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Evolution of lacewings and allied orders using anchored phylogenomics (Neuroptera, Megaloptera, Raphidioptera)

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Abstract. Analysis of anchored hybrid enrichment (AHE) data under a variety of analytical parameters for a broadly representative sample of taxa (136 species representing all extant families) recovered a well-resolved and strongly supported tree for the higher phylogeny of Neuropterida that is highly concordant with previous estimates based on DNA sequence data. Important conclusions include: Megaloptera is sister to Neuroptera; Coniopterygidae is sister to all other lacewings; Osmylidae, Nevrorthidae and Sisyridae are recovered as a monophyletic Osmyloidea, and Rhachiberothidae and Berothidae were recovered within a paraphyletic Mantispidae. Contrary to previous studies, Chrysopidae and Hemerobiidae were not recovered as sister families and morphological similarities between larvae of both families supporting this assumption are reinterpreted as symplesiomorphies. Relationships among myrmeleontoid families are similar to recent studies except Ithonidae are placed as sister to Nymphidae. Notably, Ascalaphidae render Myrmeleontidae paraphyletic, again calling into question the status of Ascalaphidae as a separate family. Using statistical binning of partitioned loci based on a branch-length proxy, we found that the diversity of phylogenetic signal across partitions was minimal from the slowest to the fastest evolving loci and varied little over time. Ancestral character-state reconstruction of the sclerotization of the gular region in the larval head found that although it is present in Coleoptera, Raphidioptera and Megaloptera, it is lost early in lacewing evolution and then regained twice as a nonhomologous gula-like sclerite in distantly related clades. Reconstruction of the ancestral larval habitat also indicates that the ancestral neuropteridan larva was aquatic, regardless of the assumed condition (i.e., aquatic or terrestrial) of the outgroup (Coleopterida).

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Introduction

Insects underwent a dramatic radiation during the late Carboniferous and Early Permian, with the origin of holometabolan insects which would later become the dominant organisms in all terrestrial and freshwater ecosystems (Grimaldi & Engel, 2005). One such clade among these highly successful insect lineages is the Neuropteroidea, comprising the orders of beetles (Coleoptera), twisted-wing parasites (Strepsiptera), alderflies and dobsonflies (Megaloptera), snakeflies (Raphidioptera) and lacewings (Neuroptera). Neuropterida is a monophyletic subset of these orders, represented by Megaloptera, Raphidioptera and the comparatively species-rich Neuroptera. Although considered minor orders today based on the number of extant species (*c*. 6500 species), the fossil record of Neuropterida is relatively rich and morphologically diverse (*c*. 930 species), with numerous fossil taxa known from various Mesozoic deposits, and many placed in now-extinct families. Based on the relative antiquity of the order, the Mesozoic origins of all families, disparate morphology of the larval stages and disjunct distributions of extant lineages, it is presumed that Neuropterida is today a relictual shadow of its pre-Cenozoic diversity (Aspöck *et al*., 2001; Winterton *et al*., 2010; Wang *et al*., 2017; Engel *et al*., 2018).

Our understanding of higher-level phylogeny amongst families of Neuropterida has been relatively limited, with phylogenetic signal obscured throughout the clade by generalized adult morphology among distantly related groups, combined conversely with highly disparate larval morphology associated with specialized life histories (Wang *et al*., 2017). Aside from some obvious close associations of a few families, broader relationships among the orders and especially among families of Neuroptera have been poorly understood and frequently controversial, with numerous widely different hypotheses proposed by various authors. Unfortunately, the rich fossil record of Neuropterida presently lacks the expected intermediate forms to bridge this disparate morphology, further obscuring the evolutionary history of the group. The traditional hypothesis of relationship among neuropteridan orders is a sister-group association between Megaloptera and Raphidioptera, and several recent molecular phylogenetic studies also support this topology (e.g. Wiegmann *et al*., 2009; Song *et al*., 2016). In contrast, a larger number of studies have instead shown with a high level of confidence that Megaloptera are sister to Neuroptera based on morphology and molecular evidence, and there remains little doubt as to their relationship (Aspöck *et al*., 2001; McKenna and Farrell, 2010; Winterton *et al*., 2010; Misof *et al*., 2014, Peters *et al*., 2014; Wang *et al*., 2017). Similarly, the reciprocal monophyly of each of the orders of Neuropterida is well-supported based on a series of morphological characters. Although some analyses have recovered Megaloptera as paraphyletic with respect to Raphidioptera (e.g. Winterton *et al*., 2010), because of the overwhelming morphological evidence for their monophyly, they have never been seriously questioned as a natural group.

Raphidioptera are a relatively species-poor order with approximately 250 extant species in two families (Raphidiidae and Inocellidae), both with a circumboreal distribution. The order has its origins in the late Carboniferous or Early Permian with an additional six snakefly families known from Triassic to Cretaceous deposits (Liu *et al*., 2014; Engel *et al*., 2018). Megaloptera are a similarly species-poor order (*c*. 380 extant species) divided into two extant families (Corydalidae and Sialidae), with relatively few species known from the fossil record (Liu *et al*., 2012; Engel *et al*., 2018). Neuroptera have the greatest species diversity with more than 6500 species currently placed in 16 extant families, although the number of families varies by author and, as we discover more about the phylogeny of the group, that number has been diminishing (e.g. Winterton & Makarkin, 2010), especially in the numerous extinct fossil families (see Engel *et al*., 2018). Neuroptera are well-supported as monophyletic based on three synapomorphies found in the larval stage, the most notable being the specialized sucking mouthparts. The buccal opening is closed with interlocking elongated mandibles and maxillae modified laterally into a pair of jaw-like sucking tubes used to seize and impale prey, delivering venom to subdue and subsequently suck out the liquid contents of the victim. By contrast, both Raphidioptera and Megaloptera have larvae with unspecialized chewing mouthparts. Larval Neuroptera also have a discontinuous gut, with the midgut and hindgut only attached by a fine filament; no waste is passed during the larval stage. Lastly, the Malpighian tubules are modified for the production of silk, which exits the body through the anus, the silk being used to prepare a protective cocoon within which pupation takes place. In many lacewing larvae, one or more of the Malpighian tubules may be intimately associated with the hindgut to form a cryptonephridium, which increases the efficiency of water resorption across the hindgut wall, particularly for larvae living in arid habitats (see New, 1989). The plesiomorphic predation type found in Raphidioptera and Megaloptera is as a generalist. Generalist predators are also common in larvae throughout Neuroptera, but certain lineages specialize on specific prey (e.g. termites, spiders, ants, wasps, freshwater sponges) or hunt prey mostly in certain habitats (e.g. arboreal, aquatic or subterranean), often with associated highly specialized morphologies and life histories.

The phylogenetic relationships among families of lacewings have been controversial, often with competing hypotheses championed by various authors based on either morphology (e.g. Aspöck *et al*., 2001; Aspöck & Aspöck, 2008), or analyses incorporating molecular evidence (e.g. Winterton *et al*., 2010; Yang *et al*., 2012; Wang *et al*., 2017). Areas of contention have largely revolved around the putative sister family to the remaining Neuroptera; placement of certain families such as Nevrorthidae, Coniopterygidae, Dilaridae and Sisyridae; and potential paraphyly of the families Myrmeleontidae and Mantispidae (Winterton *et al*., 2010; Wang *et al*., 2017; Engel *et al*., 2018). Regarding the sister group to the rest of Neuroptera, there is a strong dichotomy between two proposed hypotheses that have been championed recently. Some authors have supported the placement of Nevrorthidae as sister to the rest of the order, based largely on larval morphology (e.g. Aspöck *et al*., 2001; Aspöck & Aspöck, 2008), whereas others support Coniopterygidae as the sister based on a total evidence approach (Winterton *et al*., 2010) or mitogenomic data (Wang *et al*., 2017). Moreover, some authors suggest that Nemopteridae are sister to

Ascalaphidae and Myrmeleontidae (e.g. Stange, 1994; Winterton *et al*., 2010; Wang *et al*., 2017; Michel *et al*., 2017), whereas others have suggested a closer relationship between Nemopteridae and Psychopsidae (e.g., Withycombe, 1925; Mansell, 1992; Aspöck *et al*., 2001; Aspöck & Aspöck, 2008; Beutel *et al*., 2010a; Zimmerman, 2011; Randolf *et al*., 2013, 2014; Badano *et al*., 2016). Recent studies using a total evidence approach, often with large amounts of sequence data, however, have provided the strongest evidence of phylogeny to date, and we may be getting close to finally converging on a consensus (Winterton *et al*., 2010; Badano *et al*., 2016; Michel *et al*., 2017; Wang *et al*., 2017; Zhang & Yang, 2017).

Furthering on from recent studies that incorporate increasingly larger amounts of DNA sequence data to address phylogenetic questions among lacewings and their allied orders (Winterton, 2003; Haring & Aspöck, 2004; Winterton *et al*., 2010; Wang *et al*., 2017), we present the first genomic approach to estimating the phylogeny of Neuropterida by comparing anchored hybrid enrichment data for 136 species representing all 20 families of the superorder. We also present a revised time tree for divergences among major lineages of the order Neuroptera. One notable line of evidence forwarded for placement of Nevrorthidae as sister to the rest of Neuroptera is the presence of a gular sclerite on the posteroventral surface of the larval head capsule, and that the larva is aquatic; these are thought to represent plesiomorphic characters that Nevrorthidae shares with related orders and which are mostly absent throughout Neuroptera. Using ancestral character-state reconstruction on our AHE phylogeny, we test hypotheses regarding the homology of the gula throughout Neuropterida, and the origin of an aquatic lifestyle for the larva.

Materials and methods

Taxon sampling

Taxa were chosen specifically to broadly represent the greatest diversity within individual families of Neuropterida; 136 species from 124 genera of Megaloptera, Raphidioptera and Neuroptera were selected representing all extant families in each order. We sampled multiple representatives to ensure as close to proportional sampling as possible, and in species-rich families we included representatives of all major subfamilies and tribes where appropriate. Hitherto this is the most extensive taxonomic sampling of Neuropterida in a phylogenetic analysis of any size, which also presented the opportunity to include various enigmatic taxa where the higher-level placement (family or subfamily) has been considered previously to be contentious (e.g. Rhachiberothidae, *Nothancyla* Navás, *Albardia* Weele, *Aeropteryx* Riek). Rapismatidae and Polystoechotidae are considered synonymous with Ithonidae following Winterton & Makarkin (2010) and most lineages of the expanded concept of the family were sampled.

A carabid beetle (Coleoptera: *Lachnophorus* Dejean) was sequenced and used as an outgroup for all analyses. Data on specimens sequenced as part of the study are presented in Table S1. Specimens were initially preserved in 95–100% ethanol and stored at −80°C. Vouchers are deposited in the California State Collection of Arthropods (CSCA) and Texas A&M University Insect Collection (TAMUIC).

DNA extraction

Genomic material was extracted from thoracic or leg muscle wherever possible. For very small organisms (e.g. Coniopterygidae), we pooled the thoracic tissue from multiple individuals of the same population into one extraction. In general, we followed either the DNeasy™ or Gentra Puregene Tissue kits (Qiagen, Redwood City, CA, U.S.A.) for the DNA extraction. Minor modifications included: (i) adding 20 μL of RNAse per 20 mg of tissue after the samples were lysed to remove RNA, and (ii) heating the elution buffer to 55°C degrees before the elusion step. We performed two separate elutions for samples with 30 and 50 μL each time. A final step of drying the DNA pellet was done in some instances. After the extraction, the resulting DNA concentration and quality of each sample were quantified using a Denovix nanodrop spectrophotometer. Samples suitable for library preparation were also confirmed by running an electrophoresis on a 2% agarose gel.

Probe design

For the purposes of probe design ten species from different families were chosen to represent the diverse lineages of Neuropterida (see Table S1). For one of these species, *Nevrorthus apatelios* Aspöck *et al*. (Nevrorthidae) an assembled transcriptome is available (Peters *et al*., 2014). Low coverage whole genome data were collected for the remaining nine species as follows. Illumina libraries were prepared at the Center for Anchored Phylogenomics (htpp://www.anchoredphylogeny.com) from extracted DNA and indexed following Lemmon *et al*. (2012) and Prum *et al*. (2015). The libraries were then pooled in equal proportion and sequenced on one PE150 Illumina lane using C-bot clustering and 8-bp indexing. Following quality filtering with the CASAVA high-chastity filter, reads from this initial lane were demultiplexed (no mismatches tolerated) then merged following Rokyta *et al*. (2012). The merged reads were then used to estimate per-sample coverage as the mean of the 30-mer count distribution. Coverage estimates were then used to determine the number of additional reads needed to obtain 15× total coverage for each sample. Libraries were then re-pooled accordingly and sequenced on two additional PE150 lanes. Reads were demultiplexed, quality-filtered and merged as indicated above.

Merged reads were mapped to 962 insect-wide anchor target regions identified by Young *et al*., (2016). In brief, spaced 20-mers from *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were compared to the merged reads for each individual. Those reads with at least 17 matches to a spaced 20-mer were aligned to the corresponding *T. castaneum* reference sequence and verified if at least 55 of 100 consecutive

bases matched. Consensus sequences including the reference region and flanks were then isolated. Homologous transcripts were identified in the *N. apatelios* transcriptome (Peters *et al*., 2014) using the approach described above (17 matches to a spaced *T. castaneum* 20-mer, verified by a 55% match to the corresponding reference sequence). In the event of multiple matches, the transcript with the best match to the reference sequence was chosen. For each locus, sequences recovered for the ten reference species (plus the *T. castaneum* reference sequence) were aligned using MAFFT v7.023b (Katoh & Standley, 2013). Alignments were inspected visually in Geneious v7 (Kearse *et al*., 2012) and problematic sequences were removed. Alignments were then reduced to appropriate anchor regions by extending out from the region occupied by the *T. castaneum* reference sequence in both directions until an intron greater than 200 bp or an exon <120 bp was encountered. Anchor regions were then checked for overlap and loci were removed to avoid overlap. The resulting set of unique targets was comprised of 570 target loci with an average length of 527 bp and an average pairwise sequence identity of 66%. Following the approach of Hamilton *et al*. (2016) repetitive regions of the sequences in the alignment were masked after identification using 15-mer counts in the raw reads / transcriptome. Probes were then tiled uniformly at 5× density (new probe began every 25 bp) across each of the ten Neuroptera reference sequences for each locus, producing 50 239 probes in total. The total target size covered by probes was 233 234 bp.

