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Phylogeny and classification of Odonata using targeted genomics

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ABSTRACT

Dragonflies and damselflies are a charismatic, medium-sized insect order (~6300 species) with a unique potential to approach comparative research questions. Their taxonomy and many ecological traits for a large fraction of extant species are relatively well understood. However, until now, the lack of a large-scale phylogeny based on high throughput data with the potential to connect both perspectives has precluded comparative evolutionary questions for these insects. Here, we provide an ordinal hypothesis of classification based on anchored hybrid enrichment using a total of 136 species representing 46 of the 48 families or *incertae sedis*, and a total of 478 target loci. Our analyses recovered the monophyly for all three suborders: Anisoptera, Anisozygoptera and Zygoptera. Although the backbone of the topology was reinforced and showed the highest support values to date, our genomic data was unable to stronglyresolve portions of the topology. In addition, a quartet sampling approach highlights the potential evolutionary scenarios that may have shaped evolutionary phylogeny (e.g., incomplete lineage sorting and introgression) of this taxon. Finally, in light of our phylogenomic reconstruction and previous morphological and molecular information we proposed an updated odonate classification and define five new families (Amanipodagrionidae fam. nov., Mesagrionidae fam. nov., Mesopodagrionidae fam. nov., Priscagrionidae fam. nov., Protolestidae fam. nov.) and reinstate another two (Rhipidolestidae stat. res., Tatocnemididae stat. res.). Additionally, we feature the problematic taxonomic groupings for examination in future studies to improve our current phylogenetic hypothesis.

1. Introduction

Dragonflies and damselflies are highly mobile predatory insects that make up the insect order Odonata. Odonata is a medium-sized insect order of > 6,300 extant species (Schorr and Paulson, 2019), with a large potential for comparative, ecological, physiological, genomic and conservation research (Bybee et al., 2016; Córdoba-Aguilar, 2008; White et al., 2015). This potential is vast because, unlike so many other

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invertebrate groups, the taxonomy and ecology of the entire order are tractable (although still in need of additional research). What is currently missing to tie the ecology and taxonomy of Odonata together, and thus the ability to ask broad evolutionary questions, is a large-scale well-supported phylogeny for the order.

The first evolutionary hypotheses of Odonata relationships were published in the early to mid 20th century (Fraser, 1954, 1957; Munz, 1919; Tillyard, 1917). The first comprehensive cladistic estimates of odonate phylogeny were produced by Trueman (1996) and Rehn (2003), although other earlier works focused on subsets of taxa within the order (Polhemus, 1997). For nearly two decades, odonates have received an increased focus on ordinal- or subordinal-level molecular phylogenetics (Bybee et al., 2008; Carle et al., 2015, 2008; Dijkstra et al., 2014; Dumont et al., 2010; Kim et al., 2014; Letsch et al., 2016ab; Saux et al., 2003). Larger, more taxonomically diverse phylogenies for the major suborders (Anisoptera and Zygoptera) using molecular data have been published with the largest taxon samplings to date being published in 2014 (Dijkstra et al., 2014) for Zygoptera (Fig. 1a) and 2015 (Carle et al., 2015) and 2016 (Letsch et al., 2016a,b) for Anisoptera (Fig. 1b). These higher-level, large taxon approaches to odonate phylogenetics have been largely congruent with other phylogenetic efforts supporting the relative position of several families, but also highlighted some difficult problems in odonate phylogenetics (Fig. 1). For example, convincing nodal support for the relationship between the dragonfly families Gomphidae and Petaluridae remains elusive, as does the relationship of the damselfly family Isostictidae to the other Zygoptera. Further, these phylogenies have had relatively poor statistical support across the nodes that make up the backbone (herein defined as interfamilial relationships). Although not always resolved with high support, the backbone among Anisoptera is relatively stable between current phylogenetic estimates. Among Zygoptera, the backbone is less stable between phylogenetic estimates and suffers from low branch support (Bybee et al., 2008; Carle et al., 2008; Dijkstra et al., 2014; Dumont et al., 2010; Kim et al., 2014). All phylogenetic efforts to establish a molecular phylogeny of Odonata to date have used largely the same suite of genes (Ballare and Ware, 2011), and in some instances the same taxa. Herein we aimed to produce a large, novel molecular dataset with a broad taxon sample representing both the taxonomic and evolutionary breadth for the order to re-evaluate the phylogeny and classification of Odonata. Our specific goal is to test if a targeted enrichment approach to DNA data generation can provide strength along the backbone, particularly within Zygoptera, and establish a novel working hypothesis of Odonata phylogeny and an updated classification based on genomic data.

2. Materials and methods

2.1. Taxon sampling

A total of 142 taxa were included herein (Supplemental Table 1). Taxa were selected across the current understanding of Odonata classification (Dijkstra et al., 2013; Schorr and Paulson, 2019) with the goal to represent each of the currently recognized families and *incertae sedis* groups among the Zygoptera (Dijkstra et al., 2014). Final taxon sampling included all but two major lineages: *Rimanella* and *Sciotropis*. *Rimanella arcana* is the sole species in the monospecific Rimanellidae and is found throughout the guiana shield. *Sciotropis* is a genus endemic to the northern portion of Venezuela. *Sciotropis* contains two species and currently is classified as *incertae sedis* group 8 (Dijkstra et al., 2013; Schorr and Paulson, 2019). In total, 133 odonate taxa representing 124 genera and 46 of the 48 families or *incertae sedis* zygopteran groups were included in this study. This represents the most phylogenetically diverse and taxonomically comprehensive reconstruction of odonate evolutionary history to date. Outgroups represented the basal most lineages of Insecta and the currently hypothesized sister group to Odonata, Ephemeroptera. In total, three members of Zygentoma (all analyses were rooted to *Atelura formicaria*) and four Ephemeroptera were included.

2.2. DNA extraction

DNA was extracted from specimens preserved in both RNAlater and 95% ETOH and preserved at −80 °C. Tissue from a leg and/or flight muscle directly above the hind legs was dissected and extracted with the Qiagen DNeasy kit following the protocol for animal tissue (Valencia, CA, USA). DNA from each sample was examined using gel electrophoresis to determine the amount of fragmentation and rough estimates of concentration were measured using a Thermo Scientific Spectrophotometer NanoDrop 2000C. Almost all extracted genomic DNA was consumed during sequencing. Any remaining genomic DNA and voucher specimens stored in RNAlater or 95% ETOH were placed for long-term storage at −80 °C in the Insect Genomics Collection (IGC), M.L. Bean Museum, BYU (Provo, UT, USA), the frozen tissue collection (FTC) at the Ware lab at RUN (Newark, NJ, USA), the DNA collection at the Naturalis Biodiversity Center (RMNH, Leiden, Netherlands), the tissue collections of the Alabama Museum of Natural History (ALMNH) or ECOEVO Lab (University of Vigo, Spain). Each voucher is cross-referenced with the corresponding genomic DNA sample within the IGC, FTC, ALMNH electronic databases.

2.3. Probe design

We aimed to develop a probe set capable of enriching any Odonate sample for a set of exons shared by other insects. To accomplish this aim, we employed anchored hybrid enrichment (AHE; Lemmon et al., 2012), following the methodology of Young et al. (2016) and Breinholt et al. (2018), and Haddad and Mckenna (2016), who developed AHE enrichment kits for Diptera, butterflies, and Coleoptera, respectively. The scripts we employed (available in the supplemental material) were derived from Young et al, (2016). We scanned genomic resources from 31 representatives of Odonata for 941 exons commonly shared across insects (see Haddad and Mckenna (2016) for details). The genomic resources we chose to represent the diversity of Odonata (Supplemental Table 2) included published data from 24 transcriptomes (Futahashi et al., 2015; Suvorov et al., 2017), two assembled genomes, as well as low-coverage (whole genome sequencing) WGS from five additional samples generated for this study. For the 5 low-coverage WGS samples DNA was extracted as described above and indexed libraries were created using a Beckman Coulter FXp liquid-handling robot (following Prum et al., 2015) then the libraries were sequenced at ~ 10x coverage (27–62 Gb each) on an Illumina HiSeq2500 with a paired-end 150 bp protocol.

Before scanning all of the genomic resources for the AHE loci we developed two Odonata references using the (unassembled and assembled) genome data, by scanning these data for sequences matching the *Tribolium castenatum* probe region sequences identified by Haddad et al. (2018). We derived the first Odonata reference from the five WGS samples, by merging the overlapping reads to remove low-quality and adapter sequences (Rokyta et al., 2012), mapping the merged reads to the *T. castenatum* sequences, then extending the matching reads into the flanks. Due to the low read coverage (owing to the large genome size), locus recovery for each of the five WGS samples was only modest. So, we combined recovered sequences across the five samples, choosing the extended sequence for each locus that had the greatest sequence similarity to the corresponding *T. castenatum* probe region sequence. We derived the second Odonata reference from the assembled *Ladona fulva* genome by scanning the genome for 20-mers found in the *T. castenatum* genome, then verifying the match if the sequence similarity in a 100 bp containing the match had at least a 55% sequence similarity between *T. castenatum* and *L. fulva*. The genomic region best matching the *T. castenatum* sequence for each locus was utilized downstream (4000 bp surrounding the match location was isolated).

Target regions were identified after aligning the *T. castenatum*, WGS,

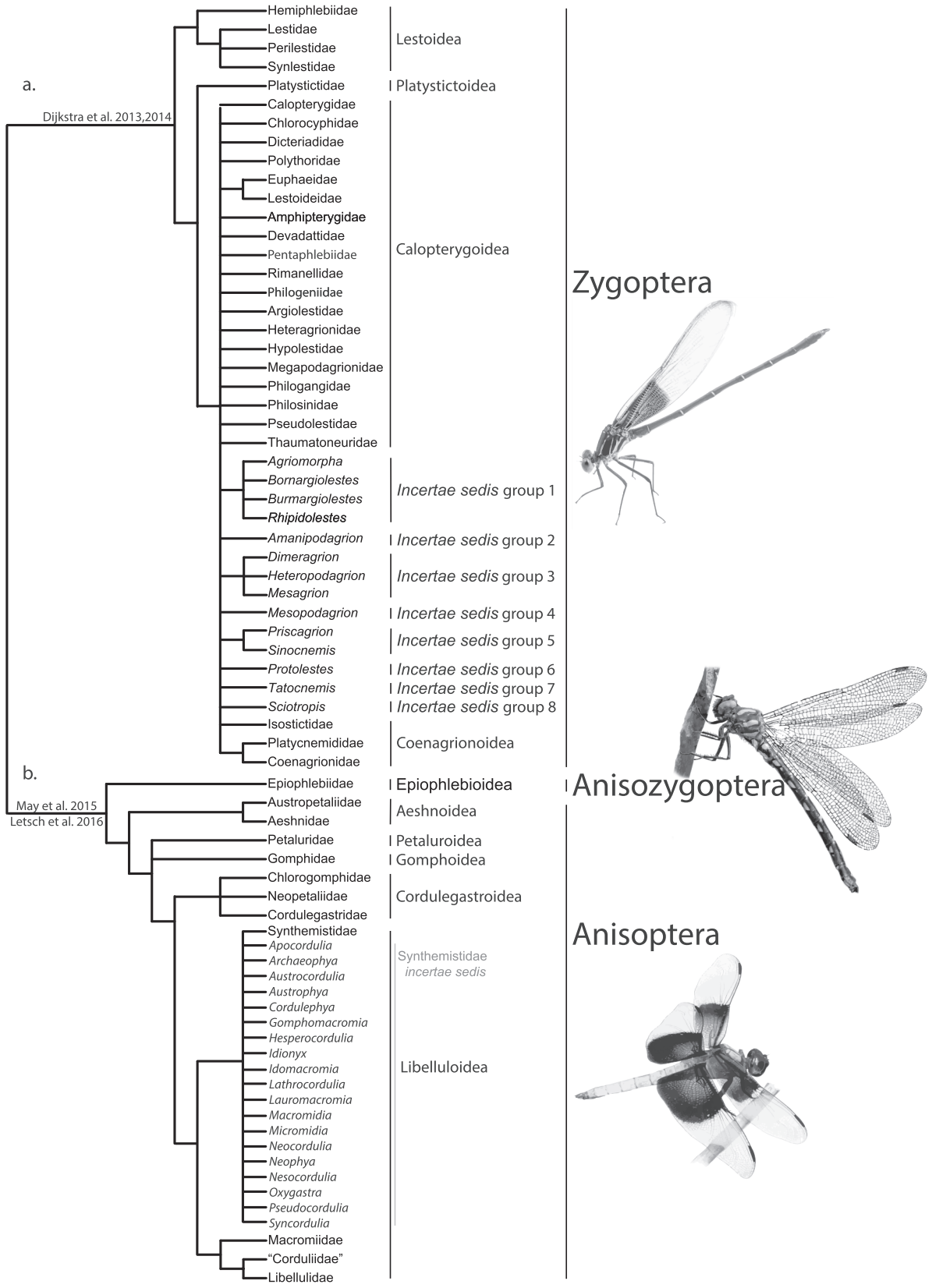

Fig. 1. Current understanding of Odonata phylogeny. Based on the most recent large-scale published phylogenetic results (see figure for citations) and nodes with support < 75% bootstrap value collapsed as well as nodes that were in conflict between the referenced phylogenetic estimates (i.e. Anisoptera). Additionally, *incertae sedis* groups for Zygoptera and *incertae sedis* genera that are currently classified as Synthemistidae are placed in the most definitive placement possible. The resulting topology demonstrates regions in need of resolution. The classification is based on Dijkstra et al. (2013).

and *L. fulva* reference sequences for each locus using MAFFT (Katoh and Standley, 2013) v7.023b (Katoh and Standley, 2013). *Tribolium castenatum* was included so that we could be sure to select regions that included exons targeted by other insect AHE kits. We inspected each alignment in Geneious (R9 Biomatters Ltd.; Kearse et al., 2012), and selected the region containing the *T. castenatum* sequence and the surrounding region that was well-aligned between the WGS reference and *L. fulva*. Loci that did not contain both Odonata references were removed. We then used sequences from these trimmed alignments as references when scanning genomic resources for all 31 of the species (Supplemental Table 2: including rescanning the WGS and *L. fulva* genome). The sequences best matching the Odonata references were selected for each locus-species combination, then aligned in MAFFT (Katoh and Standley, 2013). These alignments are referred to below as the raw AHE alignments.

