Evolution of green lacewings (Neuroptera: Chrysopidae): an anchored phylogenomics approach

Shaun L. Winterton[1*], Jessica P. Gillung[2], Ivonne J. Garzón-Orduña [3], Davide Badano[4], Laura C.V. Breitkreuz[5], Peter Duelli[6], Michael S. Engel[5], Xingyue Liu[7], Renato J.P. Machado[8], Mervyn Mansell[9], Atsushi Mochizuki[10†], Norman D. Penny[11†], Catherine A. Tauber[2,12] & John D. Oswald[13]

1. California State Collection of Arthropods, Sacramento, USA.
2. Department of Entomology and Nematology, University of California, Davis, USA.
3. Colección Nacional de Insectos (CNIN), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), México City, México.
4. Università degli Studi di Genova, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Genova, Italia.
5. Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, USA.
6. Swiss Federal Research Institute WSL, Birmensdorf, Switzerland.
7. Department of Entomology, China Agricultural University, Beijing, China.
8. Instituo de Biociências, Universidade Federal de Mato Grosso, Cuiabá, Brazil.
9. Department of Zoology & Entomology, University of Pretoria, Hatfield, South Africa.
10. National Institute for Agro-Environmental Sciences, Tsukuba City, Japan.
11. California Academy of Science, San Francisco, USA.
12. Department of Entomology, Cornell University, Ithaca, NY, USA.
13. Department of Entomology, Texas A&M University, College Station, USA

*Corresponding author: California State Collection of Arthropods, 3294 Meadowview Road, Sacramento, CA 95832, USA.
E-mail address: wintertonshaun@gmail.com (S.L. Winterton).

† Deceased.

Abstract. A phylogeny of green lacewings (Neuroptera: Chrysopidae) using anchored hybrid enrichment data is presented. Using this phylogenomic approach we analysed 137 KB of sequence data (with <10% missing) for 82 species in 50 genera of Chrysopidae under Bayesian and Maximum Likelihood criteria. We recovered a strongly supported tree topologically congruent with recently published phylogenies, especially relationships amongst higher-level groups. The subfamily Nothochrysinae was recovered as paraphyletic, with one clade sister to the rest of Chrysopidae, and the second clade containing the nominal genus (*Nothochrysa* Navás) as sister to the subfamily Apochrysinae. Chrysopinae was recovered as a monophyletic with the monobasic Nothancylini tribe nov. sister to the rest of the subfamily. Leucochrysini was recovered sister to Belonopterygini and Chrysopini rendered paraphyletic with respect to Ankylopterygini. Divergence times and diversification estimates indicate a major shift in rate in ancestral Chrysopini at the end of the Cretaceous and the extensive radiation of Chrysopinae, the numerically dominant clade of green lacewings, began in the Mid Paleogene (*ca.* 45 Ma).

Keywords. phylogeny; genomics; anchored hybrid enrichment; divergence times.

Introduction

Green lacewing (Neuroptera: Chrysopidae) adults are delicate insects generally typified by green bodies and broad, transparent wings with intricately laced venation. With few exceptions, chrysopid larvae are generalist arboreal predators with a campodeiform body shape. The thorax and abdomen of many species possess elongate lateral processes and long setae used to entangle a packet of debris, usually containing plant fragments, insect wax, carcasses or dirt (reviewed by Tauber *et al.*, 2014). This debris packet is used in both camouflage and as a physical defence against predation and parasitism and appears to be an archaic feature of the broader Chrysopoidea, with the behaviour well-developed in Mesozoic fossil examples (Pérez-de la Fuente *et al.*, 2012; Tauber *et al.*, 2014; Wang *et al.*, 2016). With at least 1416 species grouped in 82 genera (Oswald, 2018), Chrysopidae are the second most species-rich family of Neuroptera. They are divided into three extant subfamilies: Apochrysinae, Nothochrysinae, Chrysopinae and the extinct subfamily Limaiinae. Mesochrysopidae are sometimes included as a subfamily of Chrysopidae (Engel *et al*., 2018) or treated as a separate family (Nel *et al.*, 2005; Liu *et al.*, 2016, 2018). Amongst the living subfamilies, Apochrysinae and Nothochrysinae are relatively species-poor, with ca. 26 species (five genera) and 20 species (nine genera) respectively. Coincidentally, Apochrysinae are almost pan-tropical, while Nothochrysinae are almost pan-temperate in their distributions, with little overlap. Chrysopinae comprise the overwhelming majority of the species diversity of the family with over 1350 species in *ca*. 68 genera distributed in all major biogeographical regions. The subfamily is further divided into four tribes: Leucochrysini, Belonopterygini, Ankylopterygini and Chrysopini (Brooks & Barnard, 1990).

Phylogenetic relationships within Chrysopidae have been the subject of various studies based on morphological (Brooks & Barnard, 1990; Brooks, 1997; Nel *et al.*, 2005; Winterton & Brooks, 2002) and molecular data (Winterton & Freitas, 2006; Haruyama *et al.*, 2008; Duelli *et al.*, 2014, 2017; Dai *et al.*, 2017; Garzón-Orduña *et al*., 2018). These studies have ranged widely in the extent of taxon sampling, and the type and amount of data used. The recent supermatrix approach by Garzón-Orduña *et al*. (2018), which incorporated data from most previous molecular studies as well as numerous additional sequences, has probably come the closest to providing a statistically robust phylogeny of the family with broad taxon sampling. They recovered Leucochrysini and Belonopterygini as sister groups, and Chrysopini rendered paraphyletic by Ankylopterygini. Yet, questions remain regarding specific clades in the green lacewing phylogeny that could not be addressed confidently by Garzón-Orduña *et al.*, (2018). These include elucidating reciprocal monophyly of both Apochrysinae and Nothochrysinae, relationships amongst the more derived genera within the tribe Chrysopini, and the seemingly perennial issue of identifying the sister group to the rest of the family.

Recent studies have begun resolving higher-level relationships within Neuroptera using large amounts of DNA sequence data, resulting in progress in understanding evolution of the order at all levels (e.g., Winterton *et al*., 2010, 2017; Liu *et al*., 2015; Shi *et al*., 2015; Garzón-Orduña *et al.*, 2016, 2017; Wang *et al*., 2017; Bakkes *et al*., 2018; Machado *et al*., 2018). Here, we present the first large-scale genomic approach to understanding green lacewing phylogeny, in this instance using anchored hybrid enrichment data sequenced for 82 species representing 50 genera of chrysopids. Based on the topology recovered from these genomic data, we estimate the timing of cladogenesis and diversification rates of major green lacewing lineages on a geological timescale and compare them with previous estimates. In addition, the enigmatic and monotypic Australian genus *Nothancyla* Navás was placed by Garzón-Orduña *et al.* (2018) as sister to the rest of Chrysopinae, supporting similar results by Dai *et al.* (2017) and Jiang *et al.* (2017) based on mitogenomic sequence data. This genus had been historically difficult to place to subfamily as it

exhibits morphological characteristics of both Chrysopinae and Apochrysinae (Brooks & Barnard, 1990; Winterton, 1995; Winterton & Brooks, 2002; Winterton & Freitas, 2006). Recent results suggest that *Nothancyla* may merit recognition as an additional tribe within Chrysopinae; we include *Nothancyla* in this analysis to confirm its position and classification in Chrysopidae.

