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Evolution of green lacewings (Neuroptera: Chrysopidae): a molecular supermatrix approach

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Abstract. We present a time-calibrated phylogeny of the charismatic green lacewings (Neuroptera: Chrysopidae). Previous phylogenetic studies on the family using DNA sequences have suffered from sparse taxon sampling and/or limited amounts of data. Here we combine all available previously published DNA sequence data and add to it new DNA sequences generated for this study. We analysed these data in a supermatrix using Bayesian and maximum likelihood methods and provide a phylogenetic hypothesis for the family that recovers strong support for the monophyly of all subfamilies and resolves relationships among a large proportion of chrysopine genera. Chrysopinae tribes Leucochrysini and Belonopterygini were recovered as monophyletic sister clades, while the species-rich tribe Chrysopini was rendered paraphyletic by Ankylopterygini. Relationships among the subfamilies were resolved, although with relatively low statistical support, and the topology varied based on the method of analysis. Greatest support was found for Apochrysinae as sister to Nothochrysinae and Chrysopinae, which is in contrast to traditional concepts that place Nothochrysinae as sister to the rest of the family. Divergence estimates suggest that the stem groups to the various subfamilies diverged during the Triassic-Jurassic, and that stem groups of the chrysopine tribes diverged during the Cretaceous.

Introduction

Green lacewings (Chrysopidae) are perhaps one of the most recognizable groups of the insect order Neuroptera. Species of the family are usually of moderate size and typically distinguished from other lacewing families by their green coloration and large membranous wings with a characteristically modified venation (Brooks & Barnard, 1990) (Fig. 1). As larvae, chrysopids are arboreal predators and their efficacy as integrated biological control agents in agricultural ecosystems has been long recognized (Duelli, 2001). Spectacularly, the campodeiform chrysopid larvae of some chrysopid species camouflage themselves by constructing a packet of exogenous debris, entangling by specialized tubercles and elongate setae, a behaviour known as trash-carrying or debris-carrying (Eisner *et al.*, 1978; Tauber *et al.*, 2014). The debris packet serves as effective camouflage, permitting some larvae to more stealthily approach their prey and also physically protecting the larvae from predators, parasites, and conspecifics (Principi, 1944; Eisner *et al.*, 1978). Diverse and spectacular chrysopoid larvae have been described from Cretaceous amber deposits, suggesting that camouflaging behaviour and morphology were

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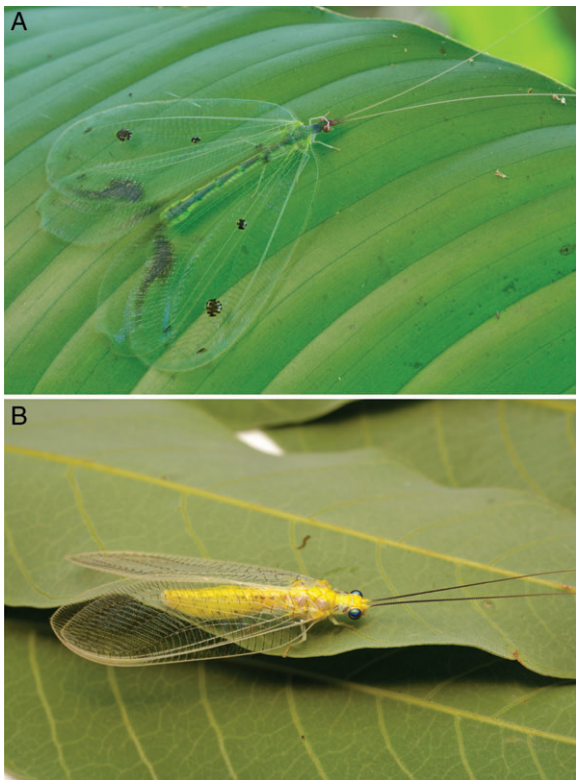

Fig. 1. Adult green lacewings. (A) *Domenechus mirificus* Navás (Apochrysinae) (Costa Rica) (copyright Paul Bertner); (B) *Italochrysa orientalis* Yang, Yang & Wang (Chrysopinae: Belonopterygini) (China) (© Shaun L. Winterton). [Colour figure can be viewed at wileyonlinelibrary.com].

important biological traits in ancestors of the clade (Pérez-de la Fuente *et al.*, 2012; Liu *et al.*, 2016; Wang *et al.*, 2016). Yet, some chrysopid larvae lack camouflaging behaviour and are considered as 'naked' or 'non-debris-carrying'. As adults, most green lacewings do not feed on insects; inspections of gut contents suggest that most chrysopids probably feed on nectar and pollen; so far only species in the genera *Chrysopa* Leach and *Plesiochrysa* Adams are believed to be carnivorous (Brooks & Barnard, 1990; Tauber *et al.*, 2001).

Chrysopidae are the second most species-rich family in the order, with 1416 species grouped in 82 genera (Oswald, 2017), although new species and genera are regularly being described (e.g. Henry *et al.*, 2012, 2015; Sosa & De Freitas, 2012; Tauber, 2012; Tauber & Garland, 2014; Winterton & Brooks, 2015; Winterton & Garzón-Orduña, 2015; Tsukaguchi & Tago, 2018). The family is traditionally divided into three extant subfamilies – Apochrysinae, Nothochrysinae, Chrysopinae – and the extinct subfamily Limaiinae. Chrysopidae sometimes includes as subfamilies what other authors treat as separate, but closely related, families – Mesochrysopinae, Allopterinae, Liassochrysinae (Nel & Henrotay, 1994; Grimaldi & Engel, 2005; Engel *et al.*, 2018). Each subfamily is distinguished by a combination of wing venation, head and genitalic characters (Brooks & Barnard, 1990). Although most genera are widely distributed geographically, some have restricted distributions within a particular biogeographic region. There also appears to be a strong division of genera between the New World and Old World, with relatively few genera found in both, and very few with truly cosmopolitan distributions.

Apochrysinae are the smallest of the three extant subfamilies with *c*. 26 species in five genera, and include typically large, delicate lacewings of pantropical distribution (Fig. 1A) (Kimmins, 1952; Brooks & Barnard, 1990; Winterton & Brooks, 2002). All species are apparently found only within closed forested habitats and no fossil representatives of this subfamily are known (Winterton & Brooks, 2002). Nothochrysinae is another small subfamily, with just over 20 extant species in nine genera of temperate occurrence (Adams, 1967; Adams & Penny, 1992; Duelli *et al.*, 2010; Tauber & Faulkner, 2015). Although represented by relatively few living species, there are numerous fossil representatives of the subfamily known from Late Cretaceous and Cenozoic deposits (Adams, 1967; Nel *et al.*, 2005; Archibald *et al.*, 2014); it remains to be determined, though, whether inclusion of these fossil species is justified cladistically as the fossils could represent a grade, with their inclusion based solely on plesiomorphies and thus possibly rendering Nothochrysinae paraphyletic. The largest subfamily is Chrysopinae, which includes 97% of all chrysopid species, distributed worldwide with over 1350 species in *c*. 70 genera. This subfamily is subdivided into four tribes, Leucochrysini, Belonopterygini (Fig. 1B), Ankylopterygini and Chrysopini (Brooks & Barnard, 1990). Chrysopini contains the greatest diversity with 32 of the 57 genera in the subfamily; next in size is the tribe Belonopterygini with *c*. 15 genera, followed by the tribes Leucochrysini and Ankylopterygini with seven and six genera, respectively. The enigmatic monotypic genus *Nothancyla* Navás has remained difficult to place to subfamily, given that it exhibits morphological characteristics of both Apochrysinae and Chrysopinae (New, 1980; Brooks & Barnard, 1990; Winterton, 1995). Winterton & Brooks (2002) placed it uneasily within Apochrysinae using morphology, and later Winterton & Freitas (2006) found a weakly supported sister relationship between Nothochrysinae and *Nothancyla* based on DNA sequence data. Recently, Dai *et al.* (2017) and Jiang *et al.* (2017) placed *Nothancyla* as sister to the rest of Chrysopinae based on mitogenomic sequence data.

