A molecular phylogeny and classification of Anisoptera (Odonata)

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Accepted 09.vi.2015.
Published online at www.senckenberg.de/arthropod-systematics on 07.viii.2015.

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.; Sympretinae (including Leucorhinnini and Rhyothermitini); Macrodiplactinae; Brachydiplactinae; Tetrathemistinae; Trameinae; Zyxommatinae; Palpopleurinae; Diastatopidinae; Pantalinae (including Trithemistini and Onychothermitini); and Libellulinae. Zygonychini is paraphyletic to and therefore included within Onychothermitini.

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

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; PF AU 1986; BRAUCKMANN & ZESSIN 1989). They have complex mating systems and unique copulatory structures (SCHMIDT 1915; CARLE 1982a; PF AU 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 (Henning 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: (Austropetalidae + Aeshnidae) (Gomphoidae (Petaluridae (Cordulegastridae (Neopetalidae (Libellulidae (Macromiidae + Corduliidae)))))). A combined analysis employing data from rRNA, EF-1α, mitochondrial DNA, and morphology, and including additional taxonomic sequences from GenBank (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; Lettsch 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 Libelluloida 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; Lettsch 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

Anisoptera rRNA sequences were relatively easy to align across the order, but are difficult to align across even closely related outgroups. Anisoptera 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; Henning 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, Systelidae, Calopterygidae, and Coenagriomidae, 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; Lettsch 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 of 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), 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 tRNA 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, congeneric taxa 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 congeneric taxa 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 congeneric or deletion of taxa (resulting in “non-consolidated” trees) were performed on three subgroups supported in the consolidated tree: Aeshnoidae, 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 & Honeycutt (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 arbitrary, 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 (Stamatakis 2006), was used for each partition, analyzed with RAxML (Stamatakis 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 Nexus file (Matrix S1).

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 assure 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 Libelluloida sensu Carle 1986) and its constituent families (Cordulegastridae, Neopetalidae, Chlorogomphidae, Synthemistidae, Macromiidae, Corduliidae, and Libellulidae). Herein the Libelluloida is restricted to the Synthemistidae, Macromiidae, Cordulidae, 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 Libellulidae. — 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 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 Aeshnoida by the mitochondrial partition (BS = 27%; Figs. 1B, S4).
3.2. Relationships within Austropetalini and Aeshnidae

All analyses split Austropetalini into Australian Austropetaliinae + Tasmanian 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).
Gomphaeschninae is well supported as sister to the remaining aeshnids, however Brachytrioninae 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 Gomphoidei (Phyllogomphoidei Belle, 1970; Figs. 1B, 3) within Ictinogomphinae (Tillyard & Fraser 1940; Carle 1986, as Lindeninae) 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 (Stylogomphus 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 – Oncyphomphinae (7a Davidioidini trib.n., 7b Oncyphomphini); 8 – Gomphinae (8a Cyclogomphini, 8b Gomphini). Node support is indicated by bootstrap values.

3.4. Relationships within Cavealabiata

Cordulegastridae, Neoptealiidae, 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 (Idomacroemia Karsch, 1896 – Archaeophya Fraser, 1959 in Fig. 1C; FRASER 1957; DAVIES & TOBIN 1985). Gomphomacroemia 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 Gomphomacroemini, Idionychinae, and Idomacroemini (TILLYARD & FRASER 1940) form a paraphyletic series within an expanded Synthemistidae s.l. In the combined analysis Idomacroemia (represented by Idomacroemia 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 Cordulidae.

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

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 pleisiotypic Anisoptera

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.

<table>
<thead>
<tr>
<th>This Study (Fig. 4)</th>
<th>Letsch 2007</th>
<th>Pilgrim &amp; van Dohlen 2007</th>
<th>Ware et al. 2008</th>
<th>Fleck et al. 2008 (fig. Z)</th>
<th>Fleck et al. 2008 (fig. G)</th>
<th>Dumont et al. 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 B</td>
<td>Dythemis – Micatharya</td>
<td>F</td>
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<tr>
<td>3 C</td>
<td>Macrodiplax – Urothemis*</td>
<td>B</td>
<td>Urothemis – Macrodiplax*</td>
<td>Macrodiplax – Urothemis*</td>
<td>—</td>
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<td>4 sub D2</td>
<td>—</td>
<td>sub E</td>
<td>Brachydiplax – Chalcostepha</td>
<td>Chalcostepha – Brachydiplax*</td>
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<tr>
<td>5 D1</td>
<td>—</td>
<td>A</td>
<td>Tetrathemis – Notiothemis</td>
<td>Notiothemis – Tetrathemis</td>
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<tr>
<td>6 C1</td>
<td>Tramea – Mitharya</td>
<td>—</td>
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<tr>
<td>7 sub D2</td>
<td>Thymis – Brachythemis*</td>
<td>sub E</td>
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<td>9 F1</td>
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<tr>
<td>10 F2</td>
<td>Onychothemis – Trithemis</td>
<td>C + sub H1</td>
<td>not recovered</td>
<td>not recovered</td>
<td>Zygonyx – Trithemis*</td>
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<tr>
<td>11 G</td>
<td>Orthemis – Libellula</td>
<td>H2</td>
<td>Orthetrum – Hadrothemis</td>
<td>Orthemis – Criatilla</td>
<td>Micromacromia – Orthetrum</td>
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</table>
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 recognized and numbered in the column to the right of the tree, they are: 1 – Dythemistinae subfam.n.; 2 – Sympetrinae; 3 – Macrodiplactinae; 4 – Brachydiplactinae; 5 – Tetramethistinae; 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).
of a biphasic sperm pump following increased size of the plesiotypic Cavealabiata, apparently via development of the libelluloid condition developed gradually from that petaloid precursors. Our topology suggests, rather, that luloids must have evolved independently from austro-