We also targeted 211 functional loci focused on vision, flight and immunity that were generated but only a few were included in our phylogenetic estimate due to low capture (supplemental Table 2). We inspected each alignment in Geneious and identified two sequences that were well-aligned and spanned the diversity seen in the alignment. Using these two sequences at each locus as references, we scanned the 31 genomic resources for matches to the reference sequences, then isolated and aligned the best-matching sequences for each species for each locus. These alignments are referred to below as the preliminary functional loci.

Using Geneious (Kearse et al., 2012), we manually inspected each of the preliminary AHE and functional loci, trimmed poorly-aligned flanks, and removed poorly-aligned sequences. In order to identify and mask repetitive elements, we estimated the coverage 15-mers found in these sequences in the assembled *L. fulva* genome. We masked alignment regions determined to be repetitive (see Hamilton et al., 2016) for details. In the end, we obtained 405 AHE loci and 209 functional loci. We tiled probes evenly across all sequences in the 614 alignments with 4x coverage. Due to the large number of probes (115,107), we divided the probes randomly into two probe sets for ordering. This probe design is referred to as AHE-Odonata.

2.4. AHE data collection and processing

We collected and analyzed AHE data in collaboration with the Center for Anchored Phylogenomics (www.anchoredphylogeny.com). Following Prum et al. (2015), we fragmented the DNA extracts to approximately 200–400 bp using a Covaris ultrasonicator, prepared indexed libraries using a Beckman Coulter FXp liquid handling robot, and enriched 16-sample pools of the libraries using from two XT kits produced by Agilent (the probe mixes were pooled prior to enrichment). We quantified the enriched library pools using Qubit prior to generating the sequencing pool, which was quantified using Bioanalyzer and Kappa qPCR. We sequenced the libraries on three Illumina HiSeq 2500 lanes with paired-end 150 bp protocol at the Translational Laboratory in the College of Medicine at Florida State University (142 Gb total). After demultiplexing with no mismatches tolerated, we filtered out low-quality reads using the Cassava high chastity filter, then merged the overlapping reads following Rokyta et al. (2012). The merging process corrected sequencing errors and removed library adapters. Supporting data can be found on Dryad (https://doi.org/10.5061/dryad.djh9w0vzr).

We assembled the reads using the quasi-de novo approach described by Hamilton et al. (2016), which includes identifying preliminary matches (17 of 20 bases required) against a set of reference sequences, confirming the match by requiring 55% similarity between the read and the reference sequence and alignment of the reads. We use six references for the assembly: the two references used for the functional locus design and sequences from four of the AHE probe region alignments (*L. fulva*, GAYO01.1.fsa_nt.txt, *Anax junius*, *Gomphus spicatus*). Note that the assembler does not require a close reference to produce high-quality assemblies. We generated consensus sequences from assembly clusters of >15 reads, with ambiguous base calls being assigned when reads produced variable characters that could not be explained by sequencing error. We established orthology among homologs at each locus using a neighbor-joining approach that utilized a pairwise sequence matrix computed using the percentage of shared kmers as the distance metric. We included at most one homolog per sample and discarded orthologous sets for which at least half of the samples were not represented. After aligning orthologous sequences, we identified poorly aligned regions by identifying sites with greater 50% consensus as conserved, then masking regions in each sequence for which 10 of the bases at 20 consecutive conserved sites did not match the majority base. Finally, we removed from the alignments those sites that contained >50 ambiguous bases. Details of this procedure are outlined in Hamilton et al. (2016).

For each unaligned locus we identified the corresponding exons from the *L. fulva* genome (https://www.ncbi.nlm.nih.gov/assembly/GCA_000376725.2/) using NCBI blastn (Camacho et al., 2009) to blast all sequences from each locus to *L. fulva* genome exons. We identified corresponding exons from *L. fulva* as the exon with the most top hits (top hit defined as highest bit score) from sequences in each locus. For loci with an identified *L. fulva* exon we added some ingroup and outgroup taxa by searching transcriptome assemblies (Misof et al., 2014) for the exons with the script genome_getprobe_TBLASTX.py (https://github.com/jessebreinholt/proteinIBA.git). The genome_getprobe_TBLASTX.py identifies all possible matches to an exon that were then screened to be orthologous following Breinholt et al. (2018) using the script ortholog_filter.py. The identified exon from *L. fulva* and orthologous sequences from the transcriptomes were added and aligned with the corresponding loci using MAFFT v7.429 (Katoh and Standley, 2013) linsi algorithm implementing the –adjustdirectionaccurately to adjust the direction of all sequences. The alignment for each locus was screened visually using ALIVIEW 1.26 (Larsson, 2014) and the *L. fulva* exon and additional transcriptomic sequences were excluded when they were obviously misaligned or added significant gaps or large insertion to the loci alignment. To remove sparse flanking regions and alignment columns with random distribution of bases each locus was cleaned with the alignment_DE_trim.py script from Breinholt et al. (Breinholt et al., 2018) using a 75% occupancy and 1.5 entropy across the entire alignment.

2.5. Maximum likelihood model selection/tree estimation

In order to reconstruct the maximum likelihood tree, we first selected an optimal partitioning scheme and nucleotide substitution models in IQ-TREE (Chernomor et al., 2016; Nguyen et al., 2015). We first merged similar subsets, with each subset corresponding to an individual locus that we targeted, using the TESTMERGEONLY option in IQ-TREE v1.6.7 (Nguyen et al., 2015), examining the top 20% of merged partitioning schemes. Models were then estimated for each merged partition using the ModelFinder algorithm as implemented in IQ-TREE v1.6.7 (Nguyen et al., 2015) with all partitions sharing the same set of branch lengths but allowing variable evolutionary rates for each partition (-spp option). Using the best model partitioning scheme estimated above, we conducted 50 partitioned maximum likelihood (ML) tree searches in IQ-TREE v1.6.7 (Nguyen et al., 2015). We used a parsimony starting tree for 25 runs and we used a random tree for the remaining 25 runs and chose the best tree based on its likelihood score. Next, we performed an exhaustive bootstrap search (Hoang et al., 2018) with ten separate runs, each with ten bootstrap replicates for a total of 100 bootstraps in IQ-TREE v1.6.7 (Nguyen et al., 2015). To determine if the bootstrap replicates had converged we used AutoMRE convergence criterion in RAXML v8.2.12 (Stamatakis, 2014).

2.6. Bayesian tree estimation

We estimated a tree using a Bayesian framework implemented in the MPI version of ExaBayes version 1.5 (Aberer et al., 2014) with four

independent runs, each with four chains and for 5,000,000 MCMC iterations, sampling every 500th generation.

2.7. Quartet sampling

In order to further investigate phylogenetic support that can be affected by such events as incomplete lineage sorting and introgression, we took the quartet sampling (QS) approach developed by Pease et al. (Pease et al., 2018). Briefly, it provides three scores for internal nodes: (i) quartet concordance (QC) score gives an estimate of how sampled quartet topologies agree with the putative species tree; (ii) quartet differential (QD) estimates frequency skewness of the discordant quartet topologies, which can be indicative of introgression if a skewed frequency is observed and (iii) quartet informativeness (QI) quantifies how informative sampled quartets are by comparing likelihood scores of alternative quartet topologies. Finally, QS provides a quartet fidelity (QF) score for terminal nodes that measures a taxon "rogueness". To run QS analysis with our putative ML species tree (random_0) and the supermatrix (FcC_smatrix.phy), we used an IQ-TREE engine for quartet likelihood calculations specifying 100 replicates (i.e. number of quartet draws per focal branch).

3. Results

3.1. Capture results

From the 614 loci targeted by the Odonata capture probe set, 478 were included in phylogenetic reconstruction (Supplemental Tables 1 & 2). A total of 83,135 parsimony informative characters resulted from the alignment and phylogenetic reconstruction. Anchored hybrid enrichment resulted in an average of 5,260,442 reads per species. Enrichment resulted in an average capture of 369 loci spanning approximately 713 base pairs per locus, with a minimum of 75 loci for *Amphipteryx agrioides* and a maximum of 465 loci for *Austroaeshna pulchra*. Only 7% of species captured<50% of loci (Supplemental Table 1).

3.2. Maximum likelihood model selection/tree estimation

After merging the 478 initial single-locus subsets using TESTMERGEONLY in IQ-TREE, we recovered 92 total subsets. The models selected and used for each subset are given in the supplementary online material (Appendix A). From the 50 independent maximum likelihood searches, the most likely tree resulted from one of the runs using a random starting tree (Supplemental Fig. 1). The AutoMRE criterion confirmed that the bootstrap values had converged after 100 runs, resulting in our final bootstrap scores.

3.3. Bayesian tree estimation

To evaluate the Bayesian analysis for convergence, the average standard deviation of split frequencies (hereafter, asdsf) was calculated every 5,000 generations. The asdsf was calculated as lower than 5% (4.17%) implying convergence had been achieved. A burn-in proportion of 0.25 of the sampled trees was removed prior to the construction of the consensus tree. The final topology (Supplemental Fig. 2) was constructed using the consensus software included in the ExaBayes package (Aberer et al., 2014) from the four independent runs, resulting in our posterior probability values.

3.4. Phylogenetic results

The ML and Bayesian topologies are highly congruent in terms of both support and phylogenetic relationships (Fig. 2, Supplemental Fig. 1, Supplemental Fig. 2). All three suborders are supported as monophyletic, including a first test for the monophyly of extant Anisozygoptera in a broader phylogenetic framework, with strong nodal support. All superfamilies (Dijkstra et al., 2013; Schorr and Paulson, 2019), with the exception of Calopterygoidea, are recovered as monophyletic including the debated Coenagrionoidea with Isostictidae recovered as sister to Platycnemididae + Coenagrionidae. Calopterygoidea is recovered as non-monophyletic and is split into three large lineages and *Priscagrion* as a separate, fourth lineage. One of these major clades contains Calopterygidae and is reconstructed as sister to the Coenagrionoidea. *Hemiphlebia* is sister to all remaining Lestoidea, with that superfamily being sister to all remaining Zygoptera. The genus *Epiophlebia* (the only extant representative of Anisozygoptera) is sister to Anisoptera and Aeshnoidea is sister to the remaining Anisoptera.

3.5. Nodal support

Bootstrap and Posterior Probabilities: Strong bootstrap and posterior probability support is found across the backbone (i.e. inter-familial relationships) of both ML and Bayesian topologies of the phylogeny (Fig. 2), with a few exceptions: Petaluridae + Gomphidae, Pentathemis + Libellulidae, Heteragrionidae + Polythoridae, (Devadattidae + Amphipterygidae) + (Thaumatoneuridae + Rhipidolestidae) and the clade that contains a portion of the Calopterygoidea and Coenagrionoidea. Below the family level, a total of 14 nodes had weak support (bootstrap < 100% and/or posterior probability < 1). This weak support was mostly isolated to Libellulidae with nine of the 14 nodes receiving well below 100% bootstrap and/or 1 posterior probability (average libellulid bootstrap/posterior probability = 73.75/84.6). Other intrafamilial relationships with nodes of lower support, each with one, include: Aeshnidae, Corduliidae, Megapodagrionidae, Calopterygidae and Coenagrionidae. However, these relationships were generally more highly supported with an average bootstrap and posterior probability of 94.8 and 99.8, respectively

3.6. Quartet sampling

We used a quartet sampling (Pease et al., 2018) approach to provide additional insight into nodal support across the phylogeny. Across the entire topology, there were a total of 24 nodes with a negative Quartet Concordance (QC) score among the ingroup. Of these, 16 were found along the backbone of the topology across both Anisoptera and Zygoptera. Specifically, there was a concentration of poor QC scores among the Libelluloidea, 11 total (~46% of all poor QC scores), with five within the libellulids alone. Other poor QC scores were spread throughout the topology. The Quartet Fidelity (QF) scores, essentially a measure of taxon stability, were relatively high across the topology, with > 80% of taxa having a QF score ≥ 75%, demonstrating an overall reliability (i.e., lack of rogueness) of taxa during quartet sampling throughout the topology. However, there was one major exception. Again, Libelullidae demonstrated the lowest overall QF values ranging from 0.66 to 0.4, with an average of 0.57. Three other taxa had a QF < 0.6: *Mesagrion leucorhinum*, *Mesopodagrion* sp. and *Amanipodagrion gilliesi*.