Sample preparation

Extracted DNA was used to produce Illumina libraries following Lemmon *et al*. (2012) and Prum *et al*. (2015). In brief, DNA was sonicated to a fragment size of ∼200–800 bp using the Covaris E220 Focused-ultrasonicator with Covaris microTUBES. Libraries were performed on a Beckman–Coulter Biomek FXp robot following a protocol originally derived from Meyer & Kircher (2010), but with library fragments being size-selected using SPRI select beads (Beckman-Coulter Inc., Brea, CA, U.S.A.) at a 0.9× ratio of bead to sample volume. Following addition of 8 bp indices, libraries were pooled in groups of ∼16 and enriched using an Agilent Custom SureSelect XT kit (ELID 3005721, Wilmington, Delaware). Following enrichment, library pools were pooled into single sequencing pool and sequenced on 1 PE150 Illumina 2500 lanes. Sequenced library fragments contained inserts between 150 and 300 bp in length. All DNA sequences generated as part of this study are deposited in the NCBI (Sequence Read Archive) depository (Table S1).

Read assembly

After quality filtering using the CASAVA high-chastity filter, raw reads were demultiplexed using the 8-bp indexes with no mismatches tolerated. Overlapping reads were merged following Rokyta *et al*. (2012). Reads were assembled using the divergent reference assembly approach (quasi-de-novo assembly) described in Prum *et al*. (2015), which recovers the probe region and flanks for each sample. References used for the assembly included *Nymphes myrmeleonoides* Leach (Nymphidae), *Thaumatosmylus delicatus* Banks (Osmylidae), *Palpares obsoletus* Gerstaecker (Myrmeleontidae) and *Nothancyla verreauxi* Navás (Chrysopidae). Assembled contigs derived from fewer than 20 reads were removed in order to reduce the effects of both rare sequencing errors in index reads and cross-contamination.

Alignment generation

After grouping homologous consensus sequences obtained during the assembly process, putative orthologues were identified for each locus following Prum *et al*. (2015), which uses a neighbour-joining-based clustering algorithm based on alignment-free pairwise sequence divergences (see Tables S2–S3). Clusters formed through this process were then screened for taxon presence. Clusters containing fewer than 50% of the species in the taxon set were removed from downstream processing. Assembled contigs derived from fewer than 50 reads were removed. Sequences in each remaining cluster were then aligned using MAFFT v7.023b (Katoh & Standley, 2013) with –genafpair and –maxiterate 1000 flags utilized. Each alignment was trimmed and masked following Prum *et al*. (2015), with 70% conservation required for each site to be considered reliable and 20-bp regions containing matches at fewer than seven reliable sites were masked. After masking, sites containing less than 67% unambiguous bases were removed from the alignment.

Dataset information content and completeness

The amount of missing data in phylogenomic datasets is generally higher than in datasets originated from traditional Sanger sequencing, so we investigated the amount and distribution of missing data in our nucleotide dataset, as well as the phylogenetic information content across partitions. Nonrandomness of missing data has been demonstrated to affect tree reconstructions (Cho *et al*., 2011; Roure *et al*., 2013; Dell'Ampio *et al*., 2014), and its impact and effect on phylogeny estimation is probably dataset-dependent (Yeates *et al*., 2016). We inspected the nucleotide alignment for uneven distribution of missing data using AliStat v1.3 (Misof *et al*., 2014) (available from: http://doi.org/10.4225/08/59309da8368e1), which generates a heat map of the distribution of missing data across the alignment based on pairwise comparisons of the sequences. We also examined the information content of the nucleotide dataset with MARE (MAtrix REduction) 0.1.2-rc (Meusemann *et al*., 2010; Meyer *et al*., 2011) to verify and visualize the relative quality of information of each single gene within the matrix. MARE calculates the information content of each partition using a quartet mapping algorithm (Nieselt-Struwe & von Haeseler, 2001) and reduces the original matrix by

repeatedly dropping the least informative genes and sequences according to an optimality criterion. Our MARE analyses were set to ensure retention of all taxa, and we used the default value for α (scaling factor, default = 3), which directly affects the size and information content of the reduced dataset – by increasing α, the resulting matrix decreases in size whereas the information content increases. Our original nucleotide dataset contained 199 partitions (i.e. loci), with an information content of 0.21 (Table S2). All alignments are deposited in TreeBase. After reduction in MARE the dataset contained 28 partitions, with an information content of 0.36. Because there was no significant increase in information content even after the removal of >85% of genes, we did not reduce the matrix by removing loci, and used the entire dataset for all subsequent analyses.

Heterogeneity of sequence divergences

Besides investigating the effects of missing data and lack of phylogenetic information content in our datasets, we also inspected the nucleotide alignment for strongly divergent nucleotide sequences that could potentially bias tree reconstruction and nodal support. We used Aligroove v1.05 (Kück *et al*., 2014), a method based on a sliding window and a Monte Carlo resampling approach to identify taxa that exhibit random sequence similarity in comparison with other taxa in the dataset. Aligroove establishes pairwise comparisons of nucleotide divergences for each terminal defined by an internal node against all other sequences in a multiple sequence alignment, and the resulting distance matrix is then compared to the similarity over the entire alignment. Score values range from −1 (indicating full random similarity) to +1 (nonrandom similarity). Aligroove was also used to explore the reliability of single branches by calculating the average similarity between taxa that are connected by a branch. This allows us to detect strongly derived sequence regions that can have a negative influence on tree reconstruction methods, as well as highlight taxa that will most likely be misplaced in trees. The default sliding window size was used, and indels in the nucleotide dataset were treated as ambiguity.

Phylogeny estimation

Both supermatrix and species tree approaches were used to estimate the relationships among taxa. For the species tree approach, RaxML (Stamatakis, 2014) was used to estimate locus-specific gene trees, with branch support values calculated by performing 100 bootstrap replicates. Gene trees were then used as input for astral-ii v4.9.7 (Mirarab & Warnow, 2015) using bootstrap replicates from the RaxML-estimated gene trees for branch support values estimation. astral tree estimation is used to mitigate the effects of incomplete lineage sorting, and although not necessarily powerful for tree estimation (especially for older divergences), it is useful for visualizing discordance among genes (or partitions). For the supermatrix approach, the concatenated amino acid and nucleotide alignments were initially partitioned by loci, and these partitions were then combined into metapartitions with PartitionFinder 2 (Lanfear *et al*., 2016) using the *rcluster* search algorithm (Lanfear *et al*., 2014) (Tables S6–S7). PartitionFinder 2 was also used to select the best-fitting model for each metapartition, using the Bayesian Information Criterion (BIC) for model selection. The best-fitting substitution model across all partitions for the nucleotide dataset was a general time-reversible substitution model (GTR; Tavaré, 1986) with rate heterogeneity described by a gamma distribution discretized into four bins (+G; Yang, 1993) and a proportion of invariant sites (+I, Fitch & Margoliash, 1967). We did not use the GTR + I + G mixture model (Gu *et al*., 1995; Waddell & Steel, 1997) because this approach has been highly criticized on both empirical and theoretical grounds (Yang, 1993, 1996, 2006; Sullivan *et al*., 1999; Mayrose *et al*., 2005; Jia *et al*., 2014). Studies indicate that some of the parameters of the +I and +G models cannot be optimized independently of each other (Yang, 1993, 2006; Jia *et al*., 2014); indeed, the estimated proportion of invariable sites was demonstrated to be highly susceptible to changes in the number of gamma rate categories of the +G model (Jia *et al*., 2014). Furthermore, it has been suggested that the assumption of a proportion of invariable sites has no obvious impact on Bayesian estimates of rates, and little to no biological meaning, especially at the intraspecific level (Jia *et al*., 2014). Thus, we employed a GTR + G mixture model separately for each metapartition. For the amino acid dataset, a partition-specific substitution model was implemented as selected by PartitionFinder 2. The final datasets and files containing the inferred partition schemes and model selection results are provided as Supplementary Files. Each dataset, with amino acid or nucleotide data, was analysed separately under both maximum-likelihood (ML) and Bayesian inference (BI). Basic alignment statistics, including percentage of missing data, A/T, C/G content, alignment length and proportion of variable sites were obtained using AMAS (Borowiec, 2016).

We used ExaML (Kozlov *et al*., 2015) to estimate phylogenies under ML, with starting trees inferred with RaxML v8.2 (Stamatakis, 2014). We used a general GTR + G mixture model as selected by PartitionFinder 2. Node support was estimated via nonparametric bootstrapping; bootstraps were also generated in RaxML, with 100 replicates per dataset. We used ExaBayes v1.4 (Aberer *et al*., 2014) to conduct phylogenetic inferences in a Bayesian framework. Two independent runs with four coupled Markov chain Monte Carlo (MCMC) chains each were performed, sampling every 1000th generation and applying uniform priors to tree topologies and an exponential prior to branch lengths. After 20 million generations, convergence of the results was assessed by computing the average standard deviation of split frequencies (ASDSP) and checking the estimated sample sizes (ESS) in Tracer v1.6 (Rambaut *et al*., 2014). We ran the chains until we obtained an ASDSF value of <1% and ESS values >200 for all parameters. Finally, the *consense* tool of the ExaBayes package was used to obtain a 50% majority rule consensus tree, discarding the first 25% of the sample topologies as *burn in*.

Gene-specific substitution rates

Phylogeny estimation can potentially be biased by a number of factors, including model mis-specification, heterotachy, compositional heterogeneity and across-site rate variation, among others (Rodríguez-Ezpeleta *et al*., 2007). One of the main sources of errors, however, is heterogeneity in rates of evolution across loci, which impacts both tree topology and branch lengths (Bininda-Emonds 2007; Goremykin *et al*., 2015). To explore the effects of substitution rate heterogeneity across loci on our tree estimation, we used a simplified binning approach as proposed by Mirarab *et al*. (2014), but grouping genes based on rate of evolution as opposed to bootstrap values on branches as originally proposed. Gene trees were estimated under ML in RaxML v8.2, using the best-fitting model identified by PartitionFinder (GTR + G) for each gene partition. A custom R script was used to compute average branch lengths of each gene partition (available from: https://github.com/marekborowiec/good_genes/blob/master/tree_props.R), used here as a proxy for rate of evolution, with short branch lengths indicating relatively slowly evolving loci, and long branch lengths indicating relatively faster evolving loci. After sorting genes based on average branch lengths (lowest to highest), we checked for clustering of loci of particular branch lengths, or abrupt changes in average branch length values across loci. As no distinct pattern of heterogeneity amongst the data emerged (Fig. 2) – evidenced as clusters or abrupt changes in distribution of branch length– we arbitrarily divided the entire set of 199 loci into three subsets (bins) of equal sizes, so that each bin consisted of a set of loci evolving roughly under the same rate. The three bins were designated as 'slow', 'intermediate' and 'fast', and – for tree estimation – we discarded the intermediate population of average branch lengths to ensure two discrete loci populations separated by a large buffer population. We concatenated the alignments of loci in each of the fast and slow bins and estimated phylogenetic trees separately for each using BI in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) and assessed their topological congruence with the tree generated from all loci.

Divergence times

Estimation of divergence times was implemented in beast v2.4.5 (Bouckaert *et al*., 2014) through the cipres science gateway v3.3 (Miller *et al*., 2010). We used the nucleotide alignment for the dating analyses, and defined the partitions and site models in BEAUti based on the output of PartitionFinder (see Phylogenetic Analyses above). We used an uncorrelated relaxed molecular clock model (Drummond *et al*., 2006) and an exponential prior, with time-tree and clock model linked across partitions. We applied a node dating approach with a birth–death tree prior (Kendall, 1948), a method that uses the age of the oldest fossil within a specific clade as a minimum age constraint for the node at which the clade, including the fossil, had diverged (i.e. calibrating node). We defined these calibrating nodes by determining a monophyletic subset of all the taxa belonging to this clade (taxon sets). A total of 32 minimum age constraints based on the ages of fossils of taxa were used for all major clades. Details of the fossils used as minimum age constraints are provided in Table 1, including fossil age, taxonomic assignment and placement in the topology. Ages of fossils were obtained from the Fossilworks database (http://www.fossilworks.org/). A prior calibration density was defined at each calibration node to account both for uncertainty underlying the age of the fossil and the possibility that the true divergence occurred earlier than defined by the fossil (Drummond & Bouckaert, 2015), and we assigned an exponential distribution for each calibration node.

The birth–death model implemented in beast 2 accounts for incomplete sampling through the combination with a model of the sampling process, resulting in the so-called birth–death sampling process (BDS) (Stadler, 2009). Two sampling scenarios are possible for the BDS: (i) sampling of lineages happens under a constant rate, or (ii) a proportion of lineages may be sampled uniformly at random at a given point in time (Stadler, 2009). For either scenario, age estimates based on BDS are subject to bias if the model of the sampling process is mis-specified. The divergence times estimates proposed by Winterton *et al*. (2010) and Wang *et al*. (2017) were highly biased towards sampling of ingroups, and only a few representatives of the outgroup (Coleoptera + Strepsiptera) were sampled. This sampling is not uniform nor random and we corrected for model violations by removing Coleoptera from our analyses as it represents extreme undersampling of that particular clade relative to the rest of the tree; we instead rooted our topology on the branch connecting Raphidioptera and Megaloptera + Neuroptera. All other clades were sampled relatively proportional to taxonomic and sequence diversity.

