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On wings of lace: phylogeny and Bayesian divergence time estimates of Neuropterida (Insecta) based on morphological and molecular data

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Abstract. Neuropterida comprise the holometabolan orders Neuroptera (lacewings, antlions and relatives), Megaloptera (alderflies, dobsonflies) and Raphidioptera (snakeflies) as a monophyletic group sister to Coleoptera (beetles). The higher-level phylogenetic relationships among these groups, as well as the family-level hierarchy of Neuroptera, have to date proved difficult to reconstruct. We used morphological data and multi-locus DNA sequence data to infer Neuropterida relationships. Nucleotide sequences were obtained for fragments of two nuclear genes (CAD, 18S rDNA) and two mitochondrial genes (COI, 16S rDNA) for 69 exemplars representing all recently recognized families of Neuropterida as well as outgroup exemplars from Coleoptera. The joint posterior probability of phylogeny and divergence times was estimated using a Bayesian relaxed-clock inference method to establish a temporal sequence of cladogenesis for the group over geological time. Megaloptera were found to be paraphyletic with respect to the rest of Neuropterida, calling into question the validity of the ordinal status for Megaloptera as presently defined. Ordinal relationships were weakly supported, and monophyly of Megaloptera was not recovered in any total-evidence analysis; Corydalidae were frequently recovered as sister to Raphidioptera. Only in relaxed-clock inferences were Raphidioptera and a paraphyletic Megaloptera recovered with strong support as a monophyletic group sister to Neuroptera. A monophyletic Neuroptera diverged from a common Raphidioptera + 'Megaloptera' ancestor during the Late Carboniferous. Contrary to some previous hypotheses, Coniopterygidae, not Nevrorthidae, were recovered as sister to the rest of Neuroptera, with Nevrorthidae recovered with Osmylidae and Sisyridae. The monophyly of the universally recognized Myrmeleontiformia was confirmed, with an origin in the mid-Triassic, but a monophyletic Hemerobiiformia was not recovered in any analysis. Dilaridae were not closely related to the clade comprising Mantispidae and Berothidae, and diverged earlier than proposed previously. The phylogenetic status and taxonomic composition of Polystoechotidae and Ithonidae are in need of re-evaluation, as *Oliarces* Carpenter (presently Ithonidae) was placed well within the present circumscription of Polystoechotidae.

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Introduction

'On wings of lace' describes the lace-like venation that is characteristic of the wings of insects in the orders Neuroptera (lacewings, antlions and relatives) (=Planipennia) (Fig. 1), Megaloptera (alderflies, dobsonflies) and Raphidioptera (snakeflies) (Grimaldi & Engel, 2005). Collectively known as Neuropterida, this clade is considered to include the oldest Endopterygota, with many members exhibiting a variety of plesiomorphic characteristics (Hennig, 1981; New, 1989; Kristensen, 1999; Wiegmann *et al.,* 2009). Both morphological and molecular evidence support placement of Neuropterida as sister to Coleoptera, which together comprise the clade called Neuropteroidea (Kristensen, 1991; Whiting *et al*., 1997; Kristensen, 1999; Wiegmann *et al.,* 2009) (although see Whiting, 2002). Traditional arrangements of neuropterid relationships place Neuroptera as sister to Megaloptera + Raphidioptera, based mainly on the plesiomorphic presence of chewing larval mouthparts in the latter two groups (Achtelig & Kristensen, 1973; Achtelig, 1976; Kristensen, 1981). This hypothesis was questioned by Boudreaux (1979), who instead suggested that Raphidioptera were sister to Megaloptera + Neuroptera, a view supported subsequently in a cladistic analysis of morphological characters by Aspöck *et al*. (2001). Recently, Wiegmann *et al*. (2009) presented a robust phylogeny of Holometabola based on multiple nuclear protein-encoding loci, wherein the traditional grouping of (Megaloptera + Raphidioptera) Neuroptera found strong support. Moreover, the monophyly of Megaloptera has not been resolved. Hennig (1953) and Achtelig (1967) suggested that Megaloptera were paraphyletic with respect to Raphidioptera, whereas a sister-group relationship between Sialidae and Raphidioptera was supported by Stys & Bilinski (1990) and Kubrakiewicz *et al*. (1998) based on striking similarities in ovariole type between the two groups. The latter conclusion was dismissed by Büning (1998) who, by providing an alternative interpretation of ovariole similarities, retained a monophyletic Megaloptera.

Megaloptera are a relatively small order comprising about 280 extant species worldwide divided into two families, Sialidae and Corydalidae (New & Theischinger, 1993). Sialidae (alderflies) (75 species in seven genera) are represented predominantly by *Sialis* Latreille, a genus common throughout the Holarctic region, although other smaller genera are found in other regions (Liu *et al*., 2008). Corydalidae (dobsonflies and fishflies) are divided into two subfamilies, Chauliodinae (*c*. 115 species in 14 extant genera) and Corydalinae (*c*. 165 species in 12 extant genera) (Oswald, 2007). Corydalidae contain some very large insects, including members of the American genus *Corydalus* Latreille, the larvae of which are major invertebrate predators in lotic ecosystems (New & Theischinger, 1993). Larvae of Megaloptera are strictly aquatic and are characterized by the presence of lateral abdominal gills.

Raphidioptera comprise 228 extant species worldwide, divided into two families, Inocellidae (27 species in 6 genera) and Raphidiidae (201 species in 12 genera) (Oswald, 2007). Snakeflies have a mostly circum-boreal distribution, usually in cooler environments such as the northern temperate zone or higher altitudes at lower latitudes. Adults are arboreal, whereas larvae live as terrestrial predators under bark or in leaf litter (Aspöck, 2002).

Neuroptera are a moderately sized order of *c*. 5750 extant species in 16 families represented in all major biogeographical regions (Oswald, 2007). The monophyly of Neuroptera is supported by three unambiguous and unique larval characteristics: (i) mouthparts with buccal cavity closed anteromedially and sucking tubes formed laterally by the interlocking of the mandibles and maxillae, (ii) mid-gut discontinuous with the hind gut – solid waste is not passed until the adult emerges from the pupal case with a fully formed digestive system, and (iii) Malpighian tubules modified for silk production from the anus (Withycombe, 1925; MacLeod, 1964; Gaumont, 1976; New, 1989). The formation of sucking mouthparts has been considered a key innovation for the success of the group (Brauer, 1852; Hennig, 1981; New, 1989).

Lacewings have a rich diversity of radically divergent morphologies and highly specialized life histories, particularly as larvae. Although many neuropteran larvae are generalist predators, some are specialized obligate predators with a narrow range of hosts or prey. For example, the aquatic larvae of spongilla-flies (Sisyridae) have highly modified mouthparts for preying upon freshwater sponges and bryozoans, and beaded lacewing larvae (Berothidae) prey on termites as termitophiles (New, 1989). Mantispid larvae (Mantispidae) undergo hypermetamorphic development. Some (e.g. Symphrasinae) are predators in social hymenopteran nests, whereas Mantispinae inhabit spider egg cases as predators, often with highly specialized obligatory behavioural cues (Redborg, 1998). Moth lacewings (Ithonidae) and giant lacewings (Polystoechotidae) have scarabaeiform, fossorial larvae with blunt mouthparts and have been associated with the root systems of plants, although the actual food source has not been confirmed (Gallard, 1932; Faulkner, 1990). This disparate diversity in larval morphology and life history of lacewings, combined with the relatively generalized adult form, has obscured structural homologies at the family level. Difficulties in identifying structural homologues and the slow pace of progress towards understanding neuropteran evolution were surmised to be '... among the principal contemporary challenges in basal endopterygote phylogenetics' by Kristensen (1999, p. 246).

Previous hypotheses of neuropteran family-level relationships are based largely on qualitative narratives. Consequently there is little consensus among phylogenetic hypotheses advanced by previous authors (Fig. 2). The first phylogram of neuropteran relationships was produced by Handlirsch (1906–1908) and was based primarily on wing venational features of fossils (Fig. 2A). Tillyard (1916, 1919) discussed the relationships among neuropteran family groups using mainly wing venation, but also larval mouthparts. Withycombe (1925) presented a detailed phylogenetic study of Neuroptera family-group relationships based on immature stages and imaginal internal anatomy (Fig. 2B), recognizing five distinct superfamilies. Martynova (1949) proposed a higher classification and phylogeny of Neuroptera, placing the numerous fossil families into context with extant families.

Fig. 1. Representative adult Neuroptera: A, *Ithone fulva* Tillyard (Ithonidae); B, *Euclimacia nuchalis* (Gerstaecker) (Mantispidae); C, *Myiodactylus osmyloides* Brauer (Nymphidae); D, *Porismus strigatus* (Burmeister) (Osmylidae); E, *Psychopsis insolens* McLachlan (Psychopsidae); F, *Chasmoptera hutti* (Westwood) (Nemopteridae); G, *Stenobiella muellerorum* Aspöck & Aspöck (Berothidae); H, *Zachobiella pallida* Banks (Hemerobiidae). Photographs by S.L. Winterton.

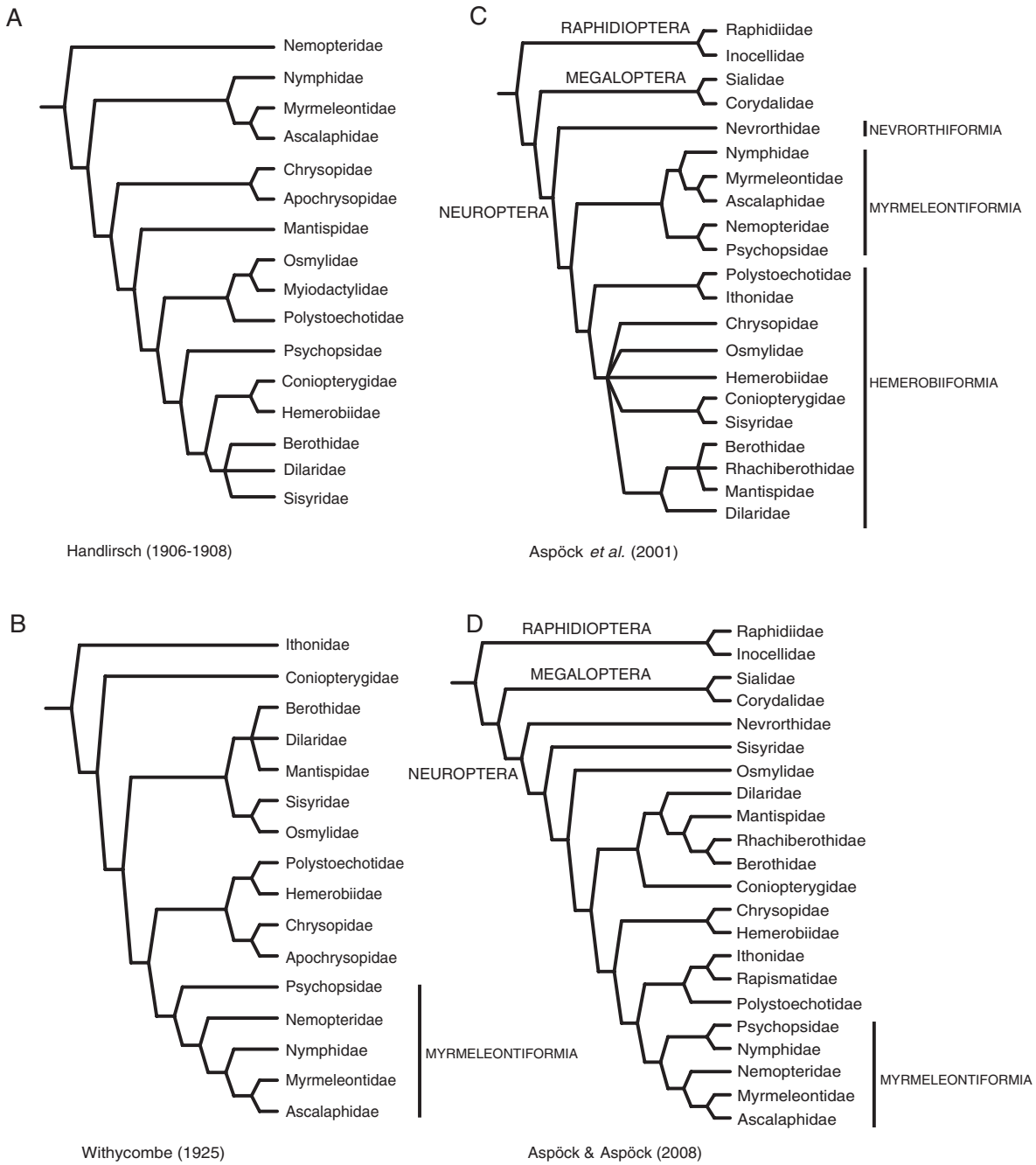

Fig. 2. Phylogenetic relationships of Neuroptera: A, after Handlirsch (1906–1908); B, after Withycombe (1925); C, after Aspöck *et al*. (2001) (strict consensus) and D, after Aspöck & Aspöck (2008).

Schlüter (1986) produced a consensus phylogeny and inferred timescale for lacewing clades, recognizing six superfamilies (Myrmeleontoidea, Hemeroboidea, Mantispoidea, Osmyloidea, Coniopteroidea, Ithonoidea), which have formed the basis for many recent classifications (e.g. New, 1991). The most significant and detailed work on neuropteran classification based on larval morphology was that by MacLeod (1964), who described and figured larvae of all families (except Nevrorthidae and Rhachiberothidae) in a comparative evolutionary context.

The first quantitative cladistic analysis of neuropteran relationships based on adult and larval morphological characters was that of Aspöck *et al*. (2001). That study advanced 36 adult and larval morphological characters in support of ten equally parsimonious trees in an unweighted analysis

(Fig. 2C – strict consensus), and two trees from a successively weighted analysis. Non-conflicting character support for most clades in these trees was limited, with only Myrmeleontiformia unambiguously supported. Myrmeleontiformia, comprising Myrmeleontidae, Ascalaphidae, Nemopteridae, Nymphidae and Psychopsidae, had been recognized in part by Handlirsch (1906–1908) almost 100 years previously and was subsequently refined by numerous workers (e.g. Withycombe, 1925; MacLeod, 1964; Henry, 1978; Mansell, 1992). Myrmeleontiformia is characterized by several synapomorphic wing venation and larval characters and is the only clade of Neuroptera whose membership is supported almost unanimously by previous authors (New, 1989). Three suborders in Neuroptera, namely Nevrorthiformia, Hemerobiiformia and the aforementioned Myrmeleontiformia, were recognized by Aspöck *et al*. (2001). Nevrorthiformia, comprising the single family Nevrorthidae, was placed as sister to the rest of the order. Hemerobiiformia, as defined by Aspöck *et al*. (2001), contained the remaining families (i.e. Hemerobiidae, Chrysopidae, Berothidae, Rhachiberothidae, Dilaridae, Osmylidae, Mantispidae, Sisyridae, Ithonidae, Polystoechotidae and Coniopterygidae), although few synapomorphies were found that support the group. A recent study by Beutel *et al*. (2009) examining head structure in Nevrorthidae presented a more detailed morphological phylogenetic analysis (64 characters) but resulted in only a slight improvement in tree resolution on the previous quantitative estimate by Aspöck *et al*. (2001).