4. Discussion

Nodal support has traditionally been difficult to assess; even before the genomics age of phylogenetics there was uncertainty regarding what bootstrap values represented (Alfaro et al., 2003; Douady et al., 2003; Mort et al., 2000). Overall, our topology was well supported by all measures of traditional nodal support (~13% with a bootstrap and/or posterior probability<100) and quartet sampling (~15% of all nodes with a QC < 0.20). Interestingly, only nine of all lower-supported nodes had both low bootstrap/posterior probability and QC support. We note some interesting observations from overall nodal support. The relationship of Petaluridae and Gomphidae to each other and the remainder of the Anisoptera has been one of the outstanding questions in Anisoptera higher-level phylogenetics. Our data demonstrate the best support for Gomphidae + Petaluridae to date and support them as sister to

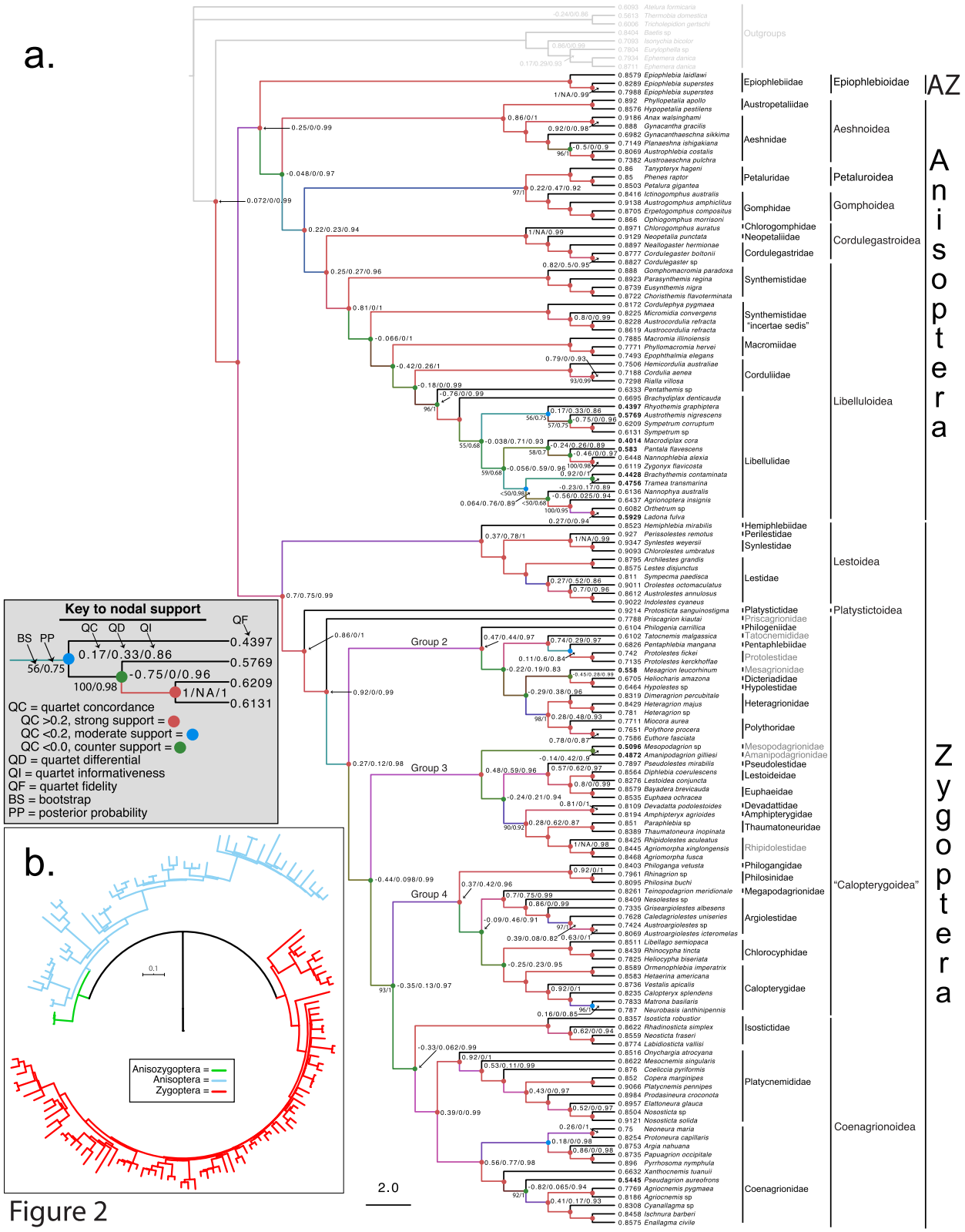

Figure 2

Fig. 2. AHE topology. Results of the phylogenetic reconstruction of Odonata using loci captured from anchored hybridization enrichment. A) ML and Bayesian phylogenetic reconstruction of the Odonata using 478 loci. AZ in the right hand column represents the suborder Anisozygoptera. See "key to nodal support" for a visual guide to nodal support. Support values of 100 bootstrap and 1.0 posterior probability are not shown. Quartet sampling (QS) that shows full support (1/NA/1) is not shown at the node but all other QS is shown at each node along with a measure of taxon rogueness (QF score) at each branch tip. Bolded GF scores represent the lowest values across the topology. Newly established or reestablished families are shown in grey text. B) A representation of the branch lengths are shown with all three suborders designated.

the Cordulegasteroidea + Libelluloidea. Libellulidae is well supported as monophyletic but intrafamilial relationships contain the lowest measures of support across the topology and in terms of a concentration of nodes with low support. This was not surprising as low support has traditionally been a problem not only within Libellulidae, but throughout the broader Libelluloidea (Ware et al., 2012, 2007). Low quartet sampling values are also observed within Aeshnidae, throughout "Calopterygoidea" and within Coenagrionidae although to a much lesser degree and usually not as low. As mentioned above the QF scores were quite robust, with the exception of Libelullidae; this mirrors past phylogenetic efforts to resolve Libellulidae relationships based on Sanger sequencing data, some of which ended up with large polytomies (e.g., Pilgrim and Von Dohlen, 2008; Ware et al., 2007). Bootstrap support for this node was 98, however the overall quartet score is considered robust (QC > 0.20) and is discussed in more detail below.

A deeper look at the quartet differential among the quartet sampling scores can give an indication of what historical evolutionary scenarios might result in the areas of low support observed in our topology. It seems the nodes that have low nodal support and/or negative quartet scores (QC) are likely driven by both introgression (QD = ~0.3) and incomplete lineage sorting (QD = ~1). Indeed, both appear to be present throughout the topology but are most pronounced in two clades of the topology: Libellulidae and group 1 of the "Calopterygoidea". This is an interesting result and one that needs further exploration with more extensive taxon sampling using an anchored approach and/or extensive genome level sequencing for both of these groups.

4.1. Taxonomic implications

We acknowledge that certain areas of the discussion that follow may be uneven, specifically concerning morphological and behavioral observations that would support the relationships recovered by the molecular data. We have chosen to focus the discussion on areas where less is known, and relationships are still uncertain. Additionally, some groups have more observations to discuss than others and/or are part of larger discussions in odonate evolution. What follows is the best presentation of the information (e.g., morphological, behavioral, biogeographical, etc.) and observations that are at the heart of the phylogenetic hypothesis presented herein. We also present the nodal support for each group discussed below adjacent to the taxonomic name for each paragraph heading (Bootstrap = BS, Posterior Probability = PP, quartet sampling = QS).

4.1.1. ***Odonata*** *(BS = 100, PP = 1, QS = 1/NA/1)*

The overall relationships along the backbone of the phylogeny are closely in line with recent phylogenetic hypotheses for Odonata using smaller, Sanger-based, molecular datasets with a similarly sized or larger taxon sample (Bybee et al., 2008; Carle et al., 2015, 2008; Dijkstra et al., 2014; Letsch et al., 2016ab). Our hypothesis differs in that nodal support, both traditional and new approaches, give robust phylogenetic support across the topology (Fig. 2). Further, our hypothesis includes nearly all major lineages of Odonata and is not only the largest molecular phylogeny to date, but the most phylogenetically inclusive hypothesis of Odonata classification. What follows is a more detailed discussion for superfamily or superfamily-level groups across the topology.

4.1.2. ***Zygoptera*** *(BS = 100, PP = 1, QS = 0.7/0.75/0.99)*

The damselflies are recovered as monophyletic with high support. There has been some uncertainty about the monophyly of Zygoptera (Hasegawa and Kasuya, 2006; Saux et al., 2003; Trueman, 1996) and particularly the relationship of Lestoidea to Anisoptera in the past (Hasegawa and Kasuya, 2006; Saux et al., 2003), but it is clear due to high support from both these data and all other recent molecular analyses (Bybee et al., 2008; Dumont et al., 2010; Dijkstra et al., 2014) that Zygoptera is monophyletic.

4.1.3. ***Lestoidea*** *(BS = 100, PP = 1, QS = 0.37/0.78/1)*

The superfamily consists of the four families Hemiphlebiidae, Perilestidae, Synlestidae and Lestidae, and is recovered as the sister group to all other damselflies with high support (BS = 100, PP = 1, QS = 0.7/0.75/0.99). The Australian monospecific family Hemiphlebiidae (*Hemiphlebia mirabilis*) was recovered as sister to the remaining Lestoidea, supporting previous studies (Davis et al., 2011; Dumont et al., 2010; Rehn, 2003). Dijkstra et al. (2014) found Perilestidae and Synlestidae to be paraphyletic in Bayesian analyses, but suggested this was due to the limited number of genes included in their study and retained both families, although the African genus *Nubiolestes* was transferred from Perilestidae to Synlestidae. Perilestidae (one species included herein) and Synlestidae (two species included herein) combined (BS = 100, PP = 1, QS = 1/NA/0.99) and Synlestidae by itself (BS = 100, PP = 1, QS = 1/NA/1) were monophyletic, but taxon sampling was too limited to say if this will hold up once more species are included. Lestidae is also recovered as monophyletic (BS = 100, PP = 1, QS = 1/NA/1). However, in contrast to Dijkstra et al. (2014) the genera *Austrolestes* and *Indolestes* form a fully supported monophyletic group with *Sympecma* when *Orolestes* is included (BS = 100, PP = 1, QS = 0.27/0.52/0.86). Thus, there is some support for the validity of the subfamilies Lestinae and Sympecmatinae. In contrast to other Lestidae, and uniquely within Zygoptera, the genera *Austrolestes*, *Indolestes* and *Sympecma* (but not *Orolestes*) fold their wings on one side of the abdomen at rest. Within Lestidae these three genera are also aberrant in surviving cold (*Sympecma*, *Indolestes*) or dry periods (*Austrolestes*) as adults; thus their adult lifespan is often longer than their nymphal phase (Corbet, 1999).

4.1.4. *Platystictoidea*

This superfamily consists entirely of the family Platystictidae and is represented by only one species herein (*Protosticta sanguinostigma*). Platystictidae, composed of 10 genera and > 280 species, is known from dense tropical forests in Asia and the Neotropics. Its monophyly was already established by previous molecular studies (Bybee et al., 2008; Davis et al., 2011; Dijkstra et al., 2014; Dumont et al., 2010; van Tol and Reijnen, 2009) and is also supported by morphology (Dijkstra et al., 2014; Garrison et al., 2010; Rehn, 2003). The phylogenetic position of Platystictidae as sister to all other Zygoptera except Lestoidea is recovered here with quite high support (BS = 100, PP = 1, QS = 0.86/0/1), confirming the findings of past efforts focused on Zygoptera (Bybee et al., 2008; Carle et al., 2008; Dijkstra et al., 2014; Dumont et al., 2010).

4.1.5. *'Calopterygoidea'*

The families previously included in the superfamily Calopterygoidea are hypothesized to form a non-monophyletic, pectinate assemblage toward Coenagrionoidea consisting of three major groupings and *Priscagrion* (Group 1). Due to low quartet sampling values along the backbone of this assemblage (see Fig. 2), we refrain from establishing new superfamilies until a more thorough taxon sampling, that includes all major genera, has taken place. This is planned for the future. In the meantime we discuss each of the four groups of "calopterygoids" individually.

