Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 41 (2006) 454–471
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of classification

Marshal Hedin [a,*], Jason E. Bond [b]

[a] San Diego State University, Department of Biology, San Diego, CA 92182, USA
[b] East Carolina University, Department of Biology, Howell Science Complex N211, Greenville, NC 27858, USA

Received 10 February 2006; revised 27 April 2006; accepted 4 May 2006
Available online 25 May 2006

Abstract

Mygalomorph spiders, which include the tarantulas, trapdoor spiders, and their kin, represent one of three main spider lineages. Mygalomorphs are currently classified into 15 families, comprising roughly 2500 species and 300 genera. The few published phylogenies of mygalomorph relationships are based exclusively on morphological data and reveal areas of both conflict and congruence, suggesting the need for additional phylogenetic research utilizing new character systems. As part of a larger combined evidence study of global mygalomorph relationships, we have gathered ~3.7 kb of rRNA data (18S and 28S) for a sample of 80 genera, representing all 15 mygalomorph families. Taxon sampling was particularly intensive across families that are questionable in composition—Cyrtaucheniidae and Nemesiidae. The following primary results are supported by both Bayesian and parsimony analyses of combined matrices representing multiple 28S alignments: (1) the Atypoidea, a clade that includes the families Atypidae, Antrodiaetidae, and Mecicobothriidae, is recovered as a basal lineage sister to all other mygalomorphs, (2) diplurids and hexathelids form a paraphyletic grade at the base of the non-atypoid clade, but neither family is monophyletic in any of our analyses, (3) a clade consisting of all sampled nemesiids, *Microstigmata* and the cyrtaucheniid genera *Kiama*, *Acontius*, and *Fufius* is consistently recovered, (4) other sampled cyrtaucheniids are fragmented across three separate clades, including a monophyletic North American Euctenizinae and a South African clade, (5) of the Domiothelina, only idiopids are consistently recovered as monophyletic; ctenizids are polyphyletic and migids are only weakly supported. The Domiothelina is not monophyletic. The molecular results we present are consistent with more recent hypotheses of mygalomorph relationship; however, additional work remains before mygalomorph classification can be formally reassessed with confidence—increased taxonomic sampling and the inclusion of additional character systems (more genes and morphology) are required.

Keywords: DNA sequence alignment; Spider phylogeny; Tree space visualization; Gondwanaland; Historical biogeography; Long-branch attraction; Bayes factor

1. Introduction

Mygalomorph spiders, which include the tarantulas, trapdoor spiders, and other less well-known groups, represent one of three main spider lineages (Fig. 1A). Although mygalomorphs retain some features that are plesiomorphic in spiders (e.g., two pairs of book lungs), several characters support mygalomorph monophyly, and this monophyly has not been seriously questioned (see Coddington et al., 2004; Platnick and Gertsch, 1976; Raven, 1985). Mygalomorphs are currently classified into 15 families, comprising roughly 2500 species and 300 genera (see Table 1; Platnick, 2006). These spiders build a diverse array of silk constructs (Coyle, 1986), range in size from the very small to the largest of all spiders, and exhibit an underappreciated diversity of morphological form (Fig. 2). Most mygalomorphs are, however, relatively large, bulky, sedentary, ground-dwelling spiders. Mygalomorphs are essentially worldwide in

* Corresponding author. Fax: +1 619 594 5676.
E-mail address: mhedin@sciences.sdsu.edu (M. Hedin).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.05.017

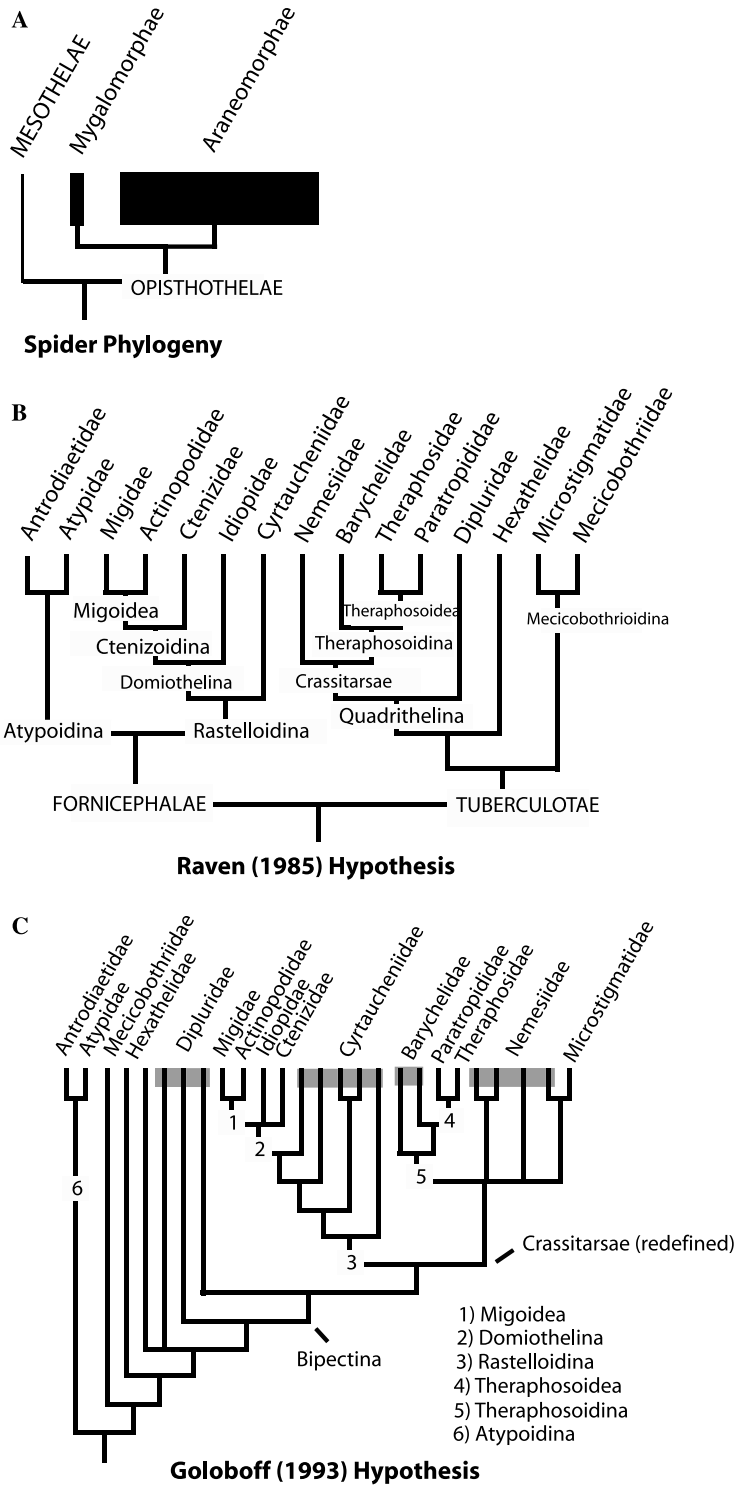

Fig. 1. Mygalomorph relatives and relationships. (A) Relationships of major spider clades, following Coddington et al. (2004). The Infraorder Araneomorphae contains the bulk of spider diversity, including spiders most familiar to the non-arachnologist (e.g., orb weavers, jumping spiders, wolf spiders, etc.). (B) Phylogenetic relationships of mygalomorph spider families, following Raven (1985, Fig. 1). (C) Phylogenetic relationships of mygalomorph families, following Goloboff (1993, Fig. 26). Paraphyly of the families Dipluridae, Cyrtaucheniidae, and Nemesiidae is indicated by grey boxes.

distribution, although all tropical regions and temperature austral regions of South America, southern Africa, and Australasia are centers of generic diversity (Table 1; Platnick, 2006; Raven, 1985).