Nucleotide sequence data, especially from nuclear ribosomal genes, have been increasingly used as part of larger studies of endopterygote phylogeny that include inferences of relationships amongst Megaloptera, Neuroptera and Raphidioptera (Carmean *et al*., 1992; Pashley *et al*., 1993; Whiting *et al*., 1997; Whiting, 2002; Wiegmann *et al*., 2009). In the first study to use molecular sequence data to examine interfamilial phylogenetic relationships of Neuroptera, Winterton (2003) used a 2.2-KB section of 18S rDNA and 56 morphological characters. Using sequence data from four genes (18S and 16S rDNA, COIII and EF1-α) Haring & Aspöck (2004) also attempted to reconstruct relationships among family groups of Neuroptera. Evidence from the individual genes they sampled was highly conflicting, demonstrating the complexities of interpretation and analysis that often accompany multigene phylogenetics, especially when sampled genes differ significantly in evolutionary rate, copy number and substitution dynamics, and when cladogenetic radiations appear both ancient and rapid (Sanderson & Schaeffer, 2002; Whitfield & Lockhart, 2007). Recently, Aspöck & Aspöck (2008) sought to reconcile differences between the results of Aspöck *et al*. (2001) and those of Haring & Aspöck (2004) by reinterpreting the genitalic morphology of adult Neuropterida and proposing new hypotheses for the homology and transformation of genitalic sclerites across the major family-level groups (Fig. 2D). From these features they identified morphological support for the monophyly of Megaloptera, and for relationships of many neuropteran families.

Previous phylogenetic analyses of Neuropterida have been hampered by limited sampling of genes and taxa, and by reliance on inference methods that poorly accommodate data heterogeneity (i.e. differences in nucleotide substitution dynamics across loci and codon positions). Here, we present results based on a total-evidence analysis combining sequence data from four genes (18S and 16S rDNA, COI and CAD) with 55 morphological characters across 67 representatives from all extant families of Neuropterida. Three phylogenetic inference methods are used: Bayesian, maximum likelihood and maximum parsimony. Using mixed substitution models we reconstructed the optimal phylogeny under maximum likelihood and integrated over possible phylogenies with Bayesian methods. In addition, we estimated relationships and divergence times with mixed substitution models in concert with a relaxed-clock model of among-lineage rate evolution to establish a timescale for the origin and diversification of the major groups of Neuropterida.

Materials and methods

Exemplar selection

Sixty-seven Neuropterida taxa were selected for the analyses, including representatives from each of the 16 recognized families of Neuroptera, and two families each of Megaloptera and Raphidioptera (Table 1). Multiple exemplars representing major subfamilial groups were included, and special effort made to sample from nominal genera. Rapismatidae and Rhachiberothidae are not recognized as separate families here (cf. Aspöck & Aspöck, 2008), as convincing arguments for the inclusion of the former in Ithonidae have been presented by Penny (1996) and Makarkin & Menon (2007), and the later is recognized as a subfamily of Berothidae following MacLeod & Adams (1968). Two representatives from Coleoptera were selected for outgroup comparison. *Calosoma scrutator* (Fabricius) (Carabidae) and *Strangalia bicolor* (Swederus) (Cerambycidae) were selected to represent the major beetle suborders Adephaga and Polyphaga, respectively. Although the sister-group relationship between Strepsiptera and Coleoptera and its inclusion in Neuropteroidea was strongly supported recently using sequence data (Wiegmann *et al*., 2009), the order was not included herein because of inherent alignment issues for ribosomal genes (e.g. Whiting *et al*., 1997). Vouchers of all specimens sequenced or examined in morphological studies are deposited in the California Academy of Sciences in San Francisco, U.S.A. (Table 1).

Morphological characters

Fifty-five morphological characters describing adult, larval and egg stages were scored for the morphological dataset. Genitalic characters were not scored in this analysis because the highly divergent morphology among families of Neuropterida made it difficult to identify homologous structures confidently, as exemplified by various previous attempts to apply consistent terminology across groups (e.g. Tjeder, 1954; Acker, 1960;

Table 1. Neuropteroidea exemplars used in this study for sequencing and/or morphological scoring.

Order/ family/ subfamily	Exemplar	Specimen voucher/code	16S	COI	18S	CAD	Collection data or reference
Coleoptera							
Carabidae							
Carabinae	*Calosoma scrutator* (Fabricius) (adult and larva)	CASENT8092152	EU734850	EU839722	AF002800	EU860105	U.S.A.: Arizona: Santa Cruz Co., Pena Blanca, 10.ix.1997, D. R. Maddison, 31.3852°N, 111.0931°W
Cerambycidae							
Lepturinae	*Strangalia bicolor* (Swederus)	CASENT8092153	EU734906	EU839773	EU815289	EU860157	U.S.A.: North Carolina: Wake Co., 6.vi.2004, B. K. Cassell
Megaloptera							
Corydalidae							
Chauliodinae	*Neohermes californicus* (Walker)	CASENT8092158	EU734880	EU839747	EU815261	FJ028812	U.S.A.: California: Placer Co., 7.viii.2005, D. Whitley, 38°54′26″N, 121°09′54″W
	Nigronia serricornis (Say)	CASENT8092157	EU734881	EU839748	EU815263	EU860133	U.S.A.: North Carolina: Smoky Mountains N.P., 13.vi.2001, S. L. Winterton [35°36.628N, 83°25.573W]
Corydalinae	*Corydalus armatus* Hagen	CASENT8092156	EU734858	EU839728	EU815238	EU860110	BOLIVIA: La Paz Prov.: Cumbre Alto Beni, 15.iv.2004, S. D. Gaimari, 15°40′19″S, 67°29′35″W
	Corydalus cornutus (Linnaeus) (adult and larva)	CASENT8092155	EU734853	EU839725	EU815239	EU860108	U.S.A.: Illinois, Urbana: 12.vi.2002, S. L. Winterton, D. Webb
Sialidae	*Sialis californica* Banks	CASENT8092162	EU734902	EU839768	EU815285	EU860153	U.S.A.: Oregon: Linn Co., Snow Peak, 07.vii.1999, K. C. Holston, 44°36′N, 122°14′W
	Sialis nr. *mohri* Ross	CASENT8092163	EU734903	EU839769	EU815286	EU860154	U.S.A.: Illinois: Madison Co., Trib. Mississippi Riv., SE Villa Ridge, 28.vi.2002, R. E. DeWalt, V. Block, 38.9103°N, 90.2178°W
Raphidioptera							
Raphidiidae	*Agulla bicolor* (Albarda) (adult and larva)	CASENT8092165	EU734847	EU839719	EU815227	EU860102	U.S.A.: California: Los Angeles Co., Mile High, 23.v.2003, J. Skevington, 34°26′52″N, 117°48′47″W
	Mongoloraphidia martynovae (Steinmann) (adult and larvae)	CASENT8092164	EU734870	EU839738	EU815252	EU860123	KAZAKHSTAN: Dzungarskij Alatau, Tyschkan valley, 6–7.vi.2001, M. Hauser, 44°30′N, 80°04′E
Inocellidae	*Parainocellia bicolor* (Costa)	CASENT8092166	EU734864	EU839733	EU815245	EU860116	ITALY: Basilicata: Maratea 10.vii.1999, A. Letardi
	Negha inflata (Hagen)	CASENT8092167	EU734877	EU839744	AF286272	EU860130	U.S.A.: California: San Bernardino Co., Wrightwood, 21–26.v.2005, S. L. Winterton, A. R. Cline, 34°19.03′N, 117°34.93′W
Neuroptera							
Ascalaphidae							
Ascalaphinae	*Libelloides rhomboideus rhomboideus* (Schneider)	CASENT8092187	EU734868	EU839736	EU815250	EU860121	GREECE: Peloponnisos Lakonia, 4.vi.2000, K. C. Holston, 37°05′N, 22°18′E
	Ululodes quadripunctatus (Burmeister) (adult and larva)	CASENT8092188	EU734854	EU839726	EU815235	EU860109	U.S.A.: North Carolina: Wake Co., Raleigh, 31.vii.2000, G. R. Balme

Table 1. Continued

Order/ family/ subfamily	Exemplar	Specimen voucher/code	16S	COI	18S	CAD	Collection data or reference
Berothidae							
Berothinae	*Lomamyia banksi* Carpenter	CASENT8092200	EU734869	EU839737	EU815251	EU860122	U.S.A.: North Carolina: Durham Co., 02.vi.2003, S. L. Winterton [36°0.739′N, 78°51.743′W]
	Podallea madegassica Aspöck & Aspöck	CASENT8092198	EU734892	EU839759	EU815276	EU860145	MADAGASCAR: Fianarantsoa Prov.: Isalo N.P., 7–22.ix.2002, R. Harin'Hala, M. E. Irwin, 22.5624°S, 45.3841°E
	Quasispermophorella ingwa Aspöck & Aspöck (adult and larva)	CASENT8092199	EU734898	EU839765	EU815281	EU860150	AUSTRALIA: Western Australia: Neerabup N.P., 29.xii.1999, J. & A. Skevington *et al.*, 31°38′25″S, 115°43′18″E
	Stenobiella muellerorum Aspöck & Aspöck	CASENT8092202	EU734900	EU839766	EU815283	EU860152	AUSTRALIA: Queensland: Taroom, 14.xi.1999, S. L. Winterton [25°36.635′S, 149°46.250′E]
Cyrenoberothinae	*Ormiscocerus nitidipennis* Blanchard	CASENT8092201	–	–	EU815267	–	CHILE: Elqui Prov.: Quebrada El Arrayán, 5.x.2003, M. E. Irwin, 30°04.26′S, 71°00.04′W
Rhachiberothinae	*Mucroberotha vesicaria* Tjeder	CASENT8092204	EU734872	EU839740	EU815254	EU860125	SOUTH AFRICA: Limpopo Prov. Beestsport Farm, 3.i.2005, C. S. Chaboo, 24°43.4′S, 28°38.3 E
Chrysopidae							
Chrysopinae	*Italochrysa insignis* (Walker) (adult and larva)	CASC210	DQ399278	DQ414485	EU815246	EU860117	AUSTRALIA: Queensland: Brisbane, Mt. Coot-tha, 14.i.2000, S. L. Winterton [27° 28.574′S, 152°57.817′E]
Nothochrysinae	*Nothochrysa californica* Banks	CASC205	DQ399283	DQ414505	EU815265	EU860135	U.S.A.: California: Monterey Co., Pfeiffer Big Sur, 2.iii.2003, J. & A. Skevington [36°14.939′N, 121°46.466′W]
	Pimachrysa nigra Adams	CASENT8092214	EU734889	EU839756	EU815273	EU860142	U.S.A.: California: Sacramento Co., Pine Hill, 24.iii.2003, J. Skevington, 38°43′N, 120°59′W
Apochrysinae	*Apochrysa lutea* (Walker) (adult and larva)	CASC203	DQ399285	EU839753	EU815269	EU860139	AUSTRALIA: Queensland: Brisbane, 13.xii.1998, S. L. Winterton [27° 28.574′S, 152° 57.817′E]
Coniopterygidae							
Coniopteryginae	*Conwentzia pineticola* Enderlein	CASENT8092172	EU734855	EU839727	EU815237	FJ028811	CANADA: Ontario: Ottawa, 17.vi.2001, J. Skevington
	Neosemidalis farinosa (Enderlein)	CASENT8092176	–	–	EU815262	–	AUSTRALIA: Queensland: Maiala N.P., Mt. Glorious, 5.i.2000, S. L. Winterton [27°20.009′S, 152°45.797′E]
	Semidalis vicina (Hagen)	CASENT8092173	EU734901	EU839767	EU815284	FJ028814	U.S.A.: North Carolina: Smoky Mountains N.P., 13.vi.2001, S. L. Winterton [35°36.628W, 83°25.573]
Aleuropteryginae	*Cryptoscenea* nr. *obscurior* Meinander	CASENT8092175	EU734860	EU839730	EU815241	EU860112	AUSTRALIA: Western Australia: Neerabup N.P., 29.xii.1999, J. & A. Skevington *et al.*, 31°38′25″S, 115°43′18″E
	Spiloconis sp.	CASENT8092174	EU734905	EU839771	EU815288	EU860156	AUSTRALIA: Queensland: Maiala N.P., Mt. Glorious, 5.i.2000, S. L. Winterton [27°20.009′S, 152°45.797′E]

Table 1. Continued

Order/ family/ subfamily	Exemplar	Specimen voucher/code	16S	COI	18S	CAD	Collection data or reference
Dilaridae							
Nallachinae	*Nallachius americanus* (MacLachlan)	CASENT8092215	EU734874	EU839742	EU815256	EU860127	U.S.A.: Texas: Brazos Co., 18.ix.2001, J. D. Oswald, 30°32′N, 96°17′W
	Nallachius pulchellus (Banks)	CASENT8092216	EU734875	EU839743	EU815257	EU860128	U.S.A.: Arizona: Cochise Co., Copper Canyon, 22.vii.2000, D. Yanega, 31°21′45′N 110°18′01′W
	Nallachius sp.	CASENT8092217	EU734876	–	EU815258	EU860129	DOMINICA: St. John: Cabrits N.P.19–20.iii.2003, M. E. Irwin *etal*., 15°35.0′N, 61°28.3′W
Dilarinae	*Dilar duelli* Aspöck & Aspöck	Dildue1	AY620164	–	AY620035	–	Haring & Aspöck (2004)
Hemerobiidae							
Notiobiellinae	*Notiobiella viridis* Tillyard (adult and larvae)	CASENT8092205	EU734883	EU839750	EU815266	EU860136	AUSTRALIA: Queensland: Rockhampton, 29.i.2000, S. L. Winterton [23°18.754′S, 150°30.966′E]
Psychobiellinae	*Psychobiella sordida* Banks	CASENT8092206	EU734896	EU839763	EU815279	FJ028813	AUSTRALIA: Queensland: Brisbane Forest Park, 29.xi.1999, S. L. Winterton [27°25.650′S, 152°50.471′E]
Drepanacrinae	*Conchopterella stangei* (Gonzalez-Olazo)	CASC202	EU734855	DQ414494	EU815236	DQ414474	CHILE: Osorna Prov.: Aguas Calientes, Puyehue N.P., 1–5.xii.2003, M. E. Irwin, 40°43.94′S, 72°18.83′W
Drepanepteryginae	*Drepanepteryx phalaenoides* (Linnaeus)	CASENT8092207	EU734861	EU839731	EU815242	EU860113	GREECE: Peloponnisos Messinia, Kardamili, 31.v.2000, K. C. Holston, 26°54′N, 22°14′E
Carobinae	*Carobius* sp.	CASENT809208	–	–	EU815231	–	AUSTRALIA: Queensland: Brisbane, 09.ii.2000, S. L. Winterton [27°28.574′S, 152°57.817′E]
Ithonidae							
	Ithone fulva Tillyard (adult and larva)	CASENT8092184	EU734865	EU839734	EU815247	EU860118	AUSTRALIA: Queensland: Rainbow Beach, J. Skevington, 25°54′S, 153°04′E
	Megalithone tillyardi Riek (adult and larva)	CASENT8092183	EU734866	EU839735	EU815248	EU860119	AUSTRALIA: New South Wales: Warrumbungle N.P., 17.x.1997, S. L. Winterton 31°16′17″S, 148°57′42″E
	Oliarces clara Banks (adult and larva)	CASENT8092185	EU734885	EU839752	AF012527	EU860138	U.S.A.: California: Imperial Co., Chocolate Mtns., 20.iv.2005, D. K. Faulkner
Mantispidae							
Drepanacinae	*Ditaxis biseriata* (Westwood) (adult and larvae)	CASENT8092194	EU734862	EU839732	EU815243	EU860114	AUSTRALIA: Queensland: Brisbane, 19.x.1999, S. L. Winterton [27°28.574′S, 152°57.817′E]
	Theristria imperfecta Lambkin	CASENT8092197	EU734909	EU839774	EU815292	EU860160	AUSTRALIA: Queensland: Brisbane, Mt. Coot-tha, 29.xi.1999, S. L. Winterton [27°28.574′S, 152°57.817′E]
Mantispinae	*Zeugomantispa minuta* (Fabricius) (adult and larvae)	CASENT8092195	EU734871	EU839739	EU815253	EU860124	U.S.A.: North Carolina: Durham Co., 12.vi.2001, S. L. Winterton [36°0.739′N, 78°51.743′W]
Symphrasinae	*Plega dactylota* Rehn	CASENT8092196	EU734891	EU839758	EU815275	EU860144	U.S.A.: Texas: Jeff Davis Co., Davis Mountains, 9.viii.2001, D. G. Marqua