Chrysopids are also rather diverse in the fossil record. A large number of Mesozoic and Cenozoic fossils have been described, with the majority of those from the Mesozoic being placed in a variety of extinct 'chrysopoid' families or subfamilies, depending on the rank classification advocated by various authors (e.g. Nel *et al.*, 2005; Grimaldi & Engel, 2005; Liu *et al.*, 2016). Some of these groups have subsequently been found to have relationships closer to other lacewing families, but it is clear that Limaiinae and Mesochrysopinae (inclusive of Allopterinae and Tachynymphinae) are part of the chrysopid clade (Adams, 1967; Makarkin, 1997; Makarkin & Archibald, 2013; Archibald *et al.*, 2014), although it remains semantic whether they are considered as independent families or as subfamilies within a broader Chrysopidae sensu lato. Neither Mesochrysopinae (or Mesochrysopidae to some authors) nor Limaiinae have been demonstrated to be monophyletic, and one or both could

represent grades leading to crown-group Chrysopidae sensu stricto (i.e. Nothochrysinae + Apochrysinae + Chrysopinae); thus their validity as stand-alone taxonomic groups rather than merely stem-group chrysopids requires further testing. Of particular interest are a variety of Chrysopoidea immatures from the Mesozoic, some of which are preserved with their debris packets intact, thus demonstrating the early evolution of such camouflaging behaviours within the clade (e.g. Pérez-de la Fuente *et al.*, 2012; Wang *et al.*, 2016; Liu *et al.*, 2018).

Historically, chrysopid taxonomy at the generic level has been challenging; numerous genera were originally based on superficial characters such as coloration (Brooks, 1997), and many monotypic genera were created based on distinctive autapomorphic characters while the bulk of nondistinctive species were dumped into the large, nominal 'wastebasket' genus (i.e. *Chrysopa* Leach). Building on the foundation established by earlier researchers, such as B. Tjeder, P. A. Adams, M. M. Principi, and others, Brooks & Barnard (1990) provided a comprehensive taxonomic framework for chrysopid genera that is both worldwide in coverage and includes all genera known at that time. It sought to identify sets of distinguishing characters from the external and genitalic morphology of each genus, and to use this information to establish a comprehensive classification of tribes and subfamilies. Although this monograph and the work that preceded it were pivotal in facilitating future work on chrysopid taxonomy, they were not undertaken under a phylogenetic framework. Thus, the question of whether the proposed groups are natural, and thereby the degree to which the classification is truly predictive, lingers. Indeed, Brooks (1997) himself brought this matter to the forefront and identified several areas where further work was needed.

Over the last 20 years, the phylogenetic relationships of Chrysopidae have received considerable attention, with studies published based on morphology (e.g. Winterton & Brooks, 2002; Nel *et al.*, 2005) and DNA sequence data (e.g. Winterton & Freitas, 2006; Haruyama *et al.*, 2008; Duelli *et al.*, 2014; Dai *et al.*, 2017). As necessary as these studies were, they too were limited; for example, morphological analyses focused either on fossil forms alone (Nel *et al.*, 2005) or on particular lineages of chrysopids (Winterton & Brooks, 2002), and none comprehensively investigated generic-level relationships across the entire family. Conversely, DNA-based studies, although typically slightly more comprehensive in taxon sampling in general, suffered from limited sequence data. As a consequence, relationships amongst subfamilies and genera were not resolved with any level of statistical confidence in any of these studies. Recent mitogenomic approaches (Dai *et al.*, 2017; Jiang *et al.*, 2017) have recovered chrysopid subfamilial and tribal relationships with a high degree of support but based on very small taxon samples.

Here we present a comprehensively sampled and well-supported phylogeny for Chrysopidae based on the concatenation of seven genes (two mitochondrial, four nuclear and the ribosomal gene 18S) and mitochondrial genomes of 12 species (representing each of the major lineages), many incorporated from the aforementioned previous studies. We use this phylogeny to explore the patterns of change in characters traditionally important for chrysopid taxonomy, such as adult wing and genitalic morphology. Based on this topology, we also estimate the timing of cladogenesis of major green lacewing lineages on a geological timescale.

Materials and methods

Exemplar sampling

By incorporating DNA sequences from previous studies and new sequences as part of this study, we sampled 84 species of Chrysopidae in 51 genera, representing all tribes, subfamilies and previously recognized groups of genera (Brooks, 1997), in the most comprehensive analysis of the family to date (Table S5). Where possible, we included multiple exemplar species per genus to ensure adequate sampling of respective lineages, as done in previous studies in related lacewing families (Garzón-Orduña *et al.*, 2016; Liu *et al.*, 2016; Badano *et al.*, 2017; Winterton *et al.*, 2017, 2018). The highest proportion of sampled genera came from Nothochrysinae (66% of the genera in the family), followed by Chrysopinae (62% including *Nothancyla*), and only a single genus (with multiple species) sampled for Apochrysinae (17%). Although green lacewings may be relatively common insects in many habitats and are one of the most species-rich families of Neuroptera, many genera are exceedingly rare and several of the monotypic genera are not known beyond their original discovery and description. Here we had the opportunity to include some uncommon genera not previously sequenced, including, among others, *Tumeochrysa* Needham, *Cacarulla* Navás, *Vieira* Navás, *Retipenna* Brooks, *Kostka* Navás, *Asthenochrysa* Adams & Penny, *Stigmachrysa* Navás and *Evanochrysa* Brooks & Barnard. We also included the enigmatic Australian genus *Nothancyla*, which has been previously placed in both Apochrysinae and Chrysopinae. Morphological on wing venation follows Breitkreuz *et al.* (2017).

DNA extraction, amplification and sequencing

Adult chrysopids were placed into 95–100% ethanol and stored at −80°C. Extraction of genomic DNA from thoracic muscle was carried out using the DNeasy® kit (Qiagen, Germantown, MD, U.S.A.) as per the manufacturer's instructions. Four partial gene loci were amplified and sequenced; they were chosen specifically to represent a range of mutational rates, thereby giving the best possibility for phylogenetically informative data across the taxa sampled. Two mitochondrial genes were sequenced [16S rDNA and cytochrome oxidase I (COI)] along with a single nuclear gene, CPSase region of carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (CAD) and 18S. Primer sequences used to amplify and sequence the three gene regions are presented in Table S1. DNA amplifications using PCR were performed using the following cycling parameters. A *c.* 550 bp fragment of 16S rDNA (3′ end) was generated using a single primer pair from Simon *et al.* (1994) with the following

PCR protocol: initial denaturation 95°C (3 min); five cycles of 92°C (15 s), 48°C (45 s), 62°C (2 min 30 s); 29 cycles of 92°C (15 s), 52°C (45 s), 62°C (2 min 30 s); and a final extension at 62°C for 7 min The 3′ end of COI DNA (*c*. 500 bp) was amplified using primers modified after Simon *et al.* (1994): initial denaturation 94°C (2 min); 35 cycles of 94°C (40 s), 55°C (50 s), 72°C (1 min); and a final extension at 72°C for 10 min. Fragment 1 of CAD [16] was generated using a touchdown PCR with the following conditions: initial denaturation at 94°C (4 min); five cycles of 94°C (30 s), 54°C (30 s) and 72°C (90 s); 37 cycles of 94°C (30 s), 51°C (30 s) and 72°C (90 s); and 72°C (3 min) for final extension. Successful PCR products were purified using ExoSap (ThermoFisher Scientific, Waltham, MA, U.S.A.). Sequences were obtained using BIG DYE TERMINATOR v3.0 (Applied Biosystems, Foster City, CA, U.S.A.). Sequences were gel-fractionated and bases called on an ABI 3730TM DNA sequencer (PE Applied Biosystems). Sequencing electropherograms were edited and contigs assembled and proofed using SEQUENCHER 5.3 (GeneCodes Corp., Ann Arbor, MI, U.S.A.) and GENEIOUS 7.1.7 (Biomatters, Auckland, New Zealand).

Supermatrix assembly

Table S3 provides the species included in our study, voucher names, locality data, identification method (including appropriate citations) and the source of the sequencing data (including GenBank accession numbers). Twelve mitochondrial genomes originally published by Haruyama *et al.* (2011), Wang *et al.* (2017), Dai *et al.* (2017) and Jiang *et al.* (2017) were sourced from GenBank and included in the analyses to provide additional support for higher-level relationships. DNA sequences for the nuclear makers phosphoenolpyruvate carboxykinase (PepCK), wingless (WG) and sodium/potassium ATPase alpha subunit (ATPase) were taken from Haruyama *et al.* (2008), and some sequences for 16S, COI and CAD were sourced from Winterton & Freitas (2006). We agree that robust phylogenetic hypotheses are the result of character congruence among all and different sources of evidence analysed simultaneously; however, we acknowledge the hindering effect that missing data may have on branch support (Brower & Garzón-Orduña, 2018) and topological resolution; thus we analysed our matrix with and without mitogenomes.

