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Resolving insect phylogeny: The significance of cephalic structures of the Nannomecoptera in understanding endopterygote relationships☆

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ABSTRACT

The Nannomecoptera are among the most enigmatic and controversial taxa of endopterygote insects, the phylogenetic resolution of which is crucial to understanding the evolution of neopteran insects. Once considered a subordinate lineage within the Mecoptera, renewed interest in nannochoristids has been prompted by evidence that the Nannomecoptera are not admissible to the clade of extant scorpion flies but are more likely to belong to the clade Siphonaptera + Nannomecoptera + Diptera. The overarching purpose of the present account is to provide novel and extensive morphological character traits in addition to those already existing for adult structures. The aim is to determine if these traits support molecular data sets that have been suggested elsewhere to clarify the phyletic position of Nannochoristidae. This account focuses on nannomecopteran larvae, which unlike those of other mecopterans have received little attention. Thus, the thrust of this investigation is to provide detailed anatomical data on nannochoristid larvae for a targeted inquiry into their phylogenetic affinities. The described characters are discussed and presented in a data matrix comprising representatives of all endopterygote orders. While the study is based primarily on the New Zealand species *Nannochorista philpotti*, it is proposed that all nannomecopteran larvae will prove to be similar to this taxon in most if not all structural features of significance to a higher-level phylogenetic context.

1. Introduction

The Nannomecoptera (=the family Nannochoristidae) are among the most enigmatic and controversial taxa of endopterygote insects. This small taxon, comprising eight described extant species currently placed in just one genus, *Nannochorista* Tillyard, 1917, has a circum-antarctic distribution: Three species occur in Argentina and Chile, four in Australia (Tasmania, one of the species also in mainland Australia), and one in New Zealand. The taxonomy and nomenclature are treated by Riek (1954), Kristensen (1989b) and Byers (1989). For a long time following its description by Tillyard (1917) the family was considered a quite subordinate lineage within the Mecoptera. However, renewed interest in nannochoristids was prompted by the discovery of their distinctive aquatic larvae (first documented in some detail by Pilgrim, 1972) and, not least, by the strong evidence from genitalia morphology of both sexes that these insects rather than the time-honoured, but obviously non-monophyletic, 'Protomecoptera' assemblage (i.e., Meropeidae and Eomeropeidae [syn. Notiothaumidae]) are outside a clade comprising the bulk of extant scorpion flies (Mickoleit, 1971, 1975, 1976, 1978; Willmann, 1981b, 1987, 1989). That the ordinal assignment of the Nannochoristidae harbours problems has been highlighted in subsequent reviews and comprehensive texts, e.g., Kristensen (1989a, 1991), Willmann (2005a,b), Grimaldi and Engel (2005) and Beutel and Pohl (2006). The rank of a separate order Nannomecoptera was assigned to the group by Hinton (1981). Wood and Borkent (1989) proposed a sistergroup relationship with Diptera (see also Oosterbroek and Theowald, 1991), while Whiting (2002) followed by Simiczyjew (2002) provided evidence for a sistergroup relationship with a clade comprising Boreidae and Siphonaptera (see also Whiting et al., 2003). Very recently Beutel and Baum (2008) tentatively proposed a (Siphonaptera + (Nannomecoptera + Diptera)) clade.

Extensive molecular data sets have been analysed with the aim of clarifying antliophoran relationships and the position of Nannochoristidae (Whiting, 2002), and a substantial amount of information on adult morphology has now been procured with a similar aim.

☆ Dedicated to emeritus Professor R.L.C. Pilgrim, Christchurch, in recognition of his achievements in pioneering research on nannomecopteran immatures. NPK adds his personal gratitude for having been introduced to living *Nannochorista* by professor Pilgrim during most enjoyable excursions in Banks Peninsula, where the bulk of the material used in this study was collected.

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Tillyard's (1917) original account of external adult structure was followed by more detailed observations on the skeleto-muscular structure of the head (Imms, 1944 [mouthparts only]; Hepburn, 1969b; Beutel and Baum, 2008), thorax (Hepburn, 1970; Friedrich and Beutel, in press) and male genital segments (Willmann, 1981a,b, 1989); there are also newer studies on wing venation (Willmann, 1989; Kristensen, 1989b), female genital segments (Mickoleit, 1975, 1976), proventriculus (Hepburn, 1969a), wing hearts (Krenn and Pass, 1993), male internal genitalia (Sinclair et al., 2007) and ovaries (Simiczyjew, 2002).

In contrast, the larvae which are very unlike those of other mecopterans have been considerably less studied. The development has been described in any detail only for the endemic New Zealand *Nannochorista philpotti* (Pilgrim, 1972), but the life-style is likely overall uniform throughout the family. The larvae occur in the silty bottom layer of shallow streams; they are predators which feed on other small invertebrates, chironomid larvae in particular. The final ('prepupal', note that according to Pilgrim the early prepupa is still not a pharate pupa) phase of the 4th larval instar is non-feeding; it usually leaves the water and may be found in the immediate vicinity of the stream in the soil, in damp (e.g., bryophyte) vegetation or under bark of decomposing, partially submerged logs. Depending on its situation it may, or may not, construct a special cell for pupation. Pilgrim's pioneer account of external morphology has so far been followed up only by work on eye structure (Melzer et al., 1994) and the uniquely specialized thoracic legs (Kluge, 2003). Much additional information about the structure of nannomecopteran larvae is therefore urgently needed.