Table 1. Continued

Order/ family/ subfamily	Exemplar	Specimen voucher/code	16S	COI	18S	CAD	Collection data or reference
Myrmeleontidae							
Palparinae	*Palpares libelloides* (Linnaeus)	CASENT8092177	EU734888	EU839755	EU815272	EU860141	ITALY: Basilicata: Maratea 10.vii.1999, A. Letardi
Stilbopteryginae	*Stilbopteryx costalis* Newman	CASENT8092178	EU734908	EU839773	EU815291	EU860159	AUSTRALIA: Queensland: Brigalow Res. Stn., 27–28.x.2000, Queensland Museum party [9804] 24°48′S, 149°45′E
Nemopteridae							
Crocinae	*Austrocroce attenuata* (Froggatt)	CASENT8092190	EU734859	EU839729	EU815240	EU860111	AUSTRALIA: Queensland: Taroom, 14.xi.1999, S. L. Winterton [25°36.635′S, 149°46.250′E]
	Dielocroce hebraea Hölzel	Dieheb1	AY620168	–	–	–	Haring & Aspöck (2004)
Nemopterinae	*Chasmoptera hutti* (Westwood)	CASENT8092192	EU734851	EU839723	EU815232	EU860106	AUSTRALIA: Western Australia: Seven Mile Beach, 15.xi.2000, T. F. Houston, 29°09′55″S, 114°53′25″E
	Lertha barbara (Klug)	CASENT8092193	EU734879	EU839746	EU815260	EU860132	TUNISIA: 15km E Tabarka, 13.vi.2000, M. Hauser, 36°58′N, 08°55′E
	Nemoptera coa (Linnaeus)	CASENT8092191	EU734878	EU839745	EU815259	EU860131	GREECE: Peloponnisos Messinia, Kardamili, 31.v.2000, K. C. Holston, 26°54′N, 22°14′E
Nevrorthidae							
	Austroneurorthus brunneipennis (Esben-Petersen) (larva)	CASENT8092189	EU734848	EU839720	EU815229	EU860103	AUSTRALIA: Queensland: Maiala N.P., Mt. Glorious, 23.xi.1999, S. L. Winterton [27° 20.009′S, 152°45.797′E]
Nymphidae							
	Myiodactylus osmyloides Brauer	CASENT8092179	EU734873	EU839741	EU815255	EU860126	AUSTRALIA: Queensland: Mt. Nebo, 15.ii.2003, G. B. Monteith [27°23.831′S, 152°47.024′E]
	Norfolius howensis (Tillyard)	CASENT8092180	EU734882	EU839749	EU815264	EU860134	AUSTRALIA: Queensland: Maiala N.P., Mt. Glorious, 5.i.2000, S. L. Winterton [27° 20.009′S, 152°45.797′E]
	Nymphes myrmeleonoides Leach (adult and larvae)	CASENT8092181	EU734884	EU839751	EU815268	EU860137	AUSTRALIA: Queensland: Brisbane: Indooroopilly, 12.xii.1999, S. L. Winterton [27°30.722′S, 152°59.787′E]
	Osmylops armatus (McLachlan) (adult and larvae)	CASENT8092182	EU734886	EU839754	EU815270	EU860140	AUSTRALIA: Queensland: Brisbane: Indooroopilly, 12.xii.1999, S. L. Winterton [27°30.722′S, 152°59.787′E]
Osmylidae							
Porismine	*Porismus strigatus* (Burmeister)	CASENT8092210	EU734894	EU839761	EU815278	EU860147	AUSTRALIA: New South Wales: Bawley Point, 3.iii.2000, O. Schmidt, 35°30′S, 150°24′E
Kempyninae	*Kempynus kimminsi* New	CASC200	EU734867	DQ515501	EU815249	EU860120	AUSTRALIA: Queensland: Springbrook, 25.iii.2003, G. B. Monteith [28°12.895′S, 153°16.174′E]
	Australysmus lacustris Kimmins	CASENT8092211	EU734849	EU839721	EU815230	EU860104	AUSTRALIA: New South Wales: Kosciuoszko N.P., 11–13.i.2002, C. L. Lambkin, N. T. Starick, 36°32′26″S, 148°15′52″E

Table 1. Continued

Order/ family/ subfamily	Exemplar	Specimen voucher/code	16S	COI	18S	CAD	Collection data or reference
Stenosmylinae	*Oedosmylus brevis* New (adult and larvae)	CASENT8092212	EU734907	–	EU815290	EU860158	AUSTRALIA: Queensland: Brisbane Forest Park, 15.xi.1999, S. L. Winterton [27°25.650′S, 152°50.471′E]
Osmylinae	*Osmlyus fulvicephalus* (Scopoli)	CASENT8092213	EU734887	–	EU815271	–	POLAND: Wroclaw, vi.2003, J. Kubrakiewicz
Polystoechotidae	*Fontecilla graphicus* Navás	CASC201	EU734863	DQ414496	EU815244	EU860115	CHILE: Elqui Prov.: Quebrada El Arrayán, 10–21.xi.2003, M. E. Irwin, F. D. Parker, 30°04.26′S, 71°00.04′W
	Platystoechotes lineatus Carpenter (adult and larvae)	CASENT8092170	EU734890	EU839757	EU815274	EU860143	U.S.A.: California: San Bernardino Co., Crestline, 2–5.vii.2001, L. M. LaPierre, 34°14′N, 117°17′W
	Polystoechotes punctatus (Fabricius)	CASENT8092171	EU734893	EU839760	EU815277	EU860146	U.S.A.: Idaho: Latah Co., Moscow, 19.viii.2001, J. B. Johnson
Psychopsidae							
Psychopsinae	*Psychopsis margarita* Tillyard (adult and larva)	CASENT8092209	EU734897	EU839764	EU815280	EU860149	AUSTRALIA: Queensland: Brigalow Res. Stn., 27–28.x.2000, Queensland Museum party [9804] 24°48′S, 149°45′E
Zygophlebiinae	*Silveira jordani* Kimmins	Siljor2	AY620171	–	AY620032	–	Haring & Aspöck (2004)
Sisyridae							
	Climacia areolaris (Hagen) (adult and larvae)	CASENT8092168	EU734852	EU839724	EU815234	EU860107	U.S.A.: Texas: Leon Co., Lake Normangee, 19.ix.2001, J. D. Oswald
	Sisyra vicaria (Walker)	CASENT8092169	EU734904	EU839770	EU815287	EU860155	U.S.A.: Texas: Leon Co., Lake Normangee, 19.ix.2001, J. D. Oswald

Accession numbers indicate specimens sequenced for various genes, with gene sequences deposited in GenBank (http://www.ncbi.nih.gov/). Voucher numbers identify individual specimens deposited in the California Academy of Sciences, San Francisco, U.S.A. All specimens are adults unless otherwise indicated.

Aspöck & Aspöck, 2008). All character states were treated as unordered in all analyses. Immature and adult life stages of sequenced exemplars were scored directly from specimens, or in some cases from the published literature (e.g. MacLeod, 1964; Henry, 1978; Gepp, 1990; Minter, 1990; Mansell, 1992; Gusten 1996; Sziraki, 1998; Aspöck *et al.,* 2001; Grebennikov, 2004; Aspöck & Aspöck, 2008). Taxa for which larvae were examined (and in most cases dissected) in this study are indicated in Table 1. Missing or unknown characters were scored as '?'. Species for which the immature stages are unknown were scored either as '?' for immature characters, or as per the states of other species in the same family if the immature stages of several such species were known not to vary with respect to the character state. In most cases, voucher specimens for DNA sequencing were used also for morphological scoring. Data on cryptonephridium structure and Malpighian tubule number were taken from the published literature (i.e. Gaumont, 1976) and from larvae dissected as part of this study (e.g. Polystoechotidae, Ithonidae, Ascalaphidae, Nymphidae, Berothidae, Mantispidae and Osmylidae). Internal dissections of larvae were carried out on specimens immersed in either distilled water or ethanol and stained with Chlorozol Black. Descriptions of character states of morphological characters are given in Table S1 in the Supporting Information, with state coding presented in Table S2.

DNA extraction and gene sequencing

Collection data, specimen voucher and Genbank accession numbers are presented in Table 1. Adult and/or larval specimens were collected by hand-netting, at mercury vapour light sheets or in Malaise traps. Individuals were subsequently placed directly into 95–100% EtOH and stored at −80°C. Genomic DNA was extracted from thoracic muscle tissue or, in the case of Coniopterygidae, entire specimens were used for extraction. Extractions were carried out using the DNeasy® kit (Qiagen, Germantown, MD) as per the manufacturer's instructions, except that specimens were incubated in the extraction buffer/proteinase-K mixture for 24–48 h. Extractions were then air-dried and resuspended in 50–150 μL of TE buffer or distilled water before storage at −80°C.

Four genes were amplified and sequenced, representing a range of rates of mutational changes over time to give the best possibility for phylogenetically informative data across a range of taxonomic levels from order to subfamily. Two ribosomal genes were used in this study (16S rDNA and 18S rDNA), along with two protein-encoding genes (cytochrome oxidase I (COI) and the CPSase region of carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (CAD)).

Primer sequences used to amplify and sequence the four sampled gene regions are presented in Table S3 . Concatenated data for these genes comprised *c*. 7.1 KB of unaligned gene sequence (CAD: 2601 bp; COI: 711 bp; 16S: 550 bp; 18S: 3173 KB). DNA amplifications using polymerase chain reaction (PCR) were performed using the following cycling parameters. A *c*. 550-bp fragment of 16S rDNA (3′-end) was generated using a single primer pair originally from Simon *et al*. (1994) with the following PCR protocol: initial denaturation at 95°C (3 min); 5 cycles of 92°C (15 s), 48°C (45 s), 62°C (2 min 30 s); 29 cycles of 92°C (15 s), 52°C (45 s), 62°C (2 min 30 s); final extension at 62°C for 7 min. A *c*. 2.7-KB section near the 3′-end of 18S rDNA was generated using primers originally from Hamby *et al*. (1988) and modified according to the published sequence of *Drosophila melanogaster* Meigen after Tautz *et al*. (1988). Three PCR fragments were generated: 20*F* to 519*R* (fragment 1), *Sai* to *Sbi* (fragment 2) and 18*H* to 18*L* (fragment 3). Fragments were amplified using a standard three-step PCR (without a touchdown step): initial denaturation at 95°C (3 min); 30 cycles of 95°C (1 min), 50°C (1 min), 72°C (2 min); final extension at 72°C for 7 min. The 3′ end of COI DNA (*c*. 500 bp) was amplified using primers modified after Simon *et al*. (1994): initial denaturation at 94°C (2 min); 35 cycles of 94°C (40 s), 55°C (50 s), 72°C (1 min); final extension at 72°C for 10 min. The *TY-J-1460* primer anneals to the tail end of the Tyrosine tRNA gene. There seems to be much variance across taxa in this region, making compete annealing difficult. In these cases *C1-J-1535* was used; this primer anneals within a more conserved COI region. Fragments 2–4 of CAD were generated using the following two-stage protocol. Stage 1: initial denaturation at 94°C (4 min); 5 cycles of 94°C (30 s), 52°C (30 s) and 72°C (2 min); 7 cycles of 94°C (30 s), 49°C (1 min) and 72°C (2 min); 37 cycles of 94°C (30 s), 45°C (20 s) and 72°C (2 min 30 s); 72°C (3 min) for final extension. To all CAD PCR mixes, 0.25 μL of DMSO (dimethyl sulphoxide) was added to improve the amplification product. Initial PCR products were run on a 1% agar electrophoresis gel, and specific bands were excised and reamplified using internal primers with reduced template concentrations and increased magnesium concentrations. TaKaRa Ex *Taq*™ DNA polymerase was used in all cases for best results. Reamplification PCR cycling protocol was: initial denaturation at 94°C (4 min); 5 cycles of 94°C (30 s), 51°C (30 s) and 72°C (1 min 30 s); 37 cycles of 94°C (30 s), 45°C (30 s) and 72°C (1 min 20 s); 72°C (3 min) for final extension. Reamplification PCR products were run out on a 1% agar electrophoresis gel and specific bands excised prior to purification using QIAquick® gel extraction kits (Qiagen).

Sequences were obtained using Applied Biosystems Big Dye Terminator ver. 3.0 (Foster City, CA). Sequences were gel-fractionated and bases called on an ABI 3730™ DNA sequencer (PE Applied Biosystems). Sequencing electropherograms were edited and contigs assembled and proofed using SEQUENCHER™ 4.1 (GeneCodes Corp., Ann Arbor, MI).

Alignment and sequence characteristics

Alignment of ribosomal gene sequences was estimated initially using the multiple-sequence alignment program CLUSTALX (ver. 1.8) (Thompson *et al*., 1997). Secondary structure-based penalties were used to improve the accuracy of these alignments (e.g. Kjer, 2004; Winterton *et al*., 2007).

Generic secondary structure models for 16S and 18S rDNA were used to insert gap penalty masks into the input file in CLUSTALX during profile alignment, raising gap penalties in relatively conserved stem regions so that gaps are inserted preferentially in highly length-variable loop regions (Thompson *et al.,* 1997). A secondary structure model was developed for 16S rDNA based on the published model of *Cicindela dorsalis* Say (GenBank accession number: AF438894) (Coleoptera: Carabidae) in Buckley *et al*. (2000) and for 18S rDNA using generalized hexapod models published in Misof *et al*. (2006) and alignment information from Kjer (2004).

Alignment of protein-encoding genes was inferred manually with reference to translated amino acid sequences using MACCLADE (ver. 4.06) (Maddison & Maddison, 2000). Introns were identified using comparative alignment in MACCLADE with limits defined using the GT-AG rule of Rogers & Wall (1980). Although there were no insertions or deletions identified in the COI alignment, some amino acid insertions and introns were identified in CAD sequences for several taxa. In this alignment, amino acid insertions or deletions were identified at various positions for a number of taxa, most notably species of Corydalidae, Raphidiidae and Coniopterygidae. Insertions in *Conwentzia* Enderlein (Coniopterygidae) sequences were unique to the species and relatively large compared with insertions identified anywhere in other lacewing sequences. Three species of *Nallachius* Navás were sequenced because initial sequencing results for *Nallachius pulchellus* (Banks) recovered some unusually divergent sequences (in all four genes examined) from taxa in presupposed sister-groups Mantispidae and Berothidae (i.e. 'dilarid clade'). Sequencing two more species of *Nallachius* confirmed our initial results of divergent sequences between *Nallachius* spp. and other Neuropterida, as well as close similarities to *Dilar* Rambur sequences used by Haring & Aspöck (2004). No evidence of multiple copies (i.e. pseudogenes) of COI or CAD was detected in the raw sequence data files. Ambiguously aligned regions of all genes, where positional homology could not be inferred with a reasonable level of confidence, were identified and excluded prior to undertaking phylogenetic analyses.

Phylogenetic analyses

Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford, 1999) using a heuristic tree search protocol with 50 replicate random addition sequences and tree bisection and reconnection (TBR). Analyses were repeated with third codon positions excluded form the dataset. Bootstrap support values (Felsenstein, 1985) for the parsimony analyses were calculated from 2000 heuristic search (TBR) pseudoreplicates of resampled datasets (with constant characters excluded), each with 20 random additions. Maximum likelihood (ML) inferences were undertaken using the program RAxML HPC 7.0.3 (Stamatakis, 2006). A separate general time-reversible nucleotide substitution model (GTR) with empirical base frequencies was applied to each ribosomal locus. A separate empirical amino acid substitution model was applied to each protein-coding locus. To select the most appropriate amino acid model, each locus was analysed first with MRBAYES, setting the amino acid model prior to *mixed*, which instructs the Markov chain to sample from each of ten empirical models according to its probability. Then we chose the amino acid model for each locus that had the highest posterior probability (PP). The *cprev* model was chosen for CAD (PP = 1.0), and the *mtrev* model was chosen for COI (PP = 1.0). We set RAxML to use empirical amino acid frequencies in the analysis. The ML search was composed of 1000 bootstrap replicates with CAT approximation of among-site rate variation, followed by optimization of every fifth bootstrap tree under the γ model for among-site rate variation. Bootstrap proportions were mapped onto the ML tree.

