

The phylogeny of the Neuropterida: long lasting and current controversies and challenges (Insecta: Endopterygota)

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Received 23.viii.2012, accepted 14.ix.2012.

Published online at www.arthropod-systematics.de on 28.ix.2012.

> Abstract

Despite numerous efforts to establish a sound phylogeny of Neuropterida and to trace their position within the tree of Endopterygota these questions up to now still appear far from being solved. The evidence for the sister group relationships among the three orders of Neuropterida is contradictory (i.e., Raphidioptera as sister group of Megaloptera + Neuroptera versus Neuroptera as sister group of Megaloptera + Raphidioptera) and recently even the monophyly of Megaloptera was challenged. Also the phylogenetic relationships among neuropteran families deduced from various studies differ basically in all aspects concerning the number and composition of suborders as well as the basal dichotomies. The morphology based division of Neuroptera into the three suborders Nevrothiformia, Myrmeleontiformia and Hemerobiiformia with the latter two being sister groups is not recovered by molecular but also some morphology based analyses – as all of them lack monophyletic Hemerobiiformia. Possible methodological problems contributing to this lack of unambiguous resolution of the phylogeny of Neuropterida are sequence saturation, lack of information in DNA marker sequences, incomplete taxon sampling and data matrices, as well as – concerning morphological characters – convergence and interpretation of ambiguous character polarity. The phylogenetic relationships of Neuropterida and their position within Endopterygota are of interest with respect to the reconstruction of character evolution and the evolution of life styles of the larvae (terrestrial in Raphidioptera, aquatic in Megaloptera and in two families of Neuroptera).

> Key words

Neuropterida, Raphidioptera, Megaloptera, Neuroptera, Nevrothiformia, Hemerobiiformia, Myrmeleontiformia, phylogeny, systematics.

1. Introduction

The superorder Neuropterida comprises three orders: Raphidioptera with two families (Raphidiidae and Inocelliidae), Megaloptera with two families (Corydalidae and Sialidae), and the heterogeneous Neuroptera with 17 families (Nevrothidae, Sisyridae, Osmyliidae,

Hemerobiidae, Chrysopidae, Ithonidae, Polystoechotidae, Coniopterygidae, Dilaridae, Berothidae, Rhachiberothidae, Mantispidae, Psychopsidae, Nemopteridae, Nymphidae, Myrmeleontidae and Ascalaphidae) (U. ASPÖCK & H. ASPÖCK 2007). Classification of Neu-

ropterida is a long history of competing hypotheses, its beginning dating back to the 1850ies. The awareness of the phylogenetic significance of the larvae is a merit of the young Austrian entomologist Friedrich BRAUER (1852, 1857). In the early 20th century, starting with Handlirsch's treatise of fossil Neuroptera (HANDLIRSCH 1906–08) and the work of COMSTOCK (1918), TILLYARD (1919) and WITHYCOMBE (1925), the basal approaches towards a phylogeny of the neuropterans were made. The term Neuropterida (for Neuropteroidea = Neuroptera = Neuroptera sensu lato) was introduced by BOUDREAUX (1979).

There is a long tradition of competing hypotheses concerning the phylogenetic position of Neuropterida within Endopterygota and on the question which group splits from the basal node: the Neuropterida + Coleoptera (KRISTENSEN 1999; WHEELER et al. 2001; KJER et al. 2006) or the Hymenoptera (CAMERON et al. 2009; WIEGMANN et al. 2009; BEUTEL et al. 2010a). Moreover, interrelationships of the three orders of Neuropterida are contradictory. Two alternative sister group relationships were proposed, i.e., Raphidioptera as sister group of Megaloptera + Neuroptera versus Neuroptera as sister group of Megaloptera + Raphidioptera. Support for one or the other hypothesis mostly came – as by-products – from analyses dealing with the Endopterygota or the intra-ordinal relationships within Neuropterida, rather than from analyses specifically targeted to this question. Besides, also the monophyly of Megaloptera has been repeatedly challenged, e.g., recently by a study proposing a clade Raphidioptera + Corydalidae (BEUTEL et al. 2010a). The phylogenetic relationships among and within neuropteridan orders are not only of taxonomic interest, but also relevant to evaluate character evolution within this group, which exhibits several extraordinary features such as pharate pupae, the complete set of male genital sclerites in Raphidioptera, complex sucking tubes, or cryptonephry of larvae in Neuroptera, and their compact larval head capsules versus heads with a mosaic-like ventral sclerite pattern. Most prominent is also the life style of the larvae, which was interpreted differently according to the one or other phylogenetic hypothesis. The larvae of Raphidioptera are terrestrial, all Megaloptera have aquatic larvae, and in Neuroptera the larvae are aquatic only in Nevrothidae and Sisyridae but terrestrial in all other families.

