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Global Phylogeny of the Water Penny Beetles Using Both Molecular and Morphological Evidence (Co

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GLOBAL PHYLOGENY OF THE WATER PENNY BEETLES USING BOTH MOLECULAR AND MORPHOLOGICAL EVIDENCE (COLEOPTERA: PSEPHENIDAE)

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Matthew Vincent Wood

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GLOBAL PHYLOGENY OF THE WATER PENNY BEETLES USING BOTH MOLECULAR AND MORPHOLOGICAL EVIDENCE (COLEOPTERA: PSEPHENIDAE)

Date Recommended 17 March 2016

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I dedicate this thesis to my parents, Markeeta and Barry Wood, who have always been there for me and supported me throughout my education and my life.

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Matthew V. Wood May 2016 68 Pages

Directed by: Keith Philips, Scott Grubbs, and Lawrence Alice

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The Psephenidae is a family of freshwater beetles usually found in swift streams worldwide. Their unique disc shaped and flattened larvae have made this a group of interest for scientists for centuries. Morphologically, this family has been relatively well researched, and systematically the family is fairly well known and supported as monophyletic. One issue with Psephenidae, and with many other insect groups, is the lack of the molecular phylogenetic analyses to test morphology hypothesizes.

For this study, the relationships among the genera of this family were studied with both molecular and morphological data as well as combined in a total evidence analysis. DNA from specimens was extracted, amplified, and sequenced for all available genera that could be acquired locally and abroad through collaborators and their contacts in other countries. The nuclear gene Wingless (Wg) and the mitochondrial gene Cytochrome Oxidase 1 (CO1) were utilized in this study; amplification of several other nuclear genes was attempted but the results were poor and they were excluded from the analysis.

After successfully sequencing these two genes from species representing nearly all of the known genera, the data were analyzed using both Bayesian and parsimony methods. Analyses were performed individually for each gene, a combined molecular analysis, using just morphological data, and a total evidence analysis using both molecular and morphological data. After analyzing the trees, definite inconsistencies

were discovered between the current data set and the previous studies performed using only morphological characters. Individual gene analysis showed low support for the monophyly of proposed subfamilies within the psephenids, but combined molecular and total evidence analysis showed much more resolution as well as support for most but not all of the proposed subfamilies.

INTRODUCTION

Although insects are the most diverse class of animals on the planet, with over one million described species and counting, the documentation of species diversity and the hypothesized evolution of many groups are still very incomplete and includes the Psephenidae. This family of aquatic beetles (Coleoptera) is commonly referred to as the water penny beetles. Their name is derived from the larval appearance which often resembles a penny in both shape and coloration (Triplehorn & Johnson 2005). Currently there are 32 known genera and, based on phylogenetic evidence, includes 4 that are undescribed and over two hundred documented species (Lee et al. 2007).

It is generally agreed that Psephenidae belongs to the infraorder Elateriformia. Insects of this infraorder tend to have a much longer larval life in comparison to their adult lives (Grimaldi & Engel, 2005). Elateriformia consists of six super-families; one of these is known as the Byrrhoidea, and including the Psephenidae, consists of most of the aquatic beetle species whose larval lifestyle is either fully aquatic or semi-aquatic. This evolutionary history is reasonably well supported by the fossil record as are many beetle phylogenies, because of their hard outer covering that fossilizes quite well (Grimaldi & Engel, 2005).

The intriguing insects that make up Psephenidae have undergone a very interesting evolutionary history. It has been learned from their adult characters reflecting terrestrial habits that they had secondarily evolved an aquatic lifestyle, but it is also known from the fossil record and from numerous adaptations that the family had been utilizing an aquatic lifestyle possibly as early as the Jurassic period (199.6-145.5 mya) (Hunt et al. 2007).

Psephenids are found globally with the exception of Antarctica. Previous proposals for an internal classification have been inconsistent (Shepard 2002). Currently they are divided into five subfamilies (Lee et al. 2007) with their distributions as follows. The Afroeubriinae are African, the Eubrianacinae are circum-Pacific, the Eubriinae are found globally, the Psepheninae are found in the New World and Asia, and the Psephenoidinae are restricted to Asia and sub-Saharan Africa.

Water penny beetles typically inhabit freshwater streams and riparian zones. The majority of their life cycle is spent as a larva attached to rocks, logs, or other debris usually in fast to semi-fast flowing streams. They feed on algae and detritus on the substrate throughout a six larval instar life cycle. Eventually the larva metamorphizes into a pupa; after emergence, beetles are typically found on plants or rocks around larval inhabited streams, or in the water during oviposition (Brown, 1976). In temperate zones, water penny adults typically emerge only in the summer months for reproduction.

With increased interest in stream ecology over the last few decades, scientists now recognize that aquatic insects can be very helpful in diagnosing the health of a watershed. Psephenid larvae are typically susceptible to organic pollution and good indicators of stream health. Hence one can study the degradation of freshwater streams reflected in the decline of psephenid and other aquatic invertebrate populations (anonymous A, 2009).

Recent phylogenetic morphological data strongly supports Psephenidae as a monophyletic group (Lawrence et al., 2011). All prior evolutionary hypotheses on water penny beetles used only morphological evidence. Until very recently, the shape of the larvae was the only synapomorphy linking all of the genera to a common ancestor. The most recent phylogeny of Lee et al. (2007) used 143 morphological characters from all life stages (larva, pupa and adult) and included representatives of all but three of the 32 known psephenid genera. This work discovered many more synapomorphies that support the monophyly of the family and also presents a new internal subfamily classification.

Studies using molecular data to either support or reject current hypotheses on internal relationships of the family have not been done. Presented here for the first time is a phylogeny of the Psephenidae using molecular data from two genes and morphology, including data from Lee et al. (2007). Also included is morphological data from three new genera (*Acneus*, *Falsodrupeus*, and Genus E) not included in the Lee et al. study. The data was analyzed using both parsimony and Bayesian algorithms to explore hypotheses on the evolution of this group in an attempt to better understand the evolution of this family.

MATERIALS AND METHODS

Sampling

Representatives of all of the subfamilies, all 36 known genera, and 40 species are represented in this study (table 1). Specimens for analysis were received from collaborators from around the world and additional genera from the United States were collected from the western USA and locally. Outgroup specimen were collected locally, or previously submitted sequences were acquired from GenBank including *Zaitzeviaria brevis* (Nomura), *Graphelmis obesa* (Ĉiampor), *Grouvellinus marginatus* (Kono). [GenBank Codes: GU816127, DQ266492, GU816152]

For a molecular phylogenetic analysis, it was imperative to have well preserved specimens for good DNA sequencing results. Hence most specimens were collected and placed into a strong (≥95%) ethanol solution for DNA preservation. Attempts were made to isolate the DNA of some dried samples from rare taxa, but this was largely unsuccessful; DNA extracted from 20 of these genera resulted in varying success for each of the five tested genes.

DNA Sequencing

DNA was extracted using the E.Z.N.A. Insect kit (Omega Bio-tek, Norcrosse, GA). Cytochrome Oxidase I (800bp fragment) and Wingless (450bp fragment) genes were amplified successfully for most taxa. Amplification of Phosphoenolpyruvate Carboxykinase (PepCK) (580bp), Arginine Kinase (720bp), and 28S (630bp) was attempted, but the resolution was typically too low when gel electrophoresis was performed. Further, less than 50% of the taxa were sequenced for each of these genes and therefore this data was not included in the analysis. All DNA sequences will be deposited in GenBank (ncbi.nlm.nih.gov) prior to publication.

Polymerase chain reaction (PCR) was used to amplify target genes for sequencing. Typical PCR cycles for CO1 consisted of an initial denaturation at 95^0 C for 2 min, followed by 40 cycles of 95^0C for 30s, 46^0C for 45s, and 72^0C for 30s, followed by a final extension at 72^0C for 5 min. The PCR cycle for Wingless consisted of an initial denaturation at 95^0C for 2 min, followed by 35 cycles of 95^0C for 30s, 60^0C for 45s, and 73^0C for 30s, followed by a final extension at 73^0C for 5 min.

All nuclear genes that were tested for use in this study were relatively new to molecular phylogenetics (Wild & Maddison, 2008), so optimization was necessary for inclusion. Most time was spent finding the optimum temperatures for PCR amplification. For each of the new nuclear genes (Wg, ArgKin, PepCK) temperature gradients were performed using local *Psephenus herricki* (DeKay) and *Ectopria nervosa* (Melsheimer) and visualizing the product using agarose gel electrophoresis. Using these gradients, the most favorable annealing temperatures were found for optimum amplification and that would also eliminate the greatest amount of non-specific fragments. In some instances where non-specific binding was a problem, gel purifications were performed using the Wizard ® SV Gel and PCR Clean-Up System (Promega, Madison, WS) to isolate a product that was uncontaminated and ready for sequencing.

The PCR and gel purified products were sequenced using ABI DYE-Terminator 3.1 mix, following the standard protocol on an ABI/3130 sequencer (Applied Biosystems, Foster City, CA). DNA sequences were edited, and the multiple CO1 and Wingless sequences were first aligned in Geneious. Each data set was then aligned in ClustalW 1.83 and Mafft using the default values to check for congruence, and adjusted manually to remove alignment artifacts. Mafft alignments using the default value were used and were the same as alignments found in ClustalW under gap penalty of 15 (default) and 10 which were identical. No further values were tested for gap open and gap extend costs.

Morphological data was from Lee et al. (2007) and with the addition of three new genera including representatives of *Acneus*, *Falsodrupeus*, and Genus E (Table 1).

Phylogenetic Analysis

Parsimony analysis was done using TNT software (Goloboff et al. 2000) with the data matrix first constructed using WinClada (Nixon 1999). All characters were coded as unordered, and the matrix was analyzed with both equal and later with implied weights using PIWE. The equal weights search was also implemented in NONA (Goloboff 1999) using the following parameters: hold 10 000, hold/50, Mult*1000 (random addition sequence, 1000 replicates and TBR branch swapping). Tree comparison was done visually and bootstrap node supports were done using WinClada. Both strict consensus and majority rules (nodes appearing ≥50% of the time) trees were used for comparisons and illustrated.

Bayesian analysis was performed using MrBayes 3.2.1 for windows 32 bit (Ronquist et al., 2012). The two individual genes were analyzed separately as well as combined. The total evidence study used both genes as well as the morphological data.

In the Bayesian analysis, JModelTest 2.1.7 (Darriba et al. 2012) was used to determine the best fit model. When the model suggested by the program was not available in the MrBayes software, the next best model was chosen. The GTR + I + G model was selected for CO1 gene and the GTR + I + G was used for codon positions 1and 2 (Nst = 6, rates = invgamma) and for codon position 3, the GTR + G (Nst = 6, rates = gamma) for the Wingless analysis (see appendix 2 for full command lines used in MrBayes). In the Bayesian analyses, the default set of priors was used (Heated Chains = 4, Heated Chains Temperature = 0.2, Rate Variation = invgamma, Subsampling frequency = 200, Burn in Length = 100,0000, Priors: Unconstrained branch lengths: Exponential = 10, Shape parameter: Exponential = 10). The topology was found with the MCMC command using two simultaneous searches. Three runs of 5,000,000 generations were performed; about 1,200,000 generations were typically needed to get below 0.01 level of the standard deviation of split frequencies. Default burn-in values used are the first 25% from the cold chain. Plots of the likelihoods of sampled trees were examined to determine when the MCMC chains had reached stationary, and the sampled trees prior to this were discarded as burn-in. The majority rule consensus tree was obtained from the remaining trees.

In the results and discussion, the character is listed first and the state second, separated by a hyphen. Node support is shown on the Bayesian analysis and the tree found with parsimony unweighted characters (bootstrap using 1,000 replications) calculated in TNT or Nona (Goloboff et. al, 2000, Goloboff 1999). Bayesian posterior probabilities and bootstrap values are displayed at all nodes supported at a level above 0.5 or 50%, respectively. Consistency and retention indices (CI and RI) for the characters in the cladogram found using unweighted data (Figs 4-5) are listed after each character within the descriptions below.

RESULTS

Most taxa were successfully amplified for the CO1 gene, and to a lesser degree for the wingless gene. Other attempted genes were not included in the phylogenetic analysis. Morphological data for adults of all species was included; pupal and larval characteristics were also included for most taxa (Table 1, Lee et al. 2007 & Chi-Feng Lee unpublished data).

For CO1 there were 824 characters total with 316 being informative. For Wingless there were 556 characters total with 249 being informative. For morphological analysis there were a total of 143 informative characters. Total evidence analysis showed 1523 total character with 708 being informative.

Table 1: Taxa included in this study with their respective origins and genetic and morphological data acquired. Numbers in parentheses indicate the number of specimens within the taxa that were successfully sequenced.

Taxa	Origin	CO1	Wingless	ArgKin	PepCK	28s	Morph
Acneus Horn (1880)	United States	Yes (1)	No	No	No	No	Yes
Afrobrianax Lee, Philips & Yang (2003)	Africa	Yes (4)	Yes (3)	Yes (1)	Yes (3)	Yes (1)	Yes
Afroeubria Villiers (1961)	Guinea, Africa	Yes (1)	No	No	No	No	Yes
Afropsephenoides Basilewsky (1959)	Africa	No	No	No	No	No	Yes
Belicinus (Genus D) Arcé-Peréz, Shepard & Morôn (2012)	Belize	No	No	No	No	No	Yes
Dicranopselaphus Guerin-Meneville (1861)	Costa Rica	Yes (1)	No	No	No	No	Yes
Ectopria LeConte (1853)	United States/Asia	Yes (3)	Yes (3)	Yes (1)	Yes (1)	Yes (1)	Yes
Eubria Latreille (1829)	United Kingdom	Yes (1)	No	No	No	No	Yes
Eubrianax Kiesenwater (1874)	China/United States	Yes (4)	Yes (3)	Yes (1)	Yes (2)	Yes (2)	Yes
Falsodrupeus Pic (1949)	Madagascar	No	No	No	No	No	Yes
Neoeubria (Genus A) Shepard & Barr (2014)	Costa Rica	No	No	No	No	No	Yes
Genus B	South Africa	Yes (1)	Yes (1)	No	No	No	Yes
Genus C	Malaysia	No	No	No	No	No	Yes
Aethioeubria (Genus E) Hajek & Lee (2014)	Senegal, Africa	No	No	No	No	No	Yes
Granuleubria Jäch & Lee (1999)	India	No	No	No	No	No	Yes
Homoeogenus Waterhouse (1880)	Taiwan	No	No	No	No	No	Yes
Jaechanax Lee, Satô, & Yang (2000)	Indonesia/Philippines	No	No	No	No	No	Yes
Jinbrianax Lee, Satô, and Yang (1999)	Vietnam/Malaysia	No	No	No	No	No	Yes
Macroeubria Pic (1916)	Vietnam	Yes (2)	Yes (2)	No	No	No	Yes
Malacopsephenoides Jeng & Satô (2006)	Vietnam	Yes (2)	Yes (2)	No	Yes (1)	No	Yes
Mataeopsephus Waterhouse (1876)	Asia	Yes (1)	Yes (1)	No	Yes (1)	Yes (1)	Yes
Microeubria Lee & Yang (1999)	Asia	Yes (1)	Yes (1)	No	No	Yes (1)	Yes
Mubrianax Lee, Satô, and Yang (1999)	Philippines/E. Malaysia	No	No	No	No	No	Yes
Neopsephenoides manuscript name	Vietnam	Yes (2)	Yes (2)	Yes (1)	Yes (2)	Yes (2)	Yes
Nipponeubria Lee & Satô (1996)	Vietnam	Yes (1)	Yes (1)	No	No	No	Yes
Odontanax Lee, Satô &Yang (1999)	Vietnam	Yes (1)	Yes (1)	No	Yes (1)	Yes (1)	Yes
Pheneps Darlington (1936)	South America	No	No	No	No	No	Yes
Psephenoides Pic (1954)	Vietnam/Taiwan	Yes (1)	Yes (1)	Yes (1)	Yes (2)	Yes (1)	Yes
Psephenops Grouvelle (1898)	Costa Rica/S. Amer.	Yes (1)	Yes (1)	No	No	No	Yes
Psephenus Haldeman (1853)	United States	Yes (1)	Yes (1)	No	No	Yes (1)	Yes
Schinostethus Waterhouse (1880)	Asia	Yes (2)	Yes (1)	No	No	Yes (1)	Yes
Sclerocyphon Blackburn (1892)	Australia	No	Yes (1)	No	No	Yes (1)	Yes
Sinopsephenoides Yang (1994)	Vietnam	Yes (1)	No	No	No	Yes (1)	Yes
Tychepsephus Waterhouse (1876)	Chile	Yes (1)	Yes (1)	No	No	No	Yes
Xylopsephenoides manuscript name	Vietnam/Malaysia	No	No	No	No	No	Yes

In the CO1 analysis using both the parsimony (single tree discovered) and Bayesian techniques, similar results were found but with some differences are present (Figs 1, 2). The most glaring issue was in the Bayesian analysis where two outgroup elmids are found within the ingroup, although in a basal clade. Another point of interest found was the location of the subfamily Psepheninae, which is monophyletic in Bayesian analysis but paraphyletic in parsimony analysis. The parsimony analysis found that the subfamily was a basal clade and was sister to all other psephenids, while the Bayesian analysis showed that Psepheninae was a more derived clade. Two genera, *Eubrianax* and *Neosephenoides*, were not monophyletic in either analysis. All other genera tested were monophyletic. The monotypic Afroeubriinae, appears within the Eubriinae. *Afroeubria* was sister to *Malacopsephenoides* in the parsimony analysis but sister to *Eubrianax* in the Bayesian analysis. In the parsimony analysis none of the recognized subfamilies were monophyletic, (i.e.), the Psepheninae are paraphyletic, both Psephenoidinae and Eubrianacinae were slightly mixed, and Eubriinae contains the proposed Afroeubriinae (Fig. 11). In contrast, the Bayesian analysis showed a monophyletic Psepheninae, but similar to parsimony as all other subfamilies were paraphyletic. Psephenoidineae and Eubrianacinae were blended in a similar manner to the parsimony analysis as well as *Afroeubria* placed within the Eubriinae. It was also worth noting that the parsimony tree was completely resolved, while the Bayesian analysis had four unresolved nodes (trichotomy to hexachotomy). This was not unexpected due to the more conservative nature of Bayesian analysis compared to Parsimony seen in previous studies (Philips, unpublished data).

Figure 1: Bayesian analysis of CO1 taxa with posterior probabilities.