Bayesian analyses were performed using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). Third codon positions were excluded. The data were partitioned by data type (DNA sequence, morphology), locus, and by the remaining two codon positions for each protein-coding locus. A separate GTR + γ nucleotide substitution model was applied to each DNA partition. The mk1 model (Lewis, 2001), with coding set to variable, was applied to the morphology partition. We were unable to experiment with mixed amino acid substitution models because the *aamodelpr* prior is not one of the parameters that can be unlinked across partitions in MRBAYES. Each analysis consisted of four Markov chain Monte Carlo (MCMC) chains run simultaneously for 5G generations. Trees were sampled every 1000th generation, resulting in 5000 trees. The first 1G trees were discarded as burn-in. A majority-rule consensus tree was computed with posterior probabilities (PP) for each node.

To investigate the relative contribution of the data partitions to the arrangement of individual nodes on the combined tree(s), total decay index and partitioned Bremer support (PBS) values were determined using TREEROT 3.0 (Sorenson & Franzosa, 2007) and PAUP* 4.0b10 using 50 random addition sequences for each constraint tree, with TBR. PBS is a useful statistic for understanding the relative contributions that individual partitions make to the combined phylogeny, but must be treated with caution as the statistic tends to be susceptible to tree topology and the number of phylogenetically informative characters within respective nodes (DeBry, 2001). In this analysis, we found that the number of random addition sequences affected the reproducibility of results, so the values presented herein are considered useful in a comparative context rather than as absolute measures. We also calculated parsimony bootstrap values with individual character partitions systematically excluded (analogous to the partition subtraction bootstrap alteration (PSBA) of Hardy (2007)) as an additional metric of partition contribution to the combined phylogeny estimate.

Divergence time estimates

Bayesian relaxed-clock estimations of divergence times and phylogeny were performed with BEAST 1.4.8 (Drummond

Table 2. Fossil taxa with ages, prior probability distributions and posterior probability densities of the calibrations used in the analysis of divergence times.

Constraint	Most recent common ancestor (MRCA)	Fossil [geological period; age (Ma)]	References	Prior distribution	Posterior – no data Mean [95% CI]	Posterior – data Mean [95% CI]
A	Berothidae + Mantispidae	*Liassochrysa stigmatica* Ansorge & Schlüter [Middle Jurassic; 180]	Ansorge & Schluter (1990); Wedmann & Makarkin (2007)	Exponential	207.2 [180.0–253.3]	201.7 [180.0–227.19]
B	Chrysopidae + Hemerobiidae	*Lembochrysa miniscula* Ren & Guo [Late Jurassic; 160]	Ren & Guo (1996); Nel *et al*. (2005)	Exponential	191.6 [160.0–243.3]	180.5 [160.0–203.8]
C	Osmylidae + (Nevrorthidae) + Sisyridae	*Epiosmylus panfilovi* Ren & Yin [Middle Jurassic; 180]	Ren & Yin (2002)	Exponential	199.2 [180.0–248.1]	236.1 [188.6–273.0]
D	Polystoechotidae + Ithonidae	*Jurapolystoechotes melanolomus* Ren, Engel & Lü [Middle Jurassic; 170]	Ren *et al*. (2002)	Exponential	197.3 [170.0–244.6]	179.5 [170.0–196.0]
E	*Corydalidae* + (Inocellidae + Raphiiidae)	*Ororaphidia megalocephala* Engel & Ren [Middle Jurassic; 180]	Engel & Ren (2008); Engel (2002)	Exponential	206.1 [180.0–250.6]	249.8 [201.8–292.0]
F	Ascalaphidae + Myrmeleontidae	*Choromyrmeleon othneius* Ren & Guo [Late Jurassic; 150]	Ren & Guo (1996)	Exponential	179.3 [150.0–229.2]	154.7 [150.0–163.6]
G	Coleoptera + Neuropterida	Coleoptera: Tschekardocoleidae [Early Permian; 280]	Grimaldi & Engel (2005); Wiegmann *et al*. (2009)	Uniform	312.2 [281.6–330.0]	322.0 [307.1–330.0]

& Rambaut, 2007). BEAST uses MCMC approximation to estimate the joint posterior probability of a tree topology, a set of branch lengths, rates of evolution along each branch and divergence times under a variety of substitution models, branching models and among-lineage rate-variation models. We ultrametricized the MRBAYES majority-rule consensus tree with R8S 1.71 (Sanderson, 2003) using penalized likelihood, and used this as the starting tree for all analyses. The published ages of deposits containing the oldest definitive fossils known for various lineages of Neuropterida served as hard minimum age constraints in exponential prior probability distributions applied to each of seven nodes (Table 2) (see Grimaldi & Engel, 2005). If no exact age was given in the publication then a mid-point during that section of the period was chosen. Selection of nodal constraints attempted to represent divergences within each of the major clades of Neuropterida. A soft maximum constraint was applied to each calibration such that 97.5% of the prior probability density would fall before 330 Ma. Priors on the ages of unconstrained nodes were derived from a birth–death tree model. The Neuropterida were constrained to be monophyletic. We ascertained the joint prior probabilities of our calibrations by analysing the seven calibration densities, monophyly constraints and birth–death prior without any sequence data. Surprisingly, the priors interacted in such a fashion as to push the probability densities for the age of the ingroup and the age of the root deep into antiquity (Silurian). From both stratum-based fossil ages and DNA-based divergence estimates (Wiegmann *et al.,* 2009) it is widely accepted that Neuropteroidea originated during the Carboniferous, despite the absence of definitive fossils from the period (Grimaldi & Engel, 2005). To reflect our strong prior belief that the age of the root should not be earlier than the mid-Carboniferous, a uniform prior was applied to the root, with a hard minimum based on the oldest Neuropteroidea stem-group fossil assignable (i.e. Coleoptera: Tschekardocoleidae; *c*. 280 Ma) (Grimaldi & Engel, 2005), and a hard maximum at 330 Ma. This is based also on divergence estimates for the Neuropterida from Coleoptera + Strepsiptera that similarly approximate this period. The dataset was partitioned by locus and by first and second codon positions, whereas third codon positions were excluded. A separate HKY + γ substitution model was applied to each partition, and we used the uncorrelated lognormal model of among-lineage rate variation (Drummond *et al*., 2006). This model relaxes the assumption that rates are autocorrelated along ancestor–descendent branches. Instead, branch rates are sampled from a lognormal distribution. Each analysis consisted of 10G steps, sampling from the MCMC chain every 1000th step. This procedure was repeated three times. We used TRACER 1.4 (Rambaut & Drummond, 2003) to parse and combine the log files, determine the point at which the MCMC chain began to sample from the stationarity distribution and to check that effective sample sizes (ESSs) were sufficient for all parameters. We created a monophyly statistic for Megaloptera + Raphidioptera to track how frequently this grouping was a feature of a sampled tree.

Results

Sequence details and nucleotide composition

The combined sequence length for our concatenated molecular dataset was 7068 bp, although this was reduced to 4154 bp (COI = 472 bp; CAD = 1358 bp; 18S = 1830 bp; 16S = 474 bp) once ambiguously aligned sites (i.e. hypervariable regions in ribosomal genes and putative introns in protein-encoding genes) and third positions were removed. The addition of morphological data increased the combined unambiguously aligned dataset to 4209 characters. The proportions of nucleotides in arthropod genomes typically are biased towards A/T, as is reflected in the gene fragments used here (Fig. 3). Base frequencies were heterogeneous across the combined sequence data for Neuropterida ($\chi^2 = 1029.998$; d.f. = 204; $P = 0.000$). Average base frequencies for 16S were A: 36.30%; C: 9.85%; G: 15.64%; T: 38.21% ($\chi^2 =$

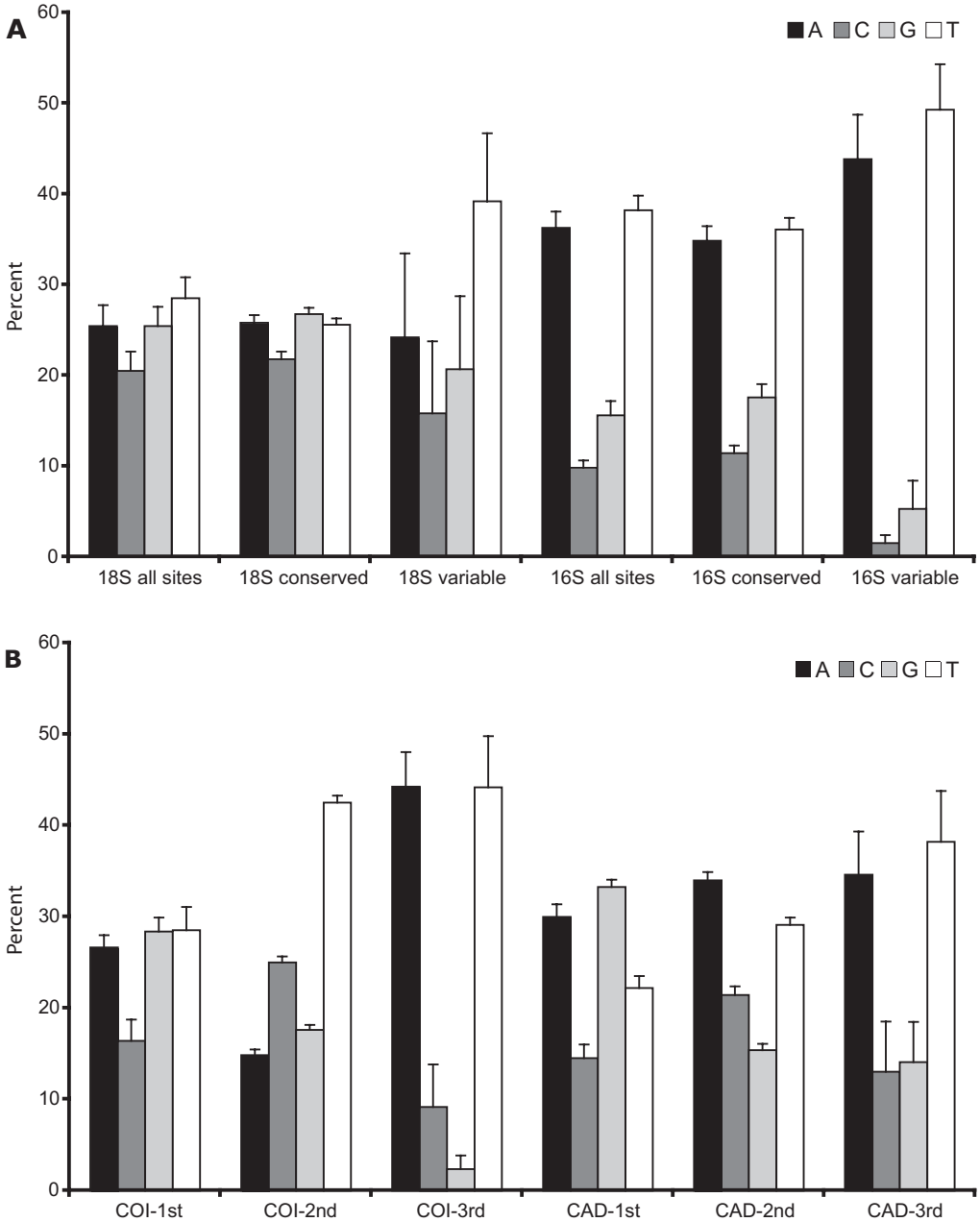

Fig. 3. Average nucleotide base frequencies for DNA sequence data: A, ribosomal genes (18S and 16S) subdivided into all sites, conserved (stem) and alignment ambiguous regions (loops) for each gene; B, COI and CAD subdivided by codon position.

89.848; d.f. = 198; $P = 1.000$) and showed an extreme A/T bias (93.16%) in length-variable regions (Fig. 3A). On average over all sites and in conserved regions, 18S showed the lowest A/T bias (54.02–51.43%), although, as in 16S, this increased in variable regions (63.46%). Base frequencies of conserved regions of 18S sequences across Neuropteroidea were relatively uniform and in relatively equal proportions, except for slightly lower cytosine frequency (i.e. A: 25.82%; C: 21.81%; G: 26.76%; T: 25.60%) ($\chi^2 = 1148.593$; d.f. = 201; $P = 0.000$). Although Haring & Aspöck (2004) described analytical difficulties as a result of severe A/T bias in many Neuropteran families and G/C bias in Nevrorthidae, Osmylidae, Sisyridae and Raphidioptera, no such biases were observed in our aligned data for the same taxonomic groupings. Length-variable regions do have significant biases in base composition in these data, such as G/C bias in Raphidioptera (A: 7.34%; C: 31.43%; G: 35.38%; T: 25.85%), Sialidae (A: 10.67%; C: 31.65%; G: 35.39%; T: 22.28%), and A/T bias in Dilaridae (A: 39.87%; C: 9.06%; G: 8.82%; T: 42.25%) and Myrmeleontiformia (A: 31.31%; C: 10.65%; G: 14.97%; T: 43.01%). Average base frequencies for CAD (all sites) were A = 32.86%; C = 16.34%; G = 20.95%; T = 29.84% ($\chi^2 = 933.158$; d.f. = 204; $P = 0.000$), and as is common with third positions, A/T bias was considerably greater (72.87%) than in either first (52.19%) or second (63.15%) codon positions (Fig. 3B). Similar trends in base frequencies were observed in COI (A: 28.59%; C: 16.87%; G: 16.13%; T: 38.42%) ($\chi^2 = 211.001$; d.f. = 177; P = 0.041), although the amount of A/T bias in third positions (88.45%) was higher than that found in CAD.

Average uncorrected sequence divergences across taxa for all genes varied between outgroup beetles and ingroup Neuropterida at 7.6–13.8%, whereas between orders of Neuropterida pairwise divergences were approximately 10% between Raphidioptera and Neuroptera and 8.0–9.0% between Megaloptera and both Raphidioptera and Neuroptera. Both species of *Corydalus* Linnaeus and *Sialis californica* Banks were notable in their higher divergences from all other taxa, regardless of distance of relationship, with divergences of 12–15%. Few neuropteran taxa showed distinctly elevated divergence distances relative to other taxa, most notably *Austrocroce* Tjeder (Nemopteridae), *Ululodes* Currie (Ascalaphidae) and *Climacia* McLachlan (Sisyridae). Comparisons within most family groups of Neuroptera showed fairly homogenous sequence divergence between taxa, most never diverging more than *c*. 12% between families or between genera within family groups.

Morphological phylogeny

Parsimony analysis of 55 parsimony-informative morphological characters yielded 222 equally parsimonious minimum-length trees (length = 151 steps, consistency index (CI) = 0.506, retention index (RI) = 0.9150); a strict consensus tree is presented in Fig. 4. Because character selection here largely reflected postulated phylogenetic utility at the family level, most taxa were scored identically within families, often resulting in polytomies for each family in the strict consensus. Coleoptera are not monophyletic based on the characters used here (i.e. Carabidae are recovered as sister to Neuroptera), although the monophyly of this order is firmly supported by many synapomorphies (Beutel & Pohl, 2006). As expected from previous published morphological studies, all orders of Neuropterida were monophyletic, with Megaloptera placed sister to Raphidioptera. Within Neuroptera a basal dichotomy was recovered between a monophyletic Myrmeleontiformia sister to Polystoechotidae and Ithonidae, and the rest of the order. Hemerobiiformia (in part) was paraphyletic with respect to Nevrorthiformia, with almost no resolution of relationships between families in this clade except for sister-groupings between Chrysopidae and Hemerobiidae, and Mantispidae and Berothidae respectively. Polystoechotidae were paraphyletic with respect to Ithonidae. Psychopsidae were recovered as a monophyletic sister-group to the rest of Myrmeleontiformia, and relationships between the other families in this superfamily were similar to those found with the molecular data. Ascalaphidae could not be separated from Myrmeleontidae as a monophyletic group based on these characters.