In the present paper we provide an overview of the various analyses performed (partly within the broader context of Endopterygota or even Hexapoda) to trace the phylogeny of Neuropterida. The following issues will be addressed: **(1)** The phylogenetic position of Neuropterida within Endopterygota. **(2)** The phylogenetic relationships among Raphidioptera, Megaloptera and Neuroptera. **(3)** The phylogenetic relationships among the 17 families of the Neuroptera.

The monophyly of Neuropterida

The monophyly of Neuropterida is generally accepted. Although it is based on rather subtle arguments (summarised in U. ASPÖCK 2002; U. ASPÖCK & H. ASPÖCK 2005), there is, up to now, no plausible alternative. This monophyly is supported by characters concerning thoracic and abdominal tergite sutures (ACHTELIG 1975, 1981; HENNIG 1981) and special musculature of the ovipositor (MICKOLEIT 1973), characters of larval stemmata (PAULUS 1986), specialisations at the wing-bases (HÖRNSCHEMEYER 1998), the presence of a field of trichobothria on the ectoproct (tenth or tenth + eleventh tergite) (U. ASPÖCK et al. 2001) and nuclear genes for 18S and 28S rRNA (WHITING et al. 1997). KRISTENSEN (1999) notes that the molecular data of WHITING et al. (1997) are doubtful as there are, e.g., almost identical sequences for Megaloptera and Raphidioptera, and there were contaminations with respect to Coleoptera. Further molecular evidence for monophyletic Neuropterida comes from WINTERTON et al. (2010), MISOF et al. (2007), WIEGMANN et al. (2009), CAMERON et al. (2009) and BEUTEL et al. (2010b).

The position of Neuropterida within Endopterygota

The traditional view of the position of Neuropterida in the phylogeny of Endopterygota postulates them as sister group of Coleoptera (+ Strepsiptera) both together being the sister group of the remaining Endopterygota. This hypothesis is thoroughly treated by KRISTENSEN (1999), who provided a very detailed treatise on the phylogeny of the Endopterygota comprising all evidence that up to then had emerged from morphological and molecular genetic approaches. In his summarizing cladogram there is a basal trichotomy of (1) Neuropterida + Coleoptera, (2) Strepsiptera, and (3) Hymenoptera + Mecopterida (a group comprising Mecoptera, Diptera, Trichoptera, and Lepidoptera). However, as Kristensen noted, there is only weak support even for this basal splitting. A general problem that becomes apparent from the discussion provided by KRISTENSEN (1999) is the fact that the different phylogenetic hypotheses resulting from the various studies had been inferred from different characters. Furthermore, differing and partially non-overlapping taxon sampling hampered straightforward comparisons of results. KRISTENSEN (1999) himself hypothesized that the principal innovation of the Endopterygota is not the pupa, but the larval stage which lacks wing buds

(and genital-appendage buds), and he accompanied this view by argumentation concerning the quiescence of the last immature (pupal) stage. He considered the specialised morphology of the pupa not as an endopterygote ground plan trait and underlined this by a picture of a pharate adult (a “running” pupa) of a raphidiid snakefly, representing the commonly neglected primitive pupal type (KRISTENSEN 1999).

A more recent study on the phylogeny of the Endopterygota surprised with a basal split between Hymenoptera and the remaining Endopterygota, while Neuropterida remained the sister group of Coleoptera + Strepsiptera (WIEGMANN et al. 2009). While the same position of Hymenoptera had been found already in the study of MISOF et al. (2007) based on sequences of the 18S rRNA gene (but without any considerable support) – Neuropterida have a different position: Neuropterida (Strepsiptera (Coleoptera, Diptera)). The trees of WIEGMANN et al. (2009) achieved well supported nodes. The latter study was based on molecular data for six nuclear protein-coding genes (AATS, CAD, TPI, SNF, PGD, RNA POL II) and supported earlier morphology based hypotheses of ROSS (1965) and ROHDENDORF & RASNITSYN (1980). Despite the impressive data set of several thousand base pairs (bp) and the mostly highly supported nodes, it has to be emphasised that there are many gaps in the data matrix. For instance, in 12 out of 29 species two to four marker genes were not sequenced and less than 50% of the species are represented by all marker sequences. WIEGMANN et al. (2009) state that there are some conflicts in their data set, which they clearly attributed to the Strepsiptera, but the authors conclude that there is no systematic error due to long branch attraction in their analyses. Whether the topology remains stable – especially with respect to the basal split – remains to be tested using a complete data matrix. Although the sister group relationship of Strepsiptera + Coleoptera recently obtained strong support in another study comprising more than 18 million nucleotides from nearly 4,500 genes (NIEHUIS et al. 2012), this result has to be tested by including Neuropterida, which were missing in that study and represent a potential sister group of Coleoptera. Furthermore, the basal split between Hymenoptera and the remaining Endopterygota remains open since in that paper neuropterans were not included.