The positive pressure phase of the penile sperm pump in 292 CARLE & KJER results closely resemble the paraphyletic topology pro-

ment of superfamilies similar to that presented by 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 paraplyetic 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 (PFARU 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 Gomphins of his family Aeschnidées. TILLYARD (1917), however, removed Petalia from the Gomphinae and placed it in his composite Petalini of the Aeshnidae [sic], until CARLE & LOUTON (1994) reconfirmed SELYS’ (1854) relative placement of Neopetalia. Here Cordulegastriidae, Neopetaliidae, Chlorogomphidae and Libellulidae are placed within Cavealabiata; this grouping is equivalent in composition to the Cordulegasteroida [sic] of TILLYARD & FRASER (1940) except that the latter excluded Neopetalia Cowley, 1934. Their group included the Cordulegastriidae 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. CARLE (1995) summarized nine morphological characters that support Cordulegastroidea, Neopetaliidae, and Chlorogomphidae as successively paraplythic 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), BLYTHE 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 Cordulegastroidea, Neopetaliidae, and Chlorogomphidae could potentially overshadow support for the short internodes of the paraplythic 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 paraplythic 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 + Tasmanian Archipetaliinae from the Hypopetaliidae + 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 Saraseschna 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 Aeshnidae. 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 Aeshnidae, 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-
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 Austropetalidae. Ictinogomphines eventually dispersed throughout the Neotropical region, with the Progomphini (Progomphus) and Gomphoidini (here represented by Phyllogomphooides) reaching the Neartic, and with the highly derived and vagile Ictinogomphini reaching tropical Africa and beyond. The implied great age of the monogenic 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 Hagenininae arise successively from the basal nodes. This molecular topology confirms the basal position of the “octogomphines” and along with strong differences in mitochodrial 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 crossovis, 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 laterobasal elongate-triangular sclerites, male epiproctal rami subparallel, male anterior lamina without raised V-shaped posterior ridge, penile prepuce globosely, 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 Afrotrropical 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 plane parallel to RP; and straight; abdominal segments 9 and 10 black, male cerci conical bright yellow and ca. as long as abdominal segment 10, epiproctal rami strongly divericate; 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 Asagomphus Asahina, 1985; placement of Gomphus (Phanogomphus) Carle & Cook, 1984 with Dromogomphus Selys, 1854; and placement of both Gomphus (Hylogomphus) Wastfall & 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 (Tachopterix 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, Neopetalidae, Chlorogomphidae