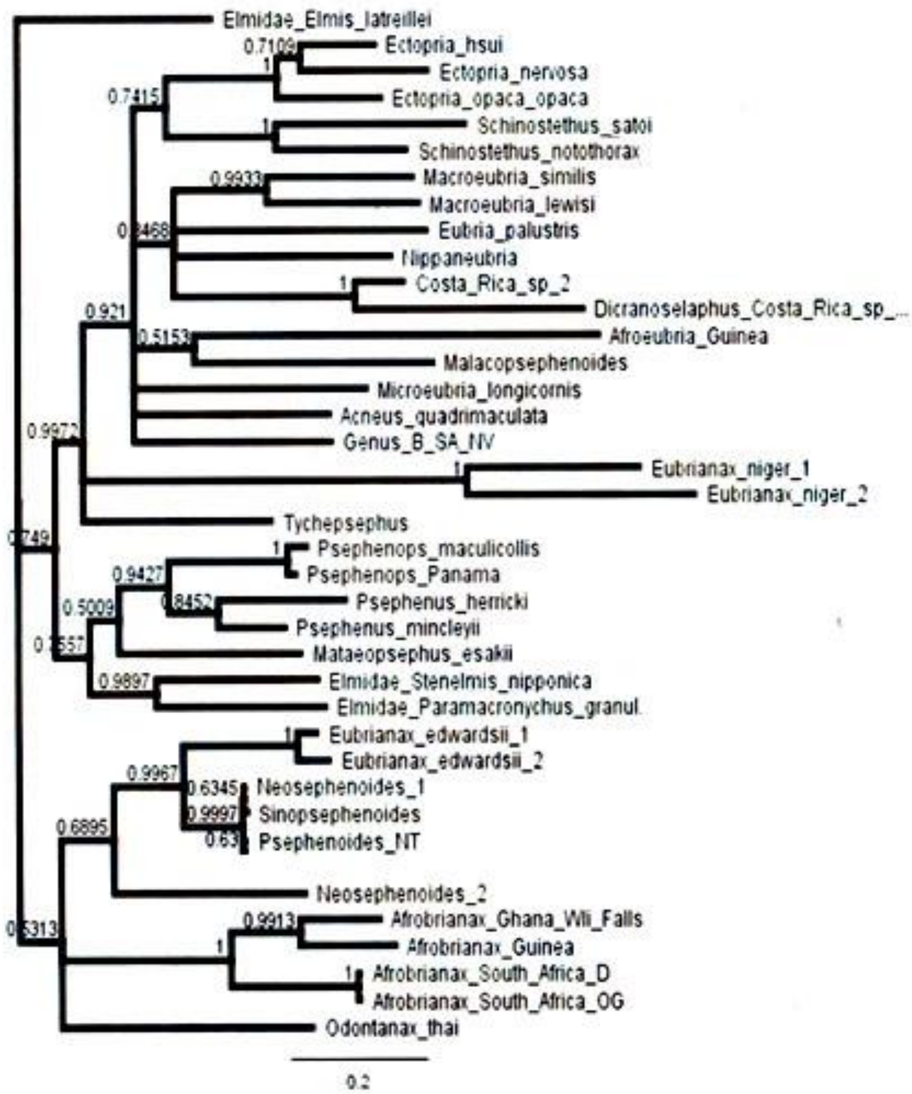
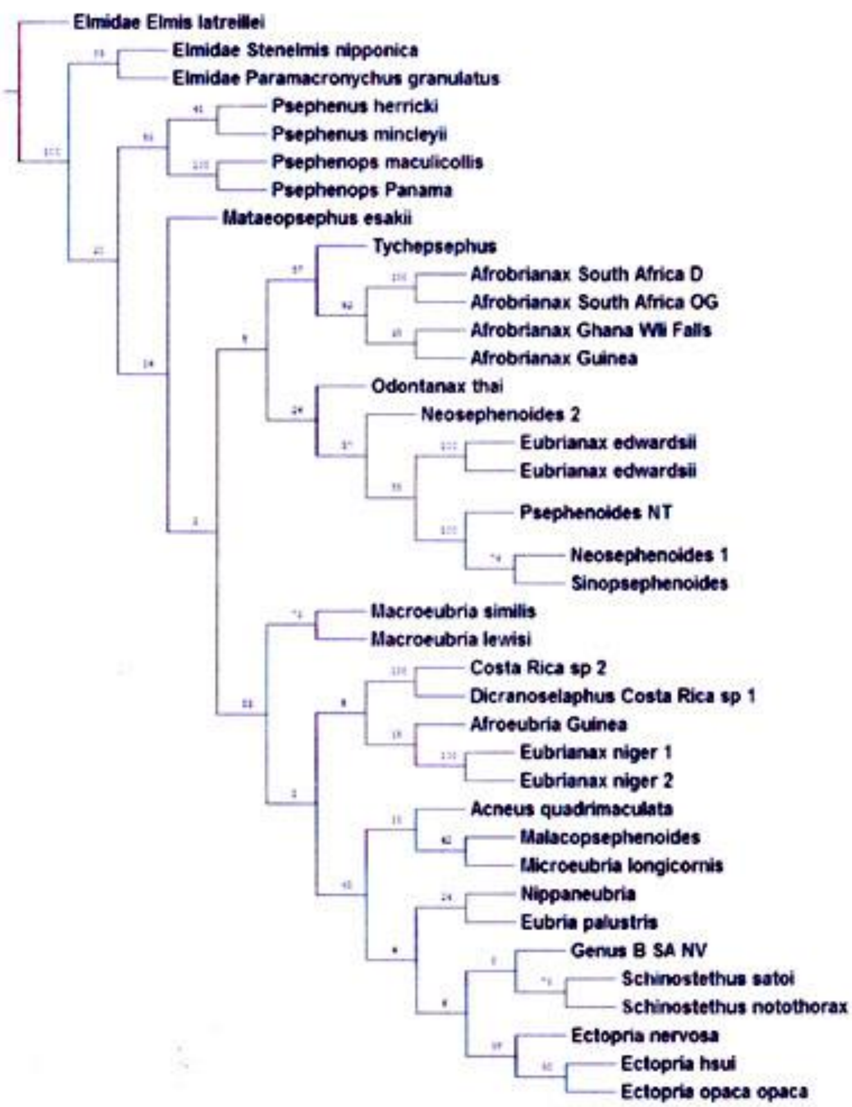


Figure 2: Parsimony analysis of CO1 taxa with bootstrap values. Total characters = 824 (485 non-informative), with total of 339 informative characters. 1 tree, tree length 2449 steps, 1,000 replications, tree found with 100 replications. CI = 25 RI = 43



When analyzing the wingless gene, both parsimony and Bayesian analyses were completely resolved (Figs 3, 4) but the only proposed subfamily that appeared monophyletic was the Psepheninae. Both the Bayesian and parsimony analysis show four very similar main clades. *Tychepsephus* plus *Sclerocyphon* were supported in both as a basal clade that was sister to all other psephenids included in this study. Similar to CO1 analysis, *Eubrianax* was not supported as monophyletic. The other non-monophyletic genus from CO1 analysis, *Neopsephenoides*, was only represented by one taxon in this study.

Wingless Analysis:

Figure 3: Bayesian analysis of wingless gene with posterior probabilities.

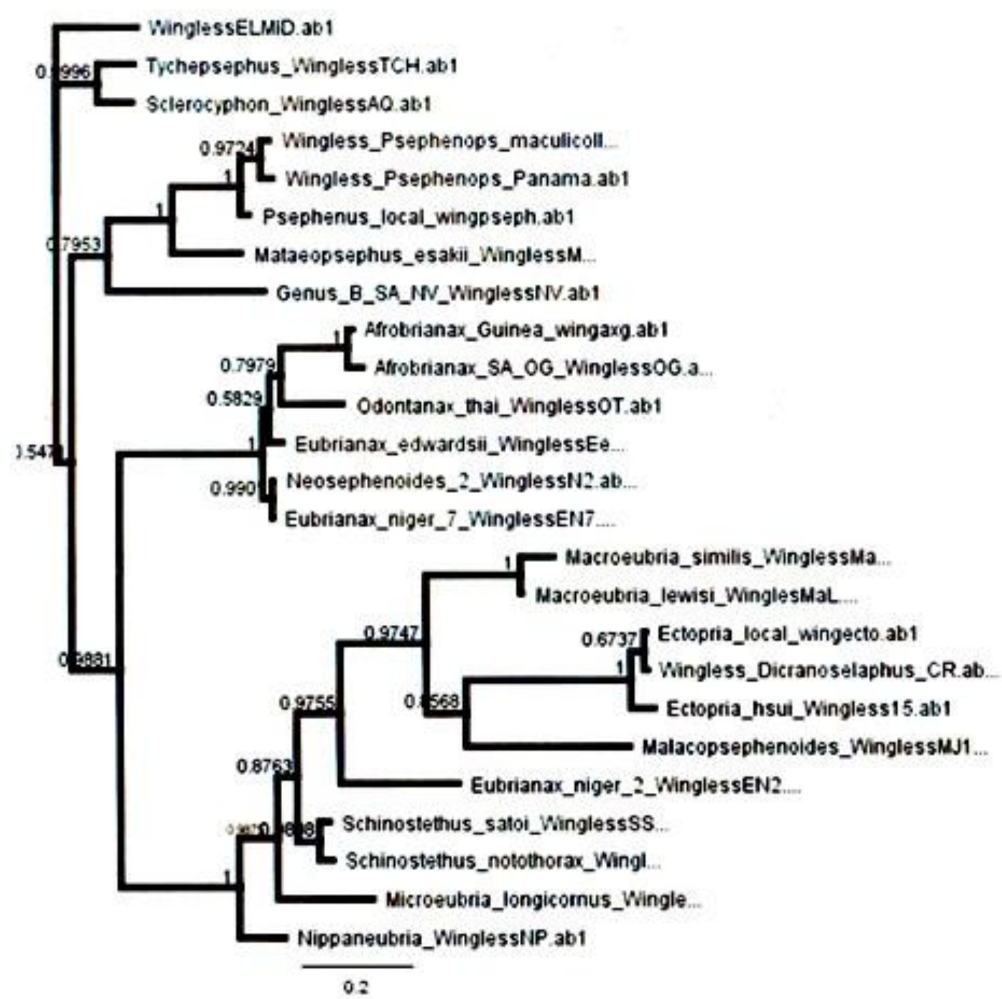
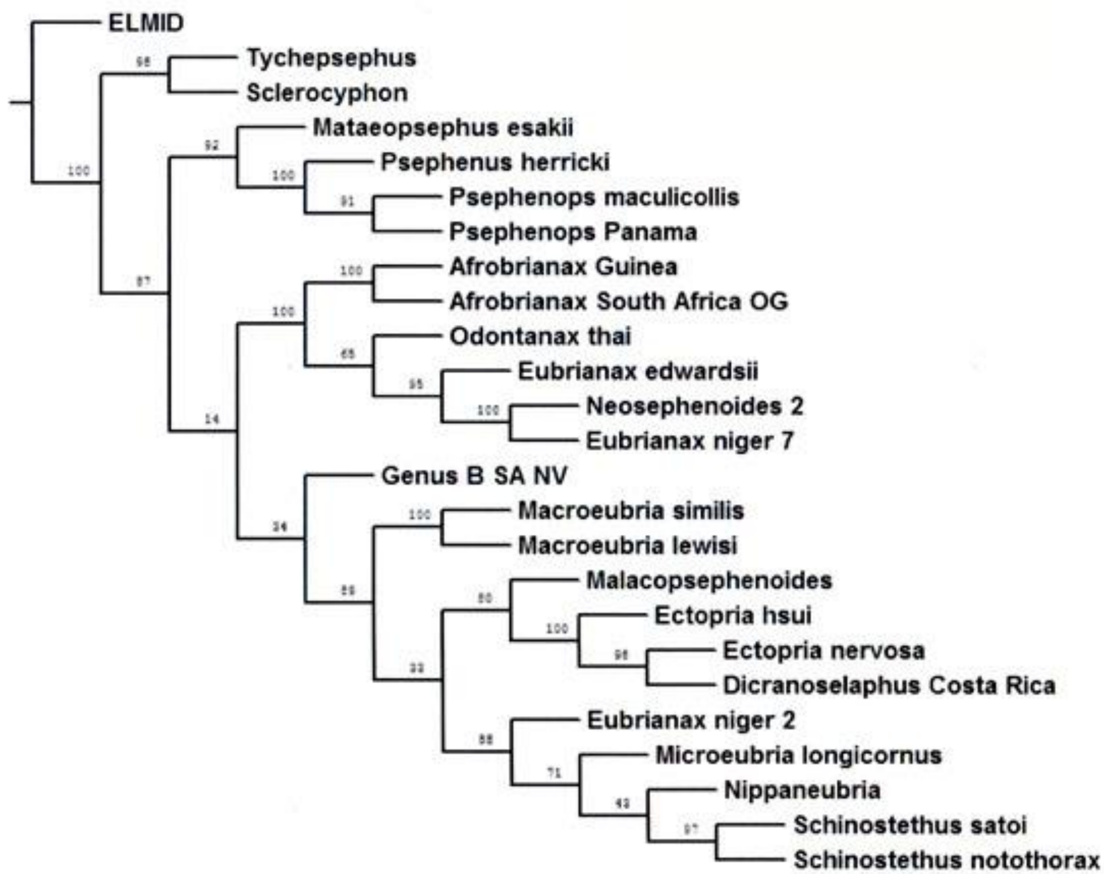


Figure 4: Parsimony analysis of wingless taxa with bootstrap values. Total characters 556 (293 not informative), with total of 263 informative characters. 1 tree, tree length 1018 steps. 1,000 replications- tree found within 100 replications. CI = 46 RI = 67



With both the CO1 and Wingless trees showing similar topologies the datasets were concatenated and analyzed as a combined dataset (Figs 5, 6, 7). This resulted in the parsimony analysis being completely resolved and the Bayesian analysis having only two trichotomies. Both analyses were very similar with some minor rearrangements of relationships among taxa. Both analyses showed members of Psepheninae as a basal water penny clade. The parsimony analysis supported the monophyly of both the Eubrianacinae and Psephenoidinae, but the analysis also found a paraphyletic Psepheninae and the Afroeubriinae was again positioned deep within Eubriinae. The Bayesian analysis supported, in contrast, the monophyly of the Psepheninae, and minor rearrangements of taxa created paraphyly in both the Eurbrianacinae and Psephenoidinae. Eubriinae was supported except that *Afroeubria* (Afroeubriinae) was once again buried fairly deep within this clade. One problem in the Bayesian analysis was one of the elmid outgroups (*Zaitzeviaria brevis* (Nomura)) fell within the ingroup. This is most likely due to all outgroups including only CO1 data and the placement of this single taxon should be ignored.

Figure 5: Bayesian analysis of combined molecular (CO1/Wingless) taxa.

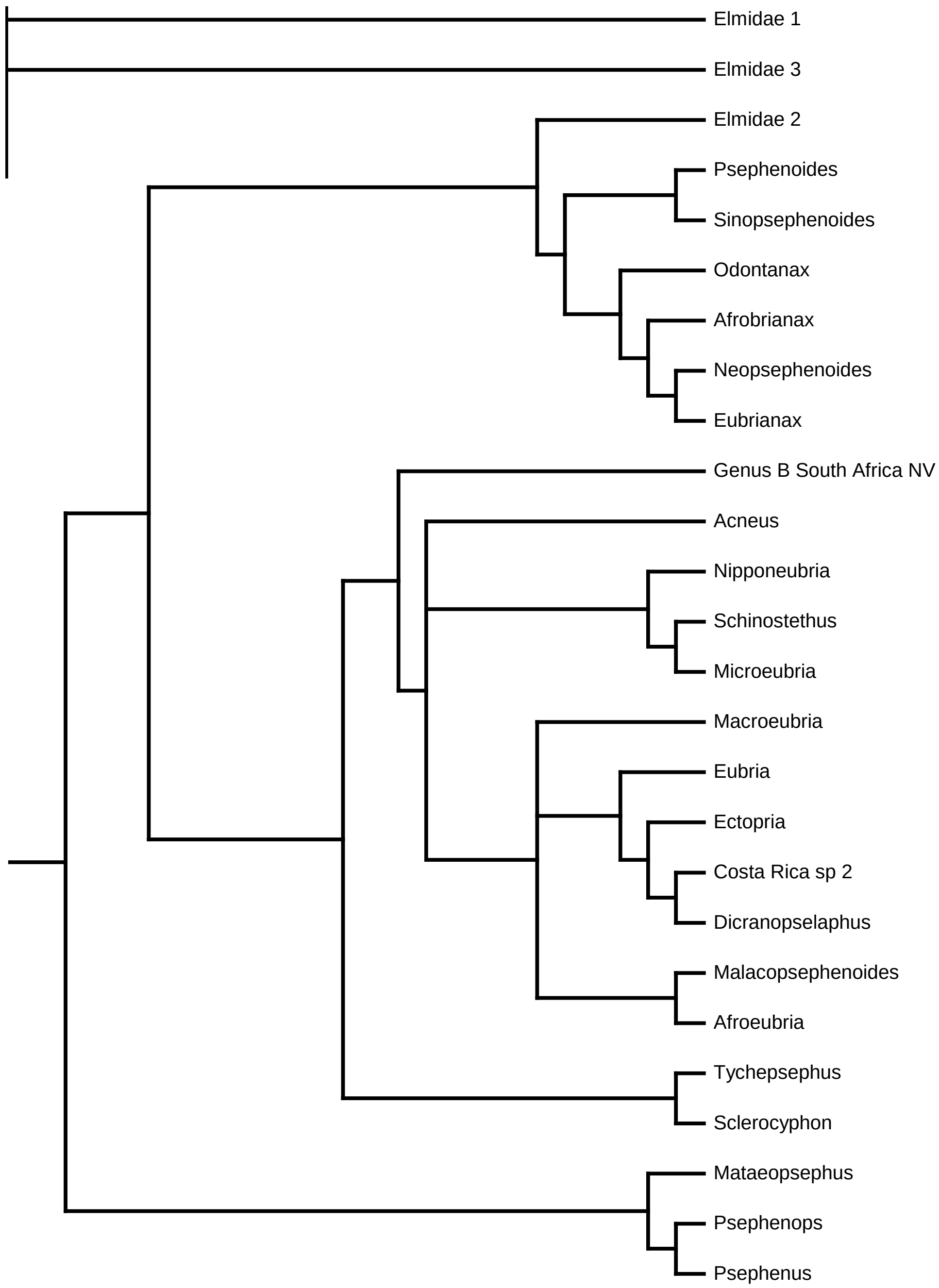


Figure 6: Parsimony analysis of combined molecular (CO1/Wingless) taxa with bootstrap values.