Our ingroup includes 84 species, representing 51 chrysopid genera (63% of the family generic diversity as currently defined). As outgroups, we included species in the closely related families Ithonidae and Hemerobiidae, the latter considered the putative extant sister group of Chrysopidae based on recent higher-level phylogenetic analyses (Winterton *et al.*, 2010; Wang *et al.*, 2017; although see Winterton *et al.*, 2017). Our taxonomic sampling aimed at covering as much generic diversity as possible, while at the same time reducing to a minimum the amount of missing data in the alignment. Towards this goal, we constructed chimeras for some terminal taxa (seven in total in the ingroup). We use the term chimera here to describe terminals that contained sequencing data from two closely related species that belong to the same genus. Although not ideal, chimeras allowed us to consolidate our dataset with that from Haruyama *et al.* (2008). The total number of chimeras were proportionally few in number, representing < 10% of total terminals. Terminals represented as chimeras are identified accordingly in each figure and table by the epithet ‘spp.’.

Sequence alignment and phylogenetic analysis

Alignment of all sequences was done manually, with coding genes aligned with reference to translated amino acid sequences using MESQUITE 3.02 (Maddison & Maddison, 2015). All alignments were relatively straightforward, with few ambiguous regions present in the ribosomal sequence data and no introns in the protein-coding genes (PCGs).

We conducted phylogenetic analyses under maximum likelihood (ML) using RAXML-HPC v.8 (Stamatakis, 2014) and Bayesian inference (BI) in MRBAYES 3.2 (Ronquist *et al.*, 2012), both on CIPRES (Miller *et al.*, 2010; https://www.phylo.org/). In MRBAYES, the *nst* command was set to mixed and *rate* to gamma, which specifies model averaging over the family of GTR models. Each analysis consisted of four Markov chain Monte Carlo analyses run simultaneously for 100 million generations. Trees were sampled every 1000th generation and the burn-in fraction was set to 0.25 (25%). Convergence was assessed using the standard deviation of split frequencies diagnostic given by MRBAYES; this was set to stop the chain once a value of 0.01 was reached. A majority-rule consensus tree was calculated with posterior probabilities (PPs) for each node. In RAXML, we conducted 11 ML searches from 11 parsimony starting trees under the GTRGAMMA model for all genes and partitions, followed by a final optimization of the best ML topology found. Branch support was assessed with 1000 pseudoreplicates of Bootstrap resampling and by calculating the nonparametric Shimodaira–Hasegawa-like (SHL) implementation of the approximate likelihood-ratio test (aLRT; Anisimova & Gascuel, 2006). We used SHL in conjunction with bootstrapping here because of its advantages in dealing with considerable amounts of missing data and its accuracy in estimating support for short branches, both relevant factors in our matrix (Pyron *et al.,* 2013). We considered SHL values of 85% or greater as strong support (for more information on how SHL is calculated, see Pyron *et al.*, 2013). Trees were visualized and edited with FIGTREE v1.3.1.

Divergence times estimation

We conducted a divergence time analysis in PHYLOBAYES 3.3 (Lartillot *et al.*, 2009) using the CAT-GTR model. Divergence times were estimated on the MRBAYES topology, which was calibrated using six fossils as minimum age constraints and a 250 Ma secondary calibration placed at the root (see Wang *et al.*, 2017). The fossil calibrations were as follows: (i) the most recent common ancestor (MRCA) of Hemerobiidae and Chrysopidae was calibrated to be minimally 155 Ma based on the age of *Mesypochrysa minuta* Jepson *et al.* (2012); (ii) the MRCA of

Nothochrysinae was constrained to an age of 57 Ma based on *Adamsochrysa aspera* Makarkin & Archibald (2013); (iii) the crown age of *Hypochrysa* Hagen was calibrated to 3.6 Ma based on the fossil *Hypochrysa hercyniensis* Schlüter (1982); (iv) the crown age of *Leucochrysa* McLachlan was constrained to 21 Ma based on *Leucochrysa (nodita) prisca* Engel & Grimaldi (2007); (v) crown age of Belonopterygini was calibrated to 38 Ma based on an undescribed specimen from late Eocene (Priabonian), Baltic amber (Weitschat & Wichard, 1998); and (vi) the crown age of *Chrysopa* was constrained to 21 Ma based on *Chrysopa glaesaria* Engel & Grimaldi and *C. vetula* Engel & Grimaldi (2007). In PHYLOBAYES, two chains of an uncorrelated gamma multipliers relaxed clock model (UGAM) (which assumes no heritability of substitution rates) were run for 7200 cycles; we assumed a birth-death speciation model for the divergence times. The chronogram was obtained after discarding the first 2000 saved cycles as burn-in.

Results and discussion

The total sequence length after alignment was 6259 bp, comprising on average 470 bp of *16S*, 835 bp of *COI*, 2101 bp of *CAD*, 483 bp of *PepCK*, 510 bp of *WG*, 410 bp of *ATPase* and 1450 bp of *18S*. For samples that included mitogenomes, the total sequence length after alignment contained 18 452 base pairs. Note that branch support is provided as PP for the BI analysis and as SHL implementation and bootstraps for the ML.

Chrysopidae higher-level relationships

As mentioned previously, the classification of Chrysopidae into three subfamilies has been long established (Brooks & Barnard, 1990), and their monophyly well supported (Brooks, 1997; Haruyama *et al.*, 2008; Tauber, 2014a,b); resolving the phylogenetic relationships among the subfamilies has consistently been elusive. Similar to previous studies, both the BI (Fig. 3) and ML (Fig. 3, inset; Figs S1–S3) topologies presented here strongly support the monophyly of the subfamilies but differ in the recovered relationships among them. The BI analysis recovered Apochrysinae as the sister to the remaining Chrysopidae (PP = 1.0) (Fig. 3), whereas the ML result recovered Apochrysinae and Nothochrysinae in a clade sister to Chrysopinae (Fig. 3, inset), although support for this alternative topology is low (SHL = 60, 38% bootstrap) relative to what is considered strong support for a particular node (i.e. SHL > 85) (Pyron *et al.*, 2013). Each of these alternative topologies agrees with two of the studies previously conducted with molecular data (Fig. 2); the BI result agrees with the results obtained from morphology only (i.e. Brooks, 1997) or including at least some mitochondrial data (i.e. Winterton & Freitas, 2006; Dai *et al.*, 2017; Jiang *et al.*, 2017), and the ML result concurs with previous studies using only nuclear genes (i.e. Haruyama *et al.*, 2008; Duelli *et al.*, 2014). Given the prevalence of mitochondrial DNA in our study and because previous studies have indicated ML to be particularly susceptible to long-branch attraction (LBA), we repeated the ML analysis on a dataset excluding third positions (Li *et al.*, 2015). Likewise, to explore the effect of the disproportionate amount of mitochondrial DNA included in the mitogenomes, we repeated both the ML and BI analyses without the extra sequencing provided by the mitogenomes (but leaving COI and 16S in the analysis). After removing third positions, we found that ML supports the same subfamilial relationships as found by BI (Figure S2), supporting our suspicion that the initial results with ML were apparently a typical case of LBA at the deepest parts of the topology. The same arrangement at the earliest splits of the tree is maintained by both analyses after removing the mitogenome sequences (Figs S3, S4), with the exception that Nothochrysinae is recovered as paraphyletic (although with very low support). Thus, as found by some previous studies based on mitochondrial DNA (Cameron *et al.*, 2009; Li *et al.*, 2015), our study can also attest to the susceptibility of mitochondrial data to recovering different topologies depending on the analytical method employed. In contrast to these studies, our BI analysis using nucleotides sequences and homogenous models was able to avoid LBA.

Chrysopid taxonomists have long considered Nothochrysinae the sister lineage to the rest of Chrysopidae because members of this subfamily not only exhibit numerous supposed plesiomorphic characteristics but also are more frequently represented in the fossil record than both Apochrysinae and Chrysopinae (Tjeder, 1966; Adams 1967; Tauber, 1975; Brooks & Barnard, 1990, Brooks, 1997; Archibald *et al.*, 2014). Yet none of the published molecular studies so far support this hypothesis (Fig. 2). The presumed plesiomorphic features exhibited by Nothochrysinae are, for example, the absence of a tympanum at the base of the wing (Fig. 4), the presence of a jugal lobe, and relatively unmodified wing venation (Adams, 1967). Our results suggest slightly higher support for Apochrysinae as sister to the rest of Chrysopidae, and thus we used the topology obtained under BI (Fig. 3) for dating analyses and character optimization.

