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

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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 n. sister to the rest of the subfamily. Leucochrysini was recovered sister to Belonopterygini, and Chrysopini was 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 (c. 45 Ma).

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 c. 26 species (five genera) and 20 species (nine genera), respectively. Coincidentally, Apochrysinae are almost pan-tropical, and 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 c. 68 genera distributed in all major biogeographical regions. The subfamily is further divided into four tribes: Leucochrysinini, 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; Winterton & Brooks, 2002; Nel et al., 2005) and molecular data (Winterton & Freitas, 2006; Haruyama et al., 2008; Duelli et al., 2014, 2017; Dai et al., 2017; Garzón-Orduña et al., 2019). 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. (2019), which incorporated data from 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 Leucochrysinini and Belonopterygini as sister groups, and Chrysopini was 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. (2019). 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., 2019). 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 of Chrysopidae, 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 previously been considered to be contentious (e.g. Nothancyla, Vieira Navás, Retipenna Brooks, Kostka Navás, Gonzaga Navás). Outgroups were selected from a wide variety of other lacewing families, including Lithinidae, Nymphidae, Psychopidae, Mantispidae, Berotidae, Rhabichrobothidae 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, 2018; Garzón-Orduña et al., 2016; Wang et al., 2017), 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 µL RNase/20 µg tissue after the samples were lysed to remove RNA; and (ii) heating the elution buffer to 55°C before the elution step. We performed two separate elutions for samples with 30 and 50 µL each
time. A final step of drying the DNA pellet was done in some instances. After the extraction, the resulting DNA concentration and quality of each sample were quantified using a Denovix nanodrop spectrophotometer (Wilmington, DE, U.S.A.). 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 −80°C. Vouchers are deposited in the California State Collection of Arthropods. 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 Neuroptera. All probes and Illumina libraries were prepared at the Center for Anchored Phylogenomics (http://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* Naváš (Chrysopidae).

**Orthology determination and alignment generation**

Putative orthologues were identified for each locus following Prum et al. (2015), which uses a neighbour joining-based clustering algorithm based on alignment-free pairwise sequence divergences. Clusters formed through this process were then screened for taxon presence. Assembled contigs derived from fewer than 17 reads were removed. Clusters containing <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 ten reliable sites were masked. After masking, sites containing <73% unambiguous bases were removed from the alignment. Sequences in each remaining cluster were then aligned using Mafft v.7.023b (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 <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 with rate heterogeneity described by a gamma distribution discretized into four bins (+G). The final alignment and partition file are presented in Supporting Information, Files S1 and S2, respectively. We estimated the phylogeny using Bayesian inference (BI) in exabayes v.1.4 (Aberer et al., 2014). We performed two independent runs with four coupled Markov chain Monte Carlo (MCMC) chains each, sampling every 1000 generations and applying uniform priors to tree topologies and an exponential prior to branch lengths. After 50 million generations, we assessed convergence by computing the average standard deviation of split frequencies (ASDSF) 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 <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 BEAST based on the partition scheme and models proposed by partitionfinder (see Phylogenetic analyses section earlier), with model selection based on the 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 for both uncer-
tainty 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 genera-
tions each. We then evaluated the convergence and mixing of the
MCMC chains in TRACER v.1.6, ensuring that the two runs converged
on the same distribution and ascertained that the ESS
were > 200. We then resampled the resulting files of the inferred
phylogenetic trees with a frequency of 100 000 in LOGCOMBINER
v.2.3.1 (BEAST package) and a burn-in of 30%. This resulted in
93 075 subsampled trees. We then summarized the subsampled
trees in a maximum clade credibility tree with common ancestor
heights as node heights using TREEANNOTATOR v.2.3.1 (BEAST
package).

