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A molecular phylogeny and classification of Anisoptera (Odonata)

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Abstract

A phylogeny of Anisoptera employing 510 representatives of 184 genera (of ca. 380) in 11 families is presented based on an analysis of over 10,000 nucleotides from portions of the large and small subunit nuclear and mitochondrial ribosomal RNA's, the mitochondrial protein coding genes COI and COII, and portions of the nuclear protein coding genes EF-1α and Histone H3. Ribosomal sequences were structurally aligned and sequences carefully checked to eliminate alignment errors, contamination, misidentification and paralogous gene amplicons. Both the RAxML and Bayesian topology based on consolidation of data at the generic level is ((Austropetaliidae, Aeshnidae), ((Gomphidae, Petaluridae), ((Chlorogomphidae, (Neopetaliidae, Cordulegastridae)), (Synthemistidae, (Macromiidae, (Corduliidae, Libellulidae)))))). As the positions of Petaluridae, Chlorogomphidae, Neopetaliidae, and Cordulegastridae are weakly supported, possible alternative hypotheses are discussed. New taxonomic groups established include: in Gomphidae, Stylogomphini **trib.n.** and Davidioidini **trib.n.**, and in Libellulidae, Dythemistinae **subfam.n.** including Dythemistini **trib.n.**, Pachydiplactini **trib.n.** and Elgini **trib.n.** New taxonomic arrangements include: placement of Hemigomphini in Ictinogomphinae, and provisional expansion of Synthemistidae to include Gomphomacromiinae and a number of genera formerly placed in several small subfamilies of Corduliidae. Idomacromiinae is placed sister to remaining Synthemistidae *s.l.* based on molecular analysis of *Idomacromia* Karsch and *Oxygastra* Selys. Hemicorduliidae and Macrodiplactidae are nested well within Corduliidae and Libellulidae, respectively, and therefore are not accorded family rank. Eleven monophyletic subdivisions of Libellulidae are tentatively recognized as subfamilies: Dythemistinae **subfam.n.**; Sympetrinae (including Leucorrhiniini and Rhyothemistini); Macrodiplactinae; Brachydiplactinae; Tetrathemistinae; Trameinae; Zyxommatinae; Palpopleurinae; Diastatopidinae; Pantalinae (including Trithemistini and Onychothemistini); and Libellulinae. Zygonychini is paraphyletic to and therefore included within Onychothemistini.

Key words

Anisoptera, Odonata, phylogeny, Austropetaliidae, Aeshnidae, Gomphidae, Petaluridae, Synthemistidae, Macromiidae, Corduliidae, Libellulidae, 18S, 28S, 16S, 12S, COI, COII, EF-1α, H3, congruence.

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Figures, Tables, and Matrices with an 'S' preceding their number are part of the Electronic Supplement. They are not included in the printed article but can be downloaded at www.arthropod-systematics.de; their legends are printed at the end of this article.

1. Introduction

Odonata are considered to be among the "charismatic megafauna" of insects: they are large, diurnal, often colorful, exhibit elaborate behaviors, and have become cultural icons in many parts of the world. Odonata were among the first animals to fly, and exhibit a unique flight mechanism and wing venation (Snodgrass 1935; Riek & Kukalová-Peck 1984; Pfau 1986; Brauckmann & Zessin 1989). They have complex mating systems and unique copulatory structures (Schmidt 1915; Carle 1982a; Pfau 1971, 2011), and have been the subjects of important

studies of behavior and ecology (Corbet 1999). They are cosmopolitan, yet many subgroups are geographically or environmentally restricted, making them useful study organisms for biogeographers (e.g., Tillyard 1917; Watson 1977; Carle 1982a, 1995; Turgeon et al. 2005) including the identification of areas of endemism (e.g., Tillyard 1917; Mitra et al. 2010; Clausnitzer et al. 2012). They are valuable indicators of water quality and useful in the determination of ecological integrity (Carle 1979; Oertli 2008). The popularity of Odonata collecting with careful recording of collection data has enabled their utilization as indicators of climate change (e.g., Hassel et al. 2007; Roble et al. 2009). Nonetheless, the exact phylogenetic placement of Odonata within Pterygota and the phylogenetic arrangement of constituent families has remained in dispute despite a rich fossil record and numerous rather recent morphological phylogenetic studies (Hennig 1981; Carle 1982b, 1986, 1995, 1996; Nel et al. 1993; Carle & Louton 1994; Bechly 1996; Lohmann 1996; Trueman 1996; Carle & Kjer 2002; Fleck et al. 2003; Rehn 2003; Klass 2008; Huang & Nel 2009; Blanke et al. 2013; Thomas et al. 2013).

Early results from rRNA (Fig. S7), employing several methods of analysis, supported the topology: ((Austropetaliidae + Aeshnidae) (Gomphoidea (Petaluroidea (Cordulegastridae (Neopetaliidae (Libellulidae (Macromiidae + Corduliidae))))))). A combined analysis employing data from rRNA, EF-1α, mitochondrial DNA, and morphology, and including additional taxon sequences from Gen Bank (Fig. S8), resulted in a topology similar to that derived from rRNA alone, except with Corduliidae as sister to Libellulidae. Subsequently, a proliferation of phylogenetic hypotheses has been generated based on molecular evidence for Anisoptera (Misof et al. 2001; Saux et al. 2003; Hasegawa & Kasuya 2006; Letsch 2007; Ware et al. 2007; Bybee et al. 2008; Carle et al. 2008; Fleck et al. 2008b; Dumont et al. 2010; Davis et al. 2011; Blanke et al. 2013). Most of these hypotheses have been consistent in finding both Anisoptera and Zygoptera monophyletic, with *Epiophlebia* Calvert, 1903 sister to Anisoptera, though relationships within suborders have eluded consensus.

In Anisoptera, except for universal recognition that the Libellulidae was one of the last major groups to evolve, nearly every possible arrangement of families has been proposed, as well as establishment of yet to be recognized families. It has become clear that many of the genera formerly placed in Corduliidae either form a group paraphyletic to Synthemistidae (the "GSI" of Ware et al. 2007) or are paraphyletic relative to remaining Libelluloidea and should be placed in one or more separate families. Broad intrafamilial relationships are also poorly understood for the most part, although some well-supported subfamilial or tribal groupings have emerged (e.g., Ware et al. 2007; Letsch 2007; Fleck et al. 2008a,b).

Our aim here has been to develop a phylogeny from selected molecular data to shed light on these persistent problems and contradictions in anisopteran phylogeny. To that end we generated new sequence data and added to it available mitochondrial, nuclear protein coding, and ribosomal sequences with sufficient taxon coverage, using structural alignment for rRNA, along with careful editing to eliminate paralogous gene copies, contamination, misidentification, and alignment errors. The resulting data matrix is the largest yet applied to anisopteran phylogeny, both in terms of the number of nucleotides and the number of taxa included.

2. Materials and methods

2.1. Outgroup selection

Odonata rRNA sequences were relatively easy to align across the order, but are difficult to align across even closely related outgroups. Odonata is almost certainly monophyletic, as is Anisoptera (e.g., Calvert 1893; Needham 1903; Tillyard 1917; Fraser 1957; Carle 1982a, 1995; Rehn 2003; Bybee et al. 2008; Carle et al. 2008). The sister taxon of Anisoptera is also very strongly corroborated as *Epiophlebia* (Fraser 1957; Hennig 1969, 1981; Carle 1982b, 1995; Trueman 1996; Pfau 1991; Bechly 1996; Rehn 2003; Bybee et al. 2008; Carle et al. 2008; Klass 2008; Davis et al. 2011). Therefore, to avoid problems with homology due to alignment ambiguity, outgroups were chosen from within Zygoptera. Analysis of Zygoptera (Carle et al. 2008) showed that the suborder could be well represented by Lestidae, Synlestidae, Calopterygidae, and Coenagrionidae, so representatives from these families were used to provide the outgroup data for (*Epiophlebia* + Anisoptera).

2.2. Data selection

Several laboratories have been working on anisopteran phylogeny using overlapping molecular data: the Misof lab (Misof et al. 2001; Letsch 2007; Fleck et al. 2008a,b); Kjer and Ware labs (Ware et al. 2007; Carle et al. 2008); Branham, Bybee and Whiting labs (Bybee et al. 2008); von Dohlen lab (Pilgrim & von Dohlen 2008); and the Dumont lab (Dumont et al. 2010), with many other papers including additional fragments. Although our group has sequenced much of the data needed for a major analysis, there are so many other data available that it was decided that it would be unjustified to exclude GenBank data. This led to a series of decisions, some of them arbitrary, that had to be made about which data, and which taxa to include in the analysis. The most commonly sequenced fragments for Odonata are the nuclear rRNA (18S, 28S), the nuclear elongation factor, subunit 1 alpha (EF-1α), and mitochondrial rRNA (12S, 16S), all which we sequenced. In addition, others have commonly sequenced the mitochondrial cytochrome oxidase, subunits 1 and 2 (COI, COII), and the nuclear Histone H3. Selected markers included the 18S and 28S rRNA (6836

sites), mitochondrial 12S-16S plus the intervening Val tRNA, along with COI and COII (4602 sites), Histone H3 (324 sites) and EF-1α (1068 exon sites), because data exist for these fragments that can completely represent all anisopteran higher groups. Other fragments in GenBank were not included because they would have resulted in mostly missing data. The degree to which missing data is problematic is still debated (Wiens 2005; Wiens & Morrill 2011; Lemmon et al. 2009), but while missing data may not be devastating to an analysis, there is general agreement that it is better to have data present and well distributed throughout the tree than to have mostly missing data with sporadic taxon representation.

2.3. Lab protocols

DNA was extracted, amplified and purified using standard protocols. Selected primers used are listed in Table S9. PCR conditions followed standard profiles, with 50°C annealing temperatures. Amplicons from both strands were purified and used as templates for cycle sequencing using Applied Biosystems BigDye ReadyMix. DNA sequencing was performed under a variety of platforms; first with an ABI 377 sequencer using acrylamide gels and later with the capillary sequencers at GeneWiz (Piscataway, NJ). Forward and reverse sequences were edited and consensus sequences created as in Kjer et al. (2001).

2.4. Alignment

Exonic portions of protein coding genes were invariant in length, and alignment was unambiguous. Ribosomal data were manually aligned according to secondary structure as described in Kjer (1995) and Kjer et al. (2007). Alignment ambiguous sites were removed from the analysis according to the following prespecified criteria: single stranded regions of rRNA were considered alignment ambiguous if they were length variable, and did not contain conserved motifs; conserved motifs are loosely defined as strings of at least 3 nucleotides conserved across 75% of the taxa; these motifs are often found in the middle of single-stranded hairpin stem loops. This approach is justified by the observation that stem-strand slippage most frequently results in the lengthening and contracting of hairpin stems, leaving both the base and the tips of the loops conserved (Gillespie et al. 2004). In cases where an otherwise alignable single stranded loop is made alignment ambiguous by a few taxa, the nucleotides from these taxa are shifted into the deleted regions, so that they would be treated as missing data.

2.5. Taxon selection and combination

In order to further minimize missing data, a multi-tiered analysis was conducted which began with a supermatrix (electronic supplement: Matrix S1), in which each species was kept as a separate taxon. Congeners were then examined. Congeners, by being placed in the same genus, have been considered by some taxonomist to be closely related, at least in some sense. However, since the purpose of our analysis is, in part, to test current taxonomic hypotheses, and to propose a phylogeny-based classification, congeners were not automatically combined. A preliminary phylogenetic analysis of the initial supermatrix (Matrix S1) was conducted using the PR-reweighting scheme described in Kjer & Honneycutt (2007) in order to assign each nucleotide to one of 5 partitions according to its substitution rate, followed by a RAxML (Stamatakis 2006) analysis using a GTR plus CAT model. Results of this preliminary analysis were examined and the data from monophyletic congeners were merged to create a more complete data matrix; markers for which sequences were contributed by more than one of the congeneric taxa were combined into a consensus sequence that included IUPAC ambiguity codes at polymorphic sites. For example, *Hemigomphus heteroclytus* plus *Hemigomphus magela* came out monophyletic in the preliminary analysis so a chimeric single *Hemigomphus* terminal was created that contains the 28S, 12S, and 16S from *H. heteroclytus* and the 18S and H3 from *H. magela*. Congeners that were not placed in an exclusive monophylum in the preliminary analysis were kept separate. This allowed a drastic reduction in the amount of missing data and reduced the number of terminal taxa. Taxa that were mostly missing data and that could not be phylogenetically linked to a congener were eliminated. These decisions were more arbitrary, in that a precise method was not used to balance decisions based upon the amount of missing data with interest in the taxon. In other words, some taxa with few data were retained because they were of critical taxonomic interest, while others were excluded because closely related taxa with more data were available. In the latter cases, taxa were favored that had large amounts of 28S and 16S data; taxa that had only a single fragment were deleted; taxa that had only a few fragments were also deleted if their putative subfamily was well represented by other taxa. Using these criteria for selection of taxa and combinations of data a "consolidated data matrix" was created, reducing the number of taxa from 510 in the preliminary analysis to 233 in the definitive analysis (including 20 outgroup taxa). This matrix, created directly from matrix in Matrix S1, is available in the electronic supplement (Matrix S2). The alignments and Nexus files for both Matrices S1 and S2 are available on Kjer's website, http://rci.rutgers.edu/~insects/pdata.htm. All new sequences have been submitted to GenBank (see supplement file 10 for accession numbers).

Analyses using a matrix without combination of congeners or deletion of taxa (resulting in "non-consolidated" trees) were performed on three subgroups supported in the consolidated tree: Aeshnoidea, Gomphoidea, and Libelluloidea *s.s.* Since the most recent common ancestor of subgroups existed more recently than the common ancestor of all of Odonata, the data exclusion decisions were re-evaluated so that fewer nucleotides were ex-

cluded because of alignment ambiguity. Data partitions (usually the COI) that were represented by three or fewer taxa were not included. These matrices were also created from Matrix S1, and the alternative (relaxed) data exclusion sites (unaligned "charsets") are listed at the end of the Nexus file (Matrix S1).