Relationships within subfamilies

Apochrysinae. Apochrysine lacewings exhibit several apomorphic characters in the adult and larvae supporting their monophyly (Kimmins, 1952; Brooks & Barnard, 1990; Winterton & Brooks, 2002; Tauber, 2014a). These tropical lacewings are generally delicate with very broad wings (Fig. 1A), and are characterized by relatively simplified male genitalic sclerites, lack of a forewing intramedial cell and highly modified, reticulated wing venation and elongate larval flagellum. Members of this subfamily are relatively rarely collected, and our study only included species from the genus *Apochrysa* Schneider, thus we could not test the monophyly of the subfamily or examine relationships amongst its other genera. The results of both ML and BI analyses recover the monophyly of *Apochrysa* with strong support (PP = 1.0, SHL = 98), and suggest that it diverged from the rest of Chrysopidae during the Middle to Late Jurassic (160 Ma) (Fig. 5). The study by Winterton & Brooks (2002), which reduced the number of genera from 13 to six [now five with the recent exclusion of *Nothancyla* by Dai *et al.* (2017)], remains

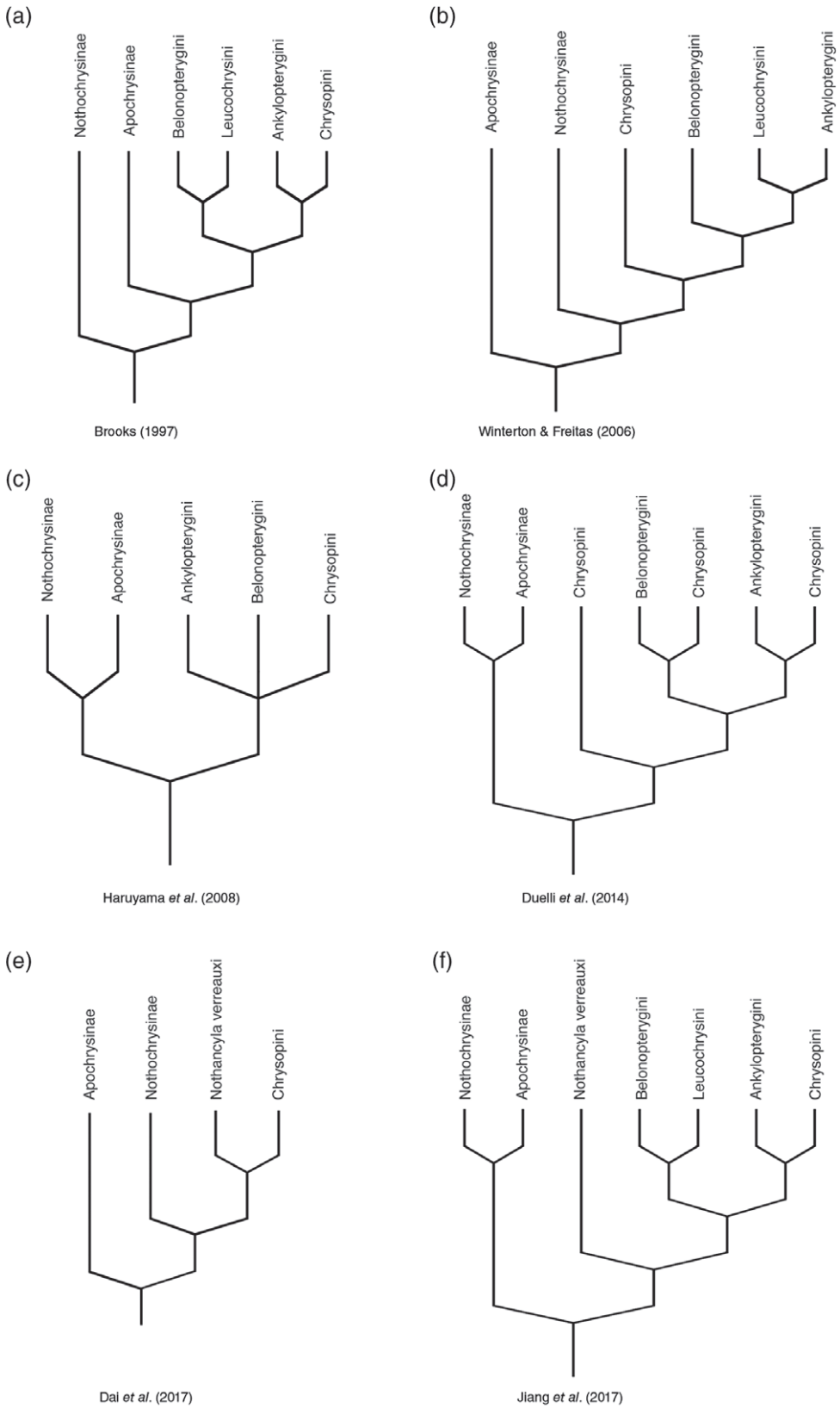

Fig. 2. Previous phylogenetic hypotheses of higher-level relationships proposed within Chrysopidae.