Materials & Methods

Taxon sampling

Taxa were selected to represent the relative diversity within individual subfamilies and tribes for Chrysopidae. We sampled 82 species of Chrysopidae in 50 genera, representing all subfamilies and tribes (Brooks, 1997). Where possible and appropriate, multiple representatives of a clade were sampled (especially for species-rich genera) to ensure close to proportional sampling, an important assumption for Bayesian analyses. We were able to sample four genera of Nothochrysinae but only a single genus of Apochrysinae; as with previous studies, representatives of the latter subfamily are exceedingly rare and other genera were not available for sequencing. The bulk of sampling was from Chrysopinae (45 genera), representing 67% of total genera in that subfamily. We also included multiple rare and/or enigmatic taxa where the higher-level placement has been considered contentious previously (e.g., *Nothancyla*, *Vieira* Navás, *Retipenna* Brooks, *Kostka* Navás, *Gonzaga* Navás). Outgroups were selected from a wide variety of other lacewing families, including Ithonidae, Nymphidae, Psychopsidae, Mantispidae, Berothidae, Rhachiberothidae, and Hemerobiidae. Hemerobiidae has long been considered the sister family to Chrysopidae, based primarily on the morphological similarity of their larval stages (including a trumpet shaped empodium in at least the first instar). This phylogenetic association has been recovered in some quantitative studies using both morphology and DNA sequence data (Winterton *et al*., 2010; Garzón-Orduña *et al.* 2016; Wang *et al*., 2017; Winterton *et al*., 2018). While other published studies using a variety of data sources have also recovered Hemerobiidae in other locations within Neuroptera, and not sister to Chrysopidae (e.g., Winterton, 2003; Yang *et al*., 2012). The large phylogenomic study by Winterton *et al*. (2018) recovered Hemerobiidae as distantly related to Chrysopidae with strong statistical support. To further test the phylogenetic association between Hemerobiidae and Chrysopidae, we sampled 11 brown lacewing genera representing all major lineages of Hemerobiidae.

DNA extraction

Genomic material was extracted from thoracic or leg muscle. We used either the DNeasy™ or Gentra Puregene Tissue kits (Qiagen, Redwood City, CA, U.S.A.) for DNA extraction. Minor modifications included: (i) adding 20 □L of RNAse per 20 mg of tissue after the samples were lysed to remove RNA, and (ii) heating the elution buffer to 55ºC degrees before the elution step. We performed two separate elutions for samples with 30 and 50 □L 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.

Sample preparation and probe design

Specimens were initially preserved in 95–100% ethanol and stored at -80º Celsius. Vouchers are deposited in the California State Collection of Arthropods (CSCA). The extracted DNA was used to produce Illumina libraries following Lemmon *et al.* (2012) and Prum *et al.* (2015), with procedures described in detail in Winterton *et al.* (2018). All DNA sequences generated as part of this study are

deposited in the NCBI (Sequence Read Archive) depository (Table S1), under BioProject PRJNA398561. Probes were designed following the methods described in Winterton *et al.* (2018). In summary, probes were produced based on published transcriptomes or newly sequenced genomes of ten representative species of different families of Neuropterida. All probes and 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). Probes were tiled uniformly at 5x 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.

Read assembly

Reads were prepared and assembled following the methods described in Winterton *et al.* (2018). Quality filtering was performed using the CASAVA high-chastity filter. 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).

Orthology determination and alignment generation

Putative orthologs 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. Clusters formed through this process were then screened for taxon presence. Assembled contigs derived from fewer than 17 reads were removed. Clusters containing fewer than 50% of the species in the taxon set were removed; 70% conservation was required for each site to be considered reliable and 20-bp regions containing matches at fewer than 10 reliable sites were masked. After masking, sites containing less than 73% unambiguous bases were removed from the alignment. 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 again 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 masked. After masking, sites containing less than 67% unambiguous bases were removed from the alignment. The final nucleotide alignment contained 372 genes, with a total length of 137,028 bp. Basic alignment statistics, including percentage of missing data, were obtained using AMAS (Borowiec, 2016).

Phylogenetic analyses

Model selection remains a very important step in phylogenomic analysis (Gillung *et al.*, 2018). The best-fitting partitioning scheme and substitution model for each partition were identified using the *rcluster* search algorithm (Lanfear *et al*., 2014) as implemented in PartitionFinder 2 (Lanfear *et al.*, 2016); the best substitution model for each partition was selected using the Bayesian Information Criterion (BIC). The best fitting substitution model across all partitions for the nucleotide dataset was a general time-reversible substitution model (GTR) with rate heterogeneity described by a gamma distribution discretised into four bins (+G). The final alignment and partition file are presented respectively in Supplementary Files S2 and S3. We estimated the phylogeny using Bayesian inference in ExaBayes v1.4 (Aberer *et al.*, 2014). We performed two independent runs with four coupled MCMC chains each, sampling every 1,000 generations and applying uniform priors to tree topologies and an exponential prior to branch lengths.

After 50,000,000 generations, we assessed convergence by computing the average standard deviation of split frequencies (ASDSP) and checking the estimated sample sizes (ESS) in Tracer v.1.6 (Rambaut *et al.*, 2014). We ran the chains until we obtained an ASDSF value lower than 1% and ESS values >200 for all parameters. Finally, we used the *consense* tool of the ExaBayes package to obtain a 50% majority rule consensus tree, discarding the first 25% of the sample topologies as *burn in*.

Divergence times

Estimation of divergence times was implemented in BEAST v.2.4.6 (Bouckaert *et al.*, 2014). We defined the partitions and site models in BEAUti based on the partition scheme and models proposed by PartitionFinder (see Phylogenetic Analyses above), with model selection based on the Bayesian Information Criterion (BIC). We used an uncorrelated relaxed molecular clock model (Drummond *et al.*, 2006) with a log-normal prior, with topology and clock model linked across partitions. We applied a node dating approach with a birth-death tree prior; we defined these calibrating nodes by determining monophyletic taxon sets at the nodes where calibrations were used. We used 16 fossils as calibration points (Table 1; Fig. S1). 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). We assigned a log-normal distribution for the calibration density at each calibration node. We ran two independent analyses in BEAST for 100 million generations each. We then evaluated the convergence and mixing of the MCMC chains in Tracer v1.6, ensuring that the two runs converged on the same distribution and ascertained that effective sample sizes (ESS) exceeded 200. We then resampled the resulting files of the inferred phylogenetic trees with a frequency of 10,0000 in LogCombiner v2.3.1 (BEAST package) and a burn-in of 30%. This resulted in 93075 subsampled trees. We then summarized the subsampled trees in a maximum clade credibility tree with common ancestor heights as node heights using TreeAnnotator v2.3.1 (BEAST package).