2.6. Phylogenetic analyses

Aligned and concatenated sequence data from reduced taxon set (Matrix S2) were partitioned into 5 site-specific rate classes according to Kjer & Honneycutt (2007). This method places individual nucleotides into discreet bins according to their estimated substitution rates, which are estimated according to their best fit on a mixture of trees generated from pseudoreplicate (bootstrap) datasets. Thus, for example, a slow third codon position (such as one coding for tryptophan) may be placed in the same partition as a second codon site. In other words, the method attempts to partition the data into bins according to similar substitution rates. Because protein coding data is often subdivided into 3 codons, but we note that there are both 2-fold and 4-fold redundant third codon sites, and Leucine first codon sites can also change without changing the amino acid state for which they code, we selected 5 rate classes to capture these potentially different rates among sites. This is admittedly aribitrary, but less so than arbitrarily partitioning into 3 codon subsets. More research is needed on selecting the optimum number of rate classes (Frandsen et al. 2015). A GTR + gamma model, approximated with the CAT model for efficiency (Stamatiakis 2006), was used for each partition, analyzed with RAxML (Stamatikis 2006), to calculate a best tree, with rapid bootstrap values, using command "-f a -q". Subgroup analyses from dataset S1 (without the consolidation of taxa) were partitioned by genes. The 28S and 18S were considered to be the same partition, and the mt rRNA, COI and COII were also pooled into the same partition. Results from individual genes should not be considered phylogenetic hypotheses, but rather, they provide independent sources for evidence of congruence or incongruence. These trees from analyses of individual partitions can be found in the electronic supplement (Figs. S3–S6). Congruence of the combined analysis with the nuclear rRNA, mt rRNA+COI/COII, EF-1α and H3 partitions is indicated on Fig. 1.

In addition, a Bayesian analysis was performed using MRBAYES 3.1.1 (HuelsenbeckwRonquist 2001) for matrix S2 only, using GTR rate model determined using the Akaike information criterion (AIC, Akaike 1974) in Modeltest 3.6 (Posada & Crandall 1998), and using the same partitions as described above. Analyses were performed with random starting trees without constraints. Two sets of four Markov chains were run simultaneously for 10,000,000 generations with sampling every 2000th. A burn-in value of 200,000 was empirically determined based on evaluation of likelihood scores converging on stable values.

2.7. Error reduction

DNA was utilized from at least 22 PCR amplicons. We recognized that sequences in GenBank may be contaminants (i.e., DNA from an organism other than the specimen intended to have been sequenced), or involve misidentification, including mislabeling. Such erroneous results can be expected even from careful workers, so it was assumed that some of our sequences as well as sequences obtained from GenBank may not have been what they were thought to be. Phylogenetically distant contaminants can be identified from a BLAST (Altschul et al. 1990) search, but this might be much less successful in detecting misidentifications and phylogenetically close contaminants (e.g., from previous rounds of DNA amplification in the same lab). A more phylogenetically based approach was preferred here for that reason, and because often there are many families represented in the top hits from a BLAST search, because fragments may not have enough variable sites to distinguish among taxa with a distance based approach. However, identifying taxonomic errors in a multi-locus dataset with phylogenetic approaches can also be difficult for the same reason as BLAST may fail (too few characters). Therefore, error detection involved an analysis of the entire dataset, but each PCR amplicon of the evaluated fragment was individually upweighted 1000-fold so that signal from each selected fragment would dominate an analysis. Using this method, where there was insufficient signal from the targeted fragment, the other data could resolve the tree, but even a single nucleotide out of place would be amplified 1000-fold, and thus, detected. For this analysis pseudoreplicate reweighted parsimony was utilized (PRP: Kjer et al. 2001; Kjer & Honeycutt 2007). While parsimony is rejected in general for molecular data, it is applicable for this method of error detection because PRP can be rapidly completed, and was found to be among the most effective weighting schemes, and as efficient as both likelihood and Bayesian approaches in terms of phylogenetic accuracy (Kjer et al. 2007). Each site from the combined data was assigned a weight according to its best fit on 1000 bootstrap trees. These weights were then imported to an Excel file, which was then used to multiply these weights by 1000 for each of the 22 PCR amplicons successively, leaving the other weights for each of the non-targeted fragments. A heuristic search was then completed for each targeted fragment. Resultant phylograms were then examined. We looked for two characteristic signs of contamination and misidentification: excessively long terminal branches or taxa placed outside their families. This method detected not only contaminants and misidentifications, but also misalignments. For example, imagine that the last 5 nucleotides in a PCR amplicon are shifted 1 nucleotide to the right of all their neighbors. This shift would be magnified to 5000 autapomorphies that are then easily detected on a phylogram by branch length. Contaminants are similarly identified, because even a few nucleotides (multiplied by 1000) will cast a taxon out of where it should belong, and

give it an easily identified long terminal branch. Fragments in question from taxa that demonstrated these long terminal branches were first examined for alignment errors, and the alignment was adjusted when errors were found. If the problem with the fragment was not the result of alignment error the fragment was submitted to a BLAST search. Only when the BLAST search resulted in strongest matches outside a given family, the data were considered to be contaminants or misidentifications, and discarded. Misidentifications inside families, however, would not be detected by this method. Paralogous gene copies can also seriously affect the results of a phylogenetic analysis (Djernæs & Damgaard 2006). EF-1α included three amplicons and in order to insure that all amplicons were from the targeted gene copy, primers were designed with substantial amplicon overlap. Non-chimeric amplicon sets from incorrect gene copies were detected by employing the phylogenetic weighting scheme utilized for detecting other contaminated amplicons. As for genetically similar species, however, our methods cannot insure distinction among very similar paralogous copies.

3. Results

Our principal results are presented in Figs. 1–4, with support values given on the trees. All analyses recovered monophyletic *Epiophlebia* + Anisoptera and a monophyletic Anisoptera with very high support by all criteria: maximum likelihood, Bayesian posterior probability, and congruence with individual gene partitions. Consequently, Anisoptera is regarded as an established taxon throughout. Comparison of Figs. 1 (based on all data) and S4 (based on mitochondrial data alone) shows that the mitochondrial partition is probably approaching saturation within the suborders (Misof et al. 2001), as it only shows appreciable congruence with the consolidated tree at the terminal branches, especially in Libellulidae. The H3 partition (Fig. S6) reveals little topology in common with the topology of the combined data tree. Visualizing congruence on Fig. 1 shows that nuclear and mitochondrial rRNA, as well as EF-1α recover many nodes that are found in the combined data topology. Note that in Fig. 1B and 1C bootstrap values and posterior probabilities appear, respectively, above and below each branch leading to the node to which the support metrics refer. Figure 1A (outgroup taxa) and Figs. 2–4 ('unconsolidated' trees) show only bootstrap support.

3.1. Major anisopteran monophyletic groups and their relationships

Results, except those from H3 (Fig. S6), consistently recover Aeshnoidea (Aeshnidae + Austropetaliidae), Petaluroidea (Petaluridae), Gomphoidea (Gomphidae), Cavealabiata (= Cavilabiata Bechly, 1996 and Libelluloidea *sensu* Carle 1986) and its constituent families (Cordulegastridae, Neopetaliidae, Chlorogomphidae, Synthemistidae, Macromiidae, Corduliidae, and Libellulidae). Herein the Libelluloidea is restricted to the Synthemistidae, Macromiidae, Corduliidae, and Libellulidae. Aeshnoidea (Austropetaliidae + Aeshnidae, Figs. 1B, 2) is sister to the remaining Anisoptera with 100% bootstrap support (= BS) for Aeshnoidea and 99% BS for the remaining Anisoptera; nuclear rRNA and EF-1α trees are congruent with this topology, as are Bayesian results

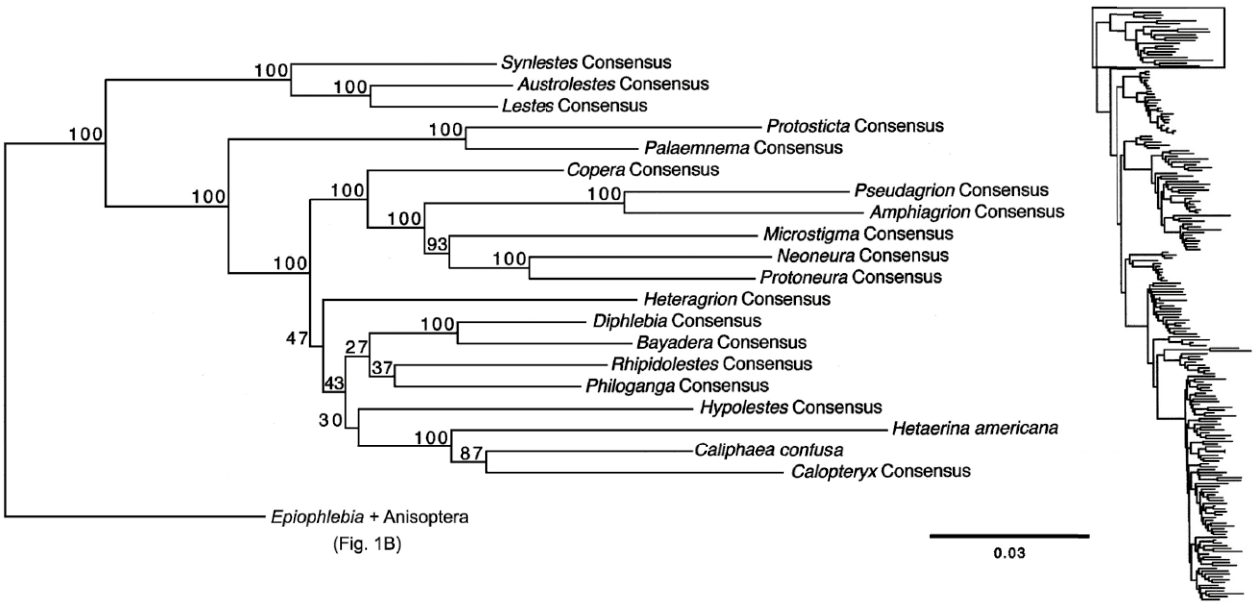

Fig. 1A. 'Consolidated' maximum likelihood (RAxML) phylogram, derived from Matrix S2, showing selection of Zygoptera outgroup taxa used to root the consolidated Anisoptera tree shown in Figs. 1B and 1C. The position of these taxa within extant Odonata as a whole is shown by the rectangular outline on the reduced tree to the right. Numbers at each node are bootstrap support values.

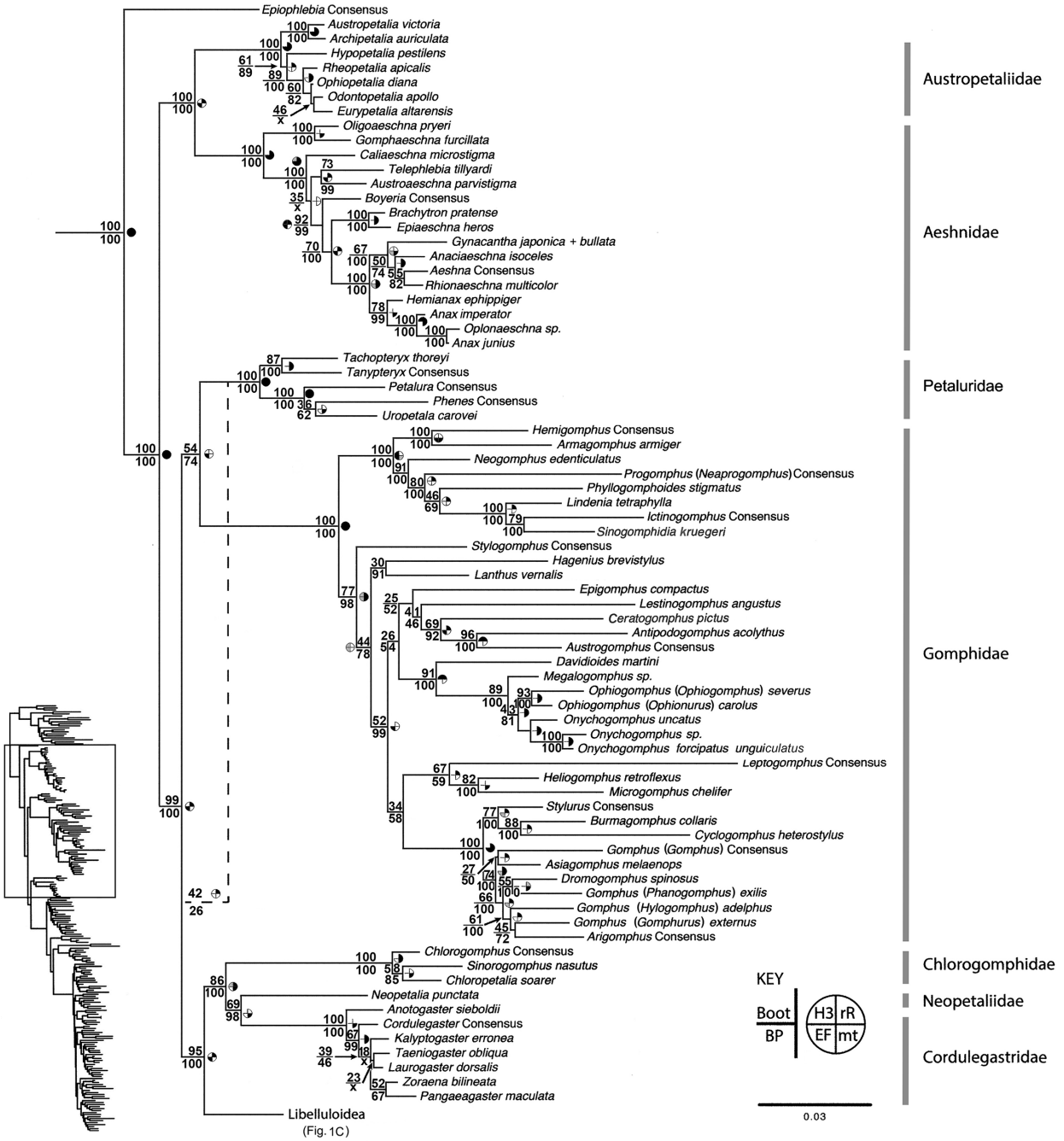

Fig. 1B. 'Consolidated' maximum likelihood (RAxML) phylogram derived from matrix S2 for *Epiophlebia* plus Anisoptera excluding Libelluloidea. — Position of each subtree within extant Odonata is shown by the rectangular outline on the reduced tree at left bottom. The extent of each family is indicated by the bar at the right. Bootstrap support, Bayesian posterior probability, and congruence with trees generated from individual data partitions are shown at each node with support as indicated in the 4partite circle key: white = none, gray = partial, black = complete or nearly so, white without a circumference line = insufficient information to categorize (H3 = histone3, EF = EF-1α, rR = nuclear rRNA, mt = mitochondrial rRNA, COI and COII); an 'x' in place of the Bayesian probability indicates that the Bayesian analysis did not recover the group in question.

with 100% posterior probabilities (= PP). The next major split is either between Gomphoidea and (Petaluroidea + Cavealabiata) or between (Petaluroidea + Gomphoidea) and Cavealabiata with the latter topology supported independently only by EF-1α. Resolution in favor of either topology is not clear cut in the consolidated analysis. Posterior probability of Petaluroidea + Gomphoidea in the Bayesian analysis is 74%, but the other 26% of trees all group Petaluridae with Cavealabiata as does the nuclear rRNA data partition (which we consider reliable; Figs. 1B, S3). Petaluridae is weakly supported as sister to Aeshnoidea by the mitochondrial partition (BS = 27%; Figs. 1B, S4).