More recently, a basal split of Hymenoptera was proposed in a further study which was based on an impressive morphological character set (BEUTEL et al. 2010a). The paper also includes a discussion in the context of recently published molecular data. In contrast to WIEGMANN et al. (2009), in the trees presented by BEUTEL et al. (2010a) the Neuropterida appear as the sister group of the remaining Endopterygota (exclusive of Hymenoptera). Thus, there is no sister group

relationship between Neuropterida and Coleoptera. In spite of the fact that the study of BEUTEL et al. (2010a) presented the largest morphological character set ever used so far, the resulting trees are poorly supported in the basal splittings. For instance, the 50% majority rule consensus tree of the Bayesian analysis presents a tetrachotomy of (1) Neuropterida, (2) Coleoptera + Strepsiptera, (3) Hymenoptera, and (4) a group containing the remaining orders. However, not even the latter group is well supported. This lack of morphological support is exemplified by the following conflicts: ‘Basal’ Hymenoptera are conflicting with the presumed ancestral characters of Raphidioptera, e.g., the pharate adult, as already accentuated by KRISTENSEN (1999). A further point of disagreement comes from the primitive male genital sclerites of Raphidioptera (U. ASPÖCK & H. ASPÖCK 2008). These key characters still point into the direction of a basal split of Neuropterida. And finally, there is a lack of convincing synapomorphies of the Endopterygota except Hymenoptera.

Phylogenetic relationships among Raphidioptera, Megaloptera, and Neuroptera

Mono- or paraphyly of Megaloptera?

Monophyly of Raphidioptera and of Neuroptera is undisputed (e.g., KRISTENSEN 1999; H. ASPÖCK et al. 1991; U. ASPÖCK et al. 2001; WINTERTON et al. 2010; HARING et al. 2011), while the monophyly of Megaloptera has been discussed repeatedly, e.g., in the context of analyses based on the ovaries (e.g., STYS & BILINSKI 1990). But BÜNING (1994, 2005), who summarised these studies, concluded that the ovaries are no suitable traits due to “... several switches between polytrophic and telotrophic ovaries ...” having occurred during the radiation of ancient insect taxa.

However, the controversy continued as in the comprehensive morphological analysis of BEUTEL et al. (2010a; comprising all Endopterygota) Megaloptera are the only non-monophyletic order: The family Corydalidae appears as sister group of Raphidioptera. However, BEUTEL et al. (2010a) themselves consider that this relationship may be an artefact, caused by the advanced predacious habits in the larvae of the two groups. Furthermore, this contradicts results presented by some of the authors in an earlier analysis resulting in monophyletic Megaloptera (BEUTEL & FRIEDRICH

2008). The latter analysis revealed several synapomorphies in the larval heads of both families, e.g., a special sensillum on the antepenultimate larval antennomere, a vestigial salivary duct, and a verticopharyngeal muscle with several subcomponents. Monophyletic Megaloptera were also supported in an analysis of male genital sclerites (U. ASPÖCK & H. ASPÖCK 2008), with the ever-visible sacs within the fused gonocoxites 11 being the important synapomorphy. The most recent monograph on the Megaloptera of China by YANG & LIU (2010) also corroborates the monophyly of this “archaic” looking group, the arguments being the fusion of Subcosta and Radius at the tip of the wing and the possession of abdominal gills in the first instar larva.

Molecular genetic analyses are not concordant concerning the monophyly of Megaloptera. The first molecular phylogeny of Neuropterida (HARING & U. ASPÖCK 2004) based on two mitochondrial genes (16S rRNA, COIII) ended up with monophyletic Megaloptera. Later, WINTERTON et al. (2010) proposed paraphyletic Megaloptera. They presented a molecular phylogeny based on two nuclear (CAD, 18S rRNA) and two mitochondrial genes (16S rRNA, COII) which was combined with morphological data. While the purely DNA based tree of WINTERTON et al. (2010) revealed Corydalidae as the sister group to all remaining Neuropterida (but without convincing support), in the combined trees either Sialidae or Corydalidae branched off from the most basal node, depending on the tree building algorithm. Interestingly, the tree based on morphology alone resulted in monophyletic Megaloptera. Thus, the contradictory results do not allow drawing unambiguous final conclusions on the question of monophyletic Megaloptera. Unfortunately, the study of WIEGMANN et al. (2009) could not contribute to this problem, as only one species of Megaloptera was included (*Nigronia* sp., Corydalidae).

Given the lack of convincing arguments for paraphyletic Megaloptera the hypothesis of monophyletic Megaloptera (HARING & U. ASPÖCK 2004; U. ASPÖCK & H. ASPÖCK 2008; BEUTEL & FRIEDRICH 2008) is the premise for the following discussion of the sister group relationships among the three orders of Neuropterida: (1) Raphidioptera + Megaloptera, (2) a trichotomy of Raphidioptera – Megaloptera – Neuroptera, (3) Megaloptera + Neuroptera.

Raphidioptera + Megaloptera?