Resolving phylogenetic relationships within the Mygalomorphae has proven to be a difficult task, for at least two reasons. First, members of this lineage have a deep evolutionary history. Well-preserved mygalomorph fossils,

Table 1
Summary of geographical distribution, recognized generic diversity, and sampled diversity for all mygalomorph families

Family	Geographic distribution	No. recognized genera	No. sampled genera
Atypidae	Nearctic (1), Ethiopian, Palearctic, Oriental	3	2
Antrodiaetidae	Nearctic (2), Japan	3	2
Mecicobothriidae	Nearctic (3), Neotropical (1)	4	2
Hexathelidae	Chile (2), Ethiopian, Palearctic, Oriental, Australasia (8)	11	6
Dipluridae	Nearctic (2), Neotropical (8), Ethiopian (3), Palearctic, Oriental (2), Australasia (7)	24	5
Cyrtaucheniidae	Nearctic (7), Neotropical (4), Ethiopian (3), Palearctic (2), Thailand (1), east Australia (1)	18	12[a]
Ctenizidae	Nearctic, Neotropical, Ethiopian (1), Palearctic (2), Oriental, Australasian	9	6
Idiopidae	Neotropical (1), Ethiopian (6), Palearctic (1), Oriental (2), Australasia (8)	20	13[b]
Actinopodidae	Neotropical (2), Australia	3	1
Migidae	temperate S. America (3), Australasia (2), Madagascar (3), Africa (1)	10	5
Nemesiidae	Nearctic (2), Neotropical (14), Ethiopian (3), Palearctic (4), Oriental (2), Australasia (12)	39[c]	16[d]
Microstigmatidae	South Africa (1), Neotropical (6)	7	1
Barychelidae	Neotropical (10), Ethiopian (8), Oriental (4), Australasia (19)	44	3
Theraphosidae	Nearctic (4), Neotropical (50), Ethiopian (28), Palearctic (1), Oriental (15), Australasia (1)	112	2
Paratropididae	Neotropical (4)	4	1

Notes: All taxonomic and geographical data are from Platnick (2006), with the following exceptions: [a]we consider *Spiroctenus* to be a cyrtaucheniid, following Bond and Opell (2002); [b]we consider *Homogona* and *Neohomogona* as distinct from *Cataxia*, following Main (1985); [c]this total does not include *Spiroctenus*, but does include *Iberesia* (Decae and Cardoso, 2005); [d]total includes *Iberesia*, and two undescribed genera included in this study. Numbers in parentheses following geographical regions designate the number of genera *endemic* to that region.

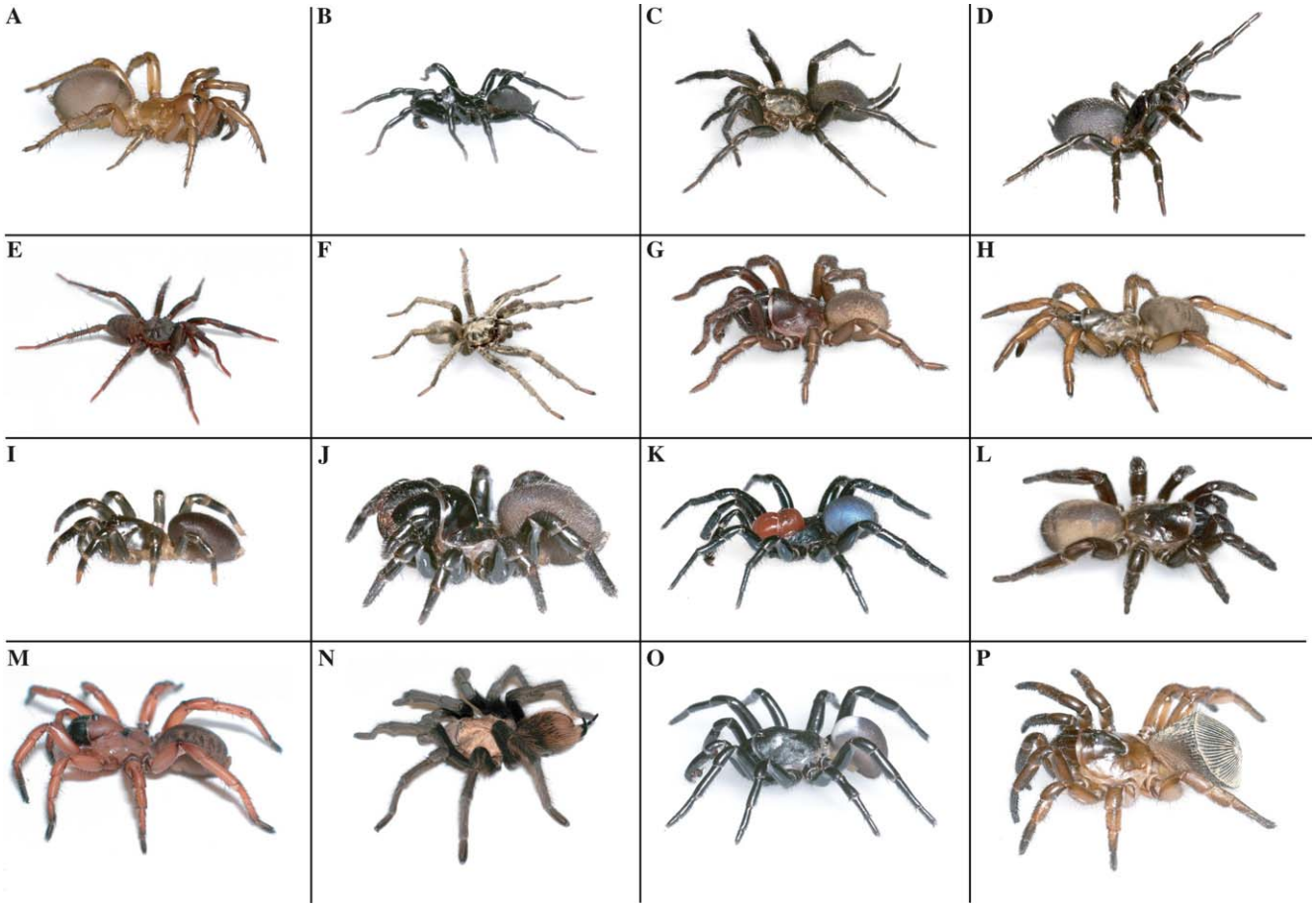

Fig. 2. Exemplary mygalomorph diversity. Pictured taxa include: (A) *Antrodiaetus unicolor* (Antrodiaetidae), (B) male *Sphodros atlanticus* (Atypidae), (C) *Namirea planipes* (Dipluridae), (D) *Atrax robustus* (Hexathelidae), (E) male *Microstigmata longipes* (Microstigmatidae), (F) male *Calisoga* sp. (Nemesiidae), (G) male *Kiama lachrymoides* (Cyrtaucheniidae), (H) *Aptostichus* sp. (Cyrtaucheniidae), (I) *Moggridgea* sp. (Migidae), (J) *Missulena* sp. (Actinopodidae), (K) male *Missulena occatoria*, (L) *Anidiops manstridgei* (Idiopidae), (M) *Homostola pardalina* (Cyrtaucheniidae), (N) *Aphonopelma* sp. (Theraphosidae), (O) male *Ummidia* sp. (Ctenizidae), (P) *Cyclocosmia truncata* (Ctenizidae).

assigned to the family Hexalthelidae, are known from as early as the lower Triassic (Selden and Gall, 1992). By the mid-Cretaceous, fossil representatives from several families can be found (see Eskov and Zonshtein, 1990; Selden, 2002; Selden et al., 2002; Penney et al., 2003). Biogeographic data are also consistent with Mesozoic divergences within several mygalomorph families. For example, the family Migidae has a classic Gondwanaland distribution, with described taxa restricted to southern South America, Africa, Madagascar, Australia, New Zealand, and New Caledonia (Griswold and Ledford, 2001). This distribution would suggest that migids, and other Gondwanan families (e.g., Idiopidae, Hexathelidae), are at least as old as the Jurassic fracturing of Gondwana itself (165–150 MYA, see Sanmartín and Ronquist, 2004).