Nucleotide sequence phylogeny

Third codon positions in both CAD and COI initially were included in analyses but always resulted in a breakdown in monophyly of many families and orders. Based on the relatively strong morphological evidence for monophyly of most families and orders of Neuropterida, changes in third positions were considered too homoplasious in their phylogenetic signal and were excluded in subsequent analyses. The concatenated and aligned sequences of the four genes included 1175 (28.3%) parsimony-informative characters and 2979 parsimony-uninformative characters, of which 2431 were constant. Parsimony analysis recovered three most-parsimonious trees (length = 7955 steps, CI = 0.326, RI = 0.510), the strict consensus of which is presented in Fig. 4. This tree is very similar in topology to those of both the Bayesian (Figure S1) and ML (Figure S5) analyses. Neuropterida were strongly supported as monophyletic (parsimony bootstrap (PB) = 100%, maximum likelihood bootstrap (MLB) = 100%, Bayesian posterior probability (PP) = 1.00), as were Raphidioptera (PB = 100%, MLB = 100%, PP = 1.00) and Neuroptera (PB = 89%, MLB = 85%, PP = 1.00). Megaloptera were not recovered as monophyletic. In the parsimony analysis Sialidae were recovered as sister to Raphidioptera as a clade sister to Neuroptera, whereas in model-based analyses Sialidae was sister to the rest of Neuropterida. All families of Neuropterida were recovered as monophyletic except for Ithonidae (always polyphyletic with respect to Polystoechotidae), Psychopsidae (paraphyletic in parsimony analysis), Ascalaphidae (monophyletic in Bayesian analysis only), Mantispidae (paraphyletic in parsimony analysis) and Berothidae (paraphyletic with respect to Mantispidae). A monophyletic Hemerobiiformia was never recovered, and Nevrorthiformia (*sensu* Aspöck *et al.,* 2001) was not recovered as sister to the rest of the order. Rather, Coniopterygidae were well supported as the sister-group to the rest of Neuroptera

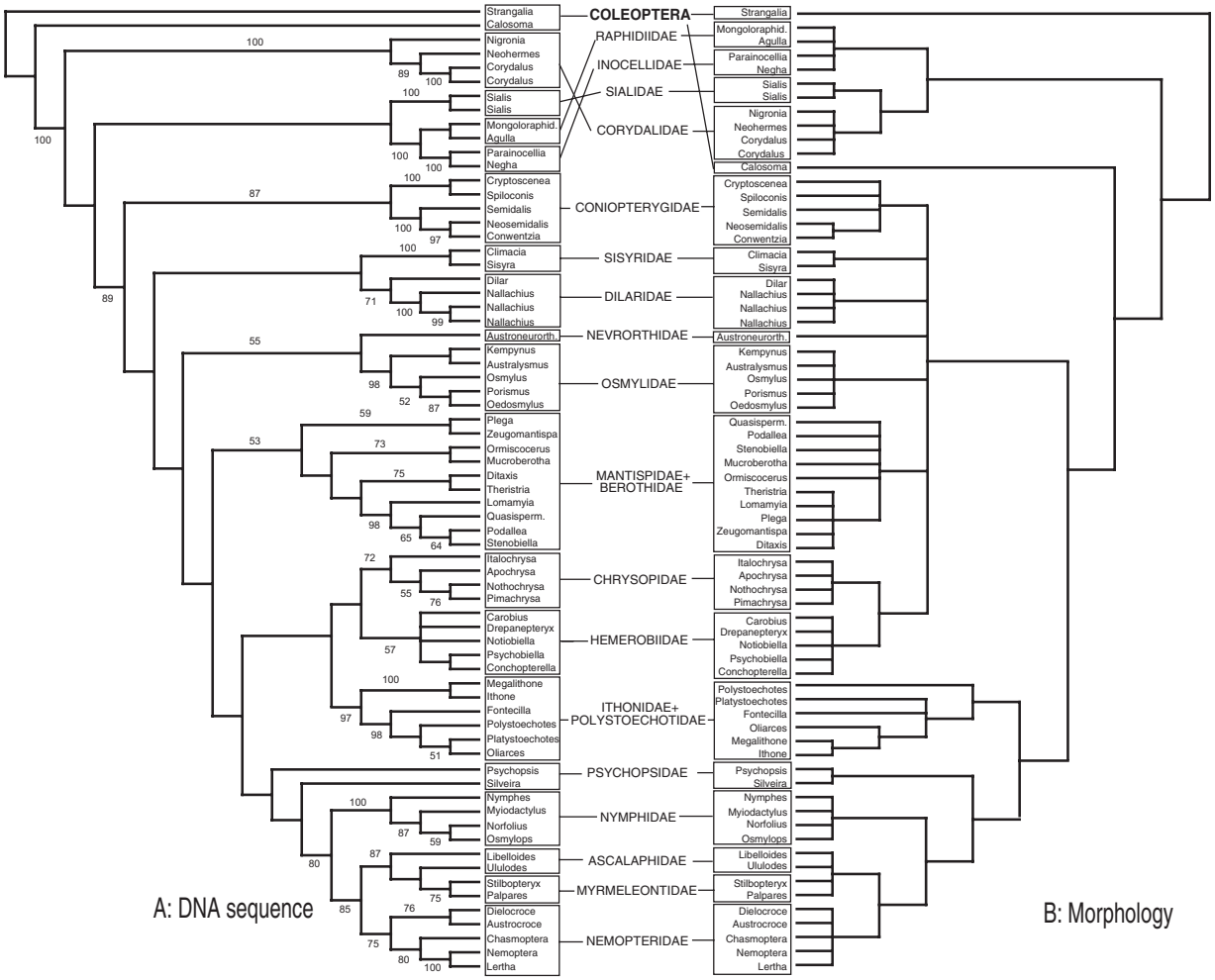

Fig. 4. Strict consensus trees recovered from parsimony analyses of combined molecular (COI, CAD, 18S, 16S) data (A), and morphological data (B). Bootstrap values (>50%) are given on respective nodes.

(PB = 89%, MLB = 85%, PP = 1.00) whereas Nevrorthidae was placed always in a clade with Osmylidae and/or Sisyridae. Myrmeleontiformia always were monophyletic except in the Bayesian analysis (Figure S1), in which support for the placement of Psychopsidae was very weak. The phylogenetic sequence of the 'Hemerobiiformia' clades was variable and statistical support was generally weak. The placement of Dilaridae varied depending on the inference method used, being placed as sister to Sisyridae (parsimony; Fig. 4) or as an intermediate clade frequently sister to the clade comprising (Berothidae + Mantispidae) + (Chrysopidae + Hemerobiidae) + (Ithonidae + Polystoechotidae) + Myrmeleontiformia). Mantispidae and Berothidae were recovered as a clade, but statistical support for the monophyly of both families was relatively low or absent. Chrysopidae were recovered as sister to Hemerobiidae, the two of which were either sister to Polystoechotidae + Ithonidae (with weak support) or sister to (Ithonidae + Polystoechotidae) + Myrmeleontiformia. The North American ithonid genus *Oliarces* Banks never grouped with Australian ithonid genera *Megalithone* Riek and *Ithone* Newman, but rather was placed deep within Polystoechotidae. Psychopsidae was sister to the rest of Myrmeleontiformia in the parsimony and ML analyses, but unresolved in the Bayesian analyses. Lack of data may explain the weak support for this arrangement, as *Silveira* Navás is represented by sequence data for only two genes (16S and 18S). Nymphidae always were supported strongly (PB = 100%) as monophyletic and sister to (Ascalaphidae + Myrmeleontidae) + Nemopteridae. Both subfamilies of Nemopteridae (Crocinae and Nemopterinae) were recovered as monophyletic with relatively strong support. This family was sister to Ascalaphidae and Myrmeleontidae, the latter two being monophyletic only in the Bayesian analysis.

Combined data phylogeny

All 69 taxa were included in the combined molecular and morphological dataset, which comprised 1234 (29.3%)

parsimony-informative characters and 2983 parsimony-uninformative characters (including 2431 constant characters). A parsimony analysis of concatenated gene sequences and morphology yielded ten most-parsimonious trees (length = 8121 steps, CI = 0.328, RI = 0.537). Bayesian analysis resulted in a tree of similar topology to the consensus parsimony tree. The most significant difference between the resultant phylogenies was that in the parsimony analysis Sialidae grouped with Raphidioptera, whereas in all model-based analyses Corydalidae grouped with Raphidioptera (always with limited support). The phylogram depicted in Fig. 5 is the Bayesian consensus tree with Bayesian posterior probability, parsimony bootstrap and decay index (DI) values presented on nodes. Only values above 50% bootstrap and 0.5 posterior probability are indicated, and nodes with bootstrap values above 70% and/or posterior probability above 0.95 are represented as thickened lines. The combined data yielded a relatively well-supported and highly resolved phylogeny with a large number of nodes with high statistical support values. Estimated branch lengths were variable throughout the tree, with a series of nodes towards the middle of the tree with relatively short branch lengths. Notable long branches were found within Nemopterinae, Coniopterygidae, Sisyridae and one species in each of Ascalaphidae and Sialidae. Most of the high support for nodes on this tree was for nodes representing family or subfamily groupings, with nodes representing higher-level clades weakly supported in general, especially along the backbone of the tree. Neuropterida were well supported as monophyletic (PP = 1.00, PB = 100%, DI = 35), as were Raphidioptera (PP = 1.00, PB = 100%, DI = 68) and Neuroptera (PP = 1.00, PB = 93%, DI = 15). Megaloptera was not recovered as a monophyletic group in any analysis, although Corydalidae and Sialidae were each recovered with very high support values. Sialidae was recovered as sister to the rest of Neuropterida whereas Corydalidae were sister to Raphidioptera. The relationships among the orders of Neuropterida were not well supported, with relatively weak support for a sister-group relationship between Neuroptera and Raphidioptera + Corydalidae (PP = 0.88, PB = <50%, DI = 0), and the sister-grouping of Raphidioptera + Corydalidae had no statistical support at all. All families of Neuroptera represented by more than one exemplar were recovered as monophyletic with high levels of support, except for Berothidae, which was paraphyletic with respect to Mantispidae (parsimony analysis) or unresolved (Bayesian), and Ithonidae, which was paraphyletic with respect to Polystoechotidae in both. Coniopterygidae were sister to the rest of the lacewings, with both subfamilies Coniopteryginae and Aleuropteryginae forming monophyletic groups with high support. Sisyridae + Nevrorthidae formed a clade with Osmylidae with no statistical support. Weak support was recovered for Dilaridae as a distinct early-branching lineage within Neuroptera. Berothidae grouped with Mantispidae with high support (PP = 1.00, PB = 91%, DI = 9), although internal family and subfamily level relationships were not well supported by these data. Berothidae were paraphyletic, with *Ormiscocerus* Blanchard (Cyrenoberothinae) grouping with *Mucroberotha* Tjeder (Rhachiberothinae) in an unresolved polytomy with Berothinae and Mantispidae. Chrysopidae were sister to Hemerobiidae (PP = 1.00, PB = 51%, DI = 7) and Ithonidae grouped with Polystoechotidae with high support (PP = 1.00, PB = 99%, DI = 17). The North American ithonid genus *Oliarces* Banks did not group with other Ithonidae genera but rather was recovered well within Polystoechotidae, sister to *Platystoechotes* Carpenter. Myrmeleontiformia were monophyletic with strong support for the following inter-familial relationships: Psychopsidae {Nymphidae [Nemopteridae (Ascalaphidae, Myrmeleontidae)]}. High support was recovered for the two nemopterid subfamilies, Nemopterinae and Crocinae, and the long branch subtending the latter subfamily parallels the highly specialized morphology of this group.

To calculate the relative contributions of individual loci and morphology to the total-evidence phylogeny, PBS values were calculated for a reduced matrix of 59 taxa for which sequence data for all gene loci (COI, CAD, 18S, 16S) and morphology were available. Partitioned Bremer support is a measure of the average individual contribution of separate data partitions to the overall (i.e. summed) decay index for each node in the context of the combined data analysis (Sorenson & Franzosa, 2007). All families were represented in the PBS analysis. Of the 4217 characters in the alignment, 2460 were constant and 1120 parsimony-informative. Analytical parameters were identical to those used in the full taxon dataset parsimony analysis with third positions excluded for CAD and COI. PBS values are difficult to interpret, and can be influenced by tree shape (DeBry, 2001). Therefore, we also explored the contribution of each locus by removing it from the dataset and noting changes in bootstrap support (Table 3) (Hardy, 2007). Parsimony analysis of this 59-taxa set yielded two equally parsimonious trees of length 7695 steps (CI = 0.339, RI = 0.531), presented as a strict consensus cladogram in Fig. 6. The topology of the pruned-taxon tree is largely congruent with that recovered from the analyses of the full taxon set. Bootstrap and summed DI values (i.e. sum of PBS values) are presented on each node, along with shaded boxes representing the PBS support (i.e. congruent, contradictory or equivocal) for that node by the individual data partitions. Average PBS values (maximum and minimum values are not listed), summed DI, and partition-exclusion bootstrap values (i.e. with individual partitions systematically excluded) corresponding to each node are presented in Table 3. Partition-specific nodal support throughout the phylogeny decreased towards the root. The partition with the highest congruence with the combined tree topology was 18S, with 76% of nodes present in the 18S tree also present in the combined tree and 18% of nodes recovered in contradictory topologies. This was followed closely by 16S, with 71% congruence and only 13% contradictory nodes, although this gene shows a greater proportion of equivocal nodes (16%) (i.e. nodes not resolved in the individual partition tree). Frequency in nodal congruence was followed in order by COI (67), CAD (51) and the morphological (45%) partitions.

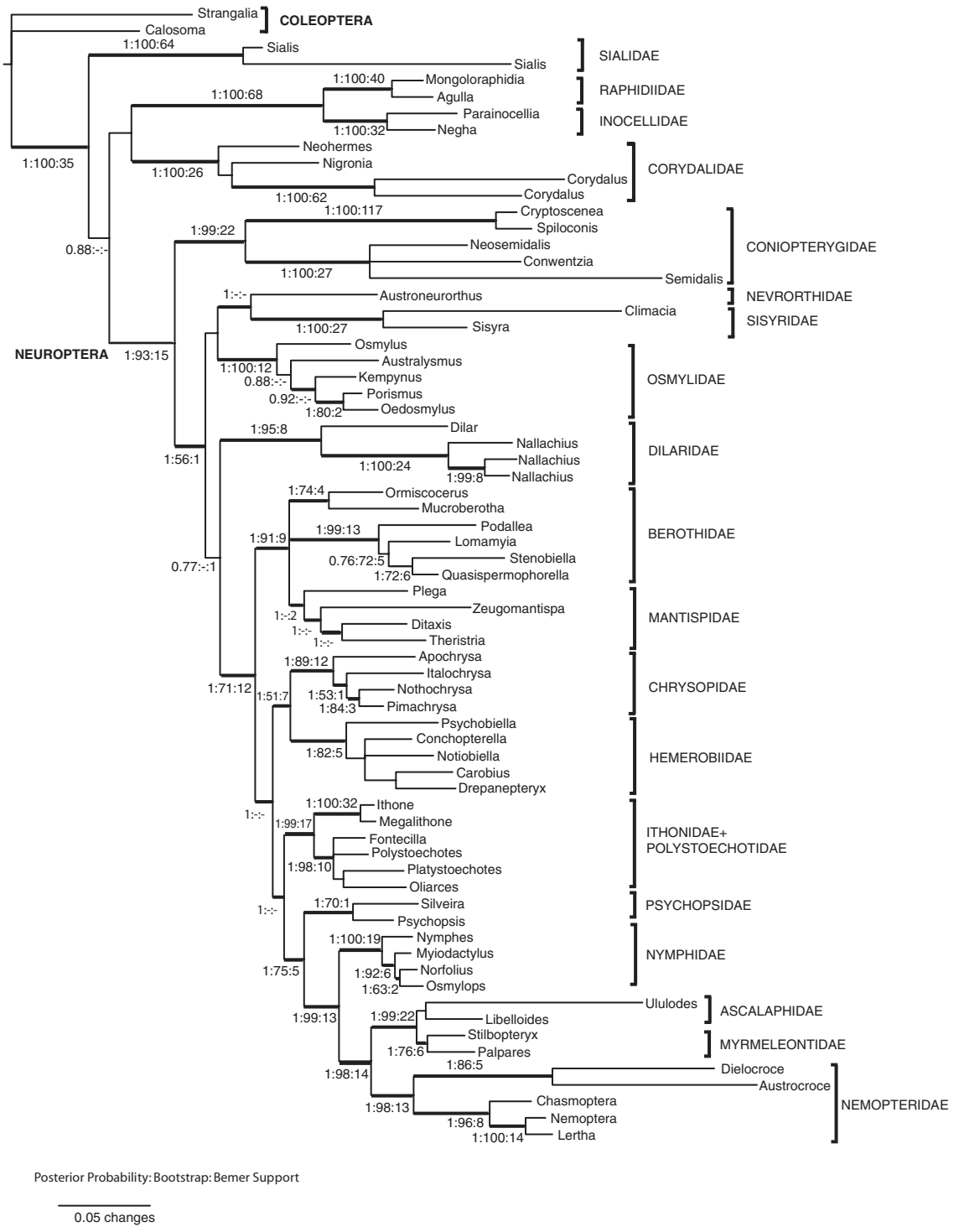

Fig. 5. Phylogram of combined COI, CAD, 18S, 16S and morphological data recovered from both parsimony and Bayesian likelihood analyses. Branch lengths correspond to the number of changes on that branch. Bayesian posterior probability, bootstrap (>50%) and decay index (>0) values are presented in order on each branch. Branches with relatively high posterior probability (>90%) and/or bootstrap (>70%) values are represented as thickened lines. Dashes (-) on nodes indicate weak support values (<50% for Bootstrap and <0.5 posterior probability) or equivocal support of parsimony decay indices. The parsimony tree was one of ten most-parsimonious trees recovered and was congruent to both the Bayesian and single, successively weighted parsimony trees.

