	-		antm	+	+	mant	+	-	?	-
M.4	0an4	53	/		-	-							-		-
M.5	0an6	+	/		-	-		-	-	-	-	-	-	-	-
M.6	0an7	+	/		-	-		-	-	-	-	-	-	-	-
M.7	0lb5	63	1		-	-		lmcp	+	+	-	-	+	-	-
M.8	0lb1	62	2		-	-		-	-	-	-	-	-	-	-
M.9	0lb2	61	3+4		M61, M61', M63	+	+	lml+ lmm	+	+	mto	R. lbr.	+	messm	+
M.10	0lb3	-	-		-	-		-	-	-	-	-	-	-	-
	0lb4	-	-		Mlr	-		-	-	-	-	-	-	-	-
M.11	0md1	21+ 24	7		M21	+	+	mdf+ mdao?	+	+	admdb	Add. md.	+	21	+
M.12	0md3	23	6		M23	+	+	mde	+	+	abmdb	Abd. md.	+	23	+
M.13	0md4	26	-		-	-		-	-	-	-	-	-	-	-
M.14	0md6, 0md8 ?	25b	-		-	-		-	-	-	-	-	-	-	-
M.15	0mx1	1	8		-	-		-	-	-	-	-	-	-	-
M.16	0mx1	1	8		-	-		-	-	-	-	-	-	-	-
M.17	0mx3	3	9		-	-		-	-	-	-	-	-	-	-
M.18	0mx4, 0mx5	4	10+1 1, 18?		M5	+	-	mxt	+	+	abmx	Abd. mx.	+	-	?
M.19	0mx2	5	12		M7	+	+	mxe+ mxf	+	+	ficc	Add. mx.	+	5	?
M.20	0mx6	6	13		-	-		-	-	-	-	-	-	-	-
M.21	0mx7	7	14		-	-		-	-	-	-	-	-	-	-
M.22	0mx8	10	15		-	-		-	-	-	-	-	-	-	-



M.23	0mx10	9	16	-	-	-	-	-	-	-	-	-	-	-
M.24	0mx12	+	-	-	-	-	-	-	-	-	-	-	-	-
M.25	0mx13	+	-	-	-	-	-	-	-	-	-	-	-	-
M.26	0mx14	+	-	-	-	-	-	-	-	-	-	-	-	-
M.27	0mx15	+	-	-	-	-	-	-	-	-	-	-	-	-
M.28	0la8	38	19	-	-	-	-	-	-	-	-	-	-	-
M.29	0la4, 0la5	34, 35	20+ 27?	Mlm	+	+	lbn	+	+	mrtlb	Add. Lhph.	+	78	-
M.30	0la6	36	21+ 29?	-	-	-	-	-	-	-	-	-	-	-
M.31	0la11	43	-	-	-	-	-	-	-	-	-	-	-	-
M.32	0la12	42	-	-	-	-	-	-	-	-	-	-	-	-
M.33	0la13	44	-	-	-	-	-	-	-	-	-	-	-	-
M.34	0la14	45	23	-	-	-	-	-	-	-	-	-	-	-
M.35	0la16	+	-	-	-	-	-	-	-	-	-	-	-	-
M.36	0la17	+	-	-	-	-	-	-	-	-	-	-	-	-
M.37	0hy12	71+ 79	24+2 5	-	-	+	sldm	+	+	-	-	-	-	+
M.38	0hy7	76+ 46+ (39?)	26?	-	-	-	-	-	-	-	-	-	-	-
M.39	0hy8	77+ 47+ (39?)	26?	-	-	-	-	-	-	-	-	-	-	-
M.40	0hy13	-	-	-	-	-	-	-	-	-	-	-	-	-
M.41	0hy1	74	-	M74	-	+	hphm2	+	+	rao	d.dil. 4-6	/	dmhyp	-
M.42	0hy3	37	5	-	-	-	-	-	-	-	-	/	-	-
M.43	0ci1	81	/	M81/82	+	+	cbm	+	+	ddcb	Add. eph.	/	cibdilm + 63	-
M.44	0bu1	82	/	-	-	+	-	-	-	-	-	/	-	-
M.45	0bu2	83	/	-	-	+	phd1	+	+	ddphy1	d.dil. 1	/	-	+
M.46	0bu3	84	/	M83-85	+	+	phd2	+	?	ddphy2	d.dil. 3	/	phdilm	+
M.47	0hy2	75	-	M74?	-	-	hphm1	+	+	msphy	d.dil. 2	/	74	-
M.48	0hy5?, 0bu5	80, 87	-	-	-	-	vphm1 +2	+	+	dvcb	v.dil. 1	/	-	+
M.49	0bu4	85	/	-	-	-	-	-	-	-	-	/	-	-
M.50	0bu6	88	/	-	-	-	-	-	-	dvphy1	-	/	-	+
M.51	0ph1	86	/	M86	-	-	phd3	+	+	dlpphy	+	/	oesdilm	-
M.52	0ph2	89+ 90	/	M87-90	-	-	vphm3	+	+	dvphy2	v.dil. 2	/	-	-
M.53	0ph3	-	/	-	-	-	-	-	-	-	-	/	-	-

