MOLECULAR AND MORPHOLOGICAL SYSTEMATICS OF LIBELLULOIDEA (ODONATA: ANISOPTERA) AND DICTYOPTERA

by

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ABSTRACT OF THE DISSERTATION MOLECULAR AND MORPHOLOGICAL SYSTEMATICS OF LIBELLULOIDEA (ODONATA: ANISOPTERA) AND DICTYOPTERA By JESSICA LEE WARE

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Libelluloidea are highly successful dragonflies with unique behavior and life histories. The systematics of Libelluloidea (Odonata: Anisoptera) has historically been in conflict, with little agreement about the number of families that are comprised in this large, heterogeneous group. For my PhD thesis, I have assembled the most comprehensive molecular and morphological libelluloid dataset to date, in an attempt to revise and simplify libelluloid taxonomy, and to answer questions about the evolutionary history of the group. I ran Bayesian and parsimony analyses to recover phylogenetic hypotheses with which I explore the success of Libelluloidea. Divergence estimation, a method by which nodes of a tree are dated, was first explored under different evolutionary models for a subset of libelluloid taxa in order to determine whether treatment of hydrogen-bonded ribosomal nucleotides affected the age of divergence estimates. Using methodology based on these

results, I was able to estimate divergence dates and diversification rates for the first large-scale dating analysis of dragonflies. On a smaller scale, I also completed a study of *Syncordulia*, a vulnerable genus of endemic South African libelluloid dragonflies whose systematics was not yet confirmed. Additional studies of phylogenetic methodology were undertaken in my thesis work for another large and heterogeneous group, the Dictyoptera (Mantodea, Blattodea and Isoptera). In this study, the effect of outgroup selection was determined using a broad, comprehensive taxon set for which we had both molecular and morphological data. These results suggest that the evolution of sociality, on which much of the recent discussion in dictyopteran systematics has focused, cannot be reliably determined when different outgroups recover dramatically conflicting topologies.

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iv

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V

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Table of Contents

Abstractpages ii-iii
Acknowledgements and/or Dedicationpages iv-vi
List of Tablespage ix
List of Illustrationspages x-xi
Chapter 1: Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an
exploration of the most speciose superfamily of dragonflies. Previously
published in Molecular Phylogenetics and Evolutionpages 1-53
Chapter 2: Divergence dates of libelluloid dragonflies (Odonata: Anisoptera)
estimated from rRNA using paired-site substitution models. Previously
published in Molecular Phylogenetics and Evolutionpages 54-78
Chapter 3: Biogeography and divergence estimation of the relic Cape
dragonfly genus Syncordulia: global significance and implications for
conservation. Submitted to the <i>Journal of Biogeography</i> pages 79-107
Chapter 4: Understanding phylogenetic relationships, homoplasy and
divergence estimates within Libelluloidea (Anisoptera: Odonata): an
exploration of the usefulness of molecular versus adult and larval
morphological characterspages 108-200

Chapter 5: Resolving internal relationships within the	Dictyoptera poses
new questions: difficulty in the placement of Polyphaga. P	Previously
published in Systematic Entomology	pages 200-257
Curriculum Vitae	pages 259-260

List of tables

Table 1, Chapter 1Page 42
Table 2, Chapter 1Page 44
Table 3, Chapter 1Page 53
Table 1, Chapter 2Page 73
Table 2, Chapter 2Page 74
Table S1, Chapter 2Page 75
Table S2, Chapter 2Page 77
Table 1, Chapter 3Page 97
Table 2, Chapter 3Page 101
Table 3, Chapter 3Page 102
Table 1, Chapter 4 Page 158
Table 2, Chapter 4Page 185
Table 3, Chapter 4Page 187
Table 4, Chapter 4 Page 189
Table 5, Chapter 4Page 189
Table 6, Chapter 4
Table 7, Chapter 4Pages 191-198
Table 8, Chapter 4Pages 198-200
Table 1, Chapter 5Page 254
Table 2, Chapter 5 Page 256
Table 3, Chapter 5

List of illustrations

Figure 1, Chapter 1Page 34	
Figure 2, Chapter 1Page 35	
Figure 3, Chapter 1Page 36	
Figure 4a, Chapter 1Page 37	
Figure 4b, Chapter 1Page 38	
Figure 5, Chapter 1Page 39	
Figure 6, Chapter 1Page 40	
Figure 7, Chapter 1Page 41	
Figure 1, Chapter 2Page 71	
Figure 2, Chapter 2Page 72	
Figure 1, Chapter 3Page 103	
Figure 2, Chapter 3Page 104	
Figure 3, Chapter 3Page 105	
Figure 4, Chapter 3Page 106	
Figure 5, Chapter 3Page 107	
Figure 1a, Chapter 4Page 151	
Figure 1b, Chapter 4Page 152	
Figure 2, Chapter 4Page 153	
Figure 3, Chapter 4Page 154	

Figure 4, Chapter 4Page 155
Figure 5, Chapter 4Page 156
Figure 6, Chapter 4Page 157
Figure 1, Chapter 5Page 249
Figure 2, Chapter 5Page 250
Figure 3, Chapter 5Page 251
Figure 4, Chapter 5Page 252
Figure 5, Chapter 5Page 253

Chapter 1:

Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies

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Abstract

Although libelluloid dragonflies are diverse, numerous, and commonly observed and studied, their phylogenetic history is uncertain. Over 150 years of taxonomic study of Libelluloidea Rambur 1842, beginning with Hagen (1840), Rambur (1842), and Selvs (1850), has failed to produce a consensus about family and subfamily relationships. The present study provides a well-substantiated phylogeny of the Libelluloidea generated from gene fragments of two independent genes, the 16S and 28S ribosomal RNA (rRNA), and using models that take into account non-independence of correlated rRNA sites. Ninety-three ingroup taxa and six outgroup taxa were amplified for the 28S fragment; seventy-eight ingroup taxa and five outgroup taxa were amplified for the 16S fragment. Bayesian, likelihood and parsimony analyses of the combined data produce well-resolved phylogenetic hypotheses and several previously suggested monophyletic groups were supported by each analysis. Macromiidae, Corduliinae, and Libellulidae are each monophyletic. The corduliid subfamilies Synthemistinae, Gomphomacromiinae, and Idionychinae form a monophyletic group, separate from the Corduliinae. Libellulidae comprises 3 previously accepted subfamilies (Urothemistinae, a very restricted Tetrathemistinae, and a modified Libellulinae) and 5 additional consistently recovered groups. None of the previously proposed subfamilies are supported. Bayesian analyses

run with an additional 71 sequences obtained from GenBank did not alter our conclusions. The evolution of adult and larval morphological characters is discussed here to suggest areas for future focus. This study shows the inherent problems in using poorly defined and sometimes inaccurately scored characters, basing groups on symplesiomorphies, and failure to recognize the widespread effects of character correlation and convergence, especially in aspects of wing venation.

1. Introduction

Dragonflies are among the most recognizable of insects, even having become subjects of extensive folklore (Sarot, 1958) and, moreover, have been used in a wide array of studies dealing with functional morphology, behavior, ecology, and evolution (Corbet, 1999). Odonata are considered to be among the earliest flying insects. Their recognizable progenitors date to the Carboniferous (360-290 million years ago) and are probably the most widely known extinct insects. Anisoptera (in their present form) arose later, with earliest known fossils from the Triassic (250-200 million years ago; Grimaldi and Engel, 2005). While clearly identifiable libelluloids are not well known from the Jurassic (206-142 million years ago), Jarzembowski and Nel (1996) suggest that libelluloids were already well established in the Early Cretaceous (142-65 million years ago). Extant libelluloids include, among others, the widespread Macromiinae and Corduliinae and the most abundant and familiar dragonflies, Libellulidae. Libellulidae are readily recognizable, often with colored or patterned wings and a boot shaped series of veins (the anal loop) in the hindwing. They are commonly seen in territorial flight around lakes and ponds, or perched along the bank.

Among libelluloids, adult reproductive and feeding behavior, larval behavior and ecology (Corbet, 1999), and biogeography (Carle, 1995) vary widely and have been investigated intensively. While it is clear that a well-supported phylogenetic hypothesis is needed in order to reach an understanding of the evolution of these traits, phylogenetic relationships among libelluloid families remain highly contentious, with numerous hypotheses proposed (Fig. 1, Table 1). For descriptive purposes, we use the taxonomy of Davies and Tobin (1985) unless otherwise indicated, since it is widely used today (Table 2).

Morphological studies of libelluloid phylogeny have relied heavily, although not exclusively, on wing vein characters (Kirby, 1890; Needham, 1903; Martin, 1907; Needham, 1908; Ris 1909-1919; Tillyard, 1910; Needham and Broughton, 1927; Fraser, 1957; Gloyd, 1959; Geijskes, 1970; Lieftinck, 1971; Theischinger and Watson, 1978; Carle, 1982a; Davies and Tobin, 1985; Carle and Louton, 1994; Carle, 1995; Bechly, 1996; Lohmann, 1996; Trueman, 1996; Jarzembowski and Nel, 1996; Carle and Kjer, 2002; Rehn, 2003). Despite progress in understanding homologies in Odonata venation (e.g. Carle, 1982b; Riek and Kukolova-Peck, 1984), many wing vein characters may support convergent relationships when used to the exclusion of other characters (Hennig, 1969; Carle, 1982b). The 11 subfamilies of Libellulidae recognized in Davies and Tobin (1985) and Bridges (1994) were largely based on wing vein morphology. Bechly's (1996) morphological study of the Odonata also relied heavily on venational characters to break up the higher Libelluloidea into numerous families: Gomphomacromiinae and Synthemistinae were split into eight families and the remaining Corduliidae and the Libellulidae were each divided into two families.

Some studies have focused on egg, genitalic, flight musculature, color, and larval characteristics (St. Quentin, 1939; Gloyd, 1959; Lieftinck, 1971; Pfau, 1971; Theischinger and Watson, 1984; Pfau, 1991, 2005; May, 1995; Bechly, 1996; Lohmann, 1996a; Carle and Kjer, 2002). Pfau's (2005) study of sperm transfer mechanisms lead him to an alternate phylogenetic hypothesis that placed Cordulegastridae, Chlorogomphidae, and Neopetaliidae within Petaluroidea rather than Libelluloidea. Much of the current confusion over libelluloid taxonomy and phylogeny may be the result of uncertain character homology and independence (reviewed in Carle, 1982b). An independent molecular dataset may help resolve conflicting phylogenetic hypotheses.

Several recent molecular studies (Kambhampati and Charlton, 1999; Artiss et al., 2001; Saux et al., 2003, Hovmoller, 2004) have included many libelluline taxa, and some (Misof et al., 2001; Misof and Fleck, 2003; Hovmoller et al., 2004; Hasegawa and Kasuya, 2006) included a broader sampling across the superfamily. Most of these studies were based on a single gene (Kambhampati and Charlton, 1999; Misof et al., 2001; Artiss et al., 2001; Hovmoller et al., 2002; Misof and Fleck, 2003; Saux et al., 2003). Because their question focused on subordinal relationships, Saux et al. (2003) used Locusta migratoria as an outgroup, which may have been too distantly related to answer questions about the internal order of the families (Farris, 1982; Lyons-Weiler et al., 1998; Graham et al., 2002). The most recent study (Fleck et al, 2006), includes a large taxon sample, primarily consisting of tetrathemistine and libelluline Libellulidae, and combines molecular data with larval morphology.

Our purpose here is to present a phylogenetic hypothesis of the higher Libelluloidea (i.e. Corduliidae and Libellulidae of Davies and Tobin, 1985), generated from two

independent gene fragments, (mitochondrial and nuclear large ribosomal RNA subunits; 16S and 28S), structurally aligned, using basal libelluloid outgroups, and the following methods that model the correlated rRNA sites as non-independent. Extensive taxon sampling has allowed us to assess several regions of contention in the higher Libelluloidea and to propose historical relationships within this group. The phylogenetic reassessment provides a basis for improving the taxonomy in the historically difficult Libelluloidea.

2. Materials and Methods

Taxon sampling

The superfamily Libelluloidea includes the Libellulidae, with 143 genera and 969 species, the most species-rich and commonly observed family of dragonflies worldwide, as well as the Corduliidae Kirby 1890 (43 genera, 406 species) (Davies and Tobin, 1985; Steinmann, 1997; number of species from Schorr et al., 2006). These are the "higher " Libelluloidea, which are the main focus of this study. We also include the basal libelluloids (sensu Carle 1995) Cordulegastridae Calvert 1893 (4 genera, 49 species), Chlorogomphidae Needham 1903 (3 genera, 46 species), and the monotypic Neopetaliidae Tillyard and Fraser 1940.

Taxa sequenced are listed in Table 2. Cordulegastridae, Chlorogomphidae, and Neopetaliidae served as outgroups, with the tree rooted using Neopetalia punctata (2 species from 2 cordulegastrid genera, 2 species from 2 chlorogomphid genera and 1 species from the monotypic Neopetaliidae). We sampled as broadly as we could across each of the libelluloid families, extracting from individuals of every subfamily in every libelluloid family (3 species from 3 macromiine genera, 14 species from 11 corduliine corduliid genera, 18 species from 17 other non-corduliine corduliid genera, and 70 species from 56 libelullid genera) (Table 2).

Gene Selection, DNA extraction and PCR amplification

Freshly collected dragonflies were used when possible; other taxa were obtained from personal and museum collections (Table 2). We amplified the second, third and seventh hypervariable (divergent) regions (D2, D3, and D7) of the nuclear large subunit rDNA (28S) and the third domain of the mitochondrial large subunit rDNA (16S).

Muscle tissue was extracted using a Qiagen Dneasy tissue kit overnight at 55°C with 180 µL of ATL Buffer and 20 µL Proteinase-K. Older specimens (collected prior to 1980) were extracted with 40 µL (twice the suggested amount) of Proteinase-K buffer for several days (a suggestion made by R. Caesar, pers. comm.). All other steps followed the manufacturer's protocol. PCR primers, and their sources, are given in Table 3. Programs used for amplifications were (a) 96°C, 3 min; 94°C, 30s; 50°C, 30s; 72°C, 45s for 35-40 cycles; 72°C, 10 min and (b) 96°C, 3 min; 94°C, 30 s; 46°C, 30s, 72°C, 45s for 10 cycles; 94°C, 30s; 48°C, 40s; 72°C, 45s for 30 cycles; 72°C, 10 min. A Qiagen PCR purification kit was used to purify amplified product, which was then sequenced on an ABI 3100 capillary sequencer. Sequences from both strands were compared and edited in Sequence

Navigator (Applied Biosystems). Lowercase letters were used to indicate nucleotides that were readable but difficult to interpret with certainty in either strand (i.e., there was competing background peaks). These lowercase letters were changed to uppercase letters only if there was agreement in the two complementary strands. When there was conflict about a single base call between reads from complementary strands, this nucleotide was coded with an R (for ambiguous purines), Y (for ambiguous pyrimidines) or N (for all other ambiguities).

Alignment and Phylogenetic Reconstruction

Initial sequence alignments were made using Clustal, and the resulting files were then aligned manually in Microsoft Word using the structural methods described in Kjer et al. (1994), Kjer (1995) Kjer et al., (2007) and secondary structure models based on Gutell et al. (1993). Ambiguously aligned regions were defined as single stranded regions with multiple insertions and deletions (indels) of variable length (and thus unclear nucleotide homology), bounded by hydrogen bonded base pairs. These regions were excluded from the dataset. For parsimony analyses, these characters were recoded as single multistate characters with the program INAASE (Lutzoni et al, 2000), with the stepmatrices applied. Alignments are available on the Kjer lab website,

www.rci.rutgers.edu/~entomology/kjer. Although we regard manual alignment based on secondary structure to be quite strongly supported (e.g., Kjer, 1995, 2004; Titus and Frost, 1996; Schnare et al., 1996; Hickson et al, 2000; Lutzoni et al, 2000; Mugridge et

al., 2000; Ellis and Morrison 1995, Morrison and Ellis, 1997, Gillespie et al., 2005) as the most accurate method for rDNA, we recognize that alignment methodology is a contentious issue. For those who prefer a different alignment procedure, however, the original sequences are, of course, deposited in GenBank and thus will be available for any reanalysis that is desired.

The data was analyzed using parsimony, maximum likelihood, and Bayesian criteria. For the parsimony reconstruction, a tree bisection-reconnection (TBR) branch swapping heuristic search was run using PAUP 4.0b10 (Swofford, 2001) with 10,000 random additions. Gaps of uniform length were each treated as presence/absence characters; other gaps were treated as missing data, except those encoded with INAASE as described above. To estimate branch support, 500 bootstrap pseudoreplicates (Felsenstein, 1985) were performed using 10 random addition searches per pseudoreplicate. Prior to maximum likelihood and Bayesian analyses, we used MODELTEST 3.06 Akaike weights (Posada and Crandall, 1998; Posada and Buckley, 2004) and DT-ModSel (Minin et al., 2003) to select an appropriate model of evolution for each of the two independent gene fragments. Both programs suggested a GTR + I + G model for the 28S and 16S (Yang, 1994; Yang et al., 1994; Gu et al., 1995). GARLI (Zwickl, 2006; available at http://www.zo.utexas.edu/ faculty/antisense/garli/ Garli.html) was used to run a rapid maximum likelihood analysis. The GARLI bootstrap analysis was run using 100 replicates of a 500,000-generation search; the heuristic search ran 500,000 generations. Datasets were unable to be partitioned in GARLI, although future versions of GARLI will be able to do so (Derrick Zwickl, pers. comm.). Because it considers the nonindependence of hydrogen-bonded rRNA sites, we also used the RNA7A seven state model available in the PHASE program (Jow et al., 2002), to run Markov-chain Monte Carlo (MCMC) analyses of partitioned RNA data (10 million generations each) and a REV model for the loop regions. Unlike the current version of Mr. Bayes, which uses a 16 by 16 rate matrix, the RNA7A seven state model (Higgs, 2000) uses a 7 by 7 rate matrix (7 frequencies, 21 rate parameters). This biologically realistic model is useful for studies of rRNA. The REV model is the most general loop model with the time reversible constraint (four frequencies, 5 rate parameters).

Using our PHASE trees, we tested the classifications of Davies and Tobin (1985) and Bechly (1996) using the constraint function in PAUP. Constraints were written for each of Davies and Tobin's subfamilies, and for each of Bechly's families. We then filtered our PHASE tree file using these constraints and recorded the number of trees that contained these clades.

To recheck our data for possible contaminants, and to incorporate additional available taxa, we ran a parsimony analysis, as described above, with 71 libelluloid sequences downloaded from GenBank and aligned them with our data (Table 7). Most of the libelluloid sequences in GenBank are mitochondrial. Some of the data available in GenBank included a longer fragment of mitochondrial rRNA that included a fragment of the 12S. These characters were included in the analysis and coded as missing for our taxa (for a discussion of missing data see Weins, 2005).

3. Results

3. 1 Molecular Data Collection

Ninety-three ingroup taxa and six outgroup taxa were amplified for the 28S fragment; seventy-eight ingroup taxa and five outgroup taxa were amplified for the 16S (Table 7) and included in the analysis. After 192 ambiguously aligned characters were excluded and coded in INAASE, 1418 nts remained from the nuclear gene fragment and 430 nts from the mitochondrial fragment. Five hundred and forty-three characters were parsimony informative (nuclear=346, mitochondrial=189, INAASE=8); 370 were parsimony uninformative and 943 characters were constant. All sequences are deposited in GenBank under accession numbers 1234567890.

3.2 Phylogenetic Relationships

Bayesian, likelihood and parsimony analyses of the combined data produced wellresolved phylogenetic hypotheses (GARLI and PHASE results: Figure 2; parsimony: Figure 3). Minor differences occur in areas for which there is low branch support (<50%). Several previously suggested monophyletic groups were supported by all three methods: (i) Libellulidae (100% support from all analyses) and Macromiidae (100% support from all analyses). These clades, plus Corduliinae, together form a monophyletic group, hereafter the MCL group in the PHASE and GARLI analyses (similar but not identical to the MCL group of Carle, 1995). (ii) Corduliidae s.l is polyphyletic. Corduliinae is monophyletic (89% PHASE posterior probability; 60% GARLI bootstrap; 86 % parsimony bootstrap). (iii) Surprisingly, Gomphomacromiinae + Synthemistinae + Cordulephyinae + Idionychinae (hereafter, the GSI group) together form a monophyletic group (100% PHASE; 79 % GARLI; 71% parsimony bootstrap) that, however, is not clearly divided into the traditional (sub)families and does not nest within Corduliidae.

The results do not support most of the 17 families proposed by Bechly (1996) and Lohmann (1996), or the subfamilies of Corduliidae suggested by Davies and Tobin (1985) and Bridges (1994). Furthermore, the puzzling Australian species *Cordulephya pygmaea*, variously placed as 1) a monogeneric subfamily (Fraser, 1957; Davies & Tobin, 1985), 2) with *Hetronaias* and *Libellulosoma* (Bridges, 1994) or 3) with *Neophya* (Bechly, 1996), is well nested within the GSI clade as sister to the nominal gomphomacromiine *Pseudocordulia circularis* (*Hetronaias*, *Libellulosoma*, and *Neophya* were not sequenced). The two genera of Idionychinae, *Macromidia* and *Idionyx*, form a monophyletic but poorly supported (35% PHASE) subclade within the GSI clade. Corduliinae and Macromiinae are separate monophyletic clades in all analyses. In terms of the MCL interfamilial relationships, the PHASE analysis placed the Corduliinae as sister to the Libellulidae, although with such low support that we would prefer to consider the relationship unresolved.

Within the Libellulidae, 3 subfamilies were consistently recovered: Leucorrhiniinae Tillyard 1917 (99% PHASE; 94% GARLI, 80% parsimony), Urothemistinae Lieftinck 1954 (96% PHASE; 79% GARLI; 85% parsimony), and Libellulinae Rambur 1842 (76% PHASE; largely, though not entirely recovered). Otherwise, members of the subfamilies listed in Davies and Tobin (1985) and Bridges (1994) are scattered throughout the Libellulidae. Conspecifics and congenerics (with the exceptions of *Libellula*, *Cordulia*, *Zyxomma* and *Orthetrum*) form monophyletic groups.

To assess congruence among independent datasets, we also ran separate PHASE analyses of the mitochondrial and nuclear genes. The nuclear PHASE data lends strong support to monophyly of the GSI (97%) but the mitochondrial data place them instead as a polytomy at the base of the tree (not shown). Both datasets support the monophyly of Macromiidae and of Libellulidae. The 16S data support the monophyly of the Corduliinae (80% 16S), but 28S support for this group was low (50%), probably due to the unstable position of the genera *Pentathemis* + *Aeschnosoma*. Within the Libellulidae, only the Libellulinae are largely supported by both genes. Subtle differences occur between the 16S and 28S in the composition and/or position of Clades B and F (Clade B includes *Hydrobasileus* in the 28S analysis while there is little support for the subfamily Urothemistinae in the 16S analysis; Clade F includes *Rhyothemis* in the 28S analysis, while Clade F is not supported by the 16S analysis). Other differences between the 16S and 28S were found only in areas with less than 50% support.

Our hypothesis testing revealed that neither Gomphomacromiinae nor Synthemistinae were present in any of the 69009 trees (after burnin discarded) created by our PHASE analysis. Idionychinae was recovered, in 15736 trees (23%). Bechly's (1996) Synthemistidae, Gomphomacromiidae, Austrocorduliidae, and Oxygastridae were never present. In addition, the monogeneric Pseudocorduliidae and Cordulephyidae are nested well within the GSI clade and thus do not form the pectinate arrangement of Bechly (Figure 1g) that would justify family status. Bechly's Hemicorduliidae (*Hemicordulia* and *Procordulia*), is recovered in 84 % of the trees (Figure 2), but its nested position does not suggest that family status is warranted. The libellulid subfamilies of Davies and Tobin (1985), with the exception of Leucorrhiniinae and Libellulinae were never recovered. We constrained *Pantala* and *Tramea* only but found that they were never recovered together. *Dasythemis* never occurred within Libellulinae.

Parsimony and PHASE analyses of the dataset that included GenBank sequences were largely consistent with our other analyses (Figure 7). The taxon sample in GenBank consists mostly of libellulids. In all cases, except for Erythemis, Pachydiplax, Plathemis, Ladona, Lyriothemis, and Libellula congenerics group together. Because the support values are so low within the Libellulinae, we consider relationships among *Plathemis*, Ladona, Lyriothemis, and Libellula to be unresolved. Erythemis and Pachydiplax GenBank sequences are available for the 12S fragment only, a fragment we did not sequence, and so they may not have enough information to place them with our congeneric taxa. Similarly, data may be insufficient to correctly place Idiataphe, for which we have only the D3 fragment sequence: in the larger dataset, it is placed in Clade A, with low support. The Urothemistinae, Leucorrhiniinae, and Libellulinae remain as monophyletic groups, and because the support along the backbone of Libellulidae is low, the other groupings (Clades A, C, D, E, F, and G) are randomly arranged (and present as a polytomy in the bootstrap analysis). The composition of the clades does not differ greatly. Not surprisingly, the differences between the phylogenetic reconstructions were found in areas with less then 50% bootstrap support.

4. Discussion

4.1. Taxonomic Implications

Our results agree with those of most previous dragonfly systematists in placing Synthemistinae as basal and Libellulidae as terminal libelluloids (e.g., Tillyard, 1917; Fraser, 1957; Carle, 1995; Bechly, 1996). Pfau's (1991, 2005) innovative morphological study of the odonate vesica spermalis (penis), however, differs radically in placing Cordulegastridae and related taxa in the Petaluroidea and the Synthemistinae as sister to the Libellulidae. In the remainder of this section, we compare in more detail our hypothesis with previous systematic treatment of Libelluloidea.

4.1a The GSI Clade

Our analysis places Synthemistinae relatively basally among higher libelluloids, as have most earlier studies (Truemann's (1989) study of egg morphology and early larval characteristics; wing venation by Tillyard (1917), Fraser (1957), Davies and Tobin (1985), Carle (1995) Lohmann (1995) and Bechly (1996)). Since Tillyard and Fraser (1940) and Fraser (1957), all authors have regarded Gomphomacromiinae and Idionychinae as distinct from Synthemistinae, although with various internal subdivisions (Carle, 1995; Bechly, 1996, and see Table 1). Our analysis, however, fails to support this distinction, with the three groups mingled within the GSI group. This is a surprising result, since Theischinger and Watson (1984) identified several convincing larval morphological characters favoring such a division. Subsequent authors (e.g., Carle and Louton (1994), Carle (1995), Lohmann (1996) and Bechly (1996)), using additional characters, also found support for the separation of these Synthemistinae and Gomphomacromiinae. In our phylogeny, support is low for many relationships within the GSI clade, but, Synthemistinae and Gomphomacromiinae were never found in any of the near optimal trees from the Bayesian treefile. Consideration of additional morphological or molecular data may suggest a more traditional structure within the clade. It would be very difficult, however, to reconcile our conclusions with the hypothesis that the Gomphomacromiinae + Idionychinae are paraphyletic with respect to Corduliinae s. s. (e.g., May and Cook 1993; Bechly, 1996; Lohmann, 1996).

4.1b Corduliidae and Macromiidae

Corduliidae s. s. and Macromiidae have long been considered closely related and have been regarded as confamilial by many workers (Martin, 1906,1909; Tillyard, 1917; Fraser, 1957; Lieftnick, 1971; Davies & Tobin, 1985; Steinmann, 1997). They share a number of characters, most of which, however, are either plesiomorphies or are also shared with Libellulidae (see below). Gloyd (1959) proposed that Macromiidae be raised to family status, but her arguments were based almost entirely on autapomorphies of Macromiidae. Nevertheless, Gloyd's suggestion is not inconsistent with our results. Our data support the monophyly of the Macromiinae; whether this monophyletic group deserves family status is a matter of taxonomic preference. The relationship of these two families to Libellulidae is not strongly supported by our data, although most morphological features suggest a corduliid-libellulid sister group relationship.

4.1c Libellulidae

Three putative libellulid subfamilies (Fraser, 1957; Davies and Tobin, 1985) are supported, with some modification, as monophyletic by all our analyses (Urothemistinae, a modified Libellulinae and a restricted Tetrathemistinae). Urothemistines are recovered as monophyletic near the base of the libellulids (Clade B): this group was considered a family, Macrodiplactidae, by Fraser (1957) and Bechly (1996), but their nested position within the Libellulidae in some analyses suggests caution should be used in elevating the urothemistines to family status (e.g., Davies and Tobin, 1985). The Libellulinae also is apparently monophyletic (Clade H) except for the exclusion of *Dasythemis esmeralda*, which falls into Clade E in all analyses. A monophyletic Libellulinae agrees with the results presented by Fleck et al., (2006), including their placement of Agrionoptera, *Misagria*, and most notably, *Neodythemis* (they did not include *Dasythemis* in their analysis). In addition, the Leucorrhininae (*Leucorrhinia* + *Celithemis*, placed in Clade D) are sister to Sympetrum, which is consistent with Pilgrim (2006) and Fleck et al., (2006). Subfamily status for Leucorrhininae is probably unwarranted because they are a small distinct group within a much larger clade (apophyletic sensu Carle (1995), i.e., its distinctive autapomorphies have resulted in its assignment to an exaggerated taxonomic rank).

Three well-established taxa are clearly polyphyletic. Species usually attributed to Tetrathemistinae, commonly regarded as the most plesiotypic of libellulid subfamilies, are scattered throughout Libellulidae, with *Tetrathemis* and *Calophlebia* in Clade A, *Nannophlebia* in Clade C, and Neodythemis in Clade H. Thus, a very restricted Tetrathemistinae might remain as the most basal Libellulidae (Clade A), but clearly its composition and delimiting characters are very different than previously defined. As noted already by Dijkstra and Vick (2006) and Fleck et al. (2006), the venational traits used heretofore to define Tetrathemistinae are correlated with narrowing of the wing base and thus are probably subject to convergence.

Second, the Trameinae is also polyphyletic, although most species cluster loosely in clade E. This clade also includes some general generally thought to be closely related to trameines but placed by Fraser (1957) and others in the Zyxommatinae (*Tholymis*, and Zyxomma). The placement of Idiataphe in Clade E is ambiguous. Morphological evidence is inconclusive about its position. Although Davies and Tobin (1985) place it in Trameinae, its position is instable in our analyses, quite possibly because only the D3 fragment was sequenced for this species. In addition, its branch is suspiciously, long compared to other nearby taxa. *Rhyothemis*, also previously thought to be closely related to trameines, is placed by PHASE (with low support) in clade D. The most surprising result with respect to the polyphyly of the trameines is that *Pantala* is very distantly related to the other trameines, falling to the base of Clade H in the smaller dataset, and nested within Clade C in the larger dataset. Again, convergent modification of the hindwing base, in this case expansion as an adaptation to extended periods of gliding, could explain the morphological similarity that has caused *Pantala* and *Tramea* to be placed together previously (Figure 4).

Finally, Diastatopidinae is distributed among Clades F (Zenithoptera), G (*Palpopleura*), and H (*Perithemis*, although with considerable uncertainty). Several

17

authors (e.g., Fraser, 1957) have noted that members of these genera have unusually patterned wings and may mimic Hymenoptera. Possibly this convergence on wasp-like color and behavior has resulted in similarities of morphology that misled previous workers. The larvae of these genera differ markedly in lateral and dorsal abdominal spine development, abdomen shape, and epiproct length (Zenithoptera, Costa et al., 2004; *Palpopleura*, Fraser, 1955; *Perithemis*, Needham et al., 2000). *Palpopleura* + *Hemistigma*, placed together in our phylogeny with high support, share several larval characteristics. They both lack dorsal spines, have prominent eyes and share a striking pale dorsal stripe (Whiteley et al., 1999). Adults of these two genera also share markedly bicolored pterostigma. None of these characters is definitive, but the combination tends to support separation of the diastatopidines and the grouping of Palpopleura and Hemistigma.

Most subclades of Libellulidae recovered in our phylogeny comprise a mixture of taxa from various previously recognized subfamilies. Members of Sympetrinae, Trithemistinae, and Brachydiplacinae are scattered throughout Libellulidae and none of these subfamilies were present among our PHASE trees.

4.2 Character Evolution

4.2a Adult characters

As in many taxa, traditional systematic treatment of Libelluloidea has typically emphasized an essentially linear transformation of characters, especially of wing veins, leading from an "archaic" to a "modern" state. As Fraser (1957) expressed it, the superfamily "... exhibits an almost unbroken chain of evolution...". Such a progression would only be expected if all phylogenies were perfectly pectinate and without homoplasy. The situation is almost certain to be less clear-cut in the real world, and such appears to be the case based on our phylogeny.

For example, the elongation of the anal loop and the development of a midrib (Figure 4) can be considered to progress from its "absence" in most non-libelluloids to its extreme (a boot-shaped structure, with a "toe" and a midrib) in Libellulidae, although with notable generic exceptions (Needham, 1903; Tillyard, 1910; Tetrathemis, *Nannothemis*, *Misagria*, and *Fylgia* for example, are libellulids with reduced anal loops). Our reconstruction, however, suggests that either elongation of the anal loop and development of a midrib occurred in parallel, perhaps multiple times, in the GSI and the MCL clades or this condition was replaced by a short broad loop independently in Synthemistinae and Macromiinae. Moreover, the loop probably has been secondarily lost several times independently in Libellulidae and possibly in members of GSI. A character often correlated with the loss of the anal loop is the presence of a "broken" costal side of the triangle, making the triangle, in fact, quadrangular; this, too, seems to have evolved multiple times. Several other characters show a considerable degree of homoplasy between the GSI and MCL clades. The supposedly increasing alignment of the antenodal crossveins (these are crossveins at the leading edge of the wings, anterior to the nodus), for example, and the proximity of the HW triangle to the arculus (a flexion point), both emphasized by Fraser (1957), and to some extent, by Bechly (1996), vary across the GSI

and MCL (although they are most strongly expressed in the Libellulidae and Corduliidae, respectively). It appears possible that all of these changes are partially correlated. They may have the effect of reducing, or at least reconfiguring, chordwise flexibility of the wing base, especially in the hindwing, and perhaps consequently increasing twisting and camber of the distal portion of the wings. Understanding of the details of wing kinematics and aerodynamics during flapping flight, however, is as yet too rudimentary to make confident predictions of these effects (Wooton and Kukalova-Peck, 2000; Combes and Daniel, 2003a, b; 2005).

Reduction in the ovipositor, convergently shared with the Gomphidae (Carle, 1995), is a prominent feature of Libelluloidea (and considered a synapomorphy of Gomphidae + Libelluloidea by Bechly, 1996 and Lohmann, 1996; Figure 5). The ovipositor of Aeshnoidea and Petaluridae (and Zygoptera) comprises three pairs of ventral processes. The first and second pairs (anterior and posterior gonapophyses) are enclosed by the third (gonoplacs). In libelluloids, including Cordulegastridae, the ovipositor is modified for exophytic oviposition (Tillyard, 1917; Carle, 1995). In Cordulegastridae, the third processes (gonoplacs) are vestigial. In the GSI clade, the third processes are absent and at least the second processes are reduced, although in some taxa the first pair is present and nearly as long as in Cordulegastridae. Our results do not allow us to conclude whether the latter GSI condition is plesiomorphic or secondarily (re)developed. In the MCL, the first processes are reduced to small flaps and the other structures are apparently absent except for the probable vestige of the styli emerging directly from the 9th sternite (Tillyard, 1917). In a few instances, the 8th (e.g., some *Somatochlora*) or 8th and 9th sternites (*Uracis*) are secondarily produced to form an ovipositor in MCL species.

Pfau's (2005) detailed morphological study of the vesica spermalis (v. s.; Figure 6), and especially of the distal "sperm pump" implies a very different phylogeny than that found here or by most other workers. Most radically, Pfau suggests that cordulegastrids are members of a larger group that includes petalurids, and gomphids (Fig. 1). He reasons that because it appears impossible to derive the complex sperm pump apparatus of libelluloids directly from the cordulegastrid sperm pump, the multiple similarities of Cordulegastridae and libelluloids are the result of convergence. Moreover, he maintains that Corduliinae s. s. are the most plesiomorphic libelluloids. Despite Pfau's (1971, 1991, 2005) conclusion, our analysis is rooted with Cordulegastridae. Given other, independent support, both morphological (Bechly, 1996; Gorb, 1999; Carle and Kjer, 2002), and molecular (Misof et al, 2001; Carle and Kjer, unpublished data), we feel some confidence that Cordulegastridae + Neopetaliidae + Chlorogomphidae are the closest relatives to other Libelluloidea, and appropriate outgroups.

Even if our tree were to be re-rooted, however, Pfau's hypothesis clearly cannot be reconciled with ours for higher libelluloids. No previous molecular dataset applies broadly to the higher Libelluloidea, but based on our results we must conclude that the v. s. morphology of *Gomphomacromia* + synthemistids is convergent to that of libellulids. It is unclear whether the synthemistid-type or the corduliid-type v. s. is plesiomorphic for higher libelluloids. Although the GSI is strongly supported, there is such low internal support that it is hard to make any conclusions about the evolution of characters within this group. To begin to understand the evolution of the v. s. in libelluloids, a more extensive taxon sampling in the GSI and MCL must be examined to uncover any intraand interfamilial differences that may shed light on v. s. development. Studies of the ontogeny of the sperm pump within libelluloids might help clarify its pattern of evolution. Pfau (1971) described its last larval instar form, but only in *Cordulegaster* and *Libellula*).

Several authors have noted the tendency toward reduction of the anterior hamule (a male secondary genetalic structure; Figure 6) in higher libelluloids. Pfau (1971) showed a loss of hamular muscle 9a in Cordulia; once 9a is lost, a correlated loss of 9b occurs in Libellulidae. Here too, however, homoplastic loss of 9a occurs in Synthemis eustalacta; other synthemistids, as well as gomphomacromiines and macromiids that have been investigated retain 9a, and loss of 9b is a unique synapomorphy of Libellulidae, as far as we know (May, unpublished).

4.2.b Larval characters

The value of larval characters in understanding odonate phylogeny has long been recognized (e.g., Fraser 1957) and often reveals relationships that are unclear from adult morphology (Carle and Louton, 1994; Novelo-Gutierrez, 1995; Fleck et al., 2006). Several larval apomorphies unite the Libelluloidea including the scoop-like prementum (lower mouthparts) with numerous setae, and an asymmetrical proventriculus (foregut) with large tooth-like lobes (Tillyard, 1917 [Libellulidae + Cordulegastridae]; Carle, 1995; Bechly, 1996 [Cavilabiata]). Dentition of the distal border of the labial palps differs among the libelluloids, however, again forming what has been interpreted as a linear transformation from plesiomorphic to apomorphic: from large, irregular teeth that lack setae in Cordulegastridae to more or less straight distal borders and shallow crenulations with setal tufts or single setae in each concavity in Libellulinae, with intermediates in Macromiidae and Corduliinae s. s. (Tillyard, 1917). Theischinger and Watson (1984) noted that synthemistid larvae, plus those of *Gomphomacromia, Archeophya* and *Pseudocordulia* (GSI) have more cordulegastrid-like dentition. Cordulegastrid and synthemistid larvae also share wing sheaths that extend apart from one-another, while in other higher libelluloids the wing sheaths lie parallel (Tillyard, 1910, 1917). Our analyses show no evidence of this divide. Furthermore, the molecular evidence uniting the GSI clade, which has strong support (Fig. 2), argues that these characters in the gomphomacromiines (other than the three genera listed above) are convergent with those of the Corduliidae s. s.

4.3 Conclusions

Based on our phylogeny we suggest that higher Libelluloidea comprises four families: the Gomphomacromiidae (including Gomphomacromiinae, Idionychinae, Cordulephyinae and Synthemistinae of Fraser, 1957, and Davies and Tobin, 1985), the Macromiidae, the Corduliidae, and the Libellulidae. The "Corduliidae" s.l. are polyphyletic. We include in Corduliidae only the taxa defined as Corduliinae in Fraser (1957), and Davies and Tobin (1985), although placement of several other genera not studied here certainly should be examined in the future (e.g., *Idomacromia, Neophya, Heteronias, Libellulosoma, Metaphy*a and *Williamsonia*; the South American *Navicordulia, Santosia, Schizocordulia*, and *Rialla*; and the geographically disjunct *Antipodochlora* of New Zealand).

Libellulidae probably comprises 3 previously accepted subfamilies, (i.e., Urothemistinae, a very restricted Tetrathemistinae, similar to that suggested by Dijkstra and Vick (2006), and, with some modification, Libellulinae) as well as 5 additional groups that are consistently recovered (one of these including an apophyletic Leucorrhiniinae). Closer examination of the morphological basis of these groupings is needed before a definitive taxonomy of the family can be proposed. It was beyond the scope of this study to sequence every genus in Libellulidae, but clearly the placement of nearly all the remaining genera remains uncertain.

Biogeographical studies may help to determine a pattern of origin with the libellulid groups and clarify the relationships among major taxa within GSI and MCL. In our taxon sample several broad geographical patterns suggested. The GSI, for example, are virtually confined to the southern Hemisphere, especially Australia (except for the Indomalayan *Idionyx* and *Macromidia* and the Palaearctic *Oxygastra*). Corduliinae and Macromiinae are predominantly Holarctic and Indomalayan, with substantial radiations and/or expansions into South America by the former and Africa by the latter. Libellulidae, as a whole, is cosmopolitan.

Future morphological work should be cautious about creating families or subfamilies based on characters prone to convergence. While some of the details of our tree are weakly supported and may change with further data and analysis, most of the framework appears sound. On a much broader scale, this reinforces suggestions that previous phylogenetic hypotheses have suffered from: 1) use of poorly defined and sometimes inaccurately scored characters (O'Grady and May, 2003); 2) groups based on symplesiomorphies; and 3) failure to recognize the widespread effects of character correlation and convergence, especially in aspects of venation (Carle, 1982b; Dijkstra and Vick, 2006; Fleck et al., 2006). An increase in the use of larval and genitalic character information is a promising step forward. Future work should use this phylogeny as a tool to focus in on phylogenetically problematic areas within the Libelluloidea. Although Libelluloidea is highly speciose, often collected, and familiar, our understanding of its phylogenetic history is only just beginning.

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References

Artiss, T., Schultz, R.T., Polhemus, D. A., Simon, C. 2001. Molecular Phylogenetic Analysis of the Dragonfly Genera Libellula, Ladona, and Plathemis (Odonata: Libellulidae) Based on Mitochondrial Cytochrome Oxidase I and 16S rRNA Sequence Data. *Molecular Phylogenetics and Evolution*, **18(3)**: 348–361.

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 vol. 2**: 402 pp.

Bridges, C. A., 1994. Catalogue of the family-group, genus-group and species-group names of the Odonata of the world (Third Edition). C. A. Bridges, Urbana, Illinois.

Carle, F. L., 1982 a. A contribution to the knowledge of the Odonata. Unpublished Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 1095 pp.

Carle, F. L., 1982 b. 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., 1995. Evolution, taxonomy, and biogeography of ancient Gondwanian libelluloides, with comments on anisopteroid evolution and phylogenetic systematics (Anisoptera: Libelluloidea). *Odonatologica*, **24(4)**: 383-506.

Carle, F.L., Louton, J.A., 1994. The larva of Neopetalia punctata and establishment of Austropetaliidae fam.nov. (Odonata). *Proceedings of the Entomological Society of Washington*, **96(1)**: 147-155.

Carle, F. L., Kjer, K. M., 2002. Phylogeny of Libellula Linnaeus (Odonata: Insecta). *Zootaxa*, **87**:1-18.

Carpenter, F.M., 1992. Superclass Hexapoda. In R. L. Kaesler (Ed.), Treatise on Invertebrate Paleontology Part R Arthropoda 4. The Geological Society of America, Boulder, Colorado. 143-153pp.

Combes, S.A. and Daniel, T.L., 2003a. Flexural stiffness in insect wings. I. Scaling and

the influence of wing venation. *Journal of Experimental Biology*, **206(17):** 2979-2987.

Combes, S.A. and Daniel, T.L., 2003b. Flexural stiffness in insect wings. II. Spatial distribution and dynamic wing bending. *Journal of Experimental Biology*, **206(17)**: 2989-2997.

Combes, S. and Daniel, T.L., 2005. Flexural stiffness in insect wings: effects of wing venation and stiffness distribution on passive bending. *American Entomotologist*, **51(1)**: 42-45.

Corbet, P. S., 1999. Dragonflies: Behavior and Ecology of Odonata. Cornell University Press, Ithaca, NY. 829pp.

Costa, J. M., Pujol-Luz, J., Regis, P.B., 2004. Descricao de larva de *Zenithoptera anceps* (Odonata, Libellulidae). *Iheringia, Series Zooloical, Prto Alegre*, **94(4)**: 421-424.

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*, *Rapid Communication* (Suppl.) No. 5., Utrecht.151 pp.

Dijkstra K.-D. D., Vick, G. S., 2006. Inflation by venation and the bankruptcy of traditional genera: the case of *Neodythemis* and *Micromacromia*, with keys to the continental African species and the description of two new *Neodythemis* species from the Albertine Rift (Odonata: Libellulidae). *International Journal of Odonatology*, **9**: 51-70.

Ellis, J., Morrison, D., 1995. Effects of sequence alignment on the phylogeny of *Sarcocystis* deduced from 18S rDNA sequences. *Parasitology Research*, **81(8)**:696-699.

Farris, J. S., 1982. Outgroups and parsimony. Systematic Zoolology, 31: 328-334.

Fleck, G., Brenk, M., Misof, B., 2006. 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. *In press*.

Fraser, F.C., 1955. Description of the nymph of *Palpopleura lucia* Drury. *Revue francaise d'Entomologie*, **22**:51-52.

Fraser, F.C., 1957. A reclassification of the order Odonata. Royal Zoological Society of New South Wales: Sydney.133 pp.

Geijskes, D.C., 1970. Generic characters of the south american Corduliidae with descriptions of the species found in the Guyanas. *Notes on Odonata of Surinam 11. Stud. Fauna Suriname*, **12(60)**: 1-42.

Gillespie, J.J., McKenna, C.H., Yoder, M.J., Gutell, R.R., Johnston, J.S., Kathirithamby, J., Cognato, A.I., 2005. Assessing the odd secondary structural properties of nuclear small subunit ribosomal RNA sequences (18S) of the twisted-wing parasites (Insecta: Strepsiptera). *Insect Molecular Biology*, **14**, 625-643.

Gloyd, L. K., 1959. Elevation of the *Macromia* group to family status (Odonata). *Entomological News*, **70**: 197-205.

Gorb, S.N., 1999. Evolution of the dragonfly head-arresting system. *Proceedings of the Royal Society of London B*, **266**: 525-535.

Graham, S. W., Olmstead, R. G., Barrett, S.C.H., 2002. Rooting phylogenetic trees with distant outgroups: A case study from the Commelinoid monocots *Molecular Biology & Evolution*, **19**: 1769-1781.

Grimaldi, D., Engel, M. S., 2005. Evolution of the Insects. Cambridge University Press, New York, NY.

Gu, X., Fu, Y.-X., Li, W.-H., 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology & Evolution*, **12**:546-557.

Guttell, R.R., Gray, M.W., Schinare, M.N., 1993. A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. *Nulcleic Acids Research*, **21**:3055-3074.

Hagen, H., 1861. Synopsis of the Neuroptera of North America, with a list of the South American species. Washington, D. C., Smithsonian Inst.

Hasegawa, E., & Kasuya, E., 2006. Phylogenetic analysis of the insect order Odonata using 28S and 16S rDNA sequences: a comparison between data sets with different evolutionary rates. *Entomological Science*, **9** (1): 55-66.

Hennig, W., 1969. Die Stammesgeschichte der Insekten. Kramer: Frankfurt A.M. 436 pp.

Hickson, R. E., C. Simon, S. W. Perrey. 2000. The performance of several multiplesequence alignment programs in relation to secondary-structure features for an rRNA sequence. Mol. Biol. Evol. 17:530-539

Higgs, P.G., 2000. RNA secondary structure: Physical and computational aspects. Q Rev Biophys **30**: 199-253.

Hovmoller, R., Pape, T., Kallersjo, M. 2002. The Palaeoptera problem: Basal pterygote phylogeny inferred from 18S and 28S rDNA sequences. *Cladistics*, **18**: 313-323.

Huelsenbeck, J. P., Ronquist, F., 2002. MrBayes 3: Bayesian analysis of phylogeny. University of California.