We ran two independent analyses in beast for 200 million generations each. We then evaluated the convergence and mixing of the MCMC chains in Tracer v1.6, ensuring that the multiple runs converged on the same distribution and ascertained that effective sample sizes (ESS) exceeded 150. We further compared the effective prior and posterior distributions of all the parameters to test whether our analyses are prior-sensitive and whether the data are informative for the MCMC analyses. We then resampled the resulting files of the inferred phylogenetic trees with a frequency of 10 000 in LogCombiner (beast package) and a burn-in of 30%. This resulted in 27 028 subsampled trees. We then summarized the subsampled trees in a maximum clade credibility tree with common ancestor heights as node heights using TreeAnnotator (beast package).

Ancestral state character reconstruction

We used the method MultiState as implemented in BayesTraits v3.0 (Pagel *et al*., 2004) under BI to reconstruct the evolution of the gula in the larval head capsule and larval habitat across Neuropterida families. For the gula, taxa were coded for the presence (state 1) or absence (state 0) of a sclerite on the ventral surface of the head capsule posterior to the posterior tentorial pit (i.e. gula) (sensu MacLeod, 1964). For larval habitat, taxa were scored as aquatic (state 0) or terrestrial

Table 1. Fossil calibrations for divergence times estimations with name of fossil, age and reference.

Node	Fossil species	Family	Placement	Age (Ma)	References
1	*Electrinocellia peculiaris*	Inocelliidae	Crown Inocelliidae	34	Carpenter (1956)
2	*Raphidia baltica*	Raphidiidae	Crown Raphidiidae	34	Carpenter (1956)
3	*Permoberotha villosa*	Permoberothidae	Stem Neuroptera + Megaloptera	280	Tillyard (1932)
4	*Dobbertinia reticulata*	Sialidae	Stem Sialidae	182	Handlirsch (1920)
5	*Juraconiopteryx zherichini*	Coniopterygidae	Crown Coniopterygidae	156	Meinander (1975)
6	*Glaesoconis baliopteryx*	Coniopterygidae	Crown Aleuroteryginae	94	Engel (2004)
7	*Libanosemidalis hammanaensis*	Coniopterygidae	Crown Coniopteryginae	125	Azar *et al*. (2000)
8	*Prosisyrina sukachevae*	Sysyridae	Crown Sysyridae	85	Handlirsch (1920)
9	*Palaeoneurorthus hoffeinsorum*	Nevrorthidae	Crown Nevrorthidae	34	Wichard *et al*. (2010)
10	*Lithosmylidia baronne*	Osmylidae	Stem Osmylidae	242	Lambkin (1988)
11	*Juraheterosmylus antiquatus*	Osmylidae	Crown Osmylidae	156	Wang *et al*. (2010)
12	*Archaeosmylidia fusca*	Osmylidae	Stem Osmylinae	156	Makarkin *et al*. (2014)
13	*Sauktangida aenigmatica*	Osmylidae	Stem Kempininae	172	Khramov (2014)
14	*Dilar cretaceus*	Dilaridae	Crown *Dilarina*	94	Liu & Zhang (2016)
15	*Sympherobius completus*	Hemerobiidae	Crown *Sympherobius*	34	Makarkin & Wedmann (2009)
16	*Notiobiella thaumasta*	Hemerobiidae	Crown *Notiobiella*	14	Oswald (1999)
17	*Promegalomus anomalus*	Hemerobiidae	Stem Hemerobiidae	156	Panfilov (1980)
18	*Cretomerobius disjunctus*	Hemerobiidae	Crown Hemerobiidae	112	Ponomarenko (1992)
19	*Clavifemora rotundata*	Mantispidae	Stem Mantispidae	156	Jepson *et al*. (2013)
20	*Liassochrysa stigmatica*	Mantispidae	Crown Mantispidae	182	Ansorge & Schlüter (1990)
21	*Mesypochrysa intermedia*	Chrysopidae	Stem Chrysopidae	156	Panfilov (1990)
22	*Paralembochrysa splendida*	Chrysopidae	Crown Chrysopidae	125	Nel *et al*. (2005)
23	Belonopterygini	Chrysopidae	Crown Belonopterygini	50	Archibald *et al*. (2014)
24	*Triassopsychops superbus*	Psychopsidae	Stem Psychopsidae	205	Tillyard (1922)
25	*Cretapsychops decipiens*	Psychopsidae	Crown Psychopsidae	156	Peng *et al*. (2010)
26	*Daonymphes bisulca*	Nymphidae	Crown Nymphidae	156	Makarkin *et al*. (2013)
27	*Liminympha makarkini*	Nymphidae	Stem Nymphidae	156	Ren & Engel (2007)
28	*Guithone bethouxi*	Ithonidae	Crown Ithonidae	156	Zheng *et al*. (2016)
29	*Principiala incerta*	Ithonidae	Crown Ithonidae	112	Makarkin & Menon (2007)
30	*Cratoscalapha electroneura*	Ascalaphidae	Crown Ascalaphidae	112	Martins-Neto & Vulcano (1997)
31	*Araripeneura gracilis*	Myrmeleontidae	Stem Myrmeleontidae	112	Martins-Neto & Vulcano (1989)
32	*Roesleriana exotica*	Nemopteridae	Crown Nemopteridae	112	Martins-Neto & Vulcano (1989)

(state 1) as done previously in a reconstruction of this state by Wang *et al*. (2017) using mitogenomic data. We treated states as unordered, assuming equal transition rates between states, and we allowed the rate of change between states to vary over each transition. The analyses were run using the MCMC implementation of MultiState for 200 million generations and a burn-in of 2 million generations, with an exponential distribution on character transition rates with a mean of 1.0. MCMC assumes that traits are allowed to evolve repeatedly between possible states on the tree branches. By sampling the character states at the internal nodes of the tree in proportion to their probability, the Markov chain is able to estimate the rate of change between states, conditioned on the values at the tips. Rate parameter values are updated in successive steps in the Markov chain resulting in a posterior sample distribution of rate coefficients and ancestral states, which in turn are visited in direct proportion to its posterior probability (PP) in the sample distribution (Pagel *et al*., 2004). We performed the analyses on the tree resulting from the total nucleotide alignment (Fig. 1). Although it is possible to take phylogenetic uncertainty into account in ancestral state reconstructions (Pagel *et al*., 2004), our main objective here was to highlight the pattern of evolution of each condition in Neuropterida, for which we believe the single tree presented here is sufficient. Convergence of runs was assessed using Tracer v1.6 to ensure that analyses had reached stationarity and that ESS values for all parameters were above 200. We re-ran the analyses under the prior to ensure that there were no unexpected interactions causing erroneous induced prior distributions. The resulted average character state probabilities per node are presented in Table S5.

Phylogenetic informativeness of binned AHE data over time

In order to measure the potential for saturation in the sequence data, the relative utility of individual loci and codon positions to resolve the phylogeny over time was evaluated a posteriori as a measure of Phylogenetic Informativeness (PI) (Townsend, 2007; López-Giráldez & Townsend, 2011). This is visualized as PI profiles representing the estimated amount of phylogenetic signal in a partition to resolve branching order of a phylogenetic tree in a particular time period. Graphs of PI values were calculated using HyPhy (Kosakovsky-Pond *et al*., 2005) in the web application PhyDesign (López-Giráldez & Townsend, 2011), which were then plotted over time against a chronogram (Fig. 4). Informativeness profiles convey the level of phylogenetic signal

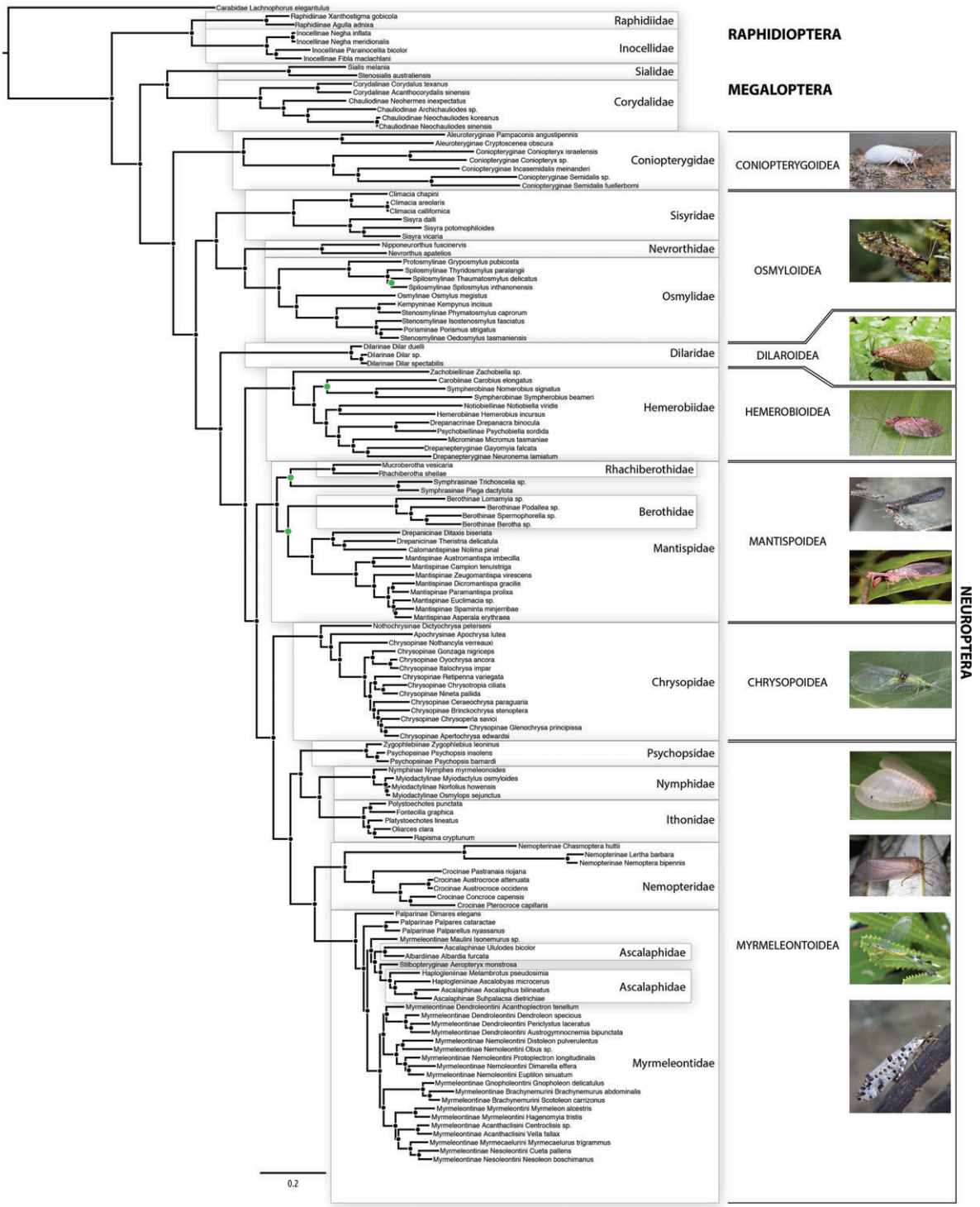

Fig. 1. Phylogram of Neuropterida relationships from the Bayesian analysis of anchored hybrid enrichment (AHE) data. All branches have a support value of 1.0 Bayesian posterior probability (PP) except where indicated by green circles (>0.95 Bayesian PP). Image credits: Photos (top to bottom): *Heteroconis* sp. (Coniopterygidae), *Thyridosmylus paralangii* (Osmylidae), *Dilar duelli* (Dilaridae), *Megalomus pictus* (Hemerobiidae), *Stenobiella muellerorum* (Berothidae), *Asperala erythraea* (Mantispidae), *Semachrysa jade* (Chrysopidae), *Zygophlebius leoninus* (Psychopsidae), *Ithone fulva* (Ithonidae), *Chasmoptera huttii* (Nemopteridae), *Periclystus aureolatus* (Myrmeleontidae). (Photo credits: all by Shaun L. Winterton, except *Semachrysa*, by Guek Hock Ping and *Dilar,* by Davide Badano.)

contributed to the tree for a particular historical epoch, but does not account for spurious effects from saturation and convergence (Townsend, 2007). Consequently, informativeness of a partition is, in general, greatest at timeframes younger than the maxima of the respective plot. Therefore, profiles which have more gradual peaks contribute more phylogenetically informative signal over a larger portion of the chronogram than profiles which peak early and high, with subsequent rapid decline.

Results

Information content and sequence heterogeneity

Overall, results from both ALISTAT and MARE indicated that our dataset did not suffer greatly from missing data effects or lack of phylogenetic information content. The heat map generated with ALISTAT (Fig. 3, right) revealed somewhat uniformly distributed completeness of data, with concentration of missing data in a few species of Megaloptera, Hemerobiidae and Osmylidae. For the included species of Coniopterygidae, we found a relatively extensive amount of missing data, which is likely due to the difficulty in DNA extracting and sequencing given their relatively small body size. Results from ALIGROOVE (Fig. 3, left) indicated overall nonrandom similarities among sequences, evidenced by the similarity scores ranging from 0 to +1 for the vast majority of pairwise comparisons. The analyses also indicated, however, strong heterogeneity in sequence divergence for those species exhibiting large amounts of missing data. In particular, pairwise sequence comparisons involving *Stenosialis australiensis* Tillyard (Megaloptera), *Porismus strigatus* (Burmeister) (Osmylidae), *Gayomyia falcata* (Blanchard) (Hemerobiidae) and five species of Coniopterygidae received lower similarity scores than pairwise comparisons between other sequences. In our dataset, large amounts of missing data seem to be highly correlated with heterogeneity in sequence divergence as suggested by ALIGROOVE. These results provide evidence that there was no apparent negative impact on phylogeny estimation due to either missing data or sequence heterogeneity, given that the large amount of PI of the dataset was enough to correctly place the aforementioned taxa in their traditionally assigned families.