Table 3. Average partition Bremer support (PBS) and partition exclusion bootstrap values for individual character sets for the combined reduced taxa set.

	Partition Bremer support						Partition exclusion bootstrap					Grouping
Node	COI	CAD	18S	16S	Morph	Total	COI	CAD	18S	16S	Morph	
1	0	9	11	13	3	36	100	100	99	100	100	NIDA
2	2	6.2	4.7	20.2	−5	28.1	100	79	100	100	100	CORY
3	0.6	5.9	−1.2	4.3	−0.6	9	82	64	80	0	83	
4	8	26	16	12	0	62	100	100	100	100	100	
5	0	5.2	−1.4	−0.2	−0.6	3	0	0	53	0	52	
6	−3	9	−3	3	−2	4	59	0	53	0	0	
7	7.8	29.2	9.4	15.4	3.2	65	100	100	100	100	100	SIAL
8	3.8	26.3	29.2	8.8	6.8	74.9	100	100	100	100	100	RAPH
9	5	20	2	14	0	41	100	100	100	100	100	
10	−7.5	23.5	8.5	−1	8.5	32	100	81	100	100	100	
11	3.3	−12	8.3	0.7	6.7	7	87	67	67	96	51	NEUR
12	−1	−2	7	15	3	22	99	97	93	86	92	CONI
13	21.5	5.8	40.5	39.3	1	108.1	100	100	100	100	100	
14	2	8	25	8	0	43	100	100	99	100	100	
15	6	−14.7	7	6.3	−0.7	3.9	0	0	0	0	0	
16	3.5	−1.8	3.5	−2.3	−1	1.9	0	0	0	0	0	
17	17	−53.3	31.5	33.8	14	43	100	100	100	100	100	SISY
18	−5.7	36.3	34.6	15.7	8	88.9	100	100	100	100	100	DILA
19	−2	−4	6	3	0	3	0	0	0	0	0	
20	−2	−4	6	3	0	3	58	69	0	0	63	
21	12.5	−0.75	15.5	−0.25	3	30	99	100	99	99	100	OSMY
22	3.3	−2.3	1.8	−0.2	−0.6	2	80	74	0	0	0	
23	−9	−10	7	11	7	6	0	0	0	0	0	
24	−9	−10	7	11	7	6	82	0	72	55	0	
25	−8.5	22.1	14.3	9.4	2.8	40.1	100	97	96	97	100	BERO
26	6.5	−0.5	−1	−1	0	4	0	70	72	60	62	
27	12	−7	−1	0	0	4	89	92	70	53	59	
28	2.5	−5.5	2.5	2.5	0	2	61	0	0	0	0	
29	2.5	−5	3	1	0.5	2	82	0	53	51	0	MANT
30	2.2	−3.8	1.8	2	−0.2	2	0	0	0	0	0	
31	1	−2	1	1	2	3	0	73	0	0	0	
32	3	−11	2	10	−3	1	0	0	0	0	0	
33	3	13.5	3	−2.5	1	18	100	76	100	100	99	
34	4.4	16	5.4	7.8	−0.6	33	100	100	100	100	100	ITHO
35	2	−1	6	7	−2	12	94	64	90	98	98	POLY
36	−1	9	0	−6	−1	1	0	0	0	73	0	
37	−1	9	0	−6	−1	1	0	0	50	53	0	
38	2.5	−2.8	6.3	0	−1	5	0	56	0	0	0	
39	12	−28.8	8.4	19.4	5	16	80	86	90	74	86	CHRY
40	3	−11	2	10	−3	1	0	96	0	0	55	
41	2	2	−1	0	0	3	75	53	92	95	81	
42	4	−46	20	25	6	9	81	100	0	0	63	HEME
43	3.3	−19.5	9.6	11.25	2.3	6.95	60	78	0	0	0	
44	0	−2	3	3	0	4	0	94	0	0	0	
45	2.5	1	6	−6	6.5	10	74	76	72	75	0	
46	2.5	−2.5	15	0	2	17	98	100	85	91	92	
47	−0.5	16.5	1	0	1	18	100	76	100	100	100	NYMP
48	0.5	4.5	0	0	0	5	89	58	86	89	87	
49	2.5	2.5	−1	11	1	16	97	100	99	77	94	
50	7.9	7.7	−14	9.6	9.8	21	86	91	100	81	82	
51	−1	1.5	−1	1.5	0	1	0	0	55	0	0	
52	5.5	2.5	−2	1	0	7	58	70	87	92	73	MYRM
53	1.5	−0.5	10	5	3	19	86	92	88	81	78	NEMO
54	9	36.5	1	6.5	0	53	100	100	100	100	100	
55	−5.5	14.5	3	8	0	20	100	95	100	100	100	
Total:	137.4	105.4	379.2	364	91.8							

Node numbers correspond to those given in Fig. 6. Monophyletic groups are listed for each family or recognized higher grouping. Abbreviations represent the first four letters of each family or ordinal group name; NIDA, Neuropterida.

Historical divergence time estimates

The maximum clade credibility tree with median node heights and the 95% high posterior density (HPD) interval on each divergence is shown in Fig. 7. The mean rate was 0.399 substitutions per site per billion years (95% HPD 0.348–0.452). The mean of the coefficient of variation (the rate variance scaled by the rate magnitude) was 0.65 (95% HPD 0.55–0.77). The difference between the birth and death rates of the tree model was estimated to be 0.0065 (95% HPD 0.0045–0.0085). The 95% HPD of the covariance statistic included zero (−0.085–0.25, mean 0.085), and thus there was no strong support for rate autocorrelation. The red bars on Fig. 7 indicate the 95% HPD for the age of each node. The mean estimated date of divergence of Neuropterida from Coleoptera was in the Late Carboniferous (324 Ma), reflecting the tendency for the analysis to push the root against the predefined hard maximum of 330 Ma. All three orders were present by the end of the Permian, and, contrary to in unrooted analyses, Megaloptera and Raphidioptera were recovered as a monophyletic group with high support (PP = 1.00), although Megaloptera was paraphyletic with respect to Raphidioptera. Coniopterygidae diverged from the rest of the lacewings around the beginning of the Permian (294 Ma), with many families of Neuroptera emerging during the Triassic and Jurassic. Only Ascalaphidae and Myrmeleontidae diverged later, during the Cretaceous period. All subfamilial groups of extant Neuropterida represented here were present by the end of the Cretaceous or early in the Palaeocene.

Discussion

This study presents the most comprehensive phylogenetic study of Neuropterida using morphology and multilocus DNA sequence data for all extant families of Neuroptera, Megaloptera and Raphidioptera. Despite the inherent difficulties of determining morphological homology, as well as of ensuring adequate genetic sampling from ancient radiations such as that of the Neuropterida, we recovered a generally well-supported phylogeny (e.g. 82% of nodes on combined tree with support of either >70% bootstrap or >90% posterior probability) that agrees in many ways with previous estimates of relationships. Each partition varied considerably in the support it lent to higher-level and lower-level groupings, exemplifying the unpredictable utility of various data types (molecular and morphological) for different levels of phylogenetic reconstruction.

Monophyly of orders and sister-grouping of Sialidae with Raphidioptera

The monophyly of Neuropterida was well supported, with high support in all analyses (Figs 4, 5, Figs S1, S2: PP = 1.00, PB = 100%, DI = 35). Similar levels of support were found for the monophyly of both Raphidioptera (PB = 100%) and Neuroptera (PB = 93%) in our analyses, a result congruent with numerous morphological synapomorphies that have been identified previously in support of the monophyly of both orders. Megaloptera was never recovered as monophyletic in any analysis, with Raphidioptera repeatedly grouping with either Corydalidae (model-based inferences) or Sialidae (parsimony) in a clade sister to Neuroptera. Higher-level relationships among neuropterid orders have been difficult to resolve, with little consensus among authors (reviewed by Kristensen, 1981). Our finding of a sister-group relationship between Corydalidae and Raphidioptera contrasts with widely held opinions of previous authors, who support a monophyletic Megaloptera sister to Raphidioptera based on various morphological characters (see Kristensen, 1999; Beutel & Pohl, 2006). This more traditional arrangement, with a monophyletic Megaloptera sister to Raphidioptera, was supported previously also by molecular and morphological data in Whiting *et al*. (1997) and Whiting (2002). Support for a paraphyletic Megaloptera, with Raphidioptera sister to either Sialidae or Corydalidae, is weak under all inference parameters used here, and we conclude that the limited phylogenetic utility of loci chosen, as well as sparse taxon sampling for these groups, preclude the defence of a specific resolution on this question. Only in our relaxed-clock tree inference did we recovered strong support for the monophyly of Megaloptera + Raphidioptera (PP = 1.00). In that analysis, a monophyletic Megaloptera was impossible because, based on our previous analyses, Raphidioptera was constrained to be sister to Corydalidae for dating purposes. Boudreaux (1979) proposed a sister-group relationship between Megaloptera and Neuroptera that was supported subsequently in a quantitative analysis of morphological data by Aspöck *et al*. (2001). The synapomorphies supporting a sister-group relationship between Megaloptera and Neuroptera identified by Aspöck & Aspöck (2008) were: male gonocoxites 9 appendix-like, attached to gonocoxites 11, and ectoprocts with trichobothria organized in a rosette pattern. The monophyly of Megaloptera is supported by morphological characters such as having an aquatic larva with lateral abdominal gills, extensive spiracle closure in early instars and eversible sacs from fused gonocoxites 11 (Kristensen, 1999; Aspöck & Aspöck, 2008). Further evidence of monophyly for Megaloptera was provided by Büning (1998) based on ovariole structure, including near-identical somatic tissue shape and organization, similar ovary and ovariole sheath integrity, ovariole arrangement and attachment of the lateral oviduct; characters not found in Raphidioptera. The monophyly of Megaloptera has been questioned repeatedly based on characters such as wing venation (Hennig, 1953) and ovariole structure (Stys & Bilinski, 1990; Kubrakiewicz *et al.,* 1998), the latter authors proposing instead that Sialidae are sister to Raphidioptera rather than to Corydalidae. Sialidae and Raphidioptera have a specialized '*Sialis'*-type telotrophic ovariole structure (Büning, 1980; Kubrakiewicz *et al*., 1998), whereas Corydalidae have secondarily panoistic-type ovarioles (Matsuzaki *et al*., 1985; Szymanska *et al*., 2001). Neuroptera have a polytropic ovariole structure common in holometabolan insects (Büning, 1998). Evidence of phylogenetic plasticity in this character

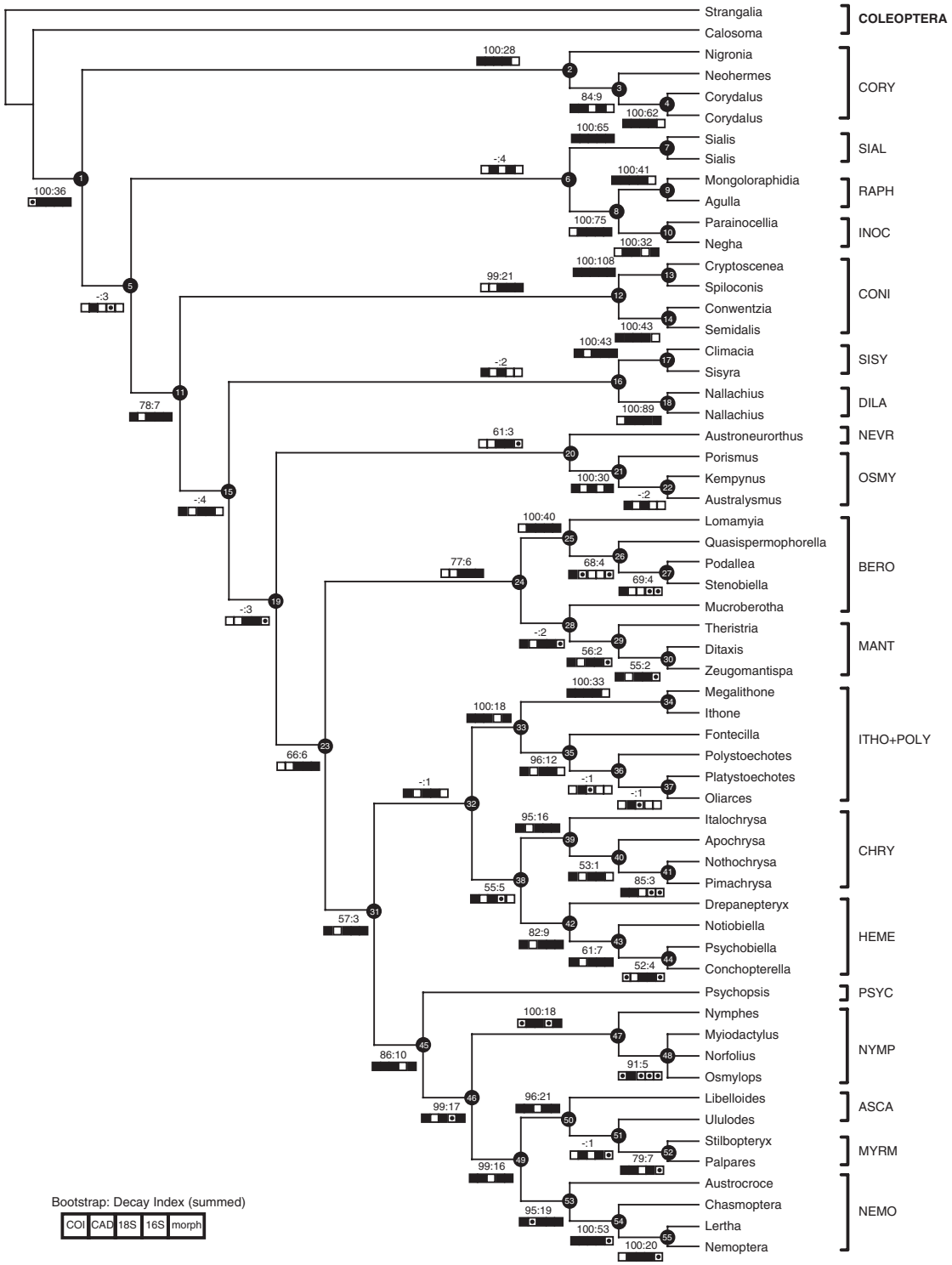

Fig. 6. Pruned tree of 59 taxa with complete morphological and sequence data (COI, CAD, 18S, 16S) for all partitions. The tree represents a strict consensus of two most-parsimonious trees. Bootstrap and decay index (i.e. sum of partition Bremer support (PBS) values) are presented on each node. Support for a particular node on the combined tree by individual partitions is represented by shaded boxes (in order: COI, CAD, 18S, 16S, morphology). Black boxes represent support for that node (i.e. positive PBS value), boxes containing a black dot represent equivocal support (i.e. PBS value of zero (±0.5)), and white boxes indicate that the node on the combined tree is not recovered in the individual analysis for that partition (i.e. negative PBS value). Actual PBS values corresponding to each numbered node (black circles) are presented in Table 3.