Mygalomorphs also present special phylogenetic difficulties because of relatively conservative, and often homoplastic, patterns of morphological evolution (summarized in Bond and Opell, 2002). Although mygalomorphs certainly exhibit morphological divergence (see Fig. 2), the group is clearly less diverse than members of the sister group Araneomorphae, particularly in character-rich systems of genitalic and spinneret morphology. This relative morphological conservation has hampered morphological systematics in the group, and as suggested by Goloboff (1993), familial limits and inter-relationships prior to circa 1980 were "*terra incognita*." This situation has changed considerably over the past 20 years, as several revisionary and systematic works have considered global level (or nearly so) relationships of mygalomorphs (see Bond and Hedin, 2006; Bond and Opell, 2002; Eskov and Zonshtein, 1990; Goloboff, 1993, 1995; Griswold and Ledford, 2001; Raven, 1985). However, despite this obvious progress, many issues remain in mygalomorph systematics. A comparison of the two most comprehensive phylogenetic hypotheses, Raven (1985) and Goloboff (1993), illustrates these issues very clearly (Figs. 1B and C). Although some clades are recovered in both works, other areas of mygalomorph phylogeny remain unresolved, including the relative placement of key families (e.g., Mecicobothriidae, Microstigmatidae, Hexathelidae), and the composition of larger clades. Several diverse families (e.g., Cyrtaucheniidae, Dipluridae, Nemesiidae, Hexathelidae, and Ctenizidae) may not even be monophyletic (Fig. 1C). Needless to say, there is much need for further phylogenetic research in this interesting and diverse clade of spiders.

In this paper, we present a molecular phylogenetic analysis of mygalomorph relationships based on nuclear ribosomal data (28S (LSU) and 18S (SSU)) sampled for approximately 80 genera, representing all 15 recognized mygalomorph families. All previous molecular phylogenetic analyses involving mygalomorphs have either considered a smaller phylogenetic problem (e.g., subfamily phylogeny, see Bond and Opell, 2002; Bond and Hedin, 2006), or have focused on species-level relationships (e.g., Bond, 2004; Bond et al., 2001; Hendrixson and Bond, 2005, 2006). Although the 28S data provide the majority of phylogenetic information in this study, analysis of these data is challenging, due to both length variation and apparent heterogeneity in rates of substitution across taxa. The resulting alignment issues are addressed using multiple 28S sequence alignments, which are ultimately combined with 18S data in both parsimony and partitioned Bayesian analyses. Comparison of these analyses shows that many consistently recovered molecular clades are incongruent with current mygalomorph taxonomy, and as suspected, several mygalomorph families (e.g., Cyrtaucheniidae, Dipluridae, Nemesiidae, Hexathelidae, and Ctenizidae) are not recovered as monophyletic. However, most well-supported molecular clades also have at least some morphological and/or biogeographical support, and some recovered clades are consistent with old hypotheses that have recently fallen out of favor (e.g., the Atypoidea). Consideration of biogeographic patterns in light of rRNA phylogeny supports ancient diversification within mygalomorphs, as corroborated by the fossil record.

2. Materials and methods

2.1. Taxon sample and data collection

The taxon sample (see Appendix A) includes 76 mygalomorph genera representing all described mygalomorph families (*sensu* Raven, 1985). Sequences representing the suborder Mesothelae were used to root all mygalomorph trees. To test familial monophyly, we sampled multiple generic representatives for all but three mygalomorph families (Actinopodidae, Microstigmatidae, and Paratropididae). The generic sample for several families of dubious monophyly (e.g., Nemesiidae, Cyrtaucheniidae) is particularly large (see Table 1). The sample is geographically biased toward North America, South Africa, and Australia, regions of high generic diversity where we conducted fieldwork. Although we included opportunistic collections from Central America, South America, Europe, and Asia, the mygalomorph diversity of these regions is poorly represented in our sample. All specimens were identified using primary taxonomic literature. Some taxa were identified to genus only, either because the sequenced specimens were females (due to morphological conservatism, morphological characters reported in the literature are often insufficient to distinguish congeneric females), or because the sequenced specimens represent undescribed species (see Appendix A). Two genera in our sample appear to represent undescribed taxa, as we were unable to identify them using available keys. In general, we follow the taxonomy of Platnick (2006), except for the family placement of the genus *Spiroctenus*, which we consider to be a cyrtaucheniid, rather than a nemesiid (following arguments made in Bond and Opell, 2002).

Voucher specimens were preserved in 80% EtOH, and tissues (disarticulated legs) for DNA work were stored either in RNAlater (Ambion, Inc.) or 100% EtOH at −80 °C. All specimens have been assigned a unique

specimen identification number and have had a label referencing this study added to their vial. Upon completion of our on-going studies, voucher specimens will be deposited in the Nation Museum of Natural History, Smithsonian Institution, Washington, DC, USA and the California Academy of Sciences, San Francisco, CA, USA. Genomic DNA was extracted and purified from 1 to 2 legs using the DNeasy Tissue Kit (Qiagen, Inc.). Procedures used to amplify (via polymerase chain reaction) and sequence the 5′ half of the 28S rRNA (~1.9 kb) and nearly the entire (~1.7 kb) 18S rRNA gene are detailed in Bond and Hedin (2006). Sequence contigs for both the 28S and 18S data sets were assembled and edited using Sequencher (Genecodes, Madison, WI), then imported into MacClade V4.06 (Maddison and Maddison, 2001) for manual alignment, matrix concatenation, and further analysis.

2.2. Sequence alignments

We observed relatively little length variation in the 18S rRNA data set (see Section 3), allowing a fairly straightforward manual alignment, which was conducted using MacClade V4.06. Length variation was extreme in the 28S rRNA data, necessitating a multifaceted alignment approach that incorporated both different alignment methods and an array of alignment parameters. We evaluated sequence alignment using three different alignment approaches, including: (1) progressive alignment using CLUSTALX (Thompson et al., 1997) evaluated across a wide range of parameters (see below), (2) a probabilistic progressive alignment approach proposed by Löytynoja and Goldman (2005) that avoids repeated penalization of insertions and has the capacity to distinguish insertions from deletions through the employment of a phylogenetic scoring function, and (3) a "manual" alignment, completed by one of the authors (MH) using MacClade V4.06. Because taxon labels were not hidden during this process, preconceived notions of mygalomorph phylogeny may have biased the manual alignment.

The computer program ClustalX (Thompson et al., 1997) was used to align the 28S data using a traditional progressive alignment approach. To investigate the sensitivity of the phylogenetic solution to alignment parameters, we explored gap-opening/gap-extension parameters over a broad range of values: 15/6–33/15 (10 alignments, parameters adjusted stepwise +2/+1). Gap-opening and extension parameters were adjusted concurrently for both multiple and pairwise alignments. Ambiguous regions of the ClustalX alignments were not modified or manually adjusted upon completion of the alignments.