Total nucleotide alignment tree

The nucleotide alignment comprised 137 taxa and 57 546 nucleotide bp sites after trimming, representing 199 loci as separate partitions. Almost all taxa contained a complete, or near-complete set of sequence data, with an overall 15.4% of missing data across the alignment. The average locus length for the nucleotide dataset was 289 bp. The resulting phylogeny (Fig. 1) was remarkably well-supported statistically throughout, with *c*. 95% of all branches supported by a Bayesian PP of 1.0 or ML bootstrap of 100%. In the Bayesian analysis, only four branches did not have a PP value of 1.0, and of those, none were lower than 0.95 PP. In the ML analysis, only nine branches had less than 80% bootstrap support and three of these corresponded to lower PP values in the Bayesian analyses. The ASTRAL tree resulting from our species tree estimation (Figure S1) was weakly supported overall, a result that probably reflects the conflict among fast versus slow evolving genes in our combined dataset (see Gene-specific substitution rates above). Approximately 20% of nodes in the ASTRAL tree have a bootstrap support value lower than 80%, and many of these poorly supported nodes were located along the backbone of the phylogeny. In discordance with our ML and BI trees, ASTRAL recovered Megaloptera as sister to Raphidioptera, with a bootstrap support value of 89%.

In the concatenated analyses under both BI (Fig. 1) and ML (Figure S8), Raphidioptera were recovered as monophyletic and sister to the rest of Neuropterida; Megaloptera were also recovered as monophyletic and sister to a monophyletic Neuroptera; and each of the families of both Raphidioptera and Megaloptera were strongly supported as reciprocally monophyletic. Coniopterygidae was recovered as the sister family to the rest of the order, and both sampled subfamilies (Aleuropteryginae and Coniopteryginae) were recovered as monophyletic. Subsequently, the next clade comprised the families Sisyridae, Nevrorthidae and Osmylidae, with Sisyridae sister to the other families. Within Osmylidae, a basal dichotomy was recovered, with Spilosmylinae and Protosmylinae in one clade, and Porismimae, Stenosmylinae, Kempyninae and Osmylinae in the other. Dilaridae and Hemerobiidae were placed as subsequent sequential clades, subtending a larger dichotomy between one clade comprising Mantispidae, Rhachiberothidae and Berothidae, and the other including the remaining families of Neuroptera. Support within Hemerobiidae was low compared with the rest of the tree and considering the paucity of weakly supported nodes, the bulk of those with weak support surprisingly were concentrated within Hemerobiidae. Within the next clade, Mantispidae was rendered paraphyletic by both Berothidae and Rhachiberothidae in all analyses, although relationships among the families were relatively weakly supported basally. In all cases, the enigmatic mantispid subfamily Symphrasinae (including here *Trichoscelia* Westwood and *Plega* Navás) was never recovered in a monophylum with the rest of Mantispidae, but instead placed with weak support as sister to either Rhachiberothidae or Berothidae, depending on the analysis. Chrysopidae were recovered as the next clade with Nothochrysinae as the sister to the rest of the family, followed by Apochrysinae. The enigmatic genus *Nothancyla* Navás was placed as sister to the rest of Chrysopinae, within which the established tribes were placed as sister pairings of Leucochrysini with Belonopterygini, and Ankylopterygini with Chrysopini. The remaining families (here collectively termed Myrmeleontoidea) were then divided into two main clades, one comprising the families Psychopsidae, Nymphidae and Ithonidae, and the other comprising Nemopteridae, Ascalaphidae and Myrmeleontidae. Bootstrap support for this clade is relatively lower than throughout the rest of the tree (1.0 PP; 53% BS) and the topology varied slightly depending on the analytical method. Moreover, under some parameters (e.g. ML; Figure S8), the basal dichotomy among the six families was not present and Psychopsidae was placed instead

as the sister to the rest of Myrmeleontoidea. Nemopteridae were always recovered as the sister to Myrmeleontidae and Ascalaphidae, with the two subfamilies of Nemopteridae (Crocinae and Nemopterinae) being reciprocally monophyletic. Myrmeleontidae were rendered as paraphyletic by Ascalaphidae in all analyses, with the latter itself rendered paraphyletic by the enigmatic subfamily Stilbopteryginae.

Amino acid tree

The translated amino acid alignment was 19 183 residues in length, with 16.8% of missing data and an average locus length of 97 residues. The tree recovered from Bayesian analysis of the amino acid translated dataset resulted in a very similar topology to the nucleotide tree, although with much fewer strongly supported nodes overall (Figure S2). The main differences between the nucleotide and amino acid trees included a sister grouping between Chrysopidae and Mantispidae + Berothidae and Rhachiberothidae, and Hemerobiidae placed equivocally as sister to Myrmeleontioidea. Ithonidae were placed as an intermediate clade between Psychopsidae and Nymphidae rather than as part of a monophyletic group with these two families. The topology obtained with species tree estimation using ASTRAL (Figure S3) was poorly supported overall, with ~40% of nodes with bootstrap support values lower than 80%. The resulted topology is generally similar to the one based on nucleotides, but with two fundamental differences: (i) Nymphidae was recovered as sister to Nemopteridae + Myrmeleontidae (including Ascalaphidae) (89% BS) and (ii) Chrysopidae and Mantispidae (including Berothidae and Rhachiberothidae) were placed in a monophyletic group sister to a clade including Psychopsidae, Ithonidae, Nymphidae, Nemopteridae and Myrmeleontidae (including Ascalaphidae) (73% BS).

Effect of substitution rate on tree estimation

Figure 2 summarizes the analytical pathway and results assessing the effect of substitution rate heterogeneity on tree estimation. Fast-evolving genes are particularly challenging for phylogenetic reconstruction, especially for deep divergences, because they are likely to have experienced multiple substitutions, potentially eroding part of the phylogenetic signal and misleading phylogenetic inference. Overall, the two topologies resulting from the inclusion of only fast- or slow-evolving genes were relatively similar to the topology we obtained when analysing the whole alignment, but with a few fundamental differences. When analysing only the slowly evolving genes (1/3 of total dataset) (Fig. 2; Figure S6), we did not recover the clade including Psychopsidae, Ithonidae and Nymphidae; instead, the three families were placed in a ladder, with Psychopsidae at the base and Nymphidae as sister to Nemopteridae + Myrmeleontidae (including Ascalaphidae). The subset of faster evolving loci (1/3 of total data) resulted in an equivocal topology with Megaloptera sister to Raphidioptera (Fig. 2; Figure S5). Also, in discordance with our total alignment topology, Rhachiberothidae was not recovered as sister to Symphrasinae (Mantispidae).

Divergence time estimation

Figure 4 shows a chronogram with the divergence time estimates (as common ancestor heights) for Neuropterida using the whole nucleotide alignment (see Fig. 1). Our results indicate an Early Permian origin for Neuropterida at approximately 267 Ma [highest posterior density (HPD) 274–258 Ma], with subsequent diversification of the group with major lineages (i.e. families) in all orders of Neuropterida diverging during the Late Permian and Triassic. The only exception to this is the subsequent divergence of Nemopteridae from Myrmeleontidae plus Ascalaphidae, and Ithonidae from Nymphidae, during the Jurassic.

The 95% HPD values for each node are given in Table S4. Some of the nodes that were calibrated by fossils had surprisingly short 95% HPD intervals (e.g. nodes 4, 54, 73 and 87 in Figure S4). That is, the age range estimates for calibration nodes are very narrow and simply mirror the fossil age used as minimum-age constraint for that particular node. This indicates that the calibration density prior had a strong effect on the age estimates, and the prior values for those nodes were likely not updated properly by the data. This artefact could be a product of the short lengths of loci that we used (average ~300 bp), which would indicate that there is not enough signal in the dataset for updating parameter values of very complex models, including the uncorrelated relaxed clock and BDS used in our dating analyses.

Phylogenetic informativeness of binned AHE data over time

In an attempt to rigorously test the robustness of the tree topologies recovered and to explore alternative topologies potentially generated from binned subsets of loci of varying evolutionary rate (fast, intermediate and slowly evolving loci) we examined the PI on the chronogram over time (Figure S7). Plotting PI on our chronogram we found that PI increased quickly to reach respective maxima during the Palaeogene (55–45 Ma) for all partitions. The PI peaks were highest for the fastest evolving loci and subsequently lower for the intermediate and slower loci. Each partition gradually declined going back in geological time. The closeness in PI maxima among all three partitions is surprising and may be an artefact of the type of data analysed here or an inability of the method to accurately resolve Informativeness for so many short sequences. Genes evolve at different rates over time under varying selective pressures and the rate of change can inform a tree or chronogram differently (PI). These differences in evolutionary rate are evident in individual loci but in this case, we sequenced a small section only for hundreds of different loci (289 bp average length) with differing evolutionary histories and only broadly similar evolutionary rates. We suppose that the PI curves for the three partitions analysed here represent an average PI for the section of numerous

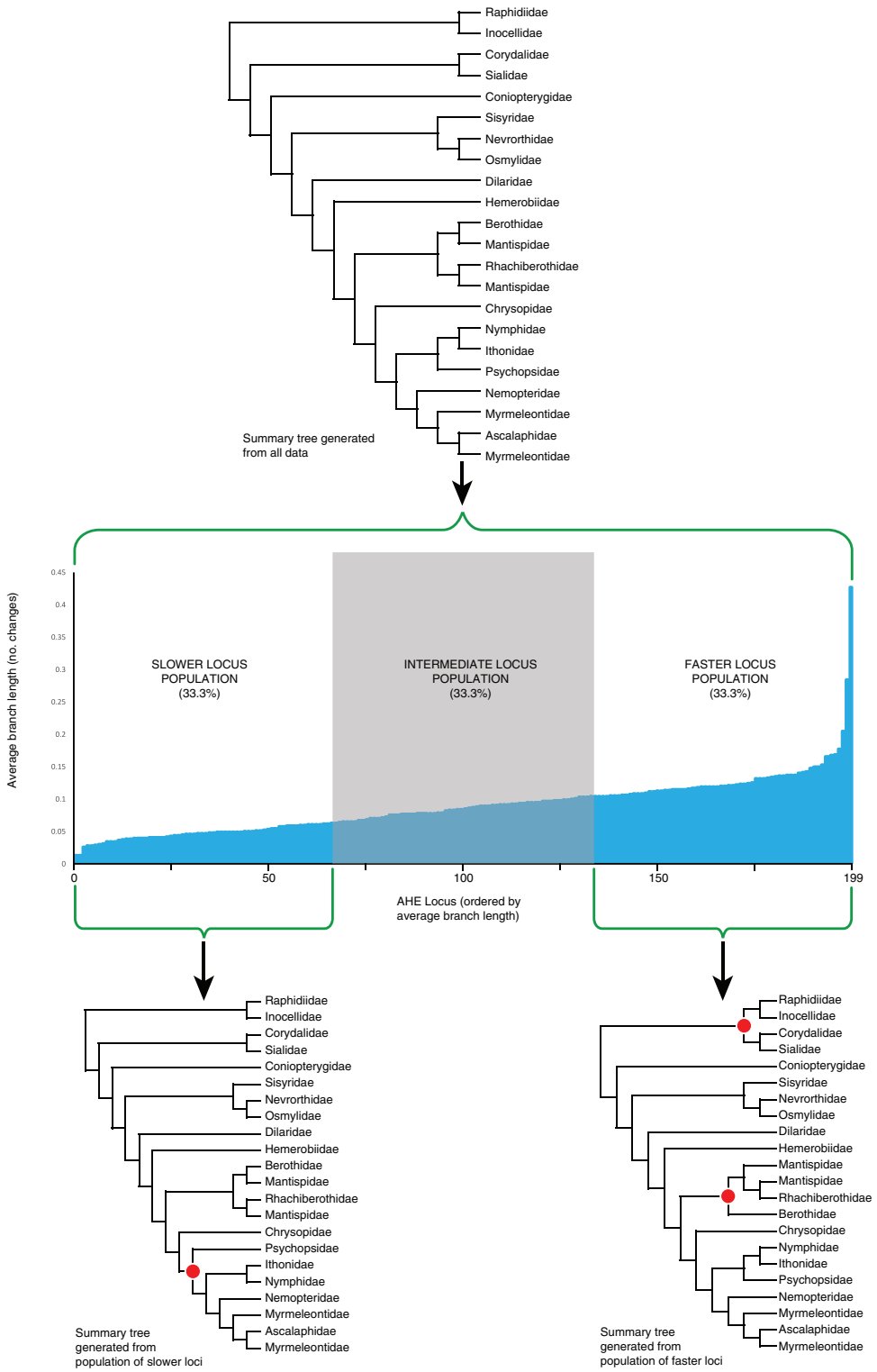

Fig. 2. Variation in tree topology attributable to mutation rate heterogeneity of anchored hybrid enrichment (AHE) loci. Upper tree generated from all loci. Lower trees subsequently generated from the slowest third (left) and fastest third (right) of loci, respectively, ranked by average branch length on the original tree (as a proxy for mutation rate). Nodes where the reduced-partition trees differ from the all-data tree are marked with red circles. Note the substantial congruence of all the trees derived from subsets of loci to the tree generated from all loci.

differing loci, each of relatively short sequence length, and that the signal was homogenized within that population. If we could separate out individual loci across the entire population, with longer extensive sequence length for each, we predict that we would see greater temporal differences in PI maxima for different individual loci.