Diversification rates estimation

We used BAMM to assess diversification rate shifts across the
Neuroptera phylogeny (Rabosky, 2014). We used the maxi-
mum clade credibility phylogeny from the BEAST analysis as
input, with sampling probabilities estimated using the extant
species diversity according to the online database Neuroptera
Species of the World (Oswald, 2018). The sampling proportions
were set as follows: Neuroptera, 0.02; Rhachiberothinae, 0.08;
Berothinae, 0.01; Mantispidae, 0.01; Psychopsidae, 0.04;
Nymphidae, 0.06; Ithonidae, 0.13; Nothochrysidinae (Chrysopi-
dae), 0.13; Nothochrysa McClachlan, 0.12; Apochrysidinae, 0.05;
Nothanchyla Navás, 1; Leucochrysa, 0.01; Belonopterygini, 0.05; Chrysopini 1 (Chrysopidae Navás + Nineta Navás), 0.034;
Ankylopterygini, 0.05; Chrysopini 2, 0.02. We used the ‘set-
BAMMPriors’ 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 1000 genera-
tions, 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 bp after trimming, representing
372 loci. The complete nucleotide alignment had 9.5% of miss-
ing data and an average locus length of 368 bp. In all analyses,
the tree topology (Fig. 1) was very strongly supported through-
out and largely congruent among astral (Fig. S3), maximum
likelihood (ML) and BI results, except in a few near terminal
nodes in Chrysopini. In the BI tree all but five nodes had poste-
rior probabilities of 1.0. In the ML tree (Fig. S2), overall support
for nodes was slightly lower, with the same five nodes hav-
ing 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. Psychopidae, Myrmeleontidae,
Ascalaphidae, Nemopteridae, Nymphidae and Ithonidae) (sensu
Winterton et al., 2018) were instead recovered as the sister
group to Chrysopidae. Chrysopidae were recovered as mono-
phyletic, originating in the Late Triassic, with one lineage of a
paraphyletic Nothochrysidinae as sister to the rest of the family.
This clade, comprising Hypochrysa Hagen, Pimachrysa Adams
and Dictyochrysa Esben-Petersen, was recovered separate to
Nothochrysa McClachlan, which itself was placed as sister to
Apochrysidinae in a clade sister to Chrysopinae. Chrysopinae
was recovered as a strongly supported monophylum diverging
from Apochrysidinae and Nothochrysidinae during the Early Cre-
taceous (102 Ma) (Figs 2, S1; Table S2). Chrysopinae was then
divided subsequently into three main lineages. Nothanchyla was

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

<table>
<thead>
<tr>
<th>Node</th>
<th>Fossil species</th>
<th>Placement</th>
<th>Age (Ma)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Cretomerothia disjuncta Ponomarenko</td>
<td>Crown Neuroptera</td>
<td>112</td>
<td>Nel et al. (2005)</td>
</tr>
<tr>
<td>5</td>
<td>Notiobiola thomasta Oswald</td>
<td>Crown Notiobiola</td>
<td>14</td>
<td>Oswald (1999)</td>
</tr>
<tr>
<td>7</td>
<td>Sympherothias compositus Makarkin &amp; Wedmann</td>
<td>Crown Sympherothias</td>
<td>14</td>
<td>Makarkin &amp; Wedmann (2009)</td>
</tr>
<tr>
<td>15</td>
<td>Liasochothia stigmatica Ansong &amp; Schlüter</td>
<td>Crown Mantispidae</td>
<td>182</td>
<td>Ansong &amp; Schlüter (1990)</td>
</tr>
<tr>
<td>18</td>
<td>Triassosphysops superbus Tillyard</td>
<td>Stem Psychopidae</td>
<td>205</td>
<td>Tillyard (1922)</td>
</tr>
<tr>
<td>20</td>
<td>Liminynpha makarkini Ren &amp; Engel</td>
<td>Stem Nymphidae</td>
<td>156</td>
<td>Ren &amp; Engel (2007)</td>
</tr>
<tr>
<td>21</td>
<td>Daonymphes bisulia Makarkin et al.</td>
<td>Crown Nymphidae</td>
<td>156</td>
<td>Makarkin et al. (2013)</td>
</tr>
<tr>
<td>26</td>
<td>Paralembochrysa splendida Nel et al.</td>
<td>Crown Chrysopidae</td>
<td>125.5</td>
<td>Nel et al. (2005)</td>
</tr>
<tr>
<td>31</td>
<td>Nothochrysa stamperini Nel &amp; Séméria</td>
<td>Crown Nothochrysa</td>
<td>23</td>
<td>Nel &amp; Séméria (1986)</td>
</tr>
<tr>
<td>35</td>
<td>Paleochrysothia montielensis Séméria &amp; Nel</td>
<td>Crown Chrysopidae</td>
<td>34</td>
<td>Séméria &amp; Nel (1990)</td>
</tr>
<tr>
<td>38</td>
<td>Leucochrysa nodita priscus Engel &amp; Grimaldi</td>
<td>Crown Leucochrysa</td>
<td>13.7</td>
<td>Engel &amp; Grimaldi (2007)</td>
</tr>
<tr>
<td>40</td>
<td>Belonopterygini larva</td>
<td>Crown Belonopterygini</td>
<td>34</td>
<td>Archibald et al. (2014)</td>
</tr>
<tr>
<td>99</td>
<td>Chrysopa glaucaria Engel &amp; Grimaldi</td>
<td>Crown Chrysopa</td>
<td>13.7</td>
<td>Engel &amp; Grimaldi (2007)</td>
</tr>
</tbody>
</table>