Table 1. Fossil calibrations used in the divergence times estimation analysis. Nodes are numbered according to Supplementary Fig. S1.

Node	Fossil species	Placement	Age (MA)	Reference
2	*Cretomerobius disjunctus* Ponomarenko	Crown Hemerobiidae	112	Nel et al. (2005)
5	*Notiobiella thaumasta* Oswald	Crown *Notiobiella*	14	Oswald (1999)
7	*Sympherobius completus* Makarkin & Wedmann	Crown *Sympherobius*	14	Makarkin & Wedmann (2009)
15	*Liassochrysa stigmatica* Ansorge & Schlüter	Crown Mantispidae	182	Ansorge & Schlüter (1990)
18	*Triassopsychops superbus* Tillyard	Stem Psychopsidae	205	Tillyard (1922)
19	*Cretapsychops decipiens* Peng *et al.*	Crown Psychopsidae	156	Peng et al. (2010)
20	*Liminympha makarkini* Ren & Engel	Stem Nymphidae	156	Ren & Engel (2007)
21	*Daonymphes bisulca* Makarkin *et al.*	Crown Nymphidae	156	Makarkin et al. (2013)
22	*Guithone bethouxi* Zheng *et al.*	Crown Ithonidae	156	Zheng et al. (2016)
26	*Paralembochrysa splendida* Nel *et al.*	Crown Chrysopidae	125.5	Nel et al. (2005)
28	*Pseudochrysopa harveyi* Makarkin & Archibald	Crown Nothochrysinae	48.6	Makarkin & Archibald (2013)
31	*Nothochrysa stampieni* Nel & Séméria	Crown *Nothochrysa*	23	Nel & Séméria (1986)
35	*Paleochrysopa monteilsensis* Séméria & Nel	Crown Chrysopinae	34	Séméria & Nel (1990)
38	*Leucochrysa (Nodita) prisca* Engel & Grimaldi	Crown *Leucochrysa*	13.7	Engel & Grimaldi 2007
40	Belonopterygini larva	Crown Belonopterygini	34	Archibald et al. (2014)
99	*Chrysopa glaesaria* Engel & Grimaldi	Crown *Chrysopa*	13.7	Engel & Grimaldi (2007)

Diversification rates estimation

We used BAMM to assess diversification rate shifts across the Neuroptera phylogeny (Rabosky, 2014). We used the maximum clade credibility phylogeny from the BEAST analysis as input, with sampling probabilities estimated using the extant species diversity according to the online database Neuropterida Species of the World (Oswald, 2018). The sampling proportions were set as follows: Hemerobiidae: 0.02, Rhachiberothinae: 0.08, Berothinae: 0.01, Mantispidae: 0.01, Psychopsidae: 0.04, Nymphidae: 0.06, Ithonidae: 0.13, Nothochrysinae (Chrysopidae): 0.13, *Nothochrysa* McLachlan: 0.12, Apochrysinae: 0.05, *Nothancyla* Navás: 1, Leucochrysini: 0.01, Belonopterygini: 0.05, Chrysopini1 (*Chrysopidia* Navás + *Nineta* Navás): 0.034, Ankylopterygini: 0.05, Chrysopini2: 0.02. We used the "setBAMMpriors" function in the R package BAMMtools (Rabosky *et al.*, 2014) to create the priors used for the analysis. We ran the MCMC for 20 million generations, sampling every 1,000 generations, and checked the convergence and plotted the analysis results using BAMMtools and CODA (Plummer *et al.*, 2006).

Results

The nucleotide alignment of 107 taxa (25 outgroup, 82 ingroup) comprised a total of 137,028 base pairs after trimming, representing 372 loci. The complete nucleotide alignment had 9.5% of missing data and an average locus length of 368 bp. In all analyses the tree topology (Fig. 1) was very strongly supported throughout and largely congruent between ML and BI results, except in a few near terminal nodes in Chrysopini. In the BI tree all but five nodes had posterior probabilities of 1.0. In the ML tree (Fig. S2) overall support for nodes was slightly lower, with the same five nodes having lower than 80% bootstrap support. Statistical support for all nodes was high, even when branch lengths were relatively short (e.g., derived clades of Chrysopini).

Hemerobiidae were not recovered as sister to Chrysopidae and were instead placed as the furthest outgroup (regardless of placement of tree root). A clade comprising the extant families Myrmeleontoidea (i.e., Psychopsidae, Myrmeleontidae, Ascalaphidae, Nemopteridae, Nymphidae and Ithonidae) (*sensu* Winterton *et al*., 2018) were instead recovered as the sister group to Chrysopidae. Chrysopidae were recovered as monophyletic, originating in the Late Triassic, with one lineage of a paraphyletic Nothochrysinae as sister to the rest of the family. This clade comprised here of *Hypochrysa* Hagen, *Pimachrysa* Adams and *Dictyochrysa* Esben-Petersen was recovered separate to *Nothochrysa* McLachlan, which itself was placed as sister to Apochrysinae in a clade sister to Chrysopinae. Chrysopinae was recovered as a strongly supported monophylum diverging from Apochrysinae and Nothochrysinae during the Early Cretaceous (102 Mya) (Fig. 2; Fig. S1; Table S2). Chrysopinae was then divided subsequently into three main lineages. *Nothancyla* was recovered as sister to the rest of Chrysopinae, diverging during the Late Cretaceous. The placement of this genus outside of the currently recognised tribes of Chrysopinae supports the establishment of a new tribe Nothancylini to accommodate it. The remaining Chrysopinae then diverged into two major clades during the early Paleogene (48 Mya) and relatively shortly after the K-T extinction event; one comprising the tribes Leucochrysini and Belonopterygini and the other comprising the tribes Chrysopini and Ankylopterygini. The node subtending this cladogenesis had a significant change in sequence rate heterogeneity as indicated by the BAMM analysis (Fig. 2, inset; Figs S4–5), suggesting a dramatic change in the rate of diversification. Subsequently, branch lengths throughout the rest of this entire clade were notably shorter on average than earlier ones in Chrysopini throughout Cenozoic, likely indicative of this rapid increase in diversification rate.