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** (1):67-85.

Jow, H., Hudelot, C., Rattray, M., Higgs, P., 2002. Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution. *Molecular Biology & Evolution*, **19(9)**:1591-1601.

Kambhampati, S., Charlton, R.E., 1999. Phylogenetic relationship among Libellula, Ladona and Plathemis (Odonata:Libellulidae) based on DNA sequence of mitochondrial 16S rRNA gene. *Systematic Entomology*, **24**: 37-49.

Kirby, W. F., 1890. A synonymic catalogue of Neuroptera Odonata. Guerney & Jackson, London, 202 pp.

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(3)** 314-330.

Kjer, K. M., 2004. Aligned 18S and insect phylogeny. Systematic Biology, 53: 506-514.

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

Lieftinck, M.A., 1960. Notes on the affinity and nomenclature of some Old World Corduliidae (Odonata). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C Zoology*, **64**: 410-23.

Lieftinck, M.A., 1971. Studies in Oriental Corduliidae (Odonata). 1. *Tijdschrift voor Entomologie*, **114(1)**:1-63.

Lieftinck, M.A., 1977, New and little known Corduliidae (Odonata: Anisoptera) from the Indo-Pacific region. *Oriental Insects*, **11(2)**:157-159.

Lohmann, H.,1996. Das phylogenetische system der Anisoptera (Insecta: Odonata). unpublished diploma thesis at the Biological Faculty of the Albert-Ludwigs-University in Freiburg i.Br. 117 + 13 pp.

Lohmann, H., 1996. Das phylogenetische system der Anisoptera (Odonata). *Entomologische Zeitschrift*, **106(6)**: 209-252 (first part); **106(7)**: 253-266 (second part); **106(9)**: 360-367 (postscript).

Lutzoni, F., Wagner, P., Reeb, V., Zoller. S., 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional

homology. Systematic Biology, 49: 628-651

Lyons-Weiler J., Hoelzer, G. A., Tausch, R. J., 1998. Optimal outgroup analysis *Biological Journal of the Linnean Society*, **64**:493-51.

Martin, R., 1907. Cordulines. *Collections Zoologiques du Baron Edm. de Selys Longchamps. Institut royal des Sciences Naturelles de Belgique: Brussels.* **17**:1-94.

Martin, R., 1909. Cordulines. Addenda. Collections zoologiques du Baron Edm. De Selys Longchamps, Catalogue systematique et descriptif. *Collections Zooogiques du Baron Edm. de Selys Longchamps* **17**:95-98.

Martin, R., 1914. Odonata, Fam. Libellulidae, subfam. Corduliinae. *Gen. Insectorium*, **155**:31pp.

May, M.L., 1995. Comparative notes on micropyle structure in "cordulegastroid" and "libelluloid" Anisoptera. *Odonatologica*, **19(1)**: 53-62.

Minin, V., Abdo, Z., Joyce, P., Sullivan, J., 2003. Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology*, **52**:674-683.
Misof, B., Fleck, G., 2003. Comparative analysis of mt LSU rRNA secondary structures of Odonates: structural variability and phylogenetic signal. *Insect Molecular Biology*. **12(6)**: 535-547.

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 & Evolution*, **18**(1):27-37.

D.A. Morrison, J.T. Ellis, 1997. Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNAs of apicomplexa. *Molecular Biology and Evolution*, **14**: 428-441.

N. B. Mugridge, D. A. Morrison, T. Jäkel, A. R. Heckeroth, A. M. Tenter, A. M. Johnson. 2000. Effects of Sequence Alignment and Structural Domains of Ribosomal DNA on Phylogeny Reconstruction for the Protozoan Family Sarcocystidae. *Molecular Biology and Evolution* **17**: 1842-1853

Nel, A., Paicheler, J.C., 1994. Les libellulidae fossiles. Un inventaire critique (Odon., Anisoptera, Libelluloidae). *Entomologica Gallica*, **4(4)**:166-190.

Needham, J.G., 1903. A genealogic study of dragonfly wing venation. *Proceedings of the U.S. National Museum*, **26(1331)**:703-764.

Needham, J. G., 1908. Critical notes on the classification of the Corduliinae (Odonata). *Annals of the Entomological Society of America*, **1**: 273-280.

Needham, J.G., Broughton, E., 1927. The venation of the Libellulinae (Odonata). *Transactions of the American Entomological Society (Philadelphia)*, **53**:157-190.

Needham, J.G., Westfall, M.J., May, M.L., 2000. Dragonflies of North America. Scientific publishers, Inc. 939 pp.

Novelo-Gutierrez, R., 1995. The larva of *Amphipteryx* and a reclassification of Amphipterygidae sensu lato based upon the larvae (Zygoptera). *Odonatologica*, **24**: 73-87.

O'Grady, E. W., May, M. L., 2003. A phylogenetic reassessment of the subfamilies of Coenagrionidae (Odonata: Zygoptera). *Journal of Natural History*, **37**:2807-2835.

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

Pfau, H.K., 1991. Contributions of functional morphology to the phylogenetic systematics of Odonata. *Advances in Odonatolology*, **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.

Pilgrim, E. Systematics of the sympetrien dragonflies with emphasis on the phylogeny, taxonomy, and historical biogeography of the genus *Sympetrum* (Odonata: Libellulidae). Utah State University, 154 pp.

Posada, D., Crandall, K. A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, **124**:817–818.

Posada, D., Buckley, T., 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology*, **53(5)**: 793-808.

Rambur, M.P., 1842. Neuropteres. Histoire naturelle des Insectes, Paris, pp.534.

Rehn, A. C., 2003. Phylogenetic analysis of higher-level relationships of Odonata. *Systematic Entomology*, **28(2)**: 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(6)**: 1150-1166.

Ris, F., 1909-1919. Collections Zoologiques du Baron Edm. de Selys Longchamps, Cat. System. et Descript., Fasc. 9-16. Bruxelles, Hayez, Impr. des Académies.

Sarot, E. E., 1958. Folklore of the Dragonfly. A Linguistic Approach. Rome, Storia e Letteratura. 80 pp.

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(6)**:693-699.

Schnare, M.N., Damberger, S.H., Gray, M.W., Gutell, R.R., 1996. Comprehensive comparison of structural characteristics in eukaryotic cytoplasmic large subunit (23S-like) ribosomal RNA. *Journal of Molecular Biology* **256**: 701-719.

Schorr, M., Lindeboom, M, Paulson, D., 2006. World Odonata List. http://www.ups.edu/x6140.xml, Last visited October 19, 2006.

Silsby, J., 2001. Dragonflies of the World. Washington, D.C.: Smithsonian Institution Press.

St. Quentin, D., 1939. Die systematische stellung der unterfamilie der Corduliinae Selys (Ordung Odonata). *Proceedings Of the International Congress of Entomology 7 (Berlin)*, 1:345-360.

Steinmann, H. 1997. World catalogue of Odonata, Anisoptera. Berlin, W. Gruyter. v. 2, 636p.

Theischinger, G., Watson, J. A. L., 1978. The Australian Gomphomacromiinae (Odonata : Corduliidae). *Australian Journal of Zoology*, **26**:399-431.

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., 1910. Monograph of the genus *Synthemis*. (Neuroptera: Odonata). *Proceedings of the Linnean Society of New South Wales*, **35**: 312-377.

Tillyard, R. J., 1917. The biology of Dragonflies. Cambridge. Cambridge Univ. Press.

Tillyard, R.J., 1928. The evolution of the order Odonata. Part I. Introduction and early history of the order. *Records of the Indian Museum*, **30**:151-172. Titus, T. A., D. R. Frost. 1996. Molecular homology assessment and phylogeny in the lizard family Opluridae (Squamata: Iguania). *Molecular Phylogenetics and Evolution* **6**:49-62.

Trueman, J. W. H., 1996. A preliminary cladistic analysis of odonate wing venation. *Odonatologica*, **25**: 59-72.

Weins, J. J., 2005. Missing data and the design of phylogenetic analyses. *Journal of Biomedical Informatics*, **39**: 34-42.

Whiteley, G., Samways, M.J., DiDomenico, M., Carchini, G., 1999. Description of the last instar of *Hemistigma albipuncta* (Rambur, 1842) and comparisions with other Brachydiplacinae (Anisoptera: Libellulidae). *Odonatologica* **28**: 433-437.

Wootton, R. J., Kukalova – Peck, J., 2000. Flight adaptations in Palaeozoic Palaeoptera (Insecta). *Biological Reviews*, **75**:129-167.

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution*, **39**:105-111.

Yang, Z., Goldman, N., Friday, A.E., 1994. Comparison of models for nucleotide substitution used in maximum likelihood estimation. *Molecular Biology & Evolution*, **11**:316-324.

Zwickl, D. J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, University of Texas at Austin.

Figure 1: Hypotheses, also listed in Table 1, of relationships within Libelluloidea. Topologies are (a) One higher Libelluloid family (Libellulidae) comprises two subfamilies: Corduliinae and Libellulinae. *Some who use this taxonomy include a third subfamily, the Macromiinae; (b) Two higher Libelluloid families (Corduliidae and Libellulidae). The Corduliidae comprises two subfamilies: Corduliinae, Macromiinae, *This is loosely based on Kirby, 1890, but he used the term 'Corduliinae'; (c) Three higher Libelluloid families (Macromiidae, Synthemistidae and Libellulidae). The Libellulidae comprises Corduliinae and Libellulinae; (d) Four higher Libelluloid families (Synthemistidae, Corduliidae, Macromiidae, Libellulidae). *This is loosely based on Fraser (1957) but excludes Macrodiplactidae; (e) Four higher Libelluloid families Synthemistidae, Corduliidae, Libellulidae, Macrodiplactidae). Fraser used the name "Synthemidae" for Synthemistidae; (f) Five higher Libelluloid families (Synthemistidae, Gomphomacromiidae, Corduliidae, Macromiidae and Libellulidae). *This is loosely based on Carle and Loutton, 1994; (g) Twelve higher Libelluloid families (Synthemistidae, Gomphomacromiidae, Idomacromiidae, Austrocorduliidae, Oxygastridae, Idionychidae, Cordulephyidae, Hemicorduliidae, Macromiidae, Corduliidae, Urothemistidae, Libellulidae). This scheme is loosely based on Bechly(1996) and Lohmann (1996 a,b) although the nomenclature differed slightly; (h) Three higher Libelluloid families (Synthemistidae, Corduliidae, and Libellulidae). Petaluroidea includes Cordulegastridae. Placement of Macromiidae not discussed. * In 2005, Pfau also includes the families Cordulephyidae and Gomphomacromiidae, placed basal to Synthemistidae + Libellulidae



Figure 2: Phylogenetic reconstruction from a 10 million generation PHASE mcmc analysis. The numbers above the branch indicate posterior probabilities. The "*" indicates GARLI support greater than 50%.



Figure 3: Phylogenetic reconstruction from ten thousand replicate parsimony heuristic search. The "*" indicates that this clade differs in composition in the PHASE analysis. Bootstrap support is written above the branches.



Figure 4color: Forewing and hindwing fragments of several libelluloids; Yellow=Anal Loop, Blue=Supra-triangle, Red=Triangle. Wings are not to scale. The "*" indicates figure modified from Bridges (1994).



Figure 4blackandwhite: Forewing and hindwing fragments of several libelluloids; Light Gray=Anal Loop, Black=Supra-triangle, Dark Gray=Triangle. Wings are not to scale. The "*" indicates figure modified from Bridges (1994).



Figure 5: Terminal abdominal segments in lateral view of (A) female *Zoraena diastatops* (Cordulegastridae) and (B) *Sympetrum costiferum* (Libellulidae). Arrows indicate the well-developed first gonapophyses typical of Cordulegastridae and some Synthemistinae and Gomphomacromiinae (A) and the nearly obsolete ovipositor, often reduced to a short extension of the sternum of S8 and/or a pair of small scales at the base of S9 in Libellulidae and most Cordulidae (B). Figures from Needham, Westfall and



May, 2000.

Figure 6: Lateral view of male secondary genitalia of (A) *Zoraena diastatops* (Cordulegastridae); (B) *Didymops transversa* (Corduliidae, Macromiinae); (C) *Libellula quadrimaculata* (Libellulidae, Libellulinae). In each case the ventral side is upward and the anterior direction to the right, and the abdominal terga are removed to reveal the genitalic structures. Accessory secondary genitalia are the anterior lamina (AL), anterior hamules (AH; absent in *L. quadrimaculata* and other Libellulidae), anterior frame (AF), posterior hamule (PH), posterior frame (PF), and genital ligula (GL); these structures engage the female ovipositor during copulation and assist movement of the vesica spermalis. Segments of the vesica spermalis, which functions both for temporary sperm storage before copulation and for intromission, are indicated by Roman numerals I, II, III, and IV from base to apex; these medial structures are most easily visible in A. Terminology follows Pfau (1971).



Figure 7: Phylogenetic reconstruction from a ten thousand replicate parsimony heuristic search. The "*" indicates a clade that differs in composition from the smaller PHASE dataset. The " \star " indicates that the taxon sequence was downloaded from GenBank. Posterior probabilities are written above the branch except on very short branches where it is written below.



Table 1: A summary of previous work in libelluloid systematics, with reference to the MCL hypotheses are listed in Figure 1.

Author	Yea	Taxa Studied	Extant Libelluloid Families	MCL	Dataset	Analysis
	ı		Studied	Hypothe sis Support ed		
Carle & Kjer	2002	9 Non-Libelluloid Anisopteran families; 4 Libelluloid families	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Computer-assisted parsimony
Carle	1982a	Odonata	Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Manual parsimony
St Quentin	1939	3 Libelluloid families	Cordulegastridae, Corduliidae, Libellulidae	A	Morphology: genetalia	Intuition
Martin	1914	1 Libelluloid family	Corduliidae	A	Morphology	Intuition
Tillyard	1917	Odonata	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Intuition
Needham	1908	2 Libelluloid families	Corduliidae, Libellulidae	A	Morphology	Intuition
Martin	1907	1 Libelluloid family	Corduliidae	Α	Morphology	Intuition
Needham	1903	Anisoptera	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Intuition
Selys	1892	Anisoptera	Cordulegastridae, Corduliidae	A	Morphology	Intuition
Kirby	1890	Odonata	Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Intuition
Hagen	1861	Odonata	Corduliidae, Libellulidae,	A	Morphology	Intuition
Rambur	1842	Odonata	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	A	Morphology	Intuition
Misof et	2001	4 non-Libelluloid	Neopetaliidae, Cordulegastridae,	В	Molecular: 16s	Computer-assisted
al.		Anisopteran families; 4 Libelluloid families	Corduliidae, Libellulidae		and 12s	parsimony and likelihood
Bridges	1994	Odonata	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	В		
Davies & Tobin	1985	Anisoptera	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	В	Morphology	Manual parsimony
Steinmann	1997	Odonata	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	В		
Lieftinck	1977	1 Libelluloid families	Corduliidae	В	Morphology	Manual parsimony
Lieftinck	1971	1 Libelluloid families	Corduliidae	В	Morphology	Intuition
Tillyard	1928	2 Libelluloid families	Corduliidae, Libellulidae	В	Morphology	Intuition

42

Intuition	Computer-assisted parsimony	Manual parsimony	Manual parsimony	Intuition	Manual parsimony	Manual parsimony	Manual parsimony	Manual parsimony	Manual parsimony	Manual parsimony	Manual parsimony
Morphology	Morphology	Morphology	Morphology	Morphology	Morphology	Morphology	Morphology	Morphology	Morphology: genitalia	Morphology: secondary genitalia	Morphology: secondary genitalia
С	D	D * No Libellulidae studied	Е	ш	Н	F* No Corduliidae studied	J	C	Н	Н	Н
Corduliidae	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	Cordulegastridae, Corduliidae,	Corduliidae	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	Neopetaliidae, Cordulegastridae, Libellulidae	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	Neopetaliidae, Cordulegastridae, Corduliidae, Libellulidae	Neopetaliidae, Cordulegastridae, Cordullidae, Libellulidae	Cordulegastridae, Cordullidae, Libellulidae	Cordulegastridae, Cordullidae, Libellulidae
1 Libelluloid family	Libelluloid fossils 4 Libelluloid families	2 Libelluloid families	1 Libelluloid families	Zygoptera + Anisozygoptera 3 Non-Libelluloid families 2 Libelluloid families	Libelluloid fossil: Nothomacromia 3 Libelluloid Anisoptera	4 Non-Libelluloid Anisoptera families 4 Libelluloid families	4 Non-LibelluloidAnisopteran families,4 Libelluloid families	Zygoptera, 4 Non- Libelluloid Anisopteran families, 4 Libelluloid families	3 Non-libelluloidfamilies4 libelluloid families	Zygoptera + Anisozygoptera 2 Non-libelluloid families 3 libelluloid families	Zygoptera + Anisozygoptera 2 Non-libelluloid families 3 libelluloid families
1959	1996	1994	1978	1957	1995	1994	1996	1996	2005	1991	1971
Glovd	Jarzembow ski & Nel	Nel & Paicheler	Theisching er & Watson	Fraser	Carle	Carle & Louton	Lohmann	Bechly	Pfau	Pfau	Pfau

Taxon	Locality	Genbank
Cordulogostridoo		Number
Taeniogaster obliqua	USA: NJ (M. L. May)	D7: EF631216 D3: EF631312 D2: EF631420
		16S: EF631533
Pangaeagaster maculata	USA: NJ (M. L. May)	N/A
Kalyptogaster erronea	USA: NJ (M. L. May)	D7: EF631245 D3: N/A D2: EF631450 16S: EF631561
Zoraena bilineata	USA: MD (M. L. May)	N/A
Chlorogomphidae		
Chloropetalia soarer	Sequences from F.L. Carle	D7: EF631248 D3: EF631339 D2: EF631453 16S: N/A
Sinorogomphus sp	Sequences from F.L. Carle	D7: EF631249 D3: EF631340 D2: EF631454 16S: EF631564
Neopetaliidae		
Neopetalia punctata	Sequences from F.L. Carle	D7: EF631247 D3: EF631338 D2: EF631452 16S: EF631563
	Corduliidae: Cordulephyinae	
Cordulephya pygmea	Australia (Theischinger)	D7: EF631255 D3: EF631346 D2: EF631460 16S: EF631570
Acashu agama fanainula	Corduliidae: Corduliinae	D7: EE(21222 D2:
Aesennosoma jorcipula	French Gulana (J. Hull)	EF631319 D2: EF631427 16S: EF631540
Cordulia shurtleffii	Canada: Ontario (J. L. Ware, J.Huff)	D7: EF631232 D3: EF631326 D2: EF631435 16S: N/A
Cordulia aenea	Sweden (K.M. Kjer)	D7: EF631286 D3: EF631383 D2: EF631500 16S: EF631603
Dorocordulia lepida	USA: NJ (M. L. May)	N/A
Epitheca princeps	USA: NJ (J. L. Ware)	D7: EF631205 D3: EF631302 D2: EF631407 16S: EF631521
Helocordulia uhleri	USA: NJ (M. L. May)	D7: EF631227 D3: N/A D2: EF631431 16S: EF631544
Hemicordulia tau	Australia (J. L. Ware, K. M. Kjer, F. L. Carle)	D7: EF631233 D3: EF631328 D2: EF631437 16S: EF631550
Neurocordulia obsoleta	USA: FL (M. L. May and K. Tennessen)	D7: EF631196 D3: N/A D2: EF631395 16S: EF631509
Metaphya elongata	New Caledonia (Tobin & Davies)	N/A
Pentathemis membranulata	Australia (F.L. Carle)	D7: EF631211 D3·

Table 2: Taxon list for the present study. Taxonomy based on Bridges (1994) and Davies and Tobin (1985).

		EF631308 D2:
		EF631415 16S:
		EF631528
Neurocordulia xanthosoma	USA: AK (M. L. May)	D7: EF631242
		D3: N/A
		D2: EF631447
Duo condulia gugui	New Zeeland (D. Dewe)	165: N/A
Procoraulia grayi	New Zealand (R. Rowe)	D7: EF031199
		D2: FF631399
		16S: EF631513
Procordulia smithi	New Zealand (R. Rowe)	D7: EF631200 D3:
		EF631295 D2:
		EF631400 16S:
		EF631514
Rialla villosa	Chile (Heppner)	D7: EF631273 D3:
		EF631364 D2:
		EF631480 16S:
<u>C 11 1</u>		EF631590
somaiocniora lenebrosa	USA. NJ (M. L. May)	D7: EF031215 D3: EF631311 D2:
		EF631419 16S
		EF631532
Tetragoneuria cvnosura	USA: NJ (M. L. May)	D7: N/A
		D3: EF631379
		D2: N/A
		16S: N/A
Tetragoneuria cynosura	USA: NJ (M. L. May)	D7: EF631231 D3:
		EF631325
		D2: N/A
		16S: N/A
Williamsonia fletcheri	USA: MA (M. L. May)	N/A
Anocordulia macrons	Australia (G. Theischinger)	N/A
Archaeophya magnifica	Australia (G. Inelschinger)	N/A
Archaeophya magnifica	Ausualia (K. M. Kjel)	D7. N/A D2: EE621256 D2:
		1113 EEUSISUU11/
		EF631470 168 [.]
		EF631470 16S: EF631580
Austrocordulia refracta	Australia (G. Theischinger)	EF631470 16S: EF631580 D7: EF631243 D3:
Austrocordulia refracta	Australia (G. Theischinger)	D3: EF631330 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2:
Austrocordulia refracta	Australia (G. Theischinger)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631448 16S:
Austrocordulia refracta	Australia (G. Theischinger)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631448 16S: EF631559
Austrocordulia refracta Austrophya mystica	Australia (G. Theischinger) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3:
Austrocordulia refracta Austrophya mystica	Australia (G. Theischinger) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631332 D2:
Austrocordulia refracta Austrophya mystica	Australia (G. Theischinger) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631348 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A
Austrocordulia refracta Austrophya mystica Gomphomacromia chilancis & paradora	Australia (G. Theischinger) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631408 16S:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631337 D2:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631449 16S:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631449 16S: EF631560
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631449 16S: EF631560 D7: EF631239 D3:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF6313449 16S: EF631560 D7: EF631239 D3: EF631334 D2:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631337 D2: EF631560 D7: D7: EF631239 D3: EF631334 D2: EF631344 16S: EF631444 16S: EF631444 16S:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle)	D3: EF631336 D2: EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631449 16S: EF631560 D7: D7: EF631239 D3: EF631334 D2: EF631344 16S: EF631556 D7:
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. May and F. L. Carle)	D3. EF631330 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631560 D7: EF631239 D3: EF631334 D2: EF631334 D2: EF631556 D7: EF631240 D3: N/A
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631560 D7: EF631239 D3: EF631334 D2: EF631334 D2: EF631556 D7: EF631240 D3: N/A D2: EF631445
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. May and F. L. Carle)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF63137 D2: EF63137 D2: EF631520 D7: EF631239 D3: EF631560 D7: EF631239 D3: EF631556 D7: EF631240 D3: N/A D2: EF631445 16S: EF631557
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons Neocordulia batesi longipollex	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. Carle) Panama (M. L. May)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631520 D7: EF631239 D3: EF631334 D2: EF631334 D2: EF631556 D7: EF631240 D3: N/A D2: EF631445 16S: EF631557 N/A
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons Neocordulia batesi longipollex Neocordulia campana	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. Carle) Panama (M. L. May) Panama (M. L. May)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631522 D7: EF631244 D3: EF631560 D7: EF631239 D3: EF631334 D2: EF631334 D2: EF631556 D7: EF631240 D3: N/A D2: EF631445 16S: EF631557 N/A N/A
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons Neocordulia batesi longipollex Neocordulia campana Oxygastra curtisii	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. Carle) Panama (M. L. May) Panama (M. L. May) Spain (P. Corbet)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF631337 D2: EF631560 D7: EF631239 D3: EF631334 D2: EF631334 D2: EF631556 D7: EF631240 D3: N/A D2: EF631445 16S: EF631557 N/A N/A D7: N/A
Austrocordulia refracta Austrophya mystica Gomphomacromia chilensis & paradoxa Hespercordulia berthoudi Lathrocordulia metallica Micromidia atrifrons Neocordulia batesi longipollex Neocordulia campana Oxygastra curtisii	Australia (G. Theischinger) Australia (F. L. Carle) Chile (F. L. Carle) Australia (F. L. Carle) Australia (F. L. Carle) Australia (M. L. Carle) Panama (M. L. May) Panama (M. L. May) Spain (P. Corbet)	D3. EF631336 D2. EF631470 16S: EF631580 D7: EF631243 D3: EF631336 D2: EF631336 D2: EF631336 D2: EF631448 16S: EF631559 D7: EF631236 D3: EF631332 D2: EF631332 D2: EF631441 16S: N/A D7: EF631206 D3: EF631303 D2: EF631408 16S: EF631522 D7: EF631244 D3: EF63137 D2: EF631522 D7: EF631239 D3: EF631560 D7: EF631239 D3: EF631556 D7: EF631240 D3: N/A D2: EF631445 16S: EF631557 N/A N/A D7: N/A D3: N/A

		16S: EF631526
Pseudocordulia circularis	Australia (G. Theischinger)	D7: EF631251 D3:
		EF631342 D2:
		EF631456 16S:
		EF631566
Syncordulia gracilis	South African (P. Grant)	D7: N/A
		D3: N/A
		D2: EF031439 16S: N/A
Cordu	liidae: Idionychinae	105. N/A
Idionvx selvsi	Hong Kong (K. Wilson)	D7: EF631193 D3:
		EF631290 D2:
		EF631391
		16S: N/A
Macromidia rapida 1	Hong Kong (K. Wilson)	D7: EF631209 D3:
		EF631306 D2:
		EF631411
		16S: N/A
Macromidia rapida 2	Hong Kong (K. Wilson)	D7: EF631271 D3:
		EF031302 D2: EF631478 16S:
		EF631588
Corduli	idae: Idomacromiinae	LI 051500
Idomacromia proavita	Cameroon (CAS)	N/A
Cordu	liidae: Macromiinae	
Didymops transversa	USA: NJ (M. L. May)	D7: N/A
		D3: EF631327 D2:
		EF631436 16S:
		EF631549
Macromia illionoiensis	USA: IL (M. L. May)	D7: EF631208 D3:
		EF631305 D2:
		EF631410 16S: EF631524
Phyllomacromia contumax	Uganda (T. W. Donnelly)	D7: EF631197 D3:
1 hytomacionia contanax	Ogundu (1. W. Donneny)	EF631293 D2:
		EF631397 16S:
		EF631511
Cordul	iidae: Synthemistinae	
Choristhemis flavoterminata	Australia (M. L. May, K. M. Kjer and F.	D7: EF631237 D3:
	L. Carle)	EF631333 D2:
		EF631442 16S:
Fusivethamic provistila	Australia (I.I. Ware K. M. Kier F. I.	EF031334
Eusyninemis Drevisiyiu	Carle)	D7. EF031230 D3. EE631323 D2.
		EF631434 16S
		EF631547
Synthemiopsis gomphomacromioides	Australia (M. L. May, K. M. Kier and F.	D7: EF631213
	L. Carle); D2 and D7 sequences from F.	D3: N/A
	L. Carle	D2: EF631417
		16S: EF631530
Synthemis eustalacta	Australia (J. L. Ware, K. M. Kjer, F. L.	D7: N/A
	Carle)	D3: EF631296 D2:
		EF031401 108: EF631515
Synthemis leachii	Australia (D. Pryce)	D7· FF631201 D3·
Synthemis teachti		EF631297 D2
		EF631402 168:
		EF631516
Cord	uliidae: Neophyinae	
Neophya rutherfordi	Liberia (J. Lempert)	N/A
Libelluli	dae: Tetrathemistinae	
Calophlebia interposita	CAS (Madagascar project)	D7: N/A
		D3: EF631381
		D2: IN/A 16S: N/A
Nannophlehia risi	Australia (M. I. May K. M. Viar and E.	D7: FF631254 D2:
	Ausualia (IVI. L. IVIAY, K. IVI. Kjel alid F.	D7. EF031234 D3.

	L. Carle)	EF631345 D2:
		EF631459 16S: EF631569
Neodythemis pauliani	CAS (Madagascar Project)	D7: EF631279 D3:
		EF631368 D2:
		EF631486
Tatrathomic polloni 1	South Africa (M. I. May)	105. IN/A D7: EE631222 D3:
Terrainemis potieni 1	South Africa (M. L. May)	EF631318 D2.
		EF631426 16S:
		EF631600
Tetrathemis polleni 2	South Africa (M. L. May)	D7: N/A
		D3: EF631376 D2:
		EF631539
Libellul	idae: Brachydiplacinae	
Anatya guttata	Trinidad (S. Dunkle)	N/A
Brachydiplax denticauda	Australia (K. M. Kjer and F. L. Carle)	D7: EF631246 D3:
		D2: EF631451
		16S: EF631562
Brachydiplax c. chalybea	Thailand (J. Michalski)	N/A
Chalcostephia flavifrons	Guinea-Bissau (J. Huff)	D7: EF631260 D3:
		EF631351 D2:
		EF631405 105: EF631575
Elga leptostyla	Trinidad (M. L. May)	D7: EF631274 D3:
		N/A
		D2: EF631481
Mierathyvia acqualis	Donomo (M. L. Moy)	168: N/A D7: EE621105 D2:
	ralialia (M. L. May)	EF631291 D2:
		EF631393 16S:
		EF631508
Micrathyria aequalis	Belize (J. L. Ware, J. Huff)	D7: EF631250 D3:
		EF631455 168 [.]
		EF631565
Hemistigma albipuncta	Senegal (J. Huff)	D7: EF631256 D3:
		EF631347 D2:
		EF631461 16S:
Nannophya dalei	Australia (J. L. Ware, K. M. Kier and F.	D7: EF631241 D3:
	L. Carle)	EF631335 D2:
		EF631446 16S:
NT .1 · 1 11		EF631558
Nannothemis bella	USA: FL (M. L. May)	D/: EF631210 D3: EF631307 D2:
		EF631412 16S:
		EF631525
Nephepeltia phyryne	Trinidad (M. L. May)	N/A
Thermochoria equivocata	Cameroon (Mbida Mbida)	N/A
Oligoclada walkeri	Trinidad (M. L. May)	N/A
Oracis imbula	Panama (M. L. May)	D/: EF031228 D3: N/A
		D2: EF631432
		16S: EF631545
Libellu	lidae: Leucorrhiniinae	
Brachymesia herbida	Venezuela (K. West)	N/A D7: EE621224 D2:
Cemmemus ensu	OSA. INJ (IVI. L. IVIAY)	EF631320 D2.
		EF631428 16S:
		EF631541
Leucorrhinia glacialis	USA: NY (M. L. May)	D7: EF631207 D3:
		EF031304 D2: FF631409 168
	<u>I</u>	LI 031707 100.

		EF631523
Libel	lulidae: Libellulinae	
Agrionoptera longitudinalis	Australia (M. L. May, K. M. Kjer and F. L. Carle)	D7: EF631235 D3: EF631331 D2: EF631440 16S: EF631552
Cannaphila viber	Panama (M. L. May)	N/A
Dasythemis esmeralda	Trinidad (J. Michalski)	D7: N/A
,		D3: N/A D2: EF631386 16S: N/A
Hadrothemis defecta	Uganda (T. W. Donnelly)	D7: EF631277 D3: EF631366 D2: EF631484 16S: EF631592
Libellula pulchella	USA: NJ (J. L. Ware)	D7: N/A D3: EF631329 D2: N/A 168: EF631551
Libellula luctuosa	USA: NJ (J. L. Ware)	D7: EF631194 D3: N/A D2: EF631392 16S: EF631507
Libellula quadrimaculata 1	USA: NJ (M. L. May)	D7: EF631272 D3: EF631363 D2: EF631479 16S: N/A
Libellula quadrimaculata 2	Sweden (K. M. Kjer)	D7: N/A D3: N/A D2: EF631497 16S: EF631589
Ladona julia	USA: WI (M. L. May)	D7: EF631219 D3: EF631315 D2: EF631423 16S: EF631536
Lyriothemis pachygastra	Japan (M. L. May)	D7: EF631276 D3: EF631365 D2: EF631483 16S: N/A
Misagria parana	French Guiana (J. Huff)	D7: EF631268 D3: EF631359 D2: EF631475 16S: EF631585
Orthemis ferruginea 1	Dominican Republic (J. Huff)	D7: EF631265 D3: EF631357 D2: EF631471 16S: EF631581
Orthemis ferruginea 2	Dominican Republic (J. Huff)	D7: EF631266 D3: N/A D2: EF631472 16S: EF631582
Orthetrum sp 1	Guinea-Bissau (J. Huff)	D7: N/A D3: N/A D2: EF631396 16S: EF631510
Orthetrum sp 2	South Africa (K. M. Kjer)	D7: EF631261 D3: EF631352 D2: EF631466 16S: EF631576
Orthetrum sp 3	South Africa (K. M. Kjer)	N/A
Orthetrum sp 4	Senegal (J. Huff)	N/A
	CAS (mauagascar)	D7. EF031275 D3: N/A D2: EF631482 168: EF631591
Orthetrum chrysis	China (X. Zhou)	D7: EF631263 D3: EF631354 D2:

		EF631468 16S:
		EF631578
Orthetrum julia 1	South Africa (K. M. Kjer)	D7: EF631285 D3: EF631380 D2:
		EF631498 16S:
		EF631601
Orthetrum julia 2	South Africa (K. M. Kjer)	D7: N/A
		D3: EF631382 D2: FF631499 168
		EF631602
Orthetrum pruinosum neglectum	CAS (Madagascar Project)	D7: EF631267 D3:
		N/A
		D2: EF6314/3 168: EF631583
Plathemis lydia	USA: NJ (F. L. Carle)	D7: EF631234 D3:
		EF631330 D2:
		EF631438 16S:
Libel	lulidae: Sympetrinae	ЕГ031332
Acisoma panorpoides	Guinea-Bissau (J. Huff)	D7: EF631229 D3:
		EF631322 D2:
		EF631433 16S:
Bradinopyga strachani	Guinea-Bissau (J. Huff)	D7: EF631257 D3
		EF631348 D2:
		EF631462 16S:
Pugebuthomia laucoatista	Cuince Discou (L. Huff)	EF631572
Brachylnemis leucoslicia	Guinea-Dissau (J. Hull)	EF631349 D2:
		EF631463 16S:
		EF631573
Crocothemis erythraea	South Africa (M. L. May)	D7: EF631225 D3:
		EF631429 16S:
		EF631542
Crocothemis servilla	USA: FL (M. L. May)	D7: EF631192 D3:
		EF631289 D2: EF631390 168
		EF631506
Crocothemis sp	Senegal (J. Huff)	N/A
Deielia phaon	Japan (M. L. May)	D7: EF631226 D3:
		EF631430 168
		N/A
Diplacodes haematodes	Australia (J. L. Ware, K. M. Kjer, F. L.	D7: EF631238 D3:
	Carle)	N/A D2: EE621442
		168: EF631555
Erythemis simplicicollis	USA: TX (M. L. May)	D7: EF631191 D3:
		EF631288 D2:
		EF631389 16S: EF631505
Erythrodiplax minuscula	USA: FL (J. L. Ware and J. Huff)	D7: EF631190 D3:
· · · · · · · · · · · · · · · · · · ·		EF631287 D2:
		EF631388 16S:
Pachydinlar longinennis	USA: NI (E. I. Carle)	EF031304 D7: FF631108 D3:
τ ασηγαιριαλ ισηχιρεπικο		EF631294 D2:
		EF631398 16S:
Dhodomunia hollan li	Trinidad (M. L. Mart)	EF631512
Knodopygia nollandi Sympetrum janeae	USA: NI (F. L. Carle)	N/A D7: EF631214 D3:
Sympon um juncue		EF631310 D2:
		EF631418 16S:
Commentation and the		EF631531
sympetrum amotguum	USA: DE (M. L. May)	D7: N/A D3: EF631324 D2:

		N/A 16S: FF631548
Libellu	lidae: Trithemistinae	105. 11 0515 10
Brechmorhoga mendax	USA: TX (M. L. May)	D7: EF631572 D3: N/A D2: EF631385 16s: EF631502
Dythemis fugax	USA: TX (M. L. May)	D7: N/A D3: N/A D2: EF631387 16S: EF631503
Dythemis multipunctata	Panama (M. L. May)	D7: EF631259 D3: EF631350 D2: EF631464 16S: EF631574
Huonia oreophila	New Guinea (J. Michalski)	D7: EF631270 D3: EF631361 D2: EF631477 16S: EF631587
Macrothemis celeno	Puerto Rico (M. L. May)	D7: EF631282 D3: EF631370 D2: EF631489 16S: EF631594
Macrothemis hemichlora	Panama (M. L. May)	D7: N/A D3: EF631292 D2: EF631394 16S: N/A
Macrothemis pulmila	Trinidad (M. L. May)	N/A
Paltothemis lineatipes	USA: CA (M. L. May)	D7: N/A D3: EF631373 D2: EF631492 16S: EF631597
Scapanea archboldi	Dominican Republic (J. Huff)	D7: EF631269 D3: EF631360 D2: EF631476 16S: EF631586
Trithemis basileri	South Africa (K. M. Kjer)	N/A
Trithemis dorsalis	South Africa (M. L. May)	D7: N/A D3: EF631299 D2: EF631404 16S: EF631518
Trithemis monardi	Guinea-Bissau (J. Huff)	D7: EF631264 D3: EF631355 D2: EF631469 16S: EF631579
Libellulio	lae: Onychothemistinae	
Onychothemis culminicola	Thailand (T. W. Donnelly)	D7: N/A D3: EF631374 D2: EF631493 16S: EF631598
Onychothemis testacea 1	Thailand (T. W. Donnelly)	D7: EF631278 D3: EF631367 D2: EF631485 16S: EF631599
Onychothemis testacea 2	Thailand (T. W. Donnelly)	D7: EF631284 D3: EF631375 D2: EF631494 16S: N/A
Libellu	lidae: Palpopleurinae	
Palpopleura jucunda	South Africa (M. L. May)	D7: N/A D3: N/A D2: EF631414 16S: EF631527
Palpopleura lucia	Senegal (J. Huff)	D7: EF631262 D3: EF631353 D2: EF631467 16S: EF631577

Perithemis tenera	New Jersey (J. L. Ware)	D7: EF631212 D3:
		EF631309 D2:
		EF631416 16S:
		EF631529
Zenithoptera fasciata 1	French Guiana (J. Huff)	D7: EF631283 D3:
		EF631371 D2:
		EF631490 168:
Zonithantong fagoiata 2	Eronah Cuiana (L. Huff)	EF031393
Zentinopiera fasciala 2	French Gulana (J. Hull)	D7: N/A D3: EE631372 D2:
		FF631491 16S
		EF631596
Libel	lulidae: Trameinae	
Tramea onusta	USA: NJ (M. L. May)	D7: EF631281 D3:
		N/A
		D2: EF631488
		16S: EF631593
Tramea lacerata	USA: NJ (J. L. Ware)	D7: EF631221 D3:
		EF631317 D2:
		EF631425 16S:
	Card Africa (M. L. Mara)	EF631538
Rhyothemis seminyalina 1	South Africa (M. L. May)	D/: EF631204 D3: EE631301 D2:
		EF631406 168
		EF631520
Rhvothemis semihvalina 2	South Africa (C. Chaboo)	D7: N/A
		D3: EF631358 D2:
		EF631474 16S:
		EF631584
Miathyria marcella	USA: FL (M. L. May)	N/A
Pantala flavescens 1	South Africa (M. L. May)	D7: EF631220 D3:
		EF631316 D2:
		EF631424 16S:
		EF631537
Pantala flavescens 2	Senegal (J. Huff)	D7: EF631280 D3:
		EF631369 D2: EF631487 168
		N/A
Hydrobasileus brevistylus	Australia (M. I., May, K. M. Kier and F.	D7: EF631252 D3:
Tyu oousteus or evistytus	L. Carle)	EF631343 D2:
		EF631457 16S:
		EF631567
Idiataphe amazonica	Bolivia (Mauffray)	D7: N/A
		D3: EF631377
		D2: N/A
	a	16S: N/A
Tholymis tillarga	Guinea-Bissau (J. Huff)	D7: EF631202 D3:
		EF631298 D2:
		EF031403 105: EE631517
Taurinhila australis	Panama (M. I. May)	N/A
Tauriphila dustralis	idae: Urothemistinge	IN/A
Aethriamanta rezia	Guinea-Bissau (J Huff)	D7: EF631188 D3·
		N/A
		D2: EF631384
		16S: EF631501
Macrodiplax balteata	USA: FL (M. L. May)	N/A
Urothemis assignata	Senegal (J. Huff)	D7: EF631217 D3:
		EF631313 D2:
		EF631421 16S:
7 1 .		EF631534
Zyxomma elgneri	Australia (K. M. Kjer, F. L. Carle)	D7: EF631253 D3:
		EF031344 D2: EF631458 168
		EF631568
Zvxomma petiolatum	Bali (A Rowat)	D7· N/A
2. Justinina ponormanin		D3: EF631378 D2:

		EF631496
		16S: N/A
Libellu	lidae: Zygonychinae	
Zygonyx torridus	South Africa (M. L. May)	D7: EF631218 D3: EF631314 D2: EF631422 16S: EF631535
Zygonyx natalensis	South Africa (M. L. May)	D7: EF631203 D3: EF631300 D2: EF631405 16S: EF631519

Table 3: Primers used in the present study.

	02 region of the	D3 region of the 28S	D7 region of the 28S	16S primers by E. Pilorim	16s primers from Misof et al (2001)
110	TGCTTGAGAG	5'ACCCGTCTTG	5'CGSGCGACGA	5'GTAAGAGTTT	LR-J-12887
Π	GCAGCCCAA3'	AAACACGGGAC3'	GTAGGAGGG3'	AAASGTCGAAC	5'GGAGCTCCGGTT
				AGA3'	TGAACTCAGATC3'
\sim	CCTTGGTCCGT	5'ATAGTTCACC	5'CTTCAGAGCC	5'AGGATTAGAT	LR-N-13398
Ú	JTTCAAGAC3'	ATCTTTCGGGTC	AATCCTTAT3'	ACCCTTTTATTT	5'CGGCCGCCTGTT
		C3'		TAAATG3'	ATCAAAACAT3'

Chapter 2:

Divergence dates of libelluloid dragonflies (Odonata: Anisoptera) estimated from rRNA using paired-site substitution models

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Abstract

Molecular dating methods make assumptions about evolutionary processes that can have a profound influence on the resulting date estimates. In this respect, the impact of substitution model selection has received little attention. This is perhaps most important in analyses of rRNA molecules, which are subject to non-independent evolution among sites due to correlated substitutions in paired stem regions. Using an rRNA data set from libelluloid dragonflies, we investigated the effect of using an explicit paired-sites model in divergence time estimation with a relaxed clock. For most nodes, the paired-sites model yielded more recent times than those produced using a model that treated all sites as independent. In several cases, the differences in date estimates resulted in divergence events being assigned to a different geological period. Disagreement in divergence date estimates complicates an evaluation of the evolutionary reduction of the libelluloid ovipositor. Accordingly, it is prudent to assess the effects of model selection on resulting date estimates, as well as on the consequent ecological and biogeographic interpretations.

1. Introduction

Molecular dating methods have provided valuable insights into the rates and timescales of evolution in a variety of organisms. All of these methods make a number of assumptions about the evolutionary process that can have a profound influence on the resulting date estimates. Accordingly, model selection is an important component not only of topological reconstruction but also of divergence time estimation; poorly chosen models can lead to errors in date estimates (e.g., Sullivan and Swofford, 1997). There has been a consistent focus on the assumption of a molecular clock, with substantial variation in substitution rates having been observed in a range of taxonomic groups (Bromham and Penny, 2003). This prompted the development of sophisticated methods for dealing with rate heterogeneity among lineages (e.g., Thorne et al., 1998; Sanderson, 2002; Drummond et al., 2006).

The impact of substitution model selection on date estimates has received considerably less attention. The potential effect of model selection is perhaps most pronounced in the analysis of rDNA molecules, the rRNA products of which are subject to distinctive substitution patterns on account of secondary structural constraints (Wheeler and Honeycutt, 1988; Dixon and Hillis, 1993). Ribosomal RNA (rRNA) folds on itself, creating a complex secondary structure maintained by bonded nucleotide pairs, which is important for ribosome function (Noller, 1984). In these stem regions, a substitution at one site is often accompanied by a compensatory substitution at its complement in order to prevent disruption to the secondary structure of the molecule (Smith et al., 2004; Tillier and Collins, 1998). For example, in an analysis of eubacterial

rRNA sequences, Savill et al. (2001) found that the rate of double substitutions was significantly non-zero.

The correlated mode of molecular evolution in RNA stems violates one of the fundamental assumptions of standard nucleotide substitution models, that of independence among sites (Wheeler and Honeycutt, 1988; Dixon and Hillis, 1993). In such cases, it is more appropriate to analyse the alignment using a paired-site model that treats the nucleotide and its complement as a single character (Jow et al., 2002). The RNA loop regions, which are unpaired, can be suitably analysed using standard substitution models. This fundamental difference in evolutionary modes also argues for data partitioning in the phylogenetic analysis of rRNA molecules based on secondary structure. These concerns have received scant attention in divergence dating studies despite the widespread use of rRNA as a phylogenetic marker: among 14 studies that used rRNA data to estimate divergence times, only one used a paired-sites model (Table 1).

A number of paired-sites substitution models are available for the phylogenetic analysis of RNA sequences. The earliest models treated all 16 possible pairs that can be formed with four nucleotides as distinct states, producing a time-reversible 16×16 rate matrix (Schöniger and von Haeseler, 1994; Muse, 1995; Rzhetsky, 1995). This may be unnecessarily complex, since only matching pairs (the four Watson-Crick pairs, along with G-U and U-G pairs) are frequent in the helix regions of ribosomal molecules (Savill et al., 2001). Subsequently, less general models have been developed. 7-state models, such as that described by Tillier and Collins (1998), consider only the six matching pairs and group the remaining 10 mismatch pairings into a single state (Higgs, 2000). These have been shown to fit the data as well as 16-state models (Savill et al., 2001).

In this study, the effect of using a paired-sites model in divergence date estimation is investigated using a libelluloid dragonfly rRNA data set. Libelluloid dragonflies are highly specialized, widely collected, and often brightly coloured members of the most recently derived part of the Anisopteran lineage. Libelluloidea comprises seven major groups (six families: Cordulegastridae, Neopetaliidae, Chlorogomphidae, Macromiidae, Corduliidae, and Libellulidae; and one monophyletic group, the GSI *sensu* Ware et al. (2007), of undetermined taxonomic status). In comparison with other dragonflies, libelluloids are considered highly derived, primarily because they possess a reduced ovipositor that allows for endophytic oviposition, a probably convergent trait shared with Gomphidae (Carle, 1995), and unique larval labial modifications. The earliest libelluloid fossils are not older than the mid-Cretaceous, roughly 120-130 Myr (e.g., Jarzembowski and Nel, 1996).

Date estimates from two relaxed-clock analyses are presented, one in which the tree is obtained using a paired-sites model for RNA stem regions, and one in which the tree is obtained using standard unpaired-sites models for all data partitions. We investigate the differences between the results of these two analyses in the context of non-independent evolution at stem sites, and examine how interpretations of biogeographical and morphological evolution are affected by differing date estimates.

2. Materials and Methods

Data Set

The rRNA sequences used in the present study were a subset of those analysed by Ware et al. (2007), with gene fragments from the nuclear large subunit (28S; D2, D3, D7) and the mitochondrial large subunit (16S) (supplementary material). Sequences were aligned manually using the structural methods described in Kjer (1995). Ambiguously aligned regions were excluded from the data set, yielding a final alignment of 1763 bp from 32 taxa. The alignment was divided into four partitions (28S stem, 28S loop, 16S stem, and 16S loop). Our alignments are available on the Kjer lab website,

www.rci.rutgers.edu/~entomology/kjer).

Model Selection

Substitution models were selected for each loop partition by comparison of Akaike Information Criterion (AIC) scores. There is no immediately apparent method for comparing the fit of paired and unpaired models for the RNA stem partition, because the most commonly used methods for model selection are not readily able to compare models with different state spaces. Consequently, analyses were performed using paired- and unpaired-sites models, and the results were compared.