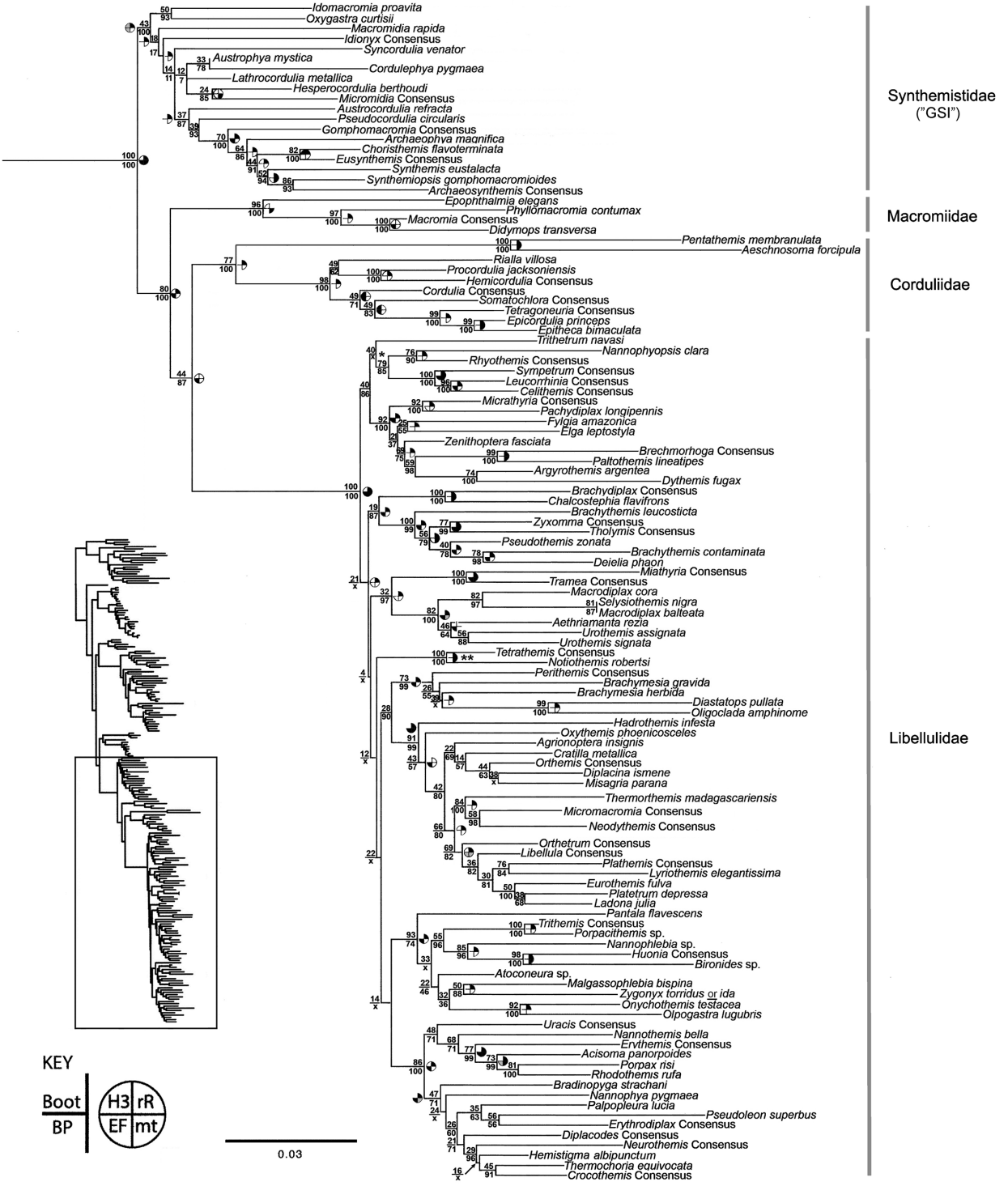

Fig. 1C. 'Consolidated' maximum likelihood (RAxML) phylogram derived from Matrix S2 for Libelluloidea. — Sub-tree position, family extent, bootstrap support, Bayesian posterior probability, and congruence of data partitions as in Fig. 1B. * = Bayesian tree excludes *Trithetrum navasi* from this group; ** = Bayesian tree includes *Trithetrum navasi* in this group.

3.2. Relationships within Austropetaliidae and Aeshnidae

All analyses split Austropetaliidae into Australian Austropetaliinae + Tasmainian Archipetaliinae (*Austropetalia* Tillyard, 1916 – *Archipetalia* Tillyard, 1917 ["–" meaning from the former to the latter taxon in phylogenetic trees]) and Chilean Hypopetaliinae + Eurypetaliinae (*Hypopetalia* McLachlan, 1870 – *Eurypetalia* Carle, 1996) with high confidence (Figs. 1B, 2). However, RAxML analyses of the mitochondrial markers (Fig. S4) and of EF-1α (Fig. S5), place Hypopetaliinae sister to (Austropetaliinae + Eurypetaliinae).

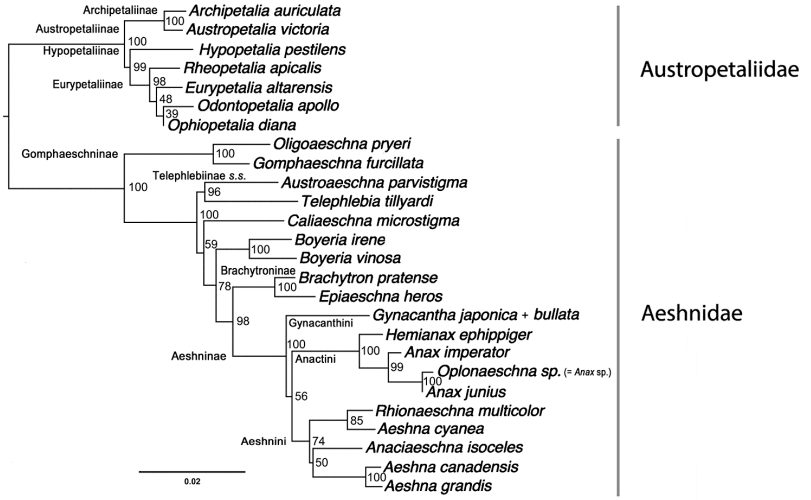

Fig. 2. 'Non-consolidated' maximum likelihood (RAxML) phylogram using relaxed exclusion criteria for sequence fragments (Matrix S1) for Aeshnoidea *sensu* CARLE & LOUTON (1994). — This superfamily arises from the most basal node of the anisopteran topology. Families are indicated as in Fig. 1B, with selected subfamilies labeled at the base of corresponding nodes. Node support is indicated by bootstrap values.

Gomphaeschninae is well supported as sister to the remaining aeshnids, however Brachytroninae as defined by FRASER (1957) is not supported and forms a paraphyletic series relative to Aeshninae. Aeshninae (*Gynacantha* Rambur, 1842 – *Anax* Leach, 1815) is a strongly supported monophyletic group represented by three of its traditional tribes: Anactini, Gynacanthini and Aeshnini. Note that *Oplonaeschna* sp. is placed within *Anax* Leach, 1815. Based on the many morphological dissimilarities between these genera, this is almost certainly incorrect and suggests that the *Oplonaeschna* sequences from GenBank were misidentified or mislabeled. Although *Oplonaeschna* was misplaced in all testing topologies, our prescribed means of detecting contaminants would not have confirmed a mislabeling in this case.

3.3. Relationships within Gomphidae and Petaluridae

The deepest phylogenetic division within Gomphidae almost always appears within the plesiotypic "Octogomphinae" (*Hemigomphus* Selys, 1854 – *Lanthus* Needham, 1895; CARLE & COOK 1984; CARLE 1986; Figs. 1B, 3); this split places a paraphyletic Hemigomphini (*Hemigomphus* – *Neogomphus* Selys, 1854) at the base of Ictinogomphinae (*Hemigomphus* – *Sinogomphidia* Carle, 1986; Fig. 1B). The exception to this arrangement occurs in the nuclear rRNA partition (Fig. S3), where all Octogomphinae are weakly clustered near the base of the other main branch of Gomphidae which also includes: Epigomphinae, Phyllogomphinae, Austrogomphinae, Onychogomphinae, and Gomphinae. Placement of New World Progomphini (*Progomphus* Selys, 1854) and Gomphoidini (*Phyllogomphoides* Belle, 1970; Figs. 1B, 3) within Ictinogomphinae (TILLYARD & FRASER 1940; CARLE 1986, as Lindeniinae) is well supported, with SELYS' (1854) Old World Légion Lindenia (*Lindenia* de Hann, 1826 – *Sinogomphidia*) firmly placed as the most highly derived group of Ictinogomphinae.

In the combined analysis the second basal branch of Gomphidae (BS = 77, PP = 98; Figs. 1B, 3), begins with two weak nodes that when collapsed result in a polytomy of the remaining Octogomphinae (*Stylogomphu*s Fraser, 1922 – *Lanthus*) and the Hageniinae (*Hagenius* Selys, 1854) placed at the base of remaining Gomphidae (BS = 52, PP = 99). At this level of the topology collapsing nodes with less than 35% bootstraps results in a polytomy of the remaining subfamilies of Gomphidae: with (Phyllogomphinae + Austrogomphinae) represented by *Lestinogomphus* Martin, 1911 – *Austrogomphus* Selys, 1854 (BS = 41, PP = 46); Onychogomphinae represented by *Davidioides* Fraser, 1924 – *Onychogomphus* Selys, 1854 (BS = 91, PP = 100); and Gomphinae represented by *Stylurus* Needham, 1897 – *Arigomphus* Needham, 1897 (BS = 100, PP = 100). In addition, Epigomphinae is represented by the remaining two branches of the polytomy, which include the New World *Epigomphus* Hagen in Selys, 1854, and the Old World *Leptogomphus* Selys, 1878, *Microgomphus* Selys, 1858 and *Heliogomphus* Laidlaw, 1922 (BS = 67, PP = 59). These branches of Epigomphinae occupy alternative sister group positions relative to Gomphinae in Figs. 1B and 3. The high support for placement of *Stylurus* Needham, 1897 within the Cyclogomphini clearly differentiates it from the Gomphini with which it has often been placed.

Petaluridae is clearly divided into two groups, corresponding to Northern Hemisphere Tachopteryginae and Southern Hemisphere Petalurinae (CARLE 1995).

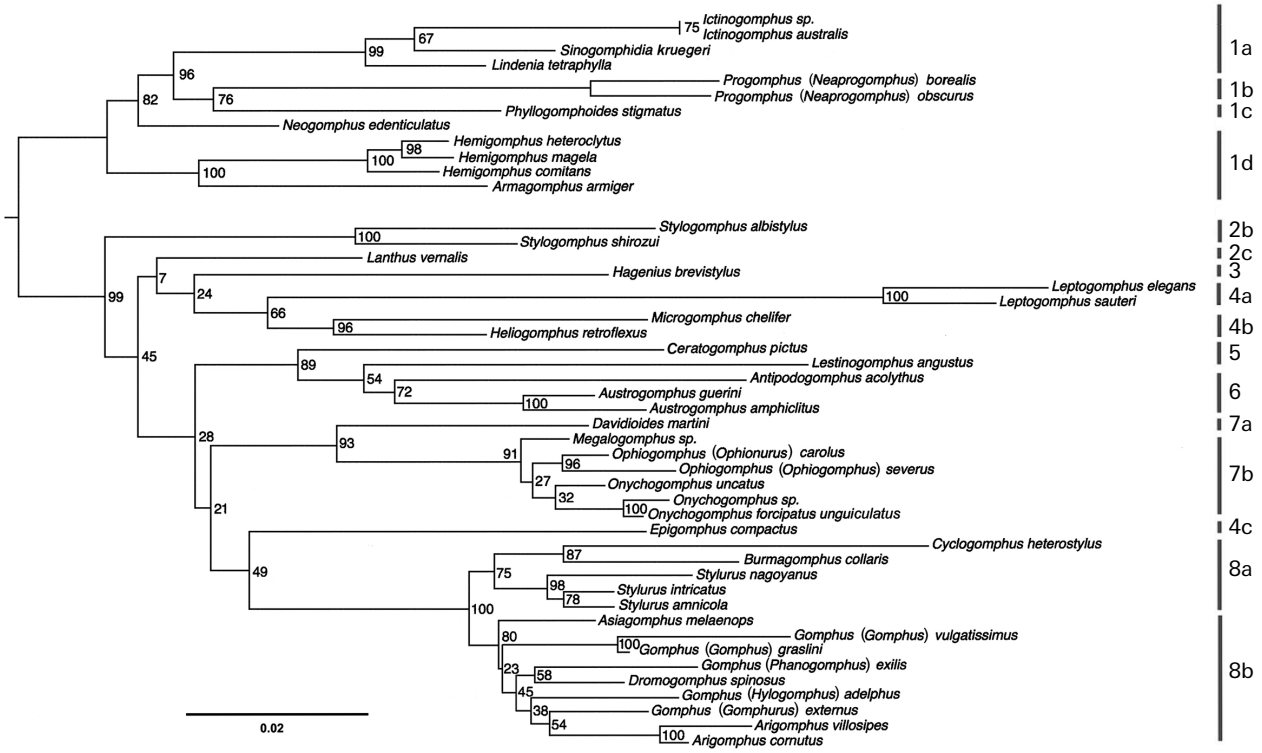

Fig. 3. 'Non-consolidated' maximum likelihood (RAxML) phylogram using relaxed exclusion criteria for sequence fragments (Matrix S1) for Gomphidae. — The eight subfamilies recognized by Carle (1986) are numbered to the right of the tree, they are: **1** – Ictinogomphinae (**1a** Ictinogomphini, **1b** Progomphini, **1c** Gomphoidini, **1d** Hemigomphini); **2** – Octogomphinae (**2a** Stylogomphini **trib.n.**, **2b** Octogomphini); **3** – Hageniinae; **4** – Epigomphinae (**4a** Leptogomphini, **4b** Microgomphini, **4c** Epigomphini); **5** – Phylogomphinae; **6** – Austrogomphinae; **7** – Onychogomphinae (**7a** Davidioidini **trib.n.**, **7b** Onychogomphini); **8** – Gomphinae (**8a** Cyclogomphini, **8b** Gomphini). Node support is indicated bybootstrap values.