Ancestral character state reconstructions

Analysis of the gula-like sclerites that occur in the larval head capsules of some neuropterans suggests that these sclerites have two independent origins, quite separate from the plesiomorphic type exhibited by Megaloptera, Raphidioptera and Coleoptera (Fig. 5; Table S5). In these latter orders, the distinction between the gular sclerite posteroventrally and the postmentum (i.e. the proximal portion of the labium lying anterior to the ventral tentorial pit) is not well-defined. In Neuroptera a well-delineated gular-like sclerite is present in the elongate head of larval Nevrorthidae and a smaller, less-delineated sclerite is present in a derived clade comprising the families Ithonidae, Psychopsidae, Nymphidae, Nemopteridae, Ascalaphidae and Myrmeleontidae. The reconstruction indicates that although the ventral sclerite in megalopteran and raphidiopteran larvae is homologous to the gula found in Coleoptera, the gular-like sclerite found in the aforementioned families of Neuroptera is not homologous and has evolved twice; in these two instances the resulting sclerites are quite disparate in morphology and in position in the tree (i.e. the nevrorthid versus myrmeleontoid gula-like sclerites). The reconstruction for the node Neuroptera + Megaloptera has an equivocal posterior probability (*c*. 0.5) for the presence or absence of a gula. Sclerotization of the gular region is not found in Osmylidae, the sister family to Nevrorthidae and the reconstruction of the common ancestor of these two families shows only a 0.328 likelihood of exhibiting that state. Sclerotization of the gular region is then absent throughout most of Neuroptera until it re-emerges in the derived clade comprising Ithonidae, Psychopsidae, Nymphidae, Nemopteridae, Ascalaphidae and Myrmeleontidae. The sister to this clade is Chrysopidae, in which any gular-like sclerotization is absent and the common ancestor is expected to a have a 0.694 posterior probability of exhibiting that feature.

The ACSR of larval habitat in the ancestral neuropteridan was undertaken using two alternative scenarios of outgroup character state conditions of either aquatic (Fig. 6A) or terrestrial (Fig. 6B) (see Table S5 for values). This was done to account for uncertainty in the correct outgroup scoring for this condition in Coleopterida. Strepsiptera are terrestrial, and it is unclear what the plesiomorphic condition is in Coleoptera due to uncertainty of relationships among major clades (exhibiting different conditions) at the base of the coleopteran tree (McKenna *et al*., 2015). Here we reconstructed both Bayesian probability and parsimony for both outgroup conditions. Under both scenarios the ancestral larva of Neuropterida was most likely aquatic using Bayesian probabilities, although PPs supporting the likelihood of a terrestrial larva throughout the basal nodes were relatively greater when the outgroup was scored as terrestrial (Fig. 6B). By contrast, under parsimony (DELTRAN optimization) we reconstructed the ancestral neuropteridan larva as aquatic when the outgroup was aquatic (Fig. 6A), and terrestrial when the outgroup was scored as terrestrial (Fig. 6B).

Discussion

Ordinal relationships among Neuropterida

Similar to many recent studies examining relationships among orders of Neuropteroidea and Neuropterida, we recovered Megaloptera as sister to Neuroptera with high statistical support (Aspöck *et al*., 2001; Misof *et al*., 2014; Peters *et al*., 2014; McKenna *et al*., 2015; Song *et al*., 2016; Wang *et al*., 2017). Some other studies have recovered Megaloptera as sister to Raphidioptera (Wiegmann *et al*., 2009; McKenna and Farrell, 2010), but the consensus based on recent large-scale genomic studies is that this is incorrect. The separate analysis of faster loci here did recover this topology with high statistical support (Fig. 3; Figure S5), but the full alignment analysis (and analysis using only slower loci) did not recover this topology. The use of large amounts of DNA sequence data in this study, similar to studies using transcriptomes (Misof *et al*., 2014; Peters *et al*., 2014) and mitochondrial genomes (Song *et al*., 2016; Wang *et al*., 2017), probably overcame any analytical artefacts inherent in previous studies that relied upon morphology alone or limited DNA sequence data (e.g. Friedrich & Beutel, 2010; Winterton *et al*., 2010; Beutel *et al*., 2011) which suggested the unlikely paraphyly of Megaloptera with respect to Raphidioptera. We recovered nearly the same tree topology with strong statistical support regardless of whether we analysed nucleotide bases or amino acids under either BI or ML. Moreover, when we analysed subsets of the nucleotide data separately based on mutation rate, we again recovered nearly identical tree topologies to the tree based on all data. Morphological characters that support a sister-group relationship between Megaloptera and Neuroptera include a rosette-like arrangement of trichobothrial alveoli on the ectoprocts, appendix-like male ninth gonocoxites (Aspöck *et al*., 2001) and weakly sclerotized posterior notal wing processes (Zhao *et al*., 2014).

Raphidioptera were recovered as monophyletic and sister to the rest of Neuropterida. Raphidiidae and Inocelliidae are the only surviving families of snakeflies, although there is a moderately-rich fossil diversity of extinct stem-group families in deposits dated to the Permian (Parasialidae, Nanosialidae) and subsequently throughout the Mesozoic (e.g. *c*. 35 genera in the families Bassiopteridae, Chrysoraphidiidae, Priscaenigmatidae, Juroraphidiidae and Mesoraphidiidae) (Engel, 2002; Liu *et al*., 2014; Oswald, 2017; Wang *et al*., 2017; Engel *et al*., 2018). Our results estimate that Inocelliidae diverged from Raphidiidae during the Cretaceous (108 Ma), although definitive fossils of both families are only known from Cenozoic deposits (see Engel, 2002; Liu *et al*., 2014). Megaloptera were recovered in all analyses as monophyletic with the sister-group relationship between Sialidae and Corydalidae estimated to have diverged

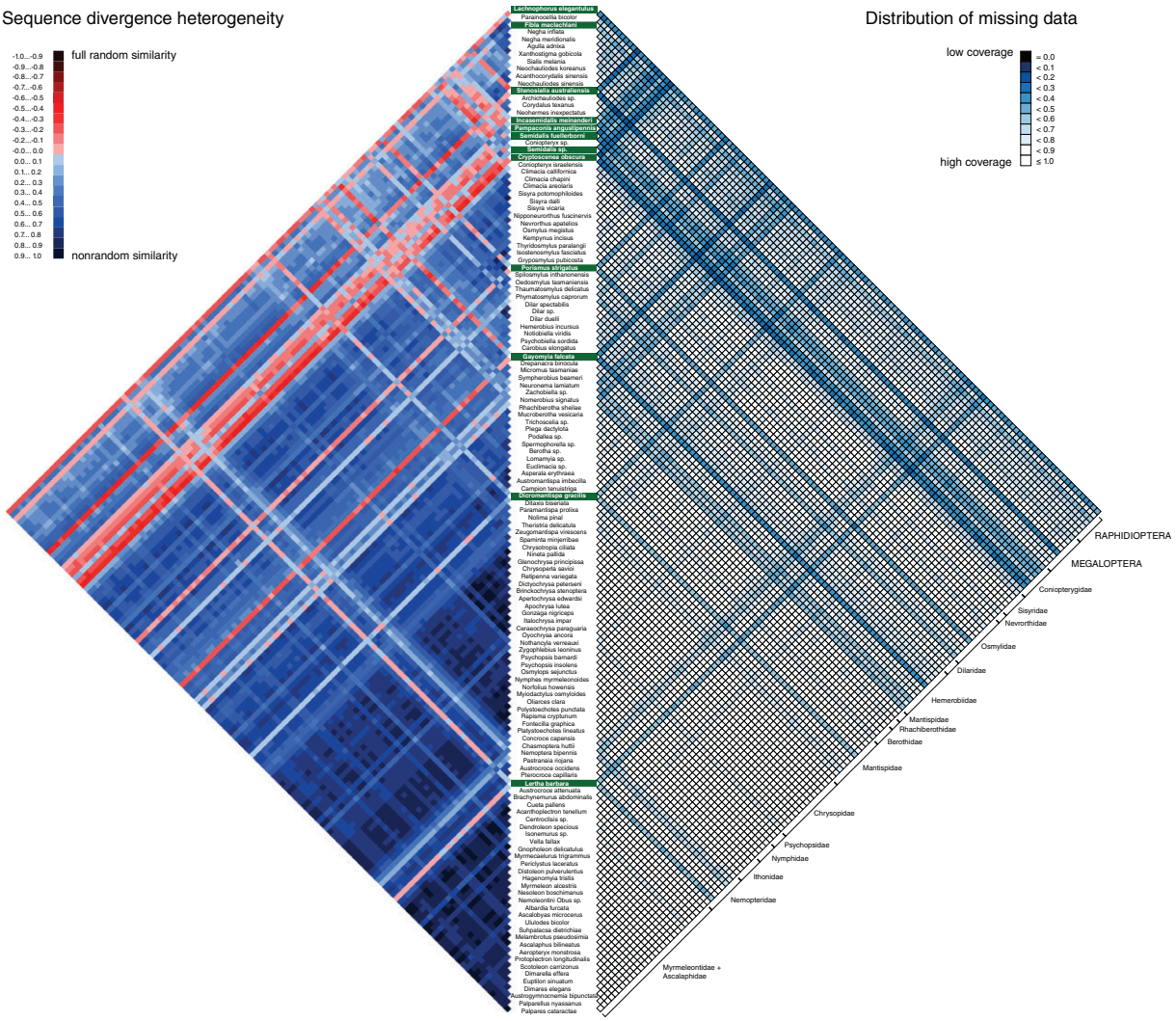

Fig. 3. AliStat (right) and AliGROOVE (left) heat maps of pairwise sequence comparisons for the nucleotide anchored hybrid enrichment (AHE) dataset. The AliStat graph shows the distribution of missing data and pairwise completeness, with dark blue meaning low shared site coverage and white meaning high shared site coverage. The AliGROOVE graph shows the mean similarity scores between sequences, with scores ranging from −1 (full random similarity) to +1 (nonrandom similarity); the darker red indicates higher randomized accordance between pairwise sequence comparisons, whereas blue indicates similarity due to descent. The patterns displayed in both heat maps together indicates that there is a strong correlation between the amount of missing data with sequence heterogeneity and uncertainty in taxon placement; taxa highlighted in green have relatively lower coverage with more random sequence similarity.

during the early Jurassic (183 Ma). The monophyly of Megaloptera has been questioned previously based on morphology (e.g. Kubrakiewicz, 1998; Stys & Bilinski, 1990) and DNA sequence data (e.g. Winterton *et al*., 2010), despite widely accepted morphological evidence to the contrary. Recently, Wang *et al*. (2017) recovered a monophyletic Megaloptera using mitogenomic data. Within Corydalidae we estimated the divergence of Corydalinae (dobsonflies) from Chauliodinae (fishflies) at 112 Ma. The divergence between the two Corydalidae subfamilies was previously estimated to be early to middle Jurassic, with stem-group fishflies known from deposits of middle Jurassic to Early Cretaceous age (Winterton *et al*., 2010; Liu *et al*., 2012).

Neuroptera family-level relationships

Neuroptera as a natural group have never been seriously questioned and we again recovered the order as monophyletic, similar to the results of other recent quantitative studies (Aspöck *et al*., 2001; Winterton *et al*., 2003, 2010; Wang *et al*., 2017). Stem-group lacewings are known from the Early Permian and our results estimate the age for the order as 278 Ma, corresponding to an estimated origin during the Cisuralian Epoch (Winterton *et al*., 2010; Wang *et al*., 2017; Engel *et al*., 2018). Other authors have estimated the origin of Neuroptera later during the Permian (Wiegmann *et al*., 2009; Misof *et al*., 2014), although based on the results presented here – and considering

the numerous derived fossil lacewing lineages already present by the Mid-Triassic – this may be an underestimate of the age of the group. Moreover, our divergence-time estimates place the diversifications of most major lineages and families occurring in rapid succession during the Permian and Triassic periods (Fig. 4); previous studies have similarly suggested a rapid diversification of lacewing families during this time (Winterton *et al*., 2010; Yang *et al*., 2012; Wang *et al*., 2017).

Phylogenetic relationship among the families of lacewings has been controversial for decades, with numerous, often widely differing relationships proposed for the constituent families (e.g. Handlirsch, 1906–1908; Withycombe, 1925; Aspöck *et al*., 2001; Winterton, 2003; Haring & Aspöck, 2004; Zimmermann *et al*., 2009, 2011; Winterton *et al*., 2010; Beutel *et al*., 2010a,b; Yang *et al*., 2012; Randolf *et al*., 2013, 2014, 2017; Wang *et al*., 2017). Recent quantitative analyses, particularly those incorporating large amounts of DNA sequence data (and those combined with morphological data), have begun to converge on specific relationships with relatively strong support (Winterton *et al*., 2010; Yang *et al*., 2012; Wang *et al*., 2017). In contrast, studies based solely on cladistic analyses of morphological data have struggled to recover a resolved, strongly supported phylogeny, often with paraphyly of previously well-established groups (Aspöck *et al*., 2001; Beutel *et al*., 2010a,b; Zimmermann *et al*., 2011; Randolf *et al*., 2013, 2014, 2017). This is likely due to difficulties in developing robust statements of homology across families due to the generalized morphology of adults and the starkly disparate morphology exhibited by the larvae, obscuring easily observable morphological evidence of common descent. Using a cladistic analysis of morphology, Aspöck *et al*. (2001) consolidated arguments for a three-suborder classification for Neuroptera, with Nevrorthiformia (comprising only Nevrorthidae), Myrmeleontiformia (comprising Psychopsidae, Nymphidae, Nemopteridae, Ascalaphidae and Myrmeleontidae) and Hemerobiiformia (comprising all remaining families). Unfortunately, this convenient classification was based on a phylogeny that was very weakly supported statistically and was not congruent with any rigorous subsequent studies using multiple sources of evidence, including importantly DNA sequences (Winterton, 2003; Winterton *et al*., 2010; Yang *et al*., 2012; Wang *et al*., 2017). Although Myrmeleontiformia is sometimes used to circumscribe a subset of derived families, the classification proposed by Aspöck *et al*. (2001) has now largely become obsolete (Winterton *et al*., 2010; Engel *et al*., 2018). A revised classification is presented here (Fig. 1), with seven major groups (superfamilies) delineated around monophyla, classified in turn as Coniopterygoidea, Osmyloidea, Dilaroidea, Hemerobioidea, Mantispoidea, Chrysopoidea and Myrmeleontoidea. Family-group names based on all of the type genera implied by this series of superfamilies have been coined by previous authors, but the family memberships for some of the superfamilies are novel here.