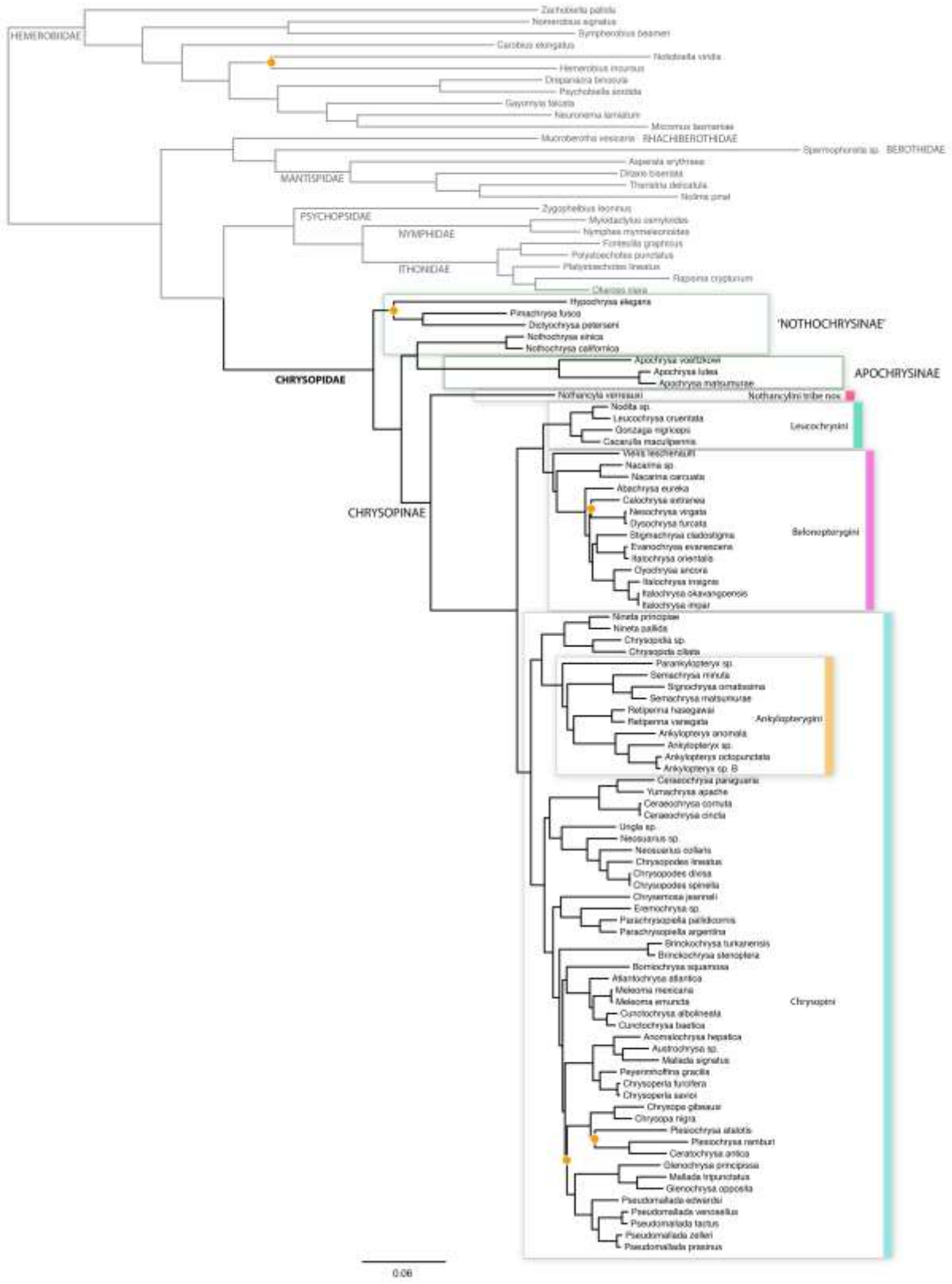

Figure 1. Bayesian phylogeny of Chrysopidae based on anchored hybrid enrichment data. All nodes have a posterior probability support value of 1.0, except for those marked with an orange disc, which have support values between 0.95 and 0.99.

Leucochrysini diverged from Belonopterygini during the mid Paleogene (41 Mya) with both tribes recovered here as reciprocally monophyletic. The New World genus *Vieira* was strongly supported as sister to the rest of Belonopterygini and not within Leucochrysini. The other two New World genera *Nacarina* Navás and *Abachrysa* Banks, also diverged during the Paleogene from Old World Belonopterygini genera. The monophyly of certain genera of Belonopterygini is questioned based on these results, with *Italochrysa* Principi rendered paraphyletic by *Stigmachrysa* Navás, *Evanochrysa* Brooks & Barnard and *Oyochrysa* Brooks.

The second major clade of Chrysopinae comprises Chrysopini rendered paraphyletic by Ankylopterygini. Within this clade is distinct basal dichotomy represented by one subclade comprising Ankylopterygini as the sister to a pair of Chrysopini genera, *Nineta* Navás and *Chrysopidia* Navás, while the other subclade contains the remaining Chrysopini genera. Ankylopterygini are recovered as monophyletic with *Parankylopteryx* Tjeder as sister to the rest of the tribe, followed by one clade comprising *Signochrysa* Brooks & Barnard with a paraphyletic *Semachrysa* Brooks, and another clade comprising *Retipenna* as sister to *Ankylopteryx* Brauer.

Within the remaining Chrysopini (*sans Nineta* and *Chrysopidia*), a group of exclusively New World genera – *Yumachrysa* Banks, *Ceraeochrysa* Adams, *Chrysopodes* Navás, *Ungla* Navás and *Neosuarius* Adams & Penny, was recovered as sister to the rest of the tribe. This was followed by a group of genera comprising *Chrysemosa* Brooks & Barnard, *Eremochrysa* Banks and *Parachrysopiella* Brooks & Barnard. Internodes in this part of the tree become increasingly shorter, but almost all retaining high branch support values (Fig. 1), indicating a period of rapid diversification (Fig. 2). The topologies of the Bayesian and divergence-time analyses vary in this part of the tree, resulting in lower subjective confidence in relationships, regardless of branch support. Several groups of genera are notable though, including one consisting of *Borniochrysa* Brooks & Barnard, *Atlantochrysa* Hölzel, *Meleoma* Fitch, and *Cunctochrysa* Hölzel, and another comprising *Chrysopa* Leach, *Ceratochrysa* Tjeder and *Plesiochrysa* Adams. The analysed species of the large genus *Pseudomallada* were recovered as monophyletic.

Diversification rate analyses in BAMM (Rabosky, 2014) identified one shift in Chrysopidae, with a significant increase in evolutionary rate identified for non-nothancyline Chrysopinae (Fig. 2, inset). A second shift in rate was identified in Hemerobiidae, with increase in evolutionary rates occurring either along the branch leading to the crown Hemerobiidae or within Hemerobiidae (the latter with low posterior probability, see Supplementary Figs S4–S5). BAMM also identified a scenario with three rate shifts, one in Hemerobiidae, another one in Chrysopinae and a third one in Apochrysinae, albeit with low probability. In all estimated scenarios the evolutionary rates found in non-nothancyline Chrysopinae were significantly higher than in any other lineage.