4.1.5.1. 'Calopterygoidea' group 1 - Priscagrionidae. This group includes only the newly established family Priscagrionidae (see below Revisions to the Classification of Zygoptera), which was represented by a single taxon in our analysis: *Priscagrion kiautai*. Previously placed tentatively in the superfamily Calopterygoidea, it is now recovered as sister to the remaining 'Calopterygoidea' + Coenagrionoidea (BS = 100, PP = 1, QS = 0.92/0/0.99). In the molecular analyses of Dijkstra et al. (2014), *Priscagrion* was found to group with *Sinocnemis* and previously called *Incertae Sedis* Group 5 (Dijkstra et al., 2014; Schorr and Paulson, 2019). Therefore we propose the family, Priscagrionidae (see below Revisions to the Classification of Zygoptera), which includes two genera, *Priscagrion* and *Sinocnemis*, with two and three species respectively. The genera

are restricted to streams in China and Vietnam, are similar in build and coloration with relatively long legs, and share the apparent apomorphy of a drawn-out internal fold on the genital ligula. Remarkably, both genera were described only in the past two decades and little has been published on their behaviour. Both perch with wings open, with *Sinocnemis* resting on broad leaves (Kalkman, 2008) and pictures in (Zhang, 2019). No nymphs are known for either genus.

4.1.5.2. 'Calopterygoidea' group 2 (BS = 100, PP = 1, QS = 0.47/0.44/0.97). Nine families make up Group 2. Included in these nine families, we propose two new families (Mesagrionidae, Protolestidae) and co recognize Tatocnemididae as at the family level (see below Family-level revisions to the Classification of Zygoptera). Seven of these families are unique morphologically and contain either a single genus (Pentaphlebiidae, Hypolestidae, Mesagrionidae, Protolestidae and Tatocnemididae) or two genera (Dicteriadidae and Philogeniidae). Our new phylogenetic hypothesis has no taxonomic consequences regarding the existing families, except for Heteragrionidae, which is expanded to include the genera *Dimeragion* and *Heteropodagrion*. With this addition to Heteragrionidae there are now two families with more than two genera; Heteragrionidae with four genera and Polythoridae with seven genera. With the exception of a well-supported cluster of three Afrotropical families (Tatocnemididae, Protolestidae and Pentaphlebiidae; BS = 100, PP = 1, QS = 0.74/0.29/0.97) Group 2 is largely Neotropical. The relationships between the families are difficult to determine from a morphological and/or behavioural perspective as they have few characters in common. Further, although the clade is well supported overall, the relationships between many of the families are not well supported due to low QS values. This is a clade where additional taxon sampling throughout the clade itself and throughout the "calopterygoidea" in general is likely to reveal much more clarity towards the overall classification.

Protolestidae (BS = 100, PP = 1, QS = 1/NA/1) and Tatocnemididae are here recognised as distinct families (see below Family-level revisions to the Classification of Zygoptera) as they have no clear relatives and are quite distinct in adult and nymphal morphology from their closest relative, Pentaphlebiidae. Both *Protolestes* (eight species described) and *Tatocnemis* (ten species) are restricted to rainforest streams in Madagascar, were until recently included in Megapodagrionidae, and are poorly known and in dire need of taxonomic revision. Adults of both genera perch with wings variably closed or (half) open and the abdomen held roughly horizontal. Protolestid nymphs have fan-shaped caudal gills, a character only shared with the distantly related Argiolestidae (Kalkman et al., 2010) and Mesopodagrionidae (Yu 2016), while adults have a rather wide and slender head, similar to some members of the unrelated Platycnemididae. Based on morphology, Tatocnemididae is not similar to other families: the potential apomorphy of crenulated wing tips is shared only with some genera of the unrelated Platycnemididae. The nymph has inflated saccoid caudal gills bearing a terminal filament as found in several other families of Zygoptera. Tatocnemididae were originally described by Rácenis (1959) as a subfamily of Megapodagrionidae to include *Tatocnemis* and *Archaeopodagrion*, but are now restricted to the genus *Tatocnemis*.

The endemic species *Mesagrion leucorhinum* from the Colombian Andes is found as sister to two small families, Dicteriadidae (two genera each with a single species from the Amazonian region) and Hypolestidae (one genus, three species from the Greater Antilles), but with low QS values (BS = 100, PP = 1, QS = -0.45/0.28/0.99). Both of these families have well defined apomorphies, are fairly distinctive and do not seem particularly close to *M. leucorhinum* from a morphological or behavioral perspective. *Mesagrion leucorhinum* was not included in previous molecular analyses (Bybee et al., 2008; Davis et al., 2011; Dijkstra et al., 2014; Dumont et al., 2010; van Tol and Reijnen, 2009), but based on morphology it was tentatively placed in an *Incertae Sedis* group together with *Dimeragrion* and *Heteropodagrion*. In our analyses *Mesagrion* is not found to be close to these genera. As there are no other likely candidates to be the closest relative of *Mesagrion*, we propose to regard it as a family in its own right Family-level revisions to the Classification of Zygoptera. Apomorphies for this family (although not unique within Zygoptera) are the scarcely sclerotized dorsum of segment eight in the female and the long paraprocts which are serrated at the distal fourth of the dorsal margin (Garrison et al., 2010; Pérez-Gutiérrez and Montes-Fontalvo, 2011). The species rests with its wings closed, which was regarded as an additional indication that *Mesagrion* was close to *Heteropodagrion,* but it is now clear that this habit evolved several times within the families previously grouped into 'Calopterygoidea'.

Recent hypotheses proposed Heteragrionidae to be composed of two South American genera: *Heteragrion* and *Oxystigma* (Bybee et al., 2008; Davis et al., 2011; Dijkstra et al., 2014; Dumont et al., 2010; van Tol and Reijnen, 2009). In their Bayesian analyses (Dijkstra et al., 2014), the South American genera *Dimeragrion* and *Heteropodagrion* were found to be sister to Heteragrionidae and would have been included too were it not for the ML analyses which showed these two genera to be close to Heteragrionidae but with *Rimanella* (Rimanellidae) and *Heliocharis* (Dicteriadidae) intermingled. In our analyses *Dimeragrion* and *Heteragrion* form a monophyletic group (BS = 100, PP = 1, QS = 1/NA/1) suggesting that *Dimeragion* and *Heteropodagrion* indeed should be included as members of Heteragrionidae. In this new definition, the Heteragrionidae include four genera from tropical South-American: *Dimeragrion* (5 species), *Heteragrion* (56 species), *Heteropodagrion* (5 species) and *Oxystigma* (3 species). With the exception of *Heteropodagrion* all these genera have their wings open at rest. The caudal gills of the nymphs of *Heteragrion*, *Heteropodagrion* and *Oxystigma* are saccoid with a constriction at about ¾ length with a slender apical filament. The caudal gills of the nymph of *Dimeragrion* are nearly flat (De Marmels, 1999), but do have a terminal filament and are slightly inflated with a thickened dorsal keel making them three-dimensional (Tennessen, 2010).

The last family included in this section of the 'Calopterygoidea' is Polythoridae (BS = 100, PP = 1, QS = 1/NA/1) which has several apomorphies in the nymphal stage such as lateral abdominal gills on the second to seventh segment, dorsal abdominal knobs and swollen caudal gills with angular or finger-like projections. The molecular revision of the family by Sanchez Herrera et al. (2018) showed that the family is monophyletic. The lateral abdominal gills of the nymphs are reminiscent of those of Euphaeidae, which has led to the suggestion that these families might be related. Our phylogeny shows clearly that these two are not close and that lateral abdominal gills evolved at least twice within Zygoptera.

4.1.5.3. 'Calopterygoidea' group 3 (BS = 100, PP = 1, QS = 0.48/0.59/0.96). This group contains nine different families with relatively little in common morphologically. Most striking is the wide variety of shapes of the nymphal caudal gills: flat and fanlike in Mesopodagrionidae, balloon-shaped in Lestoideidae and Thaumatoneuridae, balloon-shaped with lateral abdominal gills on abdominal segments two to eight in Euphaeidae, balloon-shaped with filamentous gill tufts in Pseudolestidae, sturdy and pyramidal with the epiproct terminating in three points and filamentous gill tufts below them in Devadattidae, and roundish and gradually tapering to a single point in both paraprocts and epiproct with filamentous gills tufts below them in Amphipterygidae. This suggests that there has been strong selection on the nymphal respiratory system in these groups, although nothing is known of the relative advantages of the different shapes of gills in their lotic habitats.

The genera *Amanipodagrion* and *Mesopodagrion* form a clade that is the sister group to all taxa in Group 3 that were previously placed as *incertae sedis* (Bybee et al., 2008; Davis et al., 2011; Dijkstra et al., 2014; Dumont et al., 2010; van Tol and Reijnen, 2009). *Amanipodagrion* is monotypic with the only known species, *A. gilliesi*, being from Tanzania. The species is confined to a single rocky forest stream in the East

Usambara Mountains where the nymph is yet to be discovered. It is a relatively large species with uncertain taxonomic affinities due to its overall morphology, banded wings and a habit of resting in a hanging position that does not match other species. *Mesopodagrion* is known from two species found in China and the northern regions of Vietnam, Thailand and Myanmar. The two *Mesopodagrion* species possess a combination of characters that does not fit any other genus: an apomorphy is the distinct extension of the terminal rim of the 10th tergite between the cerci (Yu and Bu, 2009). The nymphs have flat horizontal caudal gills (Yu, 2016), which are otherwise only found in the unrelated Argiolestidae and Protolestidae (Kalkman et al. 2010). The unique character set of both adults and nymphs, combined with the molecular results, lead us to establish a new family to accommodate this genus (see below Family-level revisions to the Classification of Zygoptera). In our analyses *Amanipodagrion* is the sister to *Mesopodagrion* but with low QS values (BS = 100, PP = 1, QS = 0.48/0.59/0.96). The two genera are clearly different in morphology and behaviour and cannot be considered members of the same family. We therefore place them in their own respective families: Amanipodagrionidae and Mesopodagrionidae (see below Family-level revisions to the Classification of Zygoptera).

Bybee et al. (2008) first established the sister-group relationship between Euphaeidae and Lestoideidae (BS = 100, PP = 1, QS = 0.8/0/0.99), which was supported with more extensive taxon sampling by Dijkstra et al. (2014). Sister to these two families is Pseudolestidae (BS = 100, PP = 1, QS = 0.57/0.62/0.97) with a single known species *Pseudolestes mirabilis*. Being known only from Hainan, *P. mirabilis,* has a very distinct adult and nymphal morphology, for example having the hindwing much shorter than the forewing, and unique behavior (Cordero-Rivera and Zhang, 2018a, 2018b; Yu and Bu, 2011). The Oriental Devadattidae and Mesoamerican Amphipterygidae are sister taxa (BS = 100, PP = 1, QS = 0.81/0/1). Their nymphs share the filamentous gill tufts below the caudal gills, also a trait of Pseudolestidae, but other morphological differences in the adults and nymphs make them distinct enough to keep them in their respective families. The last two families of this group are the Mesoamerican Thaumatoneuridae (BS = 100, PP = 1, QS = 1/NA/1) and the Oriental Rhipidolestidae (BS = 100, PP = 1, QS = 1/NA/0.98). The latter includes four genera (*Agriomorpha*, *Burmargiolestes*, *Bornargiolestes* and *Rhipidolestes*), which Dijkstra et al. (2014) regarded as *incertae sedis* as they were found to be paraphyletic when Thaumatoneuridae was not included. *Agriomorpha* and *Rhipidolestes* form a monophyletic group in our analyses and as *Burmargiolestes* and *Bornargiolestes* are closely related to *Agriomorpha*, we assume the four to form a monophyletic group. The name Rhipidolestinae was first used by Silsby (2001) although it seems that she did so accidentally, using it for a group that included *Pseudolestes* for which the name Pseudolestidae was already available. Nonetheless we propose to consider Silsby (2001) as the author for this family as her description, while brief, complies with the code of zoological nomenclature, including a citation of the name of the type genus, *Rhipidolestes*. The name is here used for the group of four mentioned genera, although *Rhipidolestes* stands apart due to different venation and a sturdy dorsal spine on the male's ninth abdominal segment. Further work might therefore show that the family should better be divided into two separate subfamilies or even families.

4.1.5.4. 'Calopterygoidea' group 4 (BS = 100, PP = 1, QS = 0.37/0.42/0.96). The fourth group contained within the 'Calopterygoidea' consists of three pairs of families. Philosinidae (BS = 100, PP = 1, QS = 1/NA/1) includes the Asian genera *Philosina* and *Rhinagrion,* which resemble each other strongly in adult morphology and have a clear apomorphy in the nymphal stage in the tube-shaped caudal gills, i.e. the outer gills are folded around the median gill (Kalkman et al., 2010). The Philogangidae with its single genus *Philoganga* is its sister group with strong support (BS = 100, PP = 1, QS = 0.92/0/1). Adult *Philoganga* resembles Philosinidae in general appearance, being relatively large and robust, and resting with wings outstretched. The main difference is in the denser venation, with two antenodal crossveins in Philosinidae, but 11 to 13 in Philogangidae. The nymphs of both families also resemble each other in general build, with long lateral outer caudal gills and a slightly shorter central caudal gill, although the lateral ones are not tube-shaped in *Philoganga*.