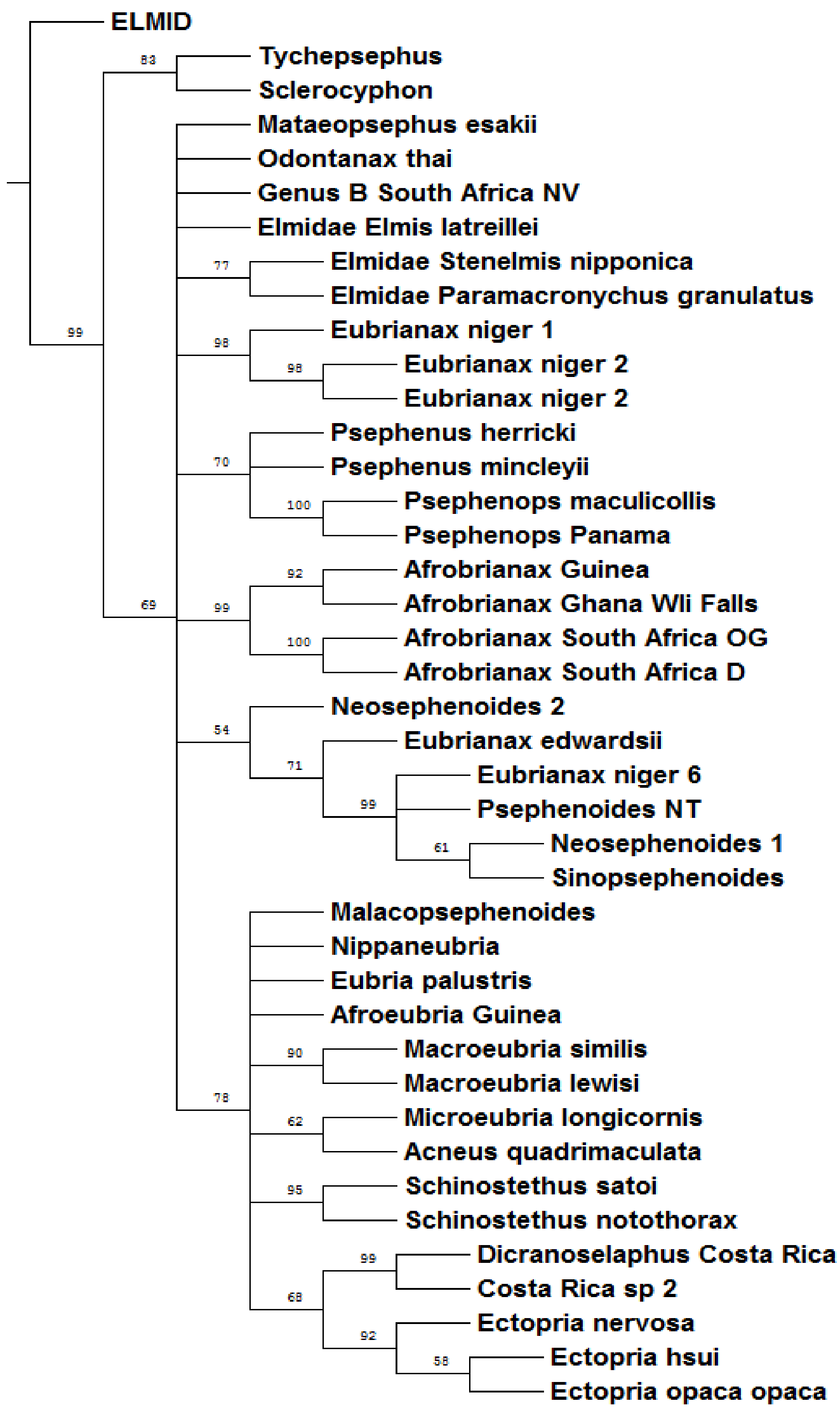
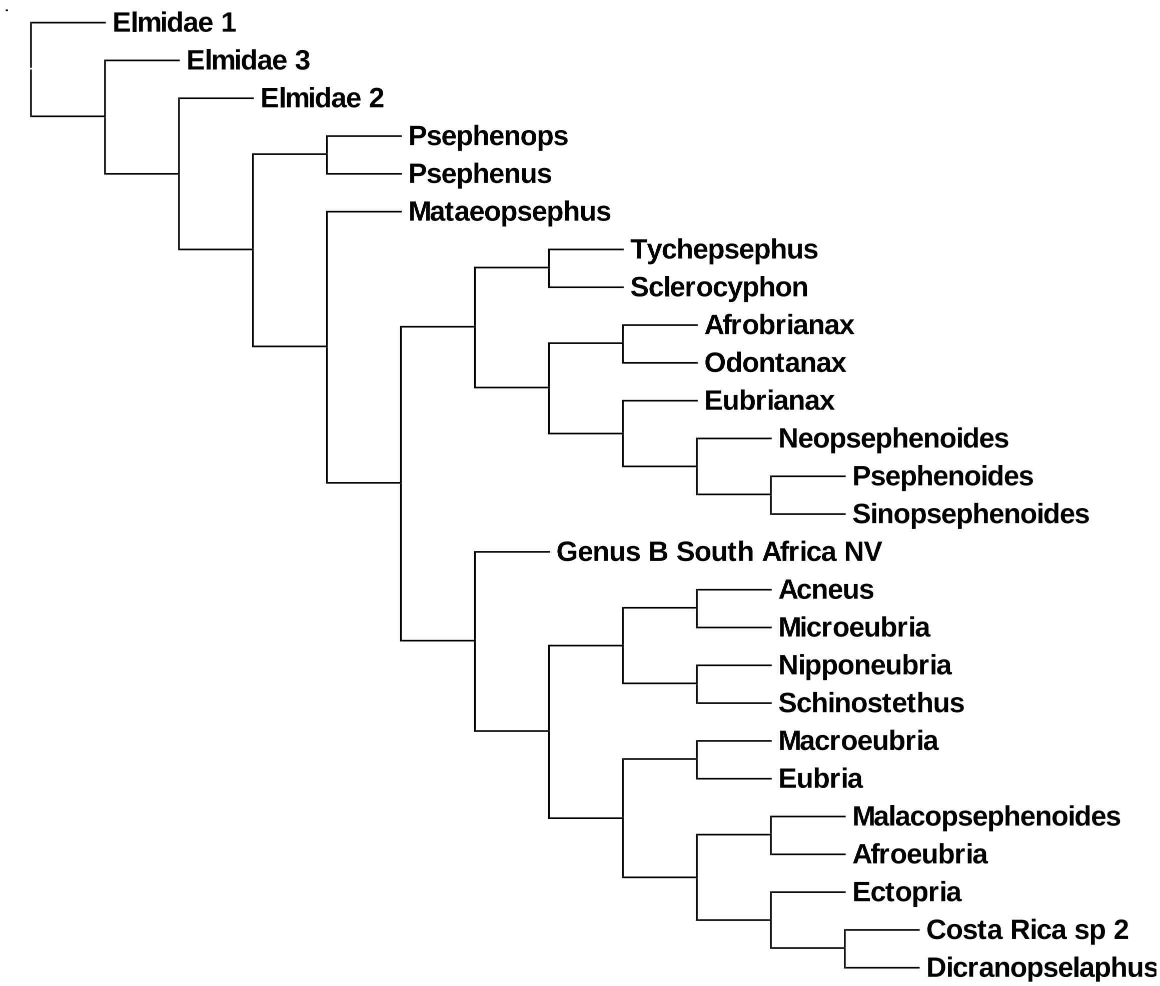


Figure 7: Parsimony analysis of combined molecular (CO1/Wingless) taxa with simplified single genus matrix using sequences from the best amplified taxon when data from more than one species for a genus was available.



When analyzing the updated morphological data set based on Lee et al. (2007- see Fig. 11), the Bayesian (Fig. 8) and parsimony analyses (Fig. 9, strict consensus topology; Fig. 10, majority rules topology) were nearly identical in relationships but included some minor differences. These analyses using morphology support the monophyly of all proposed subfamilies.

Figure 8: Bayesian analysis of morphology

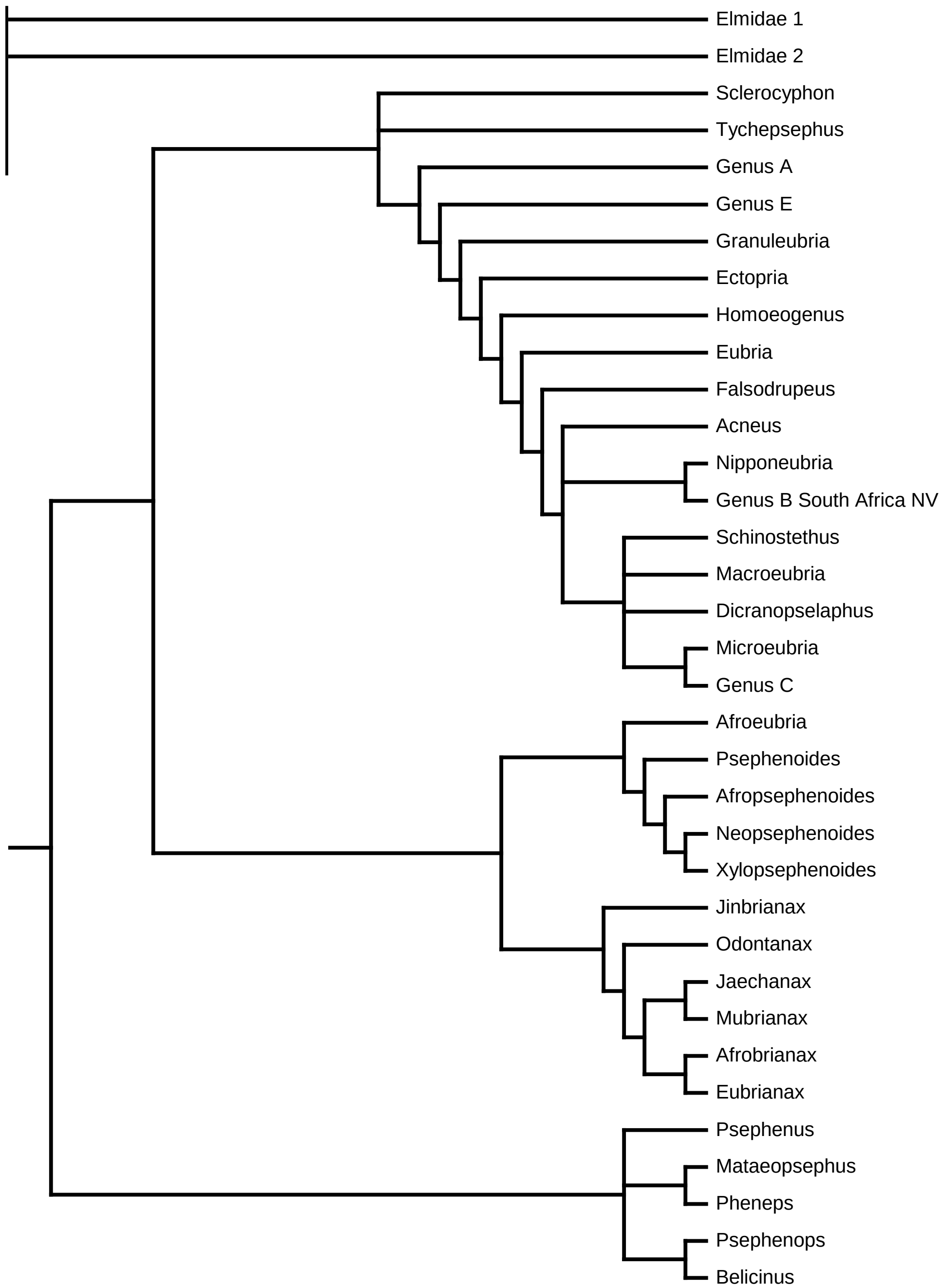


Figure 9: Parsimony analysis of morphology, strict consensus topology of nine trees, with bootstrap values. Two additional clades are not present but are supported by the bootstrap >50% involving *Mataeopsephus* + *Psephenus*, and *Macroeubria* + *Dicranopselaphus*.

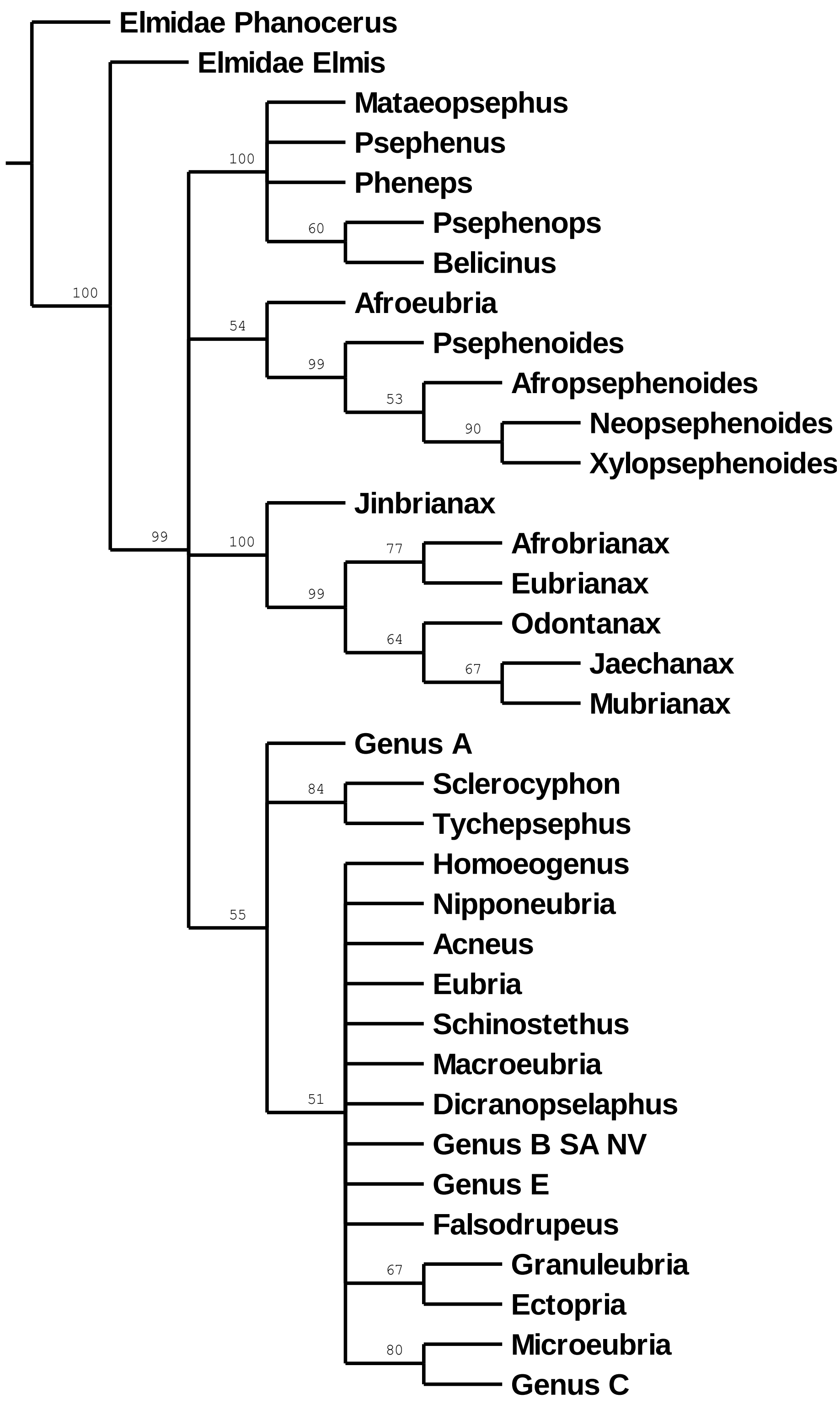


Figure 10: Parsimony analysis of morphology, majority rules consensus topology. Clade values indicate percentage that the clade appears.