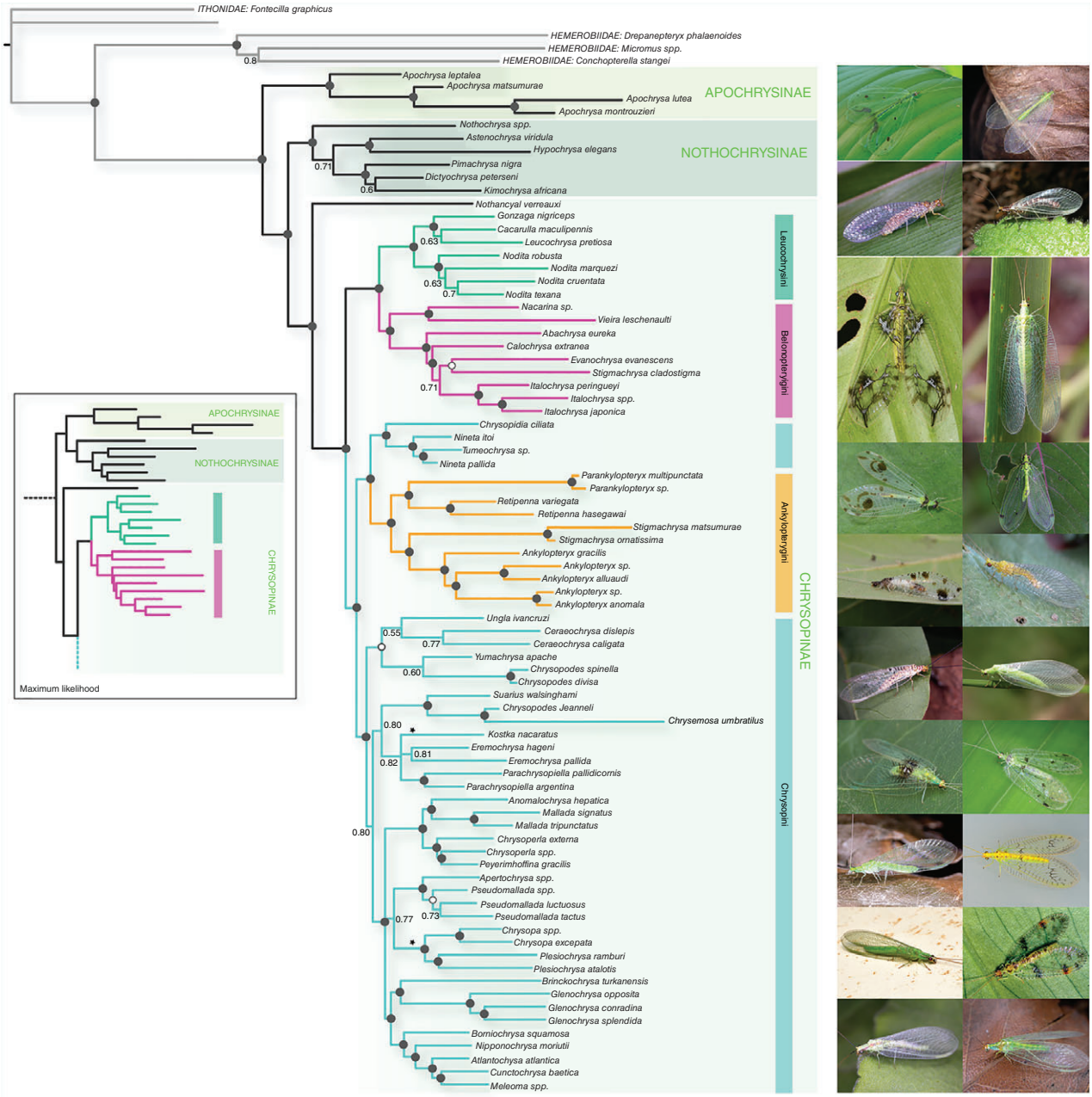

Fig. 3. Bayesian phylogeny of Chrysopidae relationships. Support values represent posterior probabilities (PP): black circles represent PP > 0.95; open circles represent nodes with PP values between 0.85 and 0.95; PP values < 0.85 are listed on individual nodes without circles. Inset: maximum likelihood topology for same dataset representing conflicting topology for subfamily relationships (full tree provided as Fig. S1). Adult chrysopid photographs (top to bottom and left to right): Apochrysinae: *Domenechus mirificus* Navás (© Paul Bertner); *Apochrysa lutea* (Walker) (© Shaun L. Winterton); Nothochrysinae: *Dictyochrysa peterseni* Kimmins (© Shaun L. Winterton); *Nothochrysa californica* Banks (© Martin Hauser); Chrysopinae: *Vieira lechenaulti* Navás (© Arthur Anker); *Nothancyla verreauxi* Navás (© Kristi Ellington); *Gonzaga nigriceps* (McLachlan) (© Arthur Anker); *Leucochrysa* sp. (© Eduardo Mena); *Chrysacanthia esbeniana* Lacroix (© Poorani Janakiraman); *Calochrysa extranea* (Esben-Petersen) (© Shaun L. Winterton); *Italochrysa exilis* Tjeder (© Shaun L. Winterton); *Nineta vittata* (Wemael) (© Giles San Martin); *Semachrysa jade* Winterton, Ping & Brooks (© Guek Hock Ping); *Ankylopteryx (Sencera) anomala* (Brauer) (© Shaun L. Winterton); *Mallada personatus* (Navás) (© Shaun L. Winterton); *Ceraeochrysa nigripedis* Penny (© Steve Marshall); *Chrysoperla savioi* (Navás) (© Shaun L. Winterton); *Glenochrysa ohlmi* Hölzel & Duelli (© Peter Duelli); *Chrysopa coloradensis* Banks (© Shaun L. Winterton); *Plesiochrysa atalotis* (Banks) (© Shaun L. Winterton). [Colour figure can be viewed at wileyonlinelibrary.com].

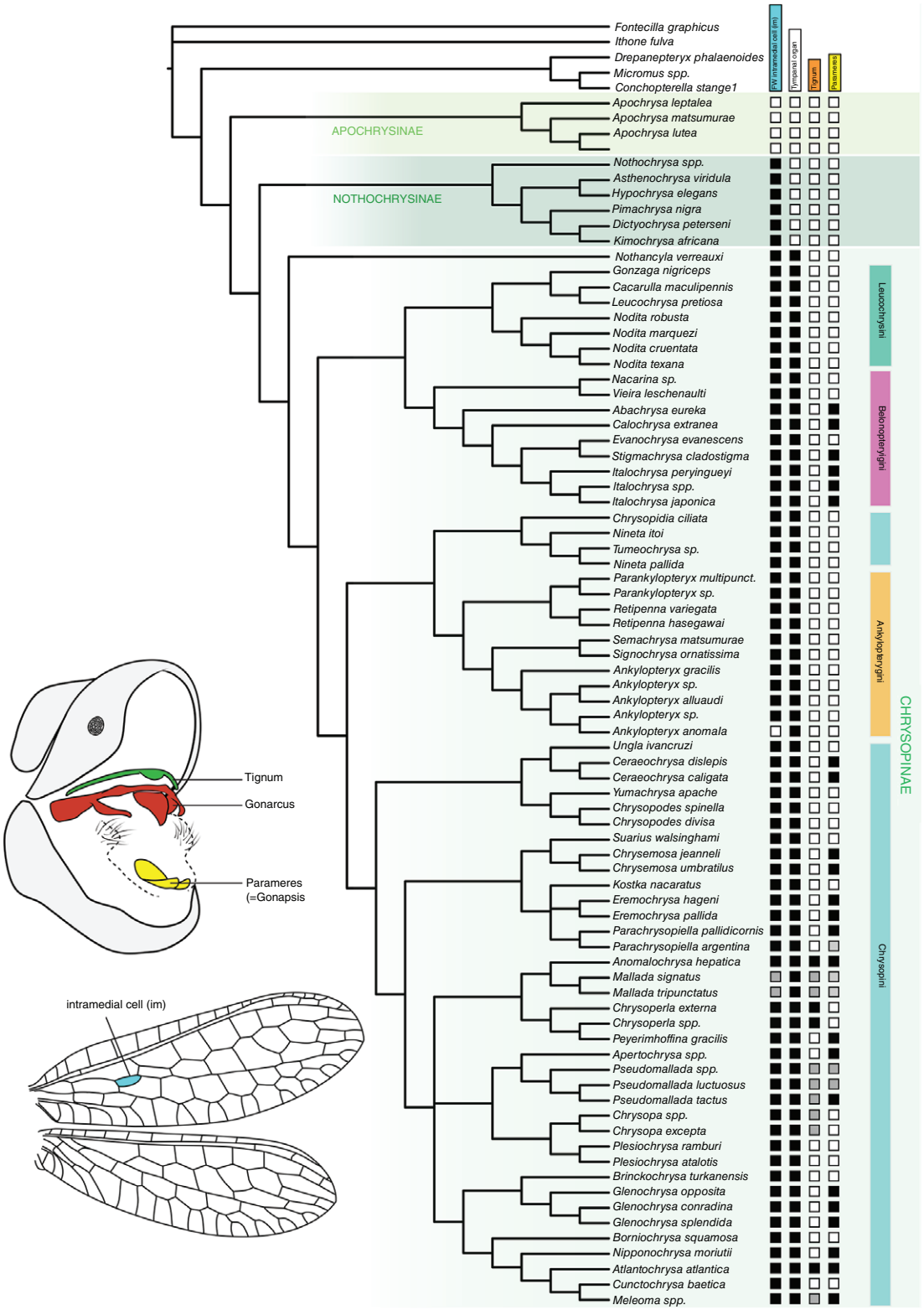

Fig. 4. Topology obtained with Bayesian inference character states of selected morphological characters mapped: black squares, state is present; white squares, state is absent; grey squares, state is polymorphic within the genus. Representative images of colour-coded states are presented as figure inset. Note that the term parameres used here is homologous with the term gonapsis. [Colour figure can be viewed at wileyonlinelibrary.com].