3.4. Relationships within Cavealabiata

Cordulegastridae, Neopetaliidae, and Chlorogomphidae form a monophyletic group, with good support (BS = 86, PP =100, Fig. 1B), including congruence with both nuclear rRNA and mitochondrial data partitions, although EF-1α weakly supports a paraphyletic topology leading to Libelluloidea (Fig. S5). The three families are each individually well supported as monophyletic and separated by moderately long internodes. Note that we follow Carle (1983) and Lohmann (1992) in recognizing cordulegastrid genera and Carle (1995) for chlorogomphid and synthemistid genera.

Libelluloidea consists of four apparent monophyletic groups in Fig. 1C; with Macromiidae, Corduliidae, and Libellulidae well-supported (BS = 96, PP = 100; BS = 77, PP = 100; BS = 100, PP = 100, respectively). The fourth and earliest offshoot, Synthemistidae (here tentatively regarded as equivalent to the GSI of Ware et al. 2007) receives fair support in the combined analysis (BS = 43, PP = 100) and is supported by the nuclear rRNA partition (BS = 67). The GSI group includes Synthemistidae (*sensu* Tillyard 1917; *Choristhemis* Tillyard, 1910 – *Archaeosynthemis* Carle, 1995 in Fig. 1C) plus genera previously placed in Corduliidae (*Idomacromia* Karsch, 1896 – *Archaeophya* Fraser, 1959 in Fig. 1C; Fraser 1957; Davies & Tobin 1985). *Gomphomacromia* Brauer, 1864 and *Archaeophya* are recovered within traditional Synthemistidae, as morphology suggests (Theischinger & Watson 1984; Carle 1995), and *Pseudocordulia* Tillyard, 1909 is sister to Synthemistidae in the restricted sense, again as suggested by morphology, but with poor support in the RAxML analysis. The Corduliphyinae of Tillyard (1917), and the Gomphomacromiinae, Idionychinae, and Idomacromiinae (Tillyard & Fraser 1940) form a paraphyletic series within an expanded Synthemistidae *s.l.* In the combined analysis Idomacromiinae (represented by *Idomacromia* and *Oxygastra* Selys, 1870; BS = 50, PP = 93) is placed as sister to remaining Synthemistidae *s.l.* The mitochondrial tree (Fig. S4) recovers Synthemistidae *s.l.* as paraphyletic relative to the remaining Libelluloidea with a partial polarity reversal relative to the combined analysis, and with *Oxygastra* Selys, 1870 in a polytomy with Macromiidae and plesiotypic Corduliidae, while *Macromidia* Martin, 1907 is placed sister to remaining Corduliidae.

Except for the tentative removal of a few synthemistids from Corduliidae, the remaining three monophyletic families of Libelluloidea correspond perfectly to the three traditional families, Macromiidae, Corduliidae and Libellulidae. *Hemicordulia* Selys, 1870 and *Procordulia* Martin, 1907, sometimes placed in their own family

Table 1. Comparison of our proposed Libellulidae subfamilies to major subdivisions proposed in the literature. For Letsch and Ware, letters are their designations for groups, numerals indicate immediate sister groups from the basal node of the lettered group, 'sub' indicates a more distal group within the labeled group. For other trees, groups were not given markers, so they are identified by the included taxa that are most distant from one another. Cells with '—' are those in which the taxon sample included no more than one of the genera in our corresponding group; 'not recovered' indicates that two or more genera of our group were sampled but did not form a monophyletic group. Groups in square brackets are nearly but not quite identical to our corresponding group, as indicated by superscript letters: [a] *Rhyothemis* excluded; [b] only two genera represented vs. at least four in present study; [c] three adjacent but paraphyletic groups.

This Study (Fig. 4)	**LETSCH 2007**	**PILGRIM & VAN DOHLEN 2007**	**WARE et al. 2008**	**FLECK et al. 2008** (fig. 2)	**FLECK et al. 2008** (fig. 3)	**DUMONT et al. 2009**
1	B	*Dythemis – Micrathyria*	F	—	—	—
2	A	[*Celithemis – Sympetrum*][a]	D	[*Celithemis – Sympetrum*][a]	*Celithemis – Sympetrum*	*Leucorrhinia – Sympetrum*[b]
3	C2	*Macrodiplax – Urothemis*[b]	B	*Urothemis – Macrodiplax*[b]	*Macrodiplax – Urothemis*	—
4	sub D2	—	sub E	*Brachydiplax – Chalcostephia*	*Chalcostephia – Brachydiplax*[b]	—
5	D1	—	A	*Tetrathemis – Notiothemis*	*Notiothemis – Tetrathemis*	—
6	C1	*Tramea – Miathyria*	–	—	—	—
7	sub D2	*Tholymis – Brachythemis*[b]	sub E	—	—	—
8	E	[*Nannothemis – Erythrodiplax*][c]	G	*Nannophya – Acisoma*	*Nannophya – Acisoma*	*Neurothemis – Diplacodes*[b]
9	F1	—	—	—	—	—
10	F2	*Onychothemis – Trithemis*	C + sub H1	not recovered	not recovered	*Zygonyx – Trithemis*[b]
11	G	*Orthemis – Libellula*	H2	*Orthetrum – Hadrothemis*	*Orthemis – Cratilla*	*Micromacromia – Orthetrum*

Hemicorduliidae, are here recovered well within Corduliidae. The somewhat aberrant corduliids *Pentathemis* Karsch, 1890 and *Aeschnosoma* Selys, 1870, long considered close relatives by WILLIAMSON (1908) and WATSON (1969), are placed sister to all other Corduliidae, with high support both in the combined analysis (BS = 77, PP = 100; Fig. 1C) and for nuclear rRNA (BS = 94; Fig. S3). This result agrees with morphological analyses by FLECK (2012) and FLECK & LEGRAND (2013).

The Macrodiplactidae of FRASER (1950) here represented by *Macrodiplax* Brauer, 1868 – *Urothemis* Brauer, 1868 (Fig. 1C) is placed well within, and therefore considered a subfamily of, Libellulidae. Results provided herein further improve the placement of genera into larger groups, most of which are placed within existing subfamilies with the exception of genera placed in a new subfamily sister to Sympetrinae. Numbers and subfamily names are as in Fig. 4 and Table 1: **1** – sister group of Sympetrinae (BS = 87, PP = 100); **2** – Sympetrinae including Leucorrhiniini and Rhyothemistini (BS = 89, PP = 85); **3** – Macrodiplactinae (BS = 86, PP = 100); **4** – Brachydiplactinae (BS = 100, PP = 100); **5** – Tetrathemistinae (BS = 35–100, PP = 67–100); **6** – Trameinae (BS = 99, PP = 100); **7** – Zyxommatinae (BS = 100, PP = 99); **8** – Palpopleurinae (BS = 86, PP = 100); **9** – Diastatopidinae (BS = 72, PP = 99); **10** – Pantalinae including Trithemistini and Onychothemistini (BS = 97, PP = 74–97); and **11** – Libellulinae (BS = 93, PP = 99). However, the composition of these redefined and sometimes provisional subfamilies is often quite different than their traditional generic make-up (e.g., FRASER 1957; BRIDGES 1994). There is also moderate indication of relationships among groups of subfamilies, but some deeper nodes remain frustratingly tenuous. Five possible groupings of subfamilies are recovered, weakly but fairly consistently, in various RAxML and Bayesian analyses (Figs. 1C, 4): Sympetrinae and its sister group (BS = 40–78, PP = 80–86), which are placed sister to remaining Libellulidae (BS = 78, PP = 80), Trameinae + Macrodiplactinae (BS = 32, PP = 97), Diastatopidinae + Libellulinae (BS = 28, PP = 90), Brachydiplactinae + Zyxommatinae (BS = 19, PP = 87), and Palpopleurinae + Diastatopidinae + Pantalinae + Libellulinae (BS = 20, PP = 69).

4. Discussion

4.1. Tree support, topology and taxonomic conclusions, with comments on biogeography

The data utilized here encompass more taxa and more sequence data than any previous analysis of Anisoptera, with particular care taken to eliminate misidentified taxa and erroneous sequences and misalignments. Among important confirmatory results is that all of the commonly recognized families are recovered, and some aspects of their internal topology are confirmed.

4.1.1. Major anisopteran monophyletic groups

Epiophlebioidea is the nearest extant sister group to Anisoptera, although several taxa, reportedly paraphyletic to modern Anisoptera, evolved during the interval between the origins of Epiophlebioidea and Aeshnoidea (CARLE 1982; BECHLY 1996), but then failed to survive the K-T extinction event, perhaps owing to a reliance on lentic habitats. *Epiophlebia* and plesiotypic Anisoptera

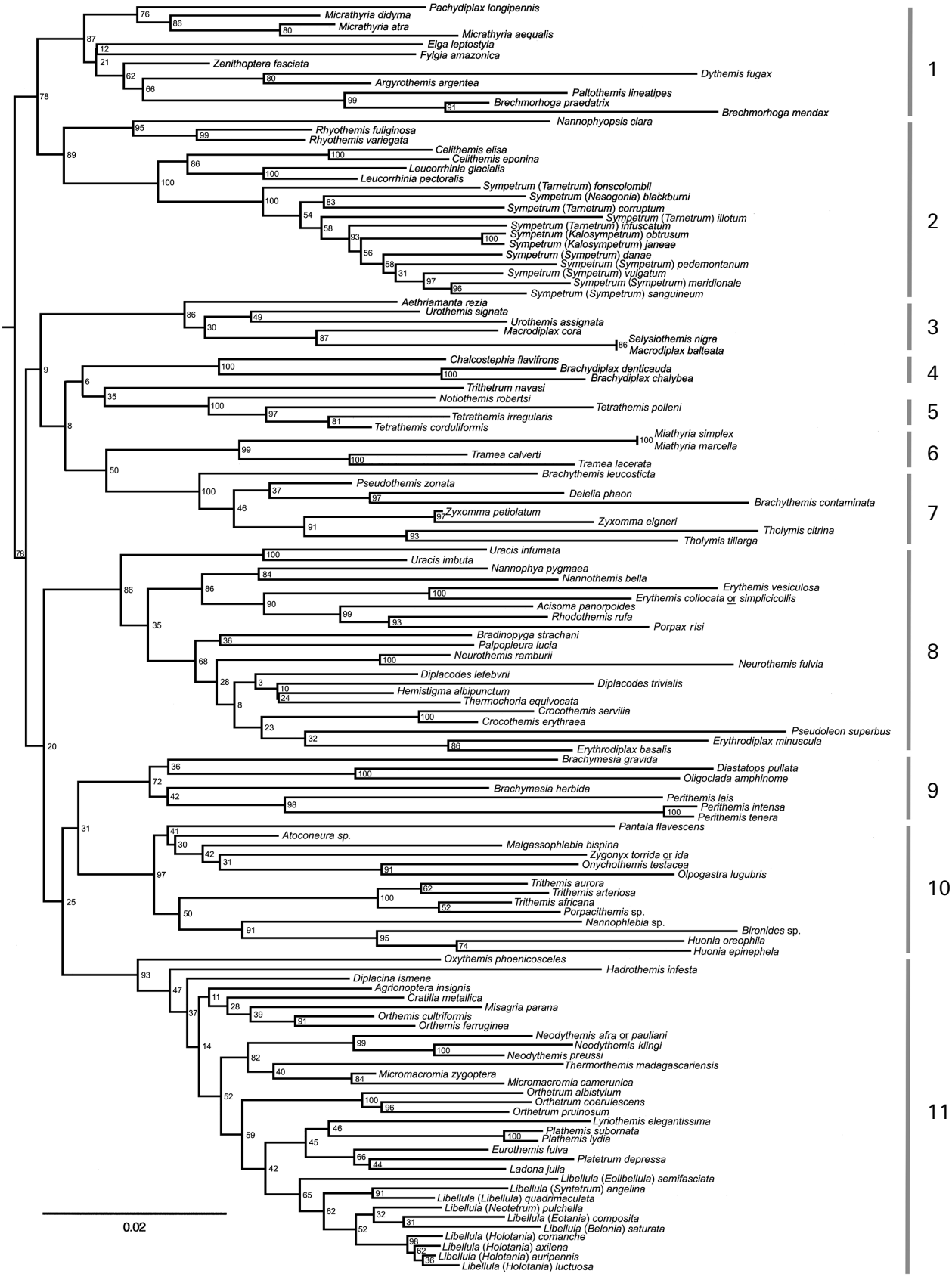

Fig. 4. 'Non-consolidated' maximum likelihood (RAxML) phylogram using relaxed exclusion criteria for sequence fragments (Matrix S1) for Libellulidae. — Based on the present analysis, as well as previous studies, 11 subfamilies are recogonized and numbered in the column to the right of the tree, they are: **1** – Dythemistinae **subfam.n.**; **2** – Sympetrinae; **3** – Macrodiplactinae; **4** – Brachydiplactinae; **5** – Tetrathemistinae; **6** – Trameinae; **7** – Zyxommatinae; **8** – Palpopleurinae; **9** – Diastatopidinae; **10** – Pantalinae; and **11** – Libellulinae. These subfamilies are compared with previous results from the literature in Table 1. Node support is indicated by bootstrap values. *Trithetrum* is likely a Sympetrinae, but its topological position is weak and varies greatly, suggesting a possible amplicon mislabeling (cf. Figs. 1C, S5).