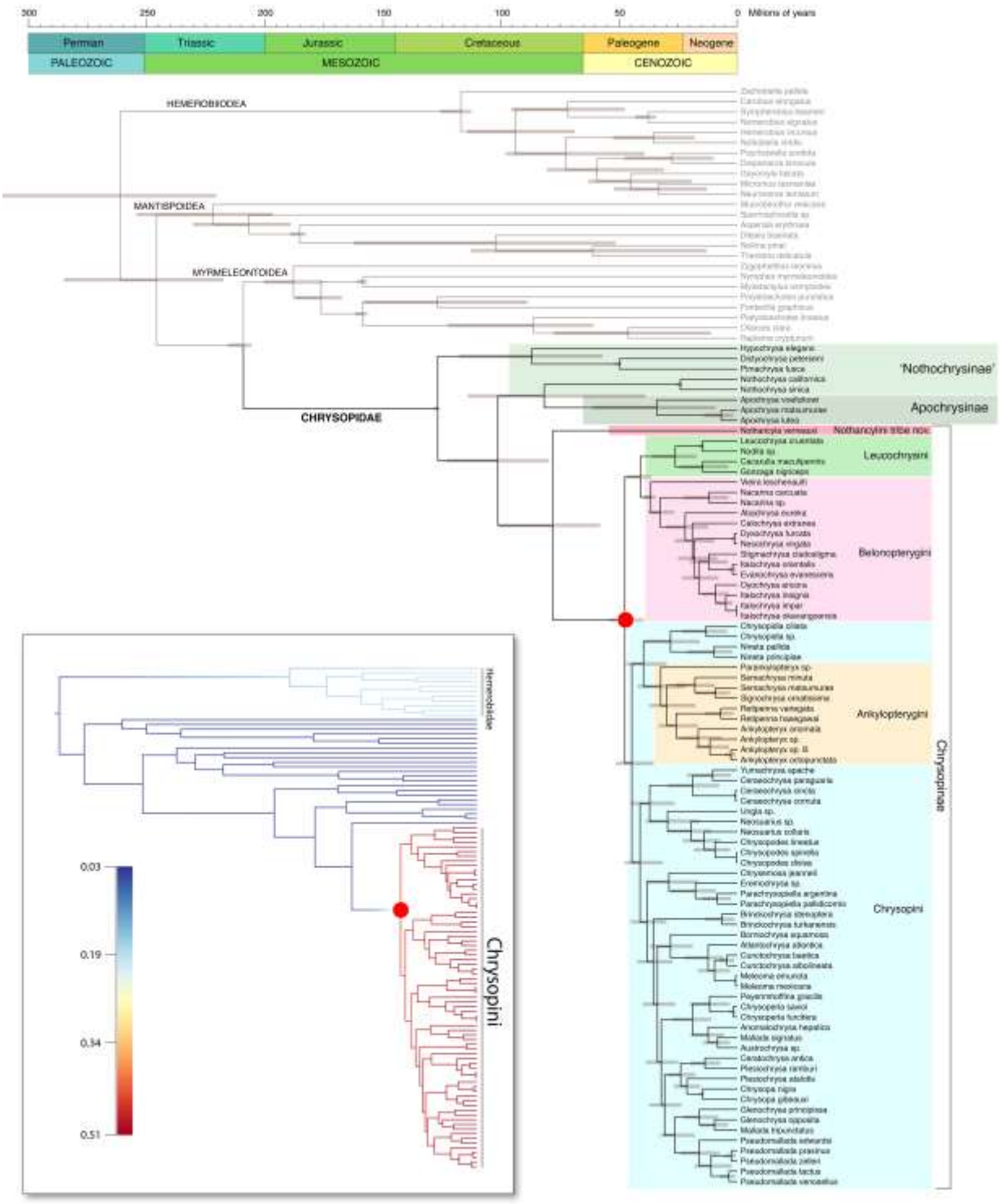

Figure 2. Chronogram of green lacewing divergence time estimates. Inset represents Phylorate plot showing net diversification rate based on the maximum clade credibility tree. Colours of branches indicate the mean evolutionary rate [relative rates from blue (slower) to red (faster)]. The red circle represents the shift in diversification rate.

Discussion

Nothochrysinae and Apochrysinae

The extant Chrysopidae have long been traditionally divided into three subfamilies, Nothochrysinae, Apochrysinae and Chrysopinae – whose relationships have been difficult to resolve conclusively. Based on traditional morphology, Nothochrysinae have usually been considered the sister to Apochrysinae + Chrysopinae (Adams, 1967; Tjeder, 1966; Brooks & Barnard, 1990, Brooks, 1997; Archibald *et al.*, 2014), but no quantitative analyses have recovered this topology with any strong statistical support. Instead, most quantitative analyses, especially those using molecular data, have recovered either Chrysopinae as sister to Apochrysinae + Nothochrysinae (i.e., Haruyama *et al.*, 2008; Duelli *et al*., 2014; Jiang *et al.*, 2017) or Apochrysinae as sister to Nothochrysinae + Chrysopinae (i.e., Winterton & Freitas, 2006; Dai *et al*., 2017; Garzón-Orduña *et al*., 2018). Our analysis of anchored enrichment data recovered another alternative (Figs 1–2). Nothochrysinae was rendered paraphyletic, with a clade comprising *Hypochrysa*, *Pimachrysa* and *Dictyochrysa* recovered as the sister group to the rest of Chrysopidae, diverging during the Early Cretaceous. Interestingly, the nominal genus *Nothochrysa* was recovered as sister to the rest of Nothochrysinae by Garzón-Orduña *et al.* (2018), while here it was recovered with strong support as the sister to Apochrysinae. It is difficult to reconcile *Nothochrysa* as sister to Apochrysinae, because their respective morphologies are relatively disparate based on wing venation and genitalia, but the separation of *Nothochrysa* from the rest of the nothochrysine genera is not surprising. Amongst the genera with larvae that are known, *Nothochrysa* is the only debris-carrier (Tauber, 2014), and it has at least some similarities in wing venation with members of Chrysopinae and Apochrysinae that are absent from the other 'nothochrysine' genera (Breitkreuz *et al.*, 2017). While novel, this result is consistent with the observation that the subfamily Nothochrysinae contains of a collection of rather heterogeneous genera that are unified only by the shared presence of a variety of plesiomorphic characters. Further study is required to fully elucidate this apparent nothochrysine paraphyly. Moreover, Apochrysinae have been shown to share multiple adult and larval characteristics with Chrysopinae (Brooks & Barnard, 1990; Tauber, 2014; Tauber *et al.*, 2014; Breitkreuz *et al.*, 2017), quite distinct from *Nothochrysa*.

Here *Hypochrysa*, *Pimachrysa* and *Dictyochrysa* represent the clade that is sister to the rest of Chrysopidae. Within this clade, *Pimachrysa* and *Dictyochrysa* were recovered as more closely related to each other than to *Hypochrysa*. Earlier, based on adult genitalic and abdominal characters and the ideas of Tjeder (1966), Brooks & Barnard (1990), and Brooks (1997), Tauber (2014) proposed two groups of genera within Nothochrysinae; now excluding *Nothochrysa*, one clade included *Asthenochrysa*, *Dictyochrysa*, *Hypochrysa* (= *Kimochrysa* Tjeder) and *Triplochrysa* Kimmins, and the second comprising the remaining genera, *Leptochrysa* Adams & Penny, *Pamochrysa* Tjeder and *Pimachrysa*. This proposal is not consistent with the results here, nor with those from the study by Garzón-Orduña et al (2018). The results of Garzón-Orduña *et al.* (2018) also do not support the synonymy of *Kimochrysa* with *Hypochrysa* as proposed by Tauber (2014) on the basis of larval similarities. Molecular data instead indicates close relationships amongst *Dictyochrysa*, *Pimachrysa* and *Kimochrysa*, as well as *Hypochrysa* with *Asthenochrysa*. Additional study will be needed to confirm or refute the nothochrysine polyphyly recovered here.