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M.67	0hy9	-	-	-	-	-	-	+	+	mtphy	QM.	/	trm	+
M.68	0st1	93	/	-	-	-	-	-	-	-	-	/	-	+
M.69	0st2	91+ 92	/	-	-	+	-	-	-	-	-	/	-	-

	<b>Culicidae</b>	<b>Culicidae</b>	<b>Culicidae</b>	<b>Culicidae</b>	<b>Culicidae</b>	<b>Culicidae</b>	<b>Corethrellidae</b>	<b>Chaoboridae</b>	<b>Bibionidae</b>	<b>Cecidomyiidae</b>
v. Kéler 1963	<i>Anopheles maculipennis</i> Schremmer 1949	<i>Anopheles maculipennis</i> Cook 1944a	<i>Anopheles quadrimaculatus</i> Fansworth 1947	<i>Culiseta</i> Cook 1944a	<i>Lutzia</i> Cook 1944a	<i>Armigeres</i> Cook 1944a	<i>Corethrella</i> present study	<i>Chaoborus</i> Schremmer 1950a	<i>Biblio</i> Perraudin 1961	<i>Phaenobremia</i> Solinas 1968
M.1										
M.2	+	?	am	antennal muscle	antennal muscle	antennal muscle	+	RAn	-	-
M.3										
M.4										
M.5	-	-	-	-	-	-	-	-	-	-
M.6	-	-	-	-	-	-	-	-	-	-
M.7	-	-	-	-	-	-	-	-	-	-
M.8	-	-	-	-	-	-	+	-	-	-
M.9	EphH+ LbM+ dDil	messorial muscle+ median palatal muscle	mpa+ em+ im	messorial muscle+ median palatal muscle	messorial muscle+ median palatal muscle	messorial muscle+ median palatal muscle	+	Ad.Eph + AbEph	mess+ mtor+ ddcb	-
M.10	-	-	-	-	-	-	-	-	-	-
M.11	Add. md.	mandibular adductor muscle	mad	mandibular adductor muscle	mandibular adductor muscle	mandibular adductor muscle	+	AdMd	admdb	MAD
M.12	Abd. md.	mandibular abductor muscle	mab	mandibular abductor muscle	mandibular abductor muscle	mandibular abductor muscle	+	AbMd	abmdb	MAB
M.13	-	-	-	-	-	-	-	-	-	-
M.14	-	-	-	-	-	-	-	-	-	-
M.15	-	-	-	-	-	-	-	-	-	-
M.16	-	-	-	-	-	-	-	-	-	-
M.17	-	-	-	-	-	-	-	-	-	-
M.18	Add. mx.	cranial flexor of stipes	cs	cranial flexor of stipes	cranial flexor of stipes	cranial flexor of stipes	+	-	abmx	MML?
M.19	Fl.mx.	maxillary muscle	mxm	maxillary muscle	maxillary muscle	maxillary muscle	+	-	flcc	MML?
M.20	-	-	-	-	-	-	-	-	-	-
M.21	-	-	-	-	-	-	-	-	-	-
M.22	-	-	-	-	-	-	-	-	-	-
M.23	-	-	-	-	-	-	-	-	-	-
M.24	-	-	-	-	-	-	-	-	-	-