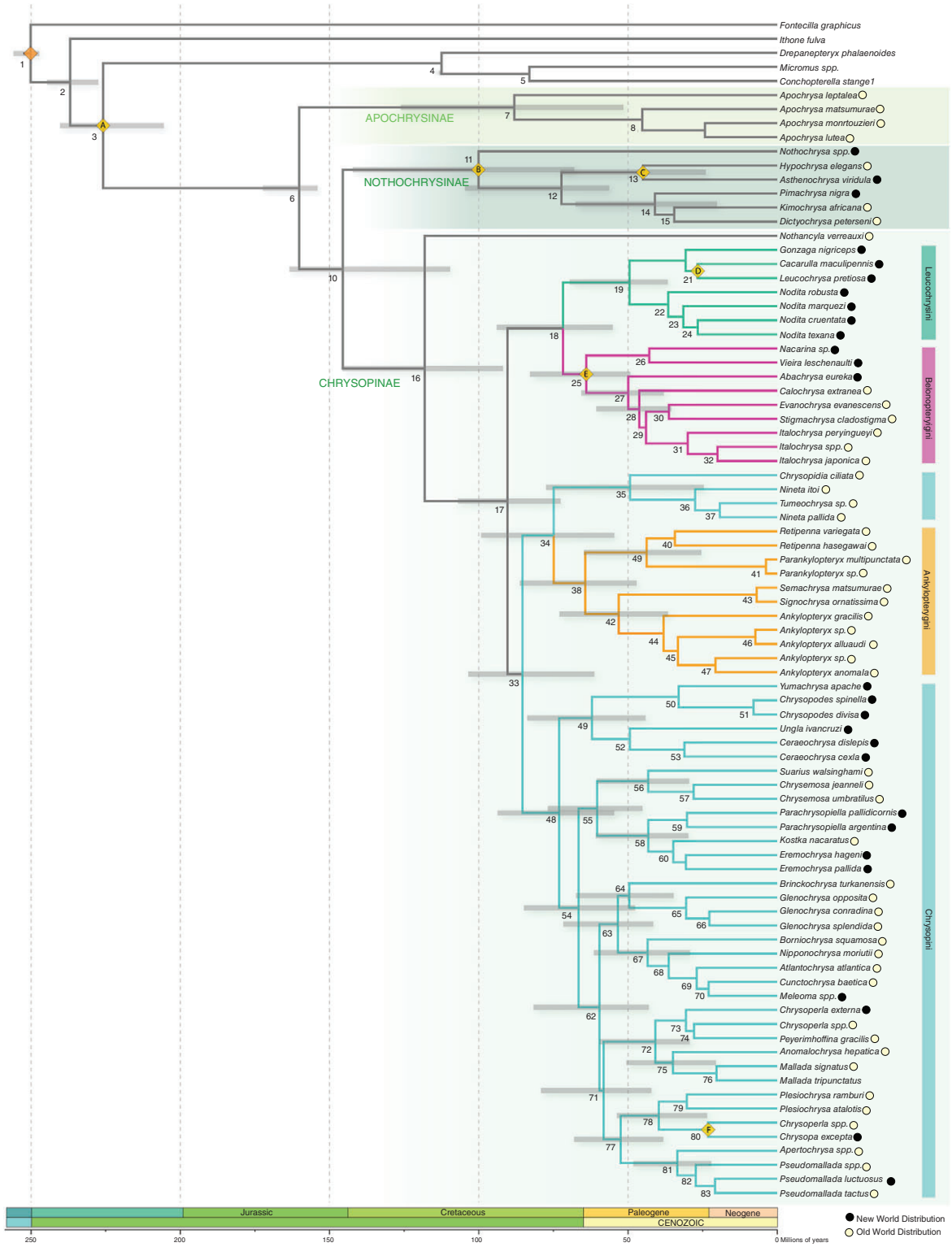

Fig. 5. Bayesian time tree divergence estimates within Chrysopidae. Yellow diamonds denote the placement of the seven minimum age calibrations; letters inside diamonds refer to the fossils discussed in the text. Grey bars represent highest posterior density ranges around the mean for major lineages, and nodes are numbered according in Table S2. Circles alongside exemplars indicate the distribution of that taxon as either New World (filled circle) or Old World (empty circle). [Colour figure can be viewed at wileyonlinelibrary.com].

the only phylogenetic analysis of relationships of genera within the subfamily.

Nothochrysinae. Nothochrysinae comprise nine genera distributed throughout the temperate regions of the world. The boreal genera include *Nothochrysa* McLachlan, *Pimachrysa* Adams, and *Hypochrysa*, whereas the austral temperate genera include *Asthenochrysa* Adams & Penny, *Leptochrysa* Adams & Penny, *Pamochrysa* Tjeder, *Kimochrysa* Tjeder, *Triplochrysa* Kimmins, and *Dictyochrysa* Esben-Petersen (Tjeder 1966; Adams 1967; Adams & Penny, 1992). Adults are characterized by lacking a tympanum at the base of the wing and often relatively unspecialized wing venation (Brooks & Barnard, 1990). These features, combined with the prevalence of nothochrysines in the fossil record, have led previous authors to propose the subfamily as sister to all other chrysopids (Adams, 1967). Although the monophyly of Nothochrysinae has, at times, been questioned (Adams, 1967; Adams & Penny, 1992; Brooks, 1997), both ML and BI analyses support it here. The Bayesian topology recovered it with very strong support (PP = 1.0), while its support in ML was slightly lower (SHL = 84). The only instance in which Nothochrysinae was found paraphyletic was when we excluded the extra sequencing data provided by the mitogenomes; however, in both instances (ML and BI) the resulting clades (i.e. excluding *Nothochrysa* from either Nothochrysinae or from Nothochrysinae + Chrysopinae) have low support for monophyly. Although, in general, larval morphology supports the monophyly of Nothochrysinae (Tauber, 2014b), recent studies (Duelli *et al.*, 2010; Tauber & Faulkner, 2015) have indicated that smaller-bodied nothochrysines [i.e. *Hypochrysa* (including *Kimochrysa*), *Pimachrysa,* and *Dictyochrysa*] are more similar to each other than to *Nothochrysa*, which is the same pattern of relationships within Nothochrysinae supported by our results. Namely, we found *Nothochrysa* sister to the rest of the subfamily, which is in turn arranged in two reciprocally monophyletic sister groups: the African genus *Kimochrysa* sister to the Australian genus *Dictyochrysa* and the South American genus *Asthenochrysa* sister to the Palaearctic *Hypochrysa*. This close relationship between *Asthenochrysa* and *Hypochrysa* was previously proposed by Brooks (1997) based on male genitalic morphology. In contrast to the results obtained by Tauber (2014b), based primarily on larval characters, our findings do not support the synonymy of *Kimochrysa* and *Hypochrysa*. Finally, our dating results indicate that most of the divergences among these genera occurred during the Late Cretaceous and Early Cenozoic, and, if some of the fossil nothochrysines are truly monophyletic with the extant diversity, then such divergences are possibly reflected in the Paleogene and later fossils of Chrysopidae (Fig. 5; Table S2). More comprehensive sampling of nothochrysine genera is needed, including *Leptochrysa*, *Pamochrysa*, and *Triplochrysa*, to elucidate relationships among these groups and to provide insights into possible biogeographic relationships.

Chrysopinae. This is the largest subfamily in species richness, with at least 1360 species in *c*. 70 genera distributed worldwide. This exceptional diversity is reflected here with the bulk of the taxonomic sampling at the genus and species level (Figs 3, 4). Results of both the ML and BI (Figs 3, S1) recover a Chrysopinae with relatively well-supported branches throughout and higher-level topological congruence between analyses. Overall, branch lengths are shorter along the backbone of this part of the tree, and some statistical uncertainty (i.e. low statistical support) is present among the most derived genera in the tribe Chrysopini, including a polytomy amongst some of the most derived clades of genera and conflicting placement of genera such as *Kostka*, *Chrysopa* and *Plesiochrysa* between analytical methods. Notable features of both analyses were the placement of *Nothancyla* as sister to all other Chrysopinae, the sister- group relationship of tribes Leucochrysini with Belonopterygini, and the paraphyly of Chrysopini relative to Ankylopterygini.

The placement of the genus *Nothancyla* as sister to all other Chrysopinae is not surprising considering that it exhibits characteristics of both Chrysopinae and Apochrysinae and, as a result, had previously been placed in either subfamily based on morphology (New, 1980; Brooks & Barnard, 1990; Winterton, 1995; Winterton & Brooks, 2002). Analyses using DNA sequence data placed the genus uneasily as sister to Nothochrysinae (Winterton & Freitas, 2006), and more recently as sister to the rest of Chrysopinae based on mitogenomic sequence data (Dai *et al.*, 2017; Jiang *et al.*, 2017). Our result expands upon Dai *et al.* (2017) and confirms Jiang *et al.* (2017), conclusively placing *Nothancyla* as sister to the rest of the subfamily, diverging during the Middle Cretaceous (Figs 3–5). Among the morphological characters supporting the inclusion of *Nothancyla* in Chrysopinae are the presence of the intramedial (im) cell in the forewing (Fig. 4, inset) (Brooks & Barnard, 1990), widely separate pseudomedial and pseudocubital veins along their length, a tympanum formed by the radial and medial vein in the forewing, and the presence of a tignum (sensu New, 1980; Winterton, 1995). The distribution of the first three characters is consistent with the phylogeny presented here (Fig. 4); however, because a tignum is present only in derived chrysopines, the reports of a tignum in *Nothancyla* led us to question the homology of the so-called tignum of *Nothancyla* with that of the other chrysopine genera (e.g. *Mallada* Navás, *Chrysoperla* Steinmann, *Nipponochrysa* Tsukaguchi). Re-examination of the male genitalia of *Nothancyla* specimens as part of this study indicates that the tignum is indeed absent and the presumed tignum is, in fact, a misinterpretation of the gonarcus by previous authors.