(Austropetaliidae) today occupy small stream or seepage habitats and have isolated, relictual distributions in opposite hemispheres. This habitat, distinguished by the environmental stability of emerging ground water and the reliable flow of energy and nutrients through detritus based ecosystems, apparently provides some protection from global extinction events and may be crucial to the long term survival of these ancient insects (Carle 2012).

Results presented here and those of many recent workers (but see, e.g., Dumont et al. 2010; Fleck et al. 2008) clearly do not support the Aeshnoidea *s.l.* of Fraser (1954, 1957) and earlier authors, which placed Aeshnidae, Gomphidae and Petaluridae together in Aeshnoidea *s.l.* based on symplesiomorphic, or so-called "primitive" characters (e.g., Tillyard 1917; Fraser 1954; Needham & Westfall 1955; Walker 1958). Rather, our molecular results closely resemble the paraphyletic topology proposed by Carle (1982a, 1986, 1996), with the arrangement of superfamilies similar to that presented by Carle & Kjer (2002), i.e., (Aeshnoidea (Gomphoidea (Petaluroidea + Cavealabiata))); at this level our molecular topology differs only in that Gomphoidea and Petaluridae are grouped together.

Aeshnoidea, as defined by Carle & Louton (1994), i.e., Austropetaliidae + Aeshnidae, is placed as sister to all other Anisoptera and supported by the unique ventral development of the apical penile section into a sperm displacement and removal organ (Carle & Kjer 2002), correlated vestigial posterior hamules, and by molecular analyses of Letsch (2007), Bybee et al. (2008), Carle et al. (2008), and Davis et al. (2011). In addition, elongate posterior hamuli between which the anisopteran penis developed are present in both fossil and extant forms except Aeshnoids, affirming that the unique apical section of the Aeshnoid penis is likely fundamentally different from that of other Anisoptera. Here Aeshnoidea *sensu* Carle & Louton alone is recovered as sister to all other Anisoptera, with 100% bootstrap and Bayesian support. This topology is congruent with at least two independent molecular partitions for both the aeshnoid and the non-aeshnoid groups (Fig. 1B), thus providing very strong evidence for the Aeshnoidea as the sister group to all other Anisoptera. Non-aeshnoid Anisoptera are typically characterized by a closed sperm duct of penile section three (Pfau 2005) and the loss of endophytic oviposition. The earliest nodes of our topology are somewhat similar to Pfau's proposed topology (1991, 2005, 2011), but his extended "Petaluroidea" is not recovered, as predicted by Carle (1995). Pfau has insisted that ejaculation during the positive pressure phase of the penile sperm pump in cordulegastroids and during the negative phase in libelluloids must have evolved independently from austropetaloid precursors. Our topology suggests, rather, that the libelluloid condition developed gradually from that of plesiotypic Cavealabiata, apparently via development of a biphasic sperm pump following increased size of the apical chamber coupled with reduced outer wall sclerotization, as in Chlorogomphidae, which allowed inflection of the outer wall and consequent ejaculation during the end of the negative penile pressure phase.

Another group suggested as sister to all other Anisoptera is Gomphoidea (Hennig 1969; Carle 1982a; Saux et al. 2003; Blanke et al. 2013). However, subsequent to Carle & Kjer (2002) employing the morphological results of Pfau (1991) and Carle & Louton (1994), only workers either excluding molecular data or using a very limited taxon sample have recovered Gomphoidea as sister to all other Anisoptera. In the present analysis, the posterior probability of Gomphoidea alone being sister to the remainder of the suborder is zero and bootstrap support for its inclusion among non-aeshnoid families is very high.

Some recent phylogenies place Petaluroidea as the sister group of all other Anisoptera (e.g., Bechly 1996; Rehn 2003; Davis et al. 2011). Others have recovered Petaluroidea + Aeshnoidea *s.s.* (Fleck et al. 2008b: parsimony) or ((Petaluroidea + Aeshnoidea) Gomphoidea) (Bybee et al. 2008: parsimony; Fleck et al. 2008b: Bayesian analysis) in this position. Our results, however, provide no support for these topologies, and in fact Bayesian posterior probabilities are zero for each.

Within our topology non-aeshnoid Anisoptera show two arrangements in Fig. 1B. The combined analysis places Petaluridae as sister to Gomphidae with moderate support (BS = 54, PP = 74; Fig. 1B), but it is supported independently only by the EF-1α partition and little morphological evidence. This result may be affected by the long branches leading to extant Gomphidae and Petaluridae relative to the short internode recovered in the paraphyletic topology. In contrast, Carle & Kjer (2002), Letsch (2007) and Carle et al. (2008) recovered Petaluroidea as sister to Cavealabiata, the latter with 100% posterior probability. Our Bayesian analysis also recovered this relationship in the remaining 26% of cases, as did the nuclear rRNA data partition, with modest bootstrap support (Fig. S3). This latter topology is also supported by several morphological apomorphies including: male penis with elongate posteriorly directed ejaculatory duct (Pfau 2005), loss of endophytic oviposition, bilaterally symmetrical proventriculus, and larval labium: with prementum ca. as wide as long, with spatulate palpal lobes, and with movable end hook shorter than palpal lobe (Carle 1995).

Originally Selys (1854) placed *Cordulegaster* Leach, 1815, *Chlorogomphus* Selys, 1854, and *Petalia* Hagen, 1854 (= *Neopetalia*) in his Division Fissilabres of the subfamily Gomphines of his family Aeschnidées. Tillyard (1917), however, removed *Petalia* from the Gomphinae and placed it in his composite Petaliini of the Aeschnidae [sic], until Carle & Louton (1994) reconfirmed Selys' (1854) relative placement of *Neopetalia.* Here Cordulegastridae, Neopetaliidae, Chlorogomphidae and Libelluloidea are placed within Cavealabiata; this grouping is equivalent in composition to the Cordulegasteroidea [sic] of Tillyard & Fraser (1940) except that the latter excluded *Neopetalia* Cowley, 1934. Their group included the Cordulegasteridae and (illogically) the Libelluloidea,

the latter equivalent to our Libelluloidea in composition. Cordulegasteroidea was later redefined by Fraser (1957) to include only Cordulegasterinae and Chlorogomphinae, and Walker (1958) emended the spelling to Cordulegastroidea. Despite many characteristics listed by both authors, only the large irregular teeth of the labial palps are a likely synapomorphy for the redefined Cordulegastroidea[1]. Carle (1995) summarized nine morphological characters that support Cordulegastridae, Neopetaliidae, and Chlorogomphidae as successively paraphyletic relative to Libelluloidea. In addition, the morphological evidence exhibits inverse character state polarities relative to the monophyletic Cordulegasteroidea topology (Carle 1983; Lohmann 1996; Bechly 1996). Better support for a monophyletic Cordulegastroidea comes from molecular results (Figs. 1B, S3, S4), Letsch (2007), Bybee et al. (2008: parsimony tree), Carle et al. (2008), and Davis et al. (2011: third tree). However, we are somewhat concerned that the long branches leading to extant Cordulegastridae, Neopetaliidae, and Chlorogomphidae could potentially overshadow support for the short internodes of the paraphyletic topology, especially here since different genes result in different, mutually exclusive topologies which may combine to support a monophyletic grouping. Molecular results that support the paraphyletic topology include Misof et al. (2001), Ware et al. (2007), Fleck et al. (2008: Bayesian tree), and Davis et al. (2011: second tree). A suitable and more extensive character set may allow for unambiguous determination of the true topology of this ancient rapid radiation. In either case these families are morphologically distinct and molecular based phylogenies reveal long branches leading to each.

4.1.2. Aeshnoidea

Within Aeshnoidea, Austropetaliidae and Aeshnidae are unequivocally distinct based on both molecular and morphological evidence including the loss of the apical sperm chamber in Aeshnidae. A North-South vicariance is likely between the austropetaliids of Australia-Chile and plesiotypic Aeshnidae (Gomphaeschninae) of the Northern Hemisphere, presumably before the dissolution of the trans-Pangaean highlands (Carle 1995). Within Austropetaliidae a clear-cut separation of Australian Austropetaliinae + Tasmainian Archipetaliinae from the Hypopetaliinae + Eurypetaliinae of Chile suggests a Mesozoic phylogenetic vicariance consequent to the breakup of southernmost Gondwana (Carle 1995, 1996). High molecular support values, morphological distinctiveness, ecological uniqueness, and isolated geographic occurrence all support the subfamily rank attributed to the groups of Austropetaliidae as proposed by Carle (1996), i.e., Austropetaliinae, Archipetaliinae, Hypopetaliinae and Eurypetaliinae. Here short internodes and terminal branch lengths could be linked to both relatively long generation times and stable environments of Temperate Zone spring seeps and spring fed streams (Carle 2012). The latter is supported by the relatively long terminal branch of the river inhabitant *Hypopetalia* (Fig. 1B).

The diversity of aeshnid taxa for which molecular data are available is currently inadequate to fully clarify the phylogenetic topology within Aeshnidae, but the morphological analysis by von Ellenrieder (2002) provides a topology for evaluation. Despite the limits of the molecular taxon sample, strong evidence supports the restricted Gomphaeschninae proposed by von Ellenrieder, comprising *Gomphaeschna* Selys, 1871, and *Oligoaeschna* Selys, 1889, and presumably also *Sarasaeschna* Karube & Yeh, 2001, and *Linaeschna* Martin, 1908, as the sister group to remaining Aeshnidae. The results also support a restricted Brachytroninae, consisting of von Ellenrieder's group 2 genera, as sister to Aeshninae. However, the topologies differ in that *Boyeria* McLachlan, 1895 and *Caliaeschna* Selys, 1883 do not form a monophyletic group, as in von Ellenrieder's trees. Within Aeshninae, Aeshnini and Gynacanthini appear to be sister groups indicating that their sister group, the Anactini (*Anax* Leach, 1815 + *Hemianax* Selys, 1871), is a valid tribe and not nested within Aeshnini as implied by von Ellenrieder and others. In addition, the new topology does not support the establishment of either Aeshnidae *s.s.* (Bechly 1996) or Telephlebiidae (Bechly 1996; Theischinger & Hawking 2006), although we do utilize both family group names for subfamilies of Aeshnidae (Fig. 2).

4.1.3. Gomphoidea

Species diversity of Gomphidae is likely higher than that of any other family of Anisoptera with the possible exception of Libellulidae, and yet no definitive phylogeny of Gomphidae has been published. Nevertheless, Carle's (1986) classification of Gomphidae provides a framework for phylogenetic evaluation and results presented herein are in substantial accord with that classification (hereafter family group names from Carle 1986 are used for concordant groupings recovered here). The diversity of gomphid taxa for which molecular data are available (Figs. 1B, 3) include representatives of all eight subfamilies, 70% of the tribes, and approximately 40% of the genera listed by Carle (1986). A basal dichotomy placing Ictinogomphinae *s.l.* (including Hemigomphini), as sister to remaining Gomphidae is strongly supported in the combined analysis, however the nuclear rRNA partition (Fig. S3) clusters all Octogomphinae together. Even so, it appears from the combined molecular topology that the "Octogomphinae *s.l.*" are even more plesiotypic than previously supposed. Putative apomorphic character states of morphological features (e.g., hamular denticulation and costal brace location) used by Carle (1986) to group the tribes of Octogomphinae are evident-

[1] However, if the expanded labial palps and elongate teeth are a duplication of the ancestral palpal armature, then this could explain the intermediate palps of various, often basal branching libelluloid genera (Tillyard 1917: fig. 32), including *Cordulephya* Selys, 1870 (Cordulephyinae), *Archaeosynthemis* (Synthemistinae) and *Epophthalmia* Burmeister, 1839 (Macromiidae).

ly plesiomorphic and the Hemigomphini (*Armagomphus* Carle, 1986 – *Neogomphus*) actually represent the sister group to all other Ictinogomphinae (*Progomphus* – *Sinogomphidia*; Fig. 1B). This split within "Octogomphinae *s.l.*" may reflect a similar history of vicariance as the split within Petaluridae between Gondwana and Laurasia, while the antipodean split within Ictinogomphinae may similarly parallel the basal vicariance within Austropetaliidae. Ictinogomphines eventually dispersed throughout the Neotropical region, with the Progomphini (*Progomphus*) and Gomphoidini (here represented by *Phyllogomphoides*) reaching the Nearctic, and with the highly derived and vagile Ictinogomphini reaching tropical Africa and beyond. The implied great age of the monogeneric Progomphini along with the morphological and ecological diversity among its 70 or so species (Carle 1986), suggest that *Progomphus* may require taxonomic revision.

Within the other major branch of Gomphidae the remaining "Octogomphinae" including the Trigomphini *s.l.* (represented by *Stylogomphus* Fraser, 1922), and Octogomphini (represented by *Lanthus*), along with the Hageniinae arise successively from the basal nodes. This molecular topology confirms the basal position of the "octogomphines" and along with strong differences in mitchondrial data, offers an explanation for the heterogeneous nature of Trigomphini, which is here divided into two tribes as follows:

Trigomphini *s.s.* – Type genus *Trigomphus* Bartenev, 1912; also including *Xenogomphus* Needham, 1944 and *Fukienogomphus* Chao, 1954 – Hind wing with 2–4 postmedian crossveins, anterobasal angle of forewing triangle acute, anal triangle typically 4–6 celled; male sternum 9 well sclerotized lateral to gonocoxae, female sternum 9 with laterobasal elongate-triangular sclerites, male epiproctal rami divergent, male anterior lamina with raised V-shaped posterior ridge, penile prepuce produced posteriorly, and apex of penis with short flagellum; and

Stylogomphini trib.n. – Type genus *Stylogomphus* Fraser, 1922 – Hind wing with 1 postmedian crossvein, anterobasal angle of forewing triangle slightly acute, anal triangle 3 celled; male sternum 9 semimembranous lateral to gonocoxae, female sternum 9 with laterobasal short-subtriangular sclerites, male epiproctal rami subparallel, male anterior lamina without raised V-shaped posterior ridge, penile prepuce globose, and apex of penis circular-flangelike.