Phylogenetic Analysis

Bayesian phylogenetic analyses were performed using the software *PHASE* (Jow et al., 2002). Two separate analyses were performed: (i) the AIC-selected substitution model was assumed for each partition; (ii) AIC-selected substitution models were

assumed for non-stem partitions, but the RNA7A model was assumed for stem partitions. This model was chosen because it is a realistic simplification of the full 16-state model (Savill et al., 2001; Jow et al., 2002). The simpler RNA6A model was not used because inspection of the alignment revealed that some paired stem sites did not contain matching pairs. Posterior distributions were approximated by Markov chain Monte Carlo (MCMC) sampling, with samples drawn every 500 steps over a total of 10,000,000 steps following a discarded burn-in of 1,000,000 steps. Convergence to the stationary distribution and acceptable mixing were checked by inspection of MCMC traces. Consensus topologies and optimal branch lengths were computed using maximum likelihood in *PHASE*. The resulting trees were used as fixed input trees for subsequent divergence dating analysis.

Following rejection of a molecular clock using a likelihood ratio test (p=0.008), divergence dates were estimated in a relaxed-clock framework by the program r8s(Sanderson, 2003) using penalized likelihood, a method that applies a penalty against large rate changes between neighboring branches. The magnitude of the penalty is dictated by a smoothing parameter, the value of which was optimized by cross-validation analysis. A logarithmic penalty was used on account of its similarity to the autocorrelated relaxed-clock models used in Bayesian phylogenetic methods (e.g., Thorne et al., 2002). Dates and substitution rates were estimated using the truncated Newton algorithm with 10 independent starts.

Calibration using fossils

We used fossil-based estimates of divergence times between Chlorogomphidae,

59

Macromiidae, Corduliidae, Libellulidae, and the Libelluloidea to calibrate the divergence dating analysis (supplementary material). Due to the difficulty in acquiring confident genus-level identifications of dragonfly fossils, age bounds were for family-level limits only.

Simulations

Analyses of simulated data were performed in order to investigate the effect of ignoring the paired nature of stem regions. Using parameter values and the tree estimated from the stem region of 28S rRNA, 50 data sets were generated by simulation under a paired-sites model, with substitution parameters and sequence length (532 bp) matching those estimated from the real data set. A strict molecular clock was assumed.

Each of the 50 alignments was analysed with paired- and unpaired-sites models in *PHASE*, with the topology fixed. In each case, the consensus tree (with average branch lengths) was used as the fixed input tree for divergence time estimation using *r8s*. The Langley-Fitch algorithm was used, which assumes a strict molecular clock. Paired *t*-tests were performed to investigate differences in divergence times inferred under paired- and unpaired-sites models.

3. Results

Model selection

Inspection of likelihoods reveals that the best tree under the paired-sites model, with branch lengths optimized using maximum likelihood, is 712 log-likelihood units more likely than the best tree under the unpaired sites model. This result, along with the known paired nature of RNA stem sites, can be taken as an indication that the paired sites model is preferred. Nevertheless, a more formal model selection framework is desirable for future studies.

Topology

As in previous studies, analyses using both paired- and unpaired-sites models strongly supported the monophyly of Corduliidae (Figure 1). The two topologies differ in their placement of Corduliidae and Macromiidae, whose mutual relationships with Libellulidae were unresolved in a previous analysis by Ware et al. (2007) and have been the subject of considerable debate (see Ware et al., 2007 for a detailed review). There were two main differences between the phylogenetic reconstructions produced using the different substitution models: the placement of the libellulid taxa Urothemis and Perithemis (Figure 1). The unpaired-sites model yielded trees in which Perithemis was closely related to *Pantala*. By contrast, the analysis using a paired-sites substitution model placed Perithemis at the base of Libellulidae, as sister taxon to all other libellulids. *Urothemis* was placed as sister to the remaining Libellulidae by the analysis using an unpaired-sites model, although with low support. The analysis using a paired-sites model reconstructed an alternate topology, with Urothemis nested well within the Libellulidae, as sister to the {Libellula + Ladona + Plathemis + Pantala} clade, again with extremely low support. The position of *Perithemis* was also variable in the analyses of Ware et al.
(2007). *Urothemis*, however, was consistently recovered in a basal position within Libellulidae in all analyses by Ware et al. (2007).

Divergence time estimates

For most nodes, divergence time estimation from the paired-sites tree yielded more recent dates than those from the unpaired-sites tree (Table 2). The estimates for the time to the most recent common ancestor (TMRCA) of (i) Libellulidae, (ii) Cordulidae, and (iii) *Cordulia* + *Somatochlora*, however, were younger in the unpaired-sites analysis.

For the *Cordulia* + *Somatochlora* node, the unpaired-sites model suggested shorter branch lengths than the paired-sites analysis (Table 2) and lower substitution rates (unpaired-sites: 6.04×10^{-4} subs/site/Myr; paired-sites: 6.04×10^{-3} subs/site/Myr). Similar patterns were observed for the Libellulidae and Corduliidae nodes (unpaired-sites: Corduliidae 5.17×10^{-4} subs/site/Myr, Libellulidae 5.02×10^{-4} subs/site/Myr; paired-sites: Corduliidae 1.22×10^{-3} subs/site/Myr, Libellulidae 1.13×10^{-3} subs/site/Myr). This resulted in older date estimates in the analysis using the paired-sites model.

In several cases, the differences in date estimates resulted in the nodal age being assigned to a different evolutionary time period (Table 2). For example, the unpairedsites dating analysis suggested a Permian/Triassic age for the root of the tree, while the paired-sites model dating analysis suggest a younger, Jurassic age (Table 2). The pairedsites model dating analysis suggests a Cretaceous age for the most recent common ancestor of Libellulidae, while the unpaired-sites model analysis suggests a younger, Cenozoic age.

Simulations

There were significant differences between the divergence times inferred under paired- and unpaired-sites models. For all major nodes (interfamilial relationships), the estimated ages were within one standard deviation of the true (simulation) ages. Age estimates made under the unpaired-sites model were older than those made under the paired-sites model, although to varying degrees (paired *t*-tests, *p*-values ranging from 9.36×10^{-5} to 0.065); this result is consistent with those obtained from the real data set.

In the trees inferred using *PHASE*, with branch lengths measured in substitutions per site, the ratio of internal to external branch lengths was significantly different (paired *t*-test, $p=2.96\times10^{-9}$) between paired- and unpaired-sites models (Figure 2). This suggests that the inappropriate use of an unpaired site model does not simply alter the total tree length, as would be expected if the model produces a consistent bias among branches.

4. Discussion

Our analyses yielded strong preference for a paired-sites model over an unpaired-sites model for the analysis of rRNA sequences from libelluloid dragonflies. Based on comparison of likelihood scores from the two analyses, along with strong support in the literature for the paired sites model in studies of rRNA (Savill et al., 2001; Jow et al., 2002; Hoyle and Higgs, 2003; Hudelot et al., 2003), and from our simulation studies, we consider divergence times obtained using the paired-sites model to be better estimates than those obtained using the unpaired-sites model. This suggests that use of the latter has led to erroneous estimates of the ages of several nodes in the tree: the age of the root of

the tree, the TMRCA of Chlorogomphidae + Libelluloidea, and the TMRCA of Corduliidae + Macromiidae were overestimated; whereas the TMRCAs of Libellulidae, Corduliidae, and Macromiidae were underestimated. Although it may be difficult to objectively assess which date estimates are unreasonable, the age of 249 Myr for the root of the unpaired sites tree is surprisingly old, and close to the upper calibration limit of 250 Myr.

Do differences in date estimates really matter?

In many cases, the dates obtained by analyses using paired- and unpaired-sites treatments did not differ greatly. Consequently, the impact on evolutionary interpretations based on these would not be substantial. For Libelluloidea, however, two important nodes of interest were inconsistent between models: the age of the root of the tree and the divergence date for the most speciose, heterogeneous, and widely studied libelluloid family, Libellulidae.

In the case of the root of the tree, the unpaired-sites analysis returned a surprisingly early date at the Permian/Triassic boundary instead of being in the Jurassic as suggested under the paired-sites model. The difference in these dates would affect assumptions of the geographical positions of continents, air temperature, sea level, and the biodiversity that would have been present when this node of interest diverged. If the Libelluloidea had diverged at the Permian/Triassic boundary, it would have been during a period where only one large continental landmass, Pangaea, existed (in what is considered a period of hot dry climate; e.g., Parish, 1993). This would have just followed one of the largest

extinctions of insects in evolutionary history (e.g., Erwin, 2006). This differs dramatically from the conditions that the Libelluloidea would have encountered had they diverged 50 million years later, in the early Jurassic, as the paired-sites tree model analysis suggests. At this younger date, Pangaea would have already begun to break apart, with an associated increase in the amount of inland water, a decrease in overall temperature, and an increase in humidity levels on the continents (e.g., Hallam, 1993). Our understanding of the biogeographical history of Libelluloidea is strongly dependent on correct assumptions about continental positions. Did the Libelluloidea diverge when there was a single continental land mass after which vicariance events involved in the break up of Pangaea led to speciation? Alternatively, did the Libelluloidea diverge during the early Jurassic in Gondwana, for example, and then disperse to other continents? Our understanding of character evolution in Libelluloidea is also strongly affected by incorrect date estimation. For example, Libelluloidea have in common a reduction in their ovipositor, convergently shared with Gomphidae (Carle, 1995). In libelluloids the ovipositor is modified for exophytic oviposition (i.e. not requiring plant tissue for egg deposition; Tillyard, 1917; Carle, 1995; see Ware et al., 2007 for figures). In the higher libelluloid taxa studied here (Macromiidae, Corduliidae and Libellulidae), the first processes are reduced to small flaps and the other structures are apparently absent except for the probable vestige of the styli emerging directly from the ninth sternite (Tillyard, 1917). The divergence estimates for Libelluloidea influence our hypotheses about the evolutionary process of this reduction. If the unpaired sites model is applied, and we assume that Libelluloidea diverged 249 Myr ago, we might favor a hypothesis proposing

ovipositor reduction and exophytic oviposition in response to limited freshwater niche space during the Triassic. Using information from the paired-sites model analysis, however, which suggests a divergence age of 205 Myr ago, we might suppose that ovipositor reduction occurred in response to an increase in the number of predators at the oviposition site. Exophytic oviposition is generally faster than endophytic oviposition, which may reduce the number of predators encountered (e.g. Corbet, 1999). Fish, frogs, and birds impose a strong predation threat on ovipositing females, particularly those that do so endophytically (Corbet, 1999). Exophytic oviposition may have evolved as a response to the diversification of these predators (modern birds diverged during the Cretaceous, Brown et al., 2007; Neobatrachid frogs diverged during the Jurassic, Roelants et al., 2007). Certainly there are numerous other hypotheses that could be supposed for the evolution of dragonfly genetalia, but without realistic dating estimates it will be hard to evaluate them effectively.

Conclusions

The results of our analyses here, coupled with the general desirability of utilizing evolutionary models that are biologically realistic, suggest that it is very important to take stem pairing into account during analyses of rRNA datasets. The impact of using pairedsites substitution models on divergence time estimates is not easily predictable, particularly when explicit models of among-lineage rate heterogeneity are used in conjunction with partitioned analyses of complex data. Paired-sites models apparently do not lead to uniformly lower dating estimates, although it is necessary to investigate a wider range of datasets before further inferences can be made. Accordingly, it is prudent to assess the effects of model selection on resulting date estimates, as well as on the consequent ecological and biogeographic interpretations.

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References

Bromham, L., Penny, D., 2003. The modern molecular clock. *Nature Rev. Genet.*, 4: 216-224.

Brown, J. W., Payne, R. B., Mindell, D. P., 2007. Comment. Nuclear DNA does not reconcile 'rocks' and 'clocks' in Neoaves: a comment on Ericson et al. *Biology Letters*, **3(3)**: 257-259.

Carle, F. L., 1995. Evolution, taxonomy, and biogeography of ancient Gondwanian libelluloides, with comments on anisopteroid evolution and phylogenetic systematics (Anisoptera: Libelluloidea). *Odonatologica*, **24**: 383-506.

Corbet, P. S., 1999. *Dragonflies: Behavior and Ecology of Odonata*. Comstock Publishing Associates, Cornell University Press. Ithaca, New York.

Dixon, M.T., Hillis, D. M., 1993. Ribosomal RNA secondary structure: compensatory mutations and implications for phylogenetic analysis. *Mol. Biol. Evol.*, **10**: 256-267.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J., Rambaut A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.*, **4:** e88.

Erwin, D. H., 2006. *Extinction: How Life on Earth Nearly Ended 250 Million Years Ago*. Princeton University Press. 296 pp.

Hallam, A. 1993. Jurassic climates as inferred from the sedimentary and fossil record. *Phil. Trans.R. Soc. Lond.* **B341**: 287–296.

Higgs, P. G., 2000. RNA secondary structure: physical and computational aspects. *Q. Rev. Biophys.*, **33**: 199-253.

Hoyle, D.C., Higgs, P.G., 2003. Factors affecting the errors in estimation of evolutionary distances between species *Mol. Biol. Evol.*, **20**: 1-9.

Hudelot, C., Gowri-Shankar, V., Jow, H., Rattray, M., Higgs, P.G., 2003. RNA-based phylogenetic methods: Application to mammalian mitochondrial RNA sequences. *Mol. Phylogenet. Evol.*, **28**: 241-252.

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 Res.*, **17**: 67-85.

Jow, H., Hudelot, C., Rattray, M., Higgs, P. G., 2002. Bayesian phylogenetics using an

RNA substitution model applied to early mammalian evolution. *Mol. Biol. Evol.*, **19**: 1591-1601.

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. *Mol. Phylogenet. Evol.*, **4**: 314-330.

Muse, S., 1995. Evolutionary analyses of DNA sequences subject to constraints on secondary structure. *Genetics*, **139**: 1429-1439.

Noller, H. F., 1984. Structure of ribosomal RNA. Ann. Rev. Biochem., 53: 119-162.

Parish, J. T., 1993. Climate of a supercontinent. Journal of Geology, 101: 215 - 233

Roelants, K., Gower, D.J., Wilkinson, M., Loader, S.P., Biju, S.D., Guillaume, K., Moriau, L., Bossuyt, F., 2007. Global patterns of diversification in the history of modern amphibians. *Proc. Natl. Acad. Sci.*, *USA*, **104** (3): 887-892

Rzhetsky, A., 1995. Estimating substitution rates in ribosomal RNA genes. *Genetics*, **141**: 771-783.

Sanderson, M. J., 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol. Biol. Evol.* **19**: 101-109.

Sanderson, M. J., 2003. r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, **19**: 301-302

Savill, N. J., Hoyle, D.C., Higgs, P.G., 2001. RNA sequence evolution with secondary structure constraints: Comparison of substitution rate models using maximum likelihood methods. *Genetics*, **157**: 399-411.

Schöniger, M., von Haeseler, A., 1994. A stochastic model for the evolution of autocorrelated DNA sequences. *Mol. Phylogenet. Evol.*, **3**: 240-247.

Smith, A., Thomas, D., Lui, W. H., Tillier, E. R. M., 2001. Empirical Models for Substitution in Ribosomal RNA. *Mol Biol Evol* **21**: 419-427.

Sullivan, J., Swofford, D.L., 1997. Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *J. Mammal. Evol.*, **4**:77-86.

Thorne, J. L., Kishino, H., Painter, I. S., 1998. Estimating the rate of evolution of the rate of molecular evolution. *Mol. Biol. Evol.* **15**: 1647-1657.

Tillier, E. R. M., Collins, R. A., 1998. High apparent rate of simultaneous compensatory base-pair substitutions in ribosomal RNA. *Genetics* **148** :1993-2002

Tillyard, R. J., 1917. The biology of Dragonflies. Cambridge. Cambridge Univ. Press.

Ware, J. L., M. L. May, K. M. Kjer, 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. *Mol. Phylogenet. Evol.*, **45(1):**289-310.

Wheeler, W. C., Honeycutt, R. L., 1988. Paired sequence difference in ribosomal RNAs: evolution and phylogenetic implications. *Mol. Biol. Evol.*, **5**: 90-96.



Figure 1: Results of r8s analyses using an unpaired-sites model (a) and a paired-sites model (b).

Figure 2: Results of simulation study: A plot of the ratios of internal to external branch lengths from analyses of 50 independent data sets using unpaired- and paired-sites models.



nation' 'naired sites' and 'doublet'

Reference ^a	Data set	Gene	Substitution model
Baker et al. (2005)	Moa	$12S, tRNA_{Lys}$	GTR+I+G
Bossuyt et al. (2006)	Frogs (Ranidae)	12S, 16S, tRNA _{Val}	GTR+I+G
Brammer & Dohlen (2007)	Flies (Stratiomyidae)	28S	GTR+I+G
Crayn et al. (2006)	Trees (Elaeocarpaceae)	trnL-trnF; ITS	GTR+G
Dumont et al. (2005)	Dragonflies	ITS1, ITS2; 18S; 28S	GTR+I+G
	(Calopterygoidea)		
Gómez-Zurita et al.	Leaf beetles	28S, 18S, 16S	F84•C8
(2007)	(Chrysomelidae)		
Jansen et al. (2006)	Fish (Clariidae)	ITS1, ITS2; 18S; 28S	GTR+G
Near et al. (2005)	Fish (Centrarchidae)		Paired- and unpaired-sites were partitioned,
		16S	but a paired-sites model was not used.
Pereira & Baker (2006)	Birds	12S, 16S, 22 mt tRNAs	HKY+G
Perez-Losada et al. (2004)	Barnacles (Thoracica)	12S, 16S, 18S, 28S	HKY+G
Wiegmann et al. (2003)	Flies (Brachycera)	28S	$HKY+G^*$
Winterton et al. (2007)	Flies (Acroceridae)	16S, 28S	GTR+I+G
Williams et al. (2003)	Periwinkles (Gastropoda)	12S, 18S	GTR+I+G (28S+12S), SYM+I+G (18S), GTR+G (12S)
Zhang et al. (2006)	Salamanders (Hynobiidae)	12S; 16S; tRNA _{Val}	ż

^aBaker et al. (2005) *Proc. Natl Acad. Sci. USA*, **102**: 8257-8262; Bossuyt et al. (2006) *Syst. Biol.*, **55**: 579-594; Brammer & Dohlen (2007) *Mol. Phylogenet. Evol.*, **43**: 660-673; Crayn et al. (2006) *Am. J. Bot.*, **93**: 1328-1342; Dumont et al. (2005) *Syst. Biol.*, **54**: 347-362; Gómez-Zurita et al. (2007) *PLoS ONE*, **2**: e360; Jansen et al. (2006) *Mol. Phylogenet. Evol.*, **38**: 65-78; Near et al. (2005) *Evolution*, **59**: 1768-1782; Pereira & Baker (2006) *Mol. Phylogenet. Evol.*, **38**: 499-509; Perez-Losada et al. (2004) *Syst. Biol.*, **53**: 244-264; Wiegmann et al. (2003) *Syst Biol.*, **52**: 745-756; Winterton et al. (2007) *Phylogenet. Evol.*, **38**: 499-509; Perez-Losada et al. (2004) *Syst. Biol.*, **53**: 244-264; Wiegmann et al. (2003) *Syst Biol.*, **52**: 745-756; Winterton et al. (2007) *Phylogenet. Evol.*, **43**: 808-832; Williams et al. (2003) *Mol. Phylogenetic. Evol.*, **28**(1):60-86: Y-Z; Zhang et al. (2006) *Proc. Natl Acad. Sci. USA*, **103**: 7360-7365.

Doumatou	Estir	nate
r ar anreuer	Paired-sites model	Unpaired-sites model
Divergence times		
Root	205.7 Myr	249.1 Myr
Macromiidae	34.2 Myr	28.2 Myr
Epitheca-Tetragoneuria	28.5 Myr	N/A
Cordulia-Somatochlora	65.7 Myr	62.3 Myr
Corduliidae	87.1 Myr	71.6 Myr
Corduliidae-Macromiidae	134.2 Myr	140.1 Myr
Libellulidae	87.6 Myr	57.7 Myr
Libellulidae+Macromiidae+Corduliidae	144.0 Myr	N/A
Chloropetaliidae+Sinorogomphidae+ingroup	186.3 Myr	192.4 Myr
Substitution rate		
Mean	7.95×10 ⁻⁴ subs/site/Myr	5.81×10 ⁻⁴ subs/site/Myr
Minimum	3.67×10 ⁻⁵ subs/site/Myr	7.49×10 ⁻⁵ subs/site/Myr
Maximum	1.85×10 ⁻³ subs/site/Myr	1.28×10 ⁻³ subs/site/Myr

Table 2: Divergence times and substitution rates estimated using the paired- and unpaired-sites models.

Table S1: Details of sequ	ences analysed		
Taxon	Species	Locality	Genbank Accession No. (D7, D3, D2, and 16S)
Cordulegastridae	Taeniogaster oblique	USA: NJ (M.L. May)	EF631216, EF631312, EF631420, EF631533
	Kalyptogaster erronea	USA: NJ (M.L. May)	EF631245, N/A, EF631450, EF631561
Chlorogomphidae	Chloropetalia soarer	Sequences from F.L. Carle	EF631248, EF631339, EF631453, N/A
	Sinorogomphus sp	Sequences from F.L. Carle	EF631249, EF631340, EF631454, EF631564
Neopetaliidae	Neopetalia punctata	Sequences from F.L. Carle	EF631247, EF631338, EF631452, EF631563
Corduliidae	Cordulia shurtleffii	Canada: ON (J.L. Ware, J.Huff)	EF631232, EF631326, EF631435, N/A
	Epitheca princeps	USA: NJ (J.L. Ware)	EF631205, EF631302, EF631407, EF631521
	Rialla villosa	Chile (Heppner)	EF631273, EF631364, EF631480, EF631590
	Somatochlora tenebrosa	USA: NJ (M.L. May)	EF631215, EF631311, EF631419, EF631532
Macromiidae	Tetragoneuria cynosura Didymons transversa	USA: NJ (M.L. May) HSA: NI (M.L. Mav)	N/A, EF631379, N/A, N/A N/A FF631377 FF631436 FF631549
	Macromia illionoiensis	USA: IL (M.L. May)	EF631208, EF631305, EF631410, FF631574
	Phyllomacromia contumax	Uganda (T.W. Donnelly)	EF631197, EF631293, EF631397, EF631511
Libellulidae: Leucorrhiniinae	Celithemis elisa	USA: NJ (M.L. May)	EF631224, EF631320, EF631428, EF631541
	Leucorrhinia glacialis	USA: NY (M.L. May)	EF631207, EF631304, EF631409, EF631523
Libellulidae: Libellulinae	Libellula pulchella Libellula luctuosa	USA: NJ (J.L. Ware) USA: NJ (J.L. Ware)	N/A, EF631329, N/A, EF631551 EF631194, N/A, EF631392, EF631507
	Libellula quadrimaculata 1 Libellula quadrimaculata 2	USA: NJ (M.L. May) Sweden (K.M. Kjer)	EF631272, EF631363, EF631479, N/A N/A, N/A, EF631497, EF631589
	Ladona julia	USA: WI (M.L. May)	EF631219, EF631315, EF631423, EF631536
	Plathemis lydia	USA: NJ (F.L. Carle)	EF631234, EF631330, EF631438,

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EF631552 EF631191, EF631288, EF631389, EF631505 EF631190, EF631287, EF631388, EF631504	EF631198, EF631294, EF631398, EF631512 EF631214, EF631310, EF631418, EF631531	N/A, EF631324, N/A, EF631548 EF631281, N/A, EF631488, EF631593 EF631221, EF631317, EF631425, EF631538	EF631220, EF631316, EF631424, EF631537	EF631280, EF631369, EF631487, N/A	EF631217, EF631313, EF631421, EF631534
USA: TX (M.L. May) USA: FL (J.L. Ware & J. Huff)	USA: NJ (F.L. Carle) USA: NJ (F.L. Carle)	USA: DE (M.L. May) USA: NJ (M.L. May) USA: NJ (J.L. Ware)	South Africa (M.L. May)	Senegal (J. Huff)	Senegal (J. Huff)
Erythemis simplicicollis Erythrodiplax minuscula	Pachydiplax longipennis Sympetrum janeae	Sympetrum ambiguum Tramea onusta Tramea lacerata	Pantala flavescens 1	Pantala flavescens 2	Urothemis assignata
Libellulidae: Sympetrinae		Libellulidae: Trameinae			Libellulidae: Urothemistinae

Node	Age constra	iint (Myr)	Kassil avidanca far calibration noints
2007	Maximum	Minimum	FUSSIL CALINCHUCE FOF CALINFAUNT POINTS
Root of tree	250	I	Date of early Triassic. Oldest anisopteran fossils are from the Triassic (Grimaldi and Engels, 2005).
Ingroup	144	120	Dates from the late Jurassic, a time period suggested by Nel et al. (1993) for the maximum age. Minimum age constraint based on the fossil dates for the most basal group of Libelluloidea, the GSI (<i>sensu</i> Ware et al, 2007; not studied here), using information from Nel et al. (1993), and the fossil Libellulidae from 120 Myr: <i>Araripelibellula</i> (Nel and Paicheler, 1994), using age information from the Chapada do Araripe formation (Viana and Neumann, 1999).
Macromiidae	I	10.1	10.4±0.3 Myr: <i>Macromia</i> (Rambur, 1842). Date for Tochigi prefecture from NoE Toru (1984), although actual fossil description lists the fossil from the Pleistocene.
Corduliidae	131	I	The split between Corduliidae and Libellulidae had already occurred by 14.5-16.5 Myr and probably occurred as early as 118-131 Myr. Date from the Wealden formation, approximate time periods suggested by Jarzembowski and Nel (1996).
Libellulidae	131	I	The split between Corduliidae and Libellulidae had already occurred by 14.5-16.5 Myr and probably occurred as early as 118-131 Myr. Date from the Wealden formation, approximate time periods suggested by Jarzembowski and Nel (1996).
Macromiidae+Corduliidae ^a	I	118	The split between Corduliidae and Libellulidae had already occurred by 14.5-16.5 Myr and probably occurred as early as 118-131 Myr. Date from the Wealden formation, approximate time periods suggested by Jarzembowski and Nel (1996).
Epitheca+Tetragoneuria ^a	Ι	5.3	7.246-5.332 Myr: <i>Epitheca</i> (Burmeister, 1839). Messinen date from Ogg (2004).
Cordulia+Somatochlora ^a	Ι	49	49 Myr: Cordulia, Pongracz 1931. Date of Monte Bolca from University of

Table S2: Fossil calibrations used in the phylogenetic analysis.

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^aFor paired site analysis only, because the *Epitheca* sequence was unusable in the unpaired site analysis; in the latter analysis, *Cordulia* did not come out as sister to *Somatochlora*, nor was Macromiidae sister to Corduliidae.

References

Jarzembowski, E.A, Nel, A., 1996. New fossil dragonflies from the Lower Cretaceous of SE England and the phylogeny of the Grimaldi, D., Engels, M. S., 2005. Evolution of the Insects, New York Cambridge University Press. superfamily Libelluloidea (Insecta: Odonata)

Cretaceous Res., **17**: 67-85.

Nel, A., Marti nez-Delclos, X., Paicheler, J. C., Henrotay, M., 1993. Les 'Anisozygoptera' fossiles. Phyloge nie et classification (Odonata). Martinia, Nume ro hors-se rie, **3**: 1–311.

Apaleo-environmental consideration Nel , A., Paicheler, J.-C., 1994. Les Libelluloidea fossiles autres que Libellulidae. Un inventaire critique (Odonata, Corduliidae, Macromiidae, Synthemistidae, Chlorogomphidae et Mesophlebiidae). *Nouvelle Revue d'Entomologie* , (N.S.), **11**: 321–334 NoE, Toru, 1984. A Pleistocene flora from Shiobara-machi, Tochigi prefecture ,Japan (1)____ Apaleo-environmental consideration under the palynological studies of the flora. *Geol. Surv. Japn.* **35(2):** 49-57.

Ogg, J., 2004. Overview of Global Boundary Stratotype Sections and Points (GSSP's). International Stratigraphic Commission

Paleontológicos do Brasil. Schobbenhaus, C., Campos, D.A., Queiroz, E.T., Winge, M., Berbert-Born, M., (Eds.). Published on Internet Website, www.stratigraphy.org/gssp.htm Viana,M.S., Neumann,V.H.L. 1999. The Crato member of the Santana formation, Ceará state, Brazil. In: Sítios Geológicos e at the address http://www.unb.br/ig/sigep/sitio005/sitio005english.htm. Last date viewed: August 8, 2007.

Chapter 3:

Biogeography and divergence estimation of the relic Cape dragonfly genus Syncordulia: global significance and implications for conservation Jessica L. Ware¹, John P. Simaika², Michael J. Samways² ¹Department of Entomology, Rutgers University, New Brunswick, NJ, 08901 (email: <u>jware42@rci.rutgers.edu</u>)²Department of Conservation Ecology and Entomology, Stellenbosch University, P Bag X1, Matieland, 7602, South Africa (email: samways@sun.ac.za)

Abstract

Syncordulia, a libelluloid dragonfly (Odonata: Anisoptera: Libelluloidea) genus, inhabits mostly cool mountainous streams in the Cape Floristic Region (CFR) of South Africa. The four species are all rare and threatened. Here we corroborate the validity of these two new species using molecular data (using 12S, 16S, 28S and COI fragments) and propose the intergeneric relationships within Syncordulia. In addition, we evaluate the phylogenetic placement of the genus within Libelluloidea, on a global basis. Finally, we relate these phylogenetic data to the importance of conservation action. We used data sets in particular from South Africa, Africa, India (Asia), Europe, and North and South America. Molecular data from two independent gene fragments (nuclear 28S and ribosomal and Cytochrome Oxidase I mitochondrial data) were sequenced (and/or downloaded from GenBank) for 7 libelluloid families, including 12 Syncordulia specimens (2 Syncordulia gracilis, 4 S. serendipator, 2 S. legator and 4 S. venator). We ran parsimony analyses using PAUP, and Bayesian analyses using phase. The phylogenetic hypotheses generated by the phase analysis were used in subsequent DIVA and r8s analyses to evaluate ancestral distributions and divergence estimates. The lower libelluloid group GSI (sensu Ware et al., 2007), a diverse group of non-corduliine taxa, is strongly supported as monophyletic. The Australian members of the GSI were recovered

as a monophyletic clade. *Syncordulia* is well supported by both methods of phylogenetic analyses as a monophyletic deeply nested within the GSI clade. All species within the genus are distinct. diva analyses constrained to two regions recovered an Indomalayan/Australian distribution for the GSI. R8s analyses estimate that *Syncordulia* diverged from other Libelluloidea in the Cretaceous. DIVA analyses suggest that the present distributions of *Syncordulia* may be the result of dispersal events. *Syncordulia* is a monophyletic genus and each species was found to be a distinct monophyletic taxon. Divergence estimates suggest that *Syncordulia* diverged well after the breakup of Gondwanaland, approximately 60 million years ago (Mya), which coincides with the divergence of several Cape fynbos taxa, between 86 – 60 Mya, specifically the Restionaceae, Proteae and Geissolomataceae. A DIVA biogeographical analysis suggests that the ancestor to the genus *Syncordulia* may have arisen consequent to the break-up of Gondwanaland (>120 Mya).

1. Introduction

Corduliid dragonflies, often known as "emeralds" for the metallic green body and eye color of many species, are members of the large superfamily, Libelluloidea. Previously considered to be one family (Rambur, 1842; Hagen, 1861; Kirby, 1890; Selys, 1892; Needham, 1908; Tillyard, 1917; Martin, 1914; Tillyard, 1928; St. Quentin, 1939; Gloyd, 1959; Lieftinck, 1971; Lieftinck, 1977; Carle, 1982; Davies & Tobin, 1985; Bridges, 1994; Steinmann, 1997) or divided into two or more families (Fraser, 1957; Carle, 1995; Bechly, 1995; Lohmann, 1996a, b; Pfau, 2005), Corduliidae has not been found to be monophyletic in modern phylogenetic analyses (May, 1995b; Misof et al., 2001; Carle

and Kjer, 2002; Ware et al., 2007; Bybee et al., 2008; Letsch in prep). 'Corduliidae' (*sensu* Fraser, 1957, and others) consists of at least three distinct clades, the Corduliinae, the Macromiinae, and a diverse group of non-corduliine taxa, informally called the "GSI" by Ware et al. (2007) after the subfamilies Gomphomacromiinae, Synthemistinae and Idionychinae. Within the GSI are Australian endemics, as well as African, South American, European and Indomalayan genera, and the relict South African genus *Syncordulia*.

Although both GSI adults and larvae have been documented in the literature (Selys-Longchamps, 1871; Geijskes, 1970; Lieftinck, 1960, 1971, 1977; Theischinger and Watson, 1978; May, 1991; Theischinger and Watson, 1984; Carle, 1995; May, 1995a, b; Carvalho et al., 2004; von Ellenrieder and Garrison, 2005), the taxa have been infrequently used in modern phylogenetic studies (Carle, 1995; Carle and Kjer, 2002; Misof et al., 2001; Ware et al., 2007; Bybee et al., 2008, Letsch, in prep; of these studies, only Ware et al. (2007) incorporated *Syncordulia* in their phylogeny). The GSI stem from a basal node in the Libelluloidea. Thus, the evolution of certain libelluloid apomorphies, such as reduction in the ovipositor for exophytic oviposition and modification of the distal sperm pump of the penis (Pfau, 2005), cannot be fully evaluated without an understanding of intergeneric relationships within GSI. In addition, several members of the GSI are rare endemics, and phylogenetic hypotheses about this group may be useful in understanding the biogeography and conservation needs of vulnerable genera such as *Syncordulia*.

Inhabiting mostly cool mountainous streams in the Cape Floristic Region (CFR) of South Africa, *Syncordulia* species generally occur in geographically-restricted areas and

at low population densities. Syncordulia species are sympatric (Dijkstra et al., 2007) and are often found dwelling in the same stream. First described by Selys-Longchamps in 1882, the genus Syncordulia comprises dragonflies endemic to South Africa and restricted almost entirely to the CFR. Until recently, only S. venator and S. gracilis were known, and both are considered Vulnerable by the World Conservation Union (IUCN) (IUCN, 2001). Dijkstra et al., (2007) described two new species, S. serendipator and S. *legator*, from previously unrecognized museum specimens and from new field collections. Intrageneric relationships within *Syncordulia*, have not yet been fully evaluated within a phylogenetic context. In addition, placement of Syncordulia within the GSI has been unresolved. Lieftinck (1961) suggested that Syncordulia was closely related to Oxygastra, Hesperocordulia, Lathrocordulia or Micromidia, but his study relied mainly on manual parsimony without a formal analysis. Ware et al. (2007) included S. gracilis in their molecular analysis, but its position within the monophyletic GSI assemblage was unstable due to low branch support. Monophyly of the genus itself, although seemingly very likely based on several morphological characters including uniform wing vein patterns and eye color, has not yet been supported by phylogenetic analysis.

The validity of the two new *Syncordulia* species, and the monophyly of the genus are assessed here in this molecular study. In addition, we attempt to determine the placement of *Syncordulia* within the GSI. Using these phylogenetic hypotheses, we estimate both divergence times and biogeographical history in order to better understand the distribution, ecology and conservation requirements of this relict genus.

2. Materials and Methods

Data collection

Specimens were collected in South Africa during the summer months of 2006 and 2007 (5 *S. serendipator*, 4 *S. legator* and 10 *S. venator*) and 2004 (4 *S. gracilis*). DNA was extracted using a Qiagen DNEasy kit with a modified protocol as described by Ware et al. (2007). Sequences from the GSI genera, *Austrocordulia, Austrophya, Lathrocordulia, Micromidia, Pseudocordulia, Cordulephya, Hesperocordulia, Oxygastra, Gomphomacromia, Macromidia,* and *Idionyx* were taken from Ware et al.'s (2007) molecular dataset. The corduliid taxon, *Hemicordulia tau,* and the macromiine taxa *Macromia illinoiensis* and *Phyllomacromia contumax* were also taken from Ware et al. (2007). We used members of the Chlorogomphidae, Chloropetaliidae, Cordulegastridae, and Neopetaliidae as outgroups. All amplified or downloaded taxa are listed in Table 1.

Alignment

We used CLUSTALX (Thompson et al., 1997) to perform a preliminary sequence alignment, with the default settings for gap costs (gap opening penalty=10.00; gap extension penalty=0.20). The resulting files were then aligned manually in Microsoft Word using the structural methods described in Kjer et al. (1995), Kjer (2004), Kjer et al. (2007) and secondary structure models based on Guttell et al. (1993). The resulting datafile contained three mitochondrial fragments from the COII gene (512 nts), the 12S (780 nts), and the 16S (435 nts), and three variable regions from the nuclear large subunit (28S), the D2 (480 nts) the D3 (369 nts) and the D7 fragment (567 nts).

Parsimony analyses

We performed parsimony analyses in PAUP (Swofford, 2000) under equal weighting of all molecular and morphological characters with 10,000 random addition sequence heuristic replicates under TBR branch swapping. Gaps of uniform length were treated as presence/absence characters; other gaps were treated as missing data. To estimate branch support, we ran 1000 parsimony nonparametric bootstrap replicates with 10 random addition sequence replicates under TBR branch swapping.

Bayesian analyses

In order to take into consideration the non-independence of hydrogen-bonded rRNA sites, we used the RNA7A seven state paired-sites model available in the PHASE program (Jow et al., 2002), to run Markov-chain Monte Carlo (MCMC) analyses of partitioned RNA data (10 million generations each) and a REV model for the loop regions. Jow et al. (2002), show that this biologically realistic model is useful for studies of rRNA and it is particularly important in estimating divergence dates for rRNA (Ware et al., 2008). The REV model is the most general loop model with the time reversible constraint (four frequencies, five rate parameters).

R8S analyses

We used a PHASE tree to run a divergence estimation analysis in R8S (Sanderson, 2003) using penalized likelihood (Sanderson, 2002), a method that applies a penalty against large-rate changes between neighboring branches. We optimized the smoothing

parameter using cross-validation analysis. Dates and substitution rates were estimated using the truncated Newton algorithm with 10 independent starts. In order to calibrate the divergence estimation analysis, we used fossil data from Grimaldi and Engel (2005), Nel et al. (1993), Nel and Paicheler (1994) and Jarzembowski and Nel (1996) (see Table 2 for a listing of the fossils we used to determine date constraints).

DIVA analyses

Using the program DIVA (Ronquist, 1996, 1997), we assessed ancestral distributions while differentiating possible vicariance and dispersal events. Since DIVA requires a fully bifurcating tree, we manually resolved the polytomy at the base of the tree for the topology (*Neopetalia*(Cordulegastridae(remaining taxa). Each taxon was assigned a distribution based on the species' collection data, Dijkstra et al. (2007) and Davies and Tobin (1985).

Taxa were scored for presence or absence in one or more of ten regions South America (A), North America (B), India/Asia (C), Europe (D), Australia (E), Africa (outside of South Africa) (F), South Africa: South-Western Cape (G), South Africa: North-Western Cape (H), South Africa: slightly east of the Hex River mountains (I), South Africa: South East (J) (Table 3)

3. Results

Phylogenetic hypotheses

The dataset had a total of 3148 characters, of which 2057 were constant, 661 were variable but parsimony uninformative, and 430 were parsimony informative. The most parsimonious tree had a length of 2334 steps. A consensus of 131 trees from the parsimony heuristic search analysis is shown in Figure 1, with bootstrap support from the separate bootstrap analysis above each branch. A consensus tree is shown from the ten million generation (20,000 trees) PHASE analysis in Figure 2, with posterior probabilities shown above each branch (we ran 11 million generations and discarded a burnin of 1 million generations). The genus *Syncordulia* was found to be monophyletic with strong bootstrap support (96%) and posterior probabilities (97%), as were all the individual species except *S. legator*, which received low to moderate support (65% posterior probability and 87% bootstrap support).

The Australian taxa, *Cordulephya, Pseudocordulia, Hesperocordulia, Austrocordulia, Micromidia, Lathrocordulia,* and *Austrophya* form a monophyletic clade, but with <50% bootstrap support and only 75 % posterior probability. This result was not found by Ware et al, (2007), with a larger GSI taxon sample. The South American taxon, *Gomphomacromia* and the Indomalayan *Macromidia* form a clade, but with low posterior probability (40%) and bootstrap support (<50%). The European *Oxygastra* and Indomalayan *Idionyx* form a monophyletic group with 95 % posterior probability but low bootstrap support (<50%).

Divergence estimation

The tree used in the R8S analysis is shown in Figure 3 and corresponding divergence estimates in Table 2. The R8S analysis recovered divergence estimates for the root of tree

at 200.53 Mya (Figure 4). The higher Libelluloidea (*sensu* Ware et al., 2007; GSI + Macromiidae + Corduliidae + Libellulidae) divergence estimate was 169.56 Mya. The GSI diverged 106.60 Mya. *Syncordulia* was estimated to have diverged from other Libelluloidea 59.58 Mya, with *S. gracilis* diverging from its ancestor 59.57 Mya, *S. serendipator* diverging from its ancestor 55.34 and *S. venator* + *S. legator* diverging from their ancestor 44.85 Mya.

DIVA biogeographical analysis

DIVA optimizations were conducted with either an unrestricted number of areas assigned to each node, or with areas per node restricted to from one to seven regions. The unrestricted DIVA analysis suggested that the ancestors to the taxa in the tree occurred in each of the ten geographical regions. The DIVA analysis resulted in multiple combinations of optimal reconstructions, and a total of 17 dispersal events. The ancestral distribution for the taxon at the root of the tree is ambiguous, but constraining the optimization to two regions resulted in an ancestral distribution that was South American and Indomalayan or North American and South American, (as opposed to North American, South American, Indomalayan, European, Australian and African, when optimization was constrained to 5 regions, and the widespread ancestral distribution when no constraints were used). When the maximum number of ancestral nodes was restricted to two, the ancestor to the 'higher Libelluloidea' has an Indomalayan distribution (Figure 4). When the optimization was constrained to 1-7 areas (i.e., the maximum number of ancestral areas possible at each node was constrained to a finite number), the ancestral distribution of Syncordulia was estimated to be in the Southwestern Cape of South Africa; with no constraints three

distribution possibilities were recovered: (a) Southwestern Cape, (b) Southwestern Cape and Southwestern Cape east of the Hex River mountains, and (c) all 4 South African locations. Current distributions of *Syncordulia* species are shown in Figure 5.

4. Discussion

The recovery of monophyletic clades for each of the *Syncordulia* species supports the morphological findings of Dijkstra et al. (2007). Corroborating the validity of each of the species may be important for conservation efforts given the Vulnerable Red List status of *S. gracilis* and *S. venator*. Both are sensitive to the presence of alien trees, and some populations have increased following removal of alien pine (Samways, 2007), as has a population of *S. legator* (Simaika and Samways, 2008). Preliminary evidence suggests that the two newly-discovered species are also threatened, with *S. legator* particularly susceptible to climate change (Samways, 2008).

Previous divergence analysis by Ware et al. (2008) did not include taxa from the GSI. The dates they recovered for the root of their Libelluloid tree using a paired-site evolutionary model were similar to the dates recovered in the present analysis (205 Mya in their analysis vs. 201 Mya in our analysis). The date they recovered for the divergence of Macromiidae + Corduliidae is also similar (20 million years younger than the date we recovered here, a difference of 10%). This might be due to a difference in taxon sample (*Macromia* is the only taxon from these families in common between our analyses). Although the DIVA analysis with 2 scenarios suggested an Indomalayan ancestral distribution for the higher Libelluloidea, the ancestor to this group is estimated to have diverged approximately 169.56 Mya, at a time when the continents were in much closer proximity to one another, and it is likely that they were present on all continents. Indeed, an unconstrained optimization in DIVA suggests 17 scenarios including a scenario with higher libelluloid presence in every geographic region. Similarly, the ancestor to Macromiidae + Corduliidae had a North American and Indomalayan or an African and Indomalayan distribution in our 2 region optimization, and optimization with no constraints recovered 6 scenarios, including presence in Indomalayan, African, North American, and Australian regions. It is possible that this ancestor's distribution may indeed have been more widespread, since these families are estimated to have diverged from other Libelluloidea at a time when the continents were in propinquity. Since Macromiidae and Corduliidae were simply used as outgroups in our analysis, however, there are probably not enough taxa to make realistic conclusions about the origins of these groups.

The R8S analysis suggests that the GSI diverged 109 Mya. This corresponds with the breakup of Gondwanaland, during the early Cretaceous (e.g., Veevers, 2004) Shortly after this, Australia, which sits on the Indo-Australian plate (e.g., Hillis and Reynolds, 2000) began to break away from Antarctica and Southern Gondwanaland. This vicariant event may have led to the isolation and speciation of the clade of Australian taxa, which were estimated to have diverged approximately 65 Mya. The DIVA optimization (with no constraints, or constrained to 1-7 regions) estimated that the ancestor to these taxa occurred in Australia, suggesting no further intercontinental dispersal events leading to the distribution of present taxa as defined here. The South African, Indomalayan, European, and South American taxa (*Syncordulia, Idionyx, Macromidia, Oxygastra* and *Gomphomacromia*,) were estimated to have diverged from their ancestor approximately

77 Mya, after Gondwanaland and Laurasia each began to break apart (e.g., Veevers, 2004). Support for the *Idionyx* and *Oxygastra* clade is surprisingly strong (95 % pp) considering that *Idionyx* has previously been suggested to be a close relative to *Macromia* and *Macromidia* (Lieftinck, 1971). Lieftinck (1971) suggested that *Macromidia* 'bridged the gap' in evolution between *Idionyx* and *Macromia* due to larval and adult morphological characters. *Idionyx* and *Oxygastra* are estimated to have diverged approximately 57 Mya.

India split from Africa and began its journey towards southern Asia around 80 Mya, eventually colliding with Eurasia approximately 50 million years ago (e.g., Zhu et al., 2005). The impact of this collision created large mountain ranges that may have isolated the ancestors to *Idionyx* and *Macromidia* and led to their divergence. The DIVA analysis suggests that the ancestor to *Oxygastra* was only present in the European region.

The divergence estimation for *Syncordulia* suggests that the genus arose during the transition from the late Cretaceous period to the Cenozoic, in agreement with Dijkstra et al.'s (2007) suggestion from morphology that the genus arose well after the break-up of Gondwanaland (>120 Mya). This hypothesis is further strengthened by evidence on the dispersal of several fynbos plant taxa, the Restionaceae, Proteae and Geissolomataceae, that are suggested to have dispersed over a proto-Indian Ocean, that was formed during the break-up of Gondwanaland (Galley and Linder, 2006).

The divergence date for the *Syncordulia* clade, 60 Mya, corresponds with the divergence range of the Restionaceae (60-70 Mya), Proteae (60-70 Mya) and Geissolomataceae (81 ± 5 Mya) (Galley and Linder, 2006). The close ecological relationship between *Syncordulia* and the architecture of these fynbos plants might

explain the strong negative impact of alien tree species on the group. The DIVA analysis constrained to two maximum areas supports an ancestral distribution for *Syncordulia* restricted to the South-western Cape of South Africa, which would imply that the current distributions of *Syncordulia* were due to dispersal. Unconstrained DIVA optimization recovers an ancestral distribution that was more widespread across South Africa. With the difficulty of conserving marginal populations (Samways, 2003), particularly in El Niño-prone eastern regions of Southern Africa, it is critically important that conservation of the relict populations of this genus in the CFR be given high priority in conservation management of the region.

In summary, *Syncordulia* is recovered in a deeply nested position in the phylogeny, as a highly derived genus well within the GSI clade. *Syncordulia* is a monophyletic genus, and its four species are each monophyletic taxa. The two newly proposed species of *Syncordulia* should be assessed further in regards to their IUCN status. This will undoubtedly be of use in shaping the future of these taxa. Population studies may provide useful information about the amount of gene flow that is occurring between the often fragmented and isolated clusters of each of the four species' populations

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References

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 vol. 2**.

Bridges, C.A., 1994. Catalogue of the family-group, genus-group and species-group names of the Odonata of the world (Third Edition). C. A. Bridges, Urbana, Illinois, USA.

Bybee, S.M., Ogden, H.T., 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*, **23**, 1-38.

Carle, F.L., 1982. A contribution to the knowledge of the Odonata. Unpublished Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Carle, F.L., 1995. Evolution, taxonomy, and biogeography of ancient Gondwanian libelluloides, with comments on anisopteroid evolution and phylogenetic systematics (Anisoptera: Libelluloidea). *Odonatologica*, **24**, 383-506.