Coniopterygidae are sister to all other lacewings

One major area of controversy among estimates of Neuroptera phylogeny has been over the identity of the sister group to the rest of the order. Coniopterygidae (herein the sole family in Coniopterygoidea) are small, typically arboreal, generalist predators with a waxy body covering and reduced wing venation that is quite unlike other lacewings. Our results recover Coniopterygidae as the sister family to the rest of Neuroptera with high support, diverging during the Permian (267 Ma). This is consistent with all recent analyses based on significant DNA sequence data, such as mitogenomes (i.e. Wang *et al*., 2017), and those also incorporating morphology in total evidence analyses (i.e. Winterton *et al*., 2010; Yang *et al*., 2012). Using morphology and external anatomy, some authors have also placed Coniopterygidae in a position either sister to the rest of Neuroptera or near to the base of the lacewing tree (Withycombe, 1925; Sziráki, 1996, 2007; Kubrakiewicz *et al*., 1998; Zizzari *et al*., 2008).

The controversy regarding the position of Coniopterygidae deserves greater scrutiny, especially considering anomalies in the published DNA sequences of members of the family, typically exhibiting highly divergent sequences with resulting long branches on phylogenetic trees (e.g. Winterton *et al*., 2010; Wang *et al*., 2017). We therefore examined our dataset to account for heterogeneity of sequence divergences – which could potentially lead to erroneous placement of taxa within the Neuropterida tree– and detected that five out of the seven species of Coniopterygidae included here had strongly divergent nucleotide sequences (Fig. 3A). However, after the examination of the distribution of missing data for taxa throughout the matrix, we observed that missing data (Fig. 3B) were strongly correlated with heterogeneity of sequence divergences. That is, taxa that exhibit patterns of divergent nucleotide sequences in our alignment were exactly the ones with a high percentage of missing data. Thus, we can attribute the high nodal support to phylogenetic signal as opposed to random similarity among taxa or long-branch attraction effects, and therefore there is little uncertainty in the placement of Coniopterygidae as sister to the rest of the Neuroptera in this instance. Moreover, two Coniopterygidae taxa included in the analyses (*Coniopteryx* spp.) did have much lower levels of missing data and were similarly recovered in the same place in the tree.

Most fossil Coniopterygidae are known from amber, presumably due to their small size and fragility, although the oldest fossil known (*Juraconiopteryx zherichini* Meinander) is a compression fossil from the late Jurassic of Kazakhstan (145–154 Ma) (Meinander, 1975). All known fossil Coniopterygidae are clearly attributable to crown groups, and no stem-group intermediates are known (Engel, 2016). Despite the extreme modifications of Coniopterygidae relative to other lacewings, presumably associated with miniaturization, dusty lacewings share a number of plesiomorphic traits with outgroup orders, divergent from the rest of Neuroptera, which support a basal placement of the family. Such characteristics include presence of only six Malpighian tubules (more than six in all other Neuroptera), female genitalic morphology, sperm microstructure and ovariole structure (Sziráki, 1996, 2007; Kubrakiewicz *et al*., 1998; Zizzari *et al*., 2008). The extreme modifications of coniopterygids have also led some authors

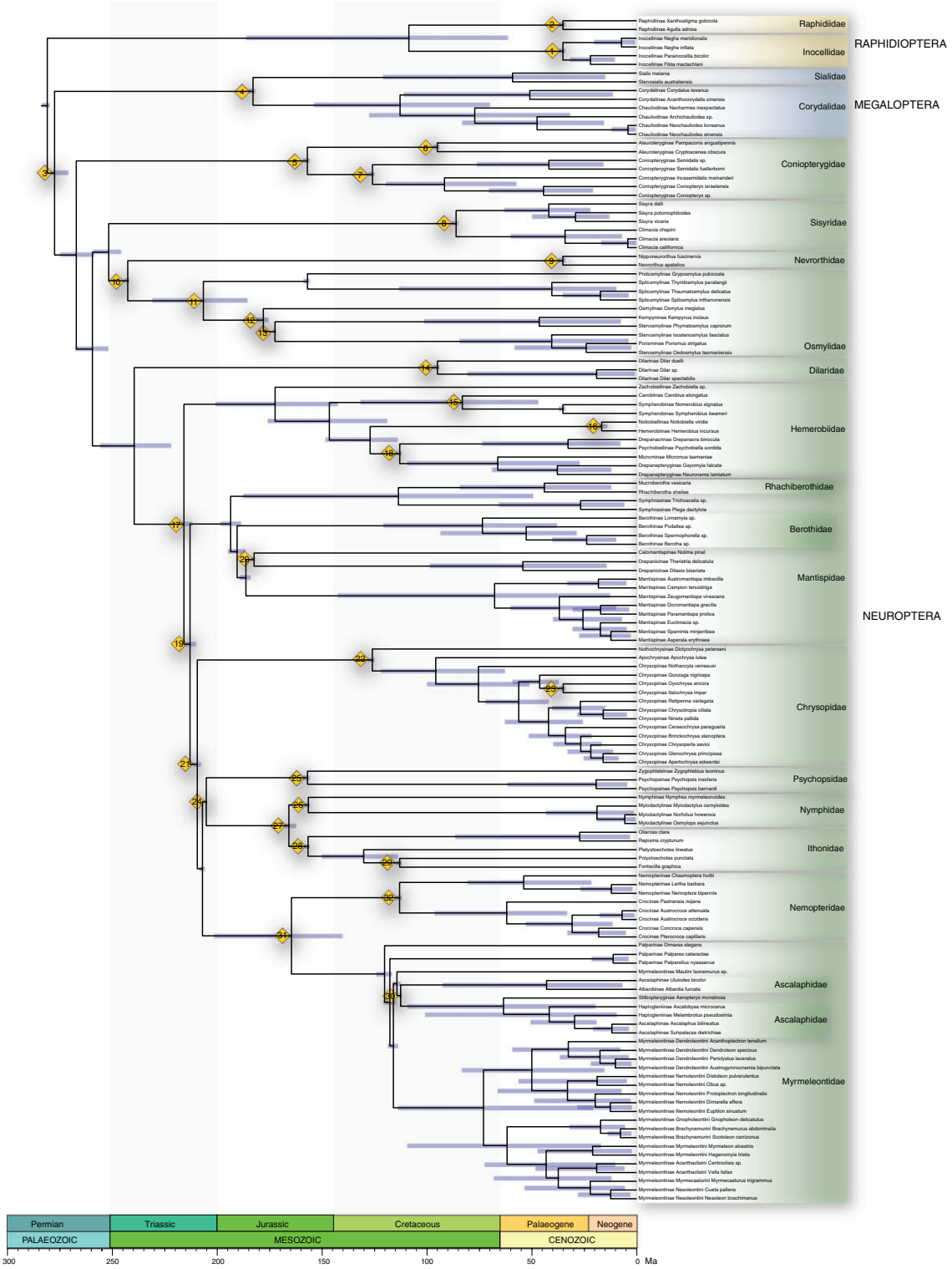

Fig. 4. Estimated divergence times among major lineages of Neuropterida. Numbered node diamonds on chronogram represent minimum age constraints for those lineages (see Table 1). Mean ages and ranges are provided in Table 1 and refer to nodes indicated in Fig. S4.

to suggest instead that they are more derived, and quantitative studies utilizing only small amounts of DNA sequence data, or morphology alone, have recovered the family in a variety of derived positions, sister to families such as Sisyridae (Aspöck *et al*., 2001; Winterton, 2003), Dilaridae (Haring & Aspöck, 2004), or the 'dilarid' clade (Zimmermann *et al*., 2009, 2011; Beutel *et al*., 2010a; Randolf *et al*., 2013, 2014, 2017). Moreover, most of these authors have thus suggested other families are sister to the rest of the order based on either qualitative or quantitative studies, including, Ithonidae s.s. (Withycombe, 1925: although he presciently placed coniopterygids as the next basal group and contrary to the more derived placement of subsequent authors), Nevrorthidae (Aspöck *et al*., 2001; Aspöck & Aspöck, 2008; Beutel *et al*., 2010a,b) and Sisyridae (Randolf *et al*., 2013, 2014; Zimmermann *et al*., 2011), among others. Considering the mounting molecular and morphological evidence for Coniopterygidae as the sister to the rest of Neuroptera, and weak evidence to the contrary, these alternative hypotheses for the placement of the family are becoming less and less plausible.

Osmyloidea

Neuroptera underwent major radiations during the Permian and especially the Triassic, with most crown family-group lineages originating during this time. Numerous fossil stem-group lineages were also very diverse during the early Mesozoic, many going extinct before the Cretaceous. Today, the extant crown-group lacewing families derived from these early lineages are represented by a depauperate fauna with largely relictual distributions. One lineage with a Late Permian origin (251 Ma) comprises the families Sisyridae, Nevrorthidae and Osmylidae (Osmyloidea sensu Engel *et al*., 2018). The positions of all three families in this clade have been highly contentious, each having been previously placed in various positions on the lacewing tree, and although sometimes placed in part as sister taxa (e.g. Sisyridae and Nevrorthidae in Wang *et al*., 2017), only rarely have they been recovered as a monophyletic group (e.g. Winterton *et al*., 2010). Here we recovered Nevrorthidae as sister to Osmylidae rather than Sisyridae, as found by Winterton *et al*. (2017) and as originally suggested by Zwick (1967) when he elevated Nevrorthidae as a separate family. One adult morphological character that may support this clade is the presence of an enlarged semi-articulated ninth gonocoxite in the female (Winterton *et al*., 2017). A larval character also supporting this clade is the location of the posterior tentorial pit ventrolateral to the subgenal ridge in the head capsule. This feature is common to larvae of this clade, whereas in all other Neuroptera the posterior tentorial pit aligns posteriorly with the subgenal ridge in the head capsule (McLeod, 1964; Beutel *et al*., 2010a). Larvae in this clade all have elongate jaws that project anteriorly. Larval Sisyridae and Nevrorthidae are entirely aquatic, and although larvae of some Osmylidae are associated with the riparian habitat along stream edges, they are not aquatic. Indeed, a fully aquatic larva appears to be the ancestral state in Osmyloidea. This aquatic lifestyle appears to have been modified to an 'amphibious' existence in most Osmylidae, and further modified to a fully terrestrial mode of life in the derived osmylid subfamilies Porismimae and Stenosmylinae, whose larvae live under bark and in leaf litter (Winterton *et al*., 2017). The stem-group of Sisyridae diverged from stem Osmylidae and Nevrorthidae during the Permian (251 Ma). Sisyrids are a small family comprising four genera whose larvae are specialist predators of freshwater sponges and bryozoans. Two genera sampled here (*Climacia* McLachlan and *Sisyra* Burmeister) are reciprocally monophyletic, diverging during the Cretaceous (86 Ma). Osmylidae contains over 225 species subdivided into seven extant and multiple extinct subfamilies (e.g. Saucrosmylinae, Mesosmylinae). Our results match the phylogeny proposed recently by Winterton *et al*. (2017) based on multi-gene DNA sequences and morphology, with a basal dichotomy during the Triassic (although later during that period), with one clade containing the subfamilies Spilosmylinae and Protosmylinae (and Gumillinae), and the other containing Osmylinae, Porismimae, Kempyninae and Stenosmylinae (inclusive of Eidoporismimae).

Nevrorthidae are a small family with a disjunct distribution in parts of Australasia, East Asia and the western Palaearctic (Aspöck *et al*., 2017). Adults have a wing venation very similar to Sisyridae, yet clearly intermediate between Sisyridae and Osmylidae. The larva is campodeiform with elongate apically curved jaws and a distinct gular-like sclerite. This sclerite also is found independently in a few other derived lacewing families (see Character state reconstructions), and is not homologous with the actual gula found in Megaloptera, Raphidioptera and Coleoptera. The presence of a gula and lack of cryptonephritic Malpighian tubules previously have been used to support the placement of Nevrorthidae as the sister to the rest of Neuroptera (Aspöck *et al*., 2001; Beutel *et al*., 2010a, etc.), although this is inconsistent with all recent quantitative analyses incorporating large amounts of data (e.g. Winterton *et al*., 2010; Yang *et al*., 2012; Wang *et al*., 2017; Winterton *et al*., 2017).

The basal position of Coniopterygidae, Osmylidae, Nevrorthidae and Sisyridae recovered here and in other studies is also supported by mitochondrial gene arrangement (Wang *et al*., 2017). The plesiomorphic condition exhibited in these families and in the rest of Neuropteroidea has $t\text{RNA}^{\text{Cys}}$ is located downstream of $t\text{RNA}^{\text{Trp}}$ (i.e. WC). In all other Neuroptera examined to date $t\text{RNA}^{\text{Cys}}$ is located upstream of $t\text{RNA}^{\text{Trp}}$ (i.e. CW) based on a single translocation occurring in the common ancestor sometime during the Late Permian (Wang *et al*., 2017). This scenario is also supported here by our AHE data, with a similar temporal estimate.