For about a century, Megapodagrionidae served as a 'dustbin' family for damselfly genera with unclear relationships. Based on present and recent work (Dijkstra et al., 2014; Kalkman and Theischinger, 2013), these are now divided across no less than fifteen families. The true Megapodagrionidae are limited to the genera *Megapodagrion, Allopodagrion* and *Teinopodagrion* with a total of only 29 species limited to tropical America. The only genus included in our study (*Teinopodagrion*) was found to be sister to Argiolestidae (BS = 100, PP = 1, QS = 0.86/0/0.99), a group restricted to the Afrotropics and Australasia. Argiolestidae was until recently considered as a subfamily of Megapodagrionidae, but raised to family level based on the morphology of caudal gills of the nymphs that are distinctively flat and fan-shaped and held in a horizontal plane (Kalkman and Theischinger 2013). True megapodagrionid caudal gills lie in a vertical plane with the lateral pair triquetral and the median foliaceous (De Marmels, 1999).

Finally, the well supported monophyletic families Calopterygidae (BS = 100, PP = 1, QS = 1/NA/1) and Chlorocyphidae (BS = 100, PP = 1, QS = 1/NA/1) form the core of a group which is colloquially often addressed as Caloptera. However, Caloptera has poor QS values (BS = 100, PP = 1, QS = -0.25/0.23/1). Nonetheless, all species are restricted to running waters and the majority of males have brightly colored (both metallic and pigmented) bodies and often wings used in wonderfully elaborate courtship displays.

*4.1.6. **Coenagrionoidea** (BS = 100, PP = 1, QS = -0.33/0.062/0.99)*

This superfamily contains three families all of which are comparatively well sampled and recovered as well supported monophyletic groups. Isostictidae has previously been recovered as either sister to Coenagrionidae and Platycnemididae combined, or as sister to members of the 'Calopterygoidea', but never with high support (Bybee et al., 2008; Dijkstra et al., 2014; Dumont et al., 2010). Our data recover a monophyletic Isostictidae (BS = 100, PP = 1, QS = 1/NA/1) as sister to the remaining Coenagrionoidea (Coenagrionidae and Platycnemididae) with high confidence among traditional measures of nodal support for the first time (BS = 100, PP = 1). However, the QS values reveal that there is counter support at this node (-0.33/0.062/0.99) and the possibility of incomplete lineage sorting (QD score close to 0.7; Pease et al., 2018), which could be resolved by additional taxon sampling and/or molecular data. The families of Coenagrionidae (BS = 100, PP = 1, QS = 0.56/0.77/0.98) and Platycnemididae (BS = 100, PP = 1, QS = 0.92/0/1) are both well supported as monophyletic, although only a fraction of taxa from both families were sampled. When combined, these two families represent one of the most diverse lineages among Odonata and include many outstanding questions of evolution and diversification in response to both ecological and sexual selection.

Our results again support the conclusion of Pessacq (2008) that the Old World genera once placed in Protoneuridae are not closely related to the New World representatives. The Old World taxa (in our hypothesis being represented by *Elattoneura*, *Nososcticta* and *Prodasineura*) form a perfectly supported monophyletic group firmly placed deep within Platycnemididae (BS = 100, PP = 1, QS = 0.43/0/0.97). The New World taxa (represented by *Neoneura* and *Protoneura*) also form a monophyletic group (BS = 100, PP = 1, QS = 0.26/0/1) and are firmly established as members of the Coenagrionidae. Our phylogenetic hypothesis for Coenagrionidae and Platycnemididae is not in conflict with that of Dijkstra et al. (2014), but sampling is too limited to make additional remarks on subfamilies. The Coenagrionidae does fall into groups that follow the notion of 'core' (BS = 100, PP = 1, QS = 1/NA/1) and 'ridge-faced' (BS = 100, PP = 1, QS = 0.18/0/0.98) Coenagrionidae. This includes high support (BS = 100, PP = 1, QS = 0.86/0/0.98) that the American genus *Argia* (probably the largest genus in the world), is part of the 'ridge-

faced' Coenagrionidae despite having a overall morphology nearer the 'core' group.

4.1.7. Family-level revisions to the classification of Zygoptera

We propose several revisions to the current classification scheme of Dijkstra et al. (2013) (see Appendix B - Family level classification of the order Odonata). We have elevated or proposed seven new families within Zygoptera. We provide the formal family diagnoses together below.

Amanipodagrionidae Dijkstra & Ware **fam.n.** (type genus: *Amanipodagrion* Pinhey, 1962) – large damselflies (hindwing 32–34 mm) restricted to East Usambara mountains of Tanzania. Wings with two Ax and no intercalated veins distally in radial fields; arculus roughly at two-thirds of distance between wing base and node; quadrangle without cross-veins; R4 originates one cell proximal to subnode and IR3 at subnode; proximal supplementary cross-vein between median vein and R4 present; broad brown bands roughly at middle of wings, somewhat closer to node than to large and very swollen pterostigma. Adult perches with wings widely spread at rest and long abdomen hanging down. Thorax and abdomen are largely black, with limited dull yellow markings and no metallic shine or bright colors, but dorsum of abdominal segments 8–10 white pruinose in mature male. Genital ligula without setae on shaft, ending in two broad and simple lobes. Adult male cerci forcipate, short and thick, with strong subbasal tooth on interior and numerous denticles on exterior margin; paraprocts simple and rather slender, about half as long as cerci, curved up- and outward. Nymph unknown. Included genera: *Amanipodagrion*.

Mesagrionidae Kalkman & Sanchez-Herrera **fam.n.** (type genus: *Mesagrion* Selys, 1885) The single species of this monotypic family is a medium sized (hindwing 28–32 mm) damselfly restricted to the central-eastern sector of the Colombian Andes were it is found at small waterfalls of forest streams. Wings clear, with two Ax; several intercalated veins distally in radial fields; wings with long petiolation and arculus slightly distal to roughly at two-thirds of distance between wing base and node. Quadrangle without cross-veins; R4 originates at subnode; IR3 originates at level of first postnodal crossvein. Pterostigma, reddish in adult males, with anterior margin about half as long as posterior margin. Adults perch with wings closed. Head black with angulated frons and extensive yellow pattern, thorax black with yellow stripes, legs with long setae, pale yellow but the first pair with red; abdomen largely red becoming dark in adult females. In females large parts of the dorsum of segment 8 are scarcely sclerotized and have distinct yellow colour. Adult male cerci with a simple forcipate shape, paraprocts about as long as cerci and serrated at the distal fourth of the dorsal margin. Genital ligula with setae on shaft which are as long as segment width; apex divided into two sideward projecting lobes; the internal fold on the genital ligula present. Nymph with relatively large head and thorax and short abdomen; saccoid abdominal gills with long terminal filaments, that of the middle gill about twice as long as that of the lateral gills. Included genera: *Mesagrion*.

Mesopodagrionidae Kalkman & Abbott **fam.n.** (type genus: *Mesopodagrion* McLachlan, 1896) — fairly large (hindwing 27–33 mm) and sturdy damselflies restricted to streams in southern China and the north of Vietnam, Thailand and Myanmar. Wings clear, with two Ax and numerous intercalated veins distally in radial fields; arculus roughly at three-fifth of distance between wing base and node; quadrangle without cross-veins; R4 originates clearly proximal of subnode; IR3 originates at subnode. Pterostigma rectangular, about three times as long as broad; yellow-reddish or black. Adult perches with wings outstretched and abdomen held in horizontal position. Head, thorax, legs and abdomen black with yellowish or blue pattern, including antehumeral stripes that cross the humeral suture to continue onto the mesepimeron and (in males) the pale dorsum of abdominal segments 9 and 10. The postocular lobes are swollen and emphasized by the largely pale (yellow) back of the head, but lack postocular spots. Adult male cerci with a simple forcipate shape, paraprocts short, about a fifth the length of the cerci. Hind rim of abdominal segment 10 modified medially with two short but sturdy spines directed distally. Genital ligula with terminal lobe reduced, deeply incised, and with two long and slender horns; its shaft with<10 setae on each side which are shorter than half the width of the shaft. Nymph is stocky and easily recognized by the large, flat and fan-like horizontal gills (only shared with the unrelated Argiolestidae and Protolestidae), as well as the occipital lobes that protrude distinctly at the side of the head and are are covered densely with strong spines. Included genera: *Mesopodagrion*.

Priscagrionidae Kalkman & Bybee **fam.n.** (type genus: *Priscagrion* Zhou & Wilson, 2001) — medium sized to fairly large (hindwing 28–30 mm in *Sinocnemis*, 34–36 mm in *Priscagrion*) damselflies with a slender appearance and long legs, restricted to streams in southern China and northern Vietnam. Wings clear except for an apical dark spot in males of Priscagrion, with two Ax in *Sinocnemis* and three (hindwing) to four (forewing) Ax in *Priscagrion*; numerous intercalated veins distally in radial fields, especially in *Priscagrion*. Arculus roughly at two-thirds of distance between wing base and node. Quadrangle without cross-veins; R4 originates clearly proximal of subnode; IR3 originates at subnode. Pterostigmata rectangular, about one and a half times as long as broad, clearly swollen in the middle; dark. Adults perch with wings outstretched and body held horizontal. Head, thorax and abdomen black with a blue pattern. The slender abdomen has a shining blue dorsal pattern on segment 8–10 (males) and a reduced and duller markings in females. Adult male cerci with a simple forcipate shape, paraprocts nearly as long as cerci and of a simple shape, carrying a tiny apical hook in *Priscagrion*. Shaft of genital ligula with >20 setae on each side which are clearly longer than half the width of the shaft. Genital ligula ends in a simple scoop that folds back against the shaft; the internal fold on the genital ligula is slightly drawn-out in *Sinocnemis* and drawn-out into a long filament in *Priscagrion*. Nymph of both genera unknown. Included genera: *Priscagrion* and *Sinocnemis*.

Protolestidae Dijkstra & Bybee **fam.n.** (type genus: *Protolestes* Förster, 1899) — medium-sized to fairly large (hindwing 21–32 mm) damselflies restricted to eastern Madagascar. Wings clear with two Ax and at most with intercalated vein between R3 and IR3; arculus roughly at two-thirds of distance between wing base and node; quadrangle without cross-veins; R4 originates roughly half a cell proximal to subnode and IR3 four to five cells distal to that; proximal supplementary cross-vein between median vein and R4 present; pterostigma swollen with very oblique proximal border. Adult variably perches with wings closed or half open, abdomen held roughly horizontal. Adult body red to black, often with bright and contrasting yellow to rufous markings, but never metallic or pruinose. Head notably wide, recalling some members of (unrelated) Platycnemididae. Genital ligula without setae on shaft, ending in two horn-like flagella. Adult male cerci forcipate, slender and rather smooth and simple, slightly widened subapically; paraprocts strongly reduced. The only available description of a nymph of *Protolestes* suggests that they have flat caudal gills which are held in a horizontal position. Included genera: *Protolestes*.

Rhipidolestidae Silsby, 2001 **stat. res.** (type genus: *Rhipidolestes* Ris, 1912) — medium sized to fairly large (hindwing 25–40 mm) damselflies with a slender appearance and relatively long legs. Wings clear with the exception of some species of *Rhipidolestes*, which have dark tips or extensive transverse bands. Wings with two Ax; numerous intercalated veins distally in radial fields. Arculus roughly at three-fourths of distance between wing base and node; quadrangle without cross-veins. R4 originates at subnode; IR3 originates slightly distal of subnode in *Agriomorpha*, *Bornargiolestes* and *Burmargiolestes*. In *Rhipidolestes* the node is much more distal in the wing with the arculus slightly distal than half of distance between wing base and node with R4 and IR3 originating about midway between second Ax and the node. Pterostigmata rectangular and slightly inflated, about one and a half to two times as long as broad; dark or (in some *Rhipidolestes*) yellow or reddish. Head, thorax and abdomen black; face in many species with extensive yellow, red, or blue pattern; thorax with limited yellow pattern and pruinose in

some species of *Rhipidolestes*; abdomen with narrow pale rings anteriorly on the segments and segments 8 to 10 with dorsal blue pattern in *Agriomorpha* and some species of *Rhipidolestes*. Legs notably brightly colored pale yellow to red. Adult male cerci are pincer-shaped and typically narrowed about a fifth of their length from their apex, thus forming a finger-like point. Paraprocts can be both short and long, reaching about two-thirds the length of the cerci. *Rhipidolestes* males carry a sturdy dorsal spine posteriorly on abdominal segment 9. Genital ligula with setae on shaft; apex divided into two lobes, which in most species are broad and directed sideward. Nymphs of *Agriomorpha*, *Bornargiolestes* and *Burmargiolestes* not formally described. Nymphs of *Rhipidolestes* with relatively short stocky abdomen and saccoid abdominal gills with terminal filament of about a fourth to a third the length of the gills. Included genera: *Rhipidolestes, Agriomorpha*, *Bornargiolestes* and *Burmargiolestes*.