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

4.1.6.1. Synthemistidae. Fraser (1954; 1957) presented the topology of Libelluloidae as: (Synthemistidae (Corduliiidae (Macrodiplactidae + Libellulidae)); he included in Corduliiidae: Cordulephyineae, Neophyinae, Idomacroniinae, Macromiinae, as Epophthalmiinae), Idionychiinae, Gomphomacroniinae, and Corduliinae. Monophyly of Fraser’s Corduliidae became questionable when Gomphomacronia, Archaeophya and possibly Pseudocordulia were shown to form the apparent sister group of Synthemistidae (Thieschinger & 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 morphologically synapomorphies and similar geographic origin, these genera include: Austrosynthemis Carle, 1995 Calesyntemis Carle, 1995 Palaeosynthemis Förster, 1903 Parasynthemis Carle, 1995 and Tonymysynthemis Thieschinger, 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 Macrodiplactidae 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 Gomphomacronia 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, Idomacronia Karsch, 1896, Macrodiida, Idonyx Selys, 1871, and Neocordulia Selys, 1882, either within Macromiidae or Corduliiidae or in a polytomy with Macromiidae, Corduliiidae, or Libellulidae. Idomacronia and Oxygastra, along with Neocordulia McEachlan, 1882, Neocordulia Selys, 1882, and Neophyta Selys, 1881, share an apparent apomorphy with Corduliiidae + 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 Corduliiidae s.s., respectively (Fig. 1B; we now refer to Corduliiidae 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, Corduliiidae. Although Fraser (1957) recognized seven subfamilies within the Corduliiidae, this analysis reveals that only genera of Corduliiidae and Macromiinae form polytypic monophyletic groups; the first of these is placed as the sister group of Libellulidae, and Macromiinae placed sister to (Corduliiidae s.s. + Libellulidae). This topology indicates that Macromiinae should also be accorded family rank, as proposed by Glyd (1959). Macromiidae and Corduliiidae s.s., are again very strongly supported except for lower bootstraps tying Aeschnosoma Selys, 1870 + Pentathemis Karsch, 1890 to the Corduliiidae s.s. The position of these two genera clearly supports Fleck’s (2012) and Fleck & Legrand’s (2013) separation of these plus Libellulosa Martin, 1907 as a very distinctive group within Corduliiidae s.s. Both Macromiidae and Corduliiidae 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 Corduliiidae 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 Corduliiidae 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 + Oligogastra Karsch, 1895 from Ris’ group 8; and placed Rhyhotemis 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; Beckly 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 Lettsch (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 Lettsch (2007), the branching order was reasonably similar to (Fig. 4). This increases confidence that most of the principal subsidiary groups have been identified and 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, Rhyothemisini, Trithemisini, Onychothemisinae 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 Onychothemisini). 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 Rhyothemisini); 3 – Macrodiplactinae; 4 – Brachydiplactinae; 5 – Tetramerisini; 6 – Trameinae; 7 – Zyxommatinae; 8 – Palpopleurinae; 9 – Diastatopodinae; 10 – Pantalinae (including Trithemisini and Onychothemisini); 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 Tetramerisini (Fraser 1957; Davies & Tobin 1985). In our molecular topology (Fig. 1C) the new subfamily is placed as the sister group of Sympetrinae and these three 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 Dythemisini 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.

Dythemisini trib.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 crosseeve (with 1 – 3 in Micrathyria), with 1 cubital-anal crosseeve (occasionally 2 in Micrathyria), and with anal loop well developed with angulated bisector, anal loop open distally in Argyrothemis.

Pachydiplactini trib.n. – Type genus Pachydiplax Brauer, 1868. – Adult with prothoracic hind margin prominently and bilobate, wings with arculus typically arising from near second antenodal, wings with R undulate, forewing intraradial crosseeves 4 – 6, radial planate well developed and medial planate vestigial, 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 supritriangle; 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.

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 crossoveins 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: Nephepelta 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 Symptremat (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 Eotania Carle & Kjer, 2002, separate from Holotainia Kirby, 1899, is supported. In Symptremat the putative subgenus Tarnetrum Needham & Fisher, 1936, forms a basal paraphyletic cluster that also includes Nesogonia Kirby, 1898 while Kalosymptrem Carle, 1993 and Symptremat 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 organisal 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 Aeshnoidae, Gomphidae, Petaluridae, Cordulegastridae, Neopetaliidae, Chlorogomphidae, and Libelluloidae. 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 & Kjer 1982). 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 dispersal 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 Libelluloidae; both groups have independently evolved similar methods of fosorial 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 Libellulidae 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 Stenophibioidea Pritykina, 1980 and Aeschnidoidea 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 Cordulidae) 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.
5. Acknowledgements

We are indebted to the many people and institutions who collected, gifted or loaned us specimens for DNA extraction: D. Carle, F. Carle, K. Carle, C. Chaboo, V. Clausnitzer, T.W. Donnelly, M. Driessen, S. Dunkle, C. Esquivel, O.S. Flint, D. Furth, R. Garrison, P. Grant, J. Lempert, B. Mauffray, M. Mbida, J. Michalski, D. Paulson, A. Rowat, R. Rowe, K. Tennesen, G. Theischinger, J. Ware, R. West, K. Wilson, the American Museum of Natural History, the California Academy of Sciences and the Smithsonian Institution. Many thanks to Dave Britton and Guenter Theischinger for assistance with Australian collecting permits, and to the Government of Australia, Dept. of Environment and Heritage, for permission to collect and export specimens (permit numbers WT2004-10767 and WITK02489604; loan number 1914). Supported by NSF DEB-0423834 and NSF DEB-0818685. New Jersey Agricultural Experiment Station publication D-08-08001-09-14, supported by state funds.

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Electronic Supplement Files

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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 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.