The hypothesis of a clade Raphidioptera + Megaloptera has been discussed repeatedly in the past and has most recently been advocated by, e.g., BEUTEL et al. (2010a) in the endopterygotan context, al-

though in their analyses Megaloptera are paraphyletic (i.e., Sialidae + (Corydalidae + Raphidioptera)). In that morphological analysis two synapomorphies of Megaloptera and Raphidioptera are emphasised: (1) The distinctly flattened larval heads. However, we consider this as a symplesiomorphy of Megaloptera and Raphidioptera, both having also simple chewing mouthparts. In contrast, the architecture of the larval heads of the Neuroptera is highly influenced by the complex sucking tubes and thus represents a derived condition. (2) The prognathous heads of adult Raphidioptera and Megaloptera. But again, this is more likely a symplesiomorphy of these orders, irrespective of para- or monophyletic Megaloptera. The orthognathous heads of the Neuroptera are characterised by the loss of the gula, which can be plausibly understood as an autapomorphy (BEUTEL et al. 2010c). Whether the loss of the gula eventually induced the enormous heterogeneity of head forms and mouth parts remains speculative.

The comprehensive analysis of the Neuropterida by WINTERTON et al. (2010) comprises a huge data set of molecular and morphological characters. However, this study does not provide clear insights concerning the relationships between Raphidioptera and Megaloptera. In most trees Megaloptera are paraphyletic (see above), but support for the various topologies is in general weak as commented by the authors themselves. Moreover, the trees in general show very low support values. The only tree showing a sister group relationship of Raphidioptera + Megaloptera is based on morphology alone and does not display any support values at all.

Only recently a sister group relationship of Megaloptera + Raphidioptera has been hypothesized in the context of a graph illustrating the evolution of the female postabdomen (HÜNEFELD et al. 2012: fig. 6). In the legend it is written “Raphidioptera, with secondary ovipositor”. However, the ovipositor of Raphidioptera can be clearly deduced from the primitive archaeognathan pattern, only the extreme elongation of the ninth gonocoxites and the complete fusion of the eighth gonapophyses are derived conditions (U. ASPÖCK & H. ASPÖCK 2008).

Unresolved trichotomy of Raphidioptera – Megaloptera – Neuroptera

An unresolved trichotomy of Neuropterida (Raphidioptera – Megaloptera – Neuroptera) was presented in a paper of WHEELER et al. (2001) in the frame of a huge analysis of Hexapoda. Despite the impressive data set – 275 morphological variables from literature (referen-

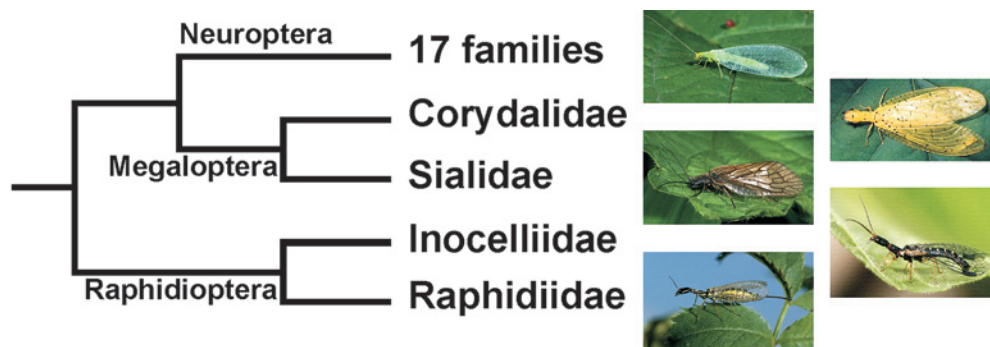


Fig. 1. Sister group relationships among the three orders of Neuropterida proposed by HARING & U. ASPÖCK (2004) and U. ASPÖCK & H. ASPÖCK (2008).

ces in WHEELER et al. 2001) were combined with DNA sequence data of 18S and 28S rRNA genes – there was no resolution of the sister group relationships of the three orders. However, this lack of resolution might be due to the quality of the morphological characters as discussed in KLASS (2007).

Megaloptera + Neuroptera?

A clade Megaloptera + Neuroptera emerged in two huge analyses in the context of a phylogeny of Hexapoda (KJER et al. 2006) or Endopterygota (CAMERON et al. 2009). KJER et al. (2006) analysed the Hexapoda based on 170 morphological characters in combination with DNA sequence data from several genes: the nuclear genes for 28S rRNA, 18S rRNA, histone H3, and elongation factor EF-1 α , as well as the mitochondrial genes for cytochrome oxidase subunits 1 and 2 (COI, COII), 12S rRNA and 16S rRNA plus their intervening tRNA. In the trees of this analysis Megaloptera + Neuroptera is well supported, although it has to be mentioned that Neuropterida are represented by one genus from each order only.

CAMERON et al. (2009) in their analysis of Hexapoda – curiously enough – emphasize the Neuropterida even in their title: “A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders”. This is unique in the extensive endopterygotan analyses. The analysis is based on complete mitochondrial genomes of seven hexapodan orders and provided good support for Megaloptera + Neuroptera irrespective of the algorithm and set of genes used. However, the three neuropteridan orders are represented by only one species each. The limited information (probably based on sequence saturation) provided by the mt sequence data is exemplified by the fact that Raphidioptera are located at different po-

sitions in the various trees, and in most cases do not cluster with Megaloptera + Neuroptera.