M.25	-	-	-	-	-	-	-	-	-	-
M.26	-	-	-	-	-	-	-	-	-	-
M.27	-	-	-	-	-	-	-	-	-	-
M.28	-	-	-	-	-	-	-	-	-	-
M.29	Fl.hph.	labial adductor muscle	lm	labial adductor muscle	labial adductor muscle	labial adductor muscle	-	AdLa	rhy	MML?
M.30	-	-	-	-	-	-	-	-	-	-
M.31	-	-	-	-	-	-	-	-	-	-
M.32	-	-	-	-	-	-	-	-	-	-
M.33	-	-	-	-	-	-	-	-	-	-
M.34	-	-	-	-	-	-	-	-	-	-
M.35	-	-	-	-	-	-	-	-	-	-
M.36	-	-	-	-	-	-	-	-	-	-
M.37	-	-	-	-	-	-	+	-	-	-
M.38	-	-	-	-	-	-	-	-	-	-
M.39	-	-	-	-	-	-	-	-	-	-
M.40	-	-	-	-	-	-	-	-	-	-
M.41	h.d.Dil.	posterior pharyngeal muscle	ppm	posterior pharyngeal muscle	posterior pharyngeal muscle	posterior pharyngeal muscle	-	RCi	rao	MDF?
M.42	-	-	-	-	-	-	-	-	-	-
M.43	Ex.Eph.	cibarial muscle	cm	cibarial muscle	cibarial muscle	cibarial muscle	?	Tr.Ro	ddcb	MDF?
M.44	-	-	-	-	-	-	?	-	-	-
M.45	-	-	-	-	-	-	+	-	ddphy	MDF?
M.46		-	mpm	median pharyngeal muscle	-	median pharyngeal muscle	+	d.Dil		
M.47	vo.d.Dil.	lateral pharyngeal muscle	pm	lateral pharyngeal muscle	lateral pharyngeal muscle	lateral pharyngeal muscle	-	l.Dil	-	-
M.48	-	ventral pharyngeal muscle	vp	M48	ventral pharyngeal muscle	ventral pharyngeal muscle	+	-	dvphy?	MDF?
M.49	-	-	-	-	-	-	-	-	-	-
M.50	-	ventral pharyngeal muscle	vp	M50	ventral pharyngeal muscle	ventral pharyngeal muscle	-	-	dvphy?	MDF?
M.51	+	-	-	-	-	-	-	d.Dil	ddphy?	-
M.52	Vp	-	vp	ventral pharyngeal muscle	ventral pharyngeal muscle	ventral pharyngeal muscle	-	-	dvphy	MDP
M.53	-	-	-	-	-	-	-	-	-	-

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M.67	QM	intrinsic pharyngeal muscle	ip	intrinsic pharyngeal muscle	intrinsic pharyngeal muscle	intrinsic pharyngeal muscle	+	-	-	-
M.68	-	-	-	-	-	-	+	-	-	-
M.69	-	-	-	-	-	-	-	-	-	-