The computer program Prank version 1508b (available at http://www.ebi.ac.uk/goldman/prank) employs the approach outlined by Löytynoja and Goldman (2005). We used the default gap-opening rate and gap-extension probabilities with the correction for insertion sites enabled and allowing the option that gaps be closed. We considered alignments based on both the JC and HKY models of molecular evolution implemented in this software. First iteration alignments were based on a guide tree taken from ClustalX using a pairwise gap opening and extension cost of 15/6. As pointed out by Löytynoja and Goldman (2005), any multiple alignment for which the correct phylogeny is unknown is going to be sensitive to alignment guide tree choice. Upon close examination, we found the guide tree generated by ClustalX to be highly suspect (i.e., recovering few expected mygalomorph relationships). Therefore, we produced a second-generation alignment from a rooted guide tree based on a distance analysis (minimum evolution) conducted in PAUP* ver. 4.0b10 (Swofford, 2002). This analysis consisted of a single heuristic search with a General Time Reversible Model of molecular evolution with a γ-shaped parameter of rate heterogeneity, the objective function set to unweighted least squares, and branch lengths constrained to be non-negative. A second iteration alignment was then produced in Prank from the newly formed guide tree, using the same parameters described for the first iteration alignments above.

2.3. Phylogenetic analyses of 28S alignments

All of the resulting individual 28S alignments (10 Clustal, 2 Prank, 1 Manual) were first analyzed as a batch parsimony search using PAUP* v. 4.0b10 (Swofford, 2002). For each alignment, we performed 10,000 random addition sequence replicates with TBR branch swapping, each search replicate limited to 1 million rearrangements, treating gaps as missing. All alignments and associated tree files are available for download at http://www.mygalomorphae.org. Due to possible inefficiencies of heuristic searches for data sets of this size (99 terminals), we repeated four searches using the improved search technologies (Goloboff, 1999; Nixon, 1999) implemented in the computer program TNT v. 1.0 (Goloboff et al., 2003). Searches in TNT consisted of 1000 random addition sequence replicates each employing default sectorial, ratchet and tree fusing parameters. Upon completion of the "New Technology search" a traditional search with TBR branch swapping was run using the trees retained in memory from the previous search.

2.4. Visualization of 28S alignment tree space

Trees obtained from parsimony analyses of all 28S alignments were compared using multi-dimensional scaling (MDS) of unweighted Robinson–Foulds topological distances. This approach helped to visualize the "tree space" implied by the various alignment procedures and parameters explored (see Hillis et al., 2005 for other examples of tree space visualization), and was used as a guide for selecting a subset of alignments for combined phylogenetic analyses. The MDS scaling was implemented using the TreeSetVis module (Amenta et al., 2005) in Mesquite V1.05 (Maddison and Maddison, 2004). We repeated the procedure four times using the "Scramble" feature to

ensure that the MDS had not become trapped in local optima.

2.5. Combined data analyses

Based on the visualization of alignment tree space (see Section 3 below) we selected a subset of 28S alignments to combine with the manually aligned 18S data matrix. Combined matrices were analyzed using both parsimony and Bayesian inference. Parsimony analyses were conducted in TNT using the same set of parameters as described for the 28S searches described above. For both the 18S and 28S data partitions, the computer program MrModeltest v. 2 (Nylander, 2004) was used to select an appropriate substitution model by Akaike Information Criterion (following Posada and Buckley, 2004). Using the model(s) of substitution indicated by AIC, partitioned analyses employing Bayesian inference were conducted with MrBayes versions 3.1.1 and 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Analyses of the combined data sets consisted of two simultaneous runs each with four simultaneous Markov Chain Monte Carlo (MCMC) chains run initially for 1,000,000 generations (numbers of generations subsequently increased as needed), saving the current tree to file every 100 generations. Default cold and heated chain parameters were used. The separate, simultaneous runs were compared every 1000th generation to assess convergence. At the end of the run we considered the sampling of the posterior distribution to be adequate if the average standard deviation of split frequencies was <0.01 (see Ronquist et al., 2005). Parameters of each MCMC run were summarized using the *sump* and *sumt* commands in MrBayes and further checked for chain stationarity and run parameter convergence using the computer program Tracer version 1.3 (Rambaut and Drummond, 2005). Trees prior to log likelihood stabilization (burnin) and convergence were discarded before producing a majority rule consensus tree.

2.6. Bayesian hypothesis testing

We used Bayes factors (see Ronquist et al., 2005) to compare the posterior odds of our preferred Bayesian tree topology (see below) to Bayesian trees that forced the monophyly of currently accepted mygalomorph groups. Monophyly constraint analyses were conducted in MrBayes ver. 3.2.1 using the command *prset* topologypr = constraint. All analyses consisted of two simultaneous runs each with an abbreviated three MCMC chains run for 2–3 million generations. Using the *sump* command in MrBayes, we sampled the stationary (post-burnin) posterior distribution to obtain the harmonic mean of tree likelihood values (following Nylander et al., 2004; Ronquist et al., 2005). Bayes factors were then computed by taking the difference between the marginal likelihood values of the preferred topology, T_1, and the constrained topology, T_0 (see Brandley et al., 2005; Nylander et al., 2004).

3. Results

3.1. Data characteristics

Ninety-nine unique 28S sequences from seventy-six mygalomorph genera were compiled to form working data matrices (see Appendix A). Of these, one *Aphonopelma* sequence was taken from GenBank, 30 are reported in Bond and Hedin (2006), and the remaining sequences were generated for this research. Nine additional redundant sequences, gathered from taxa already represented in the matrix, were found to be identical to those reported and therefore excluded from further analysis (see Appendix A).

We generated 28S sequences that are homologous to the 5′-end of the 28S gene, starting at the B20 region and extending to the E17 region (see Ben Ali et al., 1999). This section includes the most variable regions known from eukaryotic 28S genes (e.g., C, D5, D14, D20; see Ben Ali et al., 1999), and these regions also vary considerably in sequence length in mygalomorphs. Whereas the majority of mygalomorph sequences are about 1.9 kb in length, sequences of the atypids (*Sphodros*, *Atypus*) antrodiaetids (*Antrodiaetus*, *Atypoides*), and mecicobothriids (*Megahexura*, *Hexura*) all include insertions that increase this length by 60–225 basepairs. These insertion events are not localized, but rather, are spread across several of the regions mentioned above. Including all mygalomorph sequences, uncorrected sequence divergence values average 6.3 percent (min = 0.01%, mean = 6.29%, max = 23.05%; calculated from manual alignment); excluding the six taxa mentioned above decreases this average to 4.87 percent. Although base composition does not vary in a significant manner across taxa ($\chi^2 = 236.46$, df = 294, $P = 0.99$; test conducted in PAUP* on manual alignment), we note that these same six sequences are noticeably AT rich (Table 2).