Chrysopinae

The majority of species richness of green lacewings resides in the subfamily Chrysopinae, with at least 1360 species placed in at least 68 genera worldwide. This subfamily has been long considered

monophyletic based on both adult (e.g., Adams, 1967; Brooks & Barnard, 1990; Winterton & Freitas, 2006; Haruyama *et al.*, 2008; Duelli *et al.*, 2014; Jiang *et al.*, 2017; Dai *et al.*, 2017; Garzón-Orduña *et al.*, 2018) and weak larval characters (Tauber *et al.*, 2014). Our results provide further support for that monophyly and place the origin of the subfamily during the Mid Cretaceous (Fig. 2; Table S2), which is later than the previous estimates by Garzón-Orduña *et al.* (2018) and Jiang *et al.* (2017). We recover the genus *Nothancyla* sister to the rest of Chrysopinae in agreement with several recent studies using DNA sequence data (Dai *et al*., 2017; Jiang *et al*., 2017; Garzón-Orduña *et al*., 2018). Previous morphological studies had placed this monotypic genus in either Apochrysinae or Chrysopinae (Brooks & Barnard, 1990; Winterton, 1995; Winterton & Brooks, 2002), but a study using DNA sequence data placed the genus uneasily as sister to Nothochrysinae (Winterton & Freitas, 2006). Based on the strong support for the placement of *Nothancyla* as sister to the rest of Chrysopinae, combined with its unique morphology, we propose the new tribe Nothancylini to accommodate the genus, a result anticipated by Brooks (1997) two decades ago. *Nothancyla* exhibits an intermediate form, with characteristics typical of both Apochrysinae and Chrysopinae, and its placement between these two subfamilies is generally supported on morphological grounds (Winterton & Brooks, 2006). Our decision to place Nothancylini as a new tribe within Chrysopinae instead of a separate subfamily is based largely on the shared presence of a forewing tympanum and similarities in genitalic morphology between *Nothancyla* and the rest of Chrysopinae.

Chrysopinae is subsequently divided into two major clades, diverging at the end of the Cretaceous to the mid Paleogene. One clade contains Belonopterygini sister to Leucochrysini, and the other clade comprising a paraphyletic tribe Chrysopini with Ankylopterygini nested within. The relatively close proximity of the beginning of this radiation to the K-T boundary is an interesting temporal juxtaposition. It is possible that the current disproportionately species-rich fauna of non-nothancyline Chrysopinae may trace its ultimate cause to a dramatic increase in niche availability following the K-T boundary event, perhaps coupled with a genetic bottle-neck in populations of the non-nothancyline chrysopine ancestor that survived the event. However, the 15–20 Ma time lag between the K-T event and the estimated age of the first post-K-T cladogenetic event of this lineage (Fig. 2, red dot) suggests restraint in overemphasizing any immediate and direct effect of the K-T event on the initiation of the subsequent chrysopine radiation, based on current knowledge. Neither of the other two chrysopid subfamilies (Nothochrysinae and Apochrysinae) exhibit a marked Paleogene radiation event, and instead, at least in the case of Nothochrysinae (with numerous fossils known), appear to have undergone a reduction in diversity at the end of the Cretaceous with no subsequent increase in diversification rate, ultimately resulting in the relatively lower species-richness of those subfamilies in the extant fauna.

Belonopterygini and Leucochrysini

The sister group relationship between Belonopterygini and Leucochrysini recovered here with strong support has been widely accepted previously based on genitalic characters (Brooks & Barnard, 1990; Brooks, 1997) and previous molecular data (Winterton & Freitas, 2006; Garzón-Orduña *et al*., 2018). The close relationship here between *Gonzaga* and *Cacarulla* Navás was also recovered by Garzón-Orduña *et al.* (2018), in a clade sister to the species-rich genera *Leucochrysa* McLachlan and *Nodita* Navás (sometimes considered subgenera). Tauber (2007) previously transferred *Vieira* from Leucochrysini to Belonopterygini based on both adult and larval characters which was supported by Garzón-Orduña *et al.* (2018) and in this analysis. As with Garzón-Orduña *et al.* (2018), we too recovered *Vieira* as the sister to the remainder of Belonopterygini, followed by the New World genera *Nacarina* and *Abachrysa*. Old World Belonopterygini genera are again recovered as more derived, with the Australian genus *Calochrysa*

Banks sister to the remainder. With the larger sampling of genera here compared to Garzón-Orduña *et al.* (2018), we recovered *Italochrysa* rendered paraphyletic by *Stigmachrysa*, *Oyochrysa* and *Evanochrysa*, suggesting a possible future need to synonymize these very similar genera with *Italochrysa*. Brooks (1984) previously suggested a possible close relationship between *Oyochrysa* and *Italochrysa*. The Afrotropical genera *Nesochrysa* and *Dysochrysa* were recovered as sister taxa in a lineage separate from *Italochrysa sensu lato*. A more extensive phylogenetic review of all Belonopterygini genera is needed to more fully understand their interrelationships and to test whether or not the removal of various genera from *Italochrysa* renders the latter paraphyletic.

Ankylopterygini and Chrysopini

The greatest species richness in green lacewings is contained in the tribe Chrysopini. However, similar to results by Garzón-Orduña *et al.* (2018), our quantitative analyses recover the tribe rendered paraphyletic by Ankylopterygini. The status and placement of Ankylopterygini has been problematic in previous studies, with it being unplaced by Brooks & Barnard (1990) and Haruyama *et al.* (2008), and as sister to Leucochrysini by Winterton & Freitas (2006). More recently, some authors have identified a close relationship between Ankylopterygini and a small group of distinctive Chrysopini genera i.e., *Nineta*, *Tumeochrysa* Needham and *Chrysopidia* (Duelli *et al*., 2014; Mochizuki *et al*., 2017; Garzón-Orduña *et al*., 2018). Consistent with that hypothesis, we recover *Nineta* + *Chrysopidia* as sister to Ankylopterygini. A close relationship between Ankylopterygini, *Nineta*, *Tumeochrysa*, and *Chrysopidia*, is also supported by their derived symmetrical adult mandibles (most chrysopids exhibit plesiomorphic asymmetrical mandibles). Hölzel (1970) and Brooks (1983) also noted this similarity, but did not accord it phylogenetic significance. Morphological characters that support the monophyly of a clade comprising *Nineta*, *Tumeochrysa*, and *Chrysopidia* include an elongated male sternite 9, the presence and unique form of the gonocornua, and proliferation of gradate crossveins in the wings; these gradate crossveins are typically in three rows in *Tumeochrysa* and *Chrysopidia* (Brooks & Barnard, 1990; Brooks, 1997).