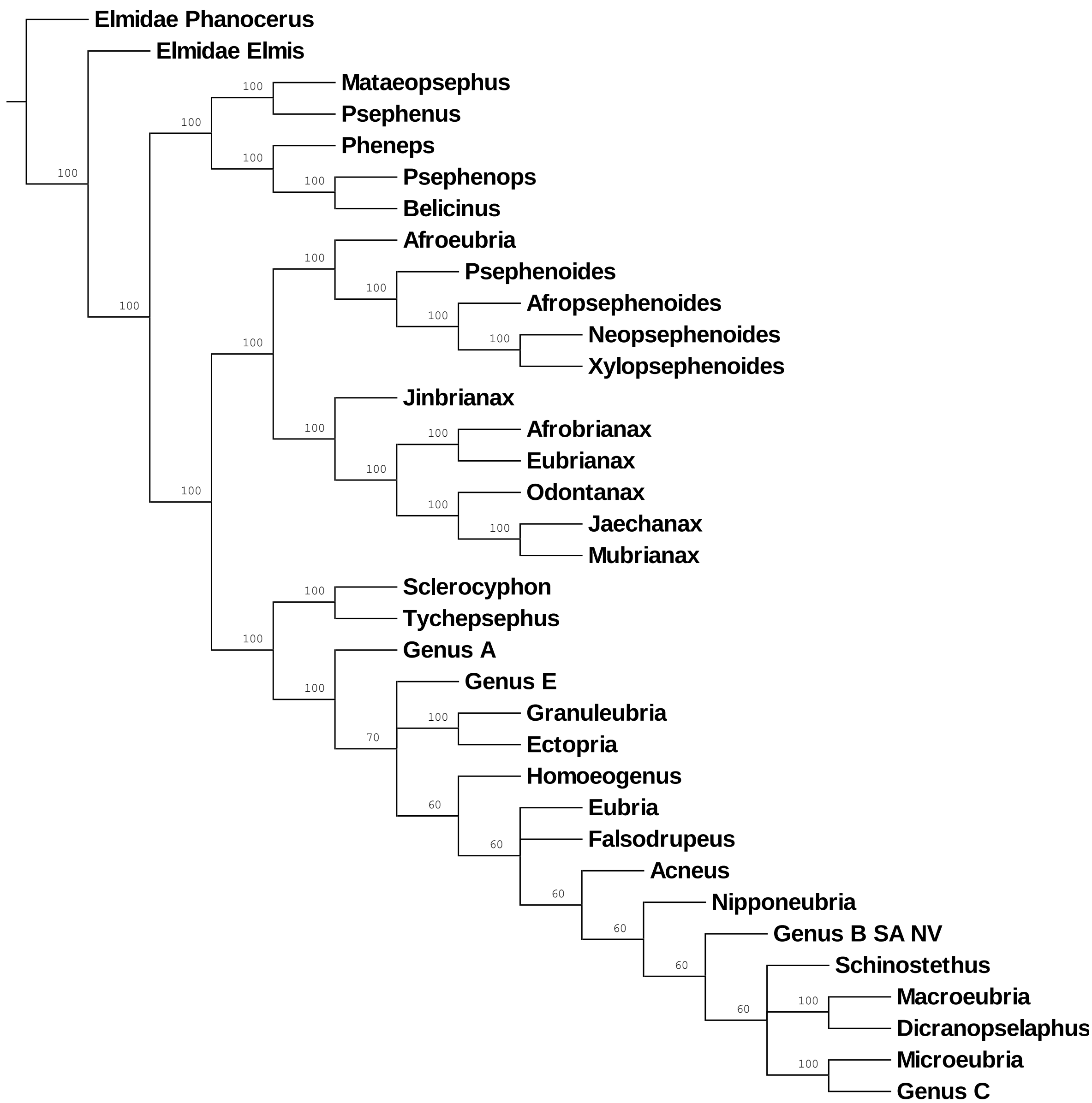


Figure 11: Morphological phylogeny of Lee et al. (2007)

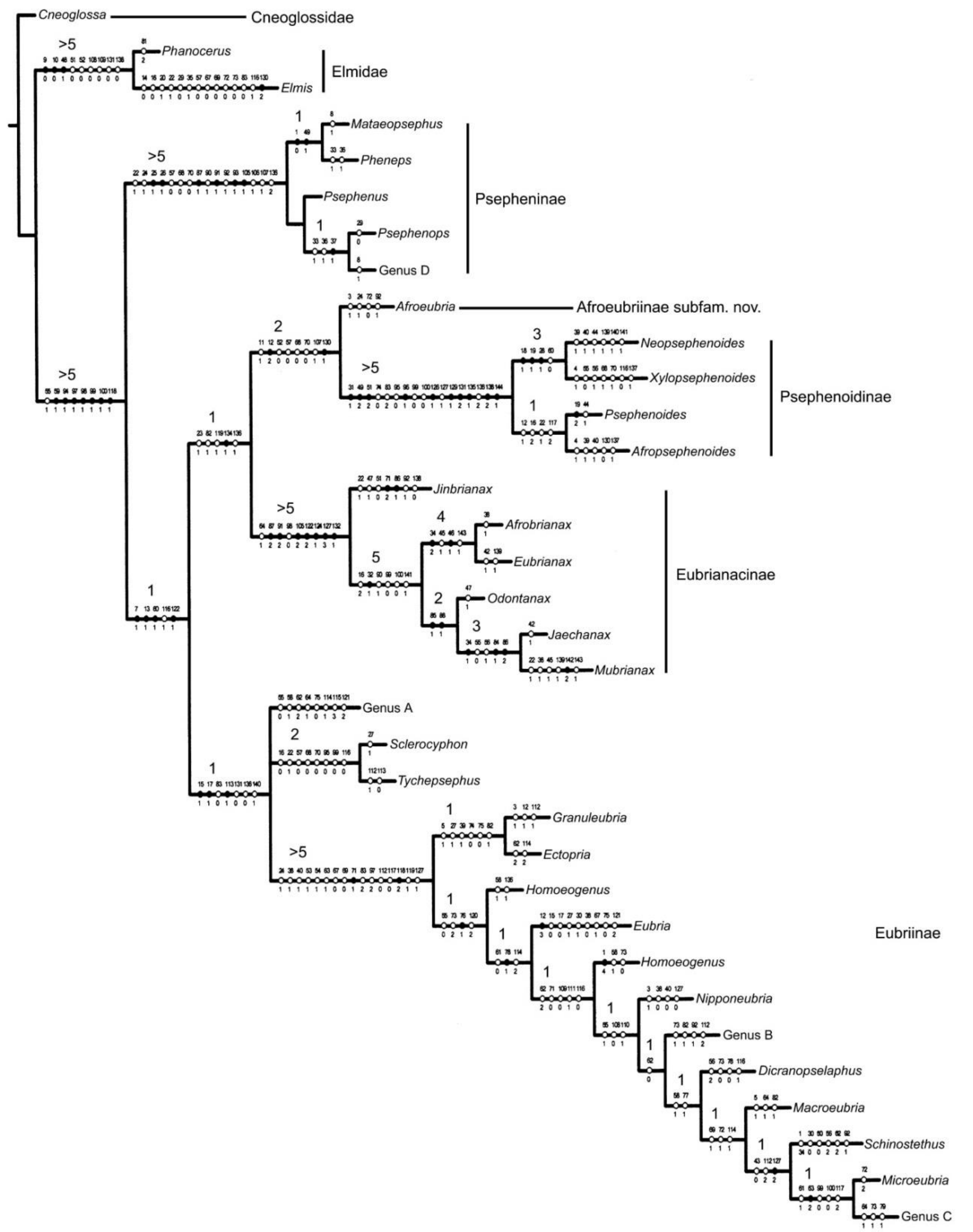


Fig. 15. Strict consensus of two most-parsimonious trees based on the full dataset (439 steps, consistency index = 0.45, retention index = 0.75). Only unambiguous changes are shown on the cladogram. Nonhomoplastic apomorphies are indicated by black circles, whereas homoplastic apomorphies are indicated by white circles. The numbers above the clades are Bremer support values (maximum Bremer support value = 5).

After finding similar relationships using both parsimony and Bayesian analysis, all data was concatenated to hopefully get an even clearer resolution with a total evidence approach (Figs 12-15). Resolution of both analyses was very good with only a single trichotomy in the parsimony, and only two trichotomies in the Bayesian analysis. These trichotomies in general involved taxa that only have a morphological dataset.

Both analyses found the Psepheninae to be monophyletic, but its position slightly altered. In the Bayesian analysis, this clade was sister to all other taxa, but in the parsimony analysis, it was sister to the Psephenoidinae + Eubrianacinae. Both analyses found that Psephenoidinae and Eubrianacinae had identical topologies and a sister relationship. *Falsodrupeus*, Genus E, and *Homoeogenus* shift position sometimes radically within the Eubriinae, but these three were only represented by morphological data. The parsimony analysis shows the Eubriinae supported as monophyletic, but only if you included the proposed Afroeubriinae as part of the larger subfamily. In contrast, the Bayesian analysis shows the Afroeubriinae as sister to all taxa included in the Eubriinae. But when analyzed in both combined molecular data (parsimony and Bayesian) and total evidence parsimony, the Afroeubriinae was well supported as part of the Eubriinae.

Figure 12: Bayesian analysis of total evidence (CO1/Wingless/Morphological) of all taxa. Branch lengths indicate amount of character difference amongst clades. 2,000,000 reps average standard deviation of split freq. = 0.016237 1,000,000 more = 0.011187 500,000 more =0.010000 500,000 more = .008964

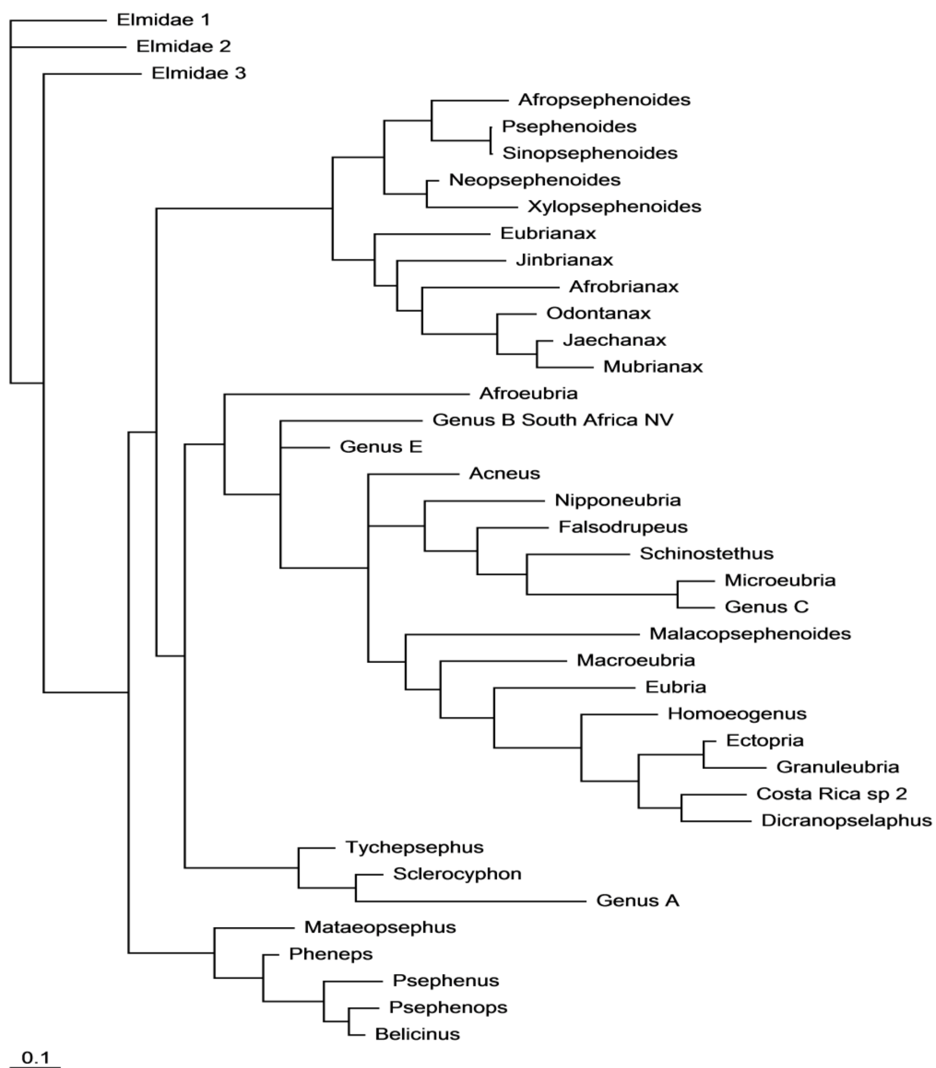


Figure 13: Bayesian analysis of total evidence (CO1/Wingless/Morphological) of taxa representing all genera. 2,000,000 reps average standard deviation of split freq. = 0.016237 1,000,000 more = 0.011187 500,000 more =0.010000 500,000 more = .008964

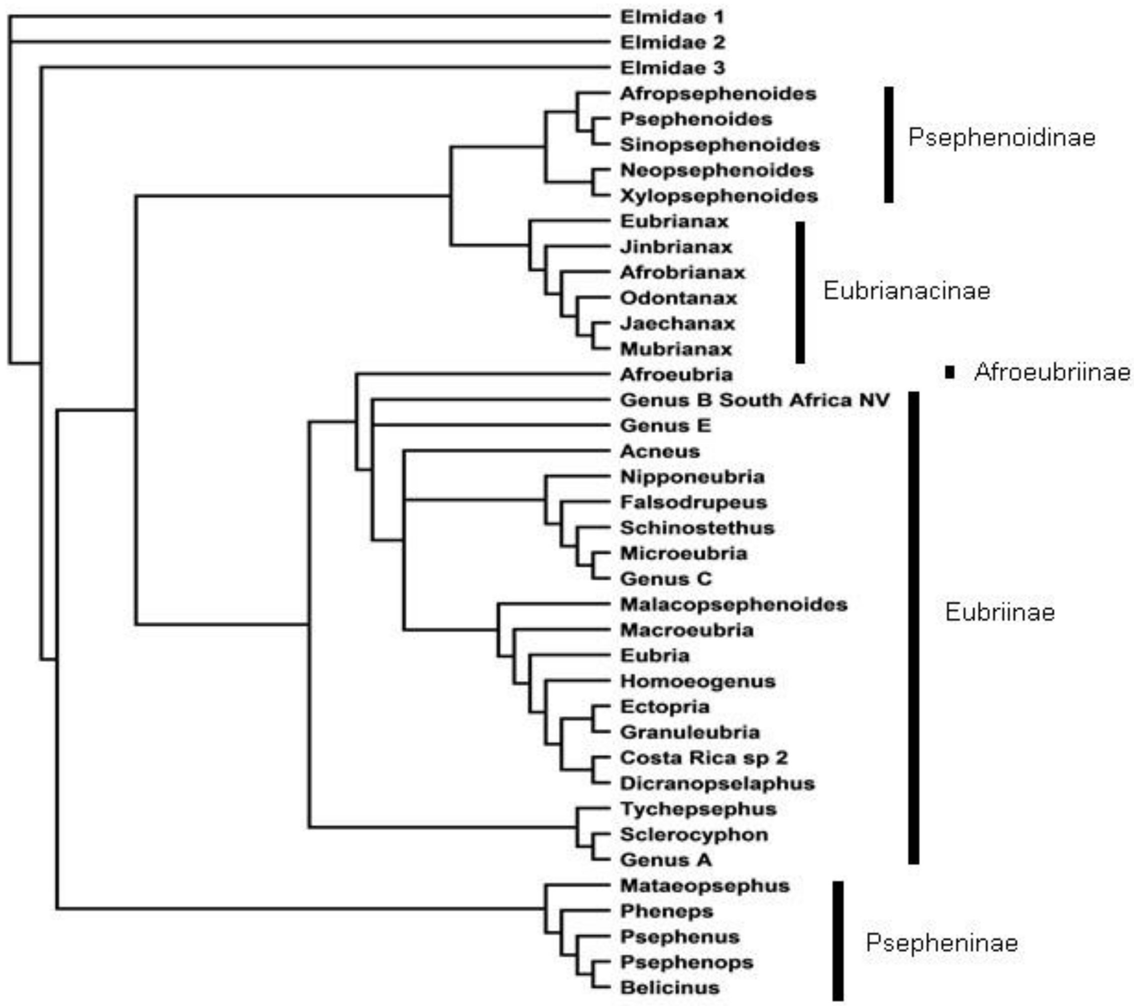


Figure 14: Strict consensus parsimony analysis of total evidence (CO1/Wingless/Morphological) of all taxa. Total characters 1523 (815 not informative), with a total of 708 informative characters. 12 trees, tree length of 3337 steps. 1,000 replication, tree found with 100 replication. CI = 40 RI = 48

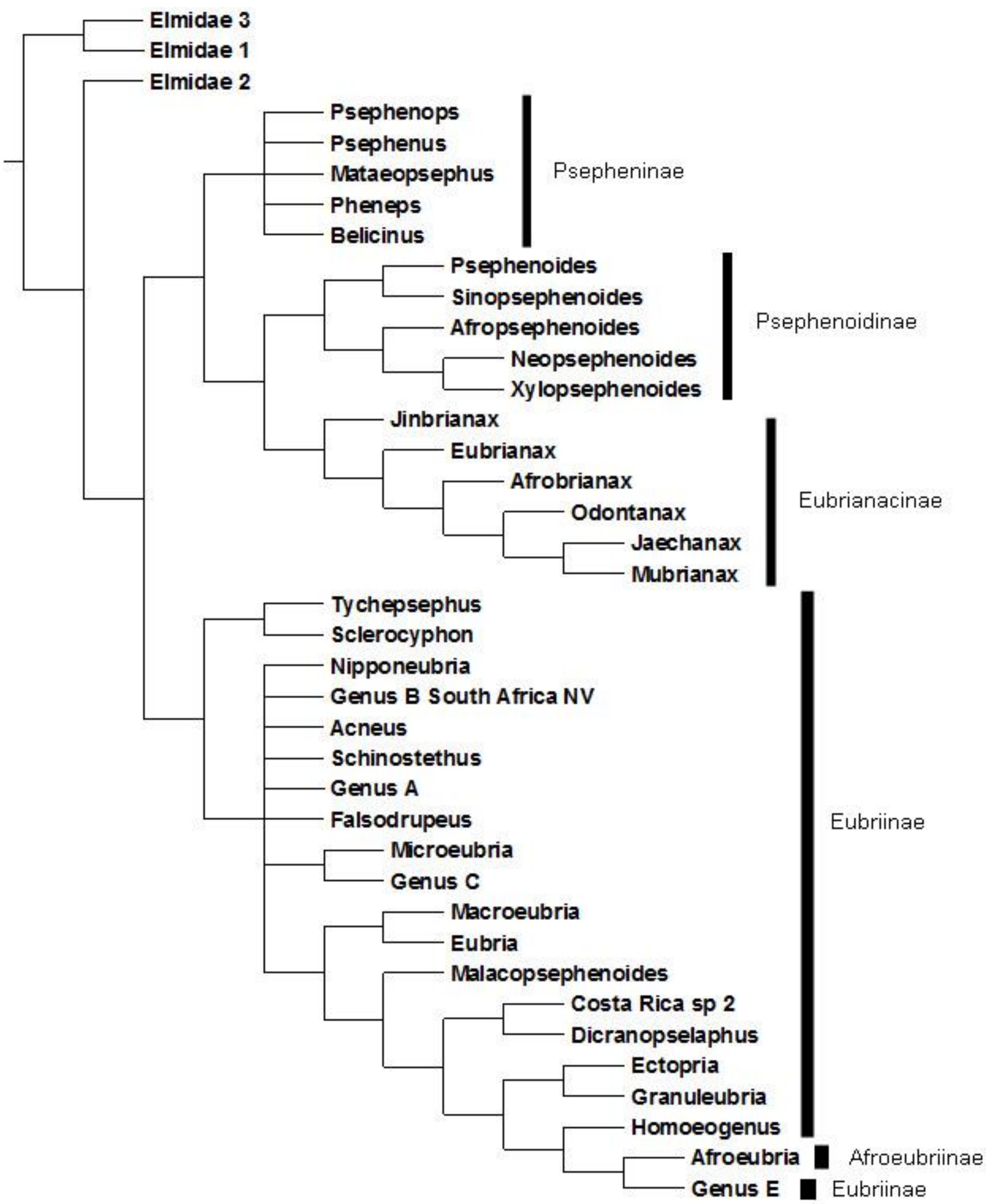


Figure 15: Majority rule parsimony analysis of total evidence (CO1/Wingless/Morphological) of all taxa with bootstrap values. Total characters 523 (815 not informative), with a total of 708 informative characters. 12 trees, tree length of 3337 steps. 1,000 replication, tree found with 100 replication. CI = 40 RI = 48. Numbers indicate the percentage of time each clade was supported