The taxonomic composition of the 18S matrix corresponds closely to the 28S matrix, but includes fewer (86) sequences. Two sequences were taken from GenBank, 28 are reported in Bond and Hedin (2006), and the remaining are newly reported here. In a majority of cases, 28S and 18S data were gathered from the same specimen. In fewer instances, these data were gathered from either conspecific (7 cases) or congeneric (6 cases) specimens, but considered equivalent for purposes of combined data analysis (see

Table 2
Summary of taxonomic variation in base composition

Data partition	Taxon set	A	C	G	T
28S	All mygalomorphs	0.208	0.272	0.332	0.188
	Atypoidea	0.233	0.247	0.317	0.203
	Non-atypoids	0.205	0.274	0.334	0.187
	Mesothelae	0.209	0.263	0.342	0.186
18S	All mygalomorphs	0.251	0.231	0.273	0.245
	Atypoidea	0.251	0.233	0.275	0.241
	Non-atypoids	0.251	0.230	0.273	0.246
	Mesothelae	0.250	0.234	0.275	0.241

Appendix A). Seventeen additional redundant 18S sequences were found to be identical to sequences already represented in the matrix and excluded from data analysis (see Appendix A).

Hendriks et al. (1988) published a complete 18S sequence from a tarantula (*Aphonopelma*) that is 1814 bp in length and includes a secondary structure with 48 universal or eukaryotic-specific helices (conserved stems and/or loops). We generated sequences that are homologous to most (~1750 bp) of this gene region, all but two (*Bymainiella* MY2045 and *Migas* MY2104) of which are more than 95% complete. Length variation across taxa is confined to one of seven regions, and again, is largely attributable to insertion events in atypids (*Atypus*, *Sphodros*), antrodiaetids (*Antrodiaetus*, *Atypoides*), and mecicobothriids (*Hexura*, *Megahexura*) that result in longer sequences. The *Megahexura* sequence is particularly long and divergent, but was validated by the sequencing of multiple individuals (see Appendix A). Apart from these long sequences, 18S is highly conserved within mygalomorphs, with uncorrected sequence divergence values averaging less than one percent across all pairwise comparisons (min = 0%, mean = 0.96%, max = 4.84%). There is no evidence for statistically significant base composition variation in these sequences ($\chi^2 = 34.64$, df = 300, $P = 1.0$; test conducted in PAUP*; Table 2).

3.2. Alignment, analysis, and tree space visualization of 28S data

Heuristic parsimony analyses were conducted on all 13 different 28S alignments, treating gaps as missing. TNT searches confirmed that the parsimony searches were finding the shortest trees possible; the only discrepancy was that PAUP* in a couple of instances found more trees of equal length. The parsimony tree space implied by these different alignments was visualized using multi-dimensional scaling (MDS) of unweighted Robinson–Foulds topological distances (Fig. 3). This visualization highlights several patterns in the data. First, the total tree space implied by all alignments is rather "diffuse," clearly demonstrating that both alignment procedure and parameterization is influencing the resulting phylogenetic estimate. Second, some alignments result in trees found in multiple, topologically distant, tree islands (e.g., Prank HKY1, 15_06, etc.), whereas others result in trees that are more cohesive in space (e.g., Prank HKY2, 33_15, etc). Third, different alignments result in different numbers of most-parsimonious trees, with some implying a single most-parsimonious tree.

3.3. Combined data analysis

We chose a subset of six 28S alignments for further combined data analysis, including Manual, Prank HKY2, Prank JC2, Clustal 19_08, Clustal 31_14, and Clustal 33_15. Alignment lengths are reported in Table 3. As a point of reference, about 15% (on average) of the positions include gaps in the shortest (31_14) alignment. All of these alignments resulted in multiple most-parsimonious trees that were found in a single tree island, and in combination, spanned the tree space implied by all thirteen alignments (see Fig. 3). We reasoned that this subset would thus represent a fair cross-section of the total implied tree space.

Results of parsimony analyses (using TNT) of the six concatenated matrices are summarized in Table 3, which indicates that 28S Prank combined matrices result in shorter trees with higher average CI and RI values. This difference likely reflects the fact that in these longer, gappier alignments, more positions are scoring as missing, decreasing both tree length and observed homoplasy. Fig. 4 shows a strict consensus of parsimony trees resulting from the Prank HKY2 combined matrix, and summarizes consistency in clade recovery across the six different combined matrices. The consistently recovered clades are thoroughly discussed below in the context of the Bayesian results.

A GTR + I + G model was chosen by the Akaike Information Criterion as the best-fit model for both 18S and 28S data partitions. Results of partitioned Bayesian analysis searches are summarized in Table 4. Although the average standard deviation of split frequencies was relatively high for two Clustal alignments (19_18, 33_15), further *sump* and Tracer comparisons of independent runs suggested convergence of all parameters. Fig. 5 shows a Bayesian majority-rule consensus tree derived from the Prank JC2 combined matrix, and summarizes consistency in clade recovery across all combined matrices. We chose to illustrate this tree because it is well resolved, generally consistent with other Bayesian results, but in addition includes clades congruent with current mygalomorph classification (migid monophyly in particular).

Bayesian analyses consistently and strongly support (posterior probability values above 0.95) the monophyly of the families Antrodiaetidae, Atypidae, Idiopidae, and Theraphosidae (Fig. 5). Migids are supported (often weakly) as monophyletic in only three of six alignments. Sampled nemesiids form a clade in three of six alignments, but only with inclusion of *Microstigmata* and the cyrtaucheniids *Kiama*, *Acontius*, and *Fufius*. Both ctenizids and cyrtaucheniids are fragmented on all Bayesian trees, represented by well-supported branches or subclades that never fall together (e.g., the cyrtauchniid subclades Euctinizinae, Ancylotrypines, *Kiama*, etc). Neither diplurids nor hexathelids are monophyletic, these groups forming a mixed grade toward the base of the tree. The only consistently supported deeper grouping is the basal separation of a clade including antrodiaetids, atypids, and mecicobothriids from all other families. A barychelid plus theraphosid grouping is also strongly supported in all Bayesian trees.

3.4. Bayesian hypothesis testing

We compared the posterior odds of our preferred Bayesian tree topology (PrankJC2, see above) to those of Bayesian trees that forced the monophyly of the groups