The major aim of the present investigation is, then, to provide detailed anatomical data on nannochoristid larvae for a targeted inquiry into their phylogenetic affinities. The head was chosen for this first study in a series because it provides a multitude of potentially relevant characters of the exo- and endoskeleton, the mouthparts, the sense organs, the digestive tract, and the nervous system. The characters are discussed and presented in a data matrix comprising representatives of all endopterygote orders. A formal cladistic character analysis has not been carried out on the basis of this limited character set, but in a subsequent study the data coded in the present contribution will be combined into a more comprehensive matrix comprising other features of larvae and characters of adults, and including appropriate non-endopterygote outgroups. The study is based primarily on the New Zealand species *N. philpotti* of which ample, well fixed material was available; unless otherwise stated descriptions apply to this taxon. Supplementary observations on the integumental structure were made on the single mainland Australian taxon *Nannochorista dipteroides*; the two were noted to differ mainly in the development of the maxillary sclerotization, and we consider it likely that all nannomecopteran larvae will prove to be similar in most, if not all structural features of significance in a higher-level phylogenetic context.

2. Material and methods

2.1. Taxa examined

The material was fixed in 70% ethanol unless otherwise noted. 'Mecoptera'–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 eboraca* Tillyard, 1917 (ethanol; whole mount preparations). The specimens examined are 4th instar larvae (judging from the head capsule measurements given by Pilgrim, 1972) in the still 'active' phase (i.e., before the 'prepupal' phase in Pilgrim's sense). 'Mecoptera'–Neomecoptera, Boreidae: *Boreus westwoodi* Hagen, 1866 (first instar, FAE = ethanol–formaldehyde–acetic acid; SEM, microtome sections). Mecoptera *s. str.*, Panorpidae: *Panorpa* sp. (FAE; dissections). Siphonaptera, Ceratophyllidae: *Ceratophyllus* sp. (SEM, microtome sections). Pulicidae: *Synosternus cleopatrae* (Rothschild, 1903). Diptera, Culicidae: *Culex* sp. (FAE; microtome sections). Bibionidae: *Bibio* sp. (SEM, microtome sections). Tipulidae: *Tipula* sp. (dissections, microtome sections). Trichoptera, Rhyacophilidae: *Rhyacophila* sp. (FAE; SEM, dissections, microtome sections). Hymenoptera, Xyelidae: *Xyela* sp. (SEM, microtome sections). Diprionidae: *Neodiprion* sp. (SEM, microtome sections). Megaloptera, Corydalinae: *Neohermes* sp. (FAE) (SEM, microtome sections). Raphidioptera, Raphidiidae: *Raphidia* (*Phlaeostigma*) *notata* Fabricius, 1781; *Raphidia* sp. (earlier instar, not identified to species level) (FAE). Neuroptera, Osmylidae: *Osmylus* sp. Nevrorthidae: *Austroneurorthus* sp. (fixation unknown, probably 100% ethanol). Zoraptera (nymphs): *Zorotypus hubbardi* Caudell, 1918 (whole mount preparations, microtome sections).

2.2. Methods of observation

Specimens of taxa listed above (microtome section) and one larval specimen of *N. philpotti* were embedded in Araldite®, cut at 1 μm (Microm microtome HM 360) and stained (Azan). Additional observations were made on paraplast embedded specimens of *N. philpotti* sectioned at 7 μm and stained with Weigert's haematoxyline/bluish erythrosine/fast green, and on whole mount preparations of heads of *N. dipteroides* mounted either in euparal after KOH maceration and chlorazol black staining, or mounted in entellan after dehydration and clearing in cedar oil. The descriptive text refers to *N. philpotti* unless otherwise stated. Drawings were made using an ocular grid, a camera lucida, or on the basis of photographs. For SEM micrographs (FEI ESEM XL 30) specimens were cleaned ultrasonically, dried (critical point) and coated with gold. Three-dimensional reconstructions based on microtome sections were made using Maya and Imaris®. The muscle nomenclature follows Kéler (1963) where possible; in muscle descriptions O denotes origin and I insertion.

3. Results

3.1. General appearance

The larval body is near-cylindrical, slender and elongated, especially the abdomen in which 10 segments are clearly identifiable (total length of final instar larvae 12.5–14.5 mm ($n = 7$), length of abdomen c. 11.5 mm [in largest specimens], maximum width c. 0.9 mm). The head capsule, mandibles, cervical sclerites, pronotum and small parts of the legs are strongly sclerotized and brownish, hence not strongly with the trunk integument which in active larvae has a speckled brown-and-whitish pigmentation pattern (Pilgrim, 1972: figs. 1–2); this pattern rapidly disappears after fixation and it also disappears in living larvae in the 'prepupal' stage. The position of the distinctly prognathous head in the living larvae is evidently somewhat variable (since the immediately following softwalled trunk region permits ample movement) but is at most moderately inclined relative to the long axis of the body. For convenience our descriptive account makes use of the orientation indicated by the arrow in Fig. 4A, i.e., with 'dorsal' and 'ventral' being relative to a horizontal long axis of the head.

3.2. Head capsule, external features

The head capsule is almost completely exposed (Figs. 1, 2, 3A, 4A, 7A). Only a trapezoid posteromedian extension of the head capsule is partly covered by (morphologically prothoracic) trunk