Support values for the arrangement of the remaining four large subfamilies of Gomphidae are weak and for now considered to form a polytomy, but a Gondwanan group of the phyllogomphine + austrogomphine tribes (*Ceratogomphus* Selys, 1892 – *Austrogomphus* Selys, 1854) is well defined, with the Neotropical Epigomphini only weakly supported as its sister group (cf. Figs. 1B, 3). The three other supported monophyletic groups are relatively diverse in the Northern Hemisphere, but also have representatives in the Afrotropical region. These are: Leptogomphini + Microgomphini (i.e., *Leptogomphus* – *Heliogomphis*, placed by morphology within Epigomphinae; Fraser 1936; Tillyard & Fraser 1940; Carle 1986); Onychogomphinae (*Davidioides* – *Onychogomphus*); and Gomphinae (*Cyclogomphus* Selys, 1854 – *Arigomphus*).

Carle's (1986) placement of the aberrant *Davidioides* Fraser within the Onychogomphinae is well supported by a 91% bootstrap and 100% posterior probability, but it is morphologically distinct and separated from other Onychogomphinae by an unusually long internode and an even longer terminal branch. Consequently, a new tribe is established for the genus, which is distinguished from other Onychogomphinae as follows:

Davidioidini trib.n. – Type genus *Davidioides* Fraser, 1924 – Occiput black and slightly concave; hind wing triangle with transverse crossvein, anterior side of hind wing triangle 2.3 times length of proximal side, apical planate parallel to RP_1 and straight; abdominal segments 9 and 10 black, male cerci conical bright yellow and ca. as long as abdominal segment 10, epiproctal rami strongly divaricate; anterior hamuli long and slender, posterior hamuli wide and sinuous.

At the generic level the transfer of *Heliogomphus* from the Leptogomphini to the Microgomphini seems warranted (BS = 82, PP = 100), as does the transfer of *Stylurus* from Gomphini to Cyclogomphini (BS = 77, PP = 100). The latter shift suggests that the simplified anterior hamuli of these genera may represent an important synapomorphy, one also typical of the African *Neurogomphus* Karsch, 1890. Within Gomphini the placement of *Gomphus* (*Gomphus*) Leach, 1815 with *Asiagomphus* Asahina, 1985; placement of *Gomphus* (*Phanogomphus*) Carle & Cook, 1984 with *Dromogomphus* Selys, 1854; and placement of both *Gomphus* (*Hylogomphus*) Westfall & May, 2000 and *Gomphus* (*Gomphurus*) Needham, 1901 with *Arigomphus* Needham, 1897 suggest that the subgenera of *Gomphus* should either be treated as genera, or all genera and subgenera of this group be considered subgenera of *Gomphus*.

4.1.4. Petaluroidea

Our molecular derived topology within the Petaluridae is nearly identical to that proposed by Ware et al. (2014), with the Northern Hemisphere Tachopteryginae (*Tachopteryx thoreyi*, *Tanypteryx* spp.), with two species from North America and one from Japan, and with at least one fossil species, *Protolindenia wittei* Giebel, 1860 of Europe; and with the Southern Hemisphere Petalurinae, with two species from Chile (*Phenes* spp.), two from New Zealand (*Uropetala* spp.) and five from Australia (*Petalura* spp.), as reported by Carle (1995); it does not support Pheninae (including only *Phenes*; Fleck 2011).

4.1.5. Cordulegastridae, Neopetaliidae, Chlorogomphidae

The internal phylogeny of Cordulegastridae was determined from morphology by Carle (1983) and Lohmann

(1992), and by Carle (1995) for the Chlorogomphidae (although the latter topology has been disputed by Karube 2002). However, morphological analyses produced inverted phylogenetic topologies as compared to the molecular results shown in Figs. 1B, S3, and S4. For example, morphology predicted that the sister groups of remaining Cordulegastridae and Chlorogomphidae were *Zoraena* Kirby, 1890 and *Chloropetalia* Carle, 1995, respectively; and that the most recently derived genera were *Anotogaster* Selys, 1854 and *Sinorogomphus* Carle, 1995, respectively. In any case the families are morphologically distinct, and molecular based phylogenies reveal long internodes leading to each.

4.1.6. Libelluloidea

4.1.6.1. Synthemistidae. Fraser (1954; 1957) presented the topology of Libelluloidea as: (Synthemistidae (Corduliidae (Macrodiplactidae + Libellulidae))); he included in Corduliidae: Cordulephyinae, Neophyinae, Idomacromiinae, Macromiinae (as Epophthalmiinae), Idionychinae, Gomphomacromiinae, and Corduliinae. Monophly of Fraser's Corduliidae became questionable when *Gomphomacromia*, *Archaeophya* and possibly *Pseudocordulia* were shown to form the apparent sister group of Synthemistidae (Theischinger & Watson 1984; Carle 1995). A further expanded Synthemistidae which includes all of Fraser's Corduliidae except Macromiinae and Corduliinae, (i.e., the GSI of Ware et al. 2007), was recovered here (BS = 43; PP = 100) and also by Letsch (2007; PP = 100) and Bybee et al. (2008; BS = 90–100, PP = 99–100). Additional genera of Synthemistidae *s.s.* not represented by molecular data, almost certainly will be included based on a number of strong morphological synapomorphies and similar geographic origin, these genera include: *Austrosynthemis* Carle, 1995 *Calesynthemis* Carle, 1995 *Palaeosynthemis* Förster, 1903 *Parasynthemis* Carle, 1995 and *Tonyosynthemis* Theischinger, 1998.

Although not recovered in all studies (e.g., Fleck et al. 2008b), and with somewhat equivocal support here (e.g., Fig. S4 with *Oxygastra* and *Macromidia* closest to Macromiidae and Corduliidae *s.s.*), we believe monophyly of a group containing at least the majority of GSI taxa is well established. The subordinate group including the traditional Synthemistidae with the addition of *Gomphomacromia* and *Archaeophya* are closely tied by morphological synapomorphies as well as molecular evidence (Fig. 1B). However, some workers (Fleck et al. 2008b; Dumont et al. 2010; J. Ware, pers. comm. 2013) have recovered one or more of the genera: *Oxygastra*, *Idomacromia* Karsch, 1896, *Macromidia*, *Idionyx* Selys, 1871, and *Neocordulia* Selys, 1882, either within Macromiidae or Corduliidae or in a polytomy with Macromiidae, Corduliidae, or Libellulidae. *Idomacromia* and *Oxygastra*, along with *Nesocordulia* McLachlan, 1882, *Neocordulia* Selys, 1882, and *Neophya* Selys, 1881, share an apparent apomorphy with Corduliidae + Libellulidae, i.e., the anal loop has an evenly curved bisector forming a pleat. Our mitochondrial data partition recovered the GSI genera, except for a slightly expanded Synthemistidae, as a paraphyletic assemblage with *Oxygastra* and *Macromidia* closest to Macromiidae and Corduliidae *s.s.*, respectively (Fig. 1B; we now refer to Corduliidae as recovered here). Here again a more extensive character set is required to achieve congruence among all results, but at least at present, it seems most reasonable to recognize an expanded monophyletic Synthemistidae *s.l.*

4.1.6.2. Macromiidae, Corduliidae. Although Fraser (1957) recognized seven subfamilies within the Corduliidae, this analysis reveals that only genera of Corduliinae and Macromiinae form polytypic monophyletic groups; the first of these is placed as the sister group of Libellulidae, and Macromiinae placed sister to (Corduliidae *s.s.* + Libellulidae). This topology indicates that Macromiinae should also be accorded family rank, as proposed by Gloyd (1959). Macromiidae and Corduliidae *s.s.*, are again very strongly supported except for lower bootstraps tying *Aeschnosoma* Selys, 1870 + *Pentathemis* Karsch, 1890 to the Corduliidae *s.s.* The position of these two genera clearly supports Fleck's (2012) and Fleck & Legrand's (2013) separation of these plus *Libellulosoma* Martin, 1907 as a very distinctive group within Corduliidae *s.s.* Both Macromiidae and Corduliidae *s.s.* have been recovered by nearly all previous molecular analyses, although here the latter two "maverick" genera are also included. Intrafamily structure is again not definitively resolved, but it is noteworthy that within Macromiidae *Phyllomacromia* Selys, 1878 is distinct from *Macromia* Rambur, 1842, and that *Hemicordulia* and *Procordulia* are grouped well within Corduliidae *s.s.* and therefore not accorded family rank. Not fully determined from the data is which of these two families is sister to the Libellulidae, although both morphology and our Bayesian analysis (PP = 87) support the common hypothesis that Corduliidae *s.s.* occupies that position.

4.1.6.3. Libellulidae. Libellulidae is one of the most successful and recently differentiated anisopteran families. These dragonflies are found worldwide and are ubiquitous in nearly every lentic habitat where Odonata occur. Numerous attempts have been made over the years to organize this family into smaller subdivisions, mostly based on the work of Ris (1909) who recognized ten numbered "Gruppen", based almost entirely on wing venation; eight of these were later named as tribes by Tillyard (1917). Fraser (1957) eventually split *Onychothemis* Brauer, 1868 and *Zygonyx* Hagen, 1867 + *Olpogastra* Karsch, 1895 from Ris' group 8; and placed *Rhyothemis* Hagen, 1867 and *Zyxomma* Rambur, 1842 + *Tholymis* Hagen, 1867 into their own subfamilies and placed *Aethriamanta* Kirby, 1889, *Macrodiplax*, *Selysiothemis* Ris, 1897, and *Urothemis* into a separate family, the Macrodiplactidae. Fraser did not consider group IX of Ris (1909). Although Fraser's Macrodiplactidae has most often been given subfamily rank, the remaining subfamilies, when used at all, have remained essentially unchanged in rank and

composition (DAVIES & TOBIN 1984; BRIDGES 1994; BECHLY 1996; STEINMANN 1997).

All libellulid genera for which data are available are here assigned to one of eleven clusters (Fig. 4) that have bootstrap support > 85% (except for group 9 at 72%), and posterior probability > 90% (except for group 2 at 85%; Figs. 1C, 4; Table 1). Most are also consistent with one or more individual data partitions (Fig. 1C) and, allowing for differences in taxon sampling, are in agreement with most previous molecular studies (Table 1). The three studies that included the most genera, those of LETSCH (2007), PILGRIM & VON DOLEN (2008), and WARE et al. (2007), recovered groups that corresponded very closely to our groups 1–5, 8, 10, and 11, and in the case of LETSCH (2007), the branching order was reasonably similar to (Fig. 4). This increases confidence that most of the principal subsidiary groups have been identified and even that their interrelationships are emerging. Although the overlap among studies in both taxa and genetic markers precludes any strong statement that congruence among these phylogenies derives from entirely independent data, it can be at least noted that similarities are unlikely to be the result of individual analytical idiosyncrasies. It is true, nonetheless; that our arrangements are not identical between Figs. 1 and 4, and the taxon sample includes only a little more than half the libellulid genera (81 of ca. 143). Clearly the final topology of the libellulid tree has yet to be completely settled. However, sampling one representative of each of our eleven clusters for transcriptome or genomic sequencing is likely to resolve the phylogeny of the libellulid radiation in the near future.

The main groups of Libellulidae have been identified here and by others from both morphological and molecular evidence, but it is difficult to characterize groups unambiguously based on morphology in this large rapidly evolving family owing to the prevalence of convergence. The molecular results suggest that as few as three subfamilies may be eventually recognized within Libellulidae (e.g., Sympetrinae, groups 1 and 2; Trameinae, groups 3–7; and Libellulinae, groups 8–11), but here intrafamily group designations currently in common use are retained as subfamilies, with the exceptions of Leucorrhiniinae, Rhyothemistinae, Trithemistinae, Onychotheminae and Zygonychinae (of which the first two are recognized as tribes of Sympetrinae, the next two as tribes of Pantalinae, and the last is combined with the Onychothemini). Ongoing morphological and molecular study to determine intergroup relationships may necessitate changes in their taxonomic rank, final composition and morphological definitions as more taxa are analyzed. Subfamilies currently recognized, listed in order of their group number from Fig. 4 are: **1** – Dythemistinae **subfam.n.**; **2** – Sympetrinae (including Leucorrhiniini and Rhyothemistini); **3** – Macrodiplactinae; **4** – Brachydiplactinae; **5** – Tetrathemistinae; **6** – Trameinae; **7** – Zyxommatinae; **8** – Palpopleurinae; **9** – Diastatopidinae; **10** – Pantalinae (including Trithemistini and Onychothemistini); and **11** – Libellulinae. A new subfamily of Libellulidae is established with three new tribes for genera previously placed in groups V, VI and IX of RIS (1909); these genera have been more recently placed in Pantalinae, Sympetrinae, Brachydiplactinae and Tetrathemistinae (FRASER 1957; DAVIES & TOBIN 1985). In our molecular topology (Fig. 1C) the new subfamily is placed as the sister group of Sympetrinae and these together form the sister group of remaining Libellulidae. Transferred genera are primarily Neotropical or southern Nearctic or both in distribution. The likely misplacement of *Zenithoptera* within Dythemistinae **subfam.n.** (Figs. 4, 1C, S3) may have resulted from the lack of mitochondrial data overlap; morphological characters suggest that *Zenithoptera* might be eventually placed elsewhere, possibly near *Rhyothemis*.

Dythemistinae subfam.n. – Type genus *Dythemis* Hagen, 1861. – Adult color non- to slightly metallic, typically with yellow markings; compound eyes in life typically bright bluish green; forewing nodus displaced distally so that antenodals are typically greater in number than postnodals and with distal antenodal typically unmatched; wings with RP, and MA (sectors of arculus) fused basally, reverse oblique vein weakly developed, apical planate weakly developed, radial planate typically well developed, typically with 1 bridge crossvein (with 1–3 in *Micrathyria*), with 1 cubital-anal crossvein (occasionally 2 in *Micrathyria*), and with anal loop well developed with angulated bisector, anal loop open distally in *Argyrothemis*.