Exclusion of *Nothancyla* from the remainder of Chrysopinae reveals the likely need for a new higher taxon (subfamily or tribe) to accommodate this peculiar genus. We identify several characteristics in the following which highlight the different combination of features unique to *Nothancyla*, which may be useful in future studies. Characters supporting this taxon include an elongate ectoproct in males, reduced gonarcus, tignum and gonapsis absent, female lacking praegenitale, forewing costal area broad, lacking a subcostal crossvein, and intramedial cell quadrangular.

Relationships among the remaining Chrysopinae exhibit a distinct basal split during the Mid- to Late Cretaceous (90 Ma), with the tribes Belonopterygini and Leucochrysini in one clade and Chrysopini and Ankylopterygini in the other. This

relationship among Chrysopini tribes was proposed by Brooks (1997) based on adult morphology, whereas other authors using DNA data (e.g. Winterton & Freitas, 2006; Haruyama *et al.*, 2008; Duelli *et al.*, 2014) found only weak support for multiple alternative topologies.

The sister-group relationship between Belonopterygini and Leucochrysini is well supported here (PP = 1.0, SHL = 100) and was previously identified (albeit with weak support) based on a combination of genitalic characters (Brooks & Barnard, 1990; Brooks, 1997; Winterton & Freitas, 2006). Our study here shows strong statistical support for the monophyly of both Belonopterygini (PP = 0.90, SHL = 87) and Leucochrysini (PP = 1.0, SHL = 100). The entirely New World tribe Leucochrysini are represented primarily by the species-rich genus *Leucochrysa*, which currently includes two subgenera, *L.* (*Leucochrysa*) McLachlan and *L.* (*Nodita*) Navás. The tribe also contains six small genera, and in general the generic classification of the tribe remains problematic and in need of a comprehensive examination. Our results recover *Gonzaga* Navás, *Cacarulla* and *Leucochrysa (Leucochrysa)* as a clade sister to *L.* (*Nodita*), suggesting that *L.* (*Nodita*) might be considered as a distinct genus. However, it should be kept in mind that we have a relatively small sampling of the two large *Leucochrysa* subgenera and no representatives from the remaining four leucochrysine genera: *Berchmansus* Navás, *Neula* Navás, *Nuvol* Navás, and *Santocellus* Tauber & Albuquerque. Clearly, future studies are needed.

Belonopterygini are estimated here diverging from stem Leucochrysini at some time during the Late Cretaceous, and as stated earlier, the tribe is well supported as monophyletic. Morphological characteristics of the tribe include the presence of enlarged parameres (note that here we consider the gonapsis as homologous with the parameres of Belonopterygini and other Neuroptera) in the male genitalia of most genera and a suite of distinctive larval features mainly in the first instar (Principi, 1944; Tauber *et al.*, 2014; Tauber & Winterton, 2014). Furthermore, the larvae of two genera are known to be associated with ant nests (Principi, 1944; Tauber & Winterton, 2014). The tribe is largely cosmopolitan in distribution, although the bulk of the generic and specific diversity is in the Old World, particularly in the Oriental, Australasian and Afrotropical regions where *Italochrysa* Principi and allied genera are dominant. New World genera such as *Nacarina* Navás, *Vieira* and *Abachrysa* Navás were recovered towards the base of the clade, indicating a single divergence between the Old and New World faunas during the Paleogene. Interestingly, the distinctive genus *Vieira* (Fig. 3) was recovered as sister to *Nacarina* in Belonopterygini. Traditionally considered as belonging to the Leucochrysini (Brooks & Barnard, 1990; Winterton & Brooks, 2015), Tauber *et al.* (2007) moved *Vieira* to Belonopterygini based on adult and larval morphology and this transfer is supported by our analysis.

Our results indicate that Chrysopini are not monophyletic as defined by Brooks & Barnard (1990) (Figs 3–5); here we find that the tribe was rendered paraphyletic by Ankylopterygini. Ankylopterygini were recovered as monophyletic in all analyses and, likewise, always sister to a strongly supported clade of three chrysopine genera (PP = 0.99, SHL = 99): *Nineta* Navás, *Tumeochrysa* and *Chrysopidia* (*Chrysotropia*) Navás. Ankylopterygini plus these three chrysopine genera are, in turn, recovered as sister to the remaining Chrysopini. The close relationship between Ankylopterygini and *Nineta* and *Chrysopidia* was also found by Duelli *et al.* (2014) using only nuclear genes, although in that case largely with equivocal support (PP < 0.8, bootstrap < 50). The monophyly of the group containing the genera *Tumeochrysa*, *Nineta* and *Chrysopidia* is supported by the shared presence of an elongated male sternite 9, unique shape of the male gonocornua, and the proliferation of wing gradates in the forewings, manifested as three rows in *Tumeochrysa* and *Chrysopidia* (Brooks & Barnard, 1990; Brooks, 1997).

Most Ankylopterygini species are characterized by highly setose wings, narrow hindwings, palpi with apically elongated palpomeres, and scythe-like mandibles that lack basal teeth (symmetrical) (Brooks, 1986; Brooks & Barnard, 1990). In Chrysopidae, the plesiomorphic condition includes broad and asymmetrical mandibles (only the left mandible exhibits a basal tooth). Thus, Brooks & Barnard (1990) hypothesized that the condition in Ankylopterygini was apomorphic, which is supported by our results. *Nineta*, *Chrysopidia (Chrysotropia)* and *Tumeochrysa* all possess broad mandibles, each with a basal tooth (symmetrical sensu Brooks & Barnard, 1990), thus also deviating from the plesiomorphic condition. It is worth noticing, however, that both characters, i.e. mandible shape and the number of teeth, appear to be variable at the genus level despite seeming consistent at the tribal level. For example, in the chrysopine genus *Chrysopodes* Navás, mandibles are scythe-like in the subgenus *Chrysopodes*, whereas the subgenus *Neosuarius* Adams & Penny expresses variability in mandible shape (Brooks & Barnard, 1990). Additionally, *Chrysopiella* Banks and *Parachrysopiella* Brooks & Barnard both have (broad) mandibles with two teeth (similar to the shape in *Nineta* and *Chrysopidia*). Thus, the significance of this character, and the status of Chrysopini relative to Ankylopterygini require more detailed examination, particularly before assessing potential changes in classification. Various authors have previously viewed the monophyly of the tribe Chrysopini with suspicion (Brooks & Barnard, 1990; Brooks, 1997), and unarguably the great morphological diversity included in the tribe has made establishing homologies a challenge. Among those authors employing molecular data, only Winterton & Freitas (2006) recovered a monophyletic Chrysopini (cf. Haruyama *et al.*, 2008; Duelli *et al.*, 2014). Winterton & Freitas (2006), however, included only nine genera in their analysis and the monophyly of Chrysopini was recovered in the absence of representatives of *Nineta*, *Chrysotropia,* or *Tumeochrysa*.

Relationships among the remaining bulk of genera in Chrysopini (Fig. 5, node 48) are less conclusive. This portion of the tree features shorter branch lengths and lower branch supports than the previously discussed nodes of the topology. Despite this, our analysis recovered several strongly supported and seemingly natural groups of genera, all apparently originating during the Late Cretaceous to Early Cenozoic. Indeed, the majority of generic divergences occurred in this clade throughout the Paleogene. The first group of genera is a clade of four New World taxa: *Yumachrysa* Banks sister to *Chrysopodes*, and *Ungla* Navás sister to *Ceraeochrysa* Adams. This clade has

relatively strong SHL support (SHL = 93), moderate PP (0.80), and no bootstrap support (BS = 20). The New World Chrysopidae have attracted strong attention from taxonomists during the last few decades, resulting in the proposal of some generic affinities. For example, the grouping of *Ungla*, *Chrysopodes,* and *Ceraeochrysa* had been previously suggested by various authors (Brooks & Barnard, 1990; Tauber, 2003; Tauber, 2010; Sosa & De Freitas, 2012; Tauber & Garland, 2014). Similarly, *Yumachrysa* and *Ceraeochrysa* were suggested to belong to the *Chrysopa* species group by Brooks (1997), although such a pattern was not recovered here. The distinction between *Chrysopodes* and *Ceraeochrysa* presented taxonomic problems (see Tauber & Flint, 2010) that have been resolved by the recognition of a distinct genus, *Kymachrysa* Tauber & Garland (Tauber & Garland, 2014); the generic relationships of this genus and the relatively new genus *Titanochrysa* Sosa & Freitas are unknown (see Tauber *et al.*, 2018). In general, the diversity and phylogeny of this group of six New World genera need additional work. Finally, the putative close relationship between *Yumachrysa* and *Meleoma* Fitch suggested by Brooks (1997) was not recovered in our analysis; here the genera were found to be distantly related.