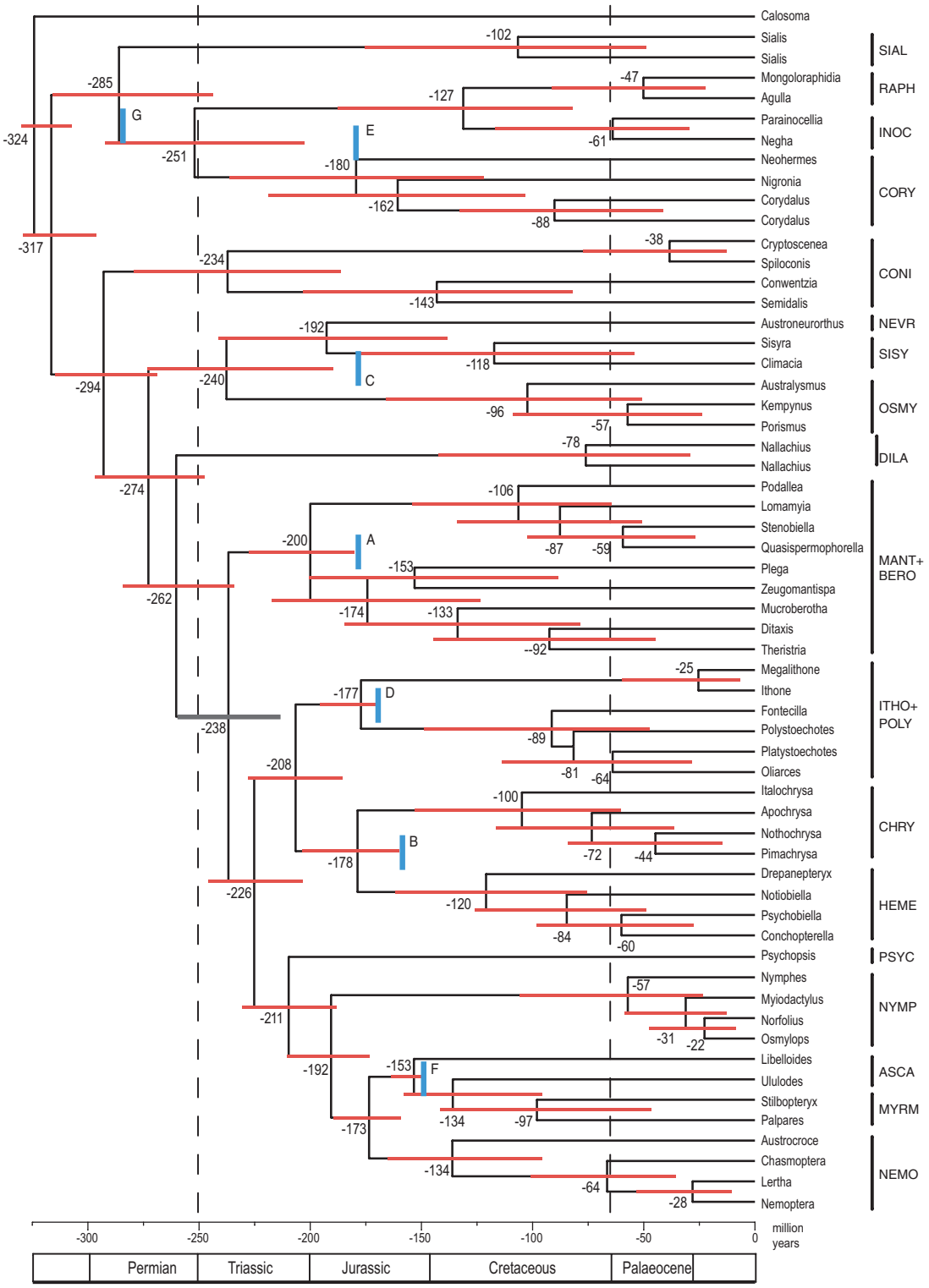

Fig. 7. Estimated divergence times of major clades of Neuropterida based on molecular sequence data. Nodes on the phylogram represent means of the probability distributions for node ages, with time intervals for 95% probability of actual age represented as red bars. Permian–Triassic (P-Tr) and Cretaceous–Paleogene (K-Pg) extinction events are indicated by dashed lines. Vertical blue bars represent the minimum age constraints used in the analysis for particular nodes based on the dates of the oldest known definitive fossils for that family (see Table 2). Time-scale units are in millions of years, and numbers on nodes represent the estimated age for that divergence. Abbreviations are the first four letters of family names.

is seen in secondarily panoistic ovarioles evolving independently in other insect lineages such as Thysanoptera and the clade Nannochoristidae+Boreidae + Siphonaptera (excluding Hystrichopsyllinae) (Büning, 1998). '*Sialis*'-type telotrophic ovarioles are recorded also in *Hydroscapha natans* LeConte (Coleoptera: Myxophaga) by Büning (2005), who, based on support for a monophyletic Megaloptera sister to Raphidioptera, implied that the secondarily panoistic ovarioles of Corydalidae are derived from the specialized (but plesiomorphic) '*Sialis*'-type telotrophic ovarioles present in the ancestral Megalopteran.

The relatively weak support we recovered for Corydalidae + Raphidioptera precludes us from discounting alternative hypotheses, and therefore our estimates of divergences times for these lineages should be considered as tentative. We needed to place a hard maximum age constraint on the root to keep our divergence times within the realm of plausibility. When we estimated the joint prior probability of our monophyly constraints, age priors and birth–death tree model (i.e. ran an analysis with no sequence data), the prior distribution for our root height had a mean in the Silurian. No soft constraint on maximum root age altered the prior. The estimated divergence date of Raphidioptera around the Permian–Triassic boundary agrees with the estimated age of the group by Grimaldi & Engel (2005), despite the absence of definitive snake-fly fossils from deposits of this period.

Neuroptera are supported strongly as monophyletic in our analyses, with only the CAD partition not supporting the group as monophyletic (Fig. 6). The monophyly of the group has never been questioned and is supported by a set of complex and highly specialized larval anatomical features such as sucking mouthparts, discontinuous gut and modified Malpighian tubules for silk production (reviewed by New, 1989; Grimaldi & Engel, 2005). Most lacewing families are highly distinctive morphologically, frequently with highly specialized morphology and life histories in the larval stages. The monophyly of most families was supported by our analyses, although notable exceptions include the paraphyly of Polystoechotidae relative to *Oliarces*, and poorly resolved cladogenesis of berothid subfamilies relative to Mantispidae. Our data suggest that Neuroptera diverged from the rest of Neuropterida during the Late Carboniferous, and proposed stem groups (e.g. Permithonidae, Permoberothidae) have been described from subsequent Early Permian deposits (Schlüter, 1986; Grimaldi & Engel, 2005). We infer that the origins of extant neuropteran families began in the Permian and Early Jurassic, with most subfamilies sampled here present by the end of the Cretaceous (Fig. 7).

Our results do not support the arrangements of Neuroptera proposed by Aspöck *et al*. (2001) and Beutel *et al*. (2009), in that we did not recover a monophyletic Hemerobiiformia, and Nevrorthiformia was not recovered as sister to the rest of the order but was placed within the paraphyletic Hemerobiiformia. As expected, the monophyly of the Myrmeleontiformia was well supported, the clade emerging during the Triassic and diversifying during the Jurassic and Cretaceous.

Coniopterygidae are sister to the rest of Neuroptera

Dustywings (Coniopterygidae) are very small lacewings characterized by reduced wing venation, a secreted waxy body covering and specialized male genitalia. Coniopterygidae have been difficult to place phylogenetically and are considered by some to be a separate superfamily branching off the main neuropteran stem near Ithonidae (Withycombe, 1925; New, 1989). Other workers have considered Coniopterygidae as sister to Hemerobiidae (Handlirsch, 1906–1908), Osmylidae (Tillyard, 1919) or Sisyridae (Aspöck *et al.,* 2001), based on varying morphological evidence, or have placed it close to Dilaridae based on molecular evidence (Haring & Aspöck, 2004). Aspöck & Aspöck (2008), Zimmermann *et al*. (2009) and Beutel *et al*. (2009) suggest that the family is sister to a clade composed of Dilaridae, Mantispidae and Berothidae, based on mouthpart shape and/or shared development of a 'penisfilum' in derived representatives of both clades. We recovered Coniopterygidae as sister to the rest of Neuroptera, diverging *c*. 294 Ma during the Late Carboniferous to Early Permian, with constituent subfamilies Aleuropteryginae and Coniopteryginae diverging during the Triassic. The oldest definitive dustywing fossils are from the Late Jurassic (*Juraconiopteryx* Meinander) and Early Cretaceous (*Libanosemidalis*) periods (Azar *et al.,* 2000), considerably younger than the divergence date we have estimated here. Authors have proposed a variety of hypotheses for the sister-group to the rest of the Neuroptera: Nemopteridae (Handlirsch, 1906–1908); Coniopterygidae (Kubrakiewicz, *et al.,* 1998; Sziráki, 2007); Berothidae (Tillyard, 1919); Ithonidae (Withycombe, 1925); and Nevrorthidae (Aspöck *et al.,* 2001; Beutel *et al.,* 2009). There is morphological evidence to support Coniopterygidae as sister to the rest of Neuroptera, although mostly as plesiomorphies. Coniopterygidae larvae have only six Malpighian tubules, a character shared with Megaloptera, Raphidioptera and Coleoptera, whereas all other neuropteran families have seven or eight Malpighian tubules. The internal genitalia of female Coniopterygidae are heterogeneous in structure, but in at least two subfamilies they are represented partially by a complex of the bursa copulatrix and ductus seminalis, a character shared with other families such as Osmylidae, Nevrorthidae, Sisyridae and Dilaridae, along with Megaloptera (Sziráki, 1996). Sziráki (2007) outlined a series of proposed symplesiomorphies shared between the enigmatic coniopterygid subfamily Brucheiserinae and Corydalidae. Lastly, sperm microstructure and ovariole structure and function in Coniopterygidae are highly divergent from in the rest of Neuroptera, and, when compared with other insect groups with polytrophic ovarioles, provide additional evidence for a sister-group position of the family to the rest of the order (Kubrakiewicz *et al.,* 1998; Zizzari *et al.,* 2008).

Sisyridae, Osmylidae and Nevrorthidae

Sisyridae (spongilla flies) and Nevrorthidae are the only families of Neuroptera whose larvae are fully aquatic. Sisyridae

are obligate predators of freshwater sponges and bryozoans, whereas Nevrorthidae are generalist benthic predators in lotic habitats. Sometimes incorrectly referred to as semiaquatic, some osmylid larvae (e.g. Osmylinae, Kempyninae) are found in moist stream-bank habitats, whereas other species (e.g. Stenosmylinae, Porisminae) live under bark in drier habitats. Our data (Figs 5, 7; Figure S1) support a clade comprising Nevrorthidae, Sisyridae and Osmylidae sister to the rest of Neuroptera after Coniopterygidae. Unfortunately, this clade has weak statistical support, and in the pruned analysis Sisyridae are recovered as sister to Dilaridae. A close relationship between these two families was supported by Sziráki (1996) based on female internal genitalia. Using molecular data, Haring & Aspöck (2004) also placed Nevrorthidae, Sisyridae and Osmylidae in sequence as sister taxa to the rest of Neuroptera. The placement of Nevrorthidae as sister to the rest of Neuroptera by these authors supported a previous proposal by Aspöck *et al*. (2001) (see also Beutel *et al*., 2009), based on morphological evidence, for an aquatic origin of the order based on the presence of plesiomorphic characters in the larva such as a well-formed gula plate and absence of cryptonephric Malpighian tubules. The presence of a gula in the larval head capsule is found throughout Neuropteroidea, but is lost in Neuroptera except for Nevrorthidae (Aspöck *et al*., 2001; Beutel *et al*., 2009), Ithonidae, Polystoechotidae and Myrmeleontiformia (MacLeod, 1964), in all of which it is probably retained as a symplesiomorphy.

Dilaridae

Pleasing lacewings (Dilaridae) are an enigmatic group of lacewings found in all biogeographic regions except Australasia. Males have distinctive pectinate antennae and females have a long ovipositor. In our combined data analyses, Dilaridae were recovered as sister to the paraphyletic Hemerobiiformia + Myrmeleontiformia. In the pruned taxa set analysis it was recovered as sister to Sisyridae, although with relatively weak statistical support. Authors have suggested a close relationship between Dilaridae, Mantispidae and Berothidae (MacLeod, 1964; Aspöck *et al.,* 2001; Aspöck & Aspöck, 2008; Beutel *et al.,* 2009), although support of this group of families is equivocal when all the evidence is considered. The molecular study of Haring & Aspöck (2004) placed Dilaridae as sister to Coniopterygidae. Morphological support for Dilaridae as an early branching lineage along with Osmylidae, Nevrorthidae and Sisyridae has been provided by Sziráki (1996) based on the structural arrangement of the female internal genitalia, whereas Handlirsch (1906–1908) grouped Sisyridae with Dilaridae based largely on fossil data. These families share with Megaloptera and Raphidioptera characters such as paired spermathecae and a bursa copulatrix forming a complex with the ductus seminalis. It appears, based on molecular data (presented herein and by Haring & Aspöck (2004)) and various morphological characteristics (e.g. Sziráki, 1996), that this anomalous family is not closely related to Mantispidae and Berothidae as was proposed previously.

Berothidae and Mantispidae

Mantispidae (mantid lacewings) are distinctive lacewings with raptorial forelegs resembling preying mantids (Mantodea). The phylogenetic placement of Rhachiberothinae (thorny lacewings) is contentious, having been proposed as a subfamily of Berothidae (Tjeder, 1959; MacLeod & Adams, 1968), a subfamily of Mantispidae (Willmann, 1990) and as a separate family entirely (Aspöck & Mansell, 1994; Grimaldi & Engel, 2005). Our analyses recovered a monophyletic clade composed of Mantispidae + Berothidae with relatively strong support (PP = 1.00, PB = 91%, DI = 9) (Fig. 5). Unfortunately, internal relationships between and within these families were not recovered with strong support and varied among analyses (Figs 4–7; Figs 4, 5). The enigmatic *Ormiscocerus* was transferred recently from Hemerobiidae to Berothidae: Cyrenoberothinae (Penny & Winterton, 2007). The oldest fossils known for Berothidae and Mantispidae are all from Late Jurassic- (Ren & Guo, 1996) and Cretaceous-aged amber deposits (Panfilov, 1980; Whalley, 1980; Klimaszewski & Kevan, 1986). Estimates of divergences times presented here indicate that the group arose during the Early Triassic (Fig. 7).