Fig. 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. [Colour figure can be viewed at wileyonlinelibrary.com].
recovered as sister to the rest of Chrysopinae, diverging during the Late Cretaceous. The placement of this genus outside of the currently recognized 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 (41 Ma) and relatively shortly after the Cretaceous–Tertiary (K–T) extinction event – one comprising the tribes Leucochrysiini 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, S5), 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, which is probably indicative of this rapid increase in diversification rate.

Leucochrysiini diverged from Belonopterygini during the Mid-Paleogene (41 Ma), with both tribes recovered here as reciprocally monophyletic. The New World genus *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 a distinct basal dichotomy, represented by one subclade comprising *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 *Chryso- pidia*), a group of exclusively New World genera – *Yumachrysa* Banks, *Ceraceochrysa* 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 retain 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 *Bormiochrysa* Brooks & Barnard, *Atlantochrysa* Hözel, *Meloeoma* Fitch, and *Cunctochrysa* Hözel, and another comprising *Chrysopea* Leach, *Ceraceochrysa* 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 an increase in evolutionary rates occurring either along the branch leading to the crown Hemerobiidae or within Hemerobiidae (the latter with low posterior probability; see Figs S4, S5). BAMM also identified a scenario with three rate shifts, one in Hemerobiidae, another in Chrysopinae and a third 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.

**Discussion**

**Nothochrysininae and Apochrysinae**

The extant Chrysopidae have long been traditionally divided into three subfamilies – Nothochrysininae, Apochrysinae and Chrysopinae – whose relationships have been difficult to resolve conclusively. Based on traditional morphology, Nothochrysininae have usually been considered as sister to Apochrysinae + Chrysopinae (Tjeder, 1966; Adams, 1967; 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 + Nothochrysininae (i.e. Haruyama et al., 2008; Duelli et al., 2014; Jiang et al., 2017) or Apochrysinae as sister to Nothochrysininae + Chrysopinae (i.e. Winterton & Freitas, 2006; Dai et al., 2017; Garzón-Orduña et al., 2019). Our analysis of anchored enrichment data recovered another alternative (Figs 1, 2). Nothochrysininae 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 Nothochrysininae by Garzón-Orduña et al. (2019), whereas here it was recovered with strong support as 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 nothochrysininae 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 ‘nothochrysininae’ genera (Breitkreuz et al., 2017). Although novel, this result is consistent with the observation that the subfamily Nothochrysininae contains 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 nothochrysininae paraphly. Moreover, Apochrysinae have been shown to share multiple adult and larval characteristics with Chrysopinae (Brooks & Barnard, 1990; Tauber, 2014; Tauber

Fig. 2. Chronogram of green lacewing divergence time estimates. Inset represents Phylorate plot showing net diversification rate based on the maximum clad 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. [Colour figure can be viewed at wileyonlinelibrary.com].

et al., 2014; Breitkreuz et al., 2017), quite distinct from Nothochorysa.