Dythemistini trib.n. – Type genus *Dythemis* Hagen, 1861. – Adult with prothoracic hind margin slightly expanded; wings with arculus typically arising from near second antenodal, wings with R_2 undulate, forewing intraradial crossveins 9–12 (i.e., crossveins between RA and RP from arculus to subnodus), radial and medial planates well developed, forewing triangle three sided with proximal side more than twice length of costal side, forewing triangle with costal side less than 3/8 length of supratriangle; hind wing triangle with proximal side slightly basal to arculus, hind wings with MP not arising from outer side of triangle, forewing trigonal interspace with 2–3 cell rows; larva with dorsal abdominal spines. Also includes: *Macrothemis* Hagen, 1868, *Scapanea* Kirby, 1889, *Paltothemis* Karsch, 1890, *Brechmorhoga* Kirby, 1894, and *Gynothemis* Calvert, 1909.

Pachydiplactini trib.n. – Type genus *Pachydiplax* Brauer, 1868. – Adult with prothoracic hind margin prominent and bilobate, wings with arculus typically arising between first and second antenodal, forewing intraradial crossveins 4–6, radial planate well developed and medial planate vestigial, forewing triangle three sided with proximal side more than twice length of costal side, costal side of forewing triangle less than 3/8 length of supratriangle; hind wing triangle with proximal side at arculus, hind wings with MP arising from outer side of triangle, forewing trigonal interspace with 2–3 cell rows; larva without dorsal abdominal spines. Also includes *Micrathria* Kirby, 1889, and *Anatya* Kirby, 1889.

Elgini trib.n. – Type genus *Elga* Ris, 1911. – Adult with prothoracic hind margin moderately to greatly ex-

panded, wings with arculus typically arising from near second antenodal, forewing intraradial crossveins 5–6, radial and medial planates vestigial or obsolete, forewing triangle four sided with proximal side less than twice length of costal side, costal side of forewing triangle nearly 1/2 length of supratriangle; hind wing triangle with proximal side slightly distal to arculus, hind wings with MP not arising from outer side of triangle, forewing trigonal interspace with one cell row. Also includes: *Nephepeltia* Kirby, 1889, *Fylgia* Kirby, 1899, *Argyrothemis* Ris, 1911 and possibly *Edonis* Needham, 1905.

Molecular results also lend support to previous morphological arrangements of subgenera within *Libellula* (24 species) and also to some extent within *Sympetrum* (62 species). The topology for *Libellula* and its near relatives is similar to that presented by Carle & Kjer (2002); significant differences include the placement of *Platetrum* Newman, 1833, with both *Ladona* Needham, 1901, and *Eurothemis* Kennedy, 1922; and the placement of *Libellula* (*Eolibellula*) Kennedy, 1922, as sister to remaining *Libellula*. In addition, establishment of *Eotainia* Carle & Kjer, 2002, separate from *Holotainia* Kirby, 1899, is supported. In *Sympetrum* the putative subgenus *Tarnetrum* Needham & Fisher, 1936, forms a basal paraphyletic cluster that also includes *Nesogonia* Kirby, 1898 while *Kalosympetrum* Carle, 1993 and *Sympetrum* Newman, 1833 both appear to be monophyletic, although still sparsely sampled.

4.2. Rapid ancient radiations

A phylogenetic topology of rapid ancient radiations that is characterized by short deep internodes followed by much longer branches is difficult to recover (Whitfield & Kjer 2008). In an ancient group like Odonata variation in the rate of evolution is very likely to leave this pattern of short and long branches deep within a phylogenetic tree. Occurrence of a rapid phylogenetic radiation is most likely when circumstances simultaneously enhance the exploitation of unoccupied niche space while increasing the rate of reproductive isolation. Environmental factors that can dramatically influence fundamental niche utilization include: continental drift and associated orogenesis (Carle 1995), climate change and its effect on ocean and rainfall levels, and meteoric impacts and extreme volcanism which can result in extinction events that free-up niche space (Alvarez et al. 1980; Knoll et al. 2007; Carle 2012). Important organismal factors include morphological preadaptations and subsequent innovations, particularly those that affect vagility and that modify the copulatory scheme (Carle 1982c).

From Fig. 1 it is clear that a rapid ancient radiation occurred among the first evolutionary splits among Anisoptera and was followed by long branches leading to extant Aeshnoidea, Gomphidae, Petaluridae, Cordulegastridae, Neopetaliidae, Chlorogomphidae, and Libelluloidea. Radiation of the anisopteran superfamilies was probably initiated by the Permian-Triassic extinction event and perhaps trimmed by the Triassic-Jurassic extinction event, with current distributions of the less vagile, stream or seepage-restricted families (Austropetaliidae, Gomphidae, Petaluridae, Cordulegastridae, Neopetaliidae, and Chlorogomphidae) explained by their radiation before the dispersion of Pangaea (Carle 1982a). This is also true of many aeshnid genera, although the modern lentic adapted *Gynacantha* Rambur, 1842, *Aeshna* Fabricius, 1775, and *Anax* Leach, 1815, are cosmopolitan and comprise nearly half the species of the family; invasion of lentic habitats apparently fosters dispersial and speciation, and undoubtedly also fossilization, but may have left the resultant species susceptible to global extinction events, and has consequently led to a disjunction between phylogenies derived from fossil and living Odonata (Carle 2012).

By far the most diverse anisopteran superfamilies are the Gomphoidea and Libelluloidea; both groups have independently evolved similar methods of fossorial larval concealment which has negated the selective link between endophytic oviposition and plant related cover for larval development (Carle 1995). Probably even more importantly from the standpoint of speciation, constraints imposed by the endophytic ovipositor were removed, thus freeing penile and ovipositor morphology to play a greater role in reproductive isolation. At the subfamily level within Gomphidae and at the family level within Libelluloidea the relatively mild Jurassic-Cretaceous extinction events may have led to gradual radiations of lotic forms in both groups which were quite similar in extent. However, the infamous Cretaceous-Paleogene extinction event at ca. 65.5 million years ago apparently marked the final demise of the gigantic and probably lentic adapted dragonflies Stenophlebioidea Pritykina, 1980 and Aeschnidioidea Carle & Wighton, 1990 (Zhang 1999; Carle 2012). Exploitation of unoccupied lentic niche space coupled with preadaptations including complex yet efficient copulatory, oviposition, and flight processes, and subsequent innovations such as secretive female behavior, may have led to a dramatic radiation of the Libellulidae. The situation within Libellulidae clearly presents the signature of a rapid radiation (Fig. 1C), with nearly all early internodes that lead to listed subfamilies short, with terminal branches within subfamilies relatively long. Ware et al. (2008) estimated the origin of the Libellulidae (its split from Corduliidae) to have occurred ca. 87–57 million years ago, depending on the nucleotide substitution model employed. The younger end of this range is shortly after the Cretaceous-Paleogene extinction event which in particular decimated lentic communities through long term daylight reduction (Carle 2012), thus opening these habitats to eventual exploitation by the rapidly evolving Libellulidae. In addition, Gingerich (2006) describes the early Eocene as a period of unusual warmth and high rainfall which could enhance lentic habitats that were expanding in both local and geographic extent.

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6. References

ALTSCHUL S., GISH W., MILLER W., MYERS E., LIPMAN D. 1990. Basic local alignment search tool. – Journal of Molecular Biology **215**: 403–410.

ALVAREZ L.W., ALVAREZ W., ASARO F., MICHEL H.V. 1980. Extraterrestrial cause for the Cretaceous-Tertiary extinction. – Science **208**: 1095–1108.

BECHLY G. 1996. Morphologische Untersuchungen am Flügelgeäder der rezenten Libellen und deren Stammgruppenvertreter (Insecta; Pterygota; Odonata), unter besonderer Berücksichtigung der Phylogenetischen Systematik und des Grundplanes der Odonata. – Petalura, special volume **2**: 402 pp.

BLANKE A., GREVE C., MOKSO R., BECKMAN F., MISOF B. 2013. An updated phylogeny of Anisoptera including formal convergence analysis of morphological characters. – Systematic Entomology **38**: 474–490. doi: 10.1111/syen.12012.

BRAUCKMANN C., ZESSIN W. 1989. Neue Meganeuridae aus dem Namurium von Hagen-Vorhalle (BRD) und die Phylogenie der Meganisoptera (Insecta: Odonata). – Deutsche Entomologische Zeitschrift **36**: 177–215.

BRIDGES C.A. 1994. Catalogue of the Family-Group, Genus-Group, and Species-Group Names of the Odonata of the World (3rd edn). – Privately published, Urbana IL.

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.

CALVERT P.P. 1893. North American Odonata. Part 1. General account of Odonata. – Transactions of the American Entomological Society **20**: 153–268.

CARLE F.L. 1979. Environmental monitoring potential of the Odonata, with a list of rare and endangered Anisoptera of Virginia, United States. – Odonatologica **8**: 319–323.

CARLE F.L. 1982a. A contribution to the knowledge of the Odonata. – Ph.D. Dissertation, Virginia Polytechnic Institute and State University Blacksburg. 1095 + xv pp.

CARLE F.L. 1982b. The wing vein homologies and phylogeny of the Odonata: a continuing debate. – Societas Internationalis Odonatologica Rapid Communications. – Rapid Communication **4**, x + 66 pp.

CARLE F.L. 1982c. Evolution of the odonate copulatory process. – Odonatologica **11**: 271–286.

CARLE F.L. 1983. A new *Zoraena* (Odonata: Cordulegastridae) from eastern North America, with a key to the adult Cordulegastridae of America. – Annals of the Entomological Society of America **76**: 61–68.

CARLE F.L., COOK C. 1984. A new *Neogomphus* from South America, with extended comments on the phylogeny and biogeography of the Octogomphini trib. nov. (Anisoptera: Gomphidae). – Odonatologica **13**: 55–70.

CARLE F.L. 1986. The classification, phylogeny and biogeography of the Gomphidae (Anisoptera). I. Classification. – Odonatologica **15**: 275–326.

CARLE F.L. 1995. Evolution, taxonomy and biogeography of ancient gondwanian libelluloids, with comments on anisopteroid evolution and phylogenetic systematics. – Odonatologica **24**: 383–424.

CARLE F.L. 1996. Revision of Austropetaliidae (Anisoptera: Aeshnoidea). – Odonatologica **25**: 231–259.

CARLE F.L. 2012. A new *Epiophlebia* (Odonata: Epiophlebioidea) from China with a review of epiophlebian taxonomy, life history, and biogeography. – Arthropod Systematics & Phylogeny **70**: 75–83.

CARLE F.L., KJER K.M. 2002. Phylogeny of *Libellula* Linnaeus (Odonata: Insecta). – Zootaxa **87**: 1–18.

CARLE F.L., KJER K.M., MAY M.L. 2008. Evolution of Odonata, with special reference to Coenagrionoidea (Zygoptera). – Arthropod Systematics & Phylogeny **66**(1): 37–44.

CARLE F.L., LOUTON J.A. 1994. The larvae of *Neopetalia punctata* and establishment of Austropetaliidae fam. nov. (Odonata). – Proceedings of Entomological Society of Washington **96**: 147–155.

CLAUSNITZER V., DIJKSTRA K.-D.B., KOCH R., BOUDOT J.P., DARWALL W.R.T., KIPPING J., SAMRAOUI B., SAMWAYS M.J., SIMAIKA J.P., SUHLING F. 2012. Focus on African Freshwaters: hotspots of dragonfly diversity and conservation concern. – Frontiers in Ecology and the Environment **10**: 129–134.

CORBET P.S. 1999. Dragonflies: Behavior and Ecology of Odonata. – Cornell University Press, Ithaca, NY.

DAVIES D.A.L., TOBIN P. 1985. The dragonflies of the world: a systematic list of extant species of Odonata. Vol. 2 Anisoptera. – Societas Internationalis Odonatologica Rapid Communications Suppl. No. 5., Utrecht.

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 Evolutionary Biology 2011 **11**: 252–261.

DJERNÆS M., DAMGAARD J. 2006. Exon-intron structure, paralogy and sequenced regions of elongation factor-1 alpha in Hexapoda. – Arthropod Systematics & Phylogeny **64**: 45–52.

DUMONT H.J., VIERSTRAETE A., VANFLETEREN J.R. 2010. A molecular phylogeny of the Odonata (Insecta). – Systematic Entomology **35**: 6–18. doi:10.1111/j.1365-3113.2009.00489.x

FLECK G. 2011. Phylogenetic affinities of Petaluridae and basal Anisoptera families (Insecta: Odonata). – Stuttgarter Beiträge zur Naturkunde A, Neue Serie **4**: 83–104.

Fleck G. 2012. Preliminary notes on the genus *Aeschnosoma* Selys 1870 (Odonata: Anisoptera: Corduliidae s. str.). – Annales de la Société Entomologique de France (N.S.) **48**: 225–228. doi: 10.1080/00379271.2012.10697766

Fleck G., Bechly G., Martinez-Delclos X., Jarzembowski E., Coram R., Nel A. 2003. Phylogeny and classification of the Stenophlebioptera (Odonata: Epiproctophora). – Annales de la Société Entomologique de France **39**: 55–94.

Fleck G., Brenk M., Misof B. 2008a. Larval and molecular characters help to solve phylogenetic puzzles in the highly diverse dragonfly family Libellulidae (Insecta: Odonata: Anisoptera): the Tetrathemistinae are a polyphyletic group. – Organisms Diversity and Evolution **8**: 1–16.

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. 2008b. A phylogeny of anisopterous dragonflies (Insecta, Odonata) using mtRNA genes and mixed nucleotide/doublet models. – Journal of Zoological Systematics and Evolutionary Research **46**: 310–322.

Frandsen P.B., Calcott B., Mayer C., Lanfear R. 2015. Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. – BMC Evolutionary Biology **15**: 13.

Fraser F.C. 1936. A note on the identifications of some obscure genera and species of the family Gomphidae. – Proceedings of the Royal Entomological Society of London Series B, Taxonomy **5**: 137–143.

Fraser F.C. 1950. The Odonata of Mauritius, suborder Anisoptera, and the description of a new species of Zygoptera. – Transactions of the Royal Entomological Society of London Series B, Taxonomy **19**: 70–77.

Fraser F.C. 1954. The origin and descent of the order Odonata based on the evidence of persistent archaic characters. – Transactions of the Royal Entomological Society of London (B) **23**: 89–94.