Tatocnemididae Rácenis, 1959 **stat. res.** (type genus: *Tatocnemis* Kirby, 1889) — medium-sized to fairly large (hindwing 22–32 mm) damselflies restricted to eastern Madagascar. Wings clear, at most stained toward tips, with two Ax and no intercalated veins distally in radial fields; arculus roughly at two-thirds of distance between wing base and node; quadrangle without cross-veins; R4 originates at subnode and IR3 one or two cells distal to that; proximal supplementary cross-vein between median vein and R4 present; pterostigma rhomboidal; wing tips crenulated, i.e. wavy rather than smoothly rounded due to two or three shallow excavations in the hind border below the pterostigma. Adult variably perches with wings closed or open, abdomen held roughly horizontal. Adult thorax black with dull yellow to reddish markings, abdomen uniformly red, body never metallic or pruinose. Genital ligula with setae on shaft, ending in two curled flagella. Adult male cerci forcipate, relatively thick, usually with distinct subapical expansion on inner margin and denticles on outer margin; paraprocts reduced to simple point. The only description available suggests that the caudal gills are long and inflated, thus appearing sausage-shaped, with a long apical filament. Included genera: *Tatocnemisdj*

4.1.8. *Anisozygoptera (Epiophlebiidae) & Anisoptera (BS = 100, PP = 1, QS = 0.25/0/0.99)*

Our phylogenetic reconstruction recovers extant Anisozygoptera as sister to Anisoptera with high support (BS = 100, PP = 1, QS = 0.25/0/0.99). Support for a sister group relationship has also been demonstrated for over two decades by both morphology and molecular data (Bybee et al., 2008; Carle et al., 2015; Dumont et al., 2010; Fleck et al., 2008; Lohmann, 1996; Letsch et al., 2016ab; Thomas et al., 2013). Quartet sampling support provides a small amount of uncertainty in this relationship (see purple color of branch) due to the QD value ("0"), thus analysis is needed to provide exact reasons for the lack of support between these two groups. Anisozygoptera have been combined with Anisoptera in a group equivalent to a suborder named "Epiprocta" (H. Lohmann, 1996) in an effort to capture all anisozygopteran fossil taxa that may form a paraphyletic grade toward Anisoptera. Further, Dijkstra et al. (2013) outline morphological reasons (i.e., the genitalia) to maintain Anisozygoptera and Anisoptera as separate suborders. Thus, we suggest that for evolutionary reasons based on both morphological and molecular data, the use of Epiprocta should be limited until further analyses combining both fossil and extant taxa are produced.

Epiophlebia is the only genus in Epiophlebiidae. The genus comprises three species, *Epiophlebia superstes, E. laidlawi* and *E. sinensis.* A fourth species, *E. diana,* was described by Carle (2012) but was subsequently synonymized with *E. sinensis* (Büsse, 2016; Dijkstra et al., 2013; Schorr and Paulson, 2019). *Epiophlebia* is distributed in Bhutan, Nepal, China, North Korea and Japan (e.g., Büsse, 2016; Büsse et al., 2012). Here, we included two specimens of the Japanese *E. superstes*, and one of the Himalayan *E. laidlawi* which are recovered as a monophyletic group with the highest possible support, confirming the work of Büsse et al. (2012).

4.1.9. ***Aeshnoidea:*** *Austropetaliidae and Aeshnidae (BS = 100, PP = 1, QS = 0.86/0/1)*

Composed of the fully supported clades (i.e., BS = 100, PP = 1, QS = 1/NA/1) Austropetaliidae and Aeshnidae, the Aeshnoidea form a monophyletic group. Aeshnoidea are recovered as sister to the remaining Anisoptera families but with suboptimal quartet sampling support (BS = 100, PP = 1, QS = -0.48/0/0.97). This lack of support among QS values was unexpected as Aeshnoidea has been recovered as sister to the remaining Anisoptera for over a decade with strong support. Outside of Libellulidae QS values show this as the least supported node within Anisoptera, including the node supporting Petaluridae and Gomphidae, although both BS and PP support provided full support for this node. A closer look at this relationship in the future with a broader taxon sampling is needed. Austropetaliidae was represented by both South American genera (*Phyllopetalia* and *Hypopetalia*). Including both Australian genera in future analyses would be ideal. Within Aeshnidae, a family comprising 456 extant species, we sequenced six. *Gynacantha* and *Anax* form a monophyletic group (BS = 100, PP = 1, QS = 0.92/0/0.98) and have commonly been recovered in a clade (e.g., Carle et al., 2015; Dumont et al., 2010; Von Ellenrieder, 2002). We also reconstruct them with full support as a clade that is sister to the remaining Aeshnidae.

4.1.10. ***Gomphidae and Petaluridae*** *(BS = 97, PP = 1, QS = 0.22/0.47/0.92)*

Past phylogenetic reconstructions have debated whether Gomphidae are sister to Libelluloidea or Petaluridae (Bybee et al., 2008; Carle et al., 2015; Dumont et al., 2010; Letsch et al., 2016ab), with even large transcriptome datasets recovering both possibilities with high probability (Kohli et al.). Despite some uncertainty in the relationship of Petaluridae + Gomphidae based on BS measures of nodal support, quartet sampling provides moderately high support for this node. It is unlikely that additional molecular data will resolve this node in such a way that traditional nodal support measures (PP, SS) will increase. The next step in higher-level classification as it relates to this node is a much deeper taxon sampling to provide a test that might finally provide both the phylogenetic signal and statistical support to reconstruct this relationship. This relationship is of interest in part because it influences our interpretation of the evolution of exophytic oviposition (not using plant material). Due to a reduction in the ovipositor, gomphids have exophytic oviposition (Sahlén, 1995). Reduction in the ovipositor, perhaps convergently shared with the Gomphidae, is a prominent feature of Libelluloidea. The ovipositor of Aeshnoidea and Petaluridae (and Zygoptera) comprises three pairs of ventral processes. The first and second pairs (anterior and posterior gonapophyses) are enclosed by the third (gonoplacs). In gomphids, libelluloids and cordulegastroids the ovipositor is modified for exophytic oviposition (Carle, 1995; Tillyard, 1917). In Cordulegastridae, the third processes (gonoplacs) are vestigial. In Synthemistidae *s.L.* clade, the third processes are absent and at least the second processes are reduced, although in some taxa the first pair is present and nearly as long as in Cordulegastridae. In Macromiidae, Corduliidae and Libellulidae, the first processes are reduced to small flaps and the other structures are apparently absent except for the probable vestige of the styli emerging directly from the 9th sternite (Tillyard, 1917). In a few instances, the 8th (e.g., some *Somatochlora*) or 8th and 9th sternites (*Uracis*) are secondarily produced to form an ovipositor in Macromiidae, Corduliidae and Libellulidae species.

The monophyly of both Petaluridae and Gomphidae were never really in doubt and both are fully supported as monophyletic. The groups stand in stark contrast to each other in terms of both diversity and distribution. The petalurids comprise 11 species, uniquely distributed towards the edges of the United States, Chile, Japan, New Zealand and Australia. Nymphs tend to have long generation times, often remaining in the nymphal stages for up to five years while living along river banks or as burrowers or rarely as semi-terrestrial hunters of high mountain bogs (Ware et al., 2014). Adults are drab in colour, usually large in size, and tend to be found near forest edges, often perching on

tree trunks. In contrast, Gomphidae are incredibly species rich, comprising over 1000 extant species. Adults are heterogeneous in their colour, shape and size, often perching on the ground or just above the water on overhanging branches or vegetation or even tree-tops. Nymphs exhibit an array of morphological forms and tend to use concealment and often burrow just below the stream substrate as ambush predators.

4.1.11. *Cordulegastroidea (BS = 100, PP = 1, QS = 1/NA/0.99)*

Chorogomphidae + Neopetaliidae + Cordulegastridae are recovered as monophyletic with high support. This relationship has been found in previous studies but with different arrangements of the three families (e.g., Carle et al., 2015; Letsch et al., 2016ab). We recover Chorogomphidae (Neopetaliidae + Cordulegastridae) with full support. Although the adults look superficially different among the families, the nymphs are quite similar and share several characters that unite this group, including the shape and dentition of the labial mask. Further, members of the group tend to inhabit streams as both adults and nymphs, but their preferred oviposition habitat is not well defined, with Neopetaliidae nymphs having been found in both muddy seeps and shallow clear streams (personal observation Bybee). Chlorogomphids may spend much of their time in the canopies, and approach streams largely to oviposit and seek a mate (personal observation A. Cordero).

4.1.12. *Libelluloidea (BS = 100, PP = 1, QS = 0.81/0/1)*

Libelluloidea traditionally includes Synthemistidae, Macromiidae, Corduliidae and Libellulidae (Fraser, 1957), and is extremely species rich (~1,500 species). We recover this as a well-supported monophyletic group.

Tillyard described Synthemistidae, which were united by synapomorphies both in the nymphal stage (e.g., premental shape, antennal segment number, tibial morphology) and adult stage (e.g., wing venation, male secondary genitalic morphology). However, the family was considered to be variable. Molecular phylogenies (Letsch et al., 2016ab; Letsch, 2007; Ware et al., 2007) have suggested that the family should be split, with *Archaeophya*, *Gomphomacromia*, *Synthemis, Synthemiopsis, Eusynthemis* often recovered as a clade, with *Austrocordulia, Micromidia, Lathrocordulia, Macromidia, Cordulephya* recovered either as a paraphyletic group or a separate clade. Here, we recover a fully supported grouping of *Gomphomacromia, Eusynthemis, Choristhemis* and *Parasynthemis*, as clade also recovered by (Letsch, 2007) and (Letsch et al., 2016a,b). *Cordulephya, Micromidia* and *Austrocordulia* are also recovered as a monophyletic clade (BS = 100, PP = 1, QS = 1/NA/1), sister to the remaining libelluloids. Our findings strongly support the idea of Synthemistidae as a complex group deserving of extensive data collection, but are unclear regarding the number and arrangement of taxa in this group; hence we await further taxon sampling before naming additional clades in this complex.

We recover *Epophthalmia, Macromia* and *Phyllomacromia* in a fully supported group (BS = 100, PP = 1, QS = 1/NA/1). *Epophthalmia, Didymops, Macromia* and *Phyllomacromia* have routinely been recovered as a monophyletic Macromiidae, first by Gloyd (1959), and again by several molecular studies (Bybee et al., 2008; Carle et al., 2015; Dumont et al., 2010; Letsch et al., 2016ab; Letsch, 2007; Ware et al., 2007, in prep.). Nymphal synapomorphies include the shape and dentition of the prementum, the relative length of the hind legs to body size (giving them a "spider-like" appearance), and a frontal horn between their eyes; adult synapomorphies include the shape and size of the anal loop in the hindwing, eyes with a small protuberance on the lateral edge, and secondary penile characters. Macromiidae adults are fast fliers known to patrol long stretches of mainly lotic habitats. *Phyllomacromia* and *Epophthalmia* are recovered as sister taxa; this relationship was suggested by May (1997) based on male penile morphology.

Even with a limited sampling of corduliids we demonstrate the complexity of the phylogenetic relationships of this group. Nymphs of the family Corduliidae are difficult to distinguish from Libellulidae, save for a small number of premental characters (Tennessen, 2019; Theischinger and Fleck, 2003). (Ware et al., 2007) recovered *Aeschnosoma* + *Pentathemis* as sister to the remaining Corduliidae; these genera have remarkably similar looking nymphs, with long spines on segment nine, despite having ranges in South America and eastern Australia, respectively. Fleck and Legrand (2013) hypothesize that *Libellulosoma* forms a clade with *Aeschnosoma* and *Pentathemis*. Here, we recover *Pentathemis* as sister to the Libellulidae, but with low bootstrap support (96%) and among the poorest of QC values (-0.76), suggesting that this relationship needs further investigation before confirmation of this grouping. In the absence of *Neocordulia*, *Lauromacromia*, *Idomacromia, Nesocordulia* and other taxa of *incertae sedis*, we cannot yet address with confidence the broader composition of Corduliidae.

Libellulidae is recovered with full support. However, there is a great amount of discord within the group demonstrated by both the highest concentration and lowest nodal supports across the topology. We recover three extremely poorly supported clades suggesting instead a polytomy among the taxa included in our analyses. However, there are clades within the libellulids that are well supported. The Libellulinae (BS = 100, PP = 0.95, QS = 1/NA/1), a large subfamily is recovered in our analyses and has been consistently recovered in past molecular work. *Nannophlebia* and *Zygonyx* were recovered in a clade previously, and here we find support for their sister relationship (BS = 100, PP = 0.96, QS = 0.92/0/1). Ware et al. (2007) found *Pantala* to be closely related to *Zygonyx* and *Nannophlebia*; here a clade comprising these three taxa is recovered with low QS values (BS = 100, PP = 1, QS = -0.46/0/0.97). Past studies have recovered *Rhyothemis* in a clade with *Sympetrum* (e.g., Ware et al., 2007). Herein we also recover this relationship but with the inclusion of never before sequenced *Austrothemis* (BS = 56, PP = 0.75, QS = 0.17/0.33/0.86). The family Libellulidae is extremely species rich, comprising well over 1,000 species. Many of the members of this family have an elongated, bisected anal loop in their hind wing, and an oblique vein immediately following the nodus. Their secondary penile characters, and general adult and nymphal morphology strongly support the monophyly of this family. A further look at this family with a much expanded taxon sampling is necessary to better understand the complex evolutionary history that certainly represents one of the most rapidly radiating lineages in Odonata.