Carle, F.L., Kjer, K.M., 2002. Phylogeny of *Libellula* Linnaeus (Odonata: Insecta). *Zootaxa*, **87**, 1-18.

Carvalho, A.L., Salgado, L.G.V., Werneck-de-Carvalho, P.C., 2004. Description of a new species of Lauromacromia Geijskes, 1970 (Odonata: Corduliidae) from Southeastern Brazil. *Zootaxa*, **666**, 1–11.

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*, *Rapid Communication* (Suppl.) No. 5., Utrecht.

Dijkstra, K-D., Samways, M.J., Simaika, J.P., 2007. Two new relict *Syncordulia* species found during museum and field studies of threatened dragonflies in the Cape Floristic Region (Odonata: Corduliidae). *Zootaxa*, **1467**, 19-34.

Fraser, F.C., 1957. A Reclassification of the Order Odonata. Royal Zoological Society of New South Wales: Sydney, Australia.

Galley, C., H.P. Linder, 2006. Geographical affinities of the Cape flora, South *African Journal of Biogeography*, **33**, 236-250.

Garrison, R.W., von Ellenrieder, N., Louton, J.A., 2006. Dragonfly genera of the New World. An illustrated and annotated key to the Anisoptera. The Johns Hopkins University Press, Baltimore, USA.

Geijskes, D.C., 1970. Generic characters of the South American Corduliidae with descriptions of the species found in the Guyanas. *Notes on Odonata of Surinam 11. Studies of the Fauna of Suriname and other Guyanas*, **12**, 1-42.

Gloyd, L. K., 1959. Elevation of the *Macromia* group to family status (Odonata). *Entomological News*, **70**, 197-205.

Grimaldi, D., Engel, M.S., 2005. *Evolution of the Insects*, Cambridge University Press, New York, USA.

Guttell, R.R., Gray, M.W., Schinare, M.N., 1993. A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. *Nucleic Acids Research*, **21**, 3055-3074.

Hagen, H., 1861. Synopsis of the Neuroptera of North America, with a List of the South American species. Smithsonian Institute, Washington, D. C., USA.

Hillis, R.R., Reynolds, S.D., 2000. The Australian Stress Map. *Journal of the Geological Society*, **157**, 915-921.

IUCN, 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland, and Cambridge, U.K.

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.

Jow, H., Hudelot, C., Rattray, M., Higgs, P.G., 2002. Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution, *Molecular Biology and Evolution*, **19**, 1591–1601.

Kirby, W.F., 1890. *A synonymic catalogue of Neuroptera Odonata*. Guerney and Jackson, London, UK.

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**, 314-330.

Kjer, K.M., 2004. Aligned 18S and insect phylogeny. Systematic Biology, 53, 506-514.

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.

Letsch, H., 2007. Phylogeny of Anisoptera (Insecta: Odonata): promises and limitations of a new alignment approach. PhD. Thesis, Universität Bonn, Bonn, Germany.

Lieftinck, M.A., 1960. Notes on the affinity and nomenclature of some Old World Corduliidae (Odonata). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C Zoology*, **64**, 410-23.

Lieftinck, M.A., 1971. Studies in Oriental Corduliidae (Odonata). 1. *Tijdschrift voor Entomologie*, **114**, 1-63.

Lieftinck, M.A., 1977. New and little known Corduliidae (Odonata: Anisoptera) from the Indo-Pacific region. *Oriental Insects*, **11**, 157-159.

Lohmann, H., 1996. Das phylogenetische system der Anisoptera (Insecta: Odonata). Unpublished Diploma Thesis at the Biological Faculty of the Albert-Ludwigs-University in Freiburg i.Br., Germany.

Martin, R., 1914. Odonata, Fam. Libellulidae, subfam. Corduliinae. *Genera Insectorium*, **155**:1-31.

May, M.L., 1991. A review of the genus *Neocordulia*, with a description of *Mesocordulia* subgen. nov. and of *Neocordulia griphus* spec. nov. from Central America, and a note on Lauromacromia (Odonata: Corduliidae). *Folia Entomologia Mexicana*, **82**, 17-67.

May, M.L., 1995a. Comparative notes on micropyle structure in "cordulegastroid" and "libelluloid" Anisoptera. *Odonatologica*, **19**, 53-62.

May, M.L., 1995b. A preliminary phylogenetic analysis of the "Corduliidae". - 13th *International Symposium of Odonatology Essential Abstracts*: 36.

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.

Needham, J.G., 1908. Critical notes on the classification of the Corduliinae (Odonata). *Annals of the Entomological Society of America*, **1**, 273-280.

Nel, A., Martínez-Delclòs, X., Paicheler, J. C., Henrotay, M., 1993. Les 'Anisozygoptera' fossiles. Phylogénie et classification (Odonata). *Martinia*, Numéro hors-série, **3**, 1–311.

Nel, A., Paicheler, J.-C., 1994. Les Libelluloidea fossiles autres que Libellulidae. Un inventaire critique (Odonata, Corduliidae, Macromiidae, Synthemistidae, Chlorogomphidae et Mesophlebiidae). *Nouvelle Revue d'Entomologie*, (N.S.), **11**, 321–334.

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

Rambur, M.P., 1842. Neuropteres. Histoire naturelle des Insectes, Paris, France.

Ronquist, F., 1996. DIVA, ver. 1.1. Computer program available at http://www.ebc.uu.se/systzoo/research/diva/diva.html. Uppsala University, Uppsala.

Samways, M.J., 2003. Marginality and national red listing of species. *Biodiversity and Conservation*, **12**, 2523-2525.

Samways, M.J., 2008. Dragonflies as focal organisms in contemporary conservation biology. In: *Dragonflies: Model Organisms for Ecological and Evolutionary Research*. (ed. A. Córdoba-Aguilar). Oxford University Press, Oxford, UK. pp. 97-108.

Samways, M.J., 2007. National Red List of South African dragonflies (Odonata). *Odonatologica*, **35**, 341-368.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach, *Molecular Biology and Evolution* **19**, 101–109.

Sanderson, M.J., 2003. R8S; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. *Bioinformatics* **19**, 301–302.

Selys-Longchamps, E. de., 1871. Synopsis des Cordulines. *Bulletin de l' Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, Bruxelles*, **31**, 238-565.

Selys-Longchamps, E. de, 1882. Note sur le genre Gomphomacromia Brauer. *Comptes*rendus des Séances de la Société entomologique de Belgique, **3**, clxvi–clxix.

Selys-Longchamps, E de, 1892. Causeries Odonatologiques. No. 6. Les Gomphines d'Afrique. *Annales de la Société Entomologique de Belgique* 36, 86–107.

Simaika J.P., Samways M.J., 2008. Valuing dragonflies as service providers. In: *Dragonflies: Model Organisms for Ecological and Evolutionary Research*. (ed. A. Córdoba-Aguilar). Oxford University Press, Oxford, UK. pp 109-123.

St. Quentin, D., 1939. Die systematische stellung der unterfamilie der Corduliinae Selys (Ordung Odonata). *Proceedings of the International Congress of Entomology 7 (Berlin)*, **1**, 345-360.

Steinmann, H., 1997. World catalogue of Odonata, Anisoptera. W. Gruyter. v. 2, Berlin, Germany.

Swofford, D.L., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

Theischinger, G., Watson, J.A.L., 1978. The Australian Gomphomacromiinae (Odonata : Corduliidae). *Australian Journal of Zoology*, **26**, 399-431.

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.

Thompson J.D., Gibson T.J., Plewniak F, Jeanmougin F., Higgins D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876-4882.

Tillyard, R.J., 1917. *The Biology of Dragonflies*. Cambridge University Press, Cambridge, UK.

Tillyard, R.J., 1928. The evolution of the order Odonata. Part I. Introduction and early history of the order. *Records of the Indian Museum*, **30**, 151-172.

Titus, T.A., D.R., Frost, 1996. Molecular homology assessment and phylogeny in the lizard family Opluridae (Squamata: Iguania). *Molecular Phylogenetics and Evolution*, **6**, 49-62.

Viana, M.S., Neumann, V.H.L., 1999. The Crato member of the Santana formation, Ceará state, Brazil. *In*: Schobbenhaus, C.; Campos, D.A.; Queiroz, E.T.; Winge, M.; Berbert-Born, M. (Edit.) *Sítios Geológicos e Paleontológicos do Brasil.* http://www.unb.br/ig/sigep/sitio005/sitio005english.htm, last viewed May 8, 2008.

Veevers, J.J., 2004. Gondwanaland from 650-500 Ma assembly through 320 Ma merger in Pangea to 185-100 Ma breakup: supercontinental tectonics via stratigraphy and radiometric dating, *Earth-Science Reviews*, **68**, 1-132.

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.

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.

Zhu, B., Kidd, W.S.F., Rowley, D.B., Currie, B.S., Shafique, N., 2005. Age of initiation of the India-Asia collision in the east-central Himalaya. *Journal of Geology*, **113**, 265–285.

Table	1	Taxon	sample	e from	the	nresent study	
Iaur	1.	галоп	Sampr		unc	present study	

Taxon Name	GenBank Accession Numbers 28S, (D7, D3, D2), 16S, 12S, COI	Collection in	formation				
Syncordulia gracilis	123456	<i>Date</i> 2004	<i>Sex</i> Female	<i>Habitat</i> In grassland away from water			
Syncordulia gracilis	123456	2004	Female	In grassland away from water			
Syncordulia legator	123456	18/10/2006	Female	Open montane stream with riffles, glides and deposition zones			
Syncordulia legator	123456	18/10/2006	Female	Open montane stream with riffles, glides and deposition zones			
Syncordulia venator	123456	11/11/1999	Female	Open montane stream with boulders			
Syncordulia venator	123456	16/11/2006	Male	Open montane stream with boulders			
Syncordulia venator	123456	16/11/2006	Male	Open montane stream with			
Syncordulia venator	123456	14/11/2006	Female	Open montane stream with			
Syncordulia serendipator	123456	18/02/2006	Male	Open montane			
Syncordulia serendipator	123456	19/03/2006	Male	stream with boulders Open montane stream with			
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Syncordulia serendipator	123456	11/03/2006	Male	boulders Open montane stream with			
Syncordulia serendipator	123456	11/03/2006	Female	boulders Open montane stream with boulders			
Sequences downlo	aded from GenBank a	and novel COI o	lata				
1	28S (D7, D3, D2),	COI Accession	n Numbers				
	16S and 12S						
	Accession Numbers						
Anotogaster	D7:N/A	N/A					
sieboldii	D3: N/A						
	D2: N/A 12S&16S:						
	AB127061						
Austrocordulia	D7: EF631243	N/A					
refracta	D3: EF631336						
5	D2: EF631448						
	16S: EF631559						
Austrophya	D7: EF631236	N/A					
mystica	D3: EF631332						
, ,	D2: EF631441						
	16S: N/A						
Chlorogomphus	D7: N/A	N/A					
brunneus	D3: N/A						
	D2: N/A						
	12S&16S:						
	AF266088						
Chloropetalia	D7: EF631248	N/A					
soarer	D3: EF631339						
	D2: EF631453						
	12S&16S:N/A						
Cordulephva	D7: EF631255	123456					
pygmaea	D3: EF631346						
	D2: EF631460						
	16S: EF631570						
Cordulegaster	D7: N/A	N/A					
boltoni	D3: N/A						
	D2: N/A						

	12S&16S:	
	AF266056	
Cordulegaster	D7: N/A	N/A
dorsalis	D3: N/A	
	D2: N/A,	
	12S&16S:	
	AY282558	
Cordulegaster	D7: N/A	N/A
picta	D3: N/A	
	D2: N/A	
	12S&16S:AF266086	
Gomphomacromia	D7: EF631206	N/A
sp.	D3: EF631303	
	D2: EF631408	
	16S: EF631522	
Hemicordulia tau	D7: EF631233	N/A
	D3: EF631328	
	D2: EF631437	
	16S: EF631550	
Hesperocordulia	D7: EF631244	N/A
berthoudi	D3: EF631337	
	D2: EF631449	
	16S: EF631560	
Idionyx selysi	EF631193	123456
	EF631290	
	EF631391	
	N/A	
Kalyptogaster	D7: EF631245	123456
erronea	D3: N/A	
	D2: EF631450	
	12S&16S:EF631561	
Lathrocordulia	D7: EF631239	N/A
metallica	D3: EF631334	
	D2: EF631444	
	16S: EF631556	
Macromia	D7: EF631208	N/A
illinoiensis	D3: EF631305	
	D2: EF631410	
	12S&16S:	
	EF631524	
Macromidia	D7: EF631209	N/A
rapida	D3: EF631306	
	D2: EF631411	
	12S&16S: NA	
Macromidia	D7: EF631271	N/A
rapida	D3: EF631362	

	D2: EF631478 12S&16S: EE621588	
Micromidia	$D7 \cdot FF631240$	N/Λ
atrifrons	$D^{2} \cdot N/\Lambda$	11/17
unijions	D3. N/A D2. EE631///5	
	16S. EE631557	
Noopotalia	$D7 \cdot EE621247$	NI/A
neopeiuliu	$D_{1}^{2} = E_{1}^{2} = E_{1$	1N/A
punciulu	D3. EF031336	
	128 & 168	
	T25&105. EE621562	
Omogetra aurticii	$D7 \cdot N/A$	NI/A
Oxygusiru curiisii	D/. IN/A D2: N/A	1N/A
	D3. IN/A D2: EE621/12	
	168. EE621526	
Omogetra aurticii	$D7 \cdot N/A$	NI/A
Oxygustru curtisti	D N/A	1N/A
	DJ: N/A DJ: N/A 12S&16S.	
	ΔΕ266103	
Phyllomacromia	D7: FF631197	N/Δ
contumar	D7: EF631293	1 1/21
сотитах	D3: EF 631295	
	128 & 168	
	EF631511	
Pseudocordulia	D7: EF631251	N/A
circularis	D3: EF631342	1 1/2 1
	D2: EF631456	
	16S [•] EF631566	
Sinorogomphus	D7 [·] EF631249	N/A
sp	D3 [•] EF631340	1 1 1 1
-P.	D2 [•] EF631454	
	12S&16S	
	EF631564	
Taeniogaster	D7: EF631216	N/A
obliqua	D3: EF631312	
	D2: FE631/20	
	128&168: EF631533	

Table 2. R8S analysis divergence estimates. 250 million year age constraint based on oldest known anisopteran fossils, which are from the Triassic; 120 million year age constraint based on *Araripelibellula* Nel and Paicheler (1994) and the age of the Chiapada do Araripe formation, Viana and Neumann (1999); 118 million year age constraint based on the Jarzembowski and Nel (1996) and dates from the Wealden formation.

Node	Constraints (mil	Age (millions of years)		
	Minimum	Maximum		
Root	120.00	250.00	200.53	
Cordulegastridae			173.73	
Chlorogomphidae +			110.60	
Chloropetaliidae				
Higher Libelluloidea	120.00	250.00	169.56	
Macromiidae +	118.00		153.08	
Corduliinae				
Gomphomacromiinae			108.60	
+ Synthemistinae +				
Idionychinae (GSI)				
Non-Australian GSI			76.90	
taxa				
Gomphomacromia +			62.59	
Macromidia				
Idionvx + Oxvgastra			56.69	
Australian GSI taxa			65.42	
(Austrocordulia.				
Cordulephva.				
Pseudocordulia.				
Hesperocordulia.				
Micromidia.				
Lathrocordulia.				
Austrophya)				
Svncordulia genus			59.58	
S. gracilis			59.57	
S. serendinator			55 34	
S_{i} legator + S_{i}			44 85	
venator			11.00	
Summary of rate	Mean	Min	Max	
variation				
(substitutions per				
site per unit time)				
•	3.294e+16	2.023e+16	4.356e+16	

J: South- eastern South Africa	S. venator							
I: South- west Cape, east of Hex River mountai	S. gracilis							
H: North -west Cape	S. legator							
G: South- west Cape	S. venator	S. serendipator	S. legator	S. gracilis				
F: Africa (outside South Africa)	Phyllomacromia contumax							
E: Australia	Hemicordulia tau	Austrocordulia refracta	Cordulephya pygmaea	Pseudocordulia circularis	Micromidia atrifrons	Hesperocordulia berthoudi	Lathrocordulia metallica	Austrophya mystica
D: Europe	Cordulegaster boltoni	Cordulegaster picta	Oxygastra curtisii					
C: India/Asia	Anotogaster sieboldii	Chloropetalia soarer	Chlorogomphu s brunneus	Sinorogomphu s sp.	Hemicordulia tau	Macromidia rapida	Idionyx selysi	
B: North America	Taeniogaster oblique	Kalyptogaste r erronea	Macromia illinoiensis	Cordulegast er dorsalis				
A: South America	Neopetalia punctata	Gomphomacro mia sp.						
	A: South B: North C: D: Europe E: Australia F: Africa G: South H: I: J: America America India/Asia (outside west North South South South South Cape -west west eastern South Cape -west west eastern Africa) Cape Cape, South East of Africa Hex River mountai	A: SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: South-H:I:J:AmericaIndia/AsiamestNorthSouthwestNorthSouth- <td>A: SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:J:AmericaIndia/AsiaIndia/Asia(outsidewestNorthSouth-S</td> <td>A: SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: South-H:I:J:AmericaIndia/AsiaIndia/Asia(outsidewestNorthSouth-</td> <td>N:SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:I:I:I:AmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthSouthAmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthSouthAmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthAmericaAmericaAfricaAfricaCapeeast ofAfricaNopealiaTaenogaserAnogaserCordulegaserHemicorduliaPhylomacromiaS. venatorI.AmericaKalypogaseColoropealiaDotomiCondulegaserAustroconduliaS. venatorS. seredipatorS. seredipatorAmericaKaroniaColoropealiaCondulegaserAustroconduliaS. venatorS. seredipatorS. seredipatorAmericaSubsocaseCondulegaseCondulegaseCondulegaseMarcongaseS. seredipatorS. seredipatorAmericaSubsocaseCondulegaseSubsocaseCondulegaseS. seredipatorS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseCondulegaseCondulegaseS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseCondulegaseCondulegaseS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseSubsocaseS. seredip</td> <td>N:SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:J:AmericaIndia/AsiaPatraSouthwestNorthSouthSouthSouthSouthSouthAmericaIndia/AsiaPPPWestNorthSouth<td< td=""><td>A:South B: North C: D: Europe E: Australia F: Africa G: South- H: I: J: America America India/Asia Curope E: Australia F: Africa G: South- B: west South- South-</td><td>A::South B::North C:: D::Europe E:.Australia F:.Africa G::South H:: I: I:</td></td<></td>	A: SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:J:AmericaIndia/AsiaIndia/Asia(outsidewestNorthSouth-S	A: SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: South-H:I:J:AmericaIndia/AsiaIndia/Asia(outsidewestNorthSouth-	N:SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:I:I:I:AmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthSouthAmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthSouthAmericaAmericaIndia/AsiaP: EuropeE: AustraliaF: AfricaSouthSouthSouthAmericaAmericaAfricaAfricaCapeeast ofAfricaNopealiaTaenogaserAnogaserCordulegaserHemicorduliaPhylomacromiaS. venatorI.AmericaKalypogaseColoropealiaDotomiCondulegaserAustroconduliaS. venatorS. seredipatorS. seredipatorAmericaKaroniaColoropealiaCondulegaserAustroconduliaS. venatorS. seredipatorS. seredipatorAmericaSubsocaseCondulegaseCondulegaseCondulegaseMarcongaseS. seredipatorS. seredipatorAmericaSubsocaseCondulegaseSubsocaseCondulegaseS. seredipatorS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseCondulegaseCondulegaseS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseCondulegaseCondulegaseS. seredipatorS. seredipatorAmericaSubsocaseSubsocaseSubsocaseS. seredip	N:SouthB: NorthC:D: EuropeE: AustraliaF: AfricaG: SouthH:I:J:AmericaIndia/AsiaPatraSouthwestNorthSouthSouthSouthSouthSouthAmericaIndia/AsiaPPPWestNorthSouth <td< td=""><td>A:South B: North C: D: Europe E: Australia F: Africa G: South- H: I: J: America America India/Asia Curope E: Australia F: Africa G: South- B: west South- South-</td><td>A::South B::North C:: D::Europe E:.Australia F:.Africa G::South H:: I: I:</td></td<>	A:South B: North C: D: Europe E: Australia F: Africa G: South- H: I: J: America America India/Asia Curope E: Australia F: Africa G: South- B: west South-	A::South B::North C:: D::Europe E:.Australia F:.Africa G::South H:: I:



Figure 1. Strict consensus tree from a PAUP parsimony heuristic search; 10,000 addition sequence replicates; bootstrap support shown above branches.



Figure 2. Consensus tree from a PHASE analysis; 10 million generations. Posterior probabilities shown above branches.



Figure 3. R8s analysis on a 26 taxon tree; Geological maps adapted from figures on rst.gsfc.nasa.gov



Figure 4. Ancestral distributions; DIVA analysis optimized with 2 regions; Larger letters indicate the scenarios discussed in the text.

Figure 5. Present distributions of *Syncordulia* species in South Africa. Uppermost box shows the distributions of all *Syncordulia* species, lower boxes show individual species distributions: *S. gracilis* (top and top left); *S. legator* (top right); *S. serendipator* (bottom left); *S. venator* (bottom right).



Chapter 4:

Phylogeny, homoplasy and divergence estimates within Libelluloidea (Anisoptera: Odonata): an exploration of the usefulness of molecular and morphological characters

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Abstract

Libelluloidea is the most speciose group in Anisoptera, but its complicated phylogenetic history has been difficult to interpret. Using the most comprehensive morphological and molecular dataset to date (166 taxa, 100 morphological characters and 9,902 molecular characters), we examine phylogenetic hypotheses of libelluloid relationships using parsimony and doublet-model Bayesian analyses. Homoplasy in dragonfly wing-based morphological datasets have been previously identified as a source of disagreement between molecular and morphological topologies, but our data suggests that other morphological larval, penile, accessory genitalic and external body datasets, which are similarly prone to convergence, recover topologies in disagreement with molecular findings. The taxonomy of non-cordulegastrid Libelluloidea is revised to include just four families (Synthemistidae sen. nov, Macromiidae, Corduliidae and Libellulidae) with Libellulidae further subdivided into at least 4 subfamilies (Trameinae sen. nov, Sympetrinae sen. nov, Pantalinae sen. nov., and Libellulinae). We use our Bayesian results to estimate both divergence times and diversification rates. Our phylogenetic hypotheses and divergence estimates are discussed in relation to the success of Libelluloidea, with a focus on the evolution of exophytic oviposition.

1. Introduction

Libelluloidea comprises at least 4 taxonomic groups including the "River Cruisers" (Macromiidae), "Emeralds" (Corduliidae), the diverse "GSI complex" (*sensu* Ware *et al.*, 2007) and the most abundant and familiar dragonflies, "Skimmers/Chasers" (Libellulidae). Libellulidae are readily recognizable, often with colored or patterned wings and a boot shaped series of veins (the anal loop) in the hindwing. They are commonly seen in territorial flight around lakes and ponds, or perched along the bank.

Of the almost 2900 species in Anisoptera (Schorr et al., 2008), a little more than half are Libelluloidea, making it the most speciose taxon in Anisoptera. Several hypotheses have been suggested to explain the success of libelluloids. Libelluloids have reduced ovipositors, modified for exophytic oviposition, a trait that they share with the second most speciose family, Gomphidae. These two groups share some larval habitats, and many of the larvae burrow in aquatic sediments (larvae in the small family Petaluridae are semiterrestrial burowers). Burrowing may have opened new niches (since muddy sediments are present in different habitats), leading to the possible development of other correlated changes in morphology and behavior (Carle et al., 2008). The similarities between gomphids and libellulids have been treated as being due to common ancestry (e.g., Bechly, 1996), which suggests that their great diversity is due to a single event. Hennig (1981), Carle (1982a), and Carle and Kjer (2002), however, considered these similarities convergent, correlated, and triggered by lost characters. Misof et al., (2001), Letsch (2007), Ware et al. (2007) and Bybee (2008) recovered odonate phylogenies in which Gomphidae and Libelluloidea are not sister groups, implying that exophytic

109

oviposition and larval burrowing traits are indeed convergences that may have contributed to the high level of speciation and diversity in these families. Our understanding of what may have led to the success of Libelluloidea would be greatly improved with a strongly supported phylogenetic hypothesis of interfamilial and intergeneric relationships.

Among libelluloids, adult reproductive and feeding behavior, larval behavior, ecology (Corbet, 1999), and biogeography (Carle, 1995) vary widely and have been investigated intensively. A well-supported phylogenetic hypothesis is needed to understand the evolution of these traits, but little agreement exists about intergeneric relationships within each of the four taxonomic groups. Furthermore, most studies focusing on Libelluloidea have relied either entirely on molecular data (e.g., Ware *et al.*, 2007) or entirely on morphological data (e.g., Carle and Kjer, 2002). Bybee et al. (2008) recently incorporated several Libelluloidea in a study of Odonata using both molecular and morphological data, but because libelluloid relationships were not the main focus of this study, libelluloid taxon sample was sparse. Pilgrim and Von Dohlen (2008) reconstructed a phylogeny using morphological and molecular data but their focus was on the single family, Libellulidae.

Most previous morphological studies of libelluloid phylogeny have relied heavily on wing vein characters (Kirby, 1890; Needham, 1903; Martin, 1907; Needham, 1908; Ris 1909-1919; Tillyard, 1910; Needham and Broughton, 1927; Fraser, 1957; Gloyd, 1959; Geijskes, 1970; Lieftinck, 1971; Theischinger & Watson, 1978; Carle, 1982a; Davies and Tobin, 1985; Carle and Louton, 1994; Carle, 1995; Bechly, 1996; Lohmann, 1996a,b; Trueman, 1996; Jarzembowski and Nel, 1996; Carle and Kjer, 2002; Rehn, 2003; Pilgrim and von Dohlen, 2008; Bybee et al., 2008). Despite progress in understanding homologies in Odonata venation (e.g. Carle, 1982b; Riek and Kukolova-Peck, 1984), many wing vein characters may support convergent relationships when used to the exclusion of other characters (Hennig, 1969; Carle, 1982b; Ware et al., in prep). For example, Carle et al. (2008) found that reduced wing venation had resulted in the erroneous grouping of protoneurines and disparoneurines (Zygoptera). The subfamilies of Libellulidae recognized in recent catalogs (Davies and Tobin, 1985; Bridges, 1994; Steinmann, 1997), largely based on wing vein morphology, have rarely been recovered with molecular or total evidence studies (Ware et al., 2007; Pilgrim and Von Dohlen, 2008). Some studies have focused on egg, genitalic, flight musculature, color, or larval characteristics (St. Quentin, 1939; Gloyd, 1959; Lieftinck, 1971; Pfau, 1971; Theischinger and Watson, 1984; Pfau, 1991, 2005; May, 1995; Bechly, 1996; Lohmann, 1996a; Carle and Kjer, 2002). Pfau's (2005) study of sperm transfer mechanisms lead him to an alternate phylogenetic hypothesis that placed Cordulegastridae, Chlorogomphidae, and Neopetaliidae within Petaluroidea rather than Libelluloidea. Much of the current confusion over libelluloid taxonomy and phylogeny may be the result of uncertain character homology and independence (reviewed in Carle, 1982b).

Here we present the first large-scale phylogenetic hypothesis of the higher Libelluloidea that includes both morphological and molecular data. We incorporate data generated from three independent gene fragments, (mitochondrial large and small (12S and 16S) ribosomal RNA subunits and the protein-coding genes Cytochrome Oxidase I (COI) and Cytochrome Oxidase II, (COII); nuclear large and small ribosomal RNA subunits (18S and 28S); , and the nuclear protein coding gene Elongation Factor 1- α (EF1-α)). We scored 100 discrete morphological characters consisting of wing venation, external body structures, accessory genitalia, penile characters and larval structures. Extensive taxon sampling has allowed us to examine several regions of contention in the higher Libelluloidea and to evaluate the findings of recent phylogenetic hypotheses (Ware *et al.*, 2007; Pilgrim and Von Dohlen, 2008). This phylogenetic reassessment provides further information for improving the taxonomy of the historically difficult Libelluloidea while evaluating the usefulness of a wide variety of morphological characters commonly used in libelluloid systematics. We compare the homoplasy present in wing venation, accessory genitalic, penile, larval and external body characters. In addition, we use our combined phylogeny to estimate divergence times for each libelluloid node using the program r8s (Sanderson, 2003). With these dates, we discuss the ecology and behavior of several libelluloid taxa, and explore the factors that may have led to Libelluloidea's success.

2. Materials and Methods

Molecular sampling

Taxon sampling

We included all of the taxa sequenced by Ware *et al.* (2007) and also sequenced congeners of several taxa for which Ware *et al.* (2007) had sequenced only a single specimen. Our analysis had a total of 166 taxa (22 GSI taxa, 7 macromiids, 21 corduliids, and 116 libellulids). All taxa sequenced or downloaded from GenBank are listed in Table 1. Cordulegastridae, Chlorogomphidae, and Neopetaliidae served as outgroups, with the

tree rooted using *Chloropetalia soarer*; outgroup taxa consisted of 4 species from 4 cordulegastrid genera, 3 species from 3 chlorogomphid genera, and 1 species from the monotypic Neopetaliidae.

Gene Selection, DNA extraction and PCR amplification

Freshly collected dragonflies were used when possible; other taxa were obtained from private and museum collections. We amplified the second, third and seventh hypervariable (divergent) regions (D2, D3, and D7) of the 28S rDNA, the third domain of the mitochondrial 16S rDNA, and a fragment of COI. We downloaded COII, 12S, 18S, 28S and EF1- α sequences. These characters were included in the analysis and, where necessary, coded as missing for our taxa (for a discussion of missing data see Weins, 2005).

Muscle tissue was extracted using a Qiagen DNEasy tissue kit overnight at 55°C with 180 μL of ATL Buffer and 20 μL Proteinase-K. Older specimens (collected prior to 1980) were extracted with 40 μL (twice the suggested amount) of Proteinase-K buffer for several days (a suggestion made by R. Caesar, pers. comm.). All other steps followed the manufacturer's protocol. PCR primers, and their sources, are listed in Ware et al. (2007). Programs used for amplifications were (a) 96°C, 3 min; 94°C, 30s; 50°C, 30s; 72°C, 45s for 35-40 cycles; 72°C, 10 min and (b) 96°C, 3 min; 94°C, 30 s; 46°C, 30s, 72°C, 45s for 10 cycles; 94°C, 30s; 48°C, 40s; 72°C, 45s for 30 cycles; 72°C, 10 min. A Qiagen PCR purification kit was used to purify amplified product, which was then sequenced on an ABI 3100 capillary sequencer. Sequences from both strands were compared and edited in Sequence Navigator (Applied Biosystems). Lowercase letters were used to indicate nucleotides that were readable but difficult to interpret with certainty in either strand (i.e., there was competing background peaks). These lowercase letters were changed to uppercase letters only if there was agreement in the two complementary strands. When there was conflict about a single base call between reads from complementary strands, this nucleotide was coded with an R (for ambiguous purines), Y (for ambiguous pyrimidines) or N (for all other ambiguities).

Alignment

Initial sequence alignments were made using CLUSTAL–X (Thompson *et al.*, 1997) and the resulting files were then aligned manually in Microsoft Word using the structural methods described in Kjer (1995) and Kjer *et al.*, (2007), and secondary structure models based on Guttell *et al.* (1993). Ambiguously aligned regions were defined as single stranded regions with multiple insertions and deletions (indels) of variable length (and thus unclear nucleotide homology), bounded by hydrogen bonded base pairs. These regions were excluded from the dataset. For parsimony analyses, these characters were recoded as single multistate characters with the program INAASE (Lutzoni *et al.*, 2000), with the stepmatrices applied. Alignments are available on the Kjer lab website, www.rci.rutgers.edu/~entomology/kjer and original sequences are deposited in GenBank.

Morphological sampling

We collected morphological data from approximately 590 specimens in the Rutgers University Collection (RUC), the California Academy of Sciences (CAS), the American Museum of Natural History (AMNH), the National Museum of Natural History (NMNH) and the private collections of Mike May (MMC) and Ken Tennessen (KTC). Each specimen was given a voucher number and treated as a separate taxon; once data collection was completed, information from multiple specimens of a particular species was combined. When multiple specimens from a single species differed in their character state, we coded all possible states present in the specimens for that particular species.

Several taxa could not be amplified with molecular techniques but were still coded for morphological characters. The taxa for which we had only morphological data were Apocordulia macrops, Anatya guttata, Brachydiplax chalybea, Cannaphila vibex, Crocothemis divisa, Diastatops intensa, Didymops floridensis, Elasmothemis cannacriodes, Fylgia sp., Idomacromia proavita, Neocordulia batesi longipollex, Neocordulia campana, Neophya rutherfordi, Nephepeltia phyryne, Oligoclada walkeri, Orthetrum chrysis, Thermochoria equivocata, Trithemis sp., Urothemis sp., and Williamsonia fletcheri. We scored 28 wing-vein, 20 external body, 14 accessory genitalic, 14 penile, and 24 larval characters (Figure 1a and 1b show selected wing vein and larval characters). Terminology for venation followed Riek and Kukavlova-Peck. For our morphological character matrix we began with previously published characters from Needham and Broughton (1927), Fraser (1957), Miller (1991), Carle (1995), Needham et al., (2000), and Garrison et al., (2007), as well as unpublished characters from Tennessen (pers. comm.) and Carle (pers. comm.) (Table 7). We could not score all of the penile characters from Miller (1991) because we usually lacked fresh material and thus were unable to inflate the lobes of the penis; these characters were scored with a "?". Finally, we scored 10 characters that, to our knowledge, have not been used previously.

Phylogenetic reconstruction

The molecular and morphological data were analyzed separately using parsimony to evaluate which nodes were independently corroborated. The combined dataset was analyzed using parsimony and Bayesian criteria. For the parsimony reconstruction, a tree bisection-reconnection (TBR) branch swapping, 10,000 replicate heuristic search, was run using PAUP 4.0b10 (Swofford, 2001). Gaps of uniform length were each treated as presence/absence characters; other gaps were treated as missing data, except those encoded with INAASE, as described above. To estimate branch support, 1000 bootstrap pseudoreplicates (Felsenstein, 1985) were performed using 10 random addition searches per pseudoreplicate.

We used a doublet model (Schoöniger and von Haeseler, 1994) in MR. BAYES 3.1.2 in our Bayesian analyses, which allows modeling of nucleotide substitutions independently in stem and loop regions of ribosomal DNA (Huelsenbeck and Ronquist, 2002). An MCMC analyses was run for 20 million generations (with four chains: one cold and three hot). Each analysis had printfreq=500. Prior to maximum likelihood and Bayesian analyses, we used MODELTEST 3.06 Akaike weights (Posada and Crandall, 1998; Posada and Buckley, 2004) and DT-MODSEL (Minin *et al.*, 2003) to select an appropriate model of evolution for each of the non-ribosomal gene fragments. Both programs suggested a GTR + I + Γ model (Yang, 1994; Yang *et al.*, 1994; Gu *et al.*, 1995). Non-stem regions of the ribosome were also analyzed with the GTR + I + Γ model. Morphological characters were analyzed with the MK model (Lewis, 2001). After the analyses were completed, we verified that each run had stabilized using Tracer 1.4 (Rambaut and Drummond, 2007), and calculated the burn in regions. The analysis had a burn in of 30% for each chain.

R8s analyses

We used a doublet-model Bayesian tree based on our combined data to run a divergence estimation analysis in R8S (Sanderson, 2003) using penalized likelihood, a method that applies a penalty against large-rate changes between neighboring branches. We optimized the smoothing parameter using cross-validation analysis. Dates and substitution rates were estimated using the truncated Newton algorithm with 10 independent starts. In order to calibrate the divergence estimation analysis, we used fossil data from Grimaldi and Engel (2005), Nel *et al.* (1993), Nel and Paicheler (1994) and Jarzembowski and Nel (1996) (Table 2). To determine whether there were differences in diversification rates among taxa, we estimated the rate of diversification, $\hat{r_e}$, of each clade using the method of moment calculation from Magallón and Sanderson (2001), where e=extinction, t=time and n=diversity measure:

$$\hat{r}_{\varepsilon} = \left(\frac{1}{t}\right) \log[n(1-\varepsilon) + \varepsilon]$$

Accelerations in the net diversification rate were estimated using the ratio calculation from Roelants *et al.* (2007), where t=time:

$$Ratio = \frac{Diversification(post - split)}{Diversification(pre - split)} = \frac{\ln\left(\frac{5}{2}\right)/t_{2-5}}{\ln\left(\frac{2}{1}\right)/t_{1-2}} = 1.322\frac{t_{1-2}}{t_{2-5}}$$

3. Results

Maximum parsimony analyses recovered 700 most parsimonious trees of 11072 steps long. Our combined analysis included 10,002 characters of which 5783 were constant, 2332 were variable but parsimony uninformative and 1887 were parsimony informative. Results from our combined parsimony analysis are shown in Figure 2, a consensus of 700 trees; results from Bayesian doublet analyses are shown in Figure 3, which is a consensus of 14,001 trees. The most likely tree had a score of -63625.45. Bootstrap support is shown above the branches in Figure 2 and posterior probability support is shown above the branches in Figure 3. A morphology-only parsimony tree is shown in Figure 4 (1 most parsimonious tree; best tree was 1025 steps long), with bootstrap support listed above the branches.

Disagreement between analytical methods

Our parsimony analyses recovered a paraphyletic GSI complex, as also found in parsimony analyses run by Ware *et al.* (2007). Corduliidae and Macromiidae are recovered in a polytomy with Libellulidae in our parsimony analysis while the Bayesian analysis strongly supports Corduliidae as sister to Libellulidae (100% posterior probability). The Libellulidae is largely a polytomy in our parsimony analysis, and clades that are recovered are not consistent with those from our Bayesian analyses. Bayesian and parsimony analyses treat data differently, (e.g. Lewis, 2001), which likely accounts for the discrepancies between the results from each analysis method. Since our dataset is comprised of several ribosomal fragments, we have chosen to focus on the results from our Bayesian analyses because it implemented a more realistic model of evolution, treating paired-hydrogen bonded sites in these fragments independently. Unless otherwise noted, the results discussed below referred to our Bayesian analysis.

The GSI complex

As in previous molecular-based analyses (Ware *et al.*, 2007; Letsch, 2007) the GSI was supported as monophyletic. The subfamily Synthemistinae was recovered as monophyletic with strong posterior probability (100% posterior probability), but this clade included the non-synthemistid taxa Gomphomacromia and Archaeophya, both of which were previously considered to be members of the subfamily Gomphomacromiinae (Davies and Tobin, 1985). Letsch (2007) recovered a similar arrangement, with the additional inclusion of *Idomacromia*. There are several morphological characters that support the monophyly of Synthemistinae, including the number of cross veins between the costal braces, the number of cross veins in the midbasal space, the number of forewing Cubital-Anal cross veins, the arrangement of larval palpal teeth, and the presence of setae on the larval palpal teeth. There are 7 morphological characters (wing vein, external body, accessory genitalic, penile, and larval) and several molecular characters (16S and COI) that support the Synthemistinae + Gomphomacromia +Archaeophya clade. Few of these are true synapomorphies, however, and most are either homoplastic derived states, or plesiomorphies shared with Chlorogomphidae, Neopetaliidae and/or Cordulegastridae. The absence of a genital lobe, shared by Synthemistinae + Gomphomacromia, for example, is a plesiomorphy shared with Chlorogomphidae. The absence of setae on larval palpal teeth, another plesiomorphy,

shared by *Archaeophya* + *Gomphomacromia* + Synthemistidae, is additionally present in *Pseudocordulia*.

The remaining gomphomacromiine taxa and members of Idionychinae and Idomacromiinae (*sensu* Davies & Tobin, 1985) are recovered in two separate clades. *Idionyx,* and *Macromidia* are recovered as a monophyletic clade, with 95% posterior probability.

As in Ware *et al.* (2007), *Cordulephya* is recovered nested well within the GSI, in a clade comprising gomphomacromiine taxa, contra to its previous assignment in Corduliinae (Davies and Tobin, 1985). *Pseudocordulia* and *Cordulephya* are strongly supported as sister taxa (92% posterior probability). These taxa are present in a clade comprising the South African endemic *Syncordulia*, the holarctic *Oxygastra* and several other Australian endemic genera with 100% posterior probability.

Corduliine Corduliidae and Macromiidae

The monophyly of the Macromiidae is strongly supported with 100% posterior probability. In addition to several molecular characters from independent datasets, Macromiidae also share morphological traits, such as a large and erect anterior hamule (although this is likely a plesiomorphy retained in this group and *Lathrocordulia*). *Phyllomacromia* is a monophyletic genus (85%). The 2 species of *Macromia* are not recovered as sisters. *Macromia urania* has only 16S molecular data so the position of these taxa relative to the other macromiids may change with the inclusion of more data.

Corduliidae (*sensu* Ware *et al.*, 2007, i.e., Corduliinae) is similarly strongly supported. *Pentathemis* + *Aeschnosoma* are sister taxa, forming a clade which is sister to the remaining Corduliidae. Unlike in Ware *et al.* (2007), *Hemicordulia* is recovered as next most apical node, a position similarly found by Letsch (2007; although with a different corduliid taxon sample, and in exclusion of *Pentathemis* and *Aeschnosoma*).

Corduliidae are recovered as sister to Libellulidae with 100% posterior probability, a position supported by a small number of morphological characters. Several Corduliidae and Libellulidae share the arrangement of their postanal cells (two or more closed cells present), for example, and both possess lateral carinae on two or more abdominal segments.

Libellulidae

The family Libellulidae is recovered as monophyletic with 96% support. The support for this monophyly is 100% in preliminary analyses excluding taxa for which only morphological data is present (results not shown). There were 7 clades that were consistently recovered in Libellulidae (Clades A-G, Figure 3). Table 3 lists the clades in this analysis that were also supported by previous molecular and/or combined data studies

The restricted Tetrathemistinae found in Ware *et al.* (2007) is recovered with 100%. Urothemistinae is not well supported (47% posterior probability).

Tramea, Hydrobasileus, Tauriphila and *Miathyria,* all previously considered to be members of the subfamily Trameinae were recovered as a monophyletic assemblage along with the libelluline taxon *Dasythemis* (97% posterior probability). Other members of the Trameinae; *Tholymis, Zyxomma, Rhyothemis, Idiataphe,* and *Pantala,* were not recovered in this clade but were instead scattered throughout the Libellulidae. A restricted Libellulinae was recovered (80% posterior probability), with the remaining libelluline taxa scattered throughout the Libellulidae, which is consistent with Ware *et al.* (2007) and Pilgrim and von Dohlen (2008).

Homoplasy in the morphological and molecular datasets

The characters in our morphological matrix were largely homoplasious. Wing vein characters had consistency indices (CI) which ranged from high (1.0) to low (0.04), but only one of our wing vein characters (the number of cross veins between costal braces in forewing) had a CI of 1.0, and 94% had CI values that were less than 0.5.

The other morphological datasets had similarly low CI values. Two external body characters had CI's of 1.0 (the presence/absence of ventrolateral processes and the presence/absence of the auricle), but most had CI values lower than 0.3333. One genitalic character had a CI of 1.0 (the size of the genital lobe), but 42% had CI's lower than 0.1. None of the penile characters scored in our morphological matrix had CI's greater than 0.67 and 85% had CI's ≤ 0.5 . Two of the larval characters had CI's of 1.0, but 33% had CI's lower than 0.1.

We compared the rescaled consistency index (Table 4) for each character in our morphological matrix using an ANOVA run in EXCEL. The ANOVA failed to demonstrate significant variation among the RC values of wing, external body, accessory-genitalic, penile and larval characters (P=0.80).

Molecular characters had higher CI and RC values than the molecular characters. Six hundred molecular characters were parsimony informative. The overall CI of the molecular characters was 0.5742, and the rescaled consistency index (RC) was 0.5776.

The morphological dataset comprising 100 characters had lower mean CI (0.2715) and RC (0.1769) values.

Divergence estimation and diversification rates

The dates recovered in our analysis were largely in agreement with the small number of previous libelluloid dating studies (most nodes differ by <25%), although certain nodes differed to a greater degree (Ware *et al.*, 2008; Ware *et al.*, submitted; Table 2). The root of the tree was estimated to have an age in the late Triassic (approximately 214 Mya; Figure 6). The ancestor to GSI + the MCL complex (Macromiidae, Corduliidae and Libellulidae) diverged approximately 132 Mya, during the Cretaceous. The GSI are estimated to have diverged from other Libelluloidea 111 Mya, and the MCL apparently diverged from other Libelluloidea 101 Mya. This date is 30% lower/than previously recovered (Ware *et al.*, 2008). Within the MCL, Macromiidae is estimated to have diverged 93 Mya, Corduliidae 85 Mya, and Libellulidae 68 Mya. The date we recovered for macromiid divergence was much older than previously recovered (e.g., 63% different from Ware *et al.*, 2008), but this is likely due to our sampling of taxa deeper into the macromiid lineage, including several non-holarctic taxa such as *Phyllomacromia* and *Macromia urania*. The libellulid divergence estimate was 23% older in Ware *et al.* (2008; 88 My) than in our analysis (68 My).

Estimates of diversification rate are dependent on assumptions about extinction rates. The diversification rate for Libelluloidea was estimated and used for comparison purposes ($r_0 = 0.0127$, $r_{0.95} = 0.0075$). Diversification estimates were lowest in Cordulegastridae ($r_0 = 0.0107$, $r_{0.95} = 0.0034$) and highest in Libellulidae ($r_0 = 0.0328$, $r_{0.95}$ =0.0219) (Table 5). The diversification rate for Libelluloidea as a whole may be lower than the actual value, since we estimated it with the age of oldest known Anisopteran fossil rather than the date from the split of Libelluloidea from it's closest sister group, since this has to yet been determined. Acceleration rates of net diversification were lowest in Cordulegastridae (0.0044) and highest in GSI (0.2998) and Libellulidae (0.4864). Acceleration rates were similar in Macromiidae and Corduliidae (0.0948 and 0.0911, respectively).

4. Discussion

The results from the current study are largely in agreement with Carle and Kjer (2002), Ware *et al.* (2007), Pilgrim and von Dohlen (2007), Letsch (2007), and Bybee (2008), in terms of the monophyly of each of the MCL families.

High posterior probability values for GSI monophyly support a taxonomic distinction between GSI and Corduliidae. Within the GSI there are three subfamilies, each one strongly supported (<95% posterior probability). As discussed in Letsch (2007) and briefly mentioned in Ware *et al.* (2007), the relative positions of *Gomphomacromia* and *Oxygastra* agree with Theischinger and Watson's (1984) suggested division of gomphomacromiines into an *Oxygastra* group and *Gomphomacromia* group. They based this distinction on *Gomphomacromia, Archaeophya* and *Pseudocordulia* larvae, which are similar in many ways to synthemistine larvae (both synthemistids and *Gomphomacromia* lack larval palpal setae, have subrectangular larval heads, broad, flat larval pronota, well developed larval frontal plates, and lack lateral spines on the larval abdominal tergites). *Gomphomacromia* + Synthemistidae was recovered with strong support by Letsch (2007; 100% posterior probability), but he did not include

Pseudocordulia or *Archaeophya*. With a more complete synthemistid taxon sample and *Archaeophya*, we recovered *Archaeophya* + *Gomphomacromia* + Synthemistidae. In our analysis, *Pseudocordulia*, suggested to be a member of the *Gomphomacromia* group, is never recovered with Synthemistidae, *Gomphomacromia* and/or *Archaeophya* but rather is sister to *Cordulephya* in a clade comprising the 'Oxygastra-group' taxa. Our results therefore support the definition of *Archaeophya* + *Gomphomacromia* +Synthemistidae as a monophyletic subfamily within the GSI only in the exclusion of *Pseudocordulia*.

Macromiidae comprises 4 genera: *Didymops, Epophthalmia, Macromia* and *Phyllomacromia* (May, 1997); taxa from each genus were included in the present study. With the exception of the strongly supported arrangement of *Epopthalmia* + *Macromia urania* (100% posterior probability), both of which are Indomalayan in their distribution, and *Didymops transvera* + *Macromia illinoiensis* (100% posterior probability), both of which are North American genera, our results do provide much information about intergeneric relationships in Macromiidae. The general lack of generic monophyly recovered here should be treated with caution until more molecular data can be incorporated for these taxa, and until more Macromiidae are sequenced.