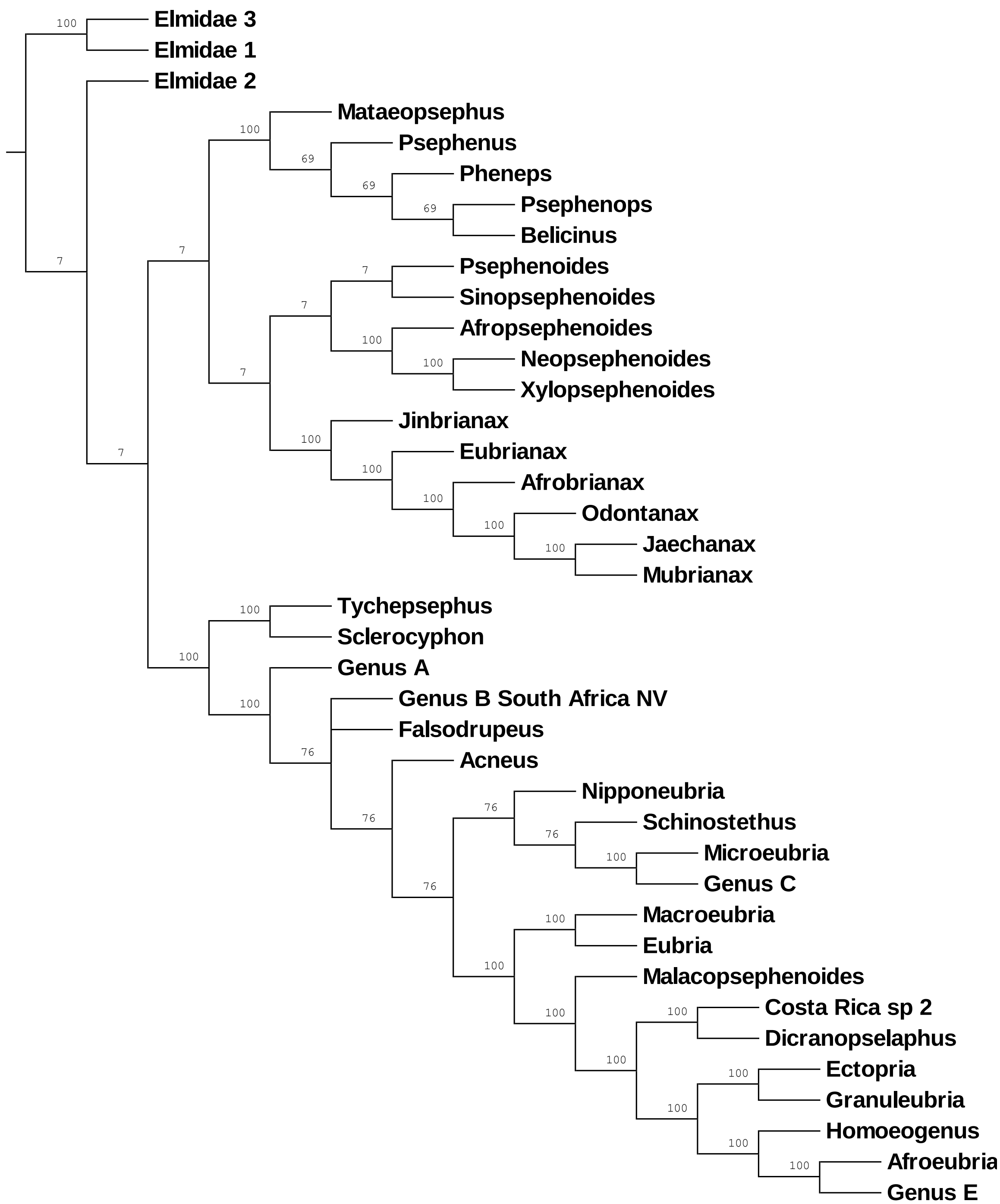


Table 2: This table shows the recognized subfamilies of the psephenids and if support for monophyly was found in the different analyses.

Taxa Analysis	**Psepheninae**	**Psephenoidinae**	**Eubrianacinae**	**Eubriinae**
CO1 Bayesian	Yes	No	No	No
CO1 Parsimony	No	No	No	No
Wingless Bayesian	Yes	N/A	No	No
Wingless Parsimony	Yes	N/A	No	Yes
CO1 + Wingless Bayesian	Yes	No	No	No
CO1 + Wingless Parsimony	Yes	Yes	Yes	No
Morphology Bayesian	Yes	Yes	Yes	Yes
Morphology Parsimony	Yes	Yes	Yes	Yes
Total Evidence Bayesian	Yes	Yes	Yes	Yes
Total Evidence Parsimony	Yes	Yes	Yes	Yes

DISCUSSION

Morphological Data

The analysis of all the evidence analyzed together supports the monophyly of all subfamilies, and was very similar to the topology found earlier by Lee et al. (2007). Some minor differences in relationships of taxa within the Psepheninae and Eubriinae were discovered. The strict consensus parsimony analysis was much less resolved than the tree published in Lee et al. (2007) that showed only one trichotomy in the Eubriinae. In this study, the same trichotomy was found as well as a tetrachotomy in the Psepheninae and a duodecachotomy (12 unresolved branches) within the Eubriinae. Considering that this was virtually the same data set (with only *Acneus*, *Falsodrupeus*, and Genus E added), this result lends credence that the better resolution in Lee et al. (2007) may be due to multistate characters being read accidentally as binary state characters. The topology seen in the majority rule consensus did resolve the basal Eubriinae and Psepheninae trichotomies as well as breaking up the duodecachotomy within the same Eubriinae subfamily, leaving only three minor trichotomies in the Eubriinae. Similarly the Bayesian topology shows a trichotomy in Psepheninae and Eubriinae as well as a tetrachotomy within the Eubriinae.

Combined Molecular

Although the morphological data set supported all subfamilies as monophyletic, the combined molecular data conflicted with the morphological data in several important ways. Parsimony analysis found the Psepheninae to be paraphyletic. Also the sister genera *Sclerocyphon* and *Tychepsephus* are positioned as the sister clade to the Eubrianacinae instead of at the base and possible sister clade to the Eubriinae subfamily as seen in Lee et al. (2007) (Fig. 11). Finally the proposed Afroeubriinae was located deep within the Eubriinae and hence not justifying its recognition as a subfamily. In the Bayesian analysis the Psepheninae are monophyletic. *Sclerocyphon* + *Tychepsephus* is sister to the Eubriinae subfamily similar to the Lee et al. (2007) topology. *Afroeubria* was also positioned relatively deep within the Eubriinae subfamily casting further doubt on the valid status of this subfamily.

Total Evidence

Based on total evidence parsimony analysis, all subfamilies were monophyletic with the exception of Afroeubriinae. All molecular data, either single gene or combined, also did not support *Afroeubria* as representing a valid subfamily. It was possible that this is due to the lack of wingless data, but the evidence herein supports the placement of *Afroeubria* in the Eubriinae. Even in the Bayesian analysis, which supported Afroeubriinae and did not create paraphyly in the Eubriinae, its placement was radically different as sister to the Psephenoidinae compared to that seen Lee et al. (2007) and morphological analyses herein. In contrast, *Afroeubria* in all of the molecular analyses was found to be either sister to or as part of the Eubriinae subfamily.

The close molecular and morphological relationship between the *Tychepsephus* and *Sclerocyphon* genera may warrant the creation of a new subfamily. This is strongly supported by the molecular data and the parsimony analysis of the morphological data. The Bayesian morphological analysis, with a basal trichotomy composed of these two genera and the remaining Eubriinae, still indicates a potential sister relationship between these two as well as a sister relationship between this pair and the Eubriinae. The sister relationship is also supported by the total evidence analysis in both analyses although in the Bayesian topology Genus A is placed within this proposed subfamily. This placement may be due to the effects of morphological convergence and the lack of any molecular data. Lastly, the genus *Malacopsephenoides* is positioned within the Eubriinae, even though it was thought to be part of the Psephenoidinae (see Jeng 2006). Although no morphological data on this genus was included in this study, all molecular evidence points to a needed reclassification.

Conclusion

These results do not support *Afroeubria* as a separate subfamily, do support the creation of a new subfamily based on *Tychepsephus* and *Sclerocyphon*, and also support the placement of *Malacopsephenoides* in the Eubriinae.

APPENDIX A:

Psephenidae: Morphology Data: Bayesian Analysis Log File

```
 Logging screen output to file "psephenidmorph.txt"
  Expecting  command

MrBayes >

Execute morph2.nex
lset rates=gamma coding=variable;
prset symdirihyperpr=fixed(infinity) ratepr=variable;

  Setting number of generations to 100000
  Running Markov chain
  MCMC stamp = 0833568490
  Seed = 768075111
  Swapseed = 1449848257
  Model settings:

    Data not partitioned --
      Datatype  = Standard
      Coding    = Variable
      # States  = Variable, up to 10
              State frequencies are fixed to be equal
      Rates     = Gamma
              Gamma shape parameter is uniformly dist-
              ributed on the interval (0.00,200.00).
              Gamma distribution is approximated using 4 categories.
              Likelihood summarized over all rate categories in each generation.

  Active parameters:

    Parameters
    ------------------
    Statefreq        1
    Shape            2
    Ratemultiplier   3
    Topology         4
    Brlens           5
    ------------------

    1 --  Parameter  = Alpha_symdir
```

```
      Type      = Symmetric diricihlet/beta distribution alpha_i parameter
      Prior     = Symmetric dirichlet with fixed(-1.00) variance parameter

   2 --  Parameter  = Alpha
      Type      = Shape of scaled gamma distribution of site rates
      Prior     = Uniform(0.00,200.00)

   3 --  Parameter  = Ratemultiplier
      Type      = Partition-specific rate multiplier
      Prior     = Fixed(1.0)

   4 --  Parameter  = Tau
      Type      = Topology
      Prior     = All topologies equally probable a priori
      Subparam.  = V

   5 --  Parameter  = V
      Type      = Branch lengths
      Prior     = Unconstrained:Exponential(10.0)


Number of taxa = 35
Number of characters = 143

The MCMC sampler will use the following moves:
   With prob.  Chain will use move
      2.13 %   Multiplier(Alpha)
      1.06 %   Dirichlet(Ratemultiplier)
      1.06 %   Slider(Ratemultiplier)
     10.64 %   ExtSPR(Tau,V)
     10.64 %   ExtTBR(Tau,V)
     10.64 %   NNI(Tau,V)
     10.64 %   ParsSPR(Tau,V)
     42.55 %   Multiplier(V)
     10.64 %   Nodeslider(V)

Division 1 has 135 unique site patterns
Initializing conditional likelihoods
Using standard non-SSE likelihood calculator for division 1 (single-precision)

Initial log likelihoods and log prior probs for run 1:
   Chain 1 -- -3097.944323 -- -23.629033
   Chain 2 -- -3298.642116 -- -23.629033
   Chain 3 -- -3114.634383 -- -23.629033
   Chain 4 -- -3159.011297 -- -23.629033
```

```
   Initial log likelihoods and log prior probs for run 2:
      Chain 1 -- -3206.499839 -- -23.629033
      Chain 2 -- -3153.801311 -- -23.629033
      Chain 3 -- -3243.115546 -- -23.629033
      Chain 4 -- -3268.312957 -- -23.629033


   Using a relative burnin of 25.0 % for diagnostics

     500000 -- (-1721.196) (-1728.533) (-1731.637) [-1709.084] * (-1741.014) (-1716.974) [-1723.791] (-1734.004) -- 0:00:00

   Average standard deviation of split frequencies: 0.011153

   Continue with analysis? (yes/no):
   Analysis completed in 17 mins 11 seconds
   Analysis used 1032.19 seconds of CPU time
   Likelihood of best state for "cold" chain of run 1 was -1700.83
   Likelihood of best state for "cold" chain of run 2 was -1701.40

   Acceptance rates for the moves in the "cold" chain of run 1:
      With prob.   (last 100)   chain accepted proposals by move
         58.5 %     ( 35 %)     Multiplier(Alpha)
        100.0 %     (100 %)     Dirichlet(Ratemultiplier)
         85.7 %     ( 85 %)     Slider(Ratemultiplier)
         12.5 %     (  7 %)     ExtSPR(Tau,V)
          4.0 %     (  4 %)     ExtTBR(Tau,V)
         16.0 %     ( 14 %)     NNI(Tau,V)
          8.6 %     ( 11 %)     ParsSPR(Tau,V)
         27.4 %     ( 29 %)     Multiplier(V)
         45.5 %     ( 47 %)     Nodeslider(V)

   Acceptance rates for the moves in the "cold" chain of run 2:
      With prob.   (last 100)   chain accepted proposals by move
         59.8 %     ( 37 %)     Multiplier(Alpha)
        100.0 %     (100 %)     Dirichlet(Ratemultiplier)
         84.7 %     ( 85 %)     Slider(Ratemultiplier)
         12.6 %     (  9 %)     ExtSPR(Tau,V)
          4.0 %     (  2 %)     ExtTBR(Tau,V)
         16.2 %     ( 10 %)     NNI(Tau,V)
          8.5 %     ( 12 %)     ParsSPR(Tau,V)
         27.6 %     ( 27 %)     Multiplier(V)
         45.6 %     ( 44 %)     Nodeslider(V)

   Chain swap information for run 1:
```

```
           1      2      3      4
     ------------------------------
   1 |         0.47   0.18   0.05
   2 |  83083         0.53   0.23
   3 |  83597  83484         0.56
   4 |  83195  83338  83303
```

Chain swap information for run 2:

```
           1      2      3      4
     ------------------------------
   1 |         0.49   0.19   0.06
   2 |  83359         0.53   0.23
   3 |  83663  83111         0.56
   4 |  83102  83332  83433
```

Upper diagonal: Proportion of successful state exchanges between chains
Lower diagonal: Number of attempted state exchanges between chains

Chain information:

```
  ID -- Heat
 -----------
   1 -- 1.00  (cold chain)
   2 -- 0.91
   3 -- 0.83
   4 -- 0.77
```

Heat = 1 / (1 + T * (ID - 1))
  (where T = 0.10 is the temperature and ID is the chain number)

MrBayes >
  Summarizing parameters in files morph2.nex.run1.p and morph2.nex.run2.p
  Writing summary statistics to file morph2.nex.pstat
  Using relative burnin ('relburnin=yes'), discarding the first 25 % of samples

Below are rough plots of the generation (x-axis) versus the log
probability of observing the data (y-axis). You can use these
graphs to determine what the burn in for your analysis should be.
When the log probability starts to plateau you may be at station-
arity. Sample trees and parameters after the log probability
plateaus. Of course, this is not a guarantee that you are at sta-
tionarity. Also examine the convergence diagnostics provided by
the 'sump' and 'sumt' commands for all the parameters in your
model. Remember that the burn in is the number of samples to dis-

card. There are a total of ngen / samplefreq samples taken during
a MCMC analysis.

Overlay plot for both runs:
(1 = Run number 1; 2 = Run number 2; * = Both runs)

```
+------------------------------------------------------------+ -1715.81
|    1                      2                      2       1 |
|                      1             1  1                   |
|2           1            11    *                           1|
|       1         1                          1              |
| 2  2 2 2                               1  2    1  2      2|
|    1   2 2           *    11          2           1 12 2    2 |
|  *2   2 1   1  12 1            1  21  1  *  2  11     1       |
|1  1        1   12   2       2  1 2   1          1            |
|    2 1  1   2   *2 *     2 1            2  *  22222    1 2    |
|                      2             22               212  |
|           22            2      2  1         2              1  |
|     1   2   1               2 12      1             1        |
| 1              2     1  2          1           1          1    |
|                                                         |
|                                   22                      |
+------+-----+-----+-----+-----+-----+-----+-----+-----+-----+ -1726.99
^                                                 ^
125000                                            500000
```

Estimated marginal likelihoods for runs sampled in files
"morph2.nex.run1.p" and "morph2.nex.run2.p":
(Use the harmonic mean for Bayes factor comparisons of models)

(Values are saved to the file morph2.nex.lstat)

```
Run   Arithmetic mean   Harmonic mean
--------------------------------------
  1     -1710.63          -1734.74
  2     -1711.09          -1738.00
--------------------------------------
TOTAL    -1710.83          -1737.35
--------------------------------------
```

Model parameter summaries over the runs sampled in files
"morph2.nex.run1.p" and "morph2.nex.run2.p":
Summaries are based on a total of 1502 samples from 2 runs.
Each run produced 1001 samples of which 751 samples were included.

Parameter summaries saved to file "morph2.nex.pstat".

			95% HPD Interval					
Parameter	Mean	Variance	Lower	Upper	Median	min ESS*	avg ESS	PSRF+
TL	6.776034	0.694031	5.163153	8.393770	6.724521	49.16	89.48	1.000
alpha	1.940332	0.790213	0.557183	3.552381	1.786240	315.43	394.16	1.001
m{1}	0.622347	0.011000	0.424533	0.829359	0.619329	30.81	71.57	1.002

* Convergence diagnostic (ESS = Estimated Sample Size); min and avg values
correspond to minimal and average ESS among runs.
ESS value below 100 may indicate that the parameter is undersampled.
\+ Convergence diagnostic (PSRF = Potential Scale Reduction Factor; Gelman
and Rubin, 1992) should approach 1.0 as runs converge.

MrBayes >
Summarizing trees in files "morph2.nex.run1.t" and "morph2.nex.run2.t"
Using relative burnin ('relburnin=yes'), discarding the first 25 % of sampled trees
Writing statistics to files morph2.nex.<parts|tstat|vstat|trprobs|con>
Examining first file ...
Found one tree block in file "morph2.nex.run1.t" with 1001 trees in last block
Expecting the same number of trees in the last tree block of all files

Tree reading status:

```
0      10      20      30      40      50      60      70      80      90     100
v-------v-------v-------v-------v-------v-------v-------v-------v-------v-------v
```

```
********************************************************************************
```

Read a total of 2002 trees in 2 files (sampling 1502 of them)
(Each file contained 1001 trees of which 751 were sampled)

General explanation:

In an unrooted tree, a taxon bipartition (split) is specified by removing a branch, thereby dividing the species into those to the left and those to the right of the branch. Here, taxa to one side of the removed branch are denoted

'.' and those to the other side are denoted '*'. Specifically, the '.' symbol is used for the taxa on the same side as the outgroup.

In a rooted or clock tree, the tree is rooted using the model and not by reference to an outgroup. Each bipartition therefore corresponds to a clade, that is, a group that includes all the descendants of a particular branch in the tree. Taxa that are included in each clade are denoted using '*', and taxa that are not included are denoted using the '.' symbol.