	<b>Mycetophilidae</b>	<b>Mecoptera</b>	<b>Mecoptera</b>	<b>Siphonaptera</b>	<b>Trichoptera</b>	<b>Lepidoptera</b>	<b>Megaloptera</b>	<b>Rhaphidioptera</b>	<b>Coleoptera</b>	<b>Coleoptera</b>	<b>Hymenoptera</b>
v. Kéler 1963	<i>Exechia</i> present study	<i>Nannochorista</i> Beutel et al. 2009	<i>Panorpa</i> Bierbrodt 1942	<i>Nosopsyllus</i> Sharif 1937	<i>Limnophilus</i> Winkler 1959	Hasenfuss & Kristensen 2003	<i>Neohermes</i> Beutel & Friedrich 2008	<i>Raphidia</i> Beutel & Ge 2008	<i>Rhipsideigma</i> Beutel & Hörschemeyer 2002	<i>Catops</i> Beutel & Molenda 1997	<i>Xyela</i> Beutel et al. 2008b
M.1	-			d.a.	-	-	M1		1	1	M1
M.2	-			l.a.	-	-	M2,4	1/2/3/4	2	2	
M.3	-	+	M.d.sc.	-	-	-	-		-	-	M2-4
M.4	-			f.a.	-	-	M2,4		4	4	
M.5	-	-	-	-	-	-	-	-	-	-	-
M.6	-	-	-	-	-	-	-	-	-	-	-
M.7	-	+	M.labr.ep.	c.l.	7	la-ep	M7	7	-	7	M7
M.8	-	+	M.retr.labr.	a.l.	8	fr-la, med	M8	8	-	-	M8
M.9	+	+	M.protr.ep.	p.l.	8	fr-la, lat	M9	9	9	9(2)	M9
M.10	-	-	-	-	-	-	-	-	-	-	-
M.11	+	+	M.add. mand.	ad.	11	cr-ma (21)	M11	11	11	11	M11
M.12	+	+	M.abd. mand.	ab.	12	cr-ma (23)	M12	12	12	12	M12
M.13	-	+	-	-	-	te-ma	M13	13	-	-	M13
M.14	-	-	-	-	-	-	-	-	-	-	-
M.15	-	-	-	ab.m.	-	-	M15	15	-	15	-
M.16	-	-	-	-	-	-	-	-	-	-	-
M.17	-	+	-	-	17	te-ca	M17	17	17	17	M17
M.18	+	+	M.add.st.1 + 2	l.ad.+ m.ad.+ u.ad.	18	te- st+cr- st?	M18	18	?	18	M18
M.19	+	+	M.prom. lac.	-	19	cr-lac	M19	19	19	19	M19
M.20	-	-	-	l.s.	20?	st-plp 1&2	M20	20	-	20	M20
M.21	-	-	-	-	-	-	M21	21	21	-	-
M.22	-	+	M.ext.p. mx.	p.m.m.?	-	-	M22	22	22	22	-
M.23	-	+			-	-	M23	23	23	23	-
M.24	-	-	-	-	-	-	-	24	-	-	-
M.25	-	-	-	-	-	-	-	-	-	-	-

M.26	-	-	-	-	-	-	-	-	-	-	-
M.27	-	-	-	-	-	-	-	-	-	-	-
M.28	-	-	-	-	-	-	M28	28	-	28	M28
M.29	-	-	-	p.l.a.	30	cr-prm?	M29	29	-	29	M29
M.30	-	-	-	-	-	-	M30	30	-	30	M30
M.31	-	-	-	-	-	-	-	-	-	-	-
M.32	-	-	-	-	-	-	-	-	-	-	-
M.33	-	-	-	-	-	-	-	-	-	-	-
M.34	-	-	M.p.lb.?	-	-	-	M34	33/34	-	-	M33/34
M.35	-	-	-	-	-	-	-	-	-	-	-
M.36	-	-	-	-	-	-	-	-	-	-	-
M.37	+	-	M.dil.spp.	d.s.m.	37	hy-sal	M37	-	-	-	-
M.38	-	-	-	-	-	-	M38	-	-	-	-
M.39	-	-	-	-	-	prm-sal	M39	38/39	-	-	-
M.40	-	-	-	-	-	-	-	-	-	-	-
M.41	-	+	-	-	43	fr-oa (retr)	M41	41	41	41	M41
M.42	-	+	-	-	29	te-ci	M42	Mslt1+2	42?	42	M42
M.43	+	+	M.dil.d.or.	d.c.+ a.d.	44	cl-ci	M43	43	-	43	M43
M.44	+	+	-	-	-	-	-	-	-	-	-
M.45		+	M.dil.d.ant. phar.	m.d.	+	-	M45	45	45	45	M45
M.46	+	+	M.dil.d. post.phar	p.d.	+	fr-ph	M46	46	46	46	M46
M.47	-	+	M.dil.l.phar .	-	-	fr-oa (susp)	-	-	-	M. trans- versalis lateralis?	-
M.48	-	-	-	v.d.a.	42, +	te-ph	M48	48	42?	-	M48
M.49	-	+	-	-	-	-	-	-	-	-	-
M.50	-	-	M.tent. phar.sup.+ M.tent. phar.inf.	v.d.a.	+	te-ph	M50	50	-	-	M50
M.51	-	-	M.par.phar. ant.+ M.par.phar. post.	d.d.p.	+	cr-ph	M51	51	-	-	M51
M.52	-	-	M.gul.phar.	v.d.p.	+	-	M52	52	52	52	M52
M.53	-	-	-	-	-	-	-	-	-	-	-