Here Hypochorysa, 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 Hypochorysa. 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 Nothochrysiinae: now excluding Nothochorysa, one clade included Asthenochrysa, Dictyochrysa, Hypochorysa (= Kimochrysa Tjeder) and Triplochrysa Kimmins, and the second comprised the remaining genera, Leptochorysa Adams & Penny, Pamochrysa Tjeder and Pimachrysa. This proposal is not consistent with the results here, or with those from the study by Garzón-Orduña et al. (2019).

The results of Garzón-Orduña et al. (2019) also do not support the synonymy of Kimochrysa with Hypochorysa as proposed by Tauber (2014) on the basis of larval similarities. Molecular data instead indicate close relationships amongst Dictyochrysa, Pimachrysa and Kimochrysa, as well as Hypochorysa with Asthenochrysa. Additional study will be needed to confirm or refute the nothochorysine 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; Dai et al., 2017; Jiang et al., 2017; Garzón-Orduña et al., 2019) 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. (2019) and Jiang et al. (2017). We recover the genus Nothancyla as 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., 2019). Previous morphological studies had placed this monotypic genus in either Apochoresinae or Chrysopinae (Brooks & Barnard, 1990; Winterton, 1995; Winterton & Brooks, 2002), but a study using DNA sequence data placed the genus uneasily as sister to Nothochorysiinae (Winterton & Freitas, 2006). Based on 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 Apochoresinae and Chrysopinae, and its placement between these two subfamilies is generally supported on morphological grounds (Winterton & Brooks, 2002). Our decision to place Nothancyla 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 genital 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 comprises 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-nothancochrysin Chrysopinae may trace its ultimate cause to a dramatic increase in niche availability following the K–T boundary event, perhaps coupled with a genetic bottleneck in populations of the non-nothancochrysin chrysopine ancestor that survived the event. However, the 15–20Ma 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 (Nothochrysiinae and Apochoresinae) exhibit a marked Paleogene radiation event, and instead, at least in the case of Nothochrysiinae (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., 2019). The close relationship here between Gonzaga and Cacarella Navás was also recovered by Garzón-Orduña et al. (2019), 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. (2019) and in this analysis. As with Garzón-Orduña et al. (2019), 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 with Garzón-Orduña et al. (2019), we recovered Italochorysa rendered paraphyletic by Stigmachrysa, Oyochrysa and Evanochrysa, suggesting a possible future need to synonymize these very similar genera with Italochorysa. Brooks (1984) previously suggested a possible close relationship between Oyochrysa and Italochorysa. The Afrotropical genera Neochrysa and Dysochrysa were recovered as sister taxa in a lineage separate from Italochorysa 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.* (2019), our quantitative analyses recover the tribe rendered paraphyletic by Ankylopterygini. The status and placement of Ankylopterygini have been problematic in previous studies; it was replaced by Brooks & Barnard (1990) and Haruyama *et al.* (2008), and as sister to Leucochrysin 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.*, 2019). 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). 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 gonocornu, and proliferation of gradate crosveins in the wings; these gradate crosveins are typically in three rows in *Tumeochrysa* and *Chrysopidia* (Brooks & Barnard, 1990; Brooks, 1997).

Within Ankylopterygini, we recovered slightly different intergeneric relationships compared with those by Garzón-Orduña *et al.* (2019), including *Parankylopteryx* as sister to the rest of the tribe instead of sister to *Reippena*. 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 *Sgonochrysa* was similarly recovered by Garzón-Orduña *et al.* (2019), but this pair was placed in a more basal position instead of sister to *Ankylopteryx*. Our result instead supported *Reippena* as sister to *Ankylopteryx*.