4.2. Taxonomic summary

No classification changes are proposed for Anisoptera or Anisozygoptera (Fig. 3). However, Anisoptera is certainly in need of further revision. Specifically the composition of the superfamily Gomphoidea containing only Gomphidae or Gomphidae + Petaluridae. There are also the notable classification issues with what we refer to as the "Synthemistidae *incertae sedis*" (Fig. 1). Research is currently planned for the near future with a much expanded taxon sampling using high throughput sequence data to essentially reconstruct a species level phylogeny that will certainly provide further insight and address these issues within Anisoptera.

Updates to the classification of Zygoptera proposed herein include the erection of five new families: Amanipodagrionidae fam. nov. (one genus, *Amanipodagrion*), Mesagrionidae fam. nov. (one genus, *Mesagrion*), Mesopodagrionidae fam. nov. (one genus, *Mesapodagrion*), Priscagrionidae fam. nov. (two genera, *Priscagrion* and *Sinocnemis*), Protolestidae fam. nov. (one genus, *Protolestes*) (Fig. 3). In addition two other families are reinstated: Rhipidolestidae stat. res. (four genera; *Agriomorpha*, *Bornargiolestes*, *Burmargiolestes*, *Rhipidolestes*) and Tatocnemididae stat. res. (one genus, *Tatocnemis*). One family, Heteragrionidae, is expanded to include four genera with *Dimeragion* and *Heteropodagrion* joining *Heteragrion* and *Oxystigma* (see Appendix B).

4.3. Conclusions

These data provide the most compelling hypothesis for Odonata phylogeny and classification to date. Odonata comprises three extant

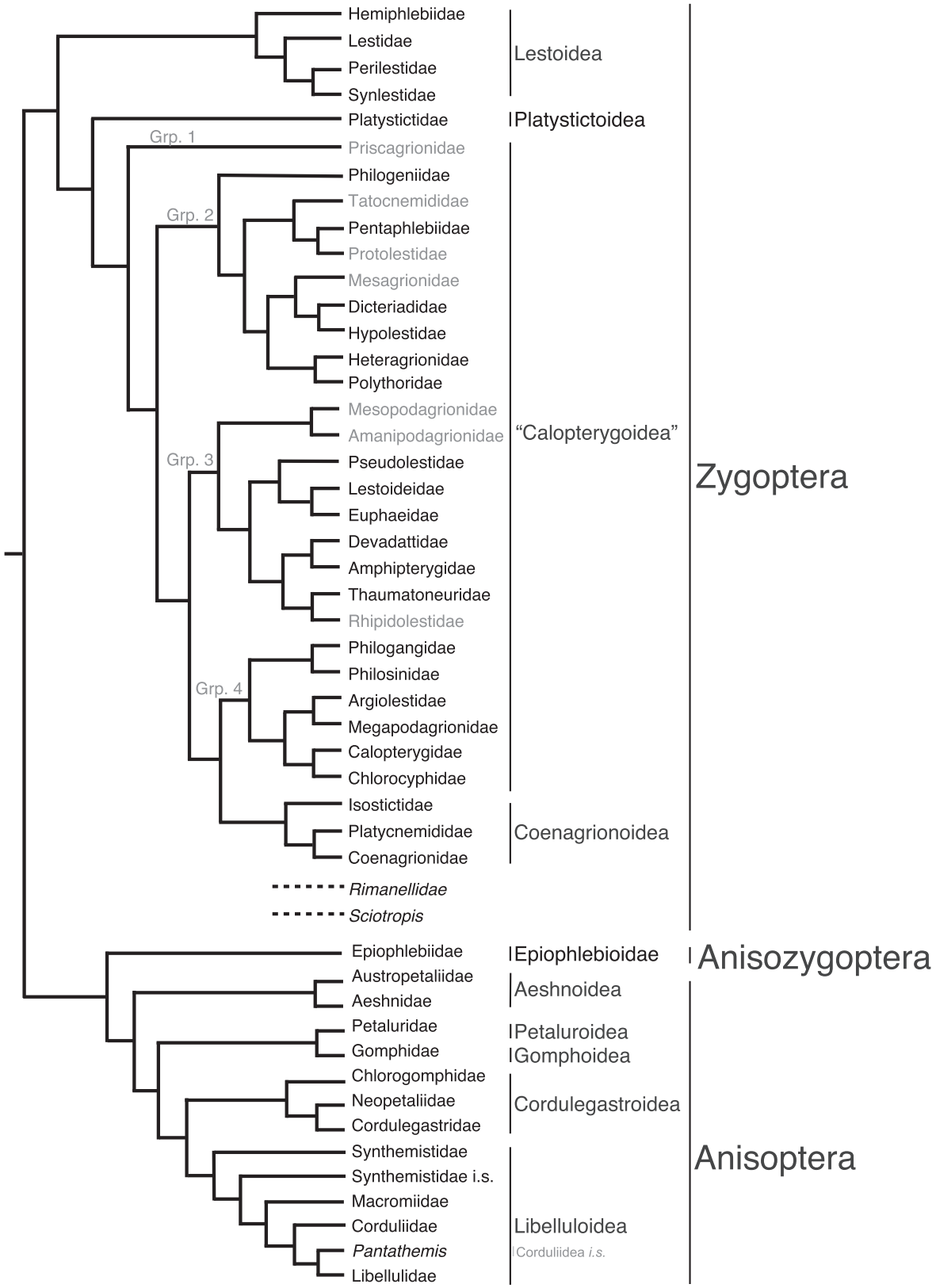

Fig. 3. Current state of odonate phylogeny. Summary of the phylogenetic hypothesis for Odonata from Fig. 2. Support values for each node can be found in Fig. 2. Grey text highlights both the reinstated (Tatocnemididae stat. res., Rhipidolestidae stat. res.) and the proposed new families (Protolestidae fam. nov., Priscagrionidae fam. nov., Mesopodagrionidae fam. nov., Mesagrionidae fam. nov., and Amanipodagrionidae fam. nov.). Discussion of Grps. 1, 2, 3, and 4 is found in the Calopterygoidea section of the manuscript. Almost all major lineages are included and now have a hypothesized phylogenetic placement. The zygopteran genera *Rimanella* (Rimanellidae) and *Sciotropis* (*Incertae Sedis* group 8) were not included in this analysis.

and morphologically distinct suborders, all of which are found to be monophyletic: Zygoptera, Anisoptera and Anisozygoptera (Fig. 3). Within the Odonata, targeted enrichment data are unable to resolve with strong support the relationships between Aeshnoidea and the rest of Anisoptera, between Petaluridae and Gomphidae, and among several Zygoptera families, mostly within the calopterygoid grade. However, these data do provide, for the first time, strong support for a majority of interfamilial zygopteran and anisopteran relationships allowing us to both support and improve the classification for the group. As a result we propose several new taxonomic groupings and highlight others to address in the future. Despite some areas of low support, both from traditional measures of nodal support and quartet sampling values, our phylogeny resulted in the highest support of odonate phylogenetic relationships and unlike in past works, relatively few nodes across the topology have low support. Further, we provide a backbone to begin to examine the ecological and morphological history for Odonata, while also with insight from QS values uncover some potential evolutionary scenarios regarding incomplete lineage sorting and introgression in portions of the topology. We suggest increased taxon sampling to reduce phylogenetic error and provide more detailed examination of odonate evolution.

CRediT authorship contribution statement

Seth M. Bybee: Conceptualization, Investigation, Resources, Data curation, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition. **Vincent J. Kalkman:** Conceptualization, Investigation, Resources, Data curation, Writing - original draft. **Robert J. Erickson:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Paul B. Frandsen:** Methodology, Software, Resources, Formal analysis, Investigation, Data curation, Writing - original draft, Supervision. **Jesse W. Breinholt:** Methodology, Software, Resources, Formal analysis, Writing - review & editing. **Anton Suvorov:** Methodology, Software, Formal analysis, Writing - review & editing. **Klaas-Douwe B. Dijkstra:** Investigation, Resources, Writing - original draft. **Adolfo Cordero-Rivera:** Investigation, Resources, Writing - review & editing. **Jeffrey H. Skevington:** Investigation, Resources, Writing - review & editing. **John C. Abbott:** Investigation, Resources, Writing - review & editing. **Melissa Sanchez Herrera:** Investigation, Resources, Writing - review & editing. **Alan R. Lemmon:** Methodology, Formal analysis, Writing - original draft. **Emily Moriarty Lemmon:** Investigation. **Jessica L. Ware:** Conceptualization, Investigation, Resources, Data curation, Writing - original draft, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Aberer, A.J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31, 2553–2556.
Alfaro, M.E., Zoller, S., Lutzoni, F., 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20, 255–266.
Ballare, E.F., Ware, J.L., 2011. Dragons fly, biologists classify: an overview of molecular odonate studies, and our evolutionary understanding of dragonfly and damselfly (Insecta: Odonata) behavior. Int. J. Odonatol.
Breinholt, J.W., Earl, C., Lemmon, A.R., Lemmon, E.M., Xiao, L., Kawahara, A.Y., 2018. Resolving Relationships among the Megadiverse Butterflies and Moths with a Novel Pipeline for Anchored Phylogenomics. Syst. Biol. 67, 78–93.
Büsse, S., 2016. Morphological re-examination of Epiophlebia laidlawi (Insecta: Odonata) including remarks on taxonomy. Int. J. Odonatol. 19, 221–238.
Büsse, S., von Grumbkow, P., Hummel, S., Shah, D.N., Tachamo Shah, R.D., Li, J., Zhang, X., Yoshizawa, K., Wedmann, S., Hörnschemeyer, T., 2012. Phylogeographic analysis elucidates the influence of the ice ages on the disjunct distribution of relict dragonflies in Asia. PLoS One 7, e38132.
Bybee, S., Córdoba-Aguilar, A., Duryea, M.C., Futahashi, R., Hansson, B., Lorenzo-Carballa, M.O., Schilder, R., Stoks, R., Suvorov, A., Svensson, E.I., Swaegers, J., Takahashi, Y., Watts, P.C., Wellenreuther, M., 2016. Odonata (dragonflies and damselflies) as a bridge between ecology and evolutionary genomics. Front. Zool. 13, 46.
Bybee, S.M., Ogden, T.H., Branham, M.A., Whiting, M.F., 2008. Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. Cladistics 24, 477–514.
Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421.
Carle, F.L., 2012. A new Epiophlebia (Odonata: Epiophlebioidea) from China with a review of epiophlebian taxonomy, life history, and biogeography. Arthropod. Systemat. Phylogeny 70, 75–83.
Carle, F.L., 1995. Evolution, taxonomy, and biogeography of ancient Gondwanian libelluloides, with comments on anisopteroid evolution and phylogenetic systematics (Anisoptera: Libelluloidea). Odonatologica 24, 383–424.
Carle, F.L., Kjer, K.M., May, M.L., 2015. A molecular phylogeny and classification of Anisoptera (Odonata). Arthropod Syst. Phylogeny 73, 281–301.
Carle, F.L., Kjer, K.M., May, M.L., 2008. Evolution of Odonata, with special reference to Coenagrionoidea (Zygoptera). Arthropod Syst. Phylogeny 66, 37–44.
Chernomor, O., von Haeseler, A., Minh, B.Q., 2016. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. Syst. Biol. 65, 997–1008.
Corbet, P.S., 1999. Dragonflies - behaviour and ecology of Odonata. Harley Books.
Cordero–Rivera, A., Zhang, H., 2018. Ethological uniqueness of a damselfly with no near relatives: the relevance of behaviour as part of biodiversity. 1 161–174.
Córdoba-Aguilar, A., 2008. Dragonflies and Damselflies: Model Organisms for Ecological and Evolutionary Research. OUP Oxford.
Davis, R.B., Nicholson, D.B., Saunders, E.L.R., Mayhew, P.J., 2011. Fossil gaps inferred from phylogenies alter the apparent nature of diversification in dragonflies and their relatives. BMC Evol. Biol. 11, 252.
De Marmels, J., 1999. A new species of Dimeragrion Calvert 1913 from Pantepui, Venezuela (Odonata: Megapodagrionidae). Boletín de Entomología Venezolana 14, 27–36.
Dijkstra, K.D.B., Bechly, G., Bybee, S.M., Dow, R.A., 2013. The classification and diversity of dragonflies and damselflies (Odonata). In: Zhang, Z.-Q.(Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey …. Zootaxa.
Dijkstra, K.-D.-B., Kalkman, V.J., Dow, R.A., Stokvis, F.R., Van Tol, J.A.N., 2014. Redefining the damselfly families: a comprehensive molecular phylogeny of Z ygoptera (O donata). Syst. Entomol. 39, 68–96.
Douady, C.J., Delsuc, F., Boucher, Y., Doolittle, W.F., Douzery, E.J.P., 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. Mol. Biol. Evol. 20, 248–254.
Dumont, H.J., Vierstraete, A., Vanfleteren, J.R., 2010. A molecular phylogeny of the Odonata (Insecta). Syst. Entomol. 35, 6–18.
Fleck, G., Legrand, J., 2013. Notes on the genus Libellulosoma Martin, 1906, and related genera (Odonata: Anisoptera: Corduliidae). Zootaxa 3745, 579–586.
Fleck, G., Ullrich, B., Brenk, M., Wallnisch, C., Orland, M., Bleidissel, S., Misof, B., 2008. A phylogeny of anisopterous dragonflies (Insecta, Odonata) using mtRNA genes and mixed nucleotide/doublet models. J. Zoolog. Syst. Evol. Res. 46, 310–322.
Fraser, F.C., 1954. The origin and descent of the order Odonata based on the evidence of persistent archaic characters. Proceedings of the Royal Entomological Society of.
Fraser, F.C., 1957. A reclassification of the order Odonata. R. zool. Soc. N. S. W. , Sydney.
Futahashi, R., Kawahara-Miki, R., Kinoshita, M., Yoshitake, K., Yajima, S., Arikawa, K., Fukatsu, T., 2015. Extraordinary diversity of visual opsin genes in dragonflies. Proc. Natl. Acad. Sci. U. S. A. 112, E1247–E1256.
Garrison, R.W., von Ellenrieder, N., Louton, J.A., 2010. Damselfly Genera of the New World: An Illustrated and Annotated Key to the Zygoptera. Johns Hopkins University Press.
Gloyd, L.K., 1959. Elevation of the Macromia group to family status (Odonata). Entomol. News 70, 197–205.
Haddad, S., Mckenna, D.D., 2016. Phylogeny and evolution of the superfamily C hrysomeloidea (C oleoptera: C ucujiformia). Syst. Entomol. 41, 697–716.