Dilaroidea

Dilaridae are an enigmatic family comprising three subfamilies with approximately 100 species, dating back from the Cretaceous and Palaeogene (Engel, 1999; Liu *et al*., 2017). They previously have been difficult to place phylogenetically; some authors have placed them with Berothidae, Mantispidae and

Rhachiberothidae as the 'dilarid' clade (Aspöck *et al*., 2001; Aspöck & Aspöck, 2008; Beutel *et al*., 2010a,b; Randolf *et al*., 2013, 2014, 2017; Zimmermann *et al*., 2011), although evidence provided for the monophyly of this group is based on frequently homoplasious morphological characters with weak statistical support. Other work, based on larger, more diverse datasets, has instead placed Dilaridae closer to the base of the lacewing tree (Haring & Aspöck, 2004; Winterton *et al*., 2010; Wang *et al*., 2017). Our results likewise place Dilaridae in an intermediate position between Osmyloidea and Hemerobiidae, diverging approximately 237 Ma. Sziráki (1996) provided morphological evidence from the female genitalia to support the placement of Dilaridae closer to Nevrorthidae and Sisyridae than to the 'dilarid' clade.

Chrysopidae and Hemerobiidae are not sister families

One surprising result of this genomic approach to lacewing phylogeny is the failure to recover Hemerobiidae as the sister to Chrysopidae, the traditional putative family pairing with a long history. Regardless of analysis type, or if we analyse subsets of the slowest or fastest evolving loci, we recovered strong support for the placement of Hemerobiidae as an intermediate clade above Dilaridae and distant from Chrysopidae (Figs 1, 2, Figures S2, S5–S6). Many previous phylogenetic studies have recovered Hemerobiidae and Chrysopidae as well-supported sister families (Beutel *et al*., 2010a,b; Winterton *et al*., 2010; Zimmermann *et al*., 2011; Randolf, 2014; Wang *et al*., 2017). Still, in both qualitative and quantitative studies, some authors have placed Hemerobiidae in various positions in the neuropteran tree, including sister to Dilaridae (Yang *et al*., 2012), Coniopterygidae (Handlirsh, 1906), Mantispidae (Winterton, 2003) and Polystoechotidae (Withycombe, 1925). Similarly, Chrysopidae, when not placed as sister to Hemerobiidae, has typically been placed as an intermediate group sister to larger clades such as Myrmeleontioidea (e.g. Yang *et al*., 2012) or a clade comprising Berothidae, Mantispidae and Hemerobiidae (Winterton *et al*., 2003). The placement of Hemerobiidae elsewhere in the tree, and not sister to Chrysopidae is surprising, yet it is by no means unreasonable. The presumed sister grouping of Hemerobiidae with Chrysopidae has historically been based on the campodeiform body shape of the larva, similar larval head shape and the presence of a trumpet-like empodium in the first instar (absent in later instars of Hemerobiidae). The current analysis suggests that this resemblance is superficial and that most of these characters are plesiomorphic (and likely ontogenetic) similarities evident in early instars of many other Neuroptera families (e.g. head shape and body form). Both larval Chrysopidae and Hemerobiidae display a typical campodeiform larval morphology, the plesiomorphic condition throughout Neuroptera. This type of elongate larvae is found also in families such as Psychopsidae, Dilaridae, Berothidae, Rhachiberothidae, Osmylidae and Nevrorthidae. Ontogenetically, this body shape is also evident in early instar larvae of Mantispidae and Ithonidae, where it is subsequently obscured in later instars specialized for a more sedentary lifestyle (McLeod, 1964; Grebennikov, 2004). Hemerobiid larvae also have a prementum with labial palpi with bases closely approximated, whereas Chrysopidae have a wider prementum and concomitantly with the bases of the labial palpi widely separated (Fig. 5). Indeed, the widely spaced bases of the labial palpi may be a uniting feature of Chrysopidae with Ithonidae and the families of Myrmeleontioidea. Importantly, the generalized form of adult Hemerobiidae shares little in common as a recognizable synapomorphy with Chrysopidae, or indeed any family of Neuroptera except maybe Sisyridae or Dilaridae, reflecting an overall amalgamation of plesiomorphic traits for hemerobiids. Also noteworthy is the presence of curved jaws (albeit shorter and blunt) in Hemerobiidae, much earlier than in Chrysopidae and other more derived families, suggesting that jaw morphology and articulation related to feeding type may be more complex than first imagined (e.g. Winterton *et al*., 2010), with potentially multiple origins of curved jaw articulation in the lateral plane.

Hemerobioidea

Internal relationships within Hemerobiidae found here overlap in part with both previous quantitative studies on the group using morphology (Oswald, 1993a) and DNA sequences combined with morphology (Garzón-Orduña *et al*., 2016). Here we recovered *Zachobiella* Banks (Zachobiellinae) as sister to the rest of Hemerobiidae, a result not found in previous studies. Carobinae and Sympherobinae are recovered as sister groups, as found in Garzón-Orduña *et al*. (2016) as well as the close relationship among Notiobiellinae, Hemerobiinae, Drepanacrinae and Psychobiellinae. The sister-group relationship between Microminae and Drepanepteryginae was recovered here with strong support, as indicated previously by Oswald (1993a) and Garzón-Orduña *et al*. (2016).

Mantispoidea

The relationships among the families Berothidae, Mantispidae and Rhachiberothidae are not fully resolved and remain ambiguous, even with the large amount of AHE data available here. Although certainly a monophyletic clade (herein Mantispoidea), the phylogeny of this group of three families (along with the extinct Dipteromantispidae) has been notoriously difficult to elucidate. Questions regarding inter- and intrafamilial relationships within this group that have been controversial previously include the monophyly of Mantispidae and the status of Rhachiberothidae (whether as a distinct family or as a subfamily of either Mantispidae or Berothidae) (Willmann, 1990; Aspöck & Mansell, 1994; Aspöck *et al*., 2001; Makarkin & Kupryjanowicz, 2010; Winterton *et al*., 2010; Liu *et al*., 2014; Wang *et al*., 2017). We recovered, with high statistical support, a Mantispidae *s.l.* that was paraphyletic without both Berothidae and Rhachiberothidae. Symphrasinae were placed as sister to Rhachiberothidae in a clade sister to the remaining Mantispidae and Berothidae (Fig. 1) in all analyses; Mantispidae, as currently circumscribed, were never recovered as monophyletic.

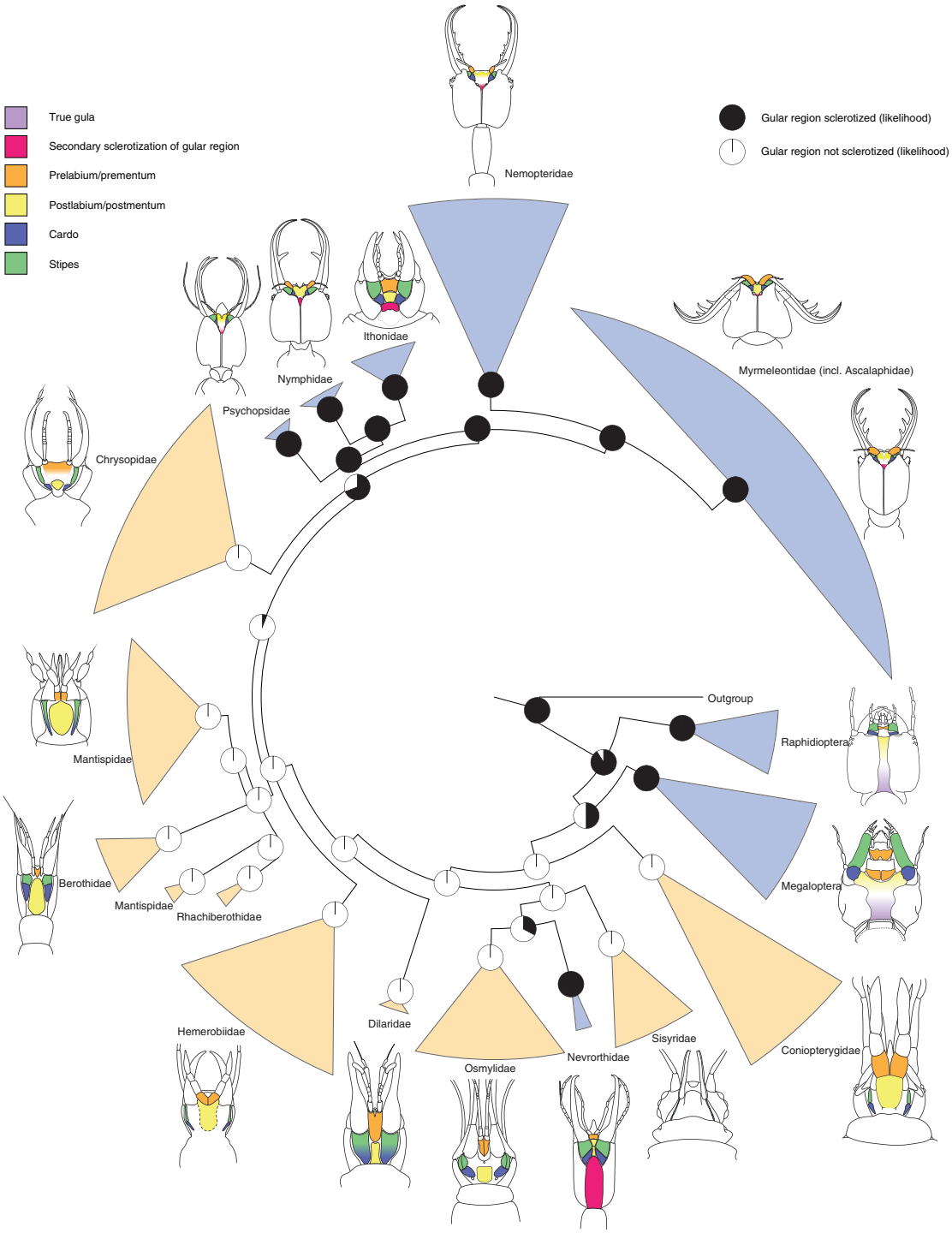

Fig. 5. Bayesian ancestral character state reconstruction of the presence of a sclerotized gular region on the ventral surface of the larval head capsule across Neuropterida families (based on tree in Fig. 1). The reconstruction suggests that the 'gula'-like sclerite found in more derived families (e.g. Nevrorthidae, Ithonidae, Nymphidae, Nemopteridae, etc.) is not homologous with the 'true' gula found in Megaloptera, Raphidioptera and Coleoptera. Pie charts on individual nodes represent the proportional likelihood for each state to be present in the common ancestor for that clade. Size of wedge represents simplified proportion of taxon sampling for that clade. Colour of the wedge and pie charts on individual branches indicates presence (blue) or absence (orange) of a gular sclerotization on the posteroventral surface of the larval head capsule.

When considering fast-evolving loci only (Fig. 2), Berothidae were placed as sister to the rest of the clade (i.e. Mantispidae was paraphyletic only without Rhachiberothidae). Based on the ambiguous results obtained here, and in other studies (Liu *et al*., 2015), it may in the future be advisable to recognize a single family – Mantispidae, with the rhachiberothids, berothids, and dipteromantispids as specialized lineages within it, although other taxonomic arrangements of multiple monophyletic families are also possible with some shifting of included genera.

Chrysopoidea

Relationships among the three subfamilies of Chrysopidae recovered here match recent studies (Winterton & Freitas, 2006; Haruyama *et al*., 2008; Jiang *et al*., 2017), although there remains dispute regarding the sister subfamily to the remaining Chrysopidae. Here we recover Nothochrysinae as the sister group to the rest of the family, followed by Apochrysinae and the enigmatic *Nothancyla*. As in Jiang *et al*. (2017), we found a basal dichotomy within Chrysopinae, with one clade comprising the tribes Leucochrysini and Belonopterygini and the other comprising Chrysopini and Ankylopterygini. We recovered stem Chrysopoidea diverging from stem Myrmeleontoidea during the late Triassic (209 Ma).

Myrmeleontoidea

Also referred to as Myrmeleontiformia, the extant families Nymphidae, Psychopsidae, Nemopteridae, Ascalaphidae and Myrmeleontidae are a well-circumscribed group based on a series of adult and larval characters (Henry, 1978). The close relationships of these five families have been described by various authors based on both morphological and molecular data (Withycombe, 1925; Henry, 1978; Aspöck *et al*., 2001; Haring & Aspöck, 2004; Winterton *et al*., 2010; Wang *et al*., 2017), and there is little doubt to their membership except that some authors place Psychopsidae as more distantly related (e.g. Yang *et al*., 2012). Here we recover Ithonidae strongly supported as the sister to Nymphidae. Moreover, Nymphidae, Ithonidae and Psychopsidae are recovered as a monophyletic group sister to a clade comprising Nemopteridae, Ascalaphidae and Myrmeleontidae. Under ML and in analyses of a subset of slower loci, a different topology was recovered, differing only in placing Psychopsidae as sister to the rest of Myrmeleontoidea (Fig. 2). Based on a series of perceived plesiomorphic characters in larval anatomy, Ithonidae were placed by Withycombe (1925) as the sister to the rest of Neuroptera, although they are now typically placed as sister to the entire Myrmeleontoidea (MacLeod, 1964; Aspöck & Aspöck, 2008; Winterton *et al*., 2010; Wang *et al*., 2017), and not embedded within it (cf. Yang *et al*., 2012). Placement of Ithonidae as sister to Nymphidae in a clade with Psychopsidae is a novel result, not previously recovered in other studies, and yet not unreasonable. Larvae of Myrmeleontoidea are typically fossorial (with some arboreal) and the larval head capsule is enlarged and heavily sclerotized to handle larger prey (Engel *et al*., 2018). Ithonid larvae are also fossorial with heavily sclerotized head capsules and have similar 'gula'-like sclerite(s). Another larval character that may support the inclusion of Ithonidae within Myrmeleontoidea is the dorsal location of the occipital foramen in the head capsule and, consequently, of the head/thorax articulation. Consequently, the head articulates with the thorax slightly below the longitudinal plane of the body. This configuration allows the partially retreat of the head under the pronotum. In all other families of Neuroptera the occipital foramen and the head–thorax articulation are placed medially on the head, with the head placed on the same coplanar axis of the body. The functional significance of this articulation is that it allows for the characteristic upward stroke performed by larval ascalaphids and myrmeleontids while catching prey and, in pit-building species to toss sand out of the trap. However, this character is not obvious in *Polystoechotes* and in the more extreme long-necked crocine Nemopteridae (e.g. *Pterocroce*), although it is recognizable in short-necked forms (e.g. *Croce*). With the placement of Ithonidae recovered here, it appears that the family represents a derived, specialized clade with subterranean larvae. Other unique aspects of the biology of this family include mass emergence of adults and instances of sexually dimorphic aptery in some taxa (i.e. *Adamsiana* Penny).