A sister group relationship of Megaloptera and Neuroptera emerged in our molecular genetic analysis (HARING & U. ASPÖCK 2004) and in our analysis of the genital segments (U. ASPÖCK & H. ASPÖCK 2008), both studies dedicated to the Neuropterida. The former analysis was based on one nuclear (18S rRNA) and two mitochondrial (COI, 16S rRNA) genes and resulted in a tree still containing several poorly supported nodes; it thus remained ambiguous in some aspects. This is due to several factors such as considerable rate differences between lineages, partly leading to long branch attraction, as well as sequence saturation in the mt genes. EF-1 α proved to be not applicable due to the existence of paralogous sequences. Nevertheless, the DNA sequence data clearly favoured a sister group relationship of Megaloptera and Neuroptera, which is in accordance with the morphology based data (U. ASPÖCK & H. ASPÖCK 2008) and the four main synapomorphies of these two orders: (1) elongation of the larval stipites, (2) integration of the larval cardines into the head capsule, (3) the complex organisation of trichobothria in the ectoproct in form of a rosette, which we regard as a derived character status, and (4) male gonocoxites 9 becoming appendices of tergite 9 (U. ASPÖCK & H. ASPÖCK 2010).

Neuropteridan basal dichotomies and character evolution

Assuming that Megaloptera and Neuroptera form a clade, Raphidioptera are ipso facto their adelphotaxon. These sister group relationships among the three orders of Neuropterida are illustrated in Fig. 1.

The sister group relationships are meaningful in the context of evolution of characters related to aquatic vs. terrestrial life style: While all Raphidioptera have ter-