Haddad, S., Shin, S., Lemmon, A.R., Lemmon, E.M., Svacha, P., Farrell, B., Ślipiński, A., Windsor, D., McKenna, D.D., 2018. Anchored hybrid enrichment provides new insights into the phylogeny and evolution of longhorned beetles (C erambycidae). Syst. Entomol. 43, 68–89.
Hamilton, C.A., Lemmon, A.R., Lemmon, E.M., Bond, J.E., 2016. Expanding anchored hybrid enrichment to resolve both deep and shallow relationships within the spider tree of life. BMC Evol. Biol. 16, 212.
Hasegawa, E., Kasuya, E., 2006. Phylogenetic analysis of the insect order Odonata using 28S and 16S rDNA sequences: a comparison between data sets with different evolutionary rates. Entomol. Sci. 9, 55–66.
Lohmann, H., 1996. Das Phylogenetische System der Anisoptera (Odonata). Entomologische Zeitschrift 106, 209–266.
Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518–522.
Kalkman, V.J., 2008. Taxonomy, behaviour, and habitat of Mesopodagrion and Sinocnemis. Notes on Old World Megapodagrionidae 3 (Odonata). Int. J. Odonatol. 11, 185–260.
Kalkman, V.J., Choong, C.Y., Orr, A.G., Schütte, K., 2010. Remarks on the taxonomy of Megapodagrionidae with emphasis on the larval gills (Odonata). Int. J. Odonatol. 13, 119–135. https://doi.org/10.1080/13887890.2010.9748366.
Kalkman, V.J., Theischinger, G., 2013. Generic revision of Argiolestidae (Odonata), with four new genera. Int. J. Odonatol. 16, 1–52.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Knapp, K., Manpreet Kohli, Michael L. May, and Jessica Ware, (In Prep.) The phylogeny of Macromiidae: molecular and morphological evaluation of intrafamilial relationships. Int. J. Odonatol.
Kim, M.J., Jung, K.S., Park, N.S., Wan, X., Kim, K.-G., Jun, J., Yoon, T.J., Bae, Y.J., Lee, S. M., Kim, I., 2014. Molecular phylogeny of the higher taxa of Odonata (Insecta) inferred from COI, 16S rRNA, 28S rRNA, and EF1-α sequences. Entomological Research 44, 65–79. https://doi.org/10.1111/1748-5967.12051.
Kohli, MKK, Letsch, H., Greve, C., Béthoux, O., Deregnaucourt, I., Gunkel, SS, Machida, R, Niehuis, O, Rust, J, Wappler, T, Ware, J, n.d. Odonata transcriptomics resolves familial relationships and allows for testing of fossil impacts on divergence time estimation. Uncertain. Artif. Intell.
Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30, 3276–3278. https://doi.org/10.1093/bioinformatics/btu531.
Lemmon, A.R., Emme, S.A., Lemmon, E.M., 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. Syst. Biol. 61, 727–744.
Letsch, H., 2007. Phylogeny of Anisoptera (Insecta: Odonata): Promises and limitations of a new alignment approach (PhD). Rheinische Friedrich-Wilhelms-Universität Bonn.
Letsch, H., Gottsberger, B., Ware, J.L., 2016a. Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Mol. Ecol. 25 (6), 1340–1353.
Letsch, H., Gottsberger, B., Ware, J.L., 2016b. Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Mol. Ecol. 25, 1340–1353.
May, M.L., 1997. Reconsideration of the status of the genera Phyllomacromia and Macromia (Anisoptera: Corduliidae). Odonatologica 26, 405–414.
Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler, T., Rust, J., Aberer, A.J., Aspöck, U., Aspöck, H., Bartel, D., Blanke, A., Berger, S., Böhm, A., Buckley, T.R., Calcott, B., Chen, J., Friedrich, F., Fukui, M., Fujita, M., Greve, C., Grobe, P., Gu, S., Huang, Y., Jermiin, L.S., Kawahara, A.Y., Krogmann, L., Kubiak, M., Lanfear, R., Letsch, H., Li, Y., Li, Z., Li, J., Lu, H., Machida, R., Mashimo, Y., Kapli, P., McKenna, D.D., Meng, G., Nakagaki, Y., Navarrete-Heredia, J.L., Ott, M., Ou, Y., Pass, G., Podsiadlowski, L., Pohl, H., von Reumont, B.M., Schütte, K., Sekiya, K., Shimizu, S., Slipinski, A., Stamatakis, A., Song, W., Su, X., Szucsich, N.U., Tan, M., Tan, X., Tang, M., Tang, J., Timelthaler, G., Tomizuka, S., Trautwein, M., Tong, X., Uchifune, T., Walzl, M.G., Wiegmann, B.M., Wilbrandt, J., Wipfler, B., Wong, T.K.F., Wu, Q., Wu, G., Xie, Y., Yang, S., Yang, Q., Yeates, D.K., Yoshizawa, K., Zhang, Q., Zhang, R., Zhang, W., Zhang, Y., Zhao, J., Zhou, C., Zhou, L., Ziesmann, T., Zou, S., Li, Y., Xu, X., Zhang, Y., Yang, H., Wang, J., Wang, J., Kjer, K.M., Zhou, X., 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346, 763–767.
Mort, M.E., Soltis, P.S., Soltis, D.E., Mabry, M.L., 2000. Comparison of three methods for estimating internal support on phylogenetic trees. Syst. Biol. 49, 160–171.
Munz, P.A., 1919. A Venational Study of the Suborder Zygoptera (Odonata): With Keys for the Identification of Genera. American Entomological Society.
Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.
Pease, J.B., Brown, J.W., Walker, J.F., Hinchliff, C.E., Smith, S.A., 2018. Quartet Sampling distinguishes lack of support from conflicting support in the green plant tree of life. Am. J. Bot. 105, 385–403.
Pérez-Gutiérrez, L.A., Montes-Fontalvo, J.M., 2011. Rediscovery of Mesagrion leucorrhinum (Zygoptera: Megapodagrionidae): a "formal" description of female and ultimate stadium of larva with notes on habits. Int. J. Odonatol. 14, 91–100.
Pessacq, P., 2008. Phylogeny of Neotropical Protoneuridae (Odonata: Zygoptera) and a preliminary study of their relationship with related families. Syst. Entomol. 33, 511–528.
Pilgrim, E.M., Von Dohlen, C.D., 2008. Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. Syst. Entomol. 33, 159–174.
Polhemus, D.A., 1997. Phylogenetic analysis of the Hawaiian damselfly genus Megalagrion (Odonata: Coenagrionidae): implications for biogeography, ecology, and conservation biology.
Prum, R.O., Berv, J.S., Dornburg, A., Field, D.J., Townsend, J.P., Lemmon, E.M., Lemmon, A.R., 2015. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. Nature 526, 569–573.
Rácenis, J., 1959. Notas taxonomicas sobre la familia Megapodagrionidae (Odonata: Zygoptera) con la sinopsis de las especies venezolanas. Acta Biologica Venezolana 2 (30), 335–367.
Rehn, A.C., 2003. Phylogenetic analysis of higher-level relationships of Odonata, 28. Syst, Entomol, pp. 181–240.
Rokyta, D.R., Lemmon, A.R., Margres, M.J., Aronow, K., 2012. The venom-gland transcriptome of the eastern diamondback rattlesnake (Crotalus adamanteus). BMC Genomics 13, 312.
Sahlén, G., 1995. Eggshell ultrastructure in Onychogomphus forcipatus unguiculatus (Vander Linden) (Odonata: Gomphidae). Int. J. Insect Morphol. Embryol. 24, 281–286.
Sanchez Herrera, M., Beatty, C., Nunes, R., Realpe, E., Salazar, C., Ware, J.L., 2018. A molecular systematic analysis of the Neotropical banner winged damselflies (Polythoridae: Odonata): Phylogenetic relationships of Polythoridae. Syst. Entomol. 43, 56–67.
Saux, C., Simon, C., Spicer, G.S., 2003. Phylogeny of the dragonfly and damselfly order Odonata as inferred by mitochondrial 12S ribosomal RNA sequences. Ann. Entomol. Soc. Am. 96, 693–699.
Schorr, M., Paulson, D.R., 2019. World Odonata List. Updated 15 August 2019.
Silsby, J., 2001. Dragonflies of the World. CSIRO publishing.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Suvorov, A., Jensen, N.O., Sharkey, C.R., Fujimoto, M.S., Bodily, P., Wightman, H.M.C., Ogden, T.H., Clement, M.J., Bybee, S.M., 2017. Opsins have evolved under the permanent heterozygote model: insights from phylotranscriptomics of Odonata. Mol. Ecol. 26, 1306–1322.
Tennessen, K.J., 2019. Dragonfly Nymphs of North America. An Identification Guide. Springer.
Tennessen, K.J., 2010. The Madicolous Nymph Of Heteropodagrion Sanguinipes Selys (Odonata: Megapodagrionidae). https://doi.org/10.5281/ZENODO.196541.
Theischinger, G., Fleck, G., 2003. A new character useful for taxonomy and phylogeny of Anisoptera (Odonata). Bull. Soc. Entomol. France 108, 409–412.
Thomas, J.A., Trueman, J.W.H., Rambaut, A., Welch, J.J., 2013. Relaxed phylogenetics and the palaeoptera problem: resolving deep ancestral splits in the insect phylogeny. Syst. Biol. 62, 285–297.
Tillyard, R.J., 1917. The biology of dragonflies : (Odonata or Paraneuroptera) / by R.J. Tillyard. https://doi.org/10.5962/bhl.title.27401.
Trueman, J.W.H., 1996. A preliminary cladistic analysis of odonate wing venation. Odonatologica 25, 59–72.
van Tol, J., Reijnen, B.T., 2009. Phylogeny and biogeography of the Platystictidae (Odonata). Leiden University, Leiden, p. PhD thesis.
Von Ellenrieder, N., 2002. A phylogenetic analysis of the extant Aeshnidae (Odonata: Anisoptera): Phylogeny of extant Aeshnidae. Syst. Entomol. 27, 437–467.
Ware, J., Karlsson, M., Sahlén, G., Koch, K., 2012. Evolution of reproductive strategies in libellulid dragonflies (Odonata: Anisoptera). Org. Divers. Evol. 12, 313–323.
Ware, J.L., Beatty, C.D., Sánchez Herrera, M., Valley, S., Johnson, J., Kerst, C., May, M.L., Theischinger, G., 2014. The petaltail dragonflies (Odonata: Petaluridae): Mesozoic habitat specialists that survive to the modern day. J. Biogeogr. 41, 1291–1300.
Ware, J., May, M., Kjer, K., 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. Mol. Phylogenet. Evol. 45, 289–310.
White, E.L., Hunt, P.D., Schlesinger, M.D., Corser, J.D., deMaynadier, P.G., 2015. Prioritizing Odonata for conservation action in the northeastern USA. Freshw. Sci. 34, 1079–1093.
Young, A.D., Lemmon, A.R., Skevington, J.H., Mengual, X., Ståhls, G., Reemer, M., Jordaens, K., Kelso, S., Lemmon, E.M., Hauser, M., De Meyer, M., Misof, B., Wiegmann, B.M., 2016. Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae). BMC Evol. Biol. 16, 143.
Yu, X., 2016. A description of the larva of Mesopodagrion tibetanum australe (Odonata: "Megapodagrionidae"). Int. J. Odonatol. 19, 275–282.
Yu, X., Bu, W., 2011. A description of the remarkable larva of Pseudolestes mirabilis Kirby (Odonata: Pseudolestidae). Int. J. Odonatol. 14, 105–110.
Yu, X., Bu, W., 2009. A Revision of Mesopodagrion McLachlan, 1896 (Odonata: Zygoptera: Megapodagrionidae). https://doi.org/10.5281/zenodo.189645.
Zhang, H.M., 2019. Dragonflies and Damselflies of China. National Publication Foundation, China. National Publication Foundation.