Curved versus straight jaws in larval Neuroptera

All of the families discussed above have larvae with straight jaws (with the exception of Nevrorthidae). The remaining Neuroptera (Chrysopidae, Hemerobiidae, Ithonidae, Polystoechotidae and members of Myrmeleontiformia) have larval jaws that are curved, with bases usually widely spaced (characters 28 and 36). The jaws of larval Ithonidae and Polystoechotidae are much shorter than those of the other families, but in at least some members the jaws are notably curved in at least one or more instars (MacLeod, 1964; Grebennikov, 2004). Larvae in this clade typically have re-enforcing modifications of the posterior rim of the head capsule. In Chrysopidae, Ithonidae and Polystoechotidae (but not Hemerobiidae) this involves a bracing of the posteroventral rim by a close approximation of the posterior tentorial arms. In Ithonidae and Polystoechotidae this also involves some sclerotization of the gula region. Larvae of Myrmeleontiformia re-enforce the posterior rim through sclerotization of the entire ventral surface of the head capsule and restriction of the maxillary and gula sclerites to a small region anteriorly (MacLeod, 1964). This latter strategy allows larvae to take larger or more active prey items and may be correlated with larger overall body size; some of the largest lacewings are found in families such as Ascalaphidae and Myrmeleontidae (MacLeod, 1964). In larvae with straight jaws and a head capsule without ventral sclerotization, the jaws move largely in the longitudinal plane and thus larvae are unable to manipulate their prey items except through movements of the entire head capsule. Larvae with straight jaws feed on small or relatively immobile prey items (Withycombe, 1925) and are more likely to be specialized feeders on a single prey type (e.g. Sisyridae, Mantispidae, Berothidae). By contrast, with the exception of highly specialized Ithonidae and Polystoechotidae, lacewing

larvae with curved and widely spaced jaws have considerable lateral traverse (often beyond 180°) and are more generalist predators that can feed on larger prey. Larval Nevrorthidae are a notable exception amongst Neuroptera as they have a well-sclerotized gula plate and the apices of the jaws are curved inwards, indicating some ability for lateral movement (as well as that they are probably generalist predators). Nevrorthidae is placed with Sisyridae and Osmylidae, both families that have straight jaws and limited sclerotization of the posterior rim.

Ithonidae and Polystoechotidae and the phylogenetic position of Oliarces

Ithonidae (moth lacewings) are a small family of robust lacewings comprising three genera from Australia (*Ithone*, *Varnia* Walker and *Megalithone*), three genera from the New World (*Oliarces*, *Narodona* Navás and *Adamsiana* Penny), and one genus from Southeast Asia (*Rapisma* McLachlan). Ithonidae have been placed as the earliest branching lineage of Neuroptera on the basis of plesiomorphic characteristics shared with Megaloptera. Tillyard (1916, 1919) considered Ithonidae sufficiently different to place them into a separate suborder, Ithonoidea, sister to all other Neuroptera. Polystoechotidae (giant lacewings) are represented by three New World genera (*Platystoechotes*, *Polystoechotes* Burmeister and *Fontecilla* Navás) and have been allied with several different neuropteran families, including Hemerobiidae (Withycombe, 1925) and Osmylidae + Myiodactylidae (Handlirsch, 1906–1908). Like Ithonidae, Polystoechotidae have been placed among the earliest branching neuropteran families based on wing morphology (Archibald & Makarkin, 2006). Using larval head capsule morphology and wing venational characters, MacLeod (1964) advanced evidence that Ithonidae and Polystoechotidae are close relatives of Myrmeleontiformia. This hypothesis was supported subsequently by Aspöck *et al*. (2001) based on morphological evidence, and by Haring & Aspöck (2004) using DNA sequence data. Our data strongly support a sister-group relationship between Ithonidae and Polystoechotidae, although Ithonidae were rendered polyphyletic with *Oliarces* placed within Polystoechotidae. Our results group these families in a clade comprising Myrmeleontiformia and Hemerobiidae + Chrysopidae, although node support for higher-level relationships among these clades is lacking. The larval empodium is secondarily lost in Sisyridae and later instar Hemerobiidae as well as in the clade comprising Ithonidae, Polystoechotidae and Myrmeleontiformia. A reduced empodium may be a characteristic of the fossorial digging habit of many larvae in this group of families. No empodium was observed in first instar *Platystoechotes* larvae examined in this study, nor in *Polystoechotes* larvae studied by Grebennikov (2004).

Carpenter (1951) provided detailed arguments for the placement of *Oliarces* in Ithonidae, although Lameere (1936) suggested previously that it belonged to Polystoechotidae. Grebennikov (2004) also described a closer morphological similarity of *Oliarces* first instar larvae to *Polystoechotes* rather than to *Ithone*. The morphological disparity between the New World and Old World ithonids has been discussed previously (Makarkin & Menon, 2007). Based on the strong statistical support for the placement of *Oliarces* in our analyses, we concur with Lameere (1936) in support of the transfer of *Oliarces* from Ithonidae to Polystoechotidae, or alternatively, in consolidating all species in both families into a single family Ithonidae.

We inferred a mean divergence time estimate for the Ithonidae + Polystoechotidae clade in the Late Triassic, with various ithonid and polystoechotid genera originating during the Jurassic and Cretaceous. Fossil Polystoechotidae are known from the Middle Jurassic (Lambkin, 1988; Ren *et al*., 2002), and a 'rapismatid'-like fossil ithonid was described recently from the Lower Cretaceous by Makarkin & Menon (2007).

Chrysopidae and Hemerobiidae

A sister-group relationship between Chrysopidae and Hemerobiidae has long been assumed based on overall larval similarities, but conclusive synapomorphies have yet to be defined (Withycombe, 1925). Our data support this grouping in most analyses, although with low statistical support, and the relationship was recovered also by Haring & Aspöck (2004). Numerous fossil chrysopid-like taxa are known from Mesozoic deposits (e.g. Makarkin, 1997; Martins-Neto & Vulcano, 1988; Nel *et al*., 2005), although relatively few similarly-aged hemerobiid fossils are known (Oswald, 1993a).

Myrmeleontiformia

This group of five families (Ascalaphidae, Myrmeleontidae, Nemopteridae, Nymphidae and Psychopsidae) gives the best-supported grouping of lacewing taxa (Grimaldi & Engel, 2005). Larvae in this clade exhibit a common set of cephalic characteristics associated with the development of large, trap-like jaws as well as of dolichasterine setae (MacLeod, 1964; Henry, 1978). Our data support a monophyletic Myrmeleontiformia in most analyses. Internal relationships within this clade were relatively strongly supported, except for, in some analyses, the sister-group placement of Psychopsidae to the rest of Myrmeleontiformia, generic arrangements of Nymphidae and paraphyly of Ascalaphidae.

Psychopsidae (silky lacewings) are a small family of spectacular lacewings represented by approximately 26 extant species narrowly distributed in Australasia, Asia and Africa (Oswald, 1993b). Numerous psychopsid, and psychopsid-like (e.g. Kalligrammatidae), fossil species are described from deposits in various biogeographical regions, ranging from the late Triassic to Miocene, indicating that the present diversity and distribution of this family is relictual (Andersen, 2001; Grimaldi & Engel, 2005). Our molecular data indicate that Psychopsidae diverged from the rest of Myrmeleontiformia during the Late Triassic. Previous authors support the placement of Psychopsidae as sister to the rest of Myrmeleontiformia

as this group exhibits numerous plesiomorphic characteristics of Myrmeleontiformia (Withycombe, 1925; MacLeod, 1964; Henry, 1978; Mansell, 1992; Haring & Aspöck, 2004; Beutel *et al.,* 2009).

Nymphidae (split-footed lacewings) are a small family endemic to the Australasian region (New, 1989). Our data strongly support a sister-group relationship between Nymphidae and Nemopteridae (Ascalaphidae + Myrmeleontidae), which concurs with a previous hypothesis of Myrmeleontiformia relationships by MacLeod (1964), although many authors place Nymphidae as sister to Ascalaphidae + Myrmeleontidae (Withycombe, 1925; Henry, 1978; Mansell, 1992; Aspöck *et al.,* 2001; Aspöck & Aspöck, 2008). *Nymphes* Leach was placed as sister to the other genera sampled, but evidence for specific relationships among 'myiodactyloid' genera (*sensu* Handlirsch, 1906–1908) was weakly supported (Figs 4–6). We inferred an origin for the Nymphidae during the early Jurassic based on our molecular data (Fig. 7), and the oldest definitive fossils are known from Jurassic-aged deposits (Carpenter, 1929).

Nemopteridae (spoon- and thread-winged lacewings) are a distinctive family, characterized by elongated, thread-like or spoon-shaped hind wings, distributed in the Afrotropical, Palaearctic, Australian and Neotropical regions (New, 1989). Nemopteridae was placed as sister to Ascalaphidae + Myrmeleontidae with strong statistical support (Figs 4–6), an arrangement proposed previously by MacLeod (1964). Two subfamilies are recognized (Nemopterinae and Crocinae) and are recovered as monophyletic clades in the full taxon analysis with strong statistical support. The long branches within Crocinae probably reflect the highly autapomorphic morphology of the adults and larvae. The estimated origin of Nemopteridae was during the mid-Jurassic, although the few fossil nemopterids known are from Cretaceous- and Tertiary-aged deposits (Carpenter, 1960; Martins-Neto, 2000). Our data contradict the results of Aspöck *et al*. (2001) and Aspöck & Aspöck (2008), who placed Nemopteridae as sister to Psychopsidae based on a single wing character, larval head sculpturing and male genitalic sclerite characters.

A sister-group relationship between Ascalaphidae (owlflies) and Myrmeleontidae (antlions) has been proposed previously, based on similarities between both adults and larvae (e.g. MacLeod, 1964; Mansell, 1992). Both families are cosmopolitan in distribution, with Ascalaphidae containing *c*. 430 species whereas Myrmeleontidae represent the most species-rich family of lacewings with c. 1630 species. We recovered strong support for the monophyly of Ascalaphidae and Myrmeleontidae in all analyses; however, in some cases, Ascalaphidae were rendered paraphyletic with respect to Myrmeleontidae (Fig. 6). We estimate an origin for Ascalaphidae + Myrmeleontidae during the late Jurassic at around 153 Ma (Fig. 7).

The ancestral neuropteran: with an aquatic or terrestrial larva?

The weak statistical support for ordinal-level relationships in these data provides little conclusive support for either an aquatic (Aspöck, 1995) or a terrestrial origin for the ancestral lacewing. The cryptonephridium, a complex formed by a close association of the Malpighian tubules and the hindgut, is found in larvae in various families of Coleoptera and Neuroptera as a mechanism for water retention in arid environments (Gaumont, 1976). The presence of a cryptonephridium in some Neuroptera larvae may represent a plesiomorphic character retained from their neuropteroid ancestry, as it is found in many coleopteran families but not in Raphidioptera or Megaloptera. Alternatively, the cryptonephridium in Neuroptera may be a secondarily derived innovation in one or more neuropteran lineages as a similar structure is present in more distantly related families of Hymenoptera, and Lepidoptera, presumably also associated with a frequent common function of water retention in arid environments. The variable number of Malpighian tubules associated with the cryptonephridium suggests that this structure is evolutionarily rather labile. Families of Neuropterida with aquatic larvae have either one (Sisyridae) or no Malpighian tubules associated with the hindgut (Nevrorthidae) whereas terrestrial families have three to six. This character system requires further detailed study in neuropteran families such as Psychopsidae and Dilaridae.

Conclusions

Phylogenetic relationships among the major clades of Neuropterida have been historically contentious (Handlirsch, 1906–1908; Tillyard, 1916, 1919; Withycombe, 1925), with consensus among hypotheses only now emerging (Aspöck *et al.,* 2001; Winterton, 2003; Haring & Aspöck, 2004; Aspöck & Aspöck, 2008; Beutel *et al*., 2009). Highly specialized larval life histories with associated disparate morphology in some groups, and generalized morphology of adults, have made the identity of homologous characters ambiguous and elusive, particularly in genitalic structures. In the morphological systems studied to date, there have been multiple hypotheses of homology proposed in certain genitalic and wing-venational structures among Neuropterida family groups (e.g. Tjeder, 1954, 1956; MacLeod, 1964; Aspöck *et al*., 2001; Grimaldi & Engel, 2005; Aspöck & Aspöck, 2008). More detailed study of additional character systems is warranted to elucidate further phylogenetically informative character systems for understanding Neuropteran phylogeny. In this study we combined morphological characters with DNA sequence data in a total-evidence analysis to recover a temporal sequence of Neuropterida family-level cladogenesis. Unfortunately, statistical support for relationships between Neuroptera, Megaloptera and Raphidioptera remains weak, although Wiegmann *et al*. (2009) recently provided strong support based on a larger set of genetic loci for (Megaloptera + Raphidioptera) Neuroptera (although without testing the monophyly of Megaloptera). Our

finding of a paraphyletic Megaloptera is not conclusive, owing to low statistical support. Kubrakiewicz *et al*. (1998) propose specialized '*Sialis'*-type ovarioles as a synapomorphy for Sialidae + Raphidioptera, but these more probably represent the plesiomorphic state within the clade comprising Megaloptera + Raphidioptera, with megalopteran monophyly still retained if neopanoistic ovarioles of Corydalidae are considered as secondarily derived (Büning, 2005). Further study is needed using increased DNA sequencing combined with increased taxon sampling within Megaloptera to determine ordinal relationships within Neuropterida conclusively.

The results of this large total-evidence study do not support the classification proposed by Aspöck *et al*. (2001). Hemerobiiformia was never recovered as monophyletic. Nevrorthiformia, containing the sole family Nevrorthidae, was not recovered as sister to the rest of Neuroptera but lies within the paraphyletic Hemerobiiformia. The monophyly of the long-established Myrmeleontiformia was well supported by both morphological and molecular data. Relationships among Myrmeleontiformia families were also well supported, with Psychopsidae sister to the rest of the suborder, although contrary to Aspöck *et al*. (2001) and subsequent reinterpretation by Aspöck & Aspöck (2008). Nemopteridae were sister to Ascalaphidae + Myrmeleontidae, not Psychopsidae.

The enigmatic family Coniopterygidae was recovered as sister to the rest of Neuroptera, diverging from the neuropteran stem group during the early Permian. Previous authors have suggested this relationship, citing the highly divergent external morphology of Coniopterygidae, such as reduced wing venation and wax-producing glands, as well as retention of several plesiomorphic characters such as Malpighian tubule number and ovariole and female internal genitalic structure (Withycombe, 1925; MacLeod, 1964; Sziráki, 1996; Kubrakiewicz *et al*., 1998). A sister relationship between Coniopterygidae and the rest of Neuroptera has not been favoured by recent authors (e.g. Aspöck *et al.,* 2001; Aspöck & Aspöck, 2008; Zimmermann *et al*., 2009), who have proposed closer relationships with Sisyridae or the 'dilarid' clade. A single character supporting the clade comprising Neuroptera exclusive of Coniopterygidae is the presence of wing trichosors (although secondarily lost in several families).

In contrast to some studies (e.g. Withycombe, 1925; Archibald & Makarkin, 2006), our data concur with MacLeod (1964) in indicating that Ithonidae and Polystoechotidae are not sister to the rest of Neuroptera but are sister to Myrmeleontiformia. Based on the strong statistical support for *Oliarces* being placed in Polystoechotidae sister to *Platystoechotes*, this genus should be transferred to Polystoechotidae, or the two families merged into a broader concept of Ithonidae. Moreover, given the phylogenetic utility of the molecular data used here for interpreting relationships in this clade, sampling of DNA sequence data is needed for additional ithonid taxa such as *Adamsiana*, *Rapsima* and *Varnia* to clarify generic relationships in this clade more rigorously and to assess the status of Ithonidae relative to Polystoechotidae.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3113.2010.00521.x

Figure S1. Phylogram of Bayesian likelihood analysis of 69 Neuropteroidea taxa for DNA sequence data for four loci (COI, CAD, 18S, 16S).

Figure S2. Phylogram of maximum likelihood analysis of 69 Neuropteroidea taxa for DNA sequence data for four loci (COI, CAD, 18S, 16S).

Table S1. Character descriptions for the morphological matrix given in Table S2.

Table S2. Matrix of scored values for the morphological characters described in Table S1.

Table S3. Sequences for primers used in this study.

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