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

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ABSTRACT

Larval head structures of *Nymphomyia dolichopeza* 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.

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 †Diplopolyneuromorpha (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), Séguy (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. Blephariceroidea (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

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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 dolichopeza* 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

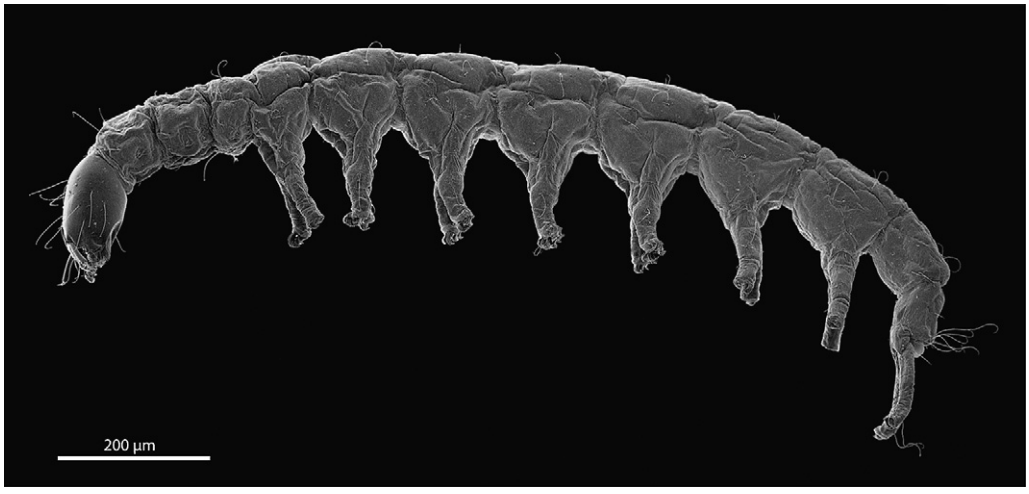

Fig. 1. *Nymphomyia dolichopeza*, larva, overview, SEM image.