Corduliidae is recovered as sister to Libellulidae, which has been found by several previous phylogenetic studies (e.g., Carle and Kjer, 2002; Bybee, 2008; Letsch, 2008). As in Letsch (2008), and Ware *et al.* (2007), some species of *Somatochlora* and *Cordulia* are recovered together, although in our phylogeny neither the 3 species of *Cordulia* nor the 2 species of *Somatochlora* are recovered in together in one clade. With the gene and taxon sample of the present study, we cannot evaluate the status of the monophyly of

Cordulia or *Somatochlora*. Species of *Cordulia* are so similar as to be difficult to distinguish morphologically, and we believe the genus is surely monophyletic (Jodicke et al., 2002). *Somatochlora*, however, is an extremely speciose and heterogeneous genus, the systematics of which are currently being evaluated using a wider taxon sample and gene fragments that evolve more rapidly than those used here (Voigt, pers. comm.).

As in Ware *et al.* (2007), and Pilgrim and von Dohlen (2007), the libellulid subfamilies Brachydiplacinae, Leucorrhiniinae, Palpopleurinae, Sympetrinae, Tetrathemistinae, Trameinae, and Trithemistinae, as defined by Fraser (1957) or Davies and Tobin (1985), are not supported as monophyletic (see Table 3 for a comparison of which nodes were supported by recent molecular analyses of Libelluloidea).

The libellulid clades recovered here are similar in composition to Ware *et al.* (2007), Letsch (2007), and Pilgrim and von Dohlen (2008), with a few notable exceptions (Table 3). All of these studies used secondary structural alignment of ribosomal data, and each ran Bayesian analyses. The present study, Ware *et al.* (2007) and Letsch (2007) each used evolutionary models that took into account the interdependence of ribosomal paired-sites, although Ware et al. used an RNA7A model to code the stem regions of their data while Letsch and the present study used the doublet model in MR. BAYES (Huselbeck and Ronquist, 2002). The aforementioned studies differed in the composition of their molecular datasets and Letsch (2007) did not incorporate morphological data. These differences may have been responsible for the minor disagreements between phylogenetic hypotheses.

Some of the taxonomic groupings that were recovered were surprising deviations from previous morphology-based assumptions. *Pantala* and *Trithemis*, for example, are

recovered as sister groups by Ware *et al.* (2007), Pilgrim and von Dohlen (2008) and the present study. This is surprising, considering striking differences in their appearance, behavior and biogeography. *Pantala* are cosmopolitan, large winged, migratory dragonflies while *Trithemis* are small to medium sized dragonflies that are non-migratory and found in Africa, Indo-Malaya and Southern Europe. The recovery of *Diastatops* + *Perithemis* and *Zenithoptera* in clades separate from *Palpopleura* was similarly surprising, since these taxa have been commonly placed in the subfamily Diastatopidinae based on their wing coloration and the presence, in some taxa, of a concave costal vein.

In general, taxa represented by morphological characters alone were treated with caution, especially in cases where these taxa were recovered outside of the family within which they were traditionally placed (i.e., *Fylgia*). Indeed, the positions of *Fylgia*, *Neocordulia*, and *Idomacromia* varied with analysis method, and between preliminary (not shown) and final Bayesian analyses. The position of these taxa did not appear to greatly affect the topology of the trees recovered, but the stochasticity of their placement is worthy of note. Many odonate collections are deficient in taxa from African, Asian and Middle Eastern regions and too often any available specimens from these regions are difficult to amplify due to age and/or preservation method. If we wish to include such taxa into our phylogenetic analyses, but are unable to obtain fresh specimens, it would be useful to be able to incorporate them into a combined analysis on the basis of morphological characters alone. The high levels of homoplasy within most morphological datasets, however, might prevent these taxa from being recovered in a position reflective of the 'true tree'. Although previous authors have suggested that the use of morphological characters other than wing venation might be useful in resolving

dragonfly relationships (e.g., Pilgrim and von Dohlen, 2008), we found that these other suites of morphological characters were similarly unable to provide strong support or resolution among genera and families within Libelluloidea.

Homoplasy among morphological and molecular characters

Differences in the evolutionary relationships proposed by libelluloid molecular and morphological datasets are numerous. In general, morphological and molecular data often disagree in phylogenetic studies, and it can be difficult to determine what to do when these datasets clash: (a) wait for more data, (b) combine the data, or (c) favor the results from one data source over another (Jenner, 2004). Awaiting more data may not be profitable in the present case, since a wide range of both small-scale (Kambhampati 1996; Misof et al., 2001; Hasegawa and Kasuya, 2006) and large-scale (Ware et al., 2007; Letsch, 2007; Pilgrim and von Dohlen, 2008; Bybee, 2008) studies have already been undertaken, incorporating anywhere from one to several independent molecular datasets, each of which has been largely in disagreement with morphological data. Traditional subfamily and even family level morphological based taxonomy is in conflict with present molecular and combined data hypotheses (Ware *et al.*, 2007; Letsch, 2007; Pilgrim and von Dohlen, 2008; Bybee, 2008). Other genes might increase resolution and branch support in molecular data, but it seems unlikely that this would also result in the resolution of conflict between morphological and molecular data. Additional morphological data should be useful if it were low in homoplasy, but, given the apparent extent of homoplasy in widely-used morphological characters, it is difficult to identify suites of non-homoplasious morphological features without reference to independent

data. A better argument may be that molecular data give more concordant results when comparing independent sequences and/or result obtained independently by different researchers. When we combined the datasets here, we recovered a phylogeny largely in agreement with previous molecular-only phylogenetic hypotheses by Ware *et al.* (2007) and Letsch (2007). Extensive homoplasy in the morphological dataset leads us to favor molecular and/or combined phylogenetic hypotheses over morphology-only datasets.

Carle (1995) recovered monophyletic families using morphological data and algorithmic manual parsimony, but he assigned character states at the family level, which may hide intrafamilial and intrageneric variation, both of which influence the amount of homoplasy within our dataset. In order to reduce homoplasy and non-independence among his characters, Carle (1995) downweighted or omitted character changes that were likely to be correlated with previously used characters ("coapomorphies") or that represented losses ("expomorphies") and thus made detection of convergence difficult. We concur that such characters can easily distort results of morphology-based phylogenetic analyses, and accounting for these effects would be beneficial, but we found it extremely difficult to determine objectively which characters are indeed functionally correlated. Moreover, if coapomorphies or exapomorphies are to be downweighted in relation to their frequency of occurrence, weights will vary depending on taxon sampling. We therefore adhere to the more common assumption that homoplasy will be revealed by conflict with presumably more frequent homologous changes. Nevertheless, we believe that efforts to identify accurately strongly coapomorphic characters, and the exercise of considerable caution in relying on putative homologies due to loss of structural features,

129

will probably be necessary to improve our ability to interpret phylogeny and character evolution in dragonflies.

Molecular-only phylogenetic hypotheses are also seldom in agreement either, and in theory an independent morphological dataset low in homoplasy would be useful in clarifying intrafamilial relationships. Unfortunately, characters commonly used in dragonfly morphology, and the new characters from the present study, are extremely homoplasious.

It is common for molecular and morphological evolution to occur at different rates (Patterson *et al.*, 1993), and interpretation of morphological characters, especially, is complicated by functionally driven convergent evolution. Wing venation features, for example, may be highly correlated (e.g., Carle, 1982b; Pilgrim and von Dohlen, 2008; Ware *et al.*, in prep), and flight behavior may influence vein density, the shape and size of the wing, and the venation pattern. As discussed in Pilgrim and von Dohlen (2008), for example, Trameinae (sensu Fraser, 1957; not supported as monophyletic by molecular data) have similarly shaped wings, with broad bases and expanded anal regions. This area of the wing has been thought to be correlated with flight behavior (e.g., Bechly, 1996; Pilgrim and von Dohlen, 2008). Similarly, as found in Ware *et al.* (2007) and Fleck *et al.* (2008), Tetrathemistinae are not found to be monophyletic. Dijkstra and Vick (2006) noted that venation in Tetrathemistinae is likely to be subject to convergence associated with narrowing of the wing base.

Several of the venation patterns in our morphological matrix (such as the number of cells in the RSPL (Figure 1b), the presence of undulation in the R₄₊₅, and the angle of the anal loop midrib) may affect chordwise flexibility, spanwise stiffness and wing twisting

130

or camber. Understanding of the details of wing kinematics and aerodynamics during flapping flight, however, is as yet too rudimentary to make confident predictions of these effects (Wooton and Kukalova-Peck, 2000; Combes and Daniel, 2003a, b; 2005) or, in general, to relate differences in venation patterns to specific flight characteristics.

Wing venation is often cited as a character-set prone to convergence (e.g., Carle, 1982b; Bechly, 1996), but we found that morphological characters unrelated to venation could also be highly homoplasious. Larval morphology may be linked to larval habitat and behavior, such as water current speed, burrowing behavior, larval position in the stream (since terrestrial, bank dwellers, rock clingers and larvae dwelling among plants in the middle of the stream may have different adaptations) and the presence of predators or competitors. Dorsal spines on the larval abdomen, for example, have been shown to differ in *Leucorrhinia* with fish predator presence (Mikolajewski and Johansson, 2003; Hovmoller and Johansson, 2004). Still other studies have suggested that spines may help stabilize the larva during recoil from labial protraction (Nestler, 1980) or when crawling through vegetation (Aguiar, 1989). Other characters, such as the prothoracic epaulets, have been suggested to protect eyes during burrowing in mayflies (Edmunds and Traver, 1959).

Male dragonflies use their secondary genitalia to remove sperm from competing males, and this behavior may have led to highly variable, often extremely elaborate penile structures, the homologies of which are sometimes difficult to assess for the purposes of morphological analyses (e.g., Miller, 1991). Miller (1991) extensively investigated the highly varied penile structures within Libellulidae. Using the classification scheme from Davies and Tobin (1985), Miller was unable to reconcile the

often complex, penile structures with libellulid taxonomy. We compared Miller's data with our combined phylogenetic hypothesis but also found no obvious phylogenetic pattern in the arrangement and length of these structures (Table 8). Additional penile characters scored in our matrix were also found to vary greatly between species within a single genus. Figure 5 displays SEM photographs showing the high variability of penile structures in Libellulidae: Figure 5b shows variation in a clade that has been widely supported as monophyletic, Sympetrum + Celithemis + Leucorrhinia, and Figure 5c, 5d, and 5e show variation among the distantly related *Brachydiplax*, *Chalcostephia* and Brachythemis. In Clade E of our phylogeny, however, there was some penile similarity between libellulid genera: the taxa Nannothemis, Erythrodiplax, Pseudoleon, Uracis and *Ypirangthemis* have in common an elongated, cylindrical distal segment of the *vesica* spermalis (Figure 5a; identified in Garrison et al., 2006). All of these taxa, with the exception of the yet to be sequenced *Ypirangthemis*, are recovered in a monophyletic clade with moderate support (83%; Figure 2; Pseudoleon was not sequenced here but found to be closely related to Erythrodiplax by Pilgrim & von Dohlen, 2008). Based on our phylogeny, one could assume that an elongated distal vesica spermalis segment was the gained several times, or that it was the ancestral condition (which has subsequently been lost in the other taxa in this clade, i.e., Acisoma, Rhodopygia, Nannophya, Bradinopyga, Hemistigma, Palpopleura, Diplacodes, and Crocothemis). Secondary losses such as this may distort our perception of the amount of disagreement between morphological and molecular datasets (Jenner, 2004).

Although color and color pattern have been used in phylogenetic analyses, especially at lower taxonomic levels (e.g., Carle and Kjer, 2002; May, 2002), its use at higher

levels, especially, is problematic because of the likelihood of strong selection for signaling, concealment, or thermal functions (e.g., *Calopteryx*: Waage, 1984, Misof *et al.*, 2000; *Chlorocypha*: Lempert, 1988; Corbet, 1999; *Plathemis*: Corbet, 1999). The libellulid subfamily Palpopleurinae (not supported as monophyletic by our molecular data), for example, share colored wings, which they may have converged upon for visual display purposes, or to act as wasp mimics (*Perithemis*: Paulson, 1966; Michalski, 1988). Further complicating the use of wing coloration in libelluloid taxonomy is the geographical variation in many coloration patterns (e.g., *Tetragoneuria*: Donnelly, pers. comm.). Some odonates use their wing or body coloration patterns for visual displays.

In general, morphological characters may not be able to answer the questions we have about interfamilial and intergeneric relationships. When we ran analyses of each morphological grouping (wing venation, external body, genitalia, penile, and larval characters) we found that there was large disagreement between each phylogenetic reconstruction recovered, which are also in conflict with the molecular data (Table 6).

Taxonomy

Given the similarity and consistency in libelluloid phylogenetic hypotheses between the combined analysis from the present study and recent analyses (Letsch, 2007; Ware *et al.*, 2007; Pilgrim and von Dohlen, 2008; Bybee, 2008), we propose names for the GSI group and 4 libellulid clades.

The GSI comprises taxa from the putative subfamilies Idionychinae, Idomacromiinae, Neophyinae, Synthemistinae and Gomphomacromiinae (Davies and Tobin, 1985). The oldest family-group name is Synthemistidae, based on Tillyard (1917), who applied it to
the subfamily Synthemistinae. We therefore propose that the GSI should be named "Synthemistidae" to include at least the taxa presently incorporated, other gomphomacromiines.

Libellulidae was previously considered to comprise at least 11 subfamilies (Davies and Tobin, 1985; Steinmann,), but given the lack of support for these subfamilies we take this opportunity to revise, in part, the taxonomy of Libellulidae based on current evidence. Here we recognize four subfamilies, each somewhat different in composition from the traditional understanding (Fraser, 1957; Davies and Tobin, 1985; Bridges, 194; Steinmann,) of these subfamilies; the remaining genera are left *incertae sedis* pending more detailed study. Where possible, we used the oldest genus name when assigning subfamily names. Part of Clade B is designated Trameinae, sens. nov., comprising at least Tramea Hagen, 1861, Hydrobasileus Kirby, 1989, Miathyia Kirby, 1889, and Tauriphila Kirby, 1889, but not Pantala Hagen, 1861, Rhyothemis Hagen, 1867, or Tholymis, Hagen, 1867, all of which have been commonly placed here previously. *Dasythemis* appears to belong here, but this is so unexpected on morphological grounds that we suggest that its position needs further study. Likewise Tetrathmistinae (sensu Fleck et al., 2008) and Urothemistinae could be included, but again we prefer to await additional data before suggesting this change, especially since support for an amalgamation of these three groups is low. Trameinae, thus restricted, was also found by Pilgrim and von Dohlen (2008), and Letsch (2007) recovered this grouping as sister to the urothemistines, but not to tetrathemistines.

We designate Clade C as Sympetrinae, *sens. nov.*, currently including the type genus *Sympetrum* Newman, 1833, as well as *Leucorrhinia* Brittinger, 1850 and *Celithemis* Hagen, 1861, and the formerly recognized subfamily Leucorrhiniinae is subsumed within Sympetrinae; a very similar result was reported by Letsch (2007), Ware et al., (2007), and Pilgrim and von Dohlen (2008). For Clade F, comprising *Trithemis* Brauer, 1968, and *Pantala* Hagen, 1861, among others, we propose the name Trithemistinae, *sens. nov*. Pilgrim and von Dohlen (2007) and Ware et al (2007) both found *Pantala* and *Trithemis* to be closely related; Letsch (2007) did not include *Pantala* in his analysis. It is highly likely that a number of genera will be added to this subfamily as research progresses, but it seems clear that it will have little resemblance to the traditional Trithemistinae, which appears to be a polyphyletic assemblage of convenience.

For Clade G, which largely comprises libelluline taxa and contains the genus *Libellula* Linnaeus 1758, we accept the current name Libellulinae, for the present excluding *Cannaphila, Dasythemis* and *Hadrothemis*, previously assigned to Libellulinae but not recovered within Clade G (confirmation of the placement of these taxa with additional molecular data would be very desirable). Letsch (2007), Ware et al., (2007), and Pilgrim and von Dohlen (2008) recovered a very similar libelluline clade. It is also reasonably well-defined by a suite of larval characters discussed by Fleck, et al. (2008). Given the complicated taxonomic history and frequent name changes of libelluloids (see Ware *et al.*, 2007 for a review of the various nomenclature changes over the last 125 years), low support for some clades that are candidates for subfamily designation, and the near certainty that composition of some clades will change with addition of more taxa to later analyses, one may question the wisdom of naming some new subfamilies now. We suggest that the formal designation of taxon names to the well-supported clades in our phylogeny will be beneficial for future study of libelluloids. Taxon name designation

provides definition to our phylogenetic hypotheses, which can be tested in the presence of additional taxa and outgroups. Named taxa provide ready indication groups that are likely to be phylogenetically justified and thus stable and, by omission, indicate the subordinate taxa for which relationships are most problematic. They also guide the study of character evolution and may facilitate recognition of morphological synapomorphies that can in turn refine and provide firmer support of succeeding phylogenetic hypotheses. To avoid unnecessary future complications in libelluloid taxonomy, however, we avoided naming clades for which support values were low (Clade E had the lowest posterior support, 59%).

Libelluloid speciation, divergence estimation, biogeography and success

Radiation in Libelluloidea, particularly in Libellulidae, was apparently rapid, as evidenced by the short branch lengths along the backbone of this section of the tree (Figure 3). This rapid radiation may be responsible for the poorly supported nodes in Libellulidae. As found previously (Misof *et al.*, 2001; Hasegawa and Kasuya, 2006; reviewed in Pilgrim and von Dohlen, 2008), the 16S and 12S fragments are not very useful in resolving the relationships within the Libelluloidea. Pilgrim and von Dohlen (2008) suggest that rapid radiation in Odonata, along with saturation of nucleotide sites has made phylogenetic analysis difficult.

Libelluloidea comprises a large number of heterogeneous taxa, and is the most speciose superfamily in Anisoptera. Our analysis had a large taxon sample, with 77% of GSI genera, 100% of macromiid genera, 63% of corduliid genera and 50% of libellulid genera represented (with a representation of 17% of GSI species, 6% of macromiid species, 14% of corduliid species and 12% of libellulid species). Using this large taxonset, we were able to assess diversification rates and estimate divergence times, in order to explore what may have led to such a rapid radiation in Libelluloidea (Figure 6). Our r8s analysis suggested the age of the root of the tree was Triassic (214). At this time, the continents were still in close proximity, following the break-up of the supercontinent of Pangea that created the southwest Indian Ocean rift, splitting South America + Africa from East Gondwana and moving India away from Antarctica, and the North Atlantic-Caribbean rift, which separated Laurasia from South America and Africa (Dietz and Holden, 1970). If the ancestor to Libelluloidea was present on all landmasses, the isolation followed by these geographical vicariant events may have influenced its divergence.

Our estimate for the divergence of non-cordulegastrid taxa (132 Mya) is similar to that of Carle (1995), who suggested that the radiation of non-cordulegastrid Libelluloidea began 'at least 140 million years ago'. During the early Cretaceous, Gondwanaland began to break apart (e.g., Veevers, 2004), which likely resulted in the creation of geographical barriers to dispersal and the isolation of populations. Vicariant events such as this have been suggested to drive the rate of speciation (e.g., Nelson, 1969; Rosen, 1975, 1978; Platnick and Nelson, 1978; Nelson and Rosen, 1980; Nelson and Platnick, 1981; Wiley, 1981). Movement of continents may have resulted in an increased amount of inland water (e.g., Hallam, 1993); the occurrence of additional water sources might have encouraged dispersal of dragonflies, virtually all of which have aquatic or semiaquatic larvae. Perhaps the ancestor of non-cordulegastrid taxa was able to exploit smaller, and/or temporary bodies of water that developed as a result of Gondwanaland's breakup, many of which may not have been able to support the wide variety of plant material present in larger water sources. Movement to new, smaller and/or temporary water sources might have been a strategy to avoid predators, such as neobatrichid frogs, which diversified during the Jurassic period, approximately 160 Mya (Roelants *et al.*, 2007). We find it hard to reconcile the evidence from our dating analysis with the hypothesis that non-cordulegastrid Libelluloidea diverged in Antarctica (Carle, 1995)

As discussed in Ware et al. (2008) divergence estimation m.ay be useful in shaping our hypotheses about the evolution of structures related to libelluloid success, such as exophytic oviposition and burrowing. Mentioned above, reduction in the ovipositor, convergently shared with the Gomphidae (Carle, 1995), is a prominent feature of Libelluloidea. The ovipositor of Aeshnoidea and Petaluridae (and Zygoptera) comprises three pairs of ventral processes. The first and second pairs (anterior and posterior gonapophyses) are enclosed by the third (gonoplacs). In libelluloids, including Cordulegastridae, the ovipositor is modified for exophytic oviposition (Tillyard, 1917; Carle, 1995; Figure 6). In Cordulegastridae, the third processes (gonoplacs) are vestigial. In the GSI clade, the third processes are absent and at least the second processes are reduced, although in some taxa the first pair is present and nearly as long as in Cordulegastridae. In the MCL, the first processes are reduced to small flaps and the other structures are apparently absent except for the probable vestige of the styli emerging directly from the 9th sternite (Tillyard, 1917). In a few instances, the 8th (e.g., some *Somatochlora*) or 8th and 9th sternites (*Uracis*) are secondarily produced to form an ovipositor in MCL species. If increased speciation is linked to a reduction in the ovipositor (Carle, 1995), we would expect the rate of diversification to be highest in the

138

clades which diverged after the ovipositor was extremely reduced. Based on their oviposition morphology, we might expect the highest diversification rates in our dataset to occur in the MCL and the lowest diversification rates to occur in the Cordulegastridae. We calculated diversification rates (r) for Libelluloidea, and for each family using the dates obtained from our r8s analysis and the numbers of species listed by Schorr et al. (2008), assuming either a low extinction rate (ε =0) or a high extinction rate (ε =95). The r values for Libelluloidea were progressively higher in the more apical nodes of Libelluloidea, under both assumptions of extinction rate (Figure 6), with Libellulidae having a tenfold higher rate than Chlorogomphidae. The net diversification rate calculations suggested that GSI at one time had higher acceleration of diversification than is presently observed which might imply that they were better adapted to previous environmental conditions, and/or that they are less able to compete for resources upon the divergence of Libellulidae. The GSI likely diverged during the Jurassic, with the ancestor to *Gomphomacromia* + *Archaeophya* + Synthemistinae diverging from other GSI taxa approximately 85 Mya. During this time period, Australia had begun to move away from Antarctica and South America (Veevers, 2004). The ancestor to Archaeophya + Synthemistidae became isolated in Australasia, which may have led to the speciation of Archaeophya and Synthemistidae. The Indomalayan GSI taxa Idionyx +Macromidia diverged around the time when India collided with Eurasia (approximately 54 Mya; e.g., Zhu et al., 2005). The recovery, with strong support, of Oxygastra and Syncordulia in a clade comprising Australian endemics diverging 47 Mya, well after the break-up of Pangea, might suggest that the current distribution of these taxa in Europe and Africa, respectively is due to dispersal.

The radiation of the MCL complex began 101 million years ago, with Macromiidae diverging 10 million years before Corduliidae and 30 million years before Libellulidae. Each of these families diverged during the Jurassic, with most of the genera within these clades diverging during the Cretaceous and Cenozoic. This suggests that the current distribution of taxa like *Libellula*, which are present on 4 continents but diverged well after the formation of the Atlantic Ocean, may be due to dispersal. Similarly, taxa such as *Orthetrum*, present in several countries in the Old World, may have dispersed into their present locations after they diverged from their libelluline ancestor during the Cenozoic.

Summary

The Libelluloidea comprise four families: Synthemistidae (*sens. nov*), Macromiidae, Corduliidae and Libellulidae. Libellulidae comprises 7 apparent clades, four of which are consistent enough in composition to be named: Trameinae (*sens. nov.*), Sympetrinae (*sens. nov.*), and Trithemistinae (*sens. nov.*), and Libellulinae (*s.s.*). The recovery of monophyletic libelluloid clades is largely due to molecular data, and our morphological data when analyzed alone is in conflict with the phylogenetic hypotheses generated by combined analyses. Homoplasy is rampant in each of the morphological datasets we examined, not just in wing venational characters. Divergence estimation suggests that Libelluloidea diverged from its ancestor during the late Triassic, with non-cordulegastrid taxa diverging during the Jurassic. Diversification rates were highest in the most speciose family, Libellulidae, which diverged during the early Cretaceous, and lowest in the Chlorogomphidae. Exophytic oviposition may have been responsible the success of Libelluloidea, and taxa with highest diversification rates have are those with the most reduction in the gonapophyses. Future work on Libelluloidea should begin to discover whether there are morphological synapomorphies which support the taxonomic groupings recovered by combined analyses. In order to best estimate the divergence times and biogeography of Libelluloidea still more taxa should be sequenced, particularly from African and Asia, to increase the number of genera represented.

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References

Aguiar, S. D. S., 1989. What is the function of the dorsal hooks and lateral spines in larval dragonflies (Anisoptera)? *Notulae Odonatologicae 3*: 43–44.

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 vol. 2*: 402 pp.

Bridges, C. A., 1994. Catalogue of the family-group, genus-group and species-group names of the Odonata of the world (Third Edition). C. A. Bridges, Urbana, Illinois.

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*, doi:10.1111/j.1096-0031.2007.00191.x

Carle, F. L., 1982 a. A contribution to the knowledge of the Odonata. Unpublished Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 1095 pp.

Carle, F. L., 1982 b. 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., 1995. Evolution, taxonomy, and biogeography of ancient Gondwanian libelluloides, with comments on anisopteroid evolution and phylogenetic systematics (Anisoptera: Libelluloidea). *Odonatologica*, 24(4): 383-506.

Carle, F.L. and Louton, J.A., 1994. The larva of Neopetalia punctata and establishment of Austropetaliidae fam.nov. (Odonata). *Proceedings of the Entomological Society of Washington*, *96(1)*: 147-155.

Carle, F. L. and Kjer, K. M., 2002. Phylogeny of Libellula Linnaeus (Odonata: Insecta). *Zootaxa*, 87:1-18.

Combes, S.A. and Daniel, T.L., 2003a. Flexural stiffness in insect wings. I. Scaling and the influence of wing venation. *Journal of Experimental Biology*, 206(17): 2979-2987.

Combes, S.A. and Daniel, T.L., 2003b. Flexural stiffness in insect wings. II. Spatial distribution and dynamic wing bending. *Journal of Experimental Biology*, 206(17): 2989-2997.

Combes, S. and Daniel, T.L., 2005. Flexural stiffness in insect wings: Effects of wing venation and stiffness distribution on passive wing bending. *American Entomologist*, 51(1): 42-45.

Corbet, P. S., 1999. Dragonflies: Behavior and Ecology of Odonata. Cornell University Press, Ithaca, NY. 829pp.

Davies, D.A.L. and Tobin P., 1985. The dragonflies of the world: a systematic list of extant species of Odonata. Vol. 2. Anisoptera. *Societas Internationalis Odonatologica Rapid Communications, Rapid Communication* (Supply.) *No. 5.*, Utrecht.151 pp.

Dietz, R. S. and Holden, J. C., 1970. Reconstruction of Pangaea: breakup and dispersion of continents, Permian to present. *Journal of Geophysical Research*, 75(26):4939-4956.

Dijkstra, K.-D.B. and Vick, G. S., 2004. Inflation by venation and the bankruptcy of traditional genera: the case of Neodythemis and Micromacromia, with keys to the continental African species and the description of two new Neodythemis species from the Albertine Rift (Odonata: Libellulidae). *International Journal of Odonatology 9*: 51-70.

Edmunds, G. F. and Traver, J. R., 1959. The classification of the Ephemeroptera II. Ephemeroidea: Behningiidae. *Annals of the Entomological Society of America* 52: 43-51.

Felsenstein J, 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Fleck, G, Brenk, M. and Misof, B., 2008. 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. 2008. *Organisms Diversity and Evolution 8(1)*:1-16.

Fraser, F.C., 1957. A reclassification of the order Odonata. Royal Zoological Society of New South Wales: Sydney.133 pp.

Garrison, R.N., von Ellenreider, N. and Louton, J. A., 2006. Dragonfly Genera of the New World: An Illustrated and Annotated Key to the Anisoptera. The Johns Hopkins University Press. 368 pp.

Geijskes, D.C., 1970. Generic characters of the South American Corduliidae with descriptions of the species found in the Guyanas. *Notes on Odonata of Surinam 11. Stud. Fauna Suriname, 12(60)*: 1-42.

Gloyd, L. K., 1959. Elevation of the *Macromia* group to family status (Odonata). *Entomological News*, 70: 197-205.

Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15(4): 415-428.

Goloboff, P.A., Mattoni, C. I. and Quinteros, A. S., 2006. Continuous characters analyzed as such. *Cladistics*, *22(6)*: 589-601.

Grimaldi, D. and Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press.

Guttell, R.R., Gray, M. W. and Schinare, M. N., 1993. A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. *Nucleic Acids Research*, *21*:3055-3074.

Gu, X., Fu, Y.-X. and Li, W.-H., 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution 12*: 546

Hallam, A., 1993. Jurassic climates as inferred from the sedimentary and fossil record. *Philosophical Transactions of the Royal Society of London B341*: 287–296.

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

Hennig, W., 1969. Stammesgeschichte der Insekten, W. Kramer, Frankfurt/M.

Hennig, W., 1981. Insect Phylogeny, Academic Press, New York.

Hovmoller R., Johansson F., 2004. A phylogenetic perspective on larval spine morphology in Leucorrhinia (Odonata: Libellulidae) based on ITS1, 5.8S, and ITS2 rDNA sequences. *Molecular Phylogenetics and Evolution*, *30 (3)*: 653-662.

Huelsenbeck, J. P., Ronquist, F., 2002. MrBayes 3: Bayesian analysis of phylogeny. University of California.

Jarzembowski, E.A. and 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.

Jenner, R. A., 2004. When molecules and morphology clash: Reconciling conflicting phylogenies of the Metazoa by considering secondary character loss. *Evolution & Development 6(5)*: 372-378.

Kennedy, C. H., 1922. The morphology of the penis in the genus Libellula (Odonata). *Entomological News*, *33*:33-40.

Kirby, W. F., 1890. A synonymic catalogue of Neuroptera Odonata. Guerney & Jackson, London, 202 pp.

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*(*3*): 314-330.

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

Lempert, J., 1988. Untersuchungen zur Fauna, Ökologie und zum Fortpflanzungsverhalten von Libellen (Odonata) an Gewässern des tropischen Regenwaldes in Liberia, Westafrika. Diplomarbeit, Universität Bonn.

Letsch, H., 2007. Phylogeny of Anisoptera (Insecta: Odonata): promises and limitations of a new alignment approach. Diplomarbeit, Universität Bonn

Lewis, P. O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, *50*:913-925.

Lieftinck, M.A., 1971. Studies in Oriental Corduliidae (Odonata). 1. *Tijdschrift voor Entomologie*, *114*(*1*):1-63.

Lohmann, H., 1996a. Das phylogenetische system der Anisoptera (Insecta: Odonata). unpublished diploma thesis at the Biological Faculty of the Albert-Ludwigs-University in Freiburg i.Br. 117 + 13 pp.

Lohmann, H., 1996b. Das phylogenetische system der Anisoptera (Odonata). *Entomologische Zeitschrift*, *106(6)*: 209-252 (first part); *106(7)*: 253-266 (second part); *106(9)*: 360-367 (postscript).

Lutzoni, F., Wagner, P., Reeb, V., Zoller. S., 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology*, *49*: 628-651

Magallón, S., Sanderson, M. J., 2001. Absolute diversification rates in angiosperm clades. *Evolution*, *55*:1762–1780.

Martin, R., 1907. Cordulines. *Collections Zoologiques du Baron Edm. de Selys Longchamps. Institut royal des Sciences Naturelles de Belgique: Brussels.* 17:1-94.

May, M.L., 1995. Comparative notes on micropyle structure in "cordulegastroid" and "libelluloid" Anisoptera. *Odonatologica*, *19(1)*: 53-62.

May, M.L., 1997. Reconsideration of the status of the genera Phyllomacromia and Macromia (Anisoptera: Corduliidae). *Odonatologica*, *26(4)*: 405-414

Michalski, J., 1988. A catalogue and guide to the dragonflies of Trinidad (Order Odonata), Occas. Pap. No. 6, Univ. of West Indies, St. Augustine, Trinidad

Mikolajewski, D.J. and Johansson, F., 2004. Morphological and behavioural defenses in dragonfly larvae: trait compensation and cospecialization, *Behavioral Ecology*, *15*: 614–620.

Miller, P.L., 1991. The structure and function of the genitalia in the Libellulidae (Odonata). *Zoological Journal of the Linnean Society*, *102(1)*: 43-74.

Minin, V., Abdo, Z., Joyce, P., Sullivan, J., 2003. Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology*, *52*:674-683.

Misof, B., Anderson, C. L., Hadrys, H., 2000. A Phylogeny of the Damselfly Genus Calopteryx (Odonata) Using Mitochondrial 16S rDNA Markers. *Molecular Phylogenetics and Evolution*, *15(1)*: 5-14.

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*(*1*):27-37.

Nel, A., X. Martinez-Delclos, Paicheler, J.-C., Henrotay, M., 1993. Les "Anisozygoptera" fossiles: phylogeneie et classification (Odonata). *Martinia* (hors ser.) *3*: 1-311.

Nel, A., Paicheler, J.C., 1994. Les libellulidae fossiles. Un inventaire critique (Odon., Anisoptera, Libelluloidae). *Entomologica Gallica*, 4(4):166-190.

Needham, J.G., 1903. A genealogic study of dragonfly wing venation. *Proceedings of the U.S. National Museum*, 26(1331):703-764.

Needham, J. G., 1908. Critical notes on the classification of the Corduliinae (Odonata). *Annals of the Entomological Society of America, 1*: 273-280.

Needham, J.G., Broughton, E., 1927. The venation of the Libellulinae (Odonata). *Transactions of the American Entomological Society (Philadelphia)*, **53**:157-190.

Needham, J.G., Westfall, M.J., May, M.L., 2000. Dragonflies of North America. Scientific publishers, Inc. 939 pp.

Nelson, G. J., 1969. The Problem of Historical Biogeography. *Systematic Zoology*, *18(2)*: 243-246

Nelson, G., Platnick, N., 1981. Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.

Nelson, G., and Rosen, D.E., 1981. Vicariance biogeography: a critique. Columbia University Press, New York. 593 pp.

Nestler, J.M., 1980. Niche relationships of the Anisoptera nymphs of Lake Isaqueena. Dissertation. Clemson University, Clemson, South Carolina, USA.

Paulson D. R., 1966. Dragonflies of South Florida (Odonata: Anisoptera). Ph.D. dissertation, University of Miami, Coral Gables, Florida. 603 pp.

Patterson, C, Williams, D. M., and Humphries, C. J., 1993. Congruence between molecular and morphological phylogenies. *Annual Review of Ecological Systematics.*, 24: 153-188.

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

Pfau, H.K., 1991. Contributions of functional morphology to the phylogenetic systematics of Odonata. *Advances in Odonatolology*, 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.

Pilgrim, E.M. and von Dohlen, C.D., 2008. Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. *Systematic Entomology*, *33*:159-174.

Platnick, N. J. and Nelson, G., 1978. A method for analysis of historical biogeography. *Systematic Zoology* 27:1-16.

Posada, D. and Crandall, K. A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, *124*:817–818.

Posada, D. and Buckley, T., 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, *53*(*5*): 793-808.

Rambaut A. and Drummond A. J., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

Rambur, M.P., 1842. Neuropteres. Histoire naturelle des Insectes, Paris, pp.534.

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

Riek, E.F. and 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(6): 1150-1166.

Ris, F., 1909-1919. Collections Zoologiques du Baron Edm. de Selys Longchamps, Cat. System. et Descript., Fasc. 9-16. Bruxelles, Hayez, Impr. des Académies.

Roelants, K., Gower, D.J., Wilkinson, M., Loader, S.P., Biju, S.D., Guillaume, K., Moriau, L., Bossuyt, F., 2007. Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 887-892.

Rosen, D. E., 1975. A vicariance model of Caribbean biogeography. *Systematic Zoology,* 24:431-464.

Rosen, D. E., 1978. Vicariant patterns and historical explanations in biogeography. *Systematic Zoology*, *27*:159-188.

Sanderson, M. J., 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, *19*: 301-302.

Schmidt, E., 1916. Vergleichende Morphologie des 2. und 3. Abdominalsegmentes bei männlichen Libellen. *Zool. Jb. (Abt. Anat.), 39*: 87-196.

Schnare, M.N., Damberger, S.H., Gray, M.W., Gutell, R.R., 1996. Comprehensive comparison of structural characteristics in eukaryotic cytoplasmic large subunit (23S-like) ribosomal RNA. *Journal of Molecular Biology*, *256*: 701-719.

Schorr, M., Lindeboom, M., Paulson, D., 2008. World Odonata List. <u>http://www.ups.edu/x6140.xml</u>, Last visited May 13, 2008.

Schoöniger, M., von Haeseler, A., 1994. Simulating efficiently the evolution of DNA sequences. *Computational Applied Bioscience 11*: 111-115.

St. Quentin, D., 1939. Die systematische stellung der unterfamilie der Corduliinae Selys (Ordung Odonata). *Proceedings Of the International Congress of Entomology 7 (Berlin)*, *1*:345-360.

Swofford, D. L., 2001. PAUP* . Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Theischinger, G., Watson, J. A. L., 1978. The Australian Gomphomacromiinae (Odonata : Corduliidae). *Australian Journal of Zoology*, *26*:399-431.

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.

Theischinger, G., Fleck, G., 2003. A new characters useful for taxonomy and phylogeny of Anisoptera (Odonata). *Bulletin de la Societe entomologique de France 108(4)*:409-412.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, 1997. The ClustalX Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. *Nucleic Acids Research*, 24:4876-4882.

Tillyard, R.J., 1910. Monograph of the genus *Synthemis*. (Neuroptera: Odonata). *Proceedings of the Linnean Society of New South Wales*, *35*: 312-377.

Tillyard, R. J., 1917. The biology of Dragonflies. Cambridge. Cambridge Univ. Press.

Trueman, J. W. H., 1996. A preliminary cladistic analysis of odonate wing venation. *Odonatologica*, 25: 59-72.

Veevers, J.J., 2004. Gondwanaland from 650–500 Ma assembly through 320 Ma merger in Pangea to 185–100 Ma breakup: supercontinental tectonics via stratigraphy and radiometric dating. *Earth-Science Reviews*, 68: 1–132.

Waage, J. K., 1984. Influence of oviposition behavior on female and male responses during courtship in Calopteryx maculata and C. dimidiata (Odonata: Calopterygidae). *Animal Behaviour 32*:400-404.

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 (1)*: 289–310.

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.

Wiens, J. J., 2001. Character analysis in morphological phylogenetics: problems and solutions. *Systematic Biology* 50:689–699.

Weins, J. J., 2005. Missing data and the design of phylogenetic analyses. *Journal of Biomedical Informatics*, *39*: 34-42.

Wiley, E.O., 1981. Phylogenetics: the theory and practice of phylogenetic systematics. New York, New York, U.S.A: John Wiley and Sons.

Wootton, R. J., Kukalova – Peck, J., 2000. Flight adaptations in Palaeozoic Palaeoptera (Insecta). *Biological Reviews*, 75:129-167.

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. Journal of Molecular

Evolution, *39*:105-111.

Yang, Z., Goldman, N., Friday, A.E., 1994. Comparison of models for nucleotide substitution used in maximum likelihood estimation. *Molecular Biology and Evolution*, *11*:316-324.

Zhu B, Kidd WSF, Rowley DB, Currie BS, Shafique N., 2005. Age of initiation of the India-Asia collision in the east-central Himalaya. *Journal of Geology* 113:265–85

Figure 1a: Selected larval morphological characters from our matrix. A: median prominence on frons; B: degree of eye protuberance; C: eyes position relative to occiput; D: coxal protuberances; E: prothoracic epaulets; F: wing pads; G: hind tarsae and claw length relative to tibial length; H: dorsal hooks present on segment 4-segment 8; I: lateral spines on abdomen; J: paraprocts; K: arrangement of labial palpal teeth and setae; L: cleft in premental hinge; M: hair presence on premental hinge; N: transverse sulcus; O: sulcus presence on 8 and 9.



Figure 1b: Selected wing vein morphological characters from our matrix; image is based on an electronic scan of *Pantala flavescens* wing. RSPL= Radial sector planate vein, MBS= Midbasal space, Cu-A= Cubito-anal cell.



Figure 2: Consensus tree from a parsimony heuristic search (10,000 addition sequence replicates; consensus of 700 trees). Numbers above branches are bootstrap support values. Taxa for which only morphological data were present are noted with a dragonfly symbol. * indicates that the clade composition differs from the Bayesian analysis.



Figure 3: Bayesian analysis consensus tree (from 14001 post "burn-in" trees). Posterior probabilities are listed above branches. Taxa for which only morphological data were present are noted with a dragonfly symbol. Inset box is a phylogram of the most likely tree.





Figure 4: Morphology based maximum parsimony tree; consensus tree.

Figure 5: Morphological characters from selected taxa in our analyses; (a) an elongated distal segment of the vesica spermalis is found in three taxa from Clade E, (b) variations in penile structure among closely related taxa from Clade C, (c) variations in penile structure between among distantly related taxa from Clade A and Clade D.





Figure 6: Results from the r8s analysis and diversification calculations. (a) Diversification rates for stem groups in our analysis under low (r_{0.0}) and high (r_{0.05}) extinction rates; (b) Cordulegaster ovipositor; (c) Synthemis ovipositor; (d) Tramea ovipositor; (e) Proportional diversity of extant clades during the Cretaceous period; (f) Proportional diversity of extant clades during the Cenozoic Paleogene period; (g) Proportional diversity of extant clades during the Cenozoic Neogene period. Ovipositor diagrams are adapted from Tillyard (1917). Geological maps modified from usgs.com.