Another clade of genera within Chrysopini recovered here includes various similar-looking taxa: *Suarius* Navás and *Chrysemosa* Brooks & Barnard from the Old World in one clade, and *Eremochrysa* Banks and *Parachrysopiella* Brooks & Barnard from the New World in the other sister clade. Overall, this clade had only moderate statistical support (SHL = 81, PP = 82) and no support from bootstrap (BS = 10). The sister-group relationship between *Suarius* and *Chrysemosa* is well supported here, and was previously identified based on morphology (Tjeder, 1966; Brooks & Barnard, 1990) and DNA sequences (Haruyama *et al.*, 2008; Duelli *et al.*, 2014). Brooks & Barnard (1990) listed several synapomorphies of *Parachrysopiella* and *Eremochrysa* and treated them as subgenera, yet Brooks (1997) subsequently associated each with distantly related genera, presumably based on their disparate male genitalic morphology. The BI analysis placed the Oriental genus *Kostka* Navás in a polytomy with *Eremochrysa* and *Parachrysopiella*, whereas the ML analysis placed it with the Eastern Hemisphere genera *Glenochrysa* Esben-Petersen and *Brinckochrysa* Tjeder. It is worth noting that these results are based on COI, which was the only fragment we had available for *Kostka*. Our results thus suggest that this fragment is clearly not sufficient alone to place this distinctive monotypic genus with any confidence. Likewise, Brooks & Barnard (1990) and Brooks (1997) could not identify any generic affinities based on morphology for *Kostka* except for a possible affinity with *Austrochrysa* Esben-Petersen.

The BI and ML analyses differ in the arrangement of the remaining genera, which in both cases featured exceptionally short branches. This clade includes mostly Old World genera and it agrees in membership with the *Mallada* and *Chrysopa* groups as proposed by Brooks (1997). In the Bayesian topology, this group is composed of three principal clades whose relationships are equivocal and collapsed in a trichotomy, although the monophyly of each of these clades is generally strongly supported (Fig. 3). One of these clades is formed by *Anomalochrysa* McLachlan sister to *Mallada*, with them in turn sister to *Chrysoperla* plus *Peyerimhoffina* Lacroix. The posterior probability support for this clade is very high, and both of these pairings are consistent with groupings previously proposed based on morphology (e.g. Brooks & Barnard, 1990; Brooks, 1997) and molecular data (Haruyama *et al.*, 2008; Duelli *et al.*, 2014). *Peyerimhoffina* render *Chrysoperla* paraphyletic, an issue noted by earlier studies (e.g. Duelli *et al.*, 2014) and that implies *Peyerimhoffina* may represent nothing more than a highly derived species of *Chrysoperla*. A second clade includes *Brinckochrysa* Tjeder sister to *Glenochrysa* Esben-Petersen, which together are sister to a laddered clade formed of five genera: *Borniochrysa* Tjeder, *Nipponochrysa*, *Atlantochrysa* Hölzel, *Cunctochrysa* Hölzel, and *Meleoma*. Relationships among these taxa were recovered with a high level of statistical support. Brooks & Barnard (1990) suggested a close relationship among *Brinckochrysa*, *Chrysoperla* and *Peyerimhoffina*, to which Brooks (1997) later added *Eremochrysa*. Although our results indicate these genera do belong to the same larger generic cluster, they do not appear to be as closely related as previously surmised. The relationship among *Cunctochrysa*, *Atlantochrysa* and *Meleoma* was based on several characters (Brooks & Barnard, 1990; Brooks, 1997; Duelli *et al.*, 2014), whereas the pairing of *Brinckochrysa* and *Glenochrysa* is novel and no clear morphological synapomorphies are evident to corroborate such a node. Specialized forms of signalling and calling behaviour are found in genera across this clade, including stridulatory structures in the males of *Brinckochrysa* and *Meleoma*, vibrational-calling behaviour in species of *Chrysoperla* and *Meleoma*, and frontal glandular attractants in *Meleoma* (Adams, 1962; Tauber, 1969; Brooks & Barnard, 1990; Henry *et al.*, 2014). *Glenochrysa* males also have a distinctive signalling behaviour involving a large eversible prothoracic gland, referred to as a 'glenofinger' (Duelli, 2004; Winterton & Garzón-Orduña, 2015). The significance of these elaborate secondary sexual glandular structures in the males of these and other genera in the diversification of these clades should be investigated in future studies. The last group of genera in this large clade includes *Apertochrysa* Tjeder sister to *Pseudomallada* Tsukaguchi, and the two in turn are sister to a reciprocally monophyletic group composed of *Chrysopa* plus *Plesiochrysa*. Although each of these sister groupings had strong PPs, the overall support for the clade was low (PP = 0.77). Indeed, under ML, *Chrysopa* and *Plesiochrysa* were recovered as sister to the most derived Chrysopini included into the trichotomy under discussion and recovered by MRBAYES. Haruyama *et al.* (2008) and Duelli *et al.* (2014) recovered a similarly close relationship among *Chrysopa* and *Plesiochrysa*, plus *Pseudomallada* and *Apertochrysa*. In fact, *Plesiochrysa* have long been considered sister to *Chrysopa* (Adams, 1982; Brooks & Barnard, 1990; Brooks, 1997) and they are the only two chrysopine genera that are known to be predaceous in the adult stage (Tauber *et al.*, 2001). With respect to *Pseudomallada* and *Apertochrysa*, the taxonomy of these genera is problematic; there are difficulties in assigning species to either, and particularly owing to the large number of species in *Pseudomallada* (Duelli *et al.*, 2014). *Pseudomallada* is unusual among chrysopids because the genus

is represented by species in both the Old and New Worlds and also because the larvae undergo an elaborate, photoperiodically regulated diapause, before they spin cocoons. Many chrysopid genera may be widely distributed but are generally restricted to one hemisphere or the other (Fig. 5). Interestingly, *Pseudomallada* species occur in both the New and Old World, and they share a distinctive overwintering stage and patterns of elaborate photoperiodic responses that regulate their hibernation; these two life-history traits appear to support the monophyly of this geographically widespread genus (Tauber & Tauber, 2015).

Conclusions

New (1984) and Brooks (1997) identified five important reasons why knowledge of chrysopids systematics lingers behind that of other lacewing families: (i) difficulties in assigning species to genera; (ii) an inadequately resolved higher-level classification; (iii) a large proportion of monotypic genera; (iv) an intrafamilial classification based on a limited range of adult features; and (v) a meagre understanding of specific variability. Our results provide a robust phylogenetic framework for future research on chrysopids and contributes towards alleviating specifically the effects of points (ii), (iii) and (iv) highlighted by these authors. This phylogeny confirms the monophyly of all previously established subfamilies and all but one of the traditionally recognized tribes. We provide important additional DNA sequence data to support taxonomic decisions presently limited by the narrow range of morphological features available for use in chrysopid taxonomy; such results present opportunities to test the monophyly of genera, place particular species to genera and challenge the validity of monotypic genera (sensu Winterton & Brooks, 2002). Furthermore, this phylogeny aids the understanding of particular misinterpretations of homology, e.g. identity of the so-called 'tignum' in *Nothancyla*, and the homology of the gonapsis of some chrysopids with parameres of the broader Neuroptera. The enigmatic *Nothancyla* is clearly deserving of much investigation given its prominent placement as sister to the rest of Chrysopinae. Future research should be directed towards the examination of morphological characters that might support the redefinition of Chrysopini relative to Ankylopterygini. This issue requires important attention as it might imply establishing a new higher grouping to accommodate the clade formed by *Tumeochrysa*, *Nineta* and *Chrysopidia*. Finally, elucidating the relationships throughout Chrysopidae is imperative to achieve a clearer picture of the evolution of larval debris-carrying throughout the broader Chrysopoidea and Myrmeleontoidea.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. ML topology. Values above branches represent Shimodaira–Hasegawa-Like (SHL) support, and those below branches are bootstrap values.

Figure S2. Topology obtained with RAXML after removing third positions from protein-coding genes.

Figure S3. Topology obtained with RAXML after removing mitogenomes.

Figure S4. Topology obtained with MRBAYES after removing mitogenomes.

Table S1. Primer sequences used for novel DNA amplification and sequencing.

Table S2. Divergence time estimates with averages around the mean and range for nodes listed in Fig. 5.

Table S3. Exemplars with GenBank accession numbers used in phylogenetic analyses.

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