Fraser F.C. 1957. A Reclassification of the Order Odonata. – Royal Zoological Society of New South Wales, Sydney. 133 pp.

Gloyd L.K. 1959. Elevation of the *Macromia* group to family status. – Entomological News **70**: 197–205.

Gillespie J.J. 2004. Characterizing regions of ambiguous alignment caused by the expansion and contraction of hairpin-stem loops in ribosomal RNA molecules. – Molecular Phylogenetics and Evolution **33**: 936–943.

Gingerich P.D. 2006. Environment and evolution through the Paleocene-Eocene thermal maximum. – Trends in Ecology and Evolution **21**: 246–253. doi 10.1016/j.tree.2006.03.006

Hasegawa E., Kasuya E. 2006. Phylogenetic analysis of the insect order Odonata using 28S and 16Sr DNA sequences: a comparison between data sets with different evolutionary rates. – Entomological Science **9**: 55–66.

Hassel C., Thompson D.J., French G.C., Harvey I.F. 2007. Historical changes in the phenology of British Odonata are related to climate change. – Global Change Biology **13**: 933–941.

Hennig W. 1969. Die Stammesgeschichte der Insekten. – Frankfurt a.M., Kramer. 436 pp.

Hennig W. 1981. Insect Phylogeny. – Chichester, John Wiley. 514 pp.

Huang D.-Y., Nel A. 2009. The first Chinese Tarsophlebiidae from the Lower Cretaceous Yixian Formation, with morphological and phylogenetic implications (Odonatoptera: Panodonata). – Cretaceous Research **30**: 429–433.

Jarzembowski E.A., Nel A. 1996. New fossil dragonflies from the Lower Cretaceous of SE England and the phylogeny of the superfamily Libelluloidea (Insecta: Odonata). – Cretaceous Research **17**: 67–85.

Karube H. 2002. *Watanabeopetalia* gen. nov., a new genus of dragonflies (Odonata, Cordulegastridae, Chlorogomphinae). – Special Bulletin of the Coleopterists Society of Japan **5**: 67–85.

Kjer K.M. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. – Molecular Phylogenetics & Evolution **4**: 314–340.

Kjer K.M., Blahnik R.J., Holzenthal R.W. 2001. Phylogeny of Trichoptera (Caddisflies): characterization of signal and noise within multiple datasets. – Systematic Biology **50**: 781–816.

Kjer K.M., Gillespie J.J., Ober K.A. 2007. Opinions on multiple sequence alignment, and an empirical comparison of repeatability and accuracy between POY and structural alignments. – Systematic Biology **56**: 133–146.

Kjer K.M., Honeycutt R.L. 2007. Site specific rates of mitochondrial genomes and the phylogeny of Eutheria. – BMC-Evolutionary Biology **7**: 8.

Klass K.-D. 2008. The female abdomen of ovipositor-bearing Odonata (Insecta: Pterygota). – Arthropod Systematics & Phylogeny **66**: 45–142.

Knoll A.H., Bambach R.K., Payne J.L., Pruss S., Fischer W.W. 2007. Paleophysiology and end-Permian mass extinction. – Earth and Planetary Science Letters **256**(3–4): 295–313.

Lemmon A.R., Brown J.M., Stanger-Hall K., Lemmon E.M. 2009. The effect of missing data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. – Systematic Biology **58**: 130–145.

Letsch H. 2007. Phylogeny of Anisoptera (Insecta: Odonata): Promises and limitations of a new alignment approach. – Ph.D. Dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn. 103 pp.

Lohmann H. 1992. Revision der Cordulegastridae. 1. Entwurf einer neuen Klassifizierung der Familie (Odonata: Anisoptera). – Opuscula Zoologica Fluminensia **96**: 1–18, figs. 1–10.

Lohmann H. 1996. Das phylogenetische System der Anisoptera (Odonata). – Entomologische Zeitschrift **106**(6): 209–252, **106**(7): 253–266, **106**(9): 360–367.

Misof B., Rickert A.M., Buckley T.R., Fleck G., Sauer K.P. 2001. Phylogenetic signal and its decay in mitochondrial SSU and LSU rRNA gene fragments of Anisoptera. – Molecular Biology and Evolution **18**: 27–37.

Mitra A., Dow R., Subramanian K.A., Sharma G. 2010. Chapter 5. The status and distribution of dragonflies and damselflies (Odonata) of the Eastern Himalaya. Pp. 54–66 in: Allen D.J., Molur S., Daniel B.A. (compilers), The Status and Distribution of Freshwater Biodiversity in the Eastern Himalaya. – Cambridge, UK and Gland, Switzerland: IUCN, and Coimbatore, India: Zoo Outreach Organisation.

Needham J.G. 1903. A genealogic study of dragonfly wing venation. – Proceedings of the United States National Museum **26**: 703–764.

NEEDHAM J.G., WESTFALL M.J. 1955. A Manual of the Dragonflies of North America (Anisoptera). – Berkeley, CA, University of California Press. 615 pp.

NEL A., MARTÍNEZ DELCLÒS X., PLACHER J.C., HENROTAY M. 1993. Les "Anisozygoptera" fossils – Phylogénie et classification (Odonata). – Martinia Numéro Hors-Série **3**: 1–311.

OERTLI B. 2008. The use of dragonflies in the assessment and monitoring of aquatic habitats. Pp. 79–95 in: CORDOBA-AGUILAR A. (ed.), Dragonflies and Damselflies, Model Organisms for Ecological and Evolutionary Research. – Oxford, UK, Oxford University Press. 290 pp.

PFAU H.K. 1971. Struktur und Funktion des sekundären Kopulationsapparates der Odonaten (Insecta, Palaeoptera), ihre Wandlung in der Stammesgeschichte und Bedeutung für die adaptive Entfaltung der Ordnung. – Zeitschrift für Morphologie der Tiere **70**: 281–371.

PFAU H.K. 1986. Untersuchungen zur Konstruktion, Funktion und Evolution des Flugapparates der Libellen (Insecta, Odonata). – Tijdschrift van de Entomologie. **129**: 35–123.

PFAU H.K. 1991. Contributions of functional morphology to the phylogenetic systematics of Odonata. – Advances in Odonatology **5**: 109–141.

PFAU H.K. 2005. Structure, function, and evolution of the 'glans' of the anisopteran vesica spermalis (Odonata). – International Journal of Odonatology **8**(2): 259–310.

PFAU H.K. 2011: Functional morphology and evolution of the male secondary copulatory apparatus of the Anisoptera (Insecta: Odonata). – Zoologica **156**: 1–103.

PILGRIM E.M., DOHLEN C. VON 2008. Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. – Systematic Entomology **3**: 159–174. doi:10.1111/j.1365-3113.2007.00401

REHN A.C. 2003. Phylogenetic analysis of higher-level relationships of Odonata. – Systematic Entomology **28**: 181–239.

RIEK E.F., KUKALOVÁ-PECK J. 1984. A new interpretation of dragonfly wing venation based upon Early Upper Carboniferous fossils from Argentina (Insecta: Odonatoidea) and basic character states in pterygote wings. – Canadian Journal of Zoology **62**: 1150–1166.

RIS F. 1909. Collections Zoologiques du Baron Edm. De Selys Longchamps, Catalogue Systematique et Descriptif, Fasc. 9. – Bruxelles, Hayez, Impr. des Academies.

ROBLE S.M., CARLE F.L., FLINT O.S. 2009. Dragonflies and Damselflies (Odonata) of the Laurel Fork Recreation Area, George Washington National Forest, Highland County, Virginia: Possible evidence for Climate Change. Pp. 365–399 in: ROBLE S.M., MITCHELL J.C. (eds), A lifetime of contributions to Myriapodology and the Natural History of Virginia. – Virginia Museum of Natural History Special Publication No. 16, Martinsville, Virginia.

SAUX C., SIMON C., SPICER G.S. 2003. Phylogeny of the dragonfly and damselfly order Odonata as inferred by mitochondrial 12S ribosomal RNA sequences. – Annals of the Entomological Society of America **96**: 693–699.

SCHMIDT E. 1915. Vergleichende Morphologie des 2. und 3. Abdominalsegmentes bei männlichen Libellen. – Zoologische Jahrbücher **39**: 87–196.

SELYS-LONGCHAMPS E. DE 1854. Synopsis des Gomphines. – Bulletin de l'Académie Royale des Sciences de Belgique **21**: 23–112.

SNODGRASS R.E. 1935. Principles of Insect Morphology. – McGraw-Hill publications in the zoological sciences, New York. 667 pp.

STAMATAKIS A. 2006. Phylogenetic models of rate heterogeneity: A high performance computing perspective. – Proceedings IPDPS.

STEINMANN H. 1997. World Catalogue of Odonata. Volume I – Anisoptera. – Walter de Gruyter, Berlin.

THEISCHINGER G., HAWKING J. 2006. The Complete Field Guide to Dragonflies of Australia. – CSIRO Publishing, Collingwood, AU.

THEISCHINGER G., WATSON J.A.L. 1984. Larvae of Australian Gomphomacromiinae, and their bearing on the status of the *Synthemis* group of genera (Odonata: Corduliidae). – Australian Journal of Zoology **32**: 67–95.

TILLYARD R.J. 1917. The Biology of Dragonflies. – Cambridge, Cambridge University Press.

TILLYARD R.J., FRASER F.C. 1940. A reclassification of the order Odonata based on some new interpretations of the venation of the dragonfly wing. Part III. Continuation and Conclusion. – Australian Zoologist **9**: 195–221.

TRUEMAN J.W.H. 1996. A preliminary cladistics analysis of odonate wing venation. – Odonatologica **25**: 59–72.

TURGEON J., STOKS R., THUM R.A., BROWN J.M., MCPEEK M.A. 2005. Simultaneous Quaternary radiations of three damselfly clades across the Holarctic. – The American Naturalist **165**: E78–E107.

VON ELLENRIEDER N. 2002. A phylogenetic analysis of the extant Aeshnidae (Odonata: Anisoptera). – Systematic Entomology **27**: 1–31.

WALKER E.M. 1958. The Odonata of Canada and Alaska. – Toronto, University of Toronto Press.

WARE J.L., BEATTY C.D., SANCHEZ 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. – Journal of Biogeography **41**: 1291–1300.

WARE J.L., HO S.Y.W., KJER K.M. 2008. Divergence dates of libelluloid dragonflies (Odonata: Anisoptera) estimated from rRNA using paired-site substitution models. – Molecular Phylogenetics and Evolution **47**: 426–432.

WARE J.L., MAY M.L., KJER K.M. 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): An exploration of the most speciose superfamily of dragonflies. – Molecular Phylogenetics and Evolution **45**: 289–310.

WATSON J.A.L. 1969. The structure and affinities of *Pentathemis membranulata* Karsch (Odonata: Corduliidae). – Proceedings of the Royal Entomological Society of London B **38**: 83–88.

WATSON J.A.L. 1977. The distribution of the Australian dragonflies (Odonata): first supplement. – Journal of the Australian Entomological Society **16**: 277–279.

WHITFIELD J.B., KJER K.M. 2008. Ancient rapid radiations of insects: challenges for phylogenetic analysis. – Annual Review of Entomology **53**: 449–472.

WIENS J.J. 2005. Missing data and the design of phylogenetic analyses. – Journal of Biomedical Informatics **39**: 34–42.

WIENS J.J., MORRILL M.C. 2011. Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. – Systematic Biology **60**: 719–731.

Williamson E.B. 1908. A new dragonfly (Odonata) belonging to the Corduliinae, and a revision of the classification of the subfamily. – Entomological News **19**: 428–234.

Zhang J. 1999. Aeschnidiid nymphs from the Jehol biota (latest Jurassic–Early Cretaceous), China, with a discussion of the family Aeschnidiidae (Insecta: Odonata). – Cretaceous Research **20**: 613–627.

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics ("Contents")

File 1: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-1.docx — **Matrix S1.** Nexus file with Matrix S1; unaligned "charset" commands to extract matrices for Figs. S3–S6 at end.

File 2: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-2.docx — **Matrix S2.** Phylip file with Matrix S2, formed from Matrix S1 by consolidation of monophyletic genera into single chimeric taxa.

File 3: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-3.pdf — **Fig. S3.** 'Non-consolidated' maximum likelihood phylogram, using all available taxa with appropriate data available in Matrix S1, constructed using data from nuclear rRNA (18S, 28S) only.

File 4: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-4.pdf — **Fig. S4**. 'Non-consolidated' maximum likelihood phylogram, using all available taxa with appropriate data available in Matrix S1, constructed using data from mitochondrial rRNA (12S, 16S) and protein coding genes COI and COII only.

File 5: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-5.pdf — **Fig. S5**. 'Non-consolidated' maximum likelihood phylogram, using all available taxa with appropriate data available in Matrix S1, constructed using data from nuclear protein coding gene EF-1α only.

File 6: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-6.pdf — **Fig. S6**. 'Non-consolidated' maximum likelihood phylogram, using all available taxa with appropriate data available in Matrix S1, constructed using data from nuclear protein coding gene H3 only.

File 7: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-7.pdf — **Fig. S7**. Maximum likelihood phylogram for Odonata with special attention to Anisoptera based on a combination of rRNA, EF-1α, mtDNA, and morphology. From the presentation by Carle F.L. & Kjer K.M. 2000. Dragonfly phylogeny: molecules, morphology and behavior. – Entomological Society of America Annual Meeting.

File 8: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-8.pdf — **Fig. S8**. Phylogenetic topology of Anisoptera for rRNA-MP and EF-1α-ML with agreement listed on nodes for various other methods of analysis. From the presentation by Carle F.L. & Kjer K.M. 2000. Dragonfly phylogeny: molecules, morphology and behavior. – Entomological Society of America Annual Meeting.

File 9: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-9.docx — **Table S9.** Selected sequencing Primers designed for 18S, 28S, 12S&16S, and EF-1α. Primer name lists the following information: Gene, fragment, direction, year, series letter. UP = Forward Primer. DN = Reverse Primer. Amplicon length is approximate and for EF-1α includes introns.

File 10: carle&al-anisopteraphylogeny-asp2015-electronicsupplement-10.docx — Gene bank accession numbers for sequences newly obtained during this study.