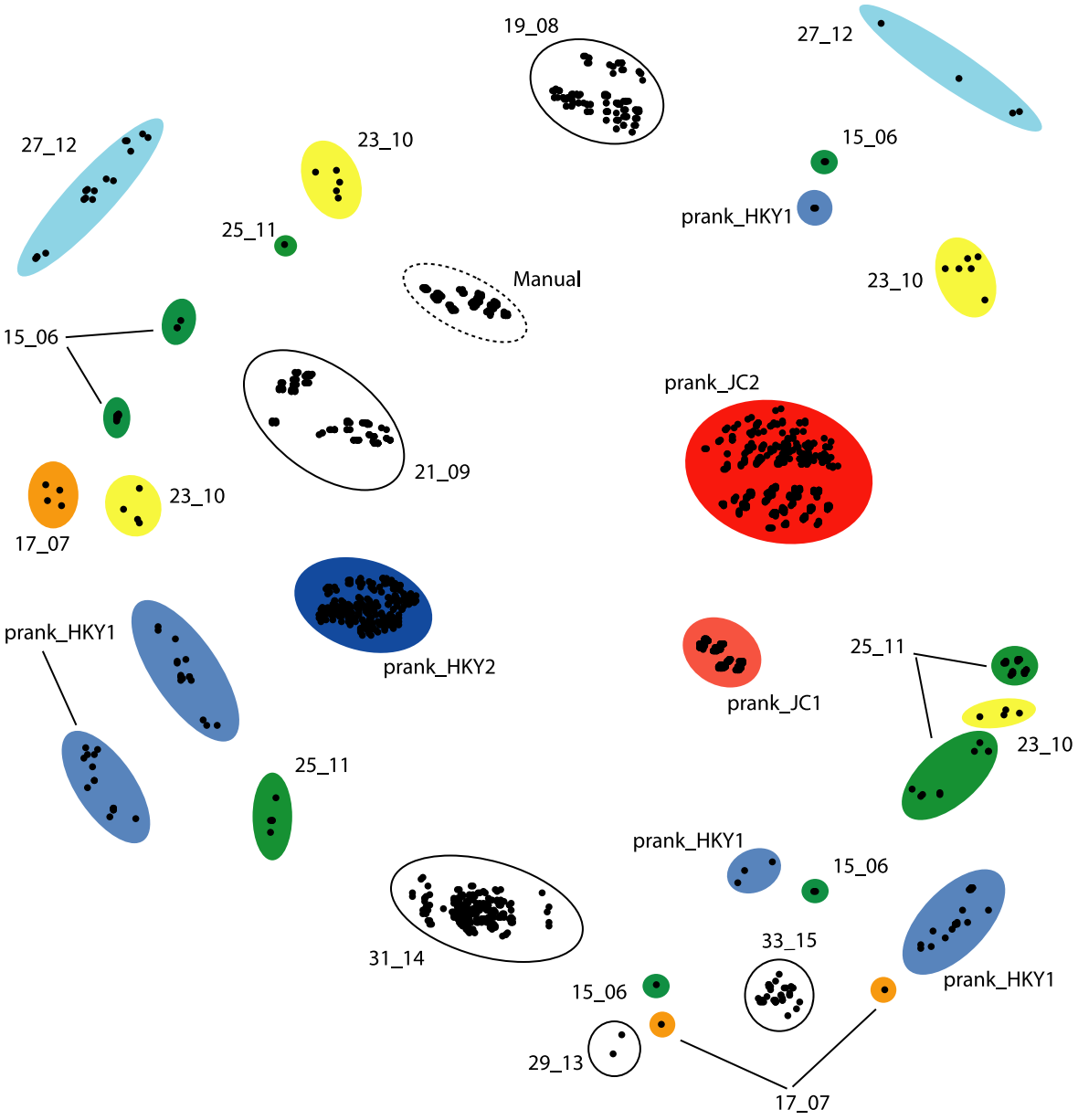

Fig. 3. 28S parsimony tree space implied by different alignment methods and parameters, visualized using multi-dimensional scaling (MDS) of unweighted Robinson–Foulds topological distances. Different alignments and/or parameter settings are circled or highlighted by unique colors.

Table 3
Summary of combined data parsimony analyses

Alignment	28S alignment length (bp)	No. of trees	Steps	CI	RI	RC
19_8	2322	9	6803	0.37	0.48	0.18
31_14	2170	207	6895	0.37	0.49	0.18
33_15	2217	9	6925	0.37	0.48	0.18
Prank JC2	2844	9	4987	0.47	0.59	0.28
Prank HKY2	3078	288	4878	0.48	0.60	0.29
Manual	2461	8	5502	0.43	0.56	0.24

Mecicobothriodina (Raven, 1985) and Bipectina (Goloboff, 1993), and the families Ctenizidae, Cyrtaucheniidae, Dipluridae, and Hexathelidae (Raven, 1985). The preferred (unconstrained) topology hypothesis results in an increase of 10–365 likelihood units, as compared to constrained topology hypotheses (Table 5). Following Kass and Rafferty, 1995, this magnitude of difference suggests that we should prefer the unconstrained hypothesis, and consider the evidence against alternative topology hypotheses as "very strong" (Table 5).

4. Discussion

4.1. Mygalomorph systematics

Of the many uncertainties evident in the higher-level systematics of mygalomorph spiders, two problems are particularly obvious. First, in several instances, it is not clear

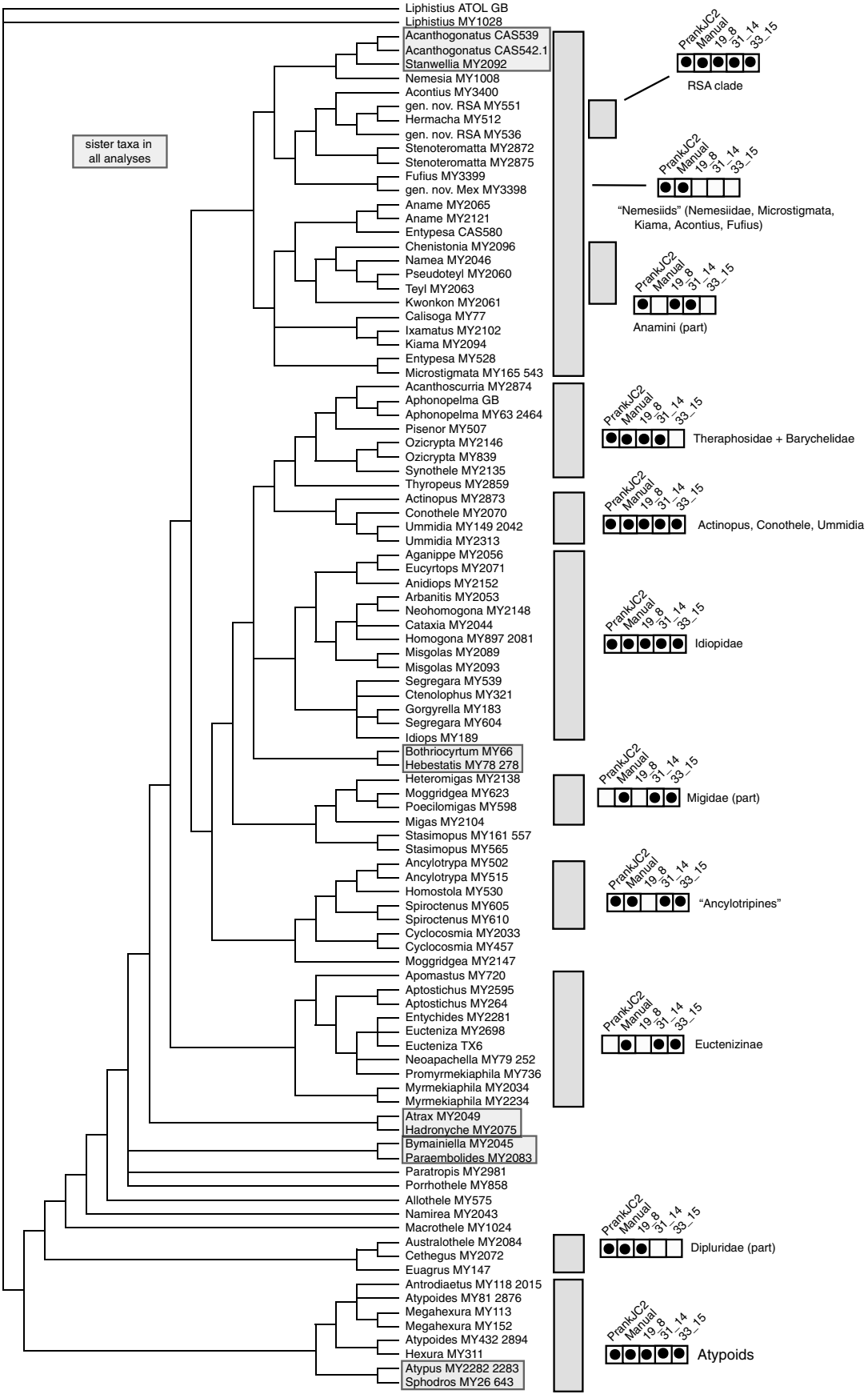

Fig. 4. Parsimony strict consensus tree based on TNT analysis of combined 18S (manual alignment) and 28S (PrankHKY2 alignment) matrices. Groups recovered in strict consensus trees of the other combined matrices (PrankJC2, Manual, 19_8, 31_14, and 33_15) are highlighted. Parsimony tree statistics for all combined matrices are found in Table 3.