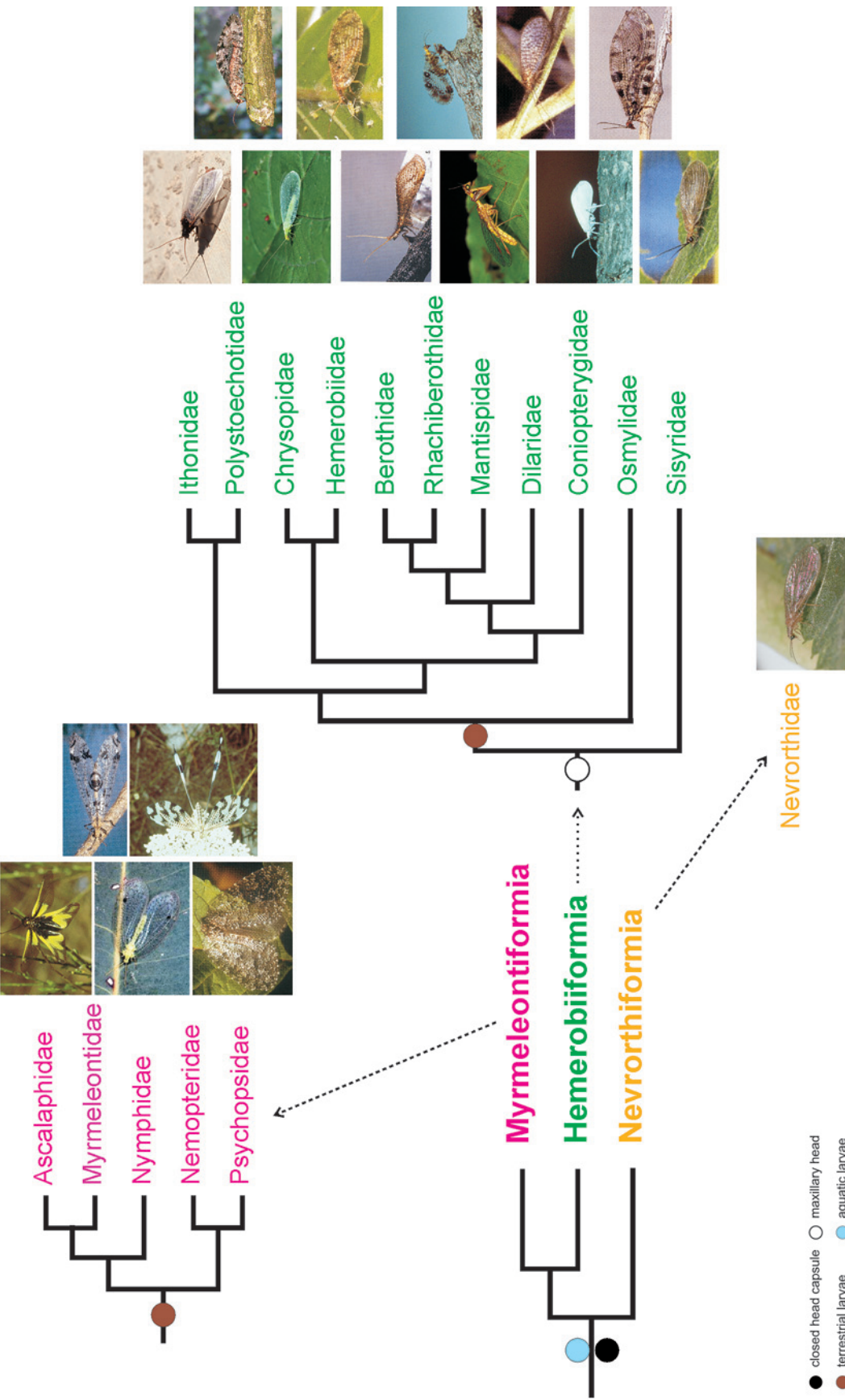


Fig. 2. Phylogenetic relationships within Neuroptera (alternative A): Summarizing tree combining previous studies (U. Aspöck et al. 2001; U. Aspöck & H. Aspöck 2008). The three-suborder-concept of Neuroptera and the sister group relationships of the families are presented.

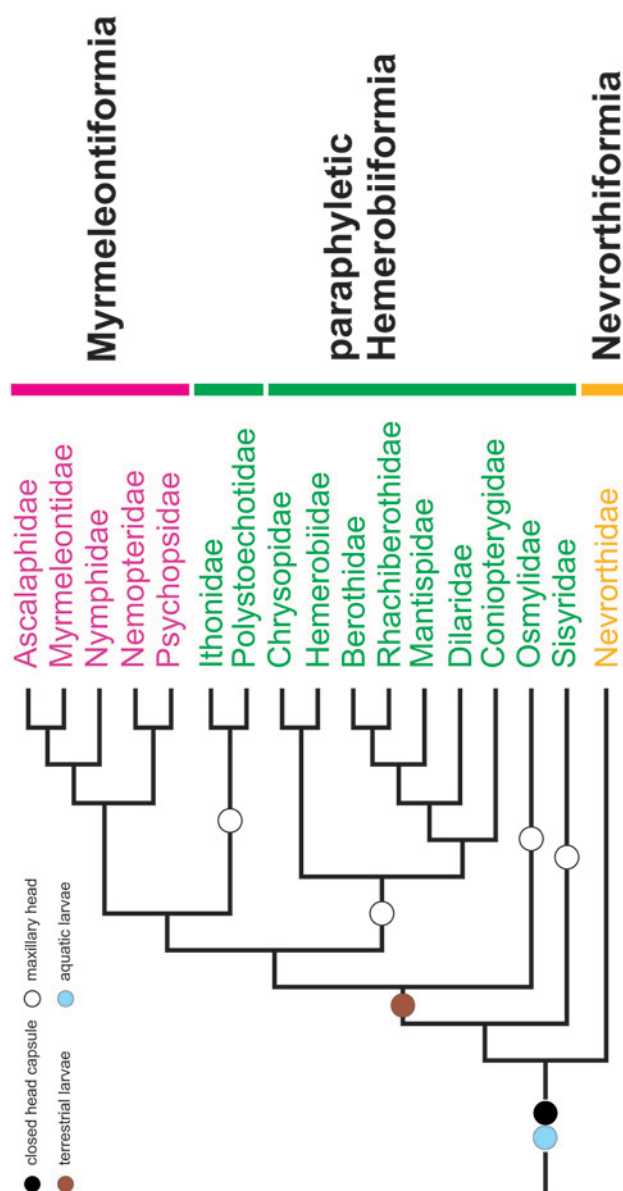


Fig. 3. Phylogenetic relationships within Neuroptera (alternative B): Summarizing tree combining previous studies (HARING & U. ASPÖCK 2004; U. ASPÖCK & H. ASPÖCK 2008). The sister group relationships among families in the context of disrupted Hemerobiiformia are presented.

restrial larvae, the larvae of Megaloptera are aquatic and generally have closed stigmata. Only certain species, e.g., *Neohermes californicus* (Corydalidae: Chaulioidinae), have larvae possessing closed as well as open stigmata. Among Neuroptera, only Nevrothidae and Sisyridae have aquatic larvae with closed stigmata, while the larvae of all others are terrestrial. ZWICK (1967) discussed the closed stigmata of Sisyridae and Nevrothidae as convergence. However, he did so without considering Megaloptera and under the assumption that Osmylidae are the sister group of Nevrothidae. He further suggested that the phylogenetic relevance of the stigmata would only be given if the concerned taxa shared an aquatic life style as a synapomorphy, but this is not congruent with his interpretation. Nonetheless, in the light of the Megaloptera + Neuroptera hypothesis, the most parsimonious assumption is that their common stem species acquired aquatic larvae and that the terrestrial life style of Neuroptera larvae evolved secondarily (U. ASPÖCK et al. 2003), which involved the reopening of the stigmata (as it has to be assumed quite clearly for *N. californicus*).