Within Ankylopterygini we recovered slightly different intergeneric relationships compared to those by Garzón-Orduña *et al.* (2018), including *Parankylopteryx* as sister to the rest of the tribe instead of sister to *Retipenna*. Brooks (1983) and Breitkreuz *et al.* (2015) had previously considered the close relationship between *Ankylopteryx* and *Sencera* Navás (as subgenera of *Ankylopteryx*) and a more distant relationship to *Parankylopteryx* as a distinct genus – a result that is confirmed here. The sister-group relationship recovered here between *Semachrysa* and *Signochrysa* was similarly recovered by Garzón-Orduña *et al.* (2018), but this pair was placed in a more basal position instead of sister to *Ankylopteryx*. Our result instead supported *Retipenna* as the sister to *Ankylopteryx*.

The largest portion of Chrysopini, comprises the remaining genera arranged in a series of smaller groups of genera in clades that are strongly supported, but with shorter branch lengths. Divergence dating for this part of the tree is slightly younger than that estimated by Garzón-Orduña *et al.* (2018), but well within the expected range in the Paleogene. The first clade to diverge is a collection of New World genera comprising *Yumachrysa*, *Ceraeochrysa* (paraphyletic without *Yumachrysa*), *Ungla*, *Chrysopodes* and *Neosuarius* (paraphyletic without *Chrysopodes*), similar to the results of Garzón-Orduña *et al.* (2018) and others (e.g., Brooks & Barnard, 1990; Tauber, 2010; Mochizuki *et al.*, 2017). Brooks & Barnard (1990) treated *Neosuarius* as a subgenus of *Chrysopodes*, but the paraphyly of *Neosuarius* recovered here suggests that the current subgeneric divisions of *Chrysopodes* may be artificial. The placement of *Yumachrysa* in *Ceraeochrysa* is unusual here, and requires further investigation of members of both genera to confirm this placement, especially *C. paraguaria*.

The next clade comprises a collection of similar-looking, physically diminutive genera, namely *Suarius* Navás, *Eremochrysa*, *Parachrysopiella* and *Chrysemosa*. While we did not include *Suarius* in our analysis (*cf.* Garzón-Orduña *et al*., 2018), we recovered *Chrysemosa* as sister to *Eremochrysa* and *Parachrysopiella*. Garzón-Orduña *et al.* (2018) also tentatively recovered the enigmatic genus *Kostka* as part of this clade, but it was not available for our analysis and we could not confirm this placement; Brooks (1997) suggested that *Kostka* may be more closely related to *Ungla*.

The widely distributed Old World genus *Brinckochrysa* is recovered next, as a monogeneric lineage. Garzon *et al.* (2018) placed *Brinckochrysa* with *Glenochrysa*, but with weak support. *Brinckochrysa* species are diminutive lacewings and some authors have suggested that the genus is closely related to other genera with male courtship songs, such as *Chrysoperla*, *Eremochrysa* and *Peyerimhoffina* (Brooks & Barnard, 1990; Brooks, 1987). Based on current results, neither *Eremochrysa* nor *Brinckochrysa* group together with *Chrysoperla* and/or *Peyerimhoffina*, suggesting the male courtship songs and male stridulatory structures have evolved independently multiple times in chrysopids. Another genus that possesses male stridulatory structures is *Meleoma*. This genus has been previously associated with *Borniochrysa*, *Nipponochrysa* Tsukaguchi, *Atlantochrysa* and *Cunctochrysa* (Brooks & Barnard, 1990); our results support with this grouping and is similar to the phylogenetic results of Duelli *et al.* (2014) and Garzón-Orduña *et al.* (2018). In contrast to Garzón-Orduña *et al.* (2018) though, we did not recover *Glenochrysa* close to these genera.

The remaining genera of Chrysopidae sampled here, with few exceptions, are those principally included in the *Mallada* and *Chrysopa* genus groups (*sensu* Brooks, 1997), whose relationships have been difficult to elucidate in all previous large-scale quantitative studies using DNA sequence data (i.e., Winterton & Freitas, 2006; Haruyama *et al*., 2010; Duelli *et al*., 2014; Garzón-Orduña *et al*., 2018). Most branches in this clade are strongly supported, even though their branch lengths are relatively very short. The close relationship amongst the genera *Chrysoperla*, *Peyerimhoffina*, *Mallada* and *Anomalochrysa*, as found at least in part by previous authors (e.g., Brooks & Barnard, 1990; Brooks, 1994, 1997; Haruyama *et al*., 2008; Duelli *et al*., 2014; Mochizuki *et al*., 2017; Garzón-Orduña *et al*., 2018), is again recovered here and is supported by a series of male genitalic characters. The inclusion of *Austrochrysa* Esben-Petersen in this clade as sister to *Mallada* is novel, although Esben-Petersen (1928) previously suggested a close relationship between *Austrochrysa* and *Anomalochrysa*. The sister group relationship between *Chrysoperla* and *Peyerimhoffina* is again well-supported, and contrary to Garzón-Orduña *et al.* (2018) and Mochizuki *et al.* (2017) we recovered *Chrysoperla* and *Peyerimhoffina* reciprocally monophyletic. The close relationship between these two genera and the possible paraphyly of *Chrysoperla* by *Peyerimhoffina* deserves additional scrutiny using an expanded taxa sampling to confirm the status of *Peyerimhoffina* as a distinct genus.

The close relationship between *Chrysopa* and *Plesiochrysa* is well supported here, which accords well with their placement as subgenera by some authors based on adult and larval morphology (Adams, 1982; Brooks & Barnard, 1990; Penny 2002). Previous molecular studies also have recovered a strong sister group relationship between the two, regardless of status (Winterton & Freitas, 2006; Haruyama *et al.*, 2008; Duelli *et al.*, 2014; Garzón-Orduña *et al*., 2018). *Ceratochrysa* has been treated as a subgenus of *Chrysopa* (Tjeder, 1966), or as a separate genus (Brooks & Barnard, 1990). Similar to Mochizuki *et al.* (2017), our analyses recover a paraphyletic *Plesiochrysa* relative to both *Ceratochrysa* and *Chrysopa*, and suggests a possible synonymy of the two smaller genera with *Chrysopa*. More study is needed for this group of genera with greater taxon sampling to further assess the status of these three genera relative to each other.

Our phylogeny grouped *Glenochrysa* with *Pseudomallada* and a species of *Mallada* (i.e., *Mallada tripunctatus* (McLachlan)). The placement of *M. tripunctatus* rendering *Glenochrysa* paraphyletic is surprising considering *M. tripunctatus* exhibits none of the typical characteristics of *Glenochrysa* (e.g., wing markings, male genitalic gonocristae and prothoracic eversible glandular sac). While *M. tripunctatus* is not typical of many species of *Mallada*, placement in *Glenochrysa* would not be supported on the basis of morphology. The sister group relationship of *Glenochrysa* and *Pseudomallada* was also recovered by Mochizuki *et al.* (2017). Various authors have displayed the polyphyletic nature of the heterogeneous genus *Apertochrysa* (e.g., Duelli *et al.*, 2017; Mochizuki *et al.*, 2017; Garzón-Orduña *et al*., 2018), providing support for the transfer of multiple species previously contained within that genus to other genera such as *Cunctochrysa* and *Pseudomallada*. In this case we recover *Apertochrysa edwardsi* (Banks) in *Pseudomallada*.