Table 4
Summary of combined data Bayesian analyses

Alignment	Ngens	Ln (Ar)	Ln (Hr)	asdsf	Burnin	99%
19_8	17.5	−39370.13	−39446.26	0.10	10	76,691
		−39370.98	−39450.88			
31_14	15.0	−39234.95	−39317.11	0.04	5	49,374
		−39237.56	−39311.16			
33_15	6.5	−39637.99	−39720.74	0.16	3	12,644
		−39659.52	−39742.51			
Prank JC2	6.863	−31954.08	−32031.99	0.01	5	21,578
		−31953.01	−32031.32			
Prank HKY2	8.385	−31790.91	−31882.88	0.01	5	40,693
		−31791.69	−31875.77			
Manual	9.711	−32194.14	−32272.88	0.04	7	23,702
		−32198.19	−32270.13			

Notes: Ngens (number of generations) and burnin are given in units of a million; asdsf = average standard deviation of split frequencies. Despite relatively high asdsf values for the 19_18 and 33_15 alignments, independent runs showed appropriate mixing for most parameters (*sump* in MrBayes and Tracer). The last column refers to the number of trees sampled from the 99% credible set.

what comprises a mygalomorph "family." Prior to the seminal work of Raven (1985), many mygalomorph families were obvious polyphyletic "dumping grounds" (e.g., Ctenizidae, Dipluridae). One of the primary goals of Raven (1985) was to redefine familial limits, both by transferring taxa amongst existing families, but also by reclassifying smaller groups (e.g., subfamilies) as families. However, Raven himself recognized that his taxonomy was likely imperfect, as many of his redefined families were supported by few unambiguous morphological synapomorphies. These suspicions were confirmed by Goloboff (1993, 1995) and Bond and Opell (2002), who, through phylogenetic analyses of morphology, suggested that the families Barychelidae, Cyrtaucheniidae, Dipluridae, Hexathelidae, and Nemesiidae were likely not monophyletic (Fig. 1C). These authors did not, however, propose formal taxonomic changes at the family level. As such, the family-level classification scheme of Raven (1985) stands as the taxonomic framework used in this study.

A second area of contention in mygalomorph systematics is the grouping of families into larger clades. For example, Raven's (1985) hypothesis of family relationships differs drastically from prior works, with the division into the clades Fornicephalae and Tuberculotae representing a completely novel hypothesis (Fig. 1B). But as repeatedly pointed out by Raven (1985), most of his proposed groupings are supported by few unambiguous synapomorphies, as morphological homoplasy at a global level is ubiquitous. Goloboff (1993) reanalyzed mygalomorph relationships using computer-assisted analyses of morphology, and recovered phylogenies that differ considerably from the Raven hypothesis (Fig. 1C). Some large clades are recovered in both analyses (e.g., Rastelloidina, Domiothelina, Theraphosoidina), but other deep clades are not held in common (e.g., Fornicephalae, Tuberculotae, Quadrithelina).

In the discussion below, we summarize similarities and differences observed between our molecular findings and prior morphology-based research and classification systems. Given the many uncertainties in mygalomorph systematics (as summarized above), incongruence between molecular and morphological hypotheses is inevitable. However, as discussed below, this incongruence is generally focused in areas of mygalomorph phylogeny that others have recognized as "weakly supported." In fact, the molecular data corroborate many current morphological hypotheses, and suggest the viability of some older, perhaps over-looked, hypotheses.

Idiopidae—Idiopids (e.g., Fig. 2L) are a mostly Gondwanan family of trapdoor-building spiders, although some taxa live in upon burrows. Monophyly of the family is fairly uncontroversial, supported by three apparently apomorphic features of the male palpus (Raven, 1985). Raven separated idiopids into three subfamilies, including the Idiopinae (South and Central America, Africa, India, western Asia), Arbanitinae (Australia and New Zealand), and Genysinae (India, Madagascar, South America). The rRNA data strongly support the monophyly of the family, and furthermore, the monophyly of sampled subfamilies (Idiopinae and Arbanitinae). Although we have only sampled African idiopines, the monophyly of this morphologically homogeneous subfamily seems secure (see Raven, 1985). Within the Australian arbinitine radiation, the rRNA data consistently recover a monophyletic Aganippini (*Aganippe*, *Eucyrtops*, *Anidiops*; see Main, 1985), a predominantly western Australian clade supported by at least one morphological synapomorphy (Raven, 1985, Fig. 7). The tribe Arbanitini is, however, not consistently recovered as a monophyletic group. Although both Raven (1985) and Goloboff (1993) hypothesize that idiopids are early-diverging Domiothelina (see Figs. 1B and C), the rRNA data suggest no consistent affinities with other domiotheline taxa (Figs. 4 and 5).

Migidae—The classic family Migidae (e.g., Fig. 2I), supported by several morphological synapomorphies, is one of the most morphologically and behaviorally distinctive mygalomorph families (Goloboff and Platnick, 1987; Gris-