The largest portion of Chrysopini comprises the remaining genera arranged in a series of smaller genera 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.* (2019), but is 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.* (2019) 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. paraguariarum*.

The next clade comprises a collection of similar-looking, physically diminutive genera, namely *Suarius* Navás, *Eremochrysa*, *Parachrysoptera* and *Chryseneida*. Although we did not include *Suarius* in our analysis (cf. Garzón-Orduña *et al.*, 2019), we recovered *Chryseneida* as sister to *Eremochrysa* and *Parachrysoptera*. Garzón-Orduña *et al.* (2019) 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 monogenic lineage. Garzón-Orduña *et al.* (2019) 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 *Chrysoerla, Eremochrysa* and *Peyerimhoffina* (Brooks, 1987; Brooks & Barnard, 1990). Based on current results, neither *Eremochrysa* nor *Brinckochrysa* group together with *Chrysoerla* 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, Nippomochrysa* Tsukaguchi, *Atlantochrysa* and *Cinquechrysa* (Brooks & Barnard, 1990); our results support this grouping and is similar to the phylogenetic results of Duelli *et al.* (2014) and Garzón-Orduña *et al.* (2019). In contrast to Garzón-Orduña *et al.* (2019), 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.*, 2008; Duelli *et al.*, 2014; Garzón-Orduña *et al.*, 2019). Most branches in this clade are strongly supported, even though their branch lengths are relatively very short. The close relationship amongst the genera *Chrysoerla, 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.*, 2019), is again recovered here and is supported by a series of male genital 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 *Chrysoerla* and *Peyerimhoffina* is again well supported, and in contrast to Garzón-Orduña *et al.* (2019) and Mochizuki *et al.* (2017) we recovered *Chrysoerla* and *Peyerimhoffina* as 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., 2019). 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 Pseudomalладa and a species of Mallada [i.e. Mallada tripunctatus (McLachlan)]. The placement of M. tripunctatus rendering Glenochrysa paraphyletic is surprising, considering that M. tripunctatus exhibits none of the typical characteristics of Glenochrysa (e.g. wing markings, male genitalic gonocristae and prothoracic eversible glandular sac). Although 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 Pseudomalладa 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., 2019), providing support for the transfer of multiple species previously contained within that genus to other genera, such as Cunctochrysa and Pseudomalладa. In this case, we recover Apertochrysa edwardsi (Banks) in Pseudomalладa.

Taxonomy

Nothancylini tribe n.
Type genus: Nothancyla Navás, 1910: 51.

Diagnosis. Antennal flagellomeres with five annular rows of setae; wings broad, ovoid; forewing costal area broad.
Supporting Information

non-nothancyline Chrysopinae. which to begin reconsideration of the higher taxonomy of the groupings of genera, provides a solid phylogenetic basis from lopterygini) and the identification of several new monophyletic chrysopine tribes (Leucochrysini, Belonopterygini and Ankylopterygini, type species: Nothancyla verreauxi Navás, 1910), which has a Bassian (south temperate) distribution in southern Australia.

Included genera. Nothancyla Navás (Fig. 3).

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. (2019). The consensus that is emerging from these works is increasing our confidence that we are making substantial progress towards a better understanding of 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 nothochrysin 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.

Supporting Information

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

File S1. Chrysopidae Anchored hybrid enrichment alignment.

File S2. Chrysopidae anchored hybrid enrichment, partition datasets.

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

Table S2. Divergence time estimates (mean ages and ranges) and branch support values for nodes in Figs 2 and S1. PP, posterior probability.

Figure S1. Chronogram node numbers and fossils.

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

Figure S3. Nucleotide Astral tree.

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.

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 in the left and upper margins of each cohort matrix.

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

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References


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