Taxonomy
Nothancylini trib. nov.
Type genus: *Nothancyla* Navás, 1910: 51.

Diagnosis. Antennal flagellomeres with five annular rows of setae; wings broad, ovoid; forewing costal area broad basally, basal sc-r crossveins absent; intramedial cell present, quadrangular; tympanum present in forewing; pseudomedial vein continuous with outer gradates series; mandibles broad, not scythe-like; male genitalia with ectoproct elongate, narrowed posteriorly; gonarcus reduced in size, gonocornua absent; parameres (=gonapsis) and tignum absent; female genitalia lacking praegenitale.

Included genera. *Nothancyla* Navás (Fig. 3).

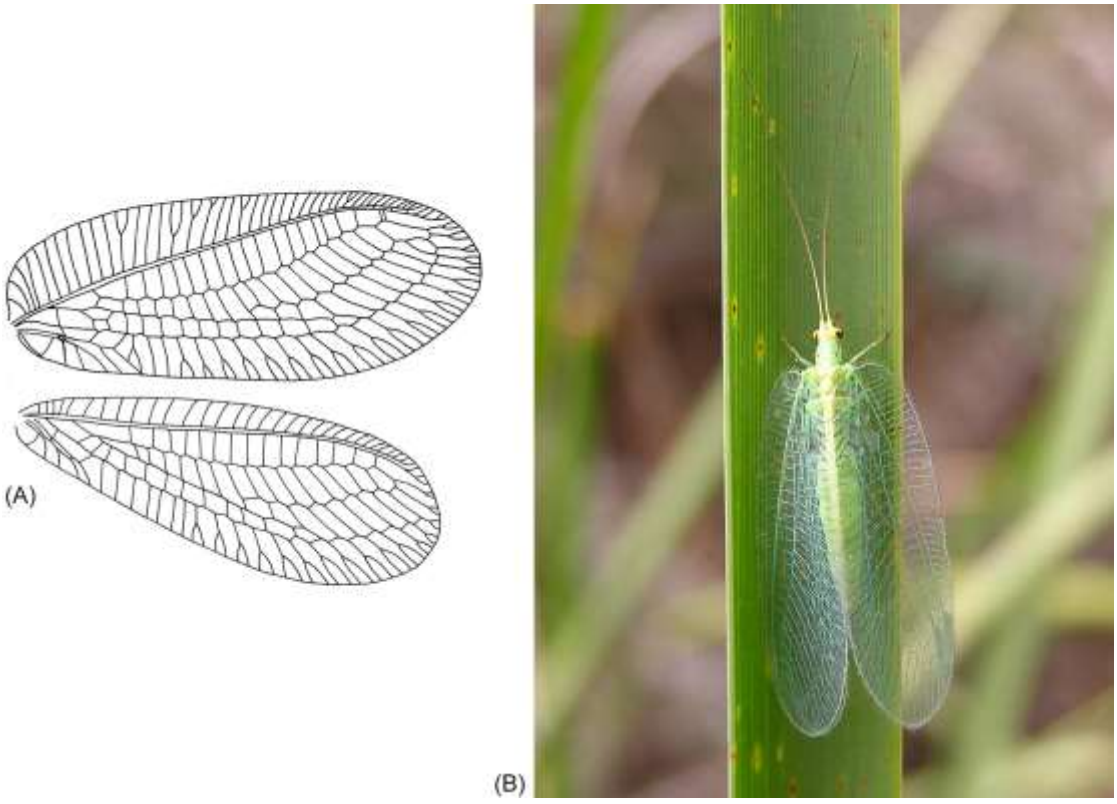

Figure 3. *Nothancyla verreauxi* Navás. (A) Wings; (B) living adult (photo © Kristy Ellington).

Comments. Nothancylini is placed in the subfamily Chrysopinae based on the presence of a forewing tympanum, a synapomorphy of the subfamily (Breitkreuz *et al*., 2017). The tribe contains only the monobasic genus *Nothancyla* (type species: *Nothancyla verreauxi* Navás, 1910), which has a Bassian (south temperate) distribution in southern Australia.

Conclusions

Using anchored hybrid enrichment phylogenomic data we recovered a strongly supported phylogeny of the family Chrysopidae that is closely congruent with other recently published molecular phylogenies, particularly those of Mochizuki *et al.* (2017) and Garzón-Orduña *et al*. (2018). The consensus that is emerging from these works is increasing our confidence that we are making substantial progress toward better understanding the deep phylogenetic relationships among green lacewings, knowledge that is a prerequisite for developing a robust, phylogenetically-based, classification for the family that can serve as a general reference scheme for the group. The current results suggest that additional phylogenetic focus is needed on nothochrysine genera, particularly those that have not yet been included in molecular phylogenetic analyses, in order to more confidently resolve basal relationships within the family. But, monophyly of the dominant radiation of green lacewings, the Chrysopinae, was strongly supported, and the erection of a new basal chrysopine tribe, the Nothancylini, together with strong support for the monophyly of several pre-existing chrysopine tribes (Leucochrysini, Belonopterygini, and Ankylopterygini) and the identification of several new monophyletic groupings of genera, provides a solid phylogenetic basis from which to begin reconsideration of the higher taxonomy of the non-nothancyline Chrysopinae.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Supplementary documents

Supplementary File S1. Chrysopidae Anchored hybrid enrichment alignment. [Note: this file will be provided for online publication but it too large for inclusion for review (500+ pages)]

Supplementary File S2. Chrysopidae Anchored hybrid enrichment, Partition data sets.

Supplementary Table S1. Taxa used in this study, including SRA Accession numbers.

Supplementary Table S2. Divergence times estimates (mean ages and ranges) and branch support values for nodes in Fig. 2 and Suppl. Fig. S1. (PP = posterior probability).

Supplementary Figure S1. Chronogram node numbers and fossils

Supplementary Figure S2. Maximum likelihood phylogeny of Chrysopidae using AHE data. Bootstrap support values are indicated on nodes and grouped by colour according to value.

Supplementary Figure S3. Nucleotide Astral tree

Supplementary Figure S4. BAMM plot showing the two most common shift configurations in the credible set. The 'f' number corresponds to the proportion of the posterior samples in which this configuration is present.

Supplementary Figure S5. Macroevolutionary cohort matrix for diversification. Each cell in the matrix is coded by a colour denoting the pairwise probability that two species share a common macroevolutionary rate regime. The maximum clade credibility tree is shown for reference on the left and upper margins of each cohort matrix.

Supplementary Figure S6. BAMM rate shift tree showing the overall best fit configuration. Red circles signify placement of shifts.