The output first includes a key to all the bipartitions with frequency larger or equual to (Minpartfreq) in at least one run. Minpartfreq is a paramiter to sumt command and currently it is set to 0.10. This is followed by a table with statistics for the informative bipartitions (those including at least two taxa), sorted from highest to lowest probability. For each bipartition, the table gives the number of times the partition or split was observed in all runs (#obs) and the posterior probability of the bipartition (Probab.), which is the same as the split frequency. If several runs are summarized, this is followed by the minimum split frequency (Min(s)), the maximum frequency (Max(s)), and the standard deviation of frequencies (Stddev(s)) across runs. The latter value should approach 0 for all bipartitions as MCMC runs converge.

This is followed by a table summarizing branch lengths, node heights (if a clock model was used) and relaxed clock parameters (if a relaxed clock model was used). The mean, variance, and 95 % credible interval are given for each of these parameters. If several runs are summarized, the potential scale reduction factor (PSRF) is also given; it should approach 1 as runs converge. Node heights will take calibration points into account, if such points were used in the analysis.

Note that Stddev may be unreliable if the partition is not present in all runs (the last column indicates the number of runs that sampled the partition if more than one run is summarized). The PSRF is not calculated at all if the partition is not present in all runs.The PSRF is also sensitive to small sample sizes and it should only be considered a rough guide to convergence since some of the assumptions allowing one to interpret it as a true potential scale reduction factor are violated in MrBayes.

```
Summary statistics for informative taxon bipartitions
  (saved to file "morph2.nex.tstat"):

ID   #obs    Probab.     Sd(s)+      Min(s)      Max(s)   Nruns
----------------------------------------------------------------
36  1502    1.000000    0.000000    1.000000    1.000000    2
37  1502    1.000000    0.000000    1.000000    1.000000    2
38  1502    1.000000    0.000000    1.000000    1.000000    2
```

39	1502	1.000000	0.000000	1.000000	1.000000	2
40	1502	1.000000	0.000000	1.000000	1.000000	2
41	1502	1.000000	0.000000	1.000000	1.000000	2
42	1500	0.998668	0.001883	0.997337	1.000000	2
43	1476	0.982690	0.007532	0.977364	0.988016	2
44	1461	0.972703	0.016006	0.961385	0.984021	2
45	1460	0.972037	0.001883	0.970706	0.973369	2
46	1381	0.919441	0.002825	0.917443	0.921438	2
47	1316	0.876165	0.001883	0.874834	0.877497	2
48	1282	0.853529	0.015065	0.842876	0.864181	2
49	1199	0.798269	0.002825	0.796272	0.800266	2
50	1162	0.773635	0.000000	0.773635	0.773635	2
51	1121	0.746338	0.008474	0.740346	0.752330	2
52	1059	0.705060	0.000942	0.704394	0.705726	2
53	1057	0.703728	0.008474	0.697736	0.709720	2
54	1005	0.669108	0.029188	0.648469	0.689747	2
55	997	0.663782	0.004708	0.660453	0.667111	2
56	904	0.601864	0.003766	0.599201	0.604527	2
57	857	0.570573	0.004708	0.567244	0.573901	2
58	848	0.564581	0.015065	0.553928	0.575233	2
59	839	0.558589	0.025422	0.540613	0.576565	2
60	788	0.524634	0.000000	0.524634	0.524634	2
61	775	0.515979	0.002825	0.513981	0.517976	2
62	764	0.508655	0.030130	0.487350	0.529960	2
63	714	0.475366	0.000000	0.475366	0.475366	2
64	710	0.472703	0.007532	0.467377	0.478029	2
65	698	0.464714	0.000000	0.464714	0.464714	2
66	698	0.464714	0.003766	0.462051	0.467377	2
67	677	0.450732	0.034837	0.426099	0.475366	2
68	602	0.400799	0.003766	0.398136	0.403462	2
69	556	0.370173	0.030130	0.348868	0.391478	2
70	555	0.369507	0.010357	0.362184	0.376831	2
71	543	0.361518	0.019773	0.347537	0.375499	2
72	541	0.360186	0.023539	0.343542	0.376831	2
73	458	0.304927	0.013182	0.295606	0.314248	2
74	443	0.294940	0.027305	0.275632	0.314248	2
75	359	0.239015	0.063084	0.194407	0.283622	2
76	355	0.236352	0.049902	0.201065	0.271638	2
77	316	0.210386	0.000000	0.210386	0.210386	2
78	316	0.210386	0.045195	0.178429	0.242344	2
79	288	0.191744	0.000000	0.191744	0.191744	2
80	273	0.181758	0.010357	0.174434	0.189081	2
81	262	0.174434	0.013182	0.165113	0.183755	2
82	243	0.161784	0.014123	0.151798	0.171771	2
83	237	0.157790	0.014123	0.147803	0.167776	2
84	226	0.150466	0.018831	0.137150	0.163782	2

85	213	0.141811	0.004708	0.138482	0.145140	2
86	207	0.137816	0.012240	0.129161	0.146471	2
87	206	0.137150	0.001883	0.135819	0.138482	2
88	204	0.135819	0.001883	0.134487	0.137150	2
89	186	0.123835	0.007532	0.118509	0.129161	2
90	165	0.109854	0.012240	0.101198	0.118509	2
91	163	0.108522	0.014123	0.098535	0.118509	2
92	152	0.101198	0.001883	0.099867	0.102530	2
93	148	0.098535	0.003766	0.095872	0.101198	2

\+ Convergence diagnostic (standard deviation of split frequencies)
should approach 0.0 as runs converge.

Summary statistics for branch and node parameters
(saved to file "morph2.nex.vstat"):

			95% HPD Interval				
Parameter	Mean	Variance	Lower	Upper	Median	PSRF+	Nruns
length[1]	0.046192	0.001314	0.000016	0.113692	0.037656	1.000	2
length[2]	0.091479	0.001948	0.008053	0.176932	0.087183	0.999	2
length[3]	0.019976	0.000354	0.000038	0.057264	0.014742	0.999	2
length[4]	0.038661	0.000845	0.000015	0.095661	0.033301	0.999	2
length[5]	0.086362	0.004281	0.000068	0.205878	0.073439	0.999	2
length[6]	0.041539	0.001067	0.000346	0.108155	0.033536	1.000	2
length[7]	0.025821	0.000555	0.000002	0.071288	0.018361	1.000	2
length[8]	0.134126	0.003001	0.027269	0.235398	0.127700	1.004	2
length[9]	0.034297	0.000826	0.000028	0.089900	0.025972	0.999	2
length[10]	0.045299	0.001147	0.000070	0.109428	0.037499	0.999	2
length[11]	0.137081	0.003073	0.033716	0.241329	0.129735	1.002	2
length[12]	0.072562	0.001856	0.001500	0.151825	0.064982	1.002	2
length[13]	0.084924	0.001777	0.012690	0.170246	0.076635	1.001	2
length[14]	0.049621	0.001339	0.000268	0.119706	0.041918	1.001	2
length[15]	0.047600	0.000888	0.000075	0.102557	0.042708	1.001	2
length[16]	0.032757	0.000829	0.000014	0.088405	0.025615	1.000	2
length[17]	0.027634	0.000646	0.000005	0.076568	0.020902	0.999	2
length[18]	0.095709	0.002143	0.017701	0.193745	0.089258	1.001	2
length[19]	0.151066	0.002733	0.057537	0.253650	0.147366	1.007	2
length[20]	0.050411	0.001678	0.000024	0.130436	0.040049	1.000	2
length[21]	0.023845	0.000374	0.000066	0.062996	0.019225	1.002	2
length[22]	0.028710	0.000487	0.000187	0.071424	0.023518	1.000	2
length[23]	0.069249	0.001566	0.000019	0.142050	0.064540	1.000	2
length[24]	0.027292	0.000495	0.000057	0.069572	0.021508	1.001	2
length[25]	0.031323	0.000428	0.000071	0.070393	0.027189	1.000	2

length[26]	0.040396	0.000626	0.001678	0.090258	0.035615	1.001	2
length[27]	0.011434	0.000127	0.000001	0.033947	0.007976	0.999	2
length[28]	0.045204	0.000710	0.000225	0.091288	0.041854	1.000	2
length[29]	0.030957	0.000454	0.000499	0.074126	0.026085	0.999	2
length[30]	0.026916	0.000408	0.000057	0.067884	0.021476	1.000	2
length[31]	0.209121	0.014224	0.005778	0.429556	0.199477	1.001	2
length[32]	0.123183	0.003303	0.018077	0.239029	0.114850	1.001	2
length[33]	0.069925	0.002226	0.000260	0.154065	0.060071	1.000	2
length[34]	0.080119	0.004203	0.000029	0.210978	0.065345	1.000	2
length[35]	0.144029	0.006111	0.005830	0.294046	0.132108	0.999	2
length[36]	0.378916	0.012628	0.192596	0.614882	0.368648	1.000	2
length[37]	0.165537	0.003862	0.044328	0.282738	0.158436	1.008	2
length[38]	0.284939	0.010029	0.118361	0.489625	0.270654	1.001	2
length[39]	0.490433	0.015098	0.262350	0.726993	0.481883	0.999	2
length[40]	0.130977	0.002391	0.045643	0.232395	0.125526	0.999	2
length[41]	0.471408	0.014792	0.265588	0.719826	0.454193	1.001	2
length[42]	0.090494	0.001629	0.021397	0.164845	0.085260	1.000	2
length[43]	0.217618	0.007682	0.060195	0.390158	0.205670	1.000	2
length[44]	0.202923	0.009231	0.027780	0.396138	0.193840	0.999	2
length[45]	0.187713	0.004682	0.063051	0.335956	0.185456	1.000	2
length[46]	0.082113	0.001640	0.013369	0.158695	0.075762	0.999	2
length[47]	0.040932	0.000776	0.001751	0.097231	0.034814	0.999	2
length[48]	0.165653	0.007362	0.013116	0.326867	0.158726	1.001	2
length[49]	0.201494	0.010042	0.016141	0.386519	0.190452	1.006	2
length[50]	0.132452	0.003084	0.040614	0.241229	0.126520	1.001	2
length[51]	0.211344	0.014594	0.000343	0.429277	0.197880	0.999	2
length[52]	0.074662	0.001444	0.006539	0.146797	0.068796	1.001	2
length[53]	0.127784	0.005365	0.001394	0.255780	0.117590	0.999	2
length[54]	0.123477	0.002897	0.010227	0.218135	0.117773	1.007	2
length[55]	0.050233	0.001122	0.000006	0.114826	0.044223	1.000	2
length[56]	0.062082	0.001221	0.002822	0.125248	0.056832	1.000	2
length[57]	0.042440	0.000575	0.004149	0.088910	0.038255	1.000	2
length[58]	0.104005	0.003055	0.000217	0.198971	0.098707	0.999	2
length[59]	0.077335	0.002658	0.000731	0.176103	0.069595	1.001	2
length[60]	0.068106	0.001056	0.014930	0.130883	0.063376	1.003	2
length[61]	0.116945	0.002715	0.021576	0.219684	0.112842	1.000	2
length[62]	0.106124	0.004815	0.000137	0.233698	0.097445	0.999	2
length[63]	0.064997	0.000934	0.012054	0.121805	0.060970	0.999	2
length[64]	0.083545	0.003147	0.000416	0.193262	0.073312	0.999	2
length[65]	0.044787	0.000866	0.001132	0.103101	0.040385	0.999	2
length[66]	0.061743	0.001174	0.002953	0.127872	0.055843	0.999	2
length[67]	0.051136	0.001173	0.000245	0.116041	0.044604	1.000	2
length[68]	0.048819	0.000721	0.011697	0.109030	0.045138	0.999	2
length[69]	0.063458	0.001430	0.000699	0.132150	0.058473	0.999	2
length[70]	0.042168	0.000791	0.000470	0.094891	0.037067	1.001	2
length[71]	0.021190	0.000406	0.000023	0.059704	0.015619	1.004	2

length[72]	0.102928	0.005568	0.000344	0.243559	0.088350	0.998	2
length[73]	0.112340	0.003061	0.009224	0.210072	0.106076	1.004	2
length[74]	0.054846	0.001010	0.002208	0.112551	0.049796	0.998	2
length[75]	0.044261	0.000868	0.000475	0.102967	0.039047	0.998	2
length[76]	0.056263	0.001242	0.000342	0.118070	0.050539	0.998	2
length[77]	0.063282	0.001685	0.000162	0.137036	0.055815	1.026	2
length[78]	0.072653	0.001664	0.002153	0.140361	0.067159	1.001	2
length[79]	0.019512	0.000328	0.000108	0.055555	0.014140	1.013	2
length[80]	0.015226	0.000259	0.000019	0.047872	0.010156	1.017	2
length[81]	0.102397	0.004397	0.000799	0.230426	0.092034	0.996	2
length[82]	0.108545	0.004275	0.001215	0.228846	0.102375	1.017	2
length[83]	0.110511	0.004389	0.003423	0.229292	0.106913	1.010	2
length[84]	0.066965	0.001864	0.001083	0.153134	0.059679	1.007	2
length[85]	0.018586	0.000303	0.000126	0.052693	0.013470	1.003	2
length[86]	0.073700	0.002532	0.001283	0.161819	0.059129	1.042	2
length[87]	0.097279	0.004066	0.002038	0.203863	0.094911	1.016	2
length[88]	0.020909	0.000370	0.000164	0.058530	0.015259	0.998	2
length[89]	0.204120	0.011096	0.042519	0.409206	0.195092	0.998	2
length[90]	0.031432	0.000605	0.000371	0.082134	0.024442	0.996	2
length[91]	0.066755	0.001802	0.000051	0.148921	0.061010	0.998	2
length[92]	0.024460	0.000313	0.000013	0.051725	0.020355	0.995	2
length[93]	0.025751	0.000552	0.000130	0.083846	0.019287	1.000	2

+ Convergence diagnostic (PSRF = Potential Scale Reduction Factor; Gelman and Rubin, 1992) should approach 1.0 as runs converge. NA is reported when deviation of parameter values within all runs is 0 or when a parameter value (a branch length, for instance) is not sampled in all runs.

Summary statistics for partitions with frequency >= 0.10 in at least one run:
Average standard deviation of split frequencies = 0.011153
Maximum standard deviation of split frequencies = 0.063084
Average PSRF for parameter values (excluding NA and >10.0) = 1.002
Maximum PSRF for parameter values = 1.042

Credible sets of trees (1495 trees sampled):
50 % credible set contains 745 trees
90 % credible set contains 1345 trees
95 % credible set contains 1420 trees
99 % credible set contains 1480 trees

MrBayes >

APPENDIX B:

Psephenidae: Molecular Data (CO1, Wingless): Bayesian Analysis Log File. First set a summary of command lines and second set an example of the output of the analysis.

Bayesian execution steps (combined with partitions)

Log file first created to store commands and output:

>log start filename = CO1wingless-partn-log.txt

1. Format data type = mixed (DNA 1-1380, standard: 1381-1523) interleave = yes gap = - missing = ?
2. Charset CO1 = 1-824
3. Charset Wingless = 825-1380
4. Charset morph = 1381-1523
5. Partition favored = 3:CO1, Wingless, morph;
6. Set Partition = favored;
7. 1 set apply to = (1,2) nst=6 rates=invgamma
8. 1 set apply to = (3) [morph data] rates = gamma
9. Unlink state freq = (all) revmat = (all) shape = (all) pinvar = (all)
10. Prset applyto= (all) rate pr = variable [Do] showmodel [see page 42]
11. mcmc filename = analysis;
12. ngen = 5000000
13. mcmc [running the Bayesian analysis]

```
  Logging screen output to file "co1wingless-partn-log.txt"

MrBayes >
  Executing file "co1wingless.nex"
  DOS line termination
  Longest line length = 1540
```

```
Parsing file
Expecting NEXUS formatted file
Reading data block
   Allocated taxon set
   Allocated matrix
   Defining new matrix with 26 taxa and 1380 characters
   Data is Mixed
      Data for partition 1 is Dna
   Data matrix is not interleaved
   Missing data coded as ?
   Gaps coded as -
   Taxon  1 -> Elmidae_1
   Taxon  2 -> Elmidae_2
   Taxon  3 -> Elmidae_3
   Taxon  4 -> Psephenoides
   Taxon  5 -> Sinopsephenoides
   Taxon  6 -> Neopsephenoides
   Taxon  7 -> Nipponeubria
   Taxon  8 -> Psephenops
   Taxon  9 -> Psephenus
   Taxon 10 -> Mataeopsephus
   Taxon 11 -> Tychepsephus
   Taxon 12 -> Afrobrianax
   Taxon 13 -> Odontanax
   Taxon 14 -> Macroeubria
   Taxon 15 -> Ectopria
   Taxon 16 -> Genus_B_South_Africa_NV
   Taxon 17 -> Acneus
   Taxon 18 -> Schinostethus
   Taxon 19 -> Eubria
   Taxon 20 -> Malacopsephenoides
   Taxon 21 -> Costa_Rica_sp_2
   Taxon 22 -> Dicranopselaphus
   Taxon 23 -> Microeubria
   Taxon 24 -> Afroeubria
   Taxon 25 -> Eubrianax
   Taxon 26 -> Sclerocyphon
   Successfully read matrix
   Setting default partition (does not divide up characters)
   Setting model defaults
   Seed (for generating default start values) = 1415822806
   Setting output file names to "co1wingless.nex.run<i>.<p|t>"
Exiting data block
Skipping "ASSUMPTIONS" block
Reached end of file
```

```
MrBayes >
   Defining charset called co1
   Expecting  command

MrBayes >
   Defining charset called wingless
   Expecting  command

MrBayes >
   Defining partition called favored
   Expecting  command

MrBayes >
   Setting favored as the partition, dividing characters into 2 parts.
   Setting model defaults
   Seed (for generating default start values) = 1565640073
   Expecting  command

MrBayes >
   Defining charset called wingless1stpos

MrBayes >
   Defining charset called wingless2ndpos

MrBayes >
   Defining charset called wingless3rdpos

MrBayes >
   Defining partition called sat-partition

MrBayes >
   Setting sat-partition as the partition, dividing characters into 4 parts.
   Setting model defaults
   Seed (for generating default start values) = 252902275

MrBayes >
   Could not find command "1"

MrBayes >
   Setting Nst to 6 for partition 1
   Setting Nst to 6 for partition 2
   Setting Nst to 6 for partition 3
   Setting Rates to Invgamma for partition 1
   Setting Rates to Invgamma for partition 2
   Setting Rates to Invgamma for partition 3
   Successfully set likelihood model parameters to
```

```
  partitions 1, 2, and 3 (if applicable)

MrBayes >
  Setting Nst to 6 for partition 4
  Setting Rates to Gamma for partition 4
  Successfully set likelihood model parameters to
  partition 4 (if applicable)

MrBayes >
  Could not find command "unlinkrevmat"

MrBayes >
  Unlinking

MrBayes >
  Could not find command "preset"

MrBayes >
  Setting Ratepr to Variable [Dirichlet(..,1,..)] for partition 1
  Setting Ratepr to Variable [Dirichlet(..,1,..)] for partition 2
  Setting Ratepr to Variable [Dirichlet(..,1,..)] for partition 3
  Setting Ratepr to Variable [Dirichlet(..,1,..)] for partition 4
  Successfully set prior model parameters to all
  applicable data partitions

MrBayes >
  Running Markov chain
  MCMC stamp = 7347055346
  Seed = 1427730688
  Swapseed = 1415822806
  Model settings:

    Settings for partition 1 --
      Datatype  = DNA
      Nucmodel  = 4by4
      Nst       = 6
                  Substitution rates, expressed as proportions
                  of the rate sum, have a Dirichlet prior
                  (1.00,1.00,1.00,1.00,1.00,1.00)
      Covarion  = No
      # States  = 4
                  State frequencies have a Dirichlet prior
                  (1.00,1.00,1.00,1.00)
      Rates     = Invgamma
                  Gamma shape parameter is uniformly dist-
                  ributed on the interval (0.00,200.00).
```

```
            Proportion of invariable sites is uniformly dist-
            ributed on the interval (0.00,1.00).
            Gamma distribution is approximated using 4 categories.
            Likelihood summarized over all rate categories in each generation.

Settings for partition 2 --
  Datatype  = DNA
  Nucmodel  = 4by4
  Nst       = 6
            Substitution rates, expressed as proportions
            of the rate sum, have a Dirichlet prior
            (1.00,1.00,1.00,1.00,1.00,1.00)
  Covarion  = No
  # States  = 4
            State frequencies have a Dirichlet prior
            (1.00,1.00,1.00,1.00)
  Rates     = Invgamma
            Gamma shape parameter is uniformly dist-
            ributed on the interval (0.00,200.00).
            Proportion of invariable sites is uniformly dist-
            ributed on the interval (0.00,1.00).
            Gamma distribution is approximated using 4 categories.
            Likelihood summarized over all rate categories in each generation.

Settings for partition 3 --
  Datatype  = DNA
  Nucmodel  = 4by4
  Nst       = 6
            Substitution rates, expressed as proportions
            of the rate sum, have a Dirichlet prior
            (1.00,1.00,1.00,1.00,1.00,1.00)
  Covarion  = No
  # States  = 4
            State frequencies have a Dirichlet prior
            (1.00,1.00,1.00,1.00)
  Rates     = Invgamma
            Gamma shape parameter is uniformly dist-
            ributed on the interval (0.00,200.00).
            Proportion of invariable sites is uniformly dist-
            ributed on the interval (0.00,1.00).
            Gamma distribution is approximated using 4 categories.
            Likelihood summarized over all rate categories in each generation.

Settings for partition 4 --
  Datatype  = DNA
  Nucmodel  = 4by4
```

```
   Nst       = 6
             Substitution rates, expressed as proportions
             of the rate sum, have a Dirichlet prior
             (1.00,1.00,1.00,1.00,1.00,1.00)
   Covarion  = No
   # States  = 4
             State frequencies have a Dirichlet prior
             (1.00,1.00,1.00,1.00)
   Rates     = Gamma
             Gamma shape parameter is uniformly dist-
             ributed on the interval (0.00,200.00).
             Gamma distribution is approximated using 4 categories.
             Likelihood summarized over all rate categories in each generation.

Active parameters:

                 Partition(s)
  Parameters       1  2  3  4
  ---------------------------
  Revmat           1  2  3  4
  Statefreq        5  6  7  8
  Shape            9 10 11 12
  Pinvar          13 14 15  .
  Ratemultiplier  16 16 16 16
  Topology        17 17 17 17
  Brlens          18 18 18 18
  ---------------------------

  Parameters can be linked or unlinked across partitions using 'link' and 'unlink'

  1 --  Parameter  = Revmat{1}
        Type       = Rates of reversible rate matrix
        Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
        Partition  = 1

  2 --  Parameter  = Revmat{2}
        Type       = Rates of reversible rate matrix
        Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
        Partition  = 2

  3 --  Parameter  = Revmat{3}
        Type       = Rates of reversible rate matrix
        Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
        Partition  = 3

  4 --  Parameter  = Revmat{4}
```

```
      Type       = Rates of reversible rate matrix
      Prior      = Dirichlet(1.00,1.00,1.00,1.00,1.00,1.00)
      Partition  = 4

 5 --  Parameter  = Pi{1}
      Type       = Stationary state frequencies
      Prior      = Dirichlet
      Partition  = 1

 6 --  Parameter  = Pi{2}
      Type       = Stationary state frequencies
      Prior      = Dirichlet
      Partition  = 2

 7 --  Parameter  = Pi{3}
      Type       = Stationary state frequencies
      Prior      = Dirichlet
      Partition  = 3

 8 --  Parameter  = Pi{4}
      Type       = Stationary state frequencies
      Prior      = Dirichlet
      Partition  = 4

 9 --  Parameter  = Alpha{1}
      Type       = Shape of scaled gamma distribution of site rates
      Prior      = Uniform(0.00,200.00)
      Partition  = 1

10 --  Parameter  = Alpha{2}
      Type       = Shape of scaled gamma distribution of site rates
      Prior      = Uniform(0.00,200.00)
      Partition  = 2

11 --  Parameter  = Alpha{3}
      Type       = Shape of scaled gamma distribution of site rates
      Prior      = Uniform(0.00,200.00)
      Partition  = 3

12 --  Parameter  = Alpha{4}
      Type       = Shape of scaled gamma distribution of site rates
      Prior      = Uniform(0.00,200.00)
      Partition  = 4

13 --  Parameter  = Pinvar{1}
      Type       = Proportion of invariable sites
```

```
      Prior      = Uniform(0.00,1.00)
      Partition  = 1

  14 --  Parameter  = Pinvar{2}
      Type       = Proportion of invariable sites
      Prior      = Uniform(0.00,1.00)
      Partition  = 2

  15 --  Parameter  = Pinvar{3}
      Type       = Proportion of invariable sites
      Prior      = Uniform(0.00,1.00)
      Partition  = 3

  16 --  Parameter  = Ratemultiplier{all}
      Type       = Partition-specific rate multiplier
      Prior      = Dirichlet(1.00,1.00,1.00,1.00)
      Partitions = All

  17 --  Parameter  = Tau{all}
      Type       = Topology
      Prior      = All topologies equally probable a priori
      Partitions = All
      Subparam.  = V{all}

  18 --  Parameter  = V{all}
      Type       = Branch lengths
      Prior      = Unconstrained:Exponential(10.0)
      Partitions = All


Number of taxa = 26
Number of characters = 1380

The MCMC sampler will use the following moves:
  With prob.  Chain will use move
    0.82 %   Dirichlet(Revmat{1})
    0.82 %   Slider(Revmat{1})
    0.82 %   Dirichlet(Revmat{2})
    0.82 %   Slider(Revmat{2})
    0.82 %   Dirichlet(Revmat{3})
    0.82 %   Slider(Revmat{3})
    0.82 %   Dirichlet(Revmat{4})
    0.82 %   Slider(Revmat{4})
    0.82 %   Dirichlet(Pi{1})
    0.82 %   Slider(Pi{1})
    0.82 %   Dirichlet(Pi{2})
```

0.82 % Slider(Pi{2})
0.82 % Dirichlet(Pi{3})
0.82 % Slider(Pi{3})
0.82 % Dirichlet(Pi{4})
0.82 % Slider(Pi{4})
1.64 % Multiplier(Alpha{1})
1.64 % Multiplier(Alpha{2})
1.64 % Multiplier(Alpha{3})
1.64 % Multiplier(Alpha{4})
1.64 % Slider(Pinvar{1})
1.64 % Slider(Pinvar{2})
1.64 % Slider(Pinvar{3})
0.82 % Dirichlet(Ratemultiplier{all})
0.82 % Slider(Ratemultiplier{all})
8.20 % ExtSPR(Tau{all},V{all})
8.20 % ExtTBR(Tau{all},V{all})
8.20 % NNI(Tau{all},V{all})
8.20 % ParsSPR(Tau{all},V{all})
32.79 % Multiplier(V{all})
8.20 % Nodeslider(V{all})

Division 1 has 385 unique site patterns
Division 2 has 84 unique site patterns
Division 3 has 165 unique site patterns
Division 4 has 96 unique site patterns
Initializing conditional likelihoods
Using standard SSE likelihood calculator for division 1 (single-precision)
Using standard SSE likelihood calculator for division 2 (single-precision)
Using standard SSE likelihood calculator for division 3 (single-precision)
Using standard SSE likelihood calculator for division 4 (single-precision)
Initializing invariable-site conditional likelihoods

Initial log likelihoods and log prior probs for run 1:
Chain 1 -- -18341.612041 -- 1.488504
Chain 2 -- -18585.828759 -- 1.488504
Chain 3 -- -18418.392833 -- 1.488504
Chain 4 -- -18428.856549 -- 1.488504

Initial log likelihoods and log prior probs for run 2:
Chain 1 -- -18774.271918 -- 1.488504
Chain 2 -- -18286.816899 -- 1.488504
Chain 3 -- -18648.788874 -- 1.488504
Chain 4 -- -18670.418993 -- 1.488504

There are results from a previous run saved using the same filename(s).

Do you want to overwrite these results? (yes/no):
Overwriting file "co1wingless.nex.run1.p"
Overwriting file "co1wingless.nex.run1.t"
Overwriting file "co1wingless.nex.run2.p"
Overwriting file "co1wingless.nex.run2.t"
Overwriting file "co1wingless.nex.mcmc"

Using a relative burnin of 25.0 % for diagnostics

[AFTER 2000000 GENERATIONS]
Average standard deviation of split frequencies: 0.005959

Continue with analysis? (yes/no):
Analysis completed in 1 hours 34 mins 44 seconds
Analysis used 5683.66 seconds of CPU time
Likelihood of best state for "cold" chain of run 1 was -12722.63
Likelihood of best state for "cold" chain of run 2 was -12722.63

Acceptance rates for the moves in the "cold" chain of run 1:

With prob.	(last 100)	chain accepted proposals by move
25.0 %	(24 %)	Dirichlet(Revmat{1})
33.8 %	(22 %)	Slider(Revmat{1})
49.4 %	(34 %)	Dirichlet(Revmat{2})
62.7 %	(34 %)	Slider(Revmat{2})
30.9 %	(25 %)	Dirichlet(Revmat{3})
43.8 %	(26 %)	Slider(Revmat{3})
47.5 %	(28 %)	Dirichlet(Revmat{4})
59.6 %	(43 %)	Slider(Revmat{4})
16.9 %	(25 %)	Dirichlet(Pi{1})
22.9 %	(31 %)	Slider(Pi{1})
35.3 %	(29 %)	Dirichlet(Pi{2})
34.1 %	(25 %)	Slider(Pi{2})
26.8 %	(33 %)	Dirichlet(Pi{3})
27.1 %	(26 %)	Slider(Pi{3})
34.6 %	(29 %)	Dirichlet(Pi{4})
34.2 %	(18 %)	Slider(Pi{4})
25.2 %	(33 %)	Multiplier(Alpha{1})
62.2 %	(28 %)	Multiplier(Alpha{2})
37.6 %	(23 %)	Multiplier(Alpha{3})
36.8 %	(24 %)	Multiplier(Alpha{4})
29.9 %	(26 %)	Slider(Pinvar{1})
39.8 %	(31 %)	Slider(Pinvar{2})
36.3 %	(26 %)	Slider(Pinvar{3})
78.3 %	(70 %)	Dirichlet(Ratemultiplier{all})
35.2 %	(28 %)	Slider(Ratemultiplier{all})
4.6 %	(5 %)	ExtSPR(Tau{all},V{all})

```
    1.2 %    (  0 %)    ExtTBR(Tau{all},V{all})
    6.0 %    (  8 %)    NNI(Tau{all},V{all})
    0.2 %    (  0 %)    ParsSPR(Tau{all},V{all})
   25.8 %    ( 25 %)    Multiplier(V{all})
   30.0 %    ( 29 %)    Nodeslider(V{all})
```

Acceptance rates for the moves in the "cold" chain of run 2:

```
 With prob.   (last 100)   chain accepted proposals by move
   25.3 %    ( 25 %)    Dirichlet(Revmat{1})
   34.7 %    ( 22 %)    Slider(Revmat{1})
   49.4 %    ( 23 %)    Dirichlet(Revmat{2})
   62.4 %    ( 45 %)    Slider(Revmat{2})
   30.5 %    ( 23 %)    Dirichlet(Revmat{3})
   43.7 %    ( 23 %)    Slider(Revmat{3})
   46.9 %    ( 25 %)    Dirichlet(Revmat{4})
   57.5 %    ( 46 %)    Slider(Revmat{4})
   16.6 %    ( 19 %)    Dirichlet(Pi{1})
   23.3 %    ( 25 %)    Slider(Pi{1})
   35.4 %    ( 28 %)    Dirichlet(Pi{2})
   34.3 %    ( 39 %)    Slider(Pi{2})
   26.3 %    ( 26 %)    Dirichlet(Pi{3})
   27.5 %    ( 26 %)    Slider(Pi{3})
   34.9 %    ( 24 %)    Dirichlet(Pi{4})
   34.0 %    ( 26 %)    Slider(Pi{4})
   25.8 %    ( 32 %)    Multiplier(Alpha{1})
   61.9 %    ( 36 %)    Multiplier(Alpha{2})
   37.7 %    ( 26 %)    Multiplier(Alpha{3})
   36.3 %    ( 32 %)    Multiplier(Alpha{4})
   30.1 %    ( 21 %)    Slider(Pinvar{1})
   40.0 %    ( 32 %)    Slider(Pinvar{2})
   36.6 %    ( 23 %)    Slider(Pinvar{3})
   79.1 %    ( 75 %)    Dirichlet(Ratemultiplier{all})
   35.4 %    ( 23 %)    Slider(Ratemultiplier{all})
    4.6 %    (  5 %)    ExtSPR(Tau{all},V{all})
    1.2 %    (  0 %)    ExtTBR(Tau{all},V{all})
    6.0 %    (  6 %)    NNI(Tau{all},V{all})
    0.2 %    (  1 %)    ParsSPR(Tau{all},V{all})
   25.8 %    ( 25 %)    Multiplier(V{all})
   30.0 %    ( 26 %)    Nodeslider(V{all})
```

Chain swap information for run 1:

```
         1       2       3       4
   ----------------------------------
 1 |           0.60    0.31    0.14
 2 |  332773           0.62    0.35
```

```
   3 |  333531  333124          0.65
   4 |  332961  333767  333844

Chain swap information for run 2:

          1       2       3       4
   ---------------------------------
   1 |          0.60    0.31    0.14
   2 |  333386          0.63    0.35
   3 |  333984  332740          0.65
   4 |  333201  333505  333184

Upper diagonal: Proportion of successful state exchanges between chains
Lower diagonal: Number of attempted state exchanges between chains

Chain information:

  ID -- Heat
 -----------
   1 -- 1.00  (cold chain)
   2 -- 0.91
   3 -- 0.83
   4 -- 0.77

Heat = 1 / (1 + T * (ID - 1))
   (where T = 0.10 is the temperature and ID is the chain number)


MrBayes >
  Summarizing parameters in files co1wingless.nex.run1.p and co1wingless.nex.run2.p
  Writing summary statistics to file co1wingless.nex.pstat
  Using relative burnin ('relburnin=yes'), discarding the first 25 % of samples

  Below are rough plots of the generation (x-axis) versus the log
  probability of observing the data (y-axis). You can use these
  graphs to determine what the burn in for your analysis should be.
  When the log probability starts to plateau you may be at station-
  arity. Sample trees and parameters after the log probability
  plateaus. Of course, this is not a guarantee that you are at sta-
  tionarity. Also examine the convergence diagnostics provided by
  the 'sump' and 'sumt' commands for all the parameters in your
  model. Remember that the burn in is the number of samples to dis-
  card. There are a total of ngen / samplefreq samples taken during
  a MCMC analysis.

  Overlay plot for both runs:
```

(1 = Run number 1; 2 = Run number 2; * = Both runs)

```
+------------------------------------------------------------+ -12744.85
|             1           2                  |
|        1                                   |
|                                   2        |
|                 1        1         1       |
|                    2 1  2 2     1          |
|*     1   2   1 2        1 1          1       2 |
|   2      1  1 1    2       2      2       2    |
|   1 1  2     *          2    1     1         |
| 22221 2  2    2    2  2  11   2        11 122   2  11   |
|  1      1*      11 2          211    2     1  22  |
| 11     1       22   1     22     2   22        2 2   2|
|     2     2 2            1        2 2     2  1  1  |
|              12   1 2     11  1        1 2 1     1 |
|      211           2 1        12       1       1|
|               2         1  2         1       |
+------+-----+-----+-----+-----+-----+-----+-----+-----+-----+ -12752.69
^                                            ^
500000                                        2000000
```

Overwriting file "co1wingless.nex.lstat"

Estimated marginal likelihoods for runs sampled in files
"co1wingless.nex.run1.p" and "co1wingless.nex.run2.p":
(Use the harmonic mean for Bayes factor comparisons of models)

(Values are saved to the file co1wingless.nex.lstat)

Run	Arithmetic mean	Harmonic mean
1	-12730.75	-12772.49
2	-12734.16	-12769.55
TOTAL	-12731.41	-12771.85

Model parameter summaries over the runs sampled in files
"co1wingless.nex.run1.p" and "co1wingless.nex.run2.p":
Summaries are based on a total of 6002 samples from 2 runs.
Each run produced 4001 samples of which 3001 samples were included.
Parameter summaries saved to file "co1wingless.nex.pstat".
Overwriting file "co1wingless.nex.pstat"