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Taxon	COI, COII, EF-1α 18S, 28S, 12S, and 16S accession numbers	Novel accession numbers
Acisoma panorpoides	COI:N/A COII: DQ166802 EF-1α: EF640489 18S:N/A 28S: EF631433, EF631322, EF631229 12S & 16S: EF631546, EF640410	V/V
Aeschnosoma forcipula	COI: N/A COII: N/A EF-1α: N/A 18S:N/A 28S: EF631427, EF631319, EF631223 12S&16S: EF631540	V/N
Aethriamanta gracilis	COI: N/A COII: N/A EF-1α: N/A 18S: N/A 28S: N/A 28S: N/A 12S&16S: N/A	16S: 123456
Aethriamanta rezia	COI: N/A COII: N/A EF-1α: N/A 18S: N/A 28S: EF631384, EF631188 28S: EF631384, EF631188 12S&16S: EF631501	V/N
Agrionoptera longitudinalis	COI: N/A COII: N/A EF-1α: N/A 18S: N/A 28S: EF631440, EF631331, EF631235 12S&16S: EF631553	V/N
Anatya guttata	Morphology only	N/A

Table 1: Taxon list for the present study: sequences downloaded from GenBank (light grey), novel molecular data (dark grey) and taxa for which only morphological data (white) are present

Anotoenaton vioholdii		NI/A
Anorogaster stevotati	COL N/A COII: N/A	V/M
	EFI-CC: N/A	
	18S: N/A	
	28S: AB127419	
	12S&16S: AB127061	
Apocordulia macrops	Morphology only	N/A
Archaeophya magnifica	COI: N/A	COI: 123456
s)	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631470. EF631356	
	12S&16S: EF631580	
Atoconeura luxata	COI: N/A	D2: 123456
	COII: N/A	16S: 123456
	FF1_0. N/A	
	11 2. N/A	
	A/VI .001	
	28S: N/A	
	12S&16S: N/A	
Austrocordulia refracta	COI: N/A	N/A
2	COII: N/A	
	EE1 or N/A	
	EFT-W. N/A	
	185: N/A	
	D7: EF631243	
	D3: EF631336	
	D2: EF631448	
	16S: EF631559	
Austrophya mystica	COI: N/A	N/A
	COII: N/A	
	EF1-02: N/A	
	18S: N/A	
	D7: EF631236	
	D3. EF631332	
	D2. FF631441	
	16S: N/A	
	A/NI .C01	
Brachydiplax c chalybea	Morphology only	N/A

Brachvdinlax denticanda	COI: N/A	N/A
	COII: EU055364	
	EF1-α: N/A	
	18S: EU055167	
	28s: EF631451, EF631246, EU055265	
	12S & 16S: EU054976, EF631562	
Brachymesia furcata (morphology)/ Brachymesia	COI: N/A	N/A
gravida (molecular)	COII: N/A	
	EF1-α: EF640470	
	18S: N/A	
	28S: N/A	
	12S&16S: EF640392	
Brachythemis leucosticta	COI: N/A	N/A
	COII: DQ166797	
	EF1-α: EF640491	
	18S: N/A	
	28S: EF631463, EF631349, EF631258	
	12S & 16S: EF640412, EF631573	
Bradinopyga strachani	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640493	
	18S: N/A	
	28S: EF631462, EF631348, EF631257	
	12S & 16S: EF640414, EF631572	
Brechmorhoga mendax	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631385, EF631189	
	12S & 16S: EF640453, EF631502	
Calophlebia interposita	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631381	
	12S & 16S: N/A	

Cannaphila vibex	Morphology only	N/A
Celithemis elisa	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640471	
	18S: N/A	
	28S: EF631428, EF631320, EF631224	
	12S&16S:, EF631541	
Celithemis elisa	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640471	
	18S: N/A	
	28S: N/A	
	12S&16S:, DQ021425	
Chalcostephia flavifrons	COI: N/A	N/A
, , , , , , , , , , , , , , , , , , ,	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631465, EF631351, EF631260	
	12S & 16S. EF640385, EF631575	
Chlorogomphus brunneus	COI: N/A	N/A
•	COII: EU055383	
	EF1-α: N/A	
	18S: EU055186	
	28S: EU055284	
	12S&16S: AF266088, EU055091	
Chloropetalia soarer	COI: N/A	N/A
	COII: N/A	
	EF1- α : N/A	
	18S: N/A	
	D7: EF631248	
	D3: EF631339	
	D2: EF631453	
	12S&16S:N/A	
Choristhemis flavoterminata	COI: N/A	COI: 123456
	COII: EU055362	
	EF1-cc: N/A	

EU055263	COI: 123456	N/A	N/A	N/A	N/A
188: EU055165 288: EF631442, EF631333, EF631237, 128 & 168: EU054974, EF631554	COI: N/A COII: EU055345 EF1-0:: N/A 18S: EU055148 D7: EF631255 D3: EF631346 D2: EF631346 D2: EF631460 16S: EF631570	COI: N/A COII: N/A EF1-0: N/A 18S: N/A D7: N/A D2: N/A D2: N/A 12S&16S: AF266056	COI: N/A COII: EU055376 EF1-0: N/A 18S: EU055179 28S: EU055277 12S&16S: AY282558	COI: N/A COII: N/A EF1-0: N/A 18S: DQ008198 D7: N/A D3: N/A D2: N/A 12S&16S:AF266086	COI:N/A COII:N/A EF1-0: N/A 18S: AF461236
	Cordulephya pygmaea	Cordulegaster boltoni	Cordulegaster dorsalis	Cordulegaster picta	Jordulia aenea

	D2: 123456 168: 123456				N/A					N/A	N/A						N/A						N/A						N/A		
28S: EF631500, EF631383, EF631286, AF461210 12S&16S: EF631603	COI: N/A COII: N/A	EF1-α: N/A	18S: N/A	285: N/A 125 & 16S: N/A	COI: N/A	COII: EU055377	EF1-α: N/A	18S: EU055180	288: EF631435 , EF631326, EF631232, EU055278 128 & 168: FU054989 EU055085	Morphology only	COI: N/A	COII: N/A	EF1-α: DQ008200	18S: DQ008200	28S: EF631429, EF631321, EF631225	12S & 16S: AF266100, EF631542	COI: N/A	COII: DQ166789	EF1-α: EF640495	18S: N/A	28S: EF631390, EF631289, EF631192, AB127415	12S&16S: EF640416, EF631506	COI: N/A	COII: N/A	EF1- α : N/A	18S: N/A	28S: EF631386	12S & 16S: N/A	COI: N/A	COII: N/A	EF1-α: EF640496
	Cordulia annurensis				Cordulia shurtleffi	3				Crocothemis divisa	Crocothemis erythraea						Crocothemis servilia						Dasythemis esmeralda						Deielia phaon		

	18S· N/A	
	28S: EF631430, EF631226	
	12S & 16S: EF640417, EF631543	
Diastatops intensa	Morphology only	N/A
Didymops floridensis	Morphology only	N/A
Didymops transversa	COI: N/A	N/A
	COII:N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631436, EF631327	
	12S &16S: EF631549	
Diplacodes haematodes	COI: N/A	N/A
7	COII: N/A	
	EF1-α: N/A	
	18S:N/A	
	28S: EF631443. EF631238	
	12S & 16S: EF631555	
Dythemis fugax	COI: N/A	N/A
	COII:N/A	
	Er1-0: Er040333	
	18S: N/A	
	28S: EF631387,	
	12S & 16S: EF640454, EF631503	
Dythemis multipunctata	COI: N/A	N/A
	COIL:N/A	
	FF1-cr. N/A	
	18S· N/A	
	796. FFK21220 FFK21750	
	200. 11 00 100, 11 00 120 102 & 163. FF63157A	
Dythemis multipunctata	COI: N/A	D7: 123456
	COIL·N/A	168-173456
	EFI-0: N/A	
	18S: N/A	
	28S: N/A	
	12S & 16S: N/A	
Elasmothemis cannacriodes	Morphology only	N/A

lga leptostyla	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631481, EF631274	
	12S & 16S: N/A	
theca princeps	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631407, EF631302, EF631205	
	12S & 16S: EF631521	
theca stella	COI: N/A	D2: 123456
	COII:N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: N/A	
	12S & 16S: N/A	
phthalmia sp.	COI: N/A	D2:123456
	COII: DQ166792	16S:123456
	EF1-α: N/A	
	18S: N/A	
	28S: N/A	
	12S & 16S: N/A	
hemis simplicicollis	COI: AF195759	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631389, EF631288, EF631191	
	12S&16S: AY282566, EF631505.	
hrodpilax minuscula	COI:	N/A
	COII: EU055340	
	EF1-α: N/A	
	18S: EU055144	
	28S: EF631388, EF631287, EF631190, EU055239	
	12S&16S: EU054950, EF631504	

COI:123456		631230	N/A	N/A								N/A				631277		N/A						N/A						
COI:N/A COII:N/A	EF1-0:: N/A 18S: N/A	28S: EF631434, EF631323, EF 12S&16S: EF631547	Morphology only	COI: N/A	COII: N/A	EF1-o: N/A	18S: N/A	D7: EF631206	D3: EF631303	D2: EF631408	16S: EF631522	COI: N/A	COII: N/A	EF1-cc: N/A	18S: N/A	28S: EF631484, EF631366, EF	12S&16S: EF631592	COI: N/A	COII: N/A	EF1-α: N/A	18S: N/A	28S: EF631431, EF631227	12S&16S: EF631544	COI: N/A	COII: N/A	EF1-o: N/A	18S: N/A	D7: EF631233	D3: EF631328	D3: EF631328 D2: EF631437
Eusynthemis brevistyla			Fylgia sp	Gomphomacromia sp.	۹ ۹							Hadrothemis defecta						Helocordulia uhleri						Hemicordulia tau						

			1347, EF631256		COI:123456									N/A				1361, EF631270		N/A				1343, EF631252		N/A							COI:123430	COI:125430	COI:125400
COII: N/A	EF1-c: N/A	18S: N/A	28S: EF631461, EF631	12S&16S: EF631571	ordulia berthoudi COI: N/A	COII: EU055357	EF1-a: N/A	18S: EU055159	28S: EU055257	D7: EF631244	D3: EF631337	D2: EF631449	16S: EF631560	reophila COI: N/A	COII: N/A	EF1-CC: N/A	18S: N/A	28S: EF631477, EF631	12S & 16S: EF631587	sileus brevistylus COI: N/A	COII: N/A	EF1-a: N/A	18S: N/A	28S: EF631457, EF631	12S & 16S: EF631567	e amazonica COI: N/A	COII: N/A	EF1-a: N/A	18S: N/A	28S: EF631377	12S & 16S: N/A	elysi COI: N/A		COII: N/A	COII: N/A

	D7. FF631193	
	D3: EF631290	
	D2: EF631391 12&16S: N/A	
Idomacromia proavita	Morphology only	N/A
Kalyptogaster erronea	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: AY082599	
	D7: EF631245	
	D3: N/A	
	D2: EF631450	
	12S&16S:EF631561	
Ladona julia	COI: AF195748	N/A
5	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631423, EF631315, EF631219	
	12S & 16S: EF631536	
Lathrocordulia metallica	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	D7: EF631239	
	D3: EF631334	
	D2: EF631444	
	16S: EF631556	
Leucorrhinia glacialis	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640472	
	18S: N/A	
	28S: EF631409, EF631304, EF631207	
	12S & 16S: EF640394, EF631523	
Leucorrhinia intacta	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640474	

	N/A	N/A	N/A	N/A	D2:123456	V/N
18S: N/A 28S: N/A 12S & 16S: EF640396,	COI: AF195749 COII: N/A EF1-c: N/A 18S: N/A 28S: EF631392, EF631194 2285: EF631392, EF631194	COI: AF195753 COII: N/A EF1-0: N/A 18S: U65109 28S: EF631329, U65168 12S & 16S: EF631551	COI: AY300816 COII: N/A EF1-0: N/A 18S: N/A 28S: EF631497 12S & 16S: AF037173	COI: AY300814 COII: N/A EF1-o: N/A 18S: N/A 28S: EF631479, EF631363, EF631272 12S & 16S: DQ021418, EF631589	COI: N.A COII: N/A EF1-c: N/A 18S: N/A 28S: N/A 12S & 16S: N/A	COI: N/A COII: DQ166800 EF1-c: N/A
	Libellula luctuosa	Libellula pulchella	Libellula quadrimaculata	Libellula quadrimaculata	Lokia incongruens	Lyriothemis pachygastra
	18S: N/A 28S: EF631483. EF631365, EF631276					
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	12S & 16S: EF032717					
Aacrodiplax balteata	COI: N/A	N/A				
	COII: EU055332					
	EF1-α: EF640538					
	18S: EU055134					
	28S: EU055229					
	12S & 16S: EU054940, EU055040					
Macromia illinoiensis	COI: N/A	N/A				
	COII: N/A					
	EF1- α : N/A					
	18S: N/A					
	D7: EF631208					
	D3: EF631305					
	D2. FF631410					
	100.120. EF621574					
	1230(103) EF031324					
Macromia urania	COI: N/A	D2:123456				
	COII:N/A	16S:123456				
	EF1-α: N/A					
	18S: N/A					
	28S· N/A					
	12S & 16S: N/A					
Macromidia rapida	COI: N/A	N/A				
	COII: N/A					
	EF1_0' N/A					
	18S- N/A					
	D7- FF631209					
	D3: EF631306					
	D2: EF631411					
	12S&16S: NA					
Macromidia rapida	COI: N/A	N/A				
	COII: N/A					
	EFT or N/A					
	10C. M/A					
	185: N/A					
	D/: EF0312/1					

	N/A	N/A	D2:123456	N/A	N/A	N/A	N/A
18S: N/A D7: EF631240 D3: N/A D2: EF631445 16S: EF631557	COI: N/A COII: N/A EFI-α: N/A 18S: N/A 28S: EF631459, EF631345, EF631254 12S & 16S: EF631569	COI: N/A COII: N/A EF1-0: N/A 18S: N/A 28S: EF631446, EF631335, EF631241 12S & 16S: EF631558	COI: N/A COII: N/A EF1-c: N/A 18S: N/A 28S: N/A 12S & 16S: N/A	COI: N/A COII: N/A EF1-0: EF640466 18S: N/A 28S: EF631412, EF631307, EF631210 12S & 16S: EF640388, EF631525	Morphology only	Morphology only	COI: N/A COII: N/A EF1-α: N/A 18S: N/A 28S: EF631486, EF631368, EF631279
	Nannophlebia risi	Nannophya dalei	Nannophyopsis clara	Nannothemis bella	Neocordulia batesi longipollex	Neocordulia campana	Neodythemis pauliani

	12S & 16S: N/A	
Neopetalia punctata	COI: N/A	N/A
	COII: N/A	
	EF1- α : N/A	
	18S: N/A	
	D7: EF631247	
	D3: EF631338	
	D2: EF631452	
	12S&16S: EF631563	
Neophya rutherfordi	Morphology only	N/A
Nephepeltia phyryne	Morphology only	N/A
Nesciothemis minor	COI: N/A	D2:123456
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: N/A	
	12S & 16S: N/A	
Neurocordula obsoleta	COI: N/A	N/A
	COII: N/A	
	FF1-α: N/A	
	18° N/A	
	103. IVA 398. EF621205 EF621106	
	203. LT 021273, LT 031170 130 8. 160. EE621500	
11 11 11 11 11 11 11 11 11 11 11 11 11	123 & 103. EF 021309	
Neurocordulia xanthosoma	C0I: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631447, EF631242	
	12S & 16S: N/A	
Oligoclada walkeri	Morphology only	N/A
Onychothemis culminicola	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631493, EF631374	
	12S & 16S: EF631598	

Onvehothomis tostaroa	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631485, EF631367, EF631278	
	12S & 16S: EF640408	
Onychothemis testacea	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631494, EF631375, EF631284	
	12S & 16S: DQ021427, EF631599	
Orthemis ferruginea	COI: AF195760	N/A
	COII: N/A	
	EF1-α: EF640482	
	18S: N/A	
	28S: EF631472, EF631357, EF631266	
	12S & 16S: EF640402, EF631582	
Orthetrum abbotti	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631482, EF631275	
	12S & 16S: EF631591	
Orthetrum austeni	COI: N/A	D2:123456
	COII: N/A	16S:123456
	EF1-α: N/A	
	18S: N/A	
	28S: N/A	
	12S & 16S: N/A	
Orthetrum chrysis sp	Morphology only	N/A
Orthetrum hintzi	COI: N/A	D2:123456
	COII: N/A	16S:123456
	EF1-α: N/A	
	18S: N/A	
	28S: N/A	

				N/A						N/A						N/A						N/A						N/A						N/A	
EF1-0: EF640512	18S: N/A	28S: EF631398, EF631294, EF631198	12S & 16S: EF640433, EF631512	COI: AY582782	COII: N/A	$EF1-\alpha$: N/A	18S: N/A	28S: EF631414, AY582763, AY582762	12S & 16S. EF631527	COI: AY582796	COII: N/A	EF1- α :N/A	18S: N/A	288. FF631467 FF631353 FF631262	128 & 168: EF631577	COI: AY582788	COII: N/A	EF1-cc: N/A	18S: N/A	28S: AY582768	12S & 16S: N/A	COI: N/A	COII: N/A	EF1-α: EF640534	18S:N/A	28S: EF631492, EF631373	12S & 16S: EF640455, EF631597	COI: DQ294650	COII: DQ166791	EF1-α: EF640529	18S: EF680326	28S: EF631487, EF631316, EF631220	12S & 16S: EF640450,	COI:N/A	COII: N/A
				Palpopleura jucunda	2					Palpopleura lucia						Palpopleura portia						Paltothemis lineatipes						Pantala flavescens						Pantala flavescens	

	N/A	N/A	N/A	D2:123456	N/A
EF1-0: N/A 18S: N/A 28S: EF631369, EF631280 12S & 16S: EF631537	COI: N/A COII: N/A EF1-0: N/A 18S: N/A 28S: EF631415, EF631308, EF631211 12S & 16S: EF631528	COI: COII: EF1-α: EF640488 18S: 28S: EF631416, EF631309, EF631212 12S & 16S: EF640409, EF631529	COI: N/A COII: N/A EF1-cc: N/A 18S: N/A D7: EF631197 D3: EF631293 D2: EF631293 D2: EF631397 12S&16S: EF631511	COI: N/A COII: N/A EF1-0: N/A 18S: N/A 28S: N/A 12S & 16S: N/A	COI: AF195750 COII: N/A EF1-0: N/A 18S: N/A 28S: EF631438, EF631330, EF631234 12S & 16S: EF631552
	Pentathemis membranulata	Perithemis tenera	Phyllomacromia contumax	Phyllomacromia hervei	Plathemis lydia

un nu dalli a cu ani		NI/A
coranna grayn	COL: N/A COII: N/A	N/N
	EF1-α: N/A	
	18S: N/A	
	28S: EF631399, EF631199	
	12S & 16S: EF631513	
cordulia smithi	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631400, EF631295, EF631200	
	12S & 16S: EF631514	
udocordulia circularis	COI: N/A	N/A
	COII: N/A	
	EF1- α : N/A	
	18S: N/A	
	D7: EF631251	
	D3: EF631342	
	D2: EF631456	
	16S: EF631566	
udothemis zonata	COI: N/A	N/A
	COII: DQ166799	
	EF1-α: N/A	
	18S: N/A	
	28S: AB127416	
	12S & 16S: EF032730	
dopygia hollandi (morphology), R. hinei (molecular)	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640516	
	18S: N/A	
	28S: N/A	
	12S & 16S: EF640437	
othemis semihyalina	COI: N/A	N/A
	COII: N/A	
	EF1-or: N/A	
	18S: N/A	

	N/A				N/A					NT/A	IN/A					N/A								D2:123456						D2:123456	
288: EF631474, EF631358 128 & 168: EF631584	COI: N/A COII: N/A	EF1-α: N/A	18S: N/A	288: EF631406, EF631301, EF631204 128 & 168: EF631520	COI: N/A	COII: N/A	EF1-α: N/A	18S: N/A	28S: EF631480, EF631364, EF631273	123 & 103. EF031390	COL: IV/A COII: N/A	EFI-0: N/A	18S: N/A	28S: EF631476, EF631360, EF631269	12S & 16S: EF631586	COI: N/A	COII: N/A	EF1-α: N/A	18S: N/A	D7: EF631249	D3: EF631340	D2: EF631454	12S&16S: EF631564	COI: N/A	COII:N/A	EF1-α: N/A	18S: N/A	28S: N/A	12S & 16S: N/A	COI: N/A	CUII:N/A
	Rhyothemis sp.				Rialla villosa					G	ocapanea frontans					Sinorogomphus sp.								Somatochlora borisi						Somatochlora graeseri	

	N/A	N/A	D2:123456	N/A	N/A	N/A
EF1-α: N/A 18S: N/A 28S: N/A 12S & 16S: N/A	COI: N/A COII: N/A EF1-0: N/A 18S: N/A 28S: EF631419, EF631311, EF631215 12S & 16S: EF631532	COI: EF636300 COII: EF1-α: 18S: EF636418 28S: EF631324 12S & 16S: EF631548	COI: N/A COII: N/A EF1-α: EF640518 18S: EU055135 28S: EU055230 12S & 16S: EU054941, EU055041	COI: EF636318 COII: EF1-0: 18S: EF636457 28S: EF631418, EF631310, EF631214 12S & 16S: EF631531	COI: N/A COII: N/A EF1-0: N/A 18S: N/A 28S: EF631439 12S & 16S: N/A	COI: N/A COII: N/A
	Somatochlora tenebrosa	Sympetrum ambiguum	Sympetrum corruptum	Sympetrum janeae	Syncordulia gracilis	Synthemiopsis gomphomacromioides

	N/A	N/A	N/A	V/N	N/A	N/A
EF1-or: N/A 18S: N/A 28S: EF631417, EF631213 12S & 16S: EF631530	COI: N/A COII: N/A EF1-c: N/A 18S: N/A 28S: EF631401, EF631296 12S & 16S: EF631515	COI: N/A COII: N/A EF1-c: N/A 18S: N/A 28S: EF631402, EF631297, EF631201 12S & 16S: EF631516	COI: N/A COII: N/A EF1-cc: N/A 18S: N/A D7: EF631216 D3: EF631312 D2: EF631420 12S&16S: EF631533	Morphology only	COI: N/A COII: N/A EF1-cc: N/A 18S: N/A 28S: EF631379, EF631325, EF631231 12S & 16S: N/A	COI:N/A COII: N/A EF1-cc: N/A
	Synthemis eustalacta	Synthemis leachii	Taeniogaster obliqua	Tauriphila sp	Tetragoneuria cynosura	Tetrathemis polleni

	18S· N/A	
	28S: EF631495, EF631376	
	12S & 16S: EF631600	
Tetrathemis polleni	COI:N/A	N/A
	COII:N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631426, EF631318, EF631222	
	12S & 16S: EF631539	
Thermochoria equivocata	Morphology only	N/A
Tholymis tillarga	COI: N/A	N/A
)	COII:N/A	
	EF1-α: EF640531	
	18S: N/A	
	28S: EF631403, EF631298, EF631202	
	12S & 16S: EF640452, EF631517	
Tramea lacerata	COI: N/A	N/A
	COII: EU055368	
	EF1-α: EF640532	
	18S: EU055171	
	28S: EF631425 , EF631317, EF631221, EU055269	
	12S & 16S. EU054980, EF631538	
Tramea onusta	COI:N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631488, EF631281	
	12S & 16S: AY282561 , EF631593	
Trithemis dorsalis	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631404, EF631299	
	12S & 16S: EF631518	
Trithemis monardi	COI:N/A	N/A
	COII: N/A	

	$FF1_{-M}$ · N/A	
	18S: N/A	
	28S: EF631469, EF631355, EF631264	
	12S & 16S: EF631579	
Trithemis sp	Morphology only	N/A
Uracis fastigiata	COI: N/A	N/A
	COII: N/A	
	EF1-α: EF640468	
	18S: N/A	
	28S: EF631432, EF631228	
	12S & 16S: EF640390, EF631545	
Urothemis assignata	COI: N/A	N/A
)	COII: N/A	
	EF1-α: EF640539	
	18S:N/A	
	28S: EF631421, EF631313, EF631217	
	12S & 16S: EF640460, EF631534	
Urothemis sp	Morphology only	N/A
Williamsonia fletcheri	Morphology only	N/A
Zenithoptera fasciata	COI: N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631491, EF631372, EF631283	
	12S & 16S: EF631596	
Zenithoptera fasciata	COI: N/A	N/A
	COII:N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631490, EF631371	
	12S & 16S: EF631595	
Zoreana bilineata	Morphology only	N/A
Zygonyx natalensis	COI: N/A	N/A
	COII: N/A	
	EF1-0: N/A	
	18S: N/A	

	28S: EF631405, EF631300, EF631203	
	12S & 16S: EF631519	
Zygonyx torridus	COI:N/A	N/A
	COII: N/A	
	EF1-α: N/A	
	18S: N/A	
	28S: EF631422, EF631314, EF631218	
	12S & 16S: EF631535	
Zyxomma elgneri	COI: N/A	N/A
	COII: N/A	
	EF1-cc: N/A	
	18S: N/A	
	28S: EF631458, EF631344, EF631253	
	12S & 16S: EF631568	
Zyxomma petiolatum	COI:N/A	N/A
	COII: N/A	
	EF1-cc: N/A	
	18S: N/A	
	28S: EF631496, EF631378	
	12S & 16S: N/A	

based on oldest known anisopteran fossils, which are from the Triassic; the split between Corduliidae and Libellulidae had analysis (LnL=-58249.808); analysis used a penalized likelihood and a TN algorithm. The 250 million year age constraint Table 2: Divergence estimates from a r8s analysis of the best tree from a reduced-taxon-sample doublet model Bayesian already occurred by 14.5-16.5 Myr and probably occurred as early as 118-131 Myr so we implemented a date from the Wealden formation (approximate time periods suggested by Jarzembowski and Nel (1996)).

Node	Max Age constraint (millions of years)	Min Age constraint (millions of years)	Estimated Age of Node from present study (millions of years)	Estimated Age of family from Ware et al. (2008; paired- site analysis) (millions	Percent difference between divergence estimates	Estimated Age of Node from Ware et al. (submitted) (millions of years)	Percent difference between divergence estimates
Root		250.00	214.15	205.7	4%	193.74	9.5 %
Chloropetalia +			158.56	186.3	15%	1	
Sinorogomphus +							
remaining Libeluluted							
Chleronotolia			100 23				
Chloropetalia + Sinorogomphus			108.33				
Cordulegastridae			157.77	1		-	
+ Neopetaliidae							
Cordulegastridae			140.51			158.15	11%
GSI + MCL			132.10			172.01	23%
GSI			110.74			132.35	16%
Gomphomacromia			84.60				
+ Archaeophya + Svnthemistidae							
Synthemistidae			51.70				

nsu stricto						
nacromia +	 	54.21			-	
cromidia +						
nyx						
naining GSI	 	83.99				
Complex	 	100.76	144.0	30%		
cromiidae	 	92.72	34.2	63%	-	-
rduliidae +	 250.00	90.88				
ellulidae						
rduliidae	 131.00	85.37	87.1	1.9%	-	
ntathemis +	 	53.25	-	1	-	-
schnosoma						
maining	 	74.70		1	-	-
rduliidae						
bellulidae	 131.00	67.68	87.6	23%	-	-
ide A	 	44.66			-	-
ide B	 	18.54				
ade C	 	22.63				
ide D	 	23.71				
ide E	 	19.75				
lde F	 	26.87				
ide G	 	21.36			-	-

Clade	Ware et	Pilgrim and von	Letsch, 2007; 12S,	Present Study;
	al., 2007;	Dohlen, 2008;	16S, tRNA	COI, COII,
	12S, 16S,	12S, 16S, EF-1a,	Valine, 28S	128, 168, 188,
	D2, D3,	wing	,	28S, EF-1a,
	D7	morphology		adult and larval
	fragments	1 00		morphology;
	of the 28S			doublet model
Synthemis +	Yes,	Not included	Yes, 100% PP,	Yes, 95% PP,
Eusynthemis +	100% PP		including only	additionally
Choristhemis +			Synthemis,	including
Cordulephya +			Eusynthemis,	Idomacromia*
Idionyx +			Gomphomacromia,	and Neocordulia
Micromidia +			Oxygastra, and	campana*
Hesperocordulia			Idomacromia	1
+				
Gomphomacromia				
+ Oxvgastra +				
Macromidia +				
Lathrocordulia +				
Pseudocordulia +				
Svncordulia +				
Austrocordulia +				
Austrophya +				
Archaeonhva+				
Synthemionsis				
Corduliinae	Ves 86%	Only Cordulia	Ves 100% PP	Ves 96% PP
Coldumnae	DD	shurtleffii	105, 100/011.	additionally
	11	included		including
		merudeu		Anocordulia*
				and Eulaia*
Maaramiidaa	Vac	Only Magnomia	Vog 1000/ DD	Voc. 1000/ DD
Macrominuae	1 es, 1000/ DD	Unity Macromia	res, 100% PP.	1 es, 100% PP
	10070 FF	included		
Sumpoting	Vag 000/	Vog 100% DD	Voc. 1000/ DD	Voc. 040/ DD:
Sympetrum +	1 CS, 9970 DD	1 es, 100 % FF	1 es, 100 % FF.	105, 9470 FF,
Leucorrninia + Colithomia	rr			but with the
Ceilinemis				possible
				Chlammant
Charles at 1:	V 000/	V 040/ DD	$V_{12} = 0.50/DD = 1$	Chlorogomphus
Cnaicostephia +	1 es, 98%	r es, 94% PP, no	res, 95% PP, also	INO;,DUI
Brachythemis +	PP	aata Ior	Including	supported by
Brachyaiplax +		Brachyaiplax,	<i>Pseudotnemis</i> , not	5/% PP 11
Deielia +		Idiataphe,	including	Brachydiplax

Table 3: Libelluloid taxonomic groups recovered among recent molecular studies; PP= posterior probability from Bayesian analysis.

<i>Idiataphe</i> +		Zyxomma.	Idiataphe or	excluded
Tholymis +			Brachythemis	
Zyxomma				
Dythemis +	Yes, 96%	Yes, 99% PP, no	Only	Yes, 96% PP
Macrothemis +	PP	data for	Brechorrhoga	
Scapanea +		<i>Macrothemis</i> or	included.	
Paltothemis +		Scapanea		
Brechmorhoga				
Diplacodes +	Smaller	No; but 100% PP	Yes, 80%,	Yes, 96% PP[?],
Bradinopyga +	analysis	also including	additionally	with the
Hemistigma +	[?]: Yes,	Neurothemis,	including	additional
Palpopleura+	60%	Nanodiplax,	Rhodothemis,	inclusion of
Nannophya +	[46%?];	Pseudoleon, and	Thermochoria and	Rhodopygia and
Uracis + Acisoma		Philonomon; not	Neurothemis.	Thermochoria*
+ Erythemis +		including		[Pachydiplax?]
Nannothemis +		Acisoma,		
Erythrodiplax +		Nannothemis,		
Crocothemis		and Erythemis;		
+Pachydiplax		no data for		
		Hemistigma,		
		Pachydiplax, or		
		Palpopleura;		
Pantala +	Yes, 68%	No, <50% PP, no	Yes, 100% PP,	No, but 58% PP,
Malgassophlebia	PP	data for	additionally	for Pantala +
+ Trithemis +		Malgassophlebia,	including	Trithemis +
Onychothemis +		Nannophlebia	Oplogastra,	Cannaphila* +
Zygonyx +		and <i>Huonia</i> .	Atoconeura, and	Nephepeltia* +
Nannophlebia			Porpacithemis, no	Atoconeura; no
+Bironides +			data for Pantala or	data for [?]
Huonia			Nannophlebia.	Malgassophlebia

*Morphological data only.

Morphological dataset	Mean RC	Variance in RC	Standard deviation in RC	Number of characters
Wing venation	0.083286138	0.06495527	0.254863238	29
External body	0.03804605	0.003719649	0.060988925	20
Accessory	0.052767143	0.004895385	0.069967031	14
genitalic				
Penile	0.098025429	0.070147723	0.264854154	14
Larval	0.108457727	0.049381177	0.22221876	22

Table 4: Rescaled-consistency indexes for each morphological dataset.

Table 5: Diversification rates among clades in Libelluloidea. *= For the diversification rate of Libelluloidea we chose an age for the stem group of 250.00, the age of the oldest Anisopteran fossils, which is a conservatively high estimate. The actual rate of diversification may be slightly higher, but not higher than the rate estimated using the root age ($r_{s=0.95}=0.0088$; $r_{s=0.0}=0.0148$).

Stem Group	Number of	Age of node	Diversification	Diversification
	species in		rate; ε=0.95	rate; ε=0
	stem group			
Libelluloidea*	1459	250.00*	0.007474578	0.012656221
Chlorogomphidae	46	214.14	0.002390414	0.007764817
Cordulegastridae +	50	158.56	0.003391896	0.010714998
Neopetaliidae				
GSI	126	132.1027	0.006512645	0.015899528
Macromiidae	122	100.7658	0.00841743	0.020705039
Corduliidae +	1116	90.8788	0.019300055	0.03353548
Libellulidae				

Table 6: Recovery of monophyletic groups in Libelluloidea using morphological datasets; Y= monophyly supported, N= monophyly not supported

			* *		
Taxonomic	Wing	Penile	Larval	Accessory	External
Group	venation	characters	characters	genitalic	body
				characters	characters
Cordulegastridae	Ν	Ν	Y	Y	Ν
GSI	Ν	Ν	Ν	Ν	Ν
Macromiidae	Ν	Ν	Ν	Ν	Ν
Corduliidae	Ν	Ν	Ν	Ν	Ν
Libellulidae	Y	Ν	Y	Ν	Y
Tetrathemistinae	Ν	Ν	Ν	Ν	Ν
Leucorrhiniinae	Ν	Ν	Ν	Ν	Ν
Libellulinae	Ν	Ν	Ν	Ν	Ν
Urothemistinae	Ν	Ν	Ν	Ν	Ν

Table 7: Morphological characters <u>Adult characters</u> -

Wing venation:

- Number of cross veins between costal braces in forewing; 0=several, 1=one, 2=none (Fraser, 1957)
 - Number of cross veins in midbasal space of forewing; 0=absent, 1=present (Fraser, 1957) Number of forewing Cubital-Anal cross veins: 0=>2, 1=<=2 (Bechly, 1994)
 - <u>vi w 4</u>
- Basal fusion of the sectors of the arculus: 0=unfused, basally separated, 1= fused only at extreme base (<1/4 arculus length), 2=fused beyond base but distinctly <1/2 arculus length in at least one pair of wings, 3= fused for >=1/2 arculus length in at least one pair of wings (Carle, 1995)
 - Cross veins in supertriangle of hindwing, 0=present, 1= absent (Fraser, 1957)
 - Cross veins in triangle of hindwing, 0=present, 1=absent (Fraser, 1957)
- Costal side of triangle in forewing, 0=straight, 1=angulated (Needham et al., 2000)
 - Hindwing triangle, 0=far distal to arculus, 1= near arculus but distal, 2= at or proximal to arculus (Needham et al., 2000)
- Form of anal loop, 0=compact, 1=elongate with no midrib, 2= elongate with zigzag midrib, 3= elongate with straight midrib, 4= elongate with expanded tip, 5= absent or greatly reduced (Needham et al., 2000)
 - 10. Forewing supertriangle 0=cross vein present, 1= cross vein absent (Fraser, 1957)
 - 11. Forewing triangle 0=cross vein present, 1= cross vein absent (Fraser, 1957)
- 12. Postanal cells, 0= not differentiated, 1= open behind, 2= one closed, 3= two or more closed, 4= absent (May, unpublished)
 - 13. Veins r4+5 and MA in forewing 0=divergent, 1=parallel or convergent (Needham et al., 2000)
- 14. Forewing discoidal field/trigonal interspace, 0=widely divergent, 1=slightly divergent, 2=parallel or convergent (Needham et al., 2000
 - 15. Vein R4+5 and/or MA 0=undulating, 1=not undulating (Needham and Westfall, 1955)
 - 16. Last antenodal cross vein in forewing complete=0; incomplete=1 (Fraser, 1957)
- 17. Arculus proximal to antenodal cross vein 2 in hindwing=0; at or distal to antenodal cross vein number 2 but closer to 2 than 3=1; barely proximal to distal to antenodal cross vein number 3=2 (Fraser, 1957)
 - Cross veins under proximal half of pterostigma=1 or not=0 (Garrison et al., 2007) 18.
 - 19. RP1, 2 concave=0 or not=1 (Garrison et al., 2007)
- 20. RSPL 1 row=0 or 2 rows=1 or absent=2 (Garrison et al., 2007)
 - 21. Anal loop toe open=0 or closed=1 (Fraser, 1957)
 - 22. Costa undulate=0; not undulate=1 (Fraser, 1957)
 - Bridge cross veins 1=0; > 1=1 (Fraser, 1957) 23.]
- Pterostigma trapezoidal=0; not trapezoidal=1 (Fraser, 1957; Needham et al., 2000) 24.
 - 25. Midrib of anal loop straight=0; obtuse=1; acute/90=2 (Garrison et al., 2007)
- Vein Cu1 (Comstock-Needham system) of hindwing arising at posterior angle of triangle =0; arising anterodistal to posterior angle of triangle=1 (Garrison et al., 2007) 26.
 - Triangle in forewing with costal side broken, short, 4 sided=0, not so, 3 sided=1 (Needham and Broughton, 1927; Fraser, 1957) 27.

28. Hindwing triangle 3 sided=0, 4 sided=1 (Needham and Broughton, 1927; Fraser, 1957) 29. Discoidal field forewing 1 row=0, 2 or more=1 (Needham and Broughton, 1927; Fraser, 1957)

External body characters

- 30. Lateral abdominal carinae: absent=0; present on one or two segments=1; present on>two segments=2 (Needham et al., 2000)
 - 31. Distal abdominal expansion: absent=0; present=1 (Carle and Kjer, 2002)
- 32. Ventrolateral processes on abdominal segment 1: absent=0; small/rudimentary=1; large=2 (Needham et al., 2000)
 - 33. Dorsal carina on abdominal segment 10: absent=0; present=1 (Needham et al., 2000) 34.
- Ventral tooth on tarsal claws: normal=0; enlarged=1; fat & short/normal length=2 (Needham et al., 2000)
 - 35. Protibial keel: short=0; long=1; absent=2 (Fraser, 1957)
 - 36. Auricles: present=0; absent=1 (Needham et al., 2000)
- 37. Male eppiproct: notched-rectangular=0; intermediate=1; distally tapered=2 (Needham et al., 2000)
 - Vertex tuberculate=0; not tuberculate=1 (Garrison et al., 2007) 38.
- 39. Posterior lobe of pronotum widest at base=0 /markedly constricted at base=1 (Garrison et al., 2007)
 - 40. Inferior tooth of tarsal claw vestigial or absent=0; well-developed=1 (Garrison et al., 2007)
- 41. Male metafemur with spines very short, stout, hooked proximately=0; mostly slender and not hooked=1 (Garrison et al., 2007) 42. Male metafemur with spines distinctly dimorphic in length=0; uniform or gradually incr. in length=1 (Garrison et al., 2007)
 - 43. Lateral carina on S9 present=0; absent=1 (Garrison et al., 2007)
- 44. Sternum of s9 not projected distally=0 or projected distally=1 (Garrison et al., 2007)
- 45. Female sternum 9 elongated to or nearly to distal margin of sternum10=0; hardly beyond base of sternum 10=1 (Garrison et al., 2007)
 - 46. Supplementary transverse carina on S3 present=1 absent=0 (Garrison et al., 2007)
- 47. Female tergum 8 with=0; without distinct ventrolateral flange=1 (Garrison et al., 2007) 48. Eye contact barely or not at all=0, longer/touching=1 (Needham and Broughton, 1927; Fraser, 1957)
- Accessory genitalic characters
- 49. Genital lobe: 0=absent, 1=rudimentary, 2=large (Schmidt, 1916)
- 50. Ventral branch of posterior hamule: 0=short, 1=long (Needham et al., 2000)
- 51. Posterior hamule: 0=not compressed, 1= laterally compressed (Needham et al., 2000)
 - 52. Median lobe of anterior hamule: 0=absent, 1=present (May, unpublished)
 - 53. Anterior hamule: 0=large and erect, 1=not so (Needham et al., 2000)
- 54. Form of ovipositor: 0=both gonapophyses elongate, 1=both gonapophyses short, 2= first pair secondarily elongate (Fraser,
- 55. Vulvar lamina with wide cleft or narrow cleft=0 or not bifid=1 (Garrison et al., 2007)
 - 56. Vulvar lamina projected ventrally=0 or not.=1 (Garrison et al., 2007)

Penile characters

57. Shape of ligula: 0- keeled, 1-scoop like, 2-curled and flattened, 3-short (Schmidt, 1916)

New Characters

- 88. Spines on genital lobe: 0= long and silk hairs no spines, or if present very rudimentary, 1= short stout spines along genital lobe and in a dense patch at the anterior most part of secondary abdominal segment, 2= short stout spines well spaced evenly along the entire margin of genital lobe up to anterior most part
 - 89. Spines on auricle: 0=no spines, 1= spines
- 90. Ventral hamule pointy=0, spatula shaped=1
- 91. Dorsal hamule pointy=0, spatula shaped=1
- 92. Length of dorsal hamule: longer than ventral or equal=0, shorter=1
- 93. Anterior lamina with projections or not: lateral projections, or one central projection=1, no projections=0
 - 94. Coxal protuberances: yes=1, no=0
- 95. Ventral neck buldge: no=0, yes=1 96. Sulci on 8 and 9: none=0, only 8=2, 8&9=1 (Tennessen, pers. comm.)
- 97. Number of teeth on inner mandible 0=1 row of serrated teeth, 1=2 rows of serrated teeth, 2= one row followed by a small row with even number of teeth l and r, 3= one row followed by a small row with uneven number of teeth l and r

 - 98. Eyes protruding?=1, flat=0 (Tennessen, pers. comm.)
 99. Hair under postmentum? (Tennessen, pers. comm.)
 100. Cleft in premental hinge? 0=no, 1=yes.

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Sympetrum .

Table 8 : Miller's penile characters arranged by our phylogenetic hypothesis. 1= Large, inflatable and usually very spinose apical capable of considerable inflation; 19= Long, narrow, spiny inner lobes that are well sclerotized, resembling flagella and inflating mainly in the lateral plane; 20= Broad inner lobes, extending very greatly in length with inflation; 21= Much reduced inner inflatable; 11 = As 10, but with a single, terminal and inflatable lobe in central plate of medial process; 12 = Less sclerotized and extensively inflatable central process of medial lobe; 13 = Single flagellum, sometimes with a terminal barb or terminal process; 14= Bifurcated flagella, each part ending in an expanded region or a barb like process; 15= Cornua long and strongly curved; 16= Cornua short and stout and sometimes partially fused together; 17= Cornua unsclerotized and weak; 18=Broad inner lobes sparingly inflatable apical lobe; 5= much reduced or absent apical lobe; 6= Large, flat, lateral plates as lateral lobes; 7= Long and blade-like lateral lobes; 8= Long, curved and rod-like lateral lobes, resembling cornua; 9= much reduced or absent lateral lobe; 2= As 1, but bilobed; 3= As 1, but with dorsally evaginating lobe; 4= Long, narrow and largely sclerotized, and at most lobes; 10= Central plate of medial process with large, prominent and well-sclerotized process; not extensively or not at all

IODES.							
Taxon	Clade	Apical Lobe	Lateral Lobes	Medial process:	Medial Process	Medial Droces:	Medial Droces:
				Central Plate	Flagellum	Cornua	Inner Lobes
Potamarcha congener	Taxon not	2	6	ć	no flagella	no cornua	18
	present in our analysis						
Parazyxomma	Taxon not	1	9	10	14	no cornua	19
flavicans	present in our						
	analysis						
Indothemis carnatica	Taxon not	1	9	11	no flagella	no cornua	<u>ر.</u>
	present in our						
	analysis						
Tetrathemis polleni	a	1	6	10	no flagella	no cornua	ć
Urothemis assignata	q	1	9	10	no flagella	no cornua	20

198

Urothemis edwardsi	р	1	6	10	no flagella	no cornua	20	
Trithemis pallidinervis	υ	1	6	10	no flagella	no cornua	18	
Trithemis annulata	U	4	6	10	no flagella	no cornua	19	
Trithemis aurora	U	4	6	10	no flagella	no cornua	19	
Trithemis donaldsoni	U	4	6	10	no flagella	no cornua	19	
Trithemis furva	U	4	9	10	no flagella	no cornua	19	
Trithemis stictica	U	4	6	10	no flagella	no cornua	19	
Trithemis arteriosa	U	e	6	12	no flagella	no cornua	19	
Trithemis kirbyi	U	4	6	12	no flagella	no cornua	19	
ardens Zygonyx natalensis	U	б	9	12	no flagella	no cornua	19	
Zygonyx torridus	υ	ε	6	12	no flagella	no cornua	19	
Pantala flavescens	U	1	6	10	-	.3 no cornua	ć.	
Sympetrum danae	p	1	6	10	no flagella	15	18	
Sympetrum	q	1	9	10	no flagella		15 18	
depressiusculum Sympetrum	q	1	ø	10	no flagella		15 18	
sanguineum					h			
Rhyothemis	q	1	9	10	no flagella	16	18	
Sympetrum	p	1	9	12	no flagella		15 18	
fonscolombei	τ	Ţ	ų	ç			10	
sympetrum meridionale	٥	Т	٥	77	no nagella		ΩT CT	
Celithemis eponina	q	1	9	ć.	no flagella		16 18	
Sympetrum striolatum	p	1	6	10	no flagella	no cornua	20	
Brachythemis	Ð	1	6	10		.3 no cornua	19	
Brachythemis	Ð	1	9	10	-	4 no cornua	19	
contaminata			ų	(Ċ	
Zyxomma petiolatum	Θ		9	10	:	4 16	ر. .	
Brachythemis Ieucosticta	θ	1	9	10	no flagella	no cornua	۰.	
Tholymis tillarga	Θ	1	9	10	no flagella	no cornua	ć	
Tramea basilaris	Ð	5	6	11	no flagella	no cornua	ć	
Tramea limbata	в	5	6	11	no flagella	no cornua	ć.	
Chalcostephia flavifrons	Ð	1	9	12	no flagella	no cornua	د.	
Diplacodes	б	5	9	10	no flagella	no cornua	15	
bipunctaata Diplacodes trivialis	б	1	9	10	no flagella		16 18	
Neurothemis tullia	б	1	6	12	no flagella	17	18	
Neurothemis fulvia	б	1	6	12	no flagella	no cornua	18	
Crocothemis	D	1	9	10	no flagella	no cornua		18
erythraea Crocothemis	D	1	6	10	no flagella	no cornua	18	
sanguinolenta		Ŧ	ļ	Q			C T	
Crocotnemis servilla	D	4	٥	ΠT	no riagella	no cornua	81	

Pachydiplax	б	1	9	12	no flagella		16 18	
Diplacodes lefebvrei	Ð	1	9	10	no flagella	16	<i>د</i> .	
Palpopleura lucia	g	1	9	10	no flagella		16 ?	
Hemistigma	б	1	9	10	no flagella	no cornua	<u>ر.</u>	
aipipuncta Acisoma panorpoides	б	1	6	10	no flagella	no cornua	د.	
Erythemis simulicicollis	б	1	9	10	no flagella	no cornua	ć	
Bradinopyga cornuta	D	1	9	11	no flagella	no cornua	ć	
Bradinopyga geminata	б	2	9	11	no flagella	no cornua	ć	
Perithemis tenera	ч	5	6	12	no flagella	no cornua	18	
Libellula incesta	ч	2	8	12	no flagella	no cornua		18
Nesciothemis farinosa	ч	2	6	12	no flagella	no cornua		18
Libellula	ч	1	7	خ	no flagella		15	18
quadrimaculata Hadrothemus coacta	Ч	1	9	ذ.	no flagella		16 20	
Hadrothemis infesta	ч	1	6	ć	no flagella		16 20	
Hadrothemis	ч	5	ć	خ	no flagella		16	20
camerensis Hadrothemis defecta	۲	1	ć	ć	no flagella		16	20
pseuaoaerecta Allorhizuca preussi	Ч	4	9	10	13	no cornua	ذ.	
Libellula depressa	ч	1	7	10	no flagella	no cornua	ć.	
Libellula lydia	ч	J	6	12	no flagella	no cornua	ر.	
Libellula cyanea	ч	2	8	12	no flagella	no cornua	خ	
Lokia coryndoni	خ.	1	6	خ	13	15	خ	
Orthetrum	ч	1	7	13	no flagella	no cornua	<i>د</i> .	
caledonicum Orthetrum	ч	1	7	13	no flagella	no cornua	ć	
cancellatum Orthetrum	ч	Ħ	7	13	no flagella	no cornua	د.	
chrysostigma Orthetrum	ч	Ţ	7	13	no flagella	no cornua	ć	
coerulescens Orthetrum pruinosum	£	1	7	13	no flagella	no cornua	<i>د</i> .	
Orthetrum sabina	Ч	1	7	13	no flagella	no cornua	د.	
Orthetrum trinacria	ч	1	7	13	no flagella	no cornua	ć	
Orthemis ferruginea	ч	5	6, 9	15	no flagella	no cornua	ځ	

Chapter 5:

Relationships among the major lineages of Dictyoptera: the effect of outgroup selection on dictyopteran tree topology

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Abstract

Dictyoptera, comprising Blattaria, Isoptera, and Mantodea, are diverse in appearance and life history, and are strongly supported as monophyletic. We downloaded COII, 16S, 18S, and 28S sequences of 39 dictyopteran species from GenBank. Ribosomal RNA sequences were manually aligned with reference to secondary structure. We included morphological data (maximum of 175 characters) for 12 of these taxa and an additional 15 dictyopteran taxa (for which we had only morphological data). We had two datasets, one 59 taxon dataset with 5 outgroup taxa including Phasmatodea (2 taxa), Mantophasmatodea (1 taxon), Embioptera (1 taxon), and Grylloblattodea (1 taxon), and a 62 taxon dataset including 3 additional outgroup taxa from Plecoptera (1 taxon), Dermaptera (1 taxon) and Orthoptera (1 taxon). We analyzed the combined molecularmorphological dataset using both the doublet and MK models in MrBayes, and using a parsimony heuristic search in PAUP. Within the monophyletic Mantodea, Mantoida and then *Chaeteessa* are confirmed as the most basal branches; the monophyly of most of the more derived families as currently defined is not supported. Unique to our study, one Bayesian analysis places Polyphagoidea as sister to all other Dictyoptera; other analyses and/or the addition of certain orthopteran sequences, however, placed Polyphagoidea

more deeply within Dictyoptera. Isoptera falls within the cockroaches, sister to the genus *Cryptocercus*. Separate parsimony analyses of independent gene fragments suggest that gene selection is an important factor in tree reconstruction. When we varied the ingroup taxa and/or outgroup taxa, the internal dictyopteran relationships differed in the position of several taxa of interest including *Cryptocercus*, *Polyphaga*, *Periplaneta* and *Supella*. This provides further evidence that the choice of both outgroup and ingroup taxa greatly affects tree topologies.

1. Introduction

The Dictyoptera includes the eusocial Isoptera (ca. 3,000 species), predatory Mantodea (ca. 2,450 species) and Blattaria (ca. 4,000 species), which show extreme divergence in both appearance and life history. Yet, the monophyly of this group is generally accepted based on morphological synapomorphies in the male and female genitalia, proventriculus, tentorium, and wings (Kristensen, 1975, 1981, 1991; Klass, 1995, 1998a, b, 2000, 2003; Grimaldi and Engel, 2005; Beutel and Gorb, 2006; Klass and Meier, 2006). This has been confirmed by many molecular-based cladistic analyses (Maekawa et al., 1999; Wheeler et al., 2001; Kjer, 2004; Terry and Whiting, 2005; Kjer et al., 2006).

Ancestral Paleozoic 'roachoids' date from the Upper Carboniferous (320 million years ago) and there are abundant fossils in the coal swamps of that period (Kukalová-Peck, 1991; Grimaldi and Engel, 2005). However, it is doubtful whether many of these fossils belong to the stem group of Dictyoptera or other major lineages of Neoptera. Extant Dictyoptera probably share a Jurassic, omnivorous, detritus-feeding ancestor (200-150 million years ago), which possessed the shortened ovipositor common to modern Dictyoptera (Grimaldi and Engel, 2005). The oldest fossils of modern families may date from the early Cretaceous (Ross, 2001; Vršanský, 2002; Grimaldi, 2003; Grimaldi and Engel, 2005). However, such systematic assignment is necessarily tentative, because the characters crucial for the phylogeny-based classification of Blattaria and Mantodea are in the genitalia (McKittrick, 1964; Klass, 1997, 1998a; Grandcolas, 1996), which are not sufficiently recognizable in fossils.

While the monophyly of Dictyoptera is generally accepted, the relationships within this group have been strongly disputed during the last 15 years (Figure 1; Table 2; see Deitz et al., 2003 and Klass and Meier, 2006 for a further summary of prior phylogenetic hypotheses).

The most striking point in this dispute has been the relationship of Isoptera and the blattarian genus *Cryptocercus*, which includes several species from North America and northeastern Asia. This relationship has generated great interest, because *Cryptocercus* shares some key life history attributes with "lower termites" (i.e., Isoptera excluding the highly derived family Termitidae): xylophagy, brood care (at least partly biparental), anal trophallaxis, and – unique among animals – a rich hindgut fauna of flagellates from the taxa Oxymonadida and Hypermastigida (e.g., Cleveland et al., 1934; Nalepa and Bandi, 2000; see Klass and Meier, 2006 for a summary and for absence of such flagellates in the cockroach genus *Parasphaeria*, contra Pellens et al., 2002, 2007). Most older contributions suggested that Isoptera is the sister-group of Blattaria or even of Blattaria + Mantodea (morphology: McKittrick, 1964; Hennig, 1981; Thorne and Carpenter, 1992; molecules: Kambhampati, 1995). Several studies have even used Isoptera as outgroup

taxa in analyses of blattarian phylogeny (morphology: Grandcolas, 1996; molecules: Kambhampati et al., 1996; Maekawa and Matsumoto, 2000; Grandcolas and D'Haese, 2001). Cryptocercus was also proposed to be deeply nested within the blattarian family Polyphagidae, close to the genus *Therea* (Grandcolas, 1994, 1997a). Later, however, when morphological datasets were revisited, enlarged/expanded, and subjected to more refined and diverse analytical treatments, a well-supported Isoptera + Cryptocercus was consistently found nested within Blattaria (morphology: Deitz et al., 2003; Klass and Meier, 2006; molecules: Lo et al., 2003; Terry and Whiting, 2005; Kjer et al., 2006; Pellens et al., 2007; Lo et al., 2007; Inward et al., 2007). In parallel, it was shown that the assignment of *Cryptocercus* to Polyphagidae cannot be upheld, because it had been based on spurious morphological interpretations (Klass, 1997, 2001), and because in molecular analyses including both *Cryptocercus* and some polyphagid(s) these taxa do not cluster (Lo et al., 2003; Kjer et al., 2006; Pellens et al., 2007; Inward et al., 2007; Lo et al., 2007). In summary, current evidence strongly supports a lineage *Cryptocercus* + Isoptera. Some authors have used "Blattodea" to include extant and extinct cockroaches, and limit the use of Blattaria to describe extant cockroaches. We refer to the clade comprising Blattaria and Isoptera as "Blattodea", following Hennig (1969, 1981). This usage of "Blattodea" is not common in recent dictyopteran phylogenetics studies (although it is used in Inward et al., 2007).