Within Myrmeleontoidea, Nemopteridae was recovered as the sister family to Myrmeleontidae and Ascalaphidae, with Myrmeleontidae rendered paraphyletic by Ascalaphidae. A synapomorphy for this clade is the close association in the forewing of veins MP and CuA basally and the presence of presectoral crossveins in the forewing (Winterton *et al*., 2010); these are absent in Ithonidae, Nymphidae and Psychopsidae. The importance of this character is not yet substantiated because many fossil species in these families lack these presectoral crossveins so it is unclear whether such a character evolved once with rampant losses, or whether it has evolved multiple times as a simple function of wing area. Another synapomorphy for the clade is the modification of the pretarsus for grasping (i.e. auxillae fused into a single plate with claws elongated) instead of walking. Various authors (e.g. Withycombe, 1925; Henry, 1978; Aspöck *et al*., 2001; Beutel *et al*., 2010a,b; Zimmermann *et al*., 2011; Randolf *et al*., 2013, 2014; Badano *et al*., 2016) have proposed Nymphidae as the sister to Ascalaphidae + Myrmeleontidae, but this topology has never been supported in any large-scale quantitative analysis incorporating molecular data (e.g. Winterton *et al*., 2010; Michel *et al*., 2017; Wang *et al*., 2017). Moreover, some suggest a closer association of Nemopteridae with Psychopsidae (sometimes as sister families) based principally on a subjective interpretation of wing venation (e.g. vena triplica), which itself could be considered plesiomorphic broadening of the costal margin (Oswald, 1993b), or simple convergence associated with wing structural aerodynamics (Engel *et al*., 2018).

The paraphyly of Myrmeleontidae by Ascalaphidae is not surprising, and has been proposed recently in analyses of DNA sequences combined with morphology (Winterton *et al*., 2010) and mitochondrial genomes (Wang *et al*., 2017). Some studies have recovered a reciprocally monophyletic Ascalaphidae and Myrmeleontidae, including Badano *et al*. (2016) using

larval morphology and Michel *et al*. (2017) and Zhang & Yang (2017) using a multi-locus approach, although support for their monophyly was statistically relatively low in each instance. We recovered the palparine genus *Dimares* Hagen as the sister to the remainder of Myrmeleontidae (inclusive of Ascalaphidae). Ascalaphidae were placed deep within Myrmeleontidae, sister to the subfamily Maulinae and paraphyletic itself relative to Stilbopteryginae (i.e. *Aeropteryx*). The taxon sampling of this analysis is particularly significant, as it includes taxa previously considered to be intermediates between the two families. Once placed in their own family (Stilbopterygidae), Stilbopteryginae (represented here by *Aeropteryx*) and Albardiinae (represented here by *Albardia*) were placed in Myrmeleontidae and Ascalaphidae, respectively, by New (1982). Both taxa exhibit a set of intermediate characteristics between the two families that have obscured the definition of each (New, 1982). With the paraphyly of Myrmeleontidae again supported here, we consider it likely that the two families will ultimately require synonymization after further study has been undertaken using more extensive taxonomic sampling from throughout all major lineages.

Re-sclerotization of the gular region in lacewings

The presence of a 'true' gula is considered to be a plesiomorphic character state in the larval head capsule of Coleoptera, Raphidioptera and Megaloptera. An apparent nonhomologous sclerite is developed in the gular region (i.e. the area between the postoccipital sutures posterior to a line drawn between the posterior tentorial pits) in some families of Neuroptera, including Nevrorthidae, Ithonidae, Psychopsidae, Nymphidae, Nemopteridae, Ascalaphidae and Myrmeleontidae. A gula, albeit clearly not homologous, is also present in other insect lineages such as Phasmatodea and Embiodea. The sclerotization of the gular region of the head capsule is likely an adaptation to a prognathous orientation in predaceous campodeiform larvae, allowing the mouthparts to project anteriorly, and is frequently exhibited by larvae throughout Neuropteroidea as well as in other insect orders and has likely evolved multiple times. The presence of an enlarged gula in the larva of Nevrorthidae was argued by some authors, in part, to support the placement of the family as the sister to Myrmeleontoidea (Aspöck 1995) or all other Neuroptera (e.g. Aspöck *et al*., 2001; Beutel *et al*., 2010a,b). Although these hypotheses have now been refuted, there remains the fact that a sclerotized gular region is present in larvae of distantly related lacewing families, which suggests at least two independent origins of this type of sclerotization in Neuroptera. Based on the ancestral-state reconstruction here (Fig. 5), we found that the sclerotization of the gular region in lacewings is indeed not homologous to the actual gula found in other orders of Neuropteroidea, and that the prognathous orientation of the head capsule is subject to repeated gains of additional supportive sclerites on the posteroventral portion of the head capsule, particularly in larvae with an elongate head (e.g. Nevrorthidae). Note that the sclerotized gula region in families of Myrmeleontoidea is usually reduced to a small sub-triangular sclerite. In this clade, only Ithonidae displays a more typical sclerite arrangement found in other lacewings, albeit the gular sclerite is still reduced and sometimes comprising multiple individual sclerites.

Ancestral Neuropterida had an aquatic larva

The reconstruction of ancestral larval habitat in Neuropterida suggests that, regardless of whether we assume that the ancestral coleopteridan larva was aquatic or terrestrial, at least the ancestral neuropteridan larva was most likely aquatic (Fig. 6). Truly aquatic larvae in extant Neuropterida are relatively uncommon and are found in Megaloptera and the lacewing families Sisyridae and Nevrorthidae; although some Osmylidae are riparian and associated with lotic water courses, they are not actually aquatic (see Winterton *et al*., 2017). Conversely, the predominant lifestyle in Neuropterida larvae is indeed terrestrial, with a multitude of adaptations for water retention in arid conditions, including notably a cryptonephridium associated with the hindgut. The greater likelihood of the ancestral lacewing having an aquatic larval stage is congruent with previous authors (Aspöck, 1995; Aspöck *et al*., 2001; Wang *et al*., 2017), although similar to Wang *et al*. (2017), we also had a discrepancy between reconstructions based on the method used. Although Wang *et al*. (2017) scored the outgroup coleopteran as having terrestrial larva, we compared both scenarios here and still recovered an aquatic ancestral lacewing larva in both Bayesian reconstructions, suggesting that this was the most likely ancestral condition.

Conclusions

Many aspects of the phylogeny of Neuropterida have been controversial in the past. With the new analysis presented herein, however, we enter a new genomic era in our efforts to infer higher-level phylogenetic relationships within the clade. The scope and scale of the new analysis greatly exceeds all previous efforts, both in terms of the magnitude of the character set employed and the breath of its taxon sampling. With very few exceptions, the strong statistical support for the nodes on the new tree provide compelling evidence that we may, finally, be nearing our goal and approaching the limits of our ability to accurately infer the true higher phylogeny of the Neuropterida, particularly with respect to deep branching events along the backbone of the Neuroptera tree for extant family-ranked taxa. The overall very generalized form of neuropterid adults, combined with the highly specialized morphologies of their larvae, has left us with an evolutionary legacy of confusing morphological evidence that has, by itself, resisted efforts for decades to produce a convincing and widely agreed-upon consensus tree of interordinal and interfamilial relationships. We hope that the new tree presented here, with its much-enhanced character and taxon sets, will help to establish a consensus view that we have previously lacked.

Key relationships that are supported on the new topology presented here include: the sister-group relationship between

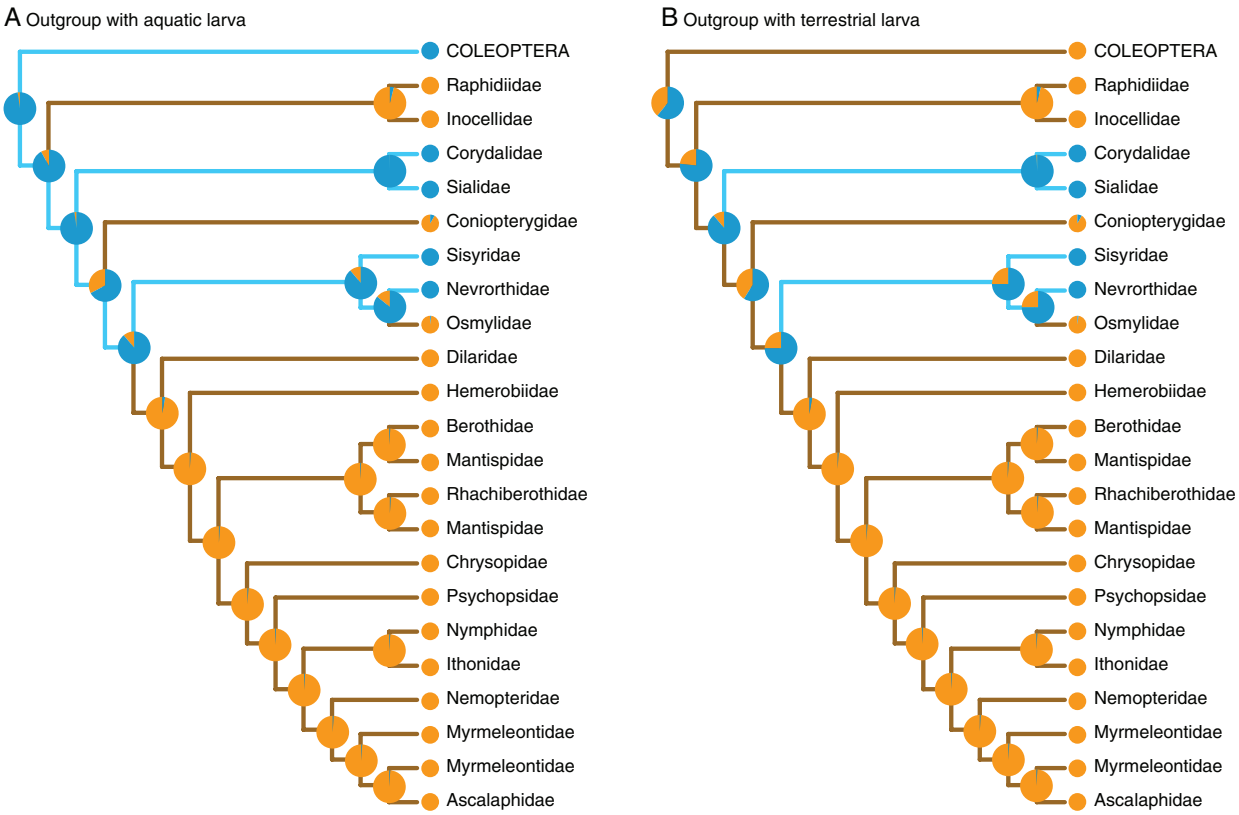

Fig. 6. Bayesian and parsimony ancestral character state reconstruction of larval habitat under different presumed ancestral outgroup (i.e. Coleopterida) conditions of either aquatic (A) or terrestrial (B). Blue indicates aquatic whereas orange indicates terrestrial. Pie charts on each node and terminal indicate likelihood of state being present in the ancestor of that clade, whereas branch colour indicates the parsimony reconstruction.

Megaloptera and Neuroptera; Coniopterygidae as sister to all other Neuroptera; Osmylidae, Nevrorthidae and Sisyridae as a monophylum; Dilaridae are not sister to the clade comprising Berothidae, Mantispidae and Rhachiberothidae; Nymphidae are not sister to Ascalaphidae and Myrmeleontidae; and Myrmeleontidae are paraphyletic relative to Ascalaphidae. Notable placements of several families that are somewhat surprising, yet not unreasonable, include: Ithonidae as sister to Nymphidae, Mantispidae as paraphyletic relative to both Berothidae and Rhachiberothidae, and Hemerobiidae as not sister to Chrysopidae. Based on these results, we replace here the sometimes-used higher classification of the Neuropterida that recognizes three suborders (i.e. Nevrorthiformia, Hemerobiiformia and Myrmeleontiformia) with a new classification that recognizes seven superfamilies, and which, in many respects, more closely reflects more traditional systematizations of the order. Major areas of future research include elucidating the monophyly and higher-level relationships among Berothidae, Mantispidae and Rhachiberothidae, and inference of subfamilial and tribal relationships within the larger families of the Neuroptera, particularly Coniopterygidae, Hemerobiidae, Chrysopidae and Myrmeleontidae (including Ascalaphidae).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12278

Figure S1. Nucleotide astral tree.

Figure S2. Amino acid tree (Bayesian).

Figure S3. Amino acid astral tree.

Figure S4. Nucleotide chronogram with nodes indicated.

Figure S5. Nucleotide Bayesian tree, fast loci only.

Figure S6. Nucleotide Bayesian tree, slow loci only.

Figure S7. Phylogenetic informativeness (PI) profiles of AHE data partitioned corresponding to fast, intermediate and slow loci populations. PI is represented as net informativeness over time (Ma) against the reference chronogram in Fig. 4. Vertical dashed lines represent the PI maxima for each partition.

Figure S8. Maximum-likelihood topology, nucleotides, all genes.

Table S1. Taxon sampling and accession numbers.

Table S2. Nucleotide alignment and tree file.

Table S3. Amino acid alignment and tree file.

Table S4. Divergence time estimated range dates for individual nodes on Fig. 4.

Table S5. Average character state probabilities per node for ancestral state character reconstructions in Figs 5, 6.

Table S6. Partition finder nucleotide model selection.

Table S7. Partition finder amino acid model selection.

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