			95% HPD Interval					
Parameter	Mean	Variance	Lower	Upper	Median	min ESS*	avg ESS	PSRF+
TL{all}	8.319939	0.389469	7.092540	9.552103	8.303349	201.94	222.57	1.000
r(A<->C){1}	0.044550	0.000151	0.020475	0.067803	0.043908	904.76	918.82	1.001
r(A<->G){1}	0.516906	0.002281	0.424033	0.611478	0.515975	421.36	492.73	1.000
r(A<->T){1}	0.023722	0.000020	0.015810	0.033043	0.023521	443.67	551.54	1.001
r(C<->G){1}	0.108977	0.000621	0.065277	0.159597	0.107234	547.21	721.14	1.000
r(C<->T){1}	0.285808	0.001682	0.208508	0.368928	0.284875	422.71	503.24	1.000
r(G<->T){1}	0.020038	0.000029	0.010360	0.031158	0.019526	961.33	1054.02	1.000
r(A<->C){2}	0.208751	0.001954	0.124590	0.293953	0.206607	1160.53	1296.86	1.000
r(A<->G){2}	0.196001	0.001514	0.125095	0.275163	0.193489	1259.97	1289.30	1.000
r(A<->T){2}	0.141899	0.001180	0.080740	0.213164	0.139102	1399.15	1471.73	1.000
r(C<->G){2}	0.120494	0.001569	0.047014	0.198315	0.117153	1271.17	1394.95	1.000
r(C<->T){2}	0.292087	0.003830	0.176824	0.417187	0.289432	1024.52	1147.98	1.000
r(G<->T){2}	0.040768	0.000550	0.001452	0.085646	0.036809	1554.17	1566.02	1.000
r(A<->C){3}	0.039122	0.000197	0.012922	0.067035	0.038478	1353.26	1505.49	1.000
r(A<->G){3}	0.386378	0.001531	0.308737	0.465338	0.384737	1075.81	1121.47	1.000
r(A<->T){3}	0.171745	0.001014	0.112075	0.236406	0.170433	1115.42	1230.59	1.001
r(C<->G){3}	0.055486	0.000157	0.031080	0.079196	0.054877	834.98	1122.93	1.000
r(C<->T){3}	0.263681	0.000935	0.208207	0.326853	0.262766	997.35	1107.61	1.000
r(G<->T){3}	0.083588	0.000407	0.043595	0.121521	0.082475	1261.12	1344.72	1.000
r(A<->C){4}	0.271471	0.002010	0.186971	0.358717	0.269462	1193.31	1256.66	1.000

r(A<->G){4} 0.183063 0.001346 0.114356 0.255998 0.180286 1474.75 1507.44 1.000

r(A<->T){4} 0.062145 0.000552 0.019908 0.109591 0.059541 1640.98 1665.04 1.001

r(C<->G){4} 0.102612 0.000969 0.043141 0.161983 0.099933 1649.49 1740.50 1.000

r(C<->T){4} 0.303887 0.003271 0.199432 0.420281 0.301499 1021.54 1056.64 1.000

r(G<->T){4} 0.076822 0.001007 0.020905 0.141012 0.073311 998.63 1138.00 1.000

pi(A){1} 0.379455 0.000148 0.354844 0.402536 0.379816 1112.93 1140.54 1.000

pi(C){1} 0.059683 0.000017 0.052187 0.068395 0.059551 685.95 886.38 1.000

pi(G){1} 0.142788 0.000040 0.129659 0.154545 0.142750 1065.98 1150.53 1.000

pi(T){1} 0.418074 0.000195 0.392087 0.446184 0.418178 1092.44 1175.43 1.000

pi(A){2} 0.388872 0.000961 0.328987 0.449238 0.388650 1618.33 1703.72 1.000

pi(C){2} 0.160206 0.000502 0.115517 0.203207 0.158903 1678.82 1702.55 1.000

pi(G){2} 0.268123 0.000878 0.210111 0.324564 0.267690 1533.80 1595.76 1.000

pi(T){2} 0.182799 0.000644 0.134770 0.232623 0.181488 1716.22 1762.20 1.000

pi(A){3} 0.144703 0.000203 0.116464 0.171564 0.144326 1225.82 1343.53 1.000

pi(C){3} 0.409444 0.000599 0.363064 0.457944 0.409789 1151.47 1201.31 1.000

pi(G){3} 0.249678 0.000433 0.209863 0.290471 0.249806 1087.54 1180.73 1.000

pi(T){3} 0.196175 0.000250 0.165696 0.226548 0.195596 1357.50 1398.24 1.000

pi(A){4} 0.375899 0.000927 0.313980 0.432793 0.375689 1492.61 1705.08 1.000

pi(C){4} 0.212914 0.000610 0.163741 0.258949 0.211881 2016.95 2129.54 1.000

pi(G){4} 0.229397 0.000729 0.174665 0.280745 0.229077 1662.33 1763.37 1.000

pi(T){4} 0.181790 0.000687 0.134338 0.235385 0.180523 1633.26 1686.54 1.000

alpha{1} 0.441151 0.009556 0.228714 0.555119 0.472944 174.15 223.19 1.000

alpha{2} 93.484748 3550.668673 0.634510 188.033552 92.065117 2712.58 2723.55 1.000

```
  alpha{3}     5.330188   50.489099    1.888708    9.611558    4.304376   2067.39
2419.25    1.000
  alpha{4}     0.806573    0.739008    0.350064    1.321248    0.738608   2245.62
2521.09    1.000
  pinvar{1}    0.462174    0.003594    0.321639    0.532922    0.482680    177.93
234.04    1.000
  pinvar{2}    0.525668    0.003230    0.420617    0.627796    0.529291   1782.57
1810.74    1.000
  pinvar{3}    0.041627    0.000512    0.000088    0.082192    0.039413   2395.53
2443.74    1.000
  m{1}        1.390342    0.000670    1.336220    1.438854    1.391696    222.37    240.22
1.000
  m{2}        0.142201    0.000413    0.105604    0.184113    0.140670    495.86    531.86
1.000
  m{3}        0.961856    0.009234    0.781210    1.161179    0.956575    251.40    265.81
1.001
  m{4}        0.161976    0.000498    0.118810    0.204499    0.160302    473.06    496.00
1.000
  -----------------------------------------------------------------------------------------------
  * Convergence diagnostic (ESS = Estimated Sample Size); min and avg values
    correspond to minimal and average ESS among runs.
    ESS value below 100 may indicate that the parameter is undersampled.
  + Convergence diagnostic (PSRF = Potential Scale Reduction Factor; Gelman
    and Rubin, 1992) should approach 1.0 as runs converge.



MrBayes >
  Summarizing trees in files "co1wingless.nex.run1.t" and "co1wingless.nex.run2.t"
  Using relative burnin ('relburnin=yes'), discarding the first 25 % of sampled trees
  Writing statistics to files co1wingless.nex.<parts|tstat|vstat|trprobs|con>
  Examining first file ...
  Found one tree block in file "co1wingless.nex.run1.t" with 4001 trees in last block
  Expecting the same number of trees in the last tree block of all files

  Tree reading status:

  0      10      20      30      40      50      60      70      80      90     100
  v-------v-------v-------v-------v-------v-------v-------v-------v-------v-------v

**********************************************************************
*********

  Read a total of 8002 trees in 2 files (sampling 6002 of them)
    (Each file contained 4001 trees of which 3001 were sampled)
  Overwriting file "co1wingless.nex.parts"
```

Overwriting file "co1wingless.nex.tstat"
Overwriting file "co1wingless.nex.vstat"
Overwriting file "co1wingless.nex.con.tre"
Overwriting file "co1wingless.nex.trprobs"

General explanation:

In an unrooted tree, a taxon bipartition (split) is specified by removing a branch, thereby dividing the species into those to the left and those to the right of the branch. Here, taxa to one side of the removed branch are denoted '.' and those to the other side are denoted '*'. Specifically, the '.' symbol is used for the taxa on the same side as the outgroup.

In a rooted or clock tree, the tree is rooted using the model and not by reference to an outgroup. Each bipartition therefore corresponds to a clade, that is, a group that includes all the descendants of a particular branch in the tree. Taxa that are included in each clade are denoted using '*', and taxa that are not included are denoted using the '.' symbol.

The output first includes a key to all the bipartitions with frequency larger or equual to (Minpartfreq) in at least one run. Minpartfreq is a paramiter to sumt command and currently it is set to 0.10. This is followed by a table with statistics for the informative bipartitions (those including at least two taxa), sorted from highest to lowest probability. For each bipartition, the table gives the number of times the partition or split was observed in all runs (#obs) and the posterior probability of the bipartition (Probab.), which is the same as the split frequency. If several runs are summarized, this is followed by the minimum split frequency (Min(s)), the maximum frequency (Max(s)), and the standard deviation of frequencies (Stddev(s)) across runs. The latter value should approach 0 for all bipartitions as MCMC runs converge.

This is followed by a table summarizing branch lengths, node heights (if a clock model was used) and relaxed clock parameters (if a relaxed clock model was used). The mean, variance, and 95 % credible interval are given for each of these parameters. If several runs are summarized, the potential scale reduction factor (PSRF) is also given; it should approach 1 as runs converge. Node heights will take calibration points into account, if such points were used in the analysis.

Note that Stddev may be unreliable if the partition is not present in all runs (the last column indicates the number of runs that sampled the partition if more than one run is summarized). The PSRF is not calculated at all if the partition is not present in all runs.The PSRF is also sensitive to small sample sizes and it should only be considered a rough guide to convergence since some of the assumptions allowing one to interpret it as a true potential scale reduction factor are violated in MrBayes.

List of taxa in bipartitions:

1 -- Elmidae_1
2 -- Elmidae_2
3 -- Elmidae_3
4 -- Psephenoides
5 -- Sinopsephenoides
6 -- Neopsephenoides
7 -- Nipponeubria
8 -- Psephenops
9 -- Psephenus
10 -- Mataeopsephus
11 -- Tychepsephus
12 -- Afrobrianax
13 -- Odontanax
14 -- Macroeubria
15 -- Ectopria
16 -- Genus_B_South_Africa_NV
17 -- Acneus
18 -- Schinostethus
19 -- Eubria
20 -- Malacopsephenoides
21 -- Costa_Rica_sp_2
22 -- Dicranopselaphus
23 -- Microeubria
24 -- Afroeubria
25 -- Eubrianax
26 -- Sclerocyphon

Summary statistics for informative taxon bipartitions
(saved to file "co1wingless.nex.tstat"):

ID	#obs	Probab.	Sd(s)+	Min(s)	Max(s)	Nruns
27	6002	1.000000	0.000000	1.000000	1.000000	2
28	6002	1.000000	0.000000	1.000000	1.000000	2
29	6002	1.000000	0.000000	1.000000	1.000000	2
30	6001	0.999833	0.000236	0.999667	1.000000	2
31	5975	0.995501	0.001649	0.994335	0.996668	2
32	5971	0.994835	0.001649	0.993669	0.996001	2
33	5960	0.993002	0.001414	0.992003	0.994002	2
34	5951	0.991503	0.002121	0.990003	0.993002	2
35	5890	0.981340	0.003770	0.978674	0.984005	2

36	5887	0.980840	0.001649	0.979673	0.982006	2
37	5823	0.970177	0.004006	0.967344	0.973009	2
38	5807	0.967511	0.004948	0.964012	0.971010	2
39	5685	0.947184	0.002121	0.945685	0.948684	2
40	5648	0.941020	0.001414	0.940020	0.942019	2
41	5496	0.915695	0.001414	0.914695	0.916694	2
42	5477	0.912529	0.003534	0.910030	0.915028	2
43	5408	0.901033	0.001885	0.899700	0.902366	2
44	4734	0.788737	0.028275	0.768744	0.808730	2
45	4601	0.766578	0.006362	0.762079	0.771076	2
46	4584	0.763745	0.017907	0.751083	0.776408	2
47	3346	0.557481	0.012252	0.548817	0.566145	2
48	2980	0.496501	0.004241	0.493502	0.499500	2
49	2181	0.363379	0.006362	0.358880	0.367877	2
50	1949	0.324725	0.004006	0.321893	0.327557	2
51	1853	0.308730	0.012488	0.299900	0.317561	2
52	1731	0.288404	0.001178	0.287571	0.289237	2
53	1412	0.235255	0.005655	0.231256	0.239254	2
54	1169	0.194768	0.006362	0.190270	0.199267	2
55	1027	0.171110	0.023327	0.154615	0.187604	2
56	970	0.161613	0.019792	0.147617	0.175608	2
57	694	0.115628	0.004712	0.112296	0.118960	2

+ Convergence diagnostic (standard deviation of split frequencies)
should approach 0.0 as runs converge.

Summary statistics for branch and node parameters
(saved to file "co1wingless.nex.vstat"):

			95% HPD Interval				
Parameter	Mean	Variance	Lower	Upper	Median	PSRF+	Nruns
length{all}[1]	0.220930	0.002101	0.131781	0.307054	0.217006	1.000	2
length{all}[2]	0.212327	0.002236	0.119158	0.300423	0.209324	1.001	2
length{all}[3]	0.201298	0.002037	0.121504	0.293701	0.198368	1.000	2
length{all}[4]	0.001437	0.000002	0.000000	0.004095	0.001015	1.000	2
length{all}[5]	0.003592	0.000004	0.000305	0.007381	0.003295	1.000	2
length{all}[6]	0.081602	0.000452	0.041773	0.124411	0.080219	1.000	2
length{all}[7]	0.252361	0.001965	0.166868	0.339732	0.250255	1.000	2
length{all}[8]	0.107951	0.000683	0.059508	0.160815	0.106026	1.000	2
length{all}[9]	0.136799	0.000782	0.084837	0.192691	0.135275	1.000	2
length{all}[10]	0.230242	0.001572	0.158552	0.311440	0.228512	1.000	2
length{all}[11]	0.080921	0.000782	0.027376	0.134059	0.078600	1.003	2
length{all}[12]	0.350971	0.002431	0.261634	0.448287	0.349000	1.000	2

length{all}[13]	0.280907	0.001797	0.201841	0.363872	0.279333	1.000	2
length{all}[14]	0.266899	0.003041	0.163741	0.381929	0.264739	1.000	2
length{all}[15]	0.159091	0.001203	0.090644	0.225920	0.156911	1.000	2
length{all}[16]	0.386080	0.003640	0.277022	0.507571	0.381598	1.000	2
length{all}[17]	0.213095	0.002724	0.115128	0.315425	0.209174	1.000	2
length{all}[18]	0.231858	0.001731	0.155575	0.315996	0.228976	1.000	2
length{all}[19]	0.304967	0.004350	0.181729	0.435179	0.298969	1.000	2
length{all}[20]	0.428496	0.008107	0.255147	0.600625	0.420717	1.000	2
length{all}[21]	0.133812	0.001248	0.070392	0.202883	0.130757	1.001	2
length{all}[22]	0.142402	0.000736	0.094313	0.198800	0.140518	1.000	2
length{all}[23]	0.372374	0.002864	0.272672	0.482525	0.368876	1.000	2
length{all}[24]	0.440917	0.008406	0.265739	0.611158	0.431898	1.000	2
length{all}[25]	0.286107	0.001512	0.216615	0.364801	0.283000	1.000	2
length{all}[26]	0.184662	0.001928	0.102116	0.271023	0.181061	1.000	2
length{all}[27]	0.118847	0.001234	0.055094	0.191613	0.116082	1.000	2
length{all}[28]	0.174141	0.001339	0.104497	0.247653	0.171521	1.000	2
length{all}[29]	0.222717	0.002035	0.136936	0.312767	0.219748	1.000	2
length{all}[30]	0.094793	0.000832	0.042548	0.153481	0.092701	1.000	2
length{all}[31]	0.296817	0.007688	0.119374	0.463328	0.297221	1.001	2
length{all}[32]	0.103646	0.002096	0.016980	0.193092	0.099401	1.000	2
length{all}[33]	0.146515	0.002981	0.036495	0.250414	0.143086	1.000	2
length{all}[34]	0.183031	0.004344	0.050395	0.312014	0.183675	1.002	2
length{all}[35]	0.076177	0.001155	0.014759	0.141248	0.072250	1.000	2
length{all}[36]	0.118544	0.002793	0.015820	0.217465	0.114608	1.000	2
length{all}[37]	0.069812	0.001184	0.009918	0.136465	0.066083	1.000	2
length{all}[38]	0.049870	0.000527	0.009256	0.094078	0.047039	1.000	2
length{all}[39]	0.088500	0.001135	0.023090	0.153610	0.086340	1.000	2
length{all}[40]	0.043029	0.000487	0.002912	0.084674	0.040408	1.000	2
length{all}[41]	0.083588	0.001497	0.006099	0.153943	0.081278	1.000	2
length{all}[42]	0.071537	0.000655	0.023060	0.121798	0.069080	1.000	2
length{all}[43]	0.122791	0.002505	0.022083	0.217967	0.123176	1.000	2
length{all}[44]	0.126863	0.003143	0.026498	0.233455	0.120148	1.000	2
length{all}[45]	0.060145	0.000789	0.009648	0.114772	0.057792	1.000	2
length{all}[46]	0.118695	0.002284	0.031515	0.216557	0.115547	1.000	2
length{all}[47]	0.117480	0.004025	0.007115	0.235512	0.112228	1.000	2
length{all}[48]	0.080374	0.002002	0.000526	0.159964	0.075086	1.000	2
length{all}[49]	0.083590	0.002335	0.000077	0.167812	0.080040	1.000	2
length{all}[50]	0.093093	0.001514	0.018426	0.166649	0.090379	1.000	2
length{all}[51]	0.081023	0.001341	0.012282	0.151543	0.080267	1.000	2
length{all}[52]	0.084129	0.001442	0.008864	0.153163	0.079622	1.000	2
length{all}[53]	0.077743	0.001967	0.000362	0.157893	0.073886	0.999	2
length{all}[54]	0.046047	0.000607	0.000175	0.089992	0.043154	0.999	2
length{all}[55]	0.124095	0.002950	0.019869	0.223600	0.120647	1.002	2
length{all}[56]	0.113123	0.002274	0.022318	0.203598	0.109449	0.999	2
length{all}[57]	0.092607	0.002730	0.000109	0.183565	0.092504	0.999	2

+ Convergence diagnostic (PSRF = Potential Scale Reduction Factor; Gelman
and Rubin, 1992) should approach 1.0 as runs converge. NA is reported when
deviation of parameter values within all runs is 0 or when a parameter
value (a branch length, for instance) is not sampled in all runs.

Summary statistics for partitions with frequency >= 0.10 in at least one run:
Average standard deviation of split frequencies = 0.005959
Maximum standard deviation of split frequencies = 0.028275
Average PSRF for parameter values (excluding NA and >10.0) = 1.000
Maximum PSRF for parameter values = 1.003

Credible sets of trees (1303 trees sampled):
50 % credible set contains 26 trees
90 % credible set contains 703 trees
95 % credible set contains 1003 trees
99 % credible set contains 1243 trees

MrBayes >

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