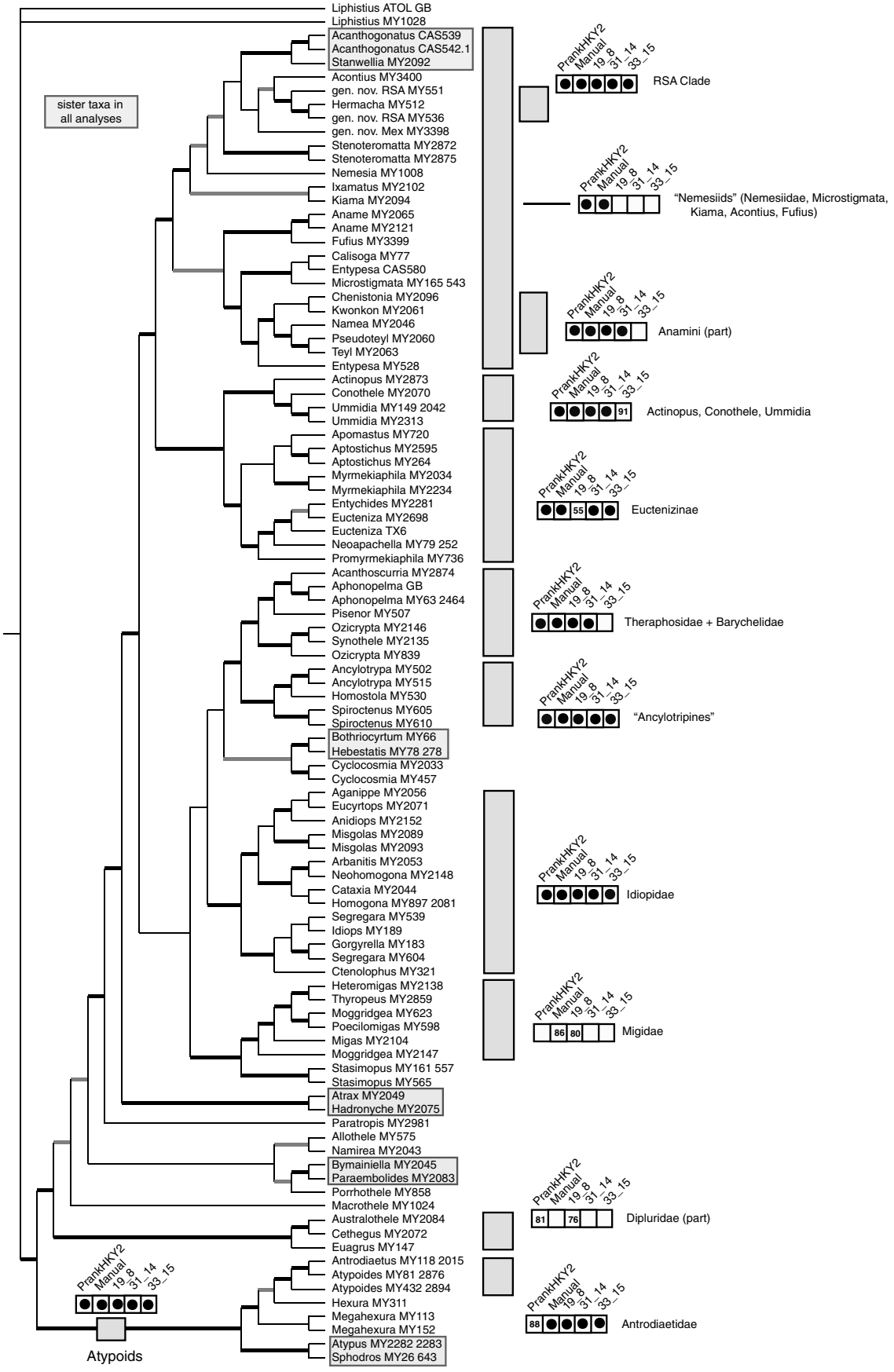
Liphistius ATOL GB
Liphistius MY1028
Acanthogonatus CAS539
Acanthogonatus CAS542.1
Stanwellia MY2092
Acontius MY3400
gen. nov. RSA MY551
Hermacha MY512
gen. nov. RSA MY536
gen. nov. Mex MY3398
Stenoteromatta MY2872
Stenoteromatta MY2875
Nemesia MY1008
Ixamatus MY2102
Kiama MY2094
Aname MY2065
Aname MY2121
Fufius MY3399
Calisoga MY77
Entypesa CAS580
Microstigmata MY165 543
Chenistonia MY2096
Kwonkon MY2061
Namea MY2046
Pseudoteyl MY2060
Teyl MY2063
Entypesa MY528
Actinopus MY2873
Conothele MY2070
Ummidia MY149 2042
Ummidia MY2313
Apomastus MY720
Aptostichus MY2595
Aptostichus MY264
Myrmekiaphila MY2034
Myrmekiaphila MY2234
Entychides MY2281
Eucteniza MY2698
Eucteniza TX6
Neoapachella MY79 252
Promyrmekiaphila MY736
Acanthoscurria MY2874
Aphonopelma GB
Aphonopelma MY63 2464
Pisenor MY507
Ozicrypta MY2146
Synothele MY2135
Ozicrypta MY839
Ancylotrypa MY502
Ancylotrypa MY515
Homostola MY530
Spiroctenus MY605
Spiroctenus MY610
Bothriocyrtum MY66
Hebestatis MY78 278
Cyclocosmia MY2033
Cyclocosmia MY457
Aganippe MY2056
Eucyrtops MY2071
Anidiops MY2152
Misgolas MY2089
Misgolas MY2093
Arbanitis MY2053
Neohomogona MY2148
Cataxia MY2044
Homogona MY897 2081
Segregara MY539
Idiops MY189
Gorgyrella MY183
Segregara MY604
Ctenolophus MY321
Heteromigas MY2138
Thyropeus MY2859
Moggridgea MY623
Poecilomigas MY598
Migas MY2104
Moggridgea MY2147
Stasimopus MY161 557
Stasimopus MY565
Atrax MY2049
Hadronyche MY2075
Paratropis MY2981
Allothele MY575
Namirea MY2043
Bymainiella MY2045
Paraembolides MY2083
Porrhothele MY858
Macrothele MY1024
Australothele MY2084
Cethegus MY2072
Euagrus MY147
Antrodiaetus MY118 2015
Atypoides MY81 2876
Atypoides MY432 2894
Hexura MY311
Megahexura MY113
Megahexura MY152
Atypus MY2282 2283
Sphodros MY26 643
sister taxa in all analyses
Atypoids
PrankHKY2 Manual 19_8 31_14 33_15
RSA Clade
"Nemesiids" (Nemesiidae, Microstigmata, Kiama, Acontius, Fufius)
Anamini (part)
Actinopus, Conothele, Ummidia
Euctenizinae
Theraphosidae + Barychelidae
"Ancylotripines"
Idiopidae
Migidae
Dipluridae (part)
Antrodiaetidae
91
55
86
80
81
76
88

Table 5
Summary of Bayes factor analyses

Constraint	Model likelihood (harmonic mean)		Evidence against T_0	
	$\log_e \hat{f}(X\|T_1)$	$\log_e \hat{f}(X\|T_0)$	$\log_e B_{10}$	
Ctenizidae	−32031.99	−32064.37	32.38	"very strong"
Mecicobothriodina	−32031.99	−32397.25	365.26	"very strong"
Cyrtaucheniidae	−32031.99	−32331.81	299.82	"very strong"
Hexathelidae	−32031.99	−32067.87	35.88	"very strong"
Dipluridae	−32031.99	−32042.07	10.08	"very strong"
Bipectina	−32031.99	−32050.40	18.41	"very strong"

wold and Ledford, 2001; Raven, 1985). Many species placed in this family build their unique sac-like, trapdoor-covered burrows on the sides of trees throughout the southern hemisphere. Migid taxa possess quadrate and keeled fangs, have a recurved thoracic fovea, and lack both anterior sternal sigilla and a rastellum. Given the strong morphological support for the family, the relatively weak support offered by the rRNA data is somewhat surprising. Migids are recovered as monophyletic in only one of six combined-data parsimony searches (19_8 alignment, Fig. 4), and only half of the combined-data Bayesian analyses (Fig. 5), sometimes with weak support. Within the Migidae, molecular data never support a monophyletic *Moggridgea*, suggesting that the Australian *Moggridgea tingle* (Main, 1991) may not be as closely related to African *Moggridgea* as previously hypothesized. This result, however, conflicts with the results of Griswold and Ledford (2001), who cite three morphological synapomorphies supporting *Moggridgea* monophyly. The superfamily Migoidea, which comprises Migidae plus Actinopodidae (Griswold and Ledford, 2001; Platnick and Shadab, 1976), is never recovered in rRNA analyses.

Ctenizidae—The family Ctenizidae (e.g., Figs. 2O and P) comprises the species typically thought of as the trapdoor or corkdoor spiders. Raven (1985) dismantled the old, clearly polyphyletic Ctenizidae, transferring many taxa to other, mostly rastelloid families. Despite this downsizing of the family, the remaining genera are still not obviously members of a single clade, as reflected in Raven's statement (1985, p 4) that a single defining apomorphy includes "possibly the single tooth on the paired claw." Our sample of ctenizid diversity includes the ctenizine genera *Bothriocyrtum*, *Cyclocosmia*, and *Stasimopus*, and the pachylomerines *Hebestatis*, *Conothele*, and *Ummidia*. Family and subfamily monophyly is not supported by the rRNA data, and constraint analyses likewise do not support a family monophyly hypothesis (Table 5). Consistently recovered placements include *Stasimopus* at the base of migids, *Conothele* and *Ummidia* (always together) with the actinopodid *Actinopus*, and the Californian *Hebestatis* plus *Bothriocyrtum* always together as sister taxa (see Figs. 4 and 5). Considering that all currently recognized ctenizid genera are robust, ground-dwelling trapdoor spiders, the anterolateral leg spination shared