When discussing the aquatic life style, the evolution of strategies for providing oxygen has to be considered. Larvae of Megaloptera have abdominal gills throughout all instars, while the larvae of Sisyridae show them only in the second and third instar. The larvae of Nevrothidae lack abdominal gills; they gain oxygen via their body surface. Whether the abdominal gills in larvae of Megaloptera and Sisyridae represent the ground pattern or are parallel adaptations remains to be discussed. Assuming that the abdominal gills evolved in the stem species of Megaloptera + Neuroptera, it must be concluded that they have been lost secondarily in Nevrothidae. This could be explained by high amount of oxygen in the fast running waters which are the typical biotopes of Nevrothidae.

In general, completely free Malpighian tubules (as found in terrestrial larvae of Raphidioptera as well as aquatic larvae of Megaloptera and Neuroptera) are considered as the ancestral state (U. ASPÖCK & H. ASPÖCK 2005). The cryptonephry of the terrestrial larvae of the Neuroptera (i.e., several or all Malpighian tubules attached to the hindgut; GAUMONT 1976) is an adaptation in response to the secondary terrestrial lifestyle and can be interpreted as a synapomorphy of the terrestrial families (U. ASPÖCK 1995, 2002; U. ASPÖCK & H. ASPÖCK 2010). The weak cryptonephry of the aquatic larvae of Sisyridae with (only) one mounted Malpighian tubule may be an adaptation to their special habitat (they are parasites of fresh water sponges and bryozoans). Our former interpretation that the single cryptonephric Malpighian tubule represents the reminiscence of a terrestrial intermezzo of the Sisyridae (U. ASPÖCK 1992) does not appear plausible in the light of recent molecular trees, but still remains an optional hypothesis.

The seventeen families of the heterogeneous Neuroptera and their promiscuous statuses

The different hypotheses on the phylogenetic relationships among neuropteran families are reflected by competing concepts concerning the number and composition of suborders as well as the basal bifurcations. The morphology based three-suborder-concept initially presented by U. ASPÖCK (1992, 1995), corroborated by U. ASPÖCK et al. (2001) and further confirmed by BEUTEL et al. (2010b) divides Neuroptera into Nevrothiformia, Myrmeleontiformia and Hemerobiiformia with the latter two being sister groups. In this concept (illustrated in Fig. 2) a compact larval head capsule with a big gula is retained in Nevrothiformia. In Myrmeleontiformia the compact head capsule became highly derived by a shift of the tentorium to the anterior part of the head and by a largely reduced gula and enlarged basal sclerites covering the huge musculature operating the big sucking tubes. In Hemerobiiformia the loss of the compact head capsule in larvae led to the formation of the so-called “maxillary head” (U. ASPÖCK 1992). Thus, the ventrally highly modified sclerites of the larval head of the Hemerobiiformia do not correlate with a primitive type of feeding as assumed by MACLEOD (1964). For the sake of completeness it should be mentioned that an alternative hypothesis proposing the relationships (Nevrothiformia + Myrmeleontiformia) + Hemerobiiformia was discussed by U. ASPÖCK (1993), based (1) on the special articulation of the larval heads of Nevrothiformia and Myrmeleontiformia as a synapomorphy, and (2) on pleuritocavae found in male specimens of some representatives which might be interpreted as an underlying synapomorphy. In this concept terrestrial larvae would have evolved twice.

Several other studies based on morphological as well as genetic data resulted in phylogenies differing in multiple aspects of interfamilial relationships. But the common feature of all these trees is that Hemerobiiformia are paraphyletic with several families splitting as independent lineages: either Sisyridae, Osmylidae and the polystoechotid clade (HARING & U. ASPÖCK 2004; U. ASPÖCK & H. ASPÖCK 2008), or Coniopterygidae, Sisyridae, Dilaridae, Osmylidae and the berothid clade (WINTERTON et al. 2010), or Sisyridae, the polystoechotid clade, and a clade comprising Osmylidae, Hemerobiidae and Chrysopidae (ZIMMERMANN et al. 2011). Among these studies the question of the most basal bifurcation in Neuroptera was discussed controversially. A basal split of Nevrothidae was supported in two studies (HARING & U. ASPÖCK