Within the Isoptera, a long accepted sister group relationship between the relic Australian termite, *Mastotermes darwiniensis*, and all other termites (e.g. Hennig, 1981) is based on the many morphological plesiomorphies in *Mastotermes* that are unique within Isoptera but reminiscent of Blattaria (Klass, 1995; Donovan et al., 2000; Klass and Meier, 2006). Examples are a complete ovipositor (Klass, 1998b), the formation of an ootheca (though somewhat reduced; Nalepa and Lenz, 2000), five tarsomeres, an anal field in the hind wing (though small; Kukalová-Peck and Peck, 1993), a high number of Malpighian tubules (see Klass and Meier, 2006: character 151), and bacteriocytes containing *Blattabacterium* in the fat body (e.g. Sacchi et al., 1998, 2000; Lo et al., 2003). Molecular analyses have overwhelmingly confirmed this hypothesized relationship (Kambhampati et al., 1996; Thompson et al., 2000a; Kjer et al., 2006; Inward et al., 2007). Only Thorne and Carpenter (1992) arrived at a clade comprising Mastotermitidae + Kalotermitidae as opposed to Termopsidae (other termite families not included), but this resulted from problems with their morphological dataset (Deitz et al., 2003).

For Blattaria, McKittrick (1964) and McKittrick and Mackkerras (1965) distinguish five families: Blattidae (with the subfamilies Blattinae, Polyzosteriinae, Tryonicinae, Lamproblattinae), Cryptocercidae, Polyphagidae, Blattellidae (with the subfamilies Anaplectinae, Plectopterinae = Pseudophyllodromiinae, Blattellinae, Ectobiinae, Nyctiborinae), and Blaberidae. In recent years, the outline and relationships of the major blattarian lineages have become a major point of debate resulting from the morphological contributions by P. Grandcolas and K.-D. Klass (Grandcolas, 1994, 1996, 1997b, 1999a, b; Klass, 1997, 2001, 2003; Klass and Meier, 2006; Fig. 1F *versus* E,K), who both predominantly use male genitalic characters. It is uncontroversial among these authors that the basal dichotomy is between Blattidae and the remaining Blattaria; that Blattellidae is paraphyletic with respect to Blaberidae; that Anaplectinae is sister to all other blattellid lineages; and that Polyphagidae and its relatives are sister to the blattellid
+ blaberid clade. One point of controversy, however, is the placement of *Cryptocercus* (see above). Another concerns the Tryonicidae and Lamproblattidae (elevated to family rank by Klass and Meier, 2006), both including only a few species, which are assigned to Blattidae by Grandcolas but considered isolated lineages by Klass (Figure 1E,K). The proposed relationships within the clade Blattellidae + Blaberidae are also very different. Furthermore, the identical clades proposed by Grandcolas and Klass are largely based on different apomorphies (see Klass, 2001). This is mainly rooted in different hypotheses of topographic homologies, i.e. which structural components of the phallomeres (e.g., sclerites, projections) correspond among different taxa and should be compared within the same character ('alignment' of structural components). This issue, which is the basis for most of the controversy, is extensively documented in Klass (2001).

From the molecular side, little had been contributed to the resolution of blattarian phylogeny until recently, mainly because the most disputed taxa (Lamproblattidae, Tryonicidae) and other key taxa (such as Anaplectinae, most subgroups of Polyphagidae, and the enigmatic Nocticolidae) had not yet been included, and dictyopteran taxon samples were generally quite poor (Kambhampati, 1995, Kambhampati et al., 1996; Maekawa et al., 1999; Maekawa and Matsumoto, 2000; Lo et al., 2003; Terry and Whiting, 2005; Kjer et al., 2006). Three very recent contributions, however, have set a new scene: Pellens et al. (2007), Lo et al. (2007) and especially Inward et al. (2007) (Figure 1L–N). The Pellens et al. (2007) taxon sample is strongly dominated by Blaberidae and Isoptera, and the results are not so relevant to deeper dictyopteran or blattarian relationships. The two other papers include Nocticolidae, and Inward et al. (2007) include a variety of Polyphagidae among a large sample of 107 dictyopteran taxa.

Yet, Lamproblattidae, Tryonicidae, and Anaplectinae are not represented in any of these analyses. Apart from the well-supported Cryptocercidae + Isoptera clade common to all three contributions, the relationships among the major dictyopteran lineages are very different (and also differ from the morphology-based hypothesis of Klass and Meier, 2006: compare Figure 1K–N). The most striking findings of these studies are: (a) the sister group relationship between Cryptocercidae + Isoptera and the remaining Dictyoptera (including Mantodea!) in Lo et al. (2007; weakly supported); (b) the clade Polyphagidae + (Nocticolidae + Mantodea) in the same paper (weakly supported); and (c) the sister group relationship between Nocticolidae + Polyphagidae ("Polyphagoidea") and the remaining Blattodea (i.e., incl. Isoptera) in Inward et al. (2007; strongly supported; Mantodea sister to all other Dictyoptera).

In contrast to Isoptera, Mantodea are usually considered to be positioned outside Blattaria, as its sister group. This is suggested both by morphological work (Klass, 1995; Klass and Meier, 2006) and by molecular analyses (Maekawa et al., 1999; Lo et al., 2000, 2003: figure 2; Terry and Whiting, 2005; Kjer et al., 2006; Pellens et al., 2007; Inward et al., 2007). However, as mentioned above, Lo et al. (2007) find Mantodea nested in Blattodea (Figure 1M). Beier (1968) divided the group into 8 families: Mantoididae, Chaeteessidae, Metallyticidae, Amorphoscelidae, Eremiaphilidae, Empusidae, Hymenopodidae, and Mantidae (maintained by Klass and Ehrmann, 2005). Other classifications (e.g. Vickery and Kevan, 1983; Balderson, 1991; Wang, 1993; Terra, 1995; Kaltenbach, 1996, 1998; Roy, 1999; Ehrmann, 2002; Otte and Spearman, 2005) differ mainly in the upranking of subfamilies of Mantidae to family-level (Mantidae *s.str.*, Acanthopidae, Iridopterygidae, Liturgusidae, Sibyllidae, Tarachodidae, Thespidae,

and Toxoderidae in Otte and Spearman, 2005), and in the inclusion of fossil taxa (Grimaldi, 2003). Common to all these systems is the consideration of the monogeneric Mantoididae (*Mantoida*), Chaeteessidae (*Chaeteessa*), and Metallyticidae (*Metallyticus*) as sister to the "higher Mantodea". Phylogenetic work based on morphology has only focused on the most basal dichotomies (Klass, 1995, 1997; Klass and Meier, 2006) and has recovered the relationships *Mantoida* + (*Chaeteessa* + (*Metallyticus* and remaining Mantodea)). The molecular study of Svenson and Whiting (2004) based on five genes (mitochondrial 16S rRNA and COII, nuclear 18S rRNA, 28S rRNA, and histone 3) and 63 genera confirmed the position of Mantoida, as did Inward et al.'s (2007) and Lo et al.'s (2007; using downloaded mantodean sequences from Svenson and Whiting, 2004) studies based on much smaller taxon samples. However, none of these three studies included Chaeteessidae, and Metallyticidae is represented only in Inward et al. (2007), where it is nested in the "higher Mantodea". Grimaldi (2003) gives an excellent revision of early fossil Mantodea, but could not provide a clear picture of the basal relationships among extant Mantodea. His tentative suggestion of a sister group relationship between Chaeteessa and all other extant Mantodea (figure 27 therein) has been discussed and rejected in Klass and Meier (2006: 16f). In general, the classification of Mantodea is in phylogenetic chaos, with many monogeneric taxa, unknown character polarities, and frequent parallel evolution of characters. Currently defined mantodean families are not effective guides for selecting exemplar taxa. The results of Svenson and Whiting (2004) also show that the current family-level classifications of higher Mantodea need extensive revision.

In the present study, apart from generally revisiting dictyopteran phylogeny, we especially want to investigate some crucial phylogenetic issues, such as the placement of Isoptera, Cryptocercus, and Polyphagidae within Dictyoptera, and Mastotermes within Isoptera. For this purpose we gathered multiple genes from several previous studies, for a fairly large taxon sample. We additionally used the morphological data matrix from Klass and Meier (2006) and thus provide the first cladistic analyses for Dictyoptera where extensive morphological and molecular data are combined. Further innovations of our study are the alignment of ribosomal RNA (rRNA) sequences with reference to secondary structure, and our selection of a paired-sites doublet model. This doublet model considers paired nucleotides in rRNA stem regions to be interdependent, and has been shown, in many cases, to be an appropriate model for rRNA datasets (Jow et al., 2002). Although such paired-sites models have been shown to be more biologically realistic for rRNA data, they have not previously been used in dictyopteran phylogenetics. We also tested the extent to which recovered ingroup relationships depend on (a) the selection of outgroup taxa, (b) the inclusion of ingroup taxa, and (c) gene selection. The phylogenetic work presented here will hopefully help shape the taxon and gene selection of future studies on dictyopteran phylogeny.

2. Materials and Methods

Taxon and character sampling

We chose mitochondrial (COII, 16S) and nuclear (18S, 28S) sequences from GenBank representing a broad sample of dictyopteran taxa from Mantodea, Blattaria and Isoptera. To limit missing molecular data, we used only dictyopteran taxa for which 18S sequences were available (the gene fragment available for the majority of the sequenced dictyopteran taxa; we chose only a representative sampling of Mantodea from Svenson and Whiting, 2004). In the case of *Blattella*, we combined genes from 3 different species into one terminal taxon. The sequences from Inward et al. (2007) were not used, because they were published after completion of our analyses. A limited number of sequences from Lo et al. (2007) and Pellens et al. (2007) were added to our dataset at the final stages of manuscript completion and the entire dataset was reanalyzed: these added taxa included a *Therea* 18S sequence from Pellens et al. (2007) and two *Nocticola* 18S sequences from Lo et al. (2007). Both *Therea* and *Nocticola* were included to increase our sampling within the Polyphagoidea (sensu Inward et al., 2007). It was beyond the scope of the current paper to add the other, non-polyphagid taxa from Pellens et al. (2007) and Lo et al. (2007).

The 175 morphological characters in our dataset were taken from Klass and Meier (2006), who scored them (or part of them) for 4 mantodean, 20 blattarian, and 3 isopteran taxa (tables 1–3 therein). Both "-" (character not applicable) and "?" (character not examined) in that matrix were here treated as "missing data" (not as gaps).

We combined molecular and morphological characters of closely related taxa in a few cases where molecular characters were available only for the one taxon and morphological characters only for the other. Such combined terminal taxa are (see Table 1): *Blattella* spp. (DNA) + *Parcoblatta lata* (morphology), *Statilia apicalis* (DNA) + *Sphodromantis* sp. (morphology), *Gromphadorhina portentosa* (DNA) + *Blaberus craniifer* (morphology), *Hodotermopsis japonica* (DNA) + Termopsinae (morphology),

Cryptotermes (DNA) + Kalotermitidae (morphology), and *Sclerophasma paresisense* (DNA) + *Karoophasma biedouwense* (morphology).

Outgroup representatives for our standard analyses were selected from Mantophasmatodea (1 taxon), Phasmatodea (2 taxa), Grylloblattodea (1 taxon) and Embioptera (1 taxon) (i.e., the 5-outgroup analysis, 5OG), and we also analyzed the data under addition of representatives of Dermaptera, Plecoptera, and Orthoptera (1 taxon each) (i.e., the 8-outgroup analysis, 8OG). This appears appropriate considering the unresolved relationships among the major lineages of Neoptera (see Klass and Meier, 2006: chapter 2.3.; Klass, 2007). In specific analyses aimed at testing the influence of the selection of taxa and markers, we included various members of the aforementioned neopteran outgroup taxa and, in addition, members of the non-neopteran Odonata and Archaeognatha as more distant outgroup taxa.

Alignment

We first used ClustalX (Thompson et al., 1997) to perform a preliminary sequence alignment, with the default settings for gap costs (gap opening penalty=10.00; gap extension penalty=0.20). The resulting files were then aligned manually in Microsoft Word using the structural methods described in Kjer et al. (1994), Kjer (1995), Kjer et al. (2006a, b) and secondary structure models based on Gutell et al. (1993). Manual alignment has strong support as the most accurate method available for aligning ribosomal data (Kjer, 1995, 2004; Ellis and Morrison 1995; Titus and Frost, 1996; Hickson et al., 1996, 2000; Morrison and Ellis, 1997; Lutzoni et al., 2000; Mugridge et al., 2000; Gillespie et al., 2005a; Deans et al., 2006; Morse and Normack, 2006). Regions of ambiguous alignment, defined as areas with multiple single gaps and flanked by hydrogen bound stem region pairs, were coded in INAASE and included along with the stepmatrices in parsimony analysis (Lutzoni et al., 2000). These ambiguous regions were not included in the Bayesian analysis. The resulting alignment contained a COII fragment of 776 nucleotides, a 16S fragment of 210 nucleotides, an 18S fragment of 1643 nucleotides, and a 28S fragment of 1708 nucleotides.

Cladistic analyses

Parsimony analyses

We performed parsimony analyses in PAUP (Swofford, 2000) under equal weighting of all molecular and morphological characters with 10,000 random addition sequence replicate heuristic analysis under TBR branch swapping. Gaps of uniform length were treated as presence/absence characters; other gaps were treated as missing data, except those encoded with INAASE as described above. To estimate branch support, we ran 1000 parsimony nonparametric bootstrap replicates with 10 random addition sequence replicates under TBR branch swapping. We also ran additional, separate bootstrap analyses on the entire dataset with each data partition weighted 1000fold, to evaluate any differences that might occur between genes and/or morphology.

Bayesian analyses

We used the doublet model (Schoniger and von Haeseler, 1994) in Mr. Bayes 3.1.2, which allows independent modeling of nucleotide substitutions in both stem and loop regions of ribosomal DNA (Huelsenbeck and Ronquist, 2001, 2002). Two MCMC

analyses were run for each of the 8OG and 5OG datasets: one ran for 20 million generations each (with four chains: one cold and three hot), and one ran for 10 million generations each (with four chains: one cold and three hot). Each analysis had printfreq=500.The morphological characters were analyzed with the MK model (Lewis et al., 2005). After the analyses were completed, we verified that each run had stabilized using Tracer 1.4 (Rambaut and Drummond, 2007), and calculated the burnin regions. The 8OG 20 million generation analysis had a burnin of 5% for each chain; the 10 million generation analysis had a burnin of 5% for one chain and 10% for the other chain. Both 5OG analyses had a burnin of 5% for each chain.

We ran both the Mr. Bayes and PAUP analyses with 39 ingroup (dictyopteran) and either 5 or 8 outgroup (non-dictyopteran) taxa for which we had downloaded nucleotide sequences, plus 15 ingroup taxa for which we had only morphological information (59 or 62 taxa total). With regard to the ingroup sampling we call this our standard dataset (see Table 1), but we varied the composition of the outgroup, conducting 5- and 8-outgroup analyses.

Tests of the influence of taxon sampling

To test preliminary results that ingroup and outgroup taxon selection affected internal dictyopteran relationships, we ran several analyses using and modifying the datasets from Maekawa and Matsumoto (2000) and from Kjer et al. (2006). All analyses were run using parsimony in PAUP, with 10,000 replicate heuristic searches under TBR branch swapping. We ran Maekawa and Matsumoto's (2000) COII dataset with *Grylloblatta* and 2 dragonfly species as outgroups, both with and without mantodean COII sequences. We analyzed Kjer et al.'s (2006) dataset (reduced to only dictyopteran taxa and the genes used in our study, i.e., COII, 16S, 18S and 28S) with outgroup taxa that were (a) close relatives and (b) distant relatives of the ingroup. Closely related outgroup taxa included members of Mantophasmatodea, Phasmatodea, Embioptera, Dermaptera, Grylloblattodea, Plecoptera and Orthoptera (i.e., various Neoptera), while more distantly related taxa included members of Odonata and Archaeognatha. To determine whether the presence/absence of the COII, 16S or 28S fragments influenced the resulting phylogenetic hypotheses, we also ran the analyses of Kjer et al.'s (2006) dictyopteran taxa with just the 18S fragment, which was present for all taxa (for further clarification of the taxa used in each analysis, see Tables 2 and 3).

3. Results

Phylogenetic analyses using our standard dataset with distantly and/or closely related outgroups

Maximum parsimony (MP) results

In the analysis with five closely related (neopteran) outgroup taxa (5-OG analysis), the dataset consisted of 2608 constant characters, 923 variable but parsimonyuninformative characters and 988 parsimony-informative characters. The score of the best tree was 4625. In the analysis with an additional three closely related outgroup taxa (8-OG analysis), there were 2396 constant characters, 1083 variable but parsimonyuninformative characters and 1040 parsimony-informative characters. The score of the best tree was 11155. Figures 2A and 2B show results from analyses including 8-OGs or 5-OGs (strict consensus trees from 206 and 156 most parsimonious trees, respectively).

As in previous analyses, Mantodea and Isoptera are monophyletic (5-OG bootstrap support, bo=94% and 100% respectively; 8-OG bo=95% and 100% respectively); within "Blattodea", Blaberoidea, *i.e.*, Blattellidae + Blaberidae is also monophyletic (5-OG bo=88%; 8-OG bo=88%), which has been previously recovered in all morphological studies. Similarly, Blattellidae is paraphyletic, and Blaberidae is monophyletic (but with bo<50% in both the 5- and 8-OG analyses); *Anaplecta* and *Nahublattella* are basal in Blaberoidea. Blattidae are also clearly obtained as monophyletic (bo=83% in both the 5- and 8-OG analyses). Unlike in Inward et al. (2007), both analyses leave the various polyphagoid taxa in a basal blattodean or dictyopteran polytomy.

Several of the results of our parsimony analyses (MP) differed depending on which of the outgroup taxa we included (Figure 2A,B). The topology incorporating only five outgroup taxa is less resolved, with a polytomy occurring between the major groups of Dictyoptera: Mantodea, Blattidae, Blattellidae + Blaberidae, Cryptocercidae + Isoptera, Tryonicidae, the 3 polyphagid taxa, 2 *Nocticola* species, and *Lamproblatta*. The analysis including the three additional outgroup taxa is more resolved. In this tree, Mantodea is sister to the remaining Dictyoptera (Figure 2A; but with bo<50%). The 8-OG tree is unresolved only within the Blaberoidea, forming a polytomy between *Nahublattella*, *Supella* and *Blattella* + *Nyctibora*. The resolution of this polytomy, which is not present in the Bayesian analysis, may be possible with the addition of more data. By contrast, the Blaberoidea were well resolved in the 5-OG analysis (Figure 2B).

Cryptocercidae is sister to the Isoptera with strong support in both analyses (5-OG bo=91%; 8-OG bo=92%). Within the Isoptera, in both the 5- and 8-OG analyses the Hodotermitidae, Rhinotermitidae, and Termitidae form a clade (bo<50%), and their relationships are identical: *Microhodotermes* + (*Nasutitermes* + (*Reticulitermes* + *Copotermes*) (5-OG bo=54% for Termitidae + Rhinotermitidae and 83% for the Rhinotermitidae; 8-OG bo=52% for Termitidae + Rhinotermitidae and 82% for Rhinotermitidae). While in the 5-OG analysis the base of the isopteran tree is an unresolved polytomy, the 8-OG analysis yields – though with bo<50% – *Mastotermes* most basal, followed by a *Hodotermopsis* + *Cryptotermes* clade and then by the aforementioned isopterans.

Although there is disagreement about the relative position of Mantodea within Dictyoptera, the inner-mantodean relationships are consistent between analyses. Seven of the 15 currently recognized extant families (Otte and Spearman, 2005) are represented. *Mantoida* is the sister to all other Mantodea (5-OG bo=75%; 8-OG bo=78% for remaining Mantodea), which agrees with both the molecular results in Svenson and Whiting (2004) and the morphological results in Klass and Meier (2006). This basal split is followed by a polytomy comprised of *Bantia*, *Hoplocorypha* (both from Thespidae), *Chaeteessa*, *Metallyticus*, and a clade including all the remaining Mantodea (the latter weakly supported by bo<50%). Following the polytomy is the clade *Litaneutria* + *Heterochaetula* (bo=57% in both analyses). We find Iridopterygidae and Amorphoscelidae forming a monophyletic group (5-OG bo=61%; 8-OG bo=59%), a result similar to that of Svenson and Whiting (2004), in which Liturgusidae was also grouped with Iridopterygidae and Amorphoscelidae (our study did not include Liturgusidae). As in Svenson and Whiting (2004), taxa from Mantidae and Thespidae are found to be broadly polyphyletic, with representative taxa found at both basal and apical positions on the tree.

Bayesian analysis (MB) results

The 20 million generation 8-OG Bayesian analysis had an average likelihood score of -2.889E4, and -2.885E4 in the 10 million generation analysis. Posterior probabilities from a majority rule consensus of 112,400 trees from the 10 million and 20 million generation analyses are shown in Figure 3A. The 20 million generation 5-OG Bayesian analysis had an average likelihood score of -2.740E4; the 10 million generation analysis had an average likelihood score of -2.741E4. Posterior probabilities from a majority rule consensus of 114,000 trees from the 10 million and 20 million generation analyses are shown in Figure 3B.

As in the MP analysis, and previous studies, Mantodea and Isoptera are monophyletic (Mantodea: 8-OG posterior probability, pp=100%; and 5-OG pp=100%; Isoptera: 8-OG pp=100% and 5-OG pp=100%). Similarly, Cryptocercidae is supported strongly as sister to the Isoptera (8-OG pp=99%; 5-OG pp=99%). Within Blaberoidea, Blattellidae is paraphyletic. Blaberidae is a monophyletic grouping in both analyses (8-OG pp=72%; 5-OG pp=58%). Blattidae is monophyletic (8-OG pp=99%; 5-OG pp=99%). In both the 5-OG and 8-OG analyses, Tryonicidae is in a polytomy with Blattidae and Cryptocercidae + Isoptera. Regarding the polyphagoid cockroaches, the 8-OG analysis finds a Polyphagidae + *Lamproblatta* + *Nocticola* clade (pp=92%) sister to the remaining Blattodea (pp=73% for Blattodea). The 5-OG analysis yields *Lamproblatta* and a clade

comprising the other polyphagoid cockroaches as separate lineages of a basal dictyopteran trichotomy, whose third clade (with pp=77%) includes the remaining Blattodea plus Mantodea.

Within Isoptera, *Mastotermes* is never recovered at the base of Isoptera, but in each analysis its position is supported with less than 60% pp. Two other groupings occur: *Microhodotermes* + *Hodotermopsis* + *Cryptotermes* and *Nasutitermes* + *Reticulitermes* + *Coptotermes* in both the 5- and 8-OG analyses (both groups with pp=100% in the 8-OG and 5-OG analyses).

Several of the relationships within Mantodea are consistent between our two Bayesian analyses, and both MP analyses. *Mantoida* is recovered in every analysis as sister to the remaining Mantodea (8-OG pp=95%; 5-OG pp=96%), there is a *Litaneutria* + *Heterochaetula* clade (8-OG pp=92%; 5-OG pp=91%), and a clade comprising all Iridopterygidae and the amorphoscelid (8-OG pp=85%; 5-OG pp=85%). As above, taxa from Mantidae and Thespidae are found to be broadly polyphyletic, with representative taxa found at both basal and apical positions on the tree. Apical to *Mantoida*, as compared to the MP analyses, only *Bantia* and *Chaeteessa* are found in a basal position (8-OG pp=84%, 5-OG pp=85% for the monophyly of the remaining Mantodea), while *Metallyticus* and *Hoplocorypha* are more deeply nested in the remaining Mantodea. *Bantia* and *Chaeteessa* are either placed in a trichotomy (8-OG analysis) or form a clade sister to the other Mantodea (5-OG analysis); the latter result is intriguing, because for *Chaeteessa* we scored only morphological data, and for *Bantia* only molecular data. Additional phylogenetic analyses for testing ingroup/outgroup influence on topology in Dictyoptera

Tests based on COII data of Maekawa and Matsumoto (2000)

To inspect the signal coming from COII sequences we ran analyses on the COII dataset used by Maekawa and Matsumoto (2000). Initially, we added *Grylloblatta* and two Odonata to the original dataset (which lacks mantodeans) in order to provide rooting (Figure 4A). The recovered dictyopteran relationships were *Cryptocercus* + (((*Ergaula* + *Polyphaga*) + Isoptera) + remaining Blattaria). This tree is consistent with the original topology in Maekawa & Matsumoto (2000: figure 1A or 1B) if rooted between *Cryptocercus* and the remaining taxa. Trees with either *Grylloblatta* or the Odonata used as outgroups showed the same relationships.

Based on the dataset with both *Grylloblatta* and Odonata used as outgroup taxa we sequentially added 1, 5, and 21 mantodean species. This had a dramatic influence on ingroup relationships. Regardless of the number or species of mantodeans that were added, the relationship Isoptera + (*Cryptocercus* + (paraphyletic roaches + ((*Polyphaga* + *Ergaula*) + Mantodea))) is recovered, i.e., termites are dragged to the base of the tree, and Polyphagidae are sister to the Mantodea.

Altogether, with our various expansions of Maekawa and Matsumoto's (2000) data set (Figure 4A,B), we recovered Polyphagidae as sister to either Mantodea or Isoptera, suggesting that *Polyphaga* and *Ergaula* are taxa whose position differs greatly when mantids are included. COII is a gene fragment that is more suited for resolving genus and species level phylogenies, and less expected to resolve inter-ordinal relationships, so we chose to evaluate the effect of ingroup/outgroup selection with additional, less rapidly evolving genes (16S, 18S and 28S) using data from Kjer et al. (2006).

Tests based on multiple gene data of Kjer et al. (2006)

Some of our results on the position of Polyphagidae conflict with Kjer et al. (2006), which is surprising since our taxon sampling within Dictyoptera was similar. We used the dataset from Kjer et al. (2006) (sequences and alignment) to try to determine the cause of the discrepancy between our studies. Our dictyopteran taxon set for molecular data comprised 11 blattarians, 7 isopterans and 21 mantodeans, whereas Kjer et al.'s (2006) dataset comprised 6 blattarians, 8 isopterans and 6 mantodeans. Thirteen of their dictyopteran taxa were also members of our ingroup taxonset (and their dataset contained all family-level taxa that we used as outgroup taxa). Thus, we chose to use these shared ingroup taxa plus outgroup taxa from the same families that we used in our 5-OG analyses as a basis to run our additional ingroup/outgroup testing analyses (taxon content as in Figure 4C with: Mantodea= *Tenodera* and *Gongylus*; Blattaria= *Polyphaga*, Blatella, Supella, Gromphadorhina, Periplaneta and Cryptocercus; and Isoptera= Mastotermes, Coptotermes, Hodotermopsis, Microhodotermes, and Termitidae). Then we sequentially added ingroup taxa and varied the set of outgroup taxa. All these analyses were conducted using maximum parsimony, running 1000 replicate heuristic searches with ten random addition searches and TBR branch swapping. For most ingroup and outgroup taxa the Genbank sequence in Kjer et al.'s (2006) dataset was the same sequence that we had downloaded for a particular species; the only exceptions are the COII sequences for *Blattella*, *Gromphadorhina*, and *Tenodera*; and the 16S sequence for Cryptocercus punctulatus.

First we excluded all dictyopteran taxa and gene fragments from Kjer et al.'s (2006) molecular taxonset except for those that we had used in our study (COII, 16S, 18S, 28S), and all outgroup taxa except for those five neopteran taxa used in our "5-OG" study (with the following exceptions: we included *Lobophasma* and *Carausius* and did not include *Timema*). This analysis recovered the clade *Polyphaga* + Mantodea sister to the remaining Dictyoptera (congruent with the tree in Lo et al. 2007), but with bootstrap support <50% for the remaining Dictyoptera (Figure 4C; bootstrap support not shown). Thus, it appears that the discrepancies between Kjer et al. (2006) and the 5-OG study are a result of outgroup rooting (Figure 4).

Using the dictyopteran sample in the aforementioned basic analysis, we assessed the effect of adding different outgroup taxa to those we had already included (starting from the sample in Figure 4C). As a first series of modifications we added orthopteran and further phasmatodean taxa into the alignment block, one by one. The addition of *Gryllus*, *Timema* and *Tettigonia*, separately or together, altered neither the position of *Polyphaga* nor the other ingroup relationships (Figure 4D).

Next we ran analyses in which only one or two outgroup taxa were added to the dictyopteran sample from in Kjer et al. (2006) (the dataset described above). Using a member of Gryllotalpidae (Figure 4E) resulted in *Polyphaga* placed as sister to the remaining Blattodea (with the exception of the oddly placed *Supella*), and Mantodea being sister to all other Dictyoptera. In addition, in contrast to the previous analyses (Figure 4C,D), this analysis yields a *Cryptocercus* + Isoptera clade. The use of a single outgroup taxon from other orders also changed ingroup relationships, in particular those of *Polyphaga* and *Cryptocercus*. An embiopteran outgroup, *Oligotoma*, results in

Cryptocercus + *Polyphaga* as sister to Mantodea (Figure 4F). The phasmatodean outgroup Carausius yields Polyphaga + (Cryptocercus (remaining Blattaria + Isoptera)) (Figure 4G). An archaeognathan outgroup, *Allomachilis*, results in (*Cryptocercus* + *Polyphaga*) + Isoptera (Figure 4H). Using the mantophasmatodean *Sclerophasma* (Figure 4I) or the dermapteran *Forficula* (Figure 4J) as outgroup taxon provided more conventional topologies, both showing a *Cryptocercus* + Isoptera clade sister to *Polyphaga*. The addition of *Grylloblatta* (Figure 4K) resulted in a clade comprising Polyphaga + Mantodea and Cryptocercus + Isoptera as sister groups, nested in paraphyletic Blattaria. An interesting aspect of some of these outgroup taxa (Figure 4F,G,I,J,K) is that either the sole blattid of the sample (*Periplaneta*; dermapteran, embiopteran, and mantophasmatodean outgroup) or one of the blattellid + blaberid representatives (Supella; archaeognathan and grylloblattodean outgroup) is dragged to the very base of the dictyopteran tree, Supella then far remote from the two other members of Blattellidae + Blaberidae (Blattella and Gromphadorhina). In some of these analyses (Figure 4F,J) the morphology-based hypothesis (*Polyphaga* + (*Cryptocercus* + Isoptera)) is recovered.

For some of the outgroup taxa that we used in these analyses sequence data for COII, 16S, and/or 28S were lacking (Table 1), and it is thus unclear to what extent differences in the results of these analyses are effects of different outgroup selection or of gene selection in the outgroup. To evaluate whether outgroup selection alone affected results on dictyopteran phylogeny, we ran the same tests as above with just the 18S gene fragment, for which all taxa had sequence information. The results are shown in Figure 5A–F. These phylogenetic reconstructions differed greatly from those above, and also among each other. The addition of a gryllotalpid (Figure 5A), or a dermapteran (Figure 5E), or an archaeognathan (Figure 5D) resulted in *Cryptocercus* being dragged to the base of the dictyopteran tree, followed by Isoptera; except for the placement of *Periplaneta*, ingroup relationships are identical using these three outgroup taxa. Using the embiopteran *Oligotoma* as an outgroup taxon (Figure 5B), Isoptera is the sister to all other Dictyoptera, followed by *Cryptocercus* as sister to cockroaches + mantodeans. Using a phasmatodean (Figure 5C) or a grylloblattodean (Figure 5F), the blattellid *Supella* is dragged to the base of the dictyopteran tree, and a clade *Cryptocercus* + Isoptera is recovered; the other dictyopteran relationships are very different between these two latter analyses. Altogether, a great variety of incongruent dictyopteran clades receives support from analyses using different outgroup taxa.

Gene selection

To evaluate further the effect of gene selection on dictyopteran phylogenetic reconstruction we used a version of Kjer et al.'s (2006) Dictyoptera dataset as described above with *Grylloblatta campodeiformis* or *Grylloblatta sculleni* as an outgroup taxon (taxon content as for Figure 4K, with *Grylloblatta campodeiformis* used in the 18S analysis). In separate analyses, we weighted each gene fragment 1000fold and ran parsimony analyses as described above (thus maintaining the same taxon list for easier comparison between genes). We found that the topology differed depending on the gene fragment. Each fragment yielded a different dictyopteran lineage as sister to the remaining Dictyoptera (topologies not shown): *Periplaneta* with 16S (but with bo<50%); *Supella* with 18S (remote from other Blattellidae + Blaberidae; this is the analysis shown in Figure 5F; bo=72%), and Mantodea + *Polyphaga* with COII (but with bo<50%). The 28S yields a more conventional topology (but note there are no polyphagoid 28S sequences): Mantodea + (paraphyletic "Blattaria" + (*Cryptocercus* + Isoptera))) (but with bo<50% for the monophyly of Dictyoptera). In all cases except COII, *Cryptocercus* is sister to the Isoptera (although with bo<50%). In the COII analysis, *Cryptocercus* is sister to *Supella* and *Periplaneta* is sister to Isoptera (but with bo<50%). The 16S analysis recovers *Polyphaga* as sister to Mantodea (again, with bo<50%). In the 18S analysis, *Polyphaga* is sister to *Periplaneta* + (Mantodea + (*Cryptocercus* + Isoptera)) (with bo<50%).

4. Discussion

Gene selection and ingroup and outgroup taxon selection

Gene selection is often a difficult decision for researchers who wish to reveal both ancient and younger cladogenetic events. Lin and Danforth (2004) extensively tested the performance of both mitochondrial and nuclear genes for a variety of insect datasets. They found that mitochondrial genes, in general, were much more heterogeneous with respect to among site rate variation, with a few sites evolving and saturating quickly, thereby leading to high levels of homoplasy. They suggest that, with the exception of studies focusing on very closely related taxa, nuclear genes are more informative than mitochondrial genes such as COII. Danforth et al. (2005) suggest that when reconstructing divergences of Mesozoic age or older, we should rely on either nuclear ribosomal genes or nuclear protein-coding genes rather than using mitochondrial genes that may be too rapidly evolving to be informative.

In our study we showed that the relationships among the dictyopteran lineages varied greatly depending on gene selection. This is in part disagreement between the mitochondrial and nuclear data. We note that COII is commonly sequenced for Dictyoptera yet it is a rapidly evolving gene and may add considerable homoplasy to the analysis. This all suggests that resolving family-level relationships in Dictyoptera, which are probably mostly due to cladogenetic events in the early to middle Mesozoic, will likely require more nuclear gene information.

The selection of outgroup taxa is a decision that should be made with care, as outgroup taxa that are too distant may erroneously polarize characters (Maddison et al., 1984; Wheeler, 1990; Maddison et al., 1992; Smith, 1994; Lyons-Weiler et al., 1998; Nylander, 2001; Sanderson and Shaffer, 2002). The selection of ingroup taxa is another vital step, one that is dependent on outgroup selection because different combinations of ingroup and outgroup taxa affect tree topology (and therefore phylogenetic hypotheses) (Milinkovitch and Lyons-Weiler, 1998). It is difficult to assess which taxon sampling will recover the true tree, but by evaluating different taxon sets during standard analyses, researches will be better able to determine which nodes are unstable with respect to taxon selection. Hypotheses, such as those regarding character evolution, should be treated with caution when tree topologies are unstable with respect to outgroup or ingroup selection.

In this study, the initial selection and addition of ingroup and outgroup taxa greatly affected the interrelationships among dictyopteran taxa as is evident from the above descriptions and subsets of Figures 4 and 5. The basal placement of *Supella* and

Periplaneta under use of certain outgroups are particularly impressive examples. Sampling taxa closely related to such rogue taxa might be a strategy to stabilize ingroup relationships under use of different outgroups. Of course, the mutual interactions between gene selection, outgroup selection, and ingroup sampling are difficult to conceive and evaluate.

Phylogenetic implications

Relationships among major dictyopteran lineages

The results on the deeper relationships within Dictyoptera are highly incongruent among the recent molecular-based phylogenetic analyses completed by Inward et al. (2007), Pellens et al. (2007), and Lo et al. (2007), and the morphology-based study of Klass and Meier (2006) finds yet different relationships (Figure 1K–N). The only consistent elements therein are the monophyly of Mantodea, of Mantodea excluding Mantoididae (see also Svenson and Whiting, 2004), of the clade Blattellidae + Blaberidae, of the clade Cryptocercidae + Isoptera, and of Isoptera; as far as decent samples are included, the Blattidae (sensu Klass and Meier, 2006: excluding Lamproblattidae and Tryonicidae) and Polyphagidae also appear as monophyla. Further, smaller taxa to be taken into consideration as "major" lineages are the Lamproblattidae, Tryonicidae, and Nocticolidae, all sparsely represented in studies, or not at all.

Our own 5-OG and 8-OG MP and Bayesian analyses (Figures 2 and 3) have added a few additional possibilities for deep dictyopteran relationships. The number of possibilities is even further increased considering our various experimental analyses (Figures 4 and 5), though these were tests not aimed at resolving the phylogeny. While it appears impossible to mutually compare and discuss all the different hypotheses inherent in our analyses and the previously published ones, we want to address some particular issues.

One major point is the most basal splitting event within Dictyoptera. This has traditionally been assumed to separate Blattodea (including cockroaches and termites) and Mantodea, but according to the aforementioned molecular studies as well as our own, the Polyphagoidea (Polyphagidae, Nocticolidae, and perhaps Lamproblattidae) may also be involved, and the Cryptocercidae + Isoptera clade is another (though unlikely) candidate to be sister to all other Dictyoptera.

The Blattodea-Mantodea dichotomy has been confirmed by Inward et al. (2007: pp=87% for Blattodea), who find the most basal inner-blattodean split between a clade Polyphagidae + Nocticolidae and the remaining Blattodea (the latter supported by pp=96%; Lamproblattidae and Tryonicidae not sampled). The same basal dichotomy is well-supported in Pellens et al. (2007: jackknife support ja=95% for Blattodea) and in Klass and Meier (2006), but a clade Polyphagidae + Lamproblattidae is more deeply subordinate in Blattodea (sister to Cryptocercidae + Isoptera in Klass and Meier, 2007; Lamproblattidae not sampled in Pellens et al., 2007, and Nocticolidae missing in both studies).

In contrast, Lo et al. (2007) find the basal dictyopteran dichotomy between the Cryptocercidae + Isoptera clade and the remaining representatives, though this is weakly supported (bo<50% for remaining Dictyoptera). In this study the Mantodea are deeply subordinate in Blattodea – inside a clade Polyphagidae + (Nocticolidae + Mantodea), which is placed in a trichotomy otherwise giving origin to Blattidae and a Blattellidae + Blaberidae clade. After re-rooting the Lo et al. (2007) tree between Mantodea and Nocticolidae, it would be quite similar to that of Inward et al. (2007), yet some differences would remain (e.g., Nocticolidae and Polyphagidae would be successive basal clades of Blattodea rather than forming one monophyletic basal clade). Nonetheless, one might suspect that outgroup choice is one crucial issue responsible for the differences between the trees.

Among our analyses, the 8-OG maximum parsimony and Bayesian analyses (Figures 2A and 3A) also yield the basal Mantodea-Blattodea dichotomy, though Blattodea is weakly supported (bo<50% in MP, pp=73% in MB); in the MP tree the sampled polyphagoid species originate all separately from a large polytomy at the base of Blattodea, while in the MB tree they are aggregated to a clade, which is sister to the remaining Blattodea. The latter result agrees with that in Inward et al. (2007), who in their Bayesian analysis use a similar set of gene fragments (they have H3 in addition, but lack 16S), but a different method for sequence aligment and a different model of evolution. The 5-OG MP analysis (Figure 2B) results in a large basal dictyopteran polytomy.

In contrast, our 5-OG Bayesian analysis (Figure 3B) obtains the polyphagoid taxa as sister group to all other Dictyoptera, the latter having a support of pp=77%. This is a topology not obtained in any previous study. With this hypothesis, from a taxonomic perspective, Mantodea (like Isoptera) would be a subgroup of Blattodea, i.e. highly derived cockroaches adapted to a predatory lifestyle, and the terms "Blattodea" and "Dictyoptera" are then synonymous. Evolutionary scenarios would also have to be revised. For instance, Blattaria and *Mastotermes* produce their ootheca in the vestibulum

(the space above the subgenital plate, coxosternum VII; McKittrick, 1964; Klass, 1998b; Nalepa and Lenz, 2000), whereas Mantodea build it externally upon some substrate. While previously the internalized production of the ootheca was parsimoniously considered apomorphic for Blattodea (see Klass and Meier, 2006: 22), this phylogenetic hypothesis would rather suggest that the external production in Mantodea is derived from internal production.

Yet we tentatively suggest that a Mantodea-Blattodea dichotomy is still the most plausible hypothesis for basal dictyopteran relationships. With this viewpoint, the next issue to discuss in the most basal splitting event in Blattodea.

The molecular results of Inward et al. (2007), and perhaps those of Lo et al. (2007; if re-rooting is advocated), as well as our combined molecular-morphological results suggest that Nocticolidae and Polyphagidae (and Lamproblattidae) are the most basal subgroups of Blattodea. They may together form a single clade sister to the remaining Blattodea (our Bayesian analyses, Figure 3; Inward et al., 2007, with *Lamproblatta* lacking), or form two successive basal clades, i.e., Nocticolidae + (Polyphagidae + other Blattodea) (Lo et al., 2007 if rerooted). The latter possibility would nicely agree with the absence of bacteriocytes in Nocticolidae (Lo et al. 2007; like in Mantodea and non-dictyopterans), the respective symbiosis being a synapomorphy of Polyphagidae and the "other Blattodea".

The basal position of Polyphagidae in Blattodea would suggest a reinterpretation of the polarity of many morphological characters in Dictyoptera, including those of the most useful character system, the male genitalia (concerning both the interpretations of Grandcolas, 1996 and Klass and Meier, 2006). In addition, much homoplasy would have to be assumed for this character system. However, related revisions should wait for more elaborate combined data sets with a more complete overlap between the taxa sampled for morphological and molecular data. In addition, the lacking inclusion of key taxa such as Lamproblattidae and Tryonicidae in large molecular analyses, and the striking instability of basal dictyopteran relationships depending on gene selection and taxon sampling (see Figures 4 and 5: *Polyphaga* either as sister to the Mantodea, Blattaria, or Isoptera) still advise caution with the molecular results.

Anyway, Polyphagidae and Nocticolidae are evidently crucial in analyses of dictyopteran phylogeny. Nocticolidae includes only a few genera and is guite homogenous; there are hardly any useful data on the morphology of their male and female genitalia, while such data would be highly desirable. Polyphagidae as currently delimited (Grandcolas, 1994; but with *Cryptocercus* excluded), however, is widespread and composed of several subfamilies that are morphologically and ecologically quite diverse. Most phylogenetic analyses (including ours) only sampled members of the subfamily Polyphaginae (*Polyphaga, Ergaula* and *Therea*). Representatives of the Euthyrrhaphinae and Latindiinae were limited to the analysis of Kambhampati (1996), who, however, sequenced only 12S. Among the recent large-scale analyses only Inward et al. (2007) included members of the polyphagid subfamilies Euthyrrhaphinae and Tiviinae, which along with Polyphaginae and Nocticolidae formed a well-supported monophyletic group (pp=100%). In our analyses the sampled Polyphaginae – *Polyphaga*, *Ergaula* and *Therea* – remain as a monophyletic group, with the exception of the MP analyses, in which they are placed separately in a large polytomy. This is not likely a hard polytomy, but rather a soft one that more data (see patchy coverage in terms of sequence

data in Table 1) and/or an increased polyphagid taxon sample might resolve. In an additional 5-OG MP analysis (not shown) that excluded Nocticolidae, Polyphaginae was recovered as sister to all remaining Dictyoptera (as in the 5-OG Bayesian analysis, Figure 3B).

One step up in the blattodean tree the next problematic issue is the relationship among the large blattodean clades Blattidae (sensu Klass and Meier, 2006), Blattellidae + Blaberidae, and Cryptocercidae + Isoptera, and the small taxon Tryonicidae (not yet sampled for molecular analyses) – and the Polyphagoidea if their basal position is not accepted. The morphology-based analysis of Klass and Meier (2007) yields Blattidae as sister to all other Blattodea; the latter have Tryonicidae as the basalmost branch, and the rest falls into a clade Blattellidae + Blaberidae and a clade including Polyphagidae + Lamproblattidae and Cryptocercidae + Isoptera (Figure 1K). In contrast, most molecular studies tend to obtain a close relationship between Blattidae and Cryptocercidae + Isoptera. This clade is strongly supported in Inward et al. (2007; pp=100%) and Pellens et al. (2007: ja=84%) and was also found in the study of Lo et al. (2003) (a clade Cryptocercidae + Blattidae, i.e. without Isoptera, was recovered in Kambhampati, 1995). The tree in Lo et al. (2007) shows relationships altogether very different from the other analyses (Figure 1M); if rerooted between Mantodea and Blattaria (see above), the clades Blattidae, Blattellidae + Blaberidae, and Cryptocercidae + Isoptera are placed in an unresolved trichotomy.

Among our standard analyses, the 5-OG and 8-OG Bayesian analyses (Figure 3) and the 8-OG MP analysis (Figure 2A) also show a Blattidae + (Cryptocercidae + Isoptera) clade, which furthermore includes Tryonicidae. However, the support for this clade is at most moderate (pp=80%, pp=74%, or bo<50%), though this may be due to the lack of molecular data for Tryonicidae. The 5-OG MP analysis (Figure 2B) obtains all these taxa as branches of the large polytomy at the base of Dictyoptera.

Altogether, the relationships among the principal lineages of Blattodea remain an area of continued conflict. The inclusion of molecular data for a few Tryonicidae (only *Lauraesilpha* or *Tryonicus*; see Klass, 2001) would be the most important improvement of the database. However, the available samples for Blattidae and basal Blattellidae + Blaberidae (mainly the basal groups Anaplectinae and Plectopterinae = Pseudophyllodromiinae) should also be increased. On the other hand, the Blaberidae are usually "oversampled" in analyses on dictyopteran phylogeny.

The Cryptocercus + Isoptera clade

Our Bayesian and parsimony analyses strongly support the clade *Cryptocercus* + Isoptera (bo=91 or 92%; pp=99%), confirming the predominant previous hypothesis (Boudreaux, 1979; Nalepa et al., 1997; Lo et al., 2000; Lo et al., 2003; Deitz et al., 2003; Terry and Whiting, 2005; Kjer 2004; Kjer et al., 2006; Lo, 2003, Klass, 1995, 2003). The cladistic analyses by Klass and Meier (2006), Lo et al. (2007), Pellens et al. (2007) and Inward et al. (2007) also recovered this relationship, all with strong support (except for Lo et al., 2007).

Some earlier molecular studies contradicted this result, but these studies were based either on small taxon samples or short sequences (Vawter, 1991; DeSalle et al., 1992), predefined Isoptera as an outgroup taxon (Maekawa and Matsumoto, 2000; Kambhampati, 1996), or have otherwise been revised by subsequent studies (e.g., Grandcolas and D'Haese, 2001 for Kambhampati, 1995). Morphological studies not recovering *Cryptocercus* + Isoptera were shown to be based on uncertain morphological data (Thorne and Carpenter, 1992 revised in Deitz et al., 2003; Grandcolas, 1994, 1996, 1997a revised in Klass, 1997, 2001) or also used Isoptera as an outgroup taxon (Grandcolas, 1996).