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M.67	+	-	M.trans. phar.	-	-	-	-	-	-	M. trans- versalis medialis?	-
M.68	+	-	Ri.M.	-	-	-	-	-	-	+	+
M.69	-	-	M.long. phar.	-	-	-	-	-	-	+	+

## 11 Publikationen

### Originalarbeiten in Fachzeitschriften

- Neugart C, **Schneeberg K**, Beutel RG. 2009. The morphology of the larval head of Tipulidae (Diptera, Insecta) – the dipteran groundplan and evolutionary trends. *Zoologischer Anzeiger* 248: 213-235.
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- Friedemann K, **Schneeberg K**, Beutel RG. 2014. Fly on the wall – attachment structures in Diptera. *Systematic Entomology*: in press.

### Buchbeitrag

- Schneeberg K**, Beutel RG. 2013. 6.35. Diptera. In: Beutel RG, Friedrich F, Ge S-Q, Yang X-K (eds). *Insect Morphology and Phylogeny*. De Gruyter, Berlin: 467-482.

**Posterpräsentation**

**Schneeberg, K.**, Friedrich, F. & Beutel, R.G., 2011. The larvae of Nymphomyiidae (Diptera, Insecta) – ancestral and highly derived? 5<sup>th</sup> Dresden Meeting on Insect Phylogeny: September 23-25, 2011.

**Schneeberg K.**, 2013. The evolution of the adult head in Diptera. 6<sup>th</sup> Dresden Meeting on Insect Phylogeny: September 27-29, 2013.

Friedemann K, **Schneeberg K.** 2013. The evolution of attachment structures in lower Diptera. 6<sup>th</sup> Dresden Meeting on Insect Phylogeny: September 27-29, 2013.

Förster M, **Schneeberg K.** 2013. Raptorial phantom midges. 6<sup>th</sup> Dresden Meeting on Insect Phylogeny: September 27-29, 2013.

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### **13 Eigenständigkeitserklärung**

Hiermit erkläre ich, dass ich die vorliegende Dissertation „The evolution of head structures in Diptera and the phylogeny of the order“ selbständig und ohne unerlaubte fremde Hilfsmittel angefertigt habe. Ich habe ausschließlich die angegebenen Quellen und Hilfsmittel verwendet. Die Arbeit habe ich bei keiner anderen Fakultät oder Universität eingereicht. Außerdem erkläre ich, dass ich bisher keine Promotionsversuche unternommen habe. Die Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt. Die Hilfe eines Promotionsberaters habe ich nicht in Anspruch genommen. Dritte haben weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, erhalten. Bei der Herstellung der in der vorliegenden Arbeit verwendeten Originalarbeiten haben Co-Autoren (Feix Beckmann, DESY Hamburg; Rolf Beutel, FSU Jena; Gregory Courtney, Iowa State University, Ames; Frank Friedrich, Universität Hamburg; Marion Harris, North Dakota State University, Fargo; Julia Herzen, DESY Hamburg; Frank Hünefeld, Jena; Katrin Krause, Jena; Andreas Löffler, Jena; Rudolf Meier, University of Singapore; Christiane Neugart, Jena; Hans Pohl, FSU Jena; Alexey Polilov, Lomonosov Moscow State University, Moskau; Benjamin Wipfler, FSU Jena) mitgewirkt. Mein Eigenanteil an den Publikationen ist jeweils angegeben.

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