2004; U. ASPÖCK & H. ASPÖCK 2008), while in other analyses Sisyridae (ZIMMERMANN et al. 2011) or Coniopterygidae (WINTERTON et al. 2010) compete for the first bifurcation. It should be noted, however, that the basal split of the Sisyridae (ZIMMERMANN et al. 2011) is apparently based on a symplesiomorphy: the larval poison gland and poison channel present in all Neuroptera except Sisyridae was plausibly lost in Sisyridae as an adaptation to their life style in freshwater sponges (no necessity of extraintestinal digestion). The position of Coniopterygidae is traditionally problematic. Their sister group relationship with Dilaridae in HARING & U. ASPÖCK (2004) was interpreted as a presumable result of long branch attraction. In other studies the Coniopterygidae appeared as sister group of the whole dilarid clade (Dilaridae + (Mantispidae + (Rhachiberothidae + Berothidae))) (U. ASPÖCK & H. ASPÖCK 2008; BEUTEL et al. 2010b; ZIMMERMANN et al. 2011) or as sister group of all remaining Neuroptera (WINTERTON et al. 2010). The latter topology obtained, however, high support only in the combined (gene sequences + morphology) tree, while in the solely DNA based tree the respective node was not supported at all. The disruption of Hemerobiiformia has emerged from various analyses, but in different ways depending on the analytical methods and markers used. This concerns mainly the families Coniopterygidae, Sisyridae, Osmylidae and Dilaridae, which are instable in their positions. A variety of topologies resulted from the studies mentioned above but the relationships remain ambiguous in many aspects as in all trees there is only weak support for most deeper nodes. A tree illustrating the hypothetical relationships emerging from our previous analyses (HARING & U. ASPÖCK 2004; U. ASPÖCK & H. ASPÖCK 2008) is shown in Fig. 3. Assuming that the secondary closure of the head capsule is an extremely improbable event (not principally, but in the special form it is present in recent Myrmeleontiformia), the opening of the head capsule (giving rise to the maxillary head) should have occurred four times independently.

Finally, there are some groups that are stable and well supported in all analyses: Myrmeleontiformia, Hemerobiidae + Chrysopidae, Ithonidae + Polystoechotidae. The result of Polystoechotidae nested within paraphyletic Ithonidae (HARING & U. ASPÖCK 2004) was recently supported by WINTERTON & MAKARKIN (2010). All analyses except HARING & U. ASPÖCK (2004) agree in accepting a berothid clade, either with Berothidae + Rhachiberothidae as sister group of Mantispidae (U. ASPÖCK & MANSELL 1994; U. ASPÖCK & H. ASPÖCK 2008) or with Rhachiberothinae nested within Berothidae (TJEDER 1959; MACLEOD & ADAMS 1967) or within Mantispidae (WINTERTON et al. 2010).

Concerning the internal relationships within Berothidae, so far six subfamilies have been promoted:

Nyrminae, Cyrenoberothinae, Protobiellinae, Trichomatinae, Nosybinae and Berothinae (U. ASPÖCK 1989; U. ASPÖCK & NEMESCHKAL 1998). In connection with the rediscovery and redescription of the enigmatic genus *Omiscocerus* Blanchard, 1851 by PENNY & WINTERTON (2007), it was overlooked that this genus apparently is related to the genus *Nyrma* Navás, 1933 from Anatolia. Only recently, in quite another context, WINTERTON (2010) mentioned the similarity of the two genera, suggesting that both belong to the Cyrenoberothinae, ignoring the already established subfamily Nyrminae (ASPÖCK & NEMESCHKAL 1998), to which *Nyrma* and the redescribed *Omiscocerus* belong.

Concerning the internal relationships within Coniopterygidae the hypothesis of ((Brucheiserinae + Coniopteryginae) + Aleuropteryginae) has been recently presented (ZIMMERMANN et al. 2009) based on the following synapomorphies: (1) The larvae of Coniopteryginae and Brucheiserinae have laterally extended heads as a result of the prominence of the ocular regions. (2) The larvae of both subfamilies possess sucking stylets which barely project beyond the tip of the labral margin. Assuming this sister group relationship, the plicatures (sac-like segmental structures on the abdomen forming two lateral rows in Aleuropteryginae and Brucheiserinae and an additional ventral row in the latter; function unknown) should be interpreted as belonging to the ground pattern of the Coniopterygidae, and their absence in Coniopteryginae as a secondary loss. Furthermore, also the presence of wax glands is understood as belonging to the ground pattern of the family, despite their reduction in Brucheiserinae.

Conclusio

The phylogeny of Neuropterida is of significance with respect to our understanding of the reconstruction of character evolution and the evolution of life styles of the larvae. It is an unsatisfying fact that, despite the numerous studies dedicated to the phylogeny of Neuropterida and their position within Endopterygota, these questions still are far from being solved. The reasons for this lack of unambiguous resolution are diverse. Morphological characters are prone to the problems resulting from convergence and interpretation of ambiguous character polarity. DNA marker sequences often are insufficient for various reasons (too short sequences, mutational saturation, lack of information). Moreover, incomplete taxon sampling and data matrices contribute to the problem. In addition it cannot

be ruled out that the difficulties to achieve a final unambiguous solution are due to the fact that the evolutionary splits leading to the various lineages presently known as orders and suborders occurred rather fast. Presently, our attention is focused on advanced molecular biological methods, in particular on on-going analyses of transcriptomes, which may answer the open questions in the near future.

Acknowledgements

We cordially thank Harald Bruckner, David Faulkner, Peter Duelli, Agostino Letardi, Hubert Rausch, Peter Sehnal and Michael Stelzl for providing photographs. We are grateful to Klaus Klass for his constructive and valuable suggestions on the manuscript.

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