While the current evidence for a *Cryptocercus* + Isoptera clade is overwhelming, in our tests based on Kjer et al.'s (2006) data we found that this clade is not robust to changes in outgroup selection. Indeed, only with either a gryllotalpid orthopteran, a mantophasmatodean, a dermapteran, or a grylloblattodean outgroup (Figure 4E,I,J,K) the clade was obtained. Most of the same analyses led to the problem that particular blattarians (*Periplaneta* or *Supella*) are dragged to the base of Dictyoptera. Nonetheless, these were tests rather than analyses optimized for resolving dictyopteran relationships.

Relationships within Isoptera

As our termite sample is fairly small, we only discuss here the most basal split inside the Isoptera. The traditional view that this is between *Mastotermes* and all other Isoptera (Hennig, 1969, 1981; Klass, 1995; see also Krishna, 1970; Thompson et al., 2000a) has been contradicted by Thorne and Carpenter (1992; but see Deitz et al., 2003 for a revision), while it has been confirmed by most subsequent studies, both morphological (Klass and Meier, 2006, but therein the only termite taxa additionally included are the lower-isopteran Termopsinae and Kalotermitidae) and molecular (Kambhampati et al., 1996; Lo et al., 2003). Among the recent large-scale molecular analyses, Pellens et al. (2007: ja=100%) and Inward et al. (2007: no support values indicated) also find this relationship, while Lo et al. (2007) obtain a polytomy of 4 clades at the base of Isoptera (one of them is *Mastotermes*).

Also from our analyses the position of *Mastotermes* cannot be determined, despite the inclusion of the morphological data from Klass and Meier (2006). In our Bayesian analyses (Figure 3) it forms a clade together with the other "lower termites", but the support is low (pp=55% or pp=60%). In our MP analyses *Mastotermes* is either placed in a basal isopteran polytomy (5-OG analysis, Figure 2B), or it indeed appears as sister to all other Isoptera (8-OG analysis, Figure 2A), but with low support (bo<50%) for the clade comprising the remaining termites. The latter result was also obtained in a 5-OG Bayesian analysis run in exclusion of Nocticolidae (not shown). A basal placement of *Mastotermes* might be more strongly favored as soon as the morphological characters have been scored for all termite taxa sampled, as the (apomorphic) states are mostly the same in all termites except for *Mastotermes*.

Altogether, the basal position of *Mastotermes* within Isoptera may be considered the most convincing hypothesis, but is currently not too strongly supported.

Relationships within Mantodea

The majority of our molecular data on Mantodea are from the previous study by Svenson and Whiting (2004). Yet, our datasets are different in several ways: Although both of us used COII, 16S, 18S, and 28S, Svenson and Whiting additionally included H3, whereas we added morphological data for 4 taxa (see Table 1); the morphological data, however, due to the limited taxon sample, can affect only the very basal mantodean dichotomies. We aligned rRNA sequences manually with reference to secondary structure, whereas Svenson and Whiting applied a manual alignment in Sequencher followed by subsequent alignment by direct optimization. In their analysis, Svenson and Whiting used an unspecified model determined by Modeltest 3.06 PPC (Posada and Crandall, 1998) whereas we used a standard GTR + I + G model for COII and a doublet model for rRNA. Nevertheless, the mantodean topologies we reconstructed are largely congruent.

One particularly interesting issue is the phylogenetic positions of the 3 genera usually considered most basal among the mantodeans (e.g. Beier, 1968): *Mantoida, Chaeteessa*, and *Metallyticus*. Notably, all three genera show characters that make them good candidates to be sister to all remaining Mantodea. In *Mantoida* the male genitalia are in several characters strongly reminiscent of blattarian genitalia and lack apomorphies shared by all other examined Mantodea, including *Chaeteessa* and *Metallyticus* (Klass, 1995, 1997). *Chaeteessa* is peculiar among Mantodea by the simple structure of the spination of its raptorial forelegs; for instance, the terminal claw of the tibia is hardly differentiated from the other tibial spines, and all spines are very slender, seta-like (Beier, 1968). *Metallyticus* shows some characters of wing venation that resemble conditions in Blattaria, mainly the extensive branching of some major veins and the presence of a 2nd anal vein in the hind wing (Smart, 1956).

Klass (1995, 1997) and Klass and Meier (2006) found the relationships *Mantoida* + (*Chaeteessa* + (*Metallyticus* and remaining Mantodea)) using morphological characters. Based on molecular data, both Svenson and Whiting (2004) and Lo et al. (2007) found *Mantoida* to be the most basal mantodean lineage, but neither study did include *Chaeteessa* or *Metallyticus*. Inward et al. (2007), who sampled *Mantoida* and *Metallyticus*, also found the former most basal, while *Metallyticus* was placed apical to the most basal "higher mantodean" (the iridopterygid *Ichromantis*).

Our analyses consistently confirm *Mantoida* to be the sister group of the remaining Mantodea (support for the latter: bo=78 or 75%, pp=95 or 96%). The positions of *Metallyticus* and *Chaeteessa* (both only represented by morphological data) are not so well resolved. In the MP analyses (Figure 2) the two are placed in a polytomy immediately apical to *Mantoida*, which additionally includes the Thespinae *Bantia* and *Hoplocorypha* (both only represented by molecular data), and a lineage comprising the remaining Mantodea. In the Bayesian analyses (Figure 3) relationships are similar but appear more resolved: *Metallyticus* and *Hoplocorypha* both fall into the large apical mantodean clade, while *Bantia* either remains in a basal trichotomy or is sister to *Chaeteessa*. All these resolutions are congruent with the results in Klass and Meier (2006; *Bantia* not sampled). Yet, the position of *Bantia* sister to *Chaeteessa* is intriguing. This is also true in a technical sense, since *Bantia* is only represented by molecular data and *Chaeteessa* only by morphological data, and they thus cannot share any matrix character state that could provide a synapomorphy. Apparently the scattered availability of data across the mantodean taxa leads to problems in the analyses.

For forthcoming studies, molecular data would be highly desirable for *Chaeteessa* (they are meanwhile available for *Metallyticus*: Inward et al., 2007). Morphological data, especially on the male genitalia, are urgently needed for the majority of the mantodean taxa, and for *Bantia* and *Hoplocorypha* in particular.

Concerning the "higher Mantodea", it is clear from Svenson and Whiting (2004) and from this study's results that the monophyly of most of its families is unlikely, and family-level classification is thus not a useful guide for taxon sampling. Extensive, broadly representative taxon sampling is still needed for the elucidation of mantodean phylogeny; the chance of missing a lineage that has been mistakenly lumped into the wrong mantodean family is high.

As mentioned above, the mantodean topologies are largely congruent between Svenson and Whiting (2004) and our study. For example, both studies recover Litaneutria + Heterochaetula, Phyllovates + Anamiopterx and Bolbe + Ima + Gyromantis. The main areas of conflict lie in the positions of Pseudocreobotra, and Stagmomantis + Tenodera. Pseudocreobotra + Gongylus + Phyllocrania is recovered in the the 8-OG Bayesian studies, and in both MP analyses. This is not found by Svenson and Whiting (2004), who instead find *Chrysomantis* to be sister to *Pseudocreobotra*. *Tenodera* and *Stagmomantis* are supported as sister taxa in both MP analyses (although with bo < 50%, with pp = 96% in our 8-OG analysis and pp = 96% in our 5-OG analysis, while their analysis recovers *Stagmomantis* with *Melliera* with 100% posterior probability and 100% nonparametric bootstrap. Although these support values cannot be directly compared, we simply wish to acknowledge that differences occur between our results. However, this is probably a result of differing model selections (the doublet model we implemented takes into account the interdependence of hydrogen-bonded stem pairs in rRNA data). When we ran an additional analysis in Mr. Bayes with a GTR + I +G model in the exclusion of morphology (5 million generations; not shown) Stagmomantis and Melliera form a clade with pp=99% posterior probability, Tenodera being its sister taxon, supported by pp=96%.

Conclusions

This study is a comprehensive analysis of Dictyoptera phylogeny, incorporating nucleotide sequences from a mitochondrial protein coding gene and both mitochondrial and nuclear rRNA genes (structurally aligned), plus morphological data. Data were analysed according to maximum parsimony and Bayesian methodology.

In our analyses as well as those of other authors, the relationships among the major dictyopteran lineages still show considerable variation. Perhaps the most instructive finding of the present study is the strong effect of ingroup and outgroup sampling on dictyopteran molecular analyses. For instance, the addition of Mantodea greatly influences the resulting dictyopteran topology; thus, studies considering only Blattodea are not unlikely to arrive at erroneous results. Studies should use both distantly and closely related outgroup taxa for rooting, and then evaluate whether the positions of dictyopteran lineages are stable under different combinations. Our outgroup analyses suggest that several blattarians are rogue taxa, whose placement in molecular studies should be viewed with caution. Our evidence for gene selection influencing ingroup topologies is more commonplace.

For further progress in the reconstruction of dictyopteran phylogeny, the sampling of additional taxa, and sequencing of additional (preferably nuclear) genes is evidently needed. While taxon sampling within Dictyoptera generally needs to be greatly expanded, important taxa still to be sampled for molecular studies are Chaeteessidae, Tryonicidae, Lamproblattidae, and Anaplectinae, as well as certain enigmatic genera such as the myrmecophilous *Atticola* and *Attaphila*, and additional polyphagid genera. Future studies should endeavor to use as many independent gene fragments as possible, with as many closely and distantly related outgroup taxa as is feasible to stabilize the position of these taxa in the tree.

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References

Balderson, J., 1991. Mantodea (praying mantids). *The Insects of Australia* (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen, J.P. Spradberry, R.W. Taylor, M.J. Whitten and M.J. Littlejohn), pp. 348–356. CSIRO Division of Entomology, Melbourne University Press, Melbourne.

Beier, M., 1968. Mantodea (Fangheuschrecken). *Handbuch der Zoologie, IV. Band Arthropoda, 2. Hälfte Insecta, Lieferung 12* (ed. by J.G. Helmcke, D. Stark and H. Wermuth), 147 pp. De Gruyter, Berlin.

Beutel, R.G., Gorb, S.N., 2006. A revised interpretation of the evolution of attachment structures in Hexapoda (Arthropoda), with special emphasis on Mantophasmatodea. *Arthropod Systematics & Phylogeny*, **64(1)**, 3–25.

Boudreaux, H.B., 1979. Arthropod Phylogeny with Special Reference to Insects. John Wiley & Sons, New York.

Cleveland, L.R., Hall, S.K., Sanders, E.P., Collier, J., 1934. The wood-feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa and roach. *Memoirs of the American Academy of Arts and Sciences*, **17**, 185–342.

Danforth, B.N., Lin, C.-P., Fang, J., 2005. How do insect nuclear ribosomal genes compare to protein-coding genes in phylogenetic utility and nucleotide substitution patterns? *Systematic Entomology*, **30**, 549–562.

Deans, Andrew R., Gillespie, Joseph J., Yoder, Matthew J., 2006. An evaluation of ensign wasp classification (Hymenoptera: Evaniidae) based on molecular data and insights from ribosomal RNA secondary structure *Systematic Entomology* **31** (**3**), 517–528.

Deitz, L.L., Nalepa, C., Klass, K.-D., 2003. Phylogeny of the Dictyoptera re-examined (Insecta). *Entomologische Abhandlungen*, **61**, 69–91.

DeSalle, R., Gatesy, J., Wheeler, W., Grimaldi, D., 1992. DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. *Science*, **257**, 1933–1936.

Donovan, S.E., Jones, D.T., Sands, W.A., Eggleton, P., 2000. Morphological phylogenetics of termites (Isoptera). *Biological Journal of the Linnean Society*, **70**, 467–513.

Eggleton, P., 2001. Termites and trees: a review of recent advances in termite phylogenetics. *Insectes Sociaux*, **48**, 187–193.

Ehrmann, R., 2002. Mantodea: Gottesanbeterinnen der Welt. Natur und Tier, Münster.

Ellis, J., Morrison, D., 1995. Effects of sequence alignment on the phylogeny of *Sarcocystis* deduced from 18S rDNA sequences. *Parasitology Research*, **81(8)**:696-699.

Gillespie, J.J., McKenna, C.H., Yoder, M.J., Gutell, R.R., Johnston, J.S., Kathirithamby, J., Cognato, A.I., 2005. Assessing the odd secondary structural properties of nuclear small subunit ribosomal RNA sequences (18S) of the twisted-wing parasites (Insecta: Strepsiptera). *Insect Molecular Biology*, **14**, 625-643.

Grandcolas, P., 1994. Phylogenetic systematics of the subfamily Polyphaginae, with the assignment of *Cryptocercus* Scudder, 1862 to this taxon (Blattaria, Blaberoidea, Polyphagidae). *Systematic Entomology*, **19**, 145–158.

Grandcolas, P., 1996. The phylogeny of cockroach families: a cladistic appraisal of morpho-anatomical data. *Canadian Journal of Zoology*, **74**, 508–527.

Grandcolas, P., 1997a. What did the ancestors of the woodroach *Cryptocercus* look like? A phylogenetic study of the origin of subsociality in the subfamily Polyphaginae (Dictyoptera, Blattaria). The origin of biodiversity in insects: phylogenetic tests of evolutionary scenarios (ed. by P. Grandcolas). *Mémoires du Muséum National d'Histoire Naturelle Paris*, **173**, 231–252.

Grandcolas, P., 1997b. Systématique phylogénétique de la sous-famille des Tryonicinae (Dictyoptera, Blattaria, Blattidae). In *Zoologia Neocaledonica* vol. 4 (ed. by J. Nait and L. Matile). *Mémoires du Muséum National d'Histoire Naturelle Paris*, **171**, 91–124.

Grandcolas, P., 1999a. Reconstructing the past of *Cryptocercus* (Blattaria: Polyphagidae): phylogenetic histories and stories. *Annals of the Entomological Society of America*, **92**, 303–307.

Grandcolas, P., 1999b. Systematics, endosymbiosis, and biogeography of *Cryptocercus clevelandi* and *C. punctulatus* (Blattaria: Polyphagidae) from North America: a phylogenetic perspective. *Annals of the Entomological Society of America*, **92**, 285–291.

Grandcolas, P., D'Haese, C., 2001. The phylogeny of cockroach families: is the current molecular hypothesis robust? *Cladistics*, **17**, 48–55.

Grimaldi, D., Engel, M.S., 2005. *Evolution of insects*. Cambridge University Press New York.

Grimaldi, D., 2003. A revision of Cretaceous mantises and their relationships, including new taxa (Insecta: Dictyoptera: Mantodea). *American Museum Novitates*, **No. 3412**, 47 pp.

Guttell, R.R., Gray, M.W., Schinare, M.N., 1993. A compilation of large subunit (23S and 23S-like) ribosomal RNA structures. *Nulcleic Acids Research*, **21**:3055-3074.

Hennig, W., 1969. Die Stammesgeschichte der Insekten. Frankfurt: Verlag Waldemar Kramer.

Hennig, W., 1981. *Insect phylogeny*. Wiley Interscience Publication, John Wiley & Sons, Chichester etc.

Hickson, R. E., Simon, C., Cooper, A., Spicer, G. S., Sullivan, J., Penny, D., 1996. Conserved sequence motifs, alignment, and secondary structure for the third domain of animal 12S rRNA. *Molecular Biology and Evolution* **13**:150-169.

Hickson, R. E., Simon, C., Perrey, S. W., 2000. The performance of several multiplesequence alignment programs in relation to secondary-structure features for an rRNA sequence. *Molecular Biology and Evolution* **17**:530-539

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Huelsenbeck, J.P., Ronquist, F., 2002. MrBayes 3: Bayesian analysis of phylogeny. Computer program distributed by the authors. Department of Ecology, Behavior and Evolution, University of California, San Diego.

Inward, D., Beccaloni, G., Eggleton, P., 2007. Death of an order: a comprehensive molecular phylogenetic study confrims that termites are eusocial cockroaches. *Biology Letters* April 5.
Jow, H., Hudelot, C., Rattray, M., Higgs, P.G., 2002. Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution. *Molecular Biology and Evolution* 19, 1591-1601.

Kaltenbach, A.P., 1996. Unterlagen für eine Monographie der Mantodea des südlichen Afrika; 1. Artenbestand, geographische Vertreitung und Ausbreitungsgrenzen. *Annalen des Naturhistorischen Museums in Wien, Serie B*, **98B**, 193–346.

Kaltenbach, A.P., 1998. Unterlagen für eine Monographie der Mantodea (Insecta) des südlichen Afrika; 2. Bestimmungstabellen für die höheren Taxa, Nachträge zum Artenbestand. *Annalen des Naturhistorischen Museums in Wien, Serie B*, **100B**, 19–59.

Kambhampati, S., 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 2017–2020.

Kambhampati, S., 1996. Phylogenetic relationship among cockroach families inferred from mitochondrial 12S rRNA gene sequence. *Systematic Entomology*, **21**, 89–98.

Kambhampati, S., Kjer, K.M., Thorne, B.L., 1996. Phylogenetic relationship among termite families based on DNA sequence of mitochondrial 16S rRNA gene. *Insect Molecular Biology*, **5**, 229–238.

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 and Evolution*, **4** (3): 314-330

Kjer, K.M., 2004. Aligned 18S and insect phylogeny. *Systematic Biology*, **53(3)**, 506–514.

Kjer, K.M., Baldridge, G.D., Fallon, A.M., 1994. Mosquito large subunit ribosomal RNA: Simultaneous alignment of primary and secondary structure. *Biochim Biophys Acta*, **1217**, 147–155.

Kjer, K.M., Carle, F.L., Litman, J., Ware, J., 2006a. A molecular phylogeny of Hexapoda. *Arthropod Systematics and Phylogeny*, **64(1)**, 35–44.

Kjer, K.M., Gillespie, J.J., Ober, K.A., 2006b. Structural homology in ribosomal RNA, and a deliberation on POY. *Arthropod Systematics and Phylogeny*. **64**, 71–76.

Klass, K.-D., 1995. *Die Phylogenie der Dictyoptera*. PhD thesis, Ludwig-Maximilians-Universität Munich. Cuvillier, Göttingen, Germany.

Klass, K.-D., 1997. The external male genitalia and the phylogeny of Blattaria and Mantodea. *Bonner Zoologische Monographien*, **42**, 1–341.

Klass, K.-D., 1998a. The proventriculus of the Dicondylia, with comments on evolution and phylogeny in Dictyoptera and Odonata (Insecta). *Zoologischer Anzeiger*, **237**, 15–42.

Klass, K.-D., 1998b. The ovipositor of Dictyoptera (Insecta): Homology and ground-plan of the main elements. *Zoologischer Anzeiger*, **236**, 69–101.

Klass, K.-D., 2000. The male abdomen of the relic termite *Mastotermes darwiniensis* (Insecta: Isoptera: Mastotermitidae). *Zoologischer Anzeiger*, **239**, 231–262.

Klass, K.-D., 2001. Morphological evidence on blattarian phylogeny: "phylogenetic histories and stories" (Insecta, Dictyoptera). *Deutsche Entomologische Zeitschrift*, **48**, 223–265.

Klass, K.-D., 2003. Relationships among the principal lineages of Dictyoptera inferred from morphological data. Proceedings of the 1st Dresden Meeting on Insect Phylogeny: "Phylogenetic Relationships within the Insect Orders" (Dresden, September 19-21, 2003), *Entomologische Abhandlungen*, **61**, 119–172.

Klass, K.-D., 2007. Die Stammesgeschichte der Hexapoden: eine kritische Diskussion neuerer Daten und Hypothesen. *Denisia* 20: 413–450.

Klass, K.-D., Ehrmann, R., 2005. 13. Ordnung Mantodea, Fangschrecken, Gottesanbeterinnen. *Lehrbuch der Speziellen Zoologie, Band I: Wirbellose, 5. Teil: Insecta, 2nd edn.* (ed. by H.H. Dathe and H.E. Gruner), pp. 182–197. Spektrum Akademischer Verlag, Heidelberg, Berlin.

Klass, K.-D., Meier, R., 2006. A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. *Entomologische Abhandlungen*, **63**, 3–50.

Krishna, K., 1970. Taxonomy, phylogeny, and distribution of termites. *Biology of Termites* (ed. by K. Krishna and F.M. Weesner), pp. 127–153. Academic Press, New York.

Kristensen, N.P., 1975. The phylogeny of hexapod "orders." A critical review of recent accounts. *Zeitschrift fuer Zoologische Systematik und Evolutionsforschung*, **13**, 1–44.

Kristensen, N.P., 1981. Phylogeny of insect orders. *Annual Review of Entomology*, **26**, 135–157.

Kristensen, N.P., 1991. Phylogeny of extant hexapods. *The Insects of Australia* (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen, J.P. Spradberry, R.W. Taylor, M.J. Whitten and M.J. Littlejohn), pp. 125–140. CSIRO Division of Entomology, Melbourne University Press, Melbourne.

Kukalová-Peck, J., 1991. Fossil history and the evolution of hexapod structures. *The Insects of Australia* (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen, J.P.

Spradberry, R.W. Taylor, M.J. Whitten and M.J. Littlejohn), pp. 141–179. CSIRO Division of Entomology, Melbourne University Press, Melbourne.

Kukalová-Peck, J., Peck, S.B., 1993. Zoraptera wing structures: evidence for new genera and relationship with the blattoid orders (Insecta: Blattoneoptera). *Systematic Entomology*, **18**, 333–350.

Lewis, P.O., Holder, M.T., Holsinger, K.E., 2005. Polytomies and Bayesian Phylogenetic Inference. *Systematic Biology*, **54(2)**, 241–253.

Lin, C.P., Danforth, B.N., 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined data sets. *Molecular Phylogenetics and Evolution*, **30**, 686–702.

Lo, N., Tokuda, G., Watanabe, H., Rose, H., Slaytor, M., Maekawa, K., Bandi, C., Noda, H., 2000. Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Current Biology*, **10**, 801–804.

Lo, N., Bandi, C., Watanabe, H., Nalepa, C., Beninati, T., 2003. Evidence for cocladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. *Molecular Biology and Evolution*, **20**, 907–913.

Lo, N., 2003. Molecular phylogenetics of Dictyoptera: insights into the evolution of termite eusociality and bacterial endosumbiosis in cockroahces. *Entomologische Abhandlungen*, **61(2)**, 137–138.

Lo, N., Beninati, T., Stone, F., Walker, J., Sacchi, L., 2007. Cockroaches that lack *Blattabacterium* endosymbionts: the phylogenetically divergent genus *Nocticola*. *Biology Letters March 21*.

Lutzoni, F., Wagener, P., Reev, V., Zoller, S., 2000. Integrating ambiguously aligned regions of DNA sequence in phylogenetic analyses without violating positional homology. *Systematic Biology*, **49**, 628–651.

Lyons-Weiler, J., Hoelzer, G.A., Tausch, R.J., 1998. Optimal outgroup analysis. *Biological Journal of the Linnean Society*, **64**, 493–511.

Maddison, D.R., Ruvolo, M., Swofford, D.L., 1992. Geographic origins of human mitochondrial DNA: phylogenetic evidence from control region sequences. *Systematic Biology*, **41**, 111–124.

Maddison, W.P., Donoghue, M.J., Maddison, D.R., 1984. Outgroup analysis and parsimony. *Systematic Zoology*, **33**, 83–103.

Maekawa, K., Kitade, O., Matsumoto, T., 1999. Molecular phylogeny of orthopteroid insect based on the mitochondrial cytochrome oxidase II gene. *Zoological Science*, **16**,

175-184.

Maekawa, K., Matsumoto, T., 2000. Molecular phylogeny of cockroaches (Blattaria) based on mitochondrial COII gene sequences. *Systematic Entomology*, **25**, 511–519.

McKittrick, F.A., 1964. Evolutionary studies of cockroaches. *Memoirs of the Cornell University Agricultural Experiment Station.*, **389**, 1–197.

McKittrick, F.A., Mackkerras, M.J., 1965. Phyletic relationships within the Blattidae. *Annals of the Entomological Society of America*, **58**, 224–230.

Milinkovitch, M.C., Lyons-Weiler, J., 1998. Finding optimal ingroup topologies and convexities when the choice of outgroups is not obvious. *Molecular Phylogenetics and Evolution*, **9(3)**, 348–357.

Morrison, D.A., Ellis, J.T., 1997. Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNAs of apicomplexa. *Molecular Biology and Evolution*, **14**: 428-441.

Morse, G.E., Normark, B.B., 2006. A molecular phylogenetic study of armoured scale insects (Hemiptera: Diaspididae), *Systematic Entomology* **31**: 338-349

Mugridge, N. B., Morrison, D. A., Jäkel, T., Heckeroth, A. R, Tenter, A. M., Johnson, A. M., 2000. Effects of Sequence Alignment and Structural Domains of Ribosomal DNA on Phylogeny Reconstruction for the Protozoan Family Sarcocystidae. *Molecular Biology and Evolution* **17**: 1842-1853

Nalepa, C.A., Byers, G.W., Bandi, C., Sironi, M., 1997. Description of *Cryptocercus clevlandi* (Dictyoptera: Cryptocercidae) from the northwestern United States, molecular analysis of bacterial symbionts in its fat body, and notes on biology, description and biogeography. *Annals of the Entomological Society of America*, **90**, 416–424.

Nalepa, C.A., Bandi, C., 2000. Characterizing the ancestors: Paedomorphosis and termite evolution. *Termites: Evolution, Sociality, Symbiosis, Ecology* (ed. by T. Abe, D.E. Bignell and M. Higashi), pp. 53–75. Kluwer Academic Publishing, Dordrecht.

Nalepa, C.A., Lenz, M., 2000. The ootheca of *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae): homology with cockroach oothecae. *Proceedings of the Royal Society London, Series B*, **267**, 1809–1813.

Nylander, J.A.A., 2001. *Taxon sampling in phylogenetic analysis: problems and strategies reviewed*. Ph.D. dissertation, Uppsala University.

Otte, D., Spearman, L.A., 2005. *Mantida Species File: A Catalog of the Mantids of the World*. Insect Diversity Association, Publication Number 1, ISBN:1-929014-06-6. Academy of Natural Sciences, Philadelphia, PA, USA.

Pellens, R., Grandcolas, P., Domingos de Silva-Neto, I., 2002. A new and independently evolved case of xylophagy and the presence of intestinal flagellates in the cockroach *Parasphaeria boleiriana* (Dictyoptera, Blaberidae, Zetoborinae) from the remnants of the Brazilian Atlantic forest. *Canadian Journal of Zoology*, **80**, 350–359.

Pellens, R., D'Haese, C.A., Bellés, X., Piulachs, M.-D., Legendre, F., Wheeler, W.C., Grandcolas, P., 2007. The evolutionary transition from subsocial to eusocial behaviour in Dictyoptera: Phylogenetic evidence for modification of the "shift-in-dependent-care" hypothesis with a new subsocial cockroach. *Molecular Phylogenetics and Evolution*, doi: 10.1016/j.ympev.2006.12.017.

Posada, D., Crandall, K.A., 1998. ModelTest: Testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

Ross, K.G., 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.

Roy, R., 1999. Morphology and Taxonomy. In *The Praying Mantids*. Eds: Prete, F.R., Wells, H., Wells, P.H. & Hurd, L.E, The Johns Hopkins University Press, London. pp. 19–40.

Sacchi, L., Nalepa, C.A., Bigliardi, E., Lenz, M., Bandi, C., Corona, S., Grigolo, A., Lambiase, S., Laudani, U., 1998. Some aspects of intracellular symbiosis during embryo development of *Mastotermes darwiniensis* (Isoptera: Mastotermitidae). *Parassitologia*, **40**, 309–316.

Sacchi, L., Nalepa, C.A., Lenz, M., Bandi, C., Corona, S., Grigolo, A., Bigliardi, E., 2000. Transovarial transmission of symbiotic bacteria in *Mastotermes darwiniensis* (Isoptera: Mastotermitidae): ultrastructural aspects and phylogenetic implications. *Annals of the Entomological Society of America*, **93**, 1308–1313.

Sanderson, M.J., Shaffer, H.B., 2002. Troubleshooting molecular phylogenetic analyses. *Annual Review of Ecological Systematics*, **33**, 49–72.

Schoniger, M., von Haeseler, A., 1994. A stochastic model and the evolution of autocorrelated DNA sequences. *Molecular Phylogenetics and Evolution*, **3**, 240–247.

Smart, J., 1956. On the wing-venation of *Chaeteessa* and other mantids (Insecta: Mantodea). *Proceedings of the Zoological Society of London*, **127**, 545–553, plate 1.

Smith, A.B., 1994. Rooting molecular trees: problems and strategies. *Biological Journal of the Linnean Society*, **51**, 279–292.

Svenson, G.J., Whiting, M.F., 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology*, **29(3)**, 342–352.

Swofford, D.L., 2000. PAUP: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.

Terra, S.P., 1995. Revisão Sistemática dos generos de Louva-adeus da Região Neotropical (Mantodea). *Revista Brasileira de Entomologia*, **39(1)**, 13–94.

Terry, M.D., Whiting, M.F., 2005. Mantophasmatodea and phylogeny of the lower neopterous insects. *Cladistics*, **21(3)**, 240–257.

Thompson, G.J., Kitade, O., Lo, N., Crozier, R.H., 2000a. Phylogenetic evidence for a single, ancestral origin of a 'true' worker caste in termites. *Journal of Evolutionary Biology*, **13**, 869–881.

Thompson, G.J., Miller, L.R., Lenz, M., Crozier, R.H., 2000b. Phylogenetic analysis and trait evolution in Australian lineages of drywood termites (Isoptera, Kalotermitidae). *Molecular Phylogenetics and Evolution*, **17(3)**, 419–429.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., D. G. Higgins, 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24:4876-4882.

Thorne, B.L., Carpenter, J.M., 1992. Phylogeny of Dictyoptera. *Systematic Entomology*, **17**, 253–268.

Titus, T. A., Frost, D. R., 1996. Molecular homology assessment and phylogeny in the lizard family Opluridae (Squamata: Iguania). *Molecular Phylogenetics Evolution* **6**:49-62

Vawter, L., 1991. Evolution of blattoid insects and of the small subunit ribosomal RNA gene. University of Michigan, Ann Arbor.

Vickery, V.R., Kevan, D.K., 1983. A Monograph of the orthopteroid insects of Canada and adjacent regions. *Mem. Lyman Ent. Mus. Res. Lab.*, **13**, 1–1462.

Vršanský, P., 2002. Origin and early evolution of mantisea. AMBA Project, 6(1), 1-16.

Wang, T., 1993. *Synopsis on the Classification of Mantodea from China*. Shangahi Scientific and Technological, Literature Publishing House, Shanghai.

Wheeler, W.C., Whiting, M., Wheeler, Q.D., Carpenter, J.M., 2001. The phylogeny of the extant hexapod orders. *Cladistics*, **17**, 113–169.

Wheeler, W.C., 1990. Nucleic acid sequence phylogeny and random outgroups. *Cladistics*, **6**, 363–367.

Yager, D.D., Hoy, R.R., 1986. The cyclopean ear: A new sense for the praying mantis. *Science*, **231**, 727–729.

Yager, D.D., Hoy, R.R., 1987. The midline metathoracic ear of the praying mantis, *Mantis religiosa. Cell Tissue Research*, **250**, 531–541.

Yager, D.D., Hoy, R.R., 1989. Audition in the praying mantis, *Mantis religiosa* L.: Identification of an interneuron meadiating ultrasonic hearing. *Journal of Comparitive Physiology*, **165**, 471–493.

Yager, D.D., May, M.L., 1990. Ultrasound-triggered flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. II. Tethered flight. *Journal of Experimental Biology*, **152**, 41–58.

Yager, D.D., May, M.L., Fenton, M.B., 1990. Ultrasound-triggered flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight. *Journal of Experimental Biology*, **152**, 17–39.

Figure 1: Previous phylogenetic hypotheses of dictyopteran relationships. (out) = taxa formally used as outgroup representatives for dictyopteran ingroup. * including the genus *Cryptocercus*; ** including the Tryonicidae and Lamproblattidae.



Figure 2: Parsimony analysis of COII, 16S, 18S, 28S and morphological data, strict consensus of most parsimonious trees; bootstrap values shown above branch are from a 1000 bootstrap replicate search using 10 random addition searches and TBR branch swapping. Mantodean families: AM Amorphoscelidae; CH Chaeteessidae; EM Empusidae; HY Hymenopodidae; IR Iridopterygidae; MD Mantoididae; ML Metallyticidae; MT Mantidae; TH Thespidae. (A) 8-OG analysis, shortest tree recovered: 11155 steps??, (B) 5-OG analysis, shortest tree recovered: 4625 steps??. See Table 1 for the data partitions included for the various terminal taxa.



Figure 3: Bayesian doublet analysis of COII, 16S, 18S, 28S and morphological data, majority rule consensus trees; posterior probabilities shown above branch. Mantodean families: AM Amorphoscelidae; CH Chaeteessidae; EM Empusidae; HY Hymenopodidae; IR Iridopterygidae; MD Mantoididae; ML Metallyticidae; MT Mantidae; TH Thespidae. (A) 8-OG analysis, (B) 5-OG analysis. See Table 1 for the data partitions included for the various terminal taxa.



Figure 4: Effect of ingroup and outgroup selection on phylogeny reconstruction (maximum parsimony): (A) Maekawa and Matsumoto (2000) COII data + Odonata (*Sympetrum* and *Argia*) sequences + *Grylloblatta* sequence; (B) Maekawa and Matsumoto (2000) COII data + Mantodea + Odonata (*Sympetrum* and *Argia*) + *Grylloblatta*; (C) Kjer et al. (2006) COII, 16S, 18S, 28S with taxon list from current study; (D) Kjer et al. (2006) COII, 16S, 18S, 28S with current taxon list plus *Timema*, *Gryllus* and *Tettigonia*; (E) Kjer et al. (2006) COII, 16S, 18S, 28S with orthopteran outgroup; (F) Kjer et al. (2006) COII, 16S, 18S, 28S with embiopteran outgroup; (G) Kjer et al. (2006) COII, 16S, 18S, 28S with phasmatodean outgroup; (H) Kjer et al. (2006) COII, 16S, 18S, 28S with dermapteran outgroup; (K) Kjer et al. (2006) COII, 16S, 18S, 28S with grylloblattodean outgroup. Note that in C–K for most outgroup taxa not all data partitions are included.



Figure 5: Effect of outgroup selection on maximum parsimony phylogeny reconstruction using only 18S sequences from taxa included in Kjer et al. (2006); composition of ingroup (Dictyoptera) constant. (A) with orthopteran-gryllotalpid outgroup; (B) with embiopteran outgroup; (C) with phasmatodean outgroup; (D) with archaeognathan outgroup; (E) with dermapteran outgroup; (F) with grylloblattodean outgroup.



Table 1: Taxon sample and data used in the present study. Morphological data taken from Klass and Meier (2006: table 2). For species belonging to our standard dataset, table entries are on grey background. Those on white background * = Taxon only used in some experimental analyses

Order	Romity	Snooimon/Cono	COIL	391	185	785	Marnholaeu
	<i>C</i> 1 1 1 1 1 1 1 1 1 1						19000 Junit
Mantodea	Mantidae	Litaneutria minor	AY491311	AY491133	AY491195	AY491254	Х
		Choeradodis rhombicollis	AY491282	AY491106	AY491167	AY491226	Х
		Tenodera australasiae	AY491291	AY491114	AY491176	AY491235	X
		Orthodera sp.	AY491299	AY491122	AY491184	AY491243	X
		Melliera brevipes	AY491293	AY491116	AY491178	AY491237	Х
		Heterochaetula sp.	AY491300	AY491123	AY491185	AY491244	X
		Statilia apicalis	AY491315	AY491136	AY491198	AY491257	Sphodromantis sp.
		Stagmomantis limbata	AY491297	AY491120	AY491182	AY491241	Х
		Phyllovates chlorophaea	AY491292	AY491115	AY491177	AY491236	Х
	Empusidae	Gongylus gongylodes	AY491274	AY491100	AY521860	AY521789	Х
	Hymenopodidae	Pseudocreobotra occellata	AY491283	AY491107	AY491168	AY491227	Х
		Chrysomantis sp.	AY491273	AY491099	AY491157	AY491216	Х
		Phyllocrania paradoxa	AY491277	Х	AY491162	AY491221	Х
	Amorphoscelidae	Gyromantis occidentalis	AY491321	AY491144	AY491206	AY491265	Х
	Mantoididae	Mantoida schraderi	AY491275	AY491101	AY491160	AY491219	Mantoida schraderi
	Iridopterygidae	Bolbe sp.	AY491305	AY491128	AY491189	AY491248	Х
		Ima fusca	AY491303	AY491126	AY491187	AY491246	Х
		Neomantis australis	AY491317	AY491140	AY491202	AY491261	Х
	Thespidae	Bantia sp.	AY491314	AY491134	AY491199	AY491258	Х
		Hoplocorypha sp.	AY491308	AY491130	AY491192	AY491251	Х
		Anamiopteryx sp.	AY491295	AY491118	AY491180	AY491239	X
	Metallyticidae	Metallyticus violacea	X	Х	Х	X	Metallyticus violacea
	Chaeteessidae	Chaeteessa caudata	Х	Х	Х	Х	Chaeteessa caudata
Blattaria	Blattidae	Periplaneta americana	M83971	AF262620	AF321248	AJ577262	Periplaneta
		Archiblatta hoeveni	X	Х	X	X	Archiblatta hoeveni
		Deropeltis sp.	X	X	Х	X	Deropeltis sp.
		Eurycotis floridana	Х	Х	X	X	Eurycotis floridana
	Polyphagidae	Polyphaga aegyptiaca	AB014069	x	AF220575	X	Polyphaga
			Δ	4 1004001	EF3(335	Λ	aegypnaca
		I herea petiveriana	A 4 Dot 1070	AJ 294952 V	EF30323	V	
		Ergauta sp.	AB0140/0	X • • • • • • • • • • • • • • • • • • •	X	X	Ergaula capucina
	Blattellidae	Supella longipalpa	AY491269	AY 491095	AY121130	AY491210	Supella longipalpa
		Blattella germanica	B. lituricollis AB005906	B. vaga U17775	AF220573	AF005243	Parcoblatta lata
		Anaplecta sp.	X	X	X	Х	Anaplecta sp.
		Euphyllodromia angustata	X	X	X	Х	Euphyllodromia
		<i>Myctihora</i> sp	X	X	X	X	Myctihora en
		Nahuhlattella sp.	X	X	X	X	Nahuhlattella sn.
	Blaberidae	Gromphadorhina portentosa	D0181523	Z97626	AY491145	AY491207	Blaberus
		Naunhoeta cinerea	X	X	X	X	Nauphoeta cinerea
		Blantica sn	X	X	X	X	Rlantica sn
		Phoetalia pallida	X	X	X	X	Phoetalia pallida
	Cryptocercidae	Cryptocercus relictus	AB005908	AY631412	AB085771	AF322489	Cryptocercus
		Cryptocercus punctulatus	AY491268	AF126773	AY491148	AF322486	Х
	Nocticolidae	Nocticola sp.	Х	Х	EF203097	Х	Х
		Nocticola australiensis	Х	Х	EF203096	Х	Х
	Lamproblattidae	Lamproblatta albipalpus	X	Х	X	X	Lamproblatta
	Trvonicidae	Tryonicus narous	X	×	X	X	Tryonicus narvus

Lauraesilpha angusta	Mastotermes	darwiniensis	Х	Х	Termopsinae	Х	Х	Kalotermitidae	Grylloblatta	X	Karoophasma	X	Х	X	X	Х	Locusta	Timema	X	X	X	Echinosoma	X	Х	X	Dinocras sp.
Х	AY125281		AY125280	Х	AY521783	X	X	Cryptotermes sp. AY521781	X	AY125279	AY521791	X	AY125274	AY521798	X	Gryllotalpidae sp. AY521803	AF370809	AY125302	AY125310	AY125301	AY521752	E. micropteryx AY707377	AY125284	AF461214	AF370806	Х
Х	AY121141		AY491151	AF220569	AF220567	AF423782	AF220564	Cryptotermes sp. AY491150	DQ457301	AY121139	X	X	AY121134	AY521869	Z97587	AF514521	AF370793	AF423806	AY121170	AY121161	AY521836	AY121132	AY121144	AF461245	AF370788	Hesperaperla pacifica AF311470
Х	AY302717		Х	AF262619	AF262575	DQ278594	AY302710	D89835	DQ457264	X	AY318884	AY318910	Х	AF248684	Z97622	AF514475	Z93294	Phyllium bioculatum Z93319	X	Z93319	Х	E. yorkense AY 144636	x	Х	AF370870	Х
Х	AB014071		Х	AF220599	AB005586	AF525355	AY536410	AF189087	DQ457368	Х	Х	AY318942	X	Х	X	AY165658	Х	X	Х	Х	Х		AY179159	DQ166804	Х	X
Lauraesilpha angusta	Mastotermes darwiniensis		Nasutitermes sp.	Microhodotermes viator	Hodotermopsis japonica	Reticulitermes tibialis	Coptotermes lacteus	Cryptotermes domesticus	Grylloblatta sculleni	Grylloblatta campodeiformis*	Sclerophasma paresisense	Lobophasma redelinghuisense*	Oligotoma nigra	Gryllus assimilis*	Tettigonia viridissima*	Gryllotalpa sp.*	Locusta sp.	Timema knulli	Carausius morosus	Phyllium bioculatum*	<i>Forficula</i> sp.*	<i>Echinosoma</i> sp.	Argia sp.*	Sympetrum sanguineum*	Allomachillis froggarti*	Dinocras sp.
	Mastotermitidae		Termitidae	Hodotermitidae	Termopsidae	Rhinotermitidae		Kalotermitidae	Grylloblattidae		Mantophasmatidae	Austrophasmatidae	Oligotomidae	Gryllidae	Tettigoniidae	Gryllotalpidae	Acrididae	Timematidae	Heteronemiidae	Phylliidae	Forficulidae		Coenagrionidae	Libellulidae	Meinertellidae	Perlidae
	Isoptera								Grylloblattodea		Mantophasmatodea		Embioptera	Orthoptera				Phasmatodea			Dermaptera		Odonata		Archaeognatha	Plecoptera

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Keterence	Focal taxon and	Data used	Methods of analysis
	sampling for Dictyoptera		
Kjer (2004)	Hexapoda (13	18S	Maximum parsimony
	dictyopteran species)		(equally and differently
			weighted)
			Maximum likelihood
Kjer et al. (2006)	Hexapoda (20	COI, COI amino acids,	Maximum parsimony,
	dictyopteran species)	COII, COII amino acids,	Maximum parsimony
		12S, 16S, 18S, 28S,	(with pseudoreplicate re-
		Morphology	weighting)
_		all rRNA aligned with	Maximum likelihood
		refer-ence to secondary	
		structure	
Terry and Whiting (2005)	Polyneopteran Pterygota	Morphology, 18S, 28S,	Maximum parsimony
_	(18 dictyopteran species)	H3	using direct optimization
			(POY), Maximum
			parsimony using
			CLUSTALX alignment,
			Bayesian analysis using
			CLUSTALX analysis
Thorne and Carpenter	Dictyoptera (6 high level	Morphology	Maximum parsimony
(1992)	taxa)		
Kambhampati (1995)	Dictyoptera (36 species)	16S, 12S	Maximum parsimony
Lo et al. (2000)	Dictyoptera (16 species)	18S, COII,	Maximum likelihood
		endo-ß-1,4 glucanase	
Deitz et al. (2003)	Dictyoptera (6 high level	Morphology	Maximum parsimony
	taxa)		
Klass and Meier (2006)	Dictyoptera (27 species)	Morphology	Maximum parsimony
Pellens et al. (2007)	Dictyoptera (50 species,	COII, 12S, 16S, 18S	Direct optimization,
	focus on Blaberidae)		Maximum likelihood

Inward et al (2007)	Dictvontera (107 species)	COII 12S 18S 28S H3	Maximum narsimonv
			Maximum likelihood
Lo et al. (2007)	Dictyoptera (50 species)	COII, 18S, H3	Maximum parsimony
			Maximum likelihood
Lo et al. (2003)	Blattaria and Isoptera (17	COII, 12S, 16S, 18S,	Maximum parsimony
	species)	16S of Blattabacterium	Maximum likelihood
Maekawa and Matsumoto (2000)	"Blattaria" (19 species)	COII	Neighbor joining
Donovan et al. (2000)	Isoptera (49 species)	Morphology	Maximum parsimony
Kambhampati et al.	Isoptera (10 species)	16S aligned with	Maximum parsimony
(1996)		reference to secondary	Neighbor joining
		structure	
Kambhampati and	Isoptera (20 species)	ND5	Maximum parsimony
Eggleton (2000)			(with successive character
			weighting)
Thompson et al. (2000)	Isoptera (12 species in	COII, COII amino acids,	Maximum likelihood
	most relevant analysis)	16S all rRNA aligned	
		with refer-ence to	
		secondary structure	
Miura et al. (1998)	Termitidae (15 species)	COII	Maximum parsimony
Miura et al. (2000)	Nasutitermes (17 species)	COII, 16S rRNA, aligned	Maximum parsimony
		with reference to	Neighbor joining
		secondary structure	
Thompson et al. (2000)	Australian Kalotermitidae	COII, Cytb	Maximum likelihood
	(25 species)		
Svenson and Whiting	Mantodea (63 species)	COII, H3, 16S, 18S, 28S	Direct optimization
(2004)			Maximum likelihood

Table 3: Analytical methods

Taxon set	Analytical method	Resulting Figure Number	Gene selection
Current 5 outgroup dataset (50 taxa)	Bayesian and	Parsimony: Figure 2b; Bavesian: Figure 3h	COII, 16S, 18S, 28S
Current 8 outgroup	Bayesian and	Parimony Figure 2a;	COII, 16S, 18S, 28S
dataset (62 taxa)	Parsimony	Bayesian: Figure 3a	к к
Kjer et al., 2006	Parsimony	Figure 4 c	COII, 16S, 18S, 28S
dictyopteran dataset			
and gene selection			
based on current study			
(14 taxa)			
Maekawa and	Parsimony	Figure 4 a, b	COII
Matsumoto (2000)		1	
dataset			
Kjer et al., 2006	Parsimony	Figure 4 d- k and Figure 5	COII, 16S, 18S, 28S
dictyopteran dataset for			
outgroup selection			
studies (20 ingroup			
taxa)			

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Publications:

(*) Denotes papers coauthored with undergraduates, which was NSF REU funded.

Refereed Publications:

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Ware, J. L., Litman, J., Klass, K-D, Spearman, L. Resolving internal relationships within the Dictyoptera poses new questions: difficulty in the placement of *Polyphaga*. *Systematic Entomology*. 33:429-450

Ware, J. L., Ho, S., Kjer, K. Divergence dates of libelluloid dragonflies (Odonata: Anisoptera) estimated from rRNA using paired-site substitution models. *Molecular Phylogenetics and Evolution*, 47(1):426-32

Ware, J. L., M. L. May, K.M. Kjer. 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. *Molecular Phylogenetics and Evolution* **45(1)**: 289-310.

Janmaat, Alida F., **Ware, Jessica**, Myers, Judy. 2007. Effects of crop type on *Bacillus thuringiensis* toxicity and residual activity against *Trichoplusia ni* in greenhouses. *Journal of Applied Entomology* **131(5)**: 333-337.

Karl M. Kjer, Frank L. Carle, Jesse Litman and Jessica Ware. 2006. A molecular phylogeny of Insecta. *Arthropod Systematics and Phylogeny* 64(1):35-44.

Myers, J. H. and **Ware, J**. 2002. Setting priorities for the biological control of weeds: What to do and how to do it. In: *Proceedings, Hawaii Biological Control Workshop. Technical Report # 129.*

Denslow, J. E., Hight, S.D. and Smith, C. W. (eds.) Honolulu: Pacific cooperative Studies Unit, University of Hawaii. Pp. 62-74.

In press publications:

Nielsen, A., **Ware, J. L.**, Mahar, J., Hamilton, G. *Entomological Society of America Student Biosecurity Debate, PRO Position.* American Entomologist. Submitted February 2008.

Ware, J. L., Simaika, J. P., Samways, M. Biogeography and divergence estimation of the relic Cape dragonfly genus Syncordulia: global significance and implications for conservation. *Journal of Biogeography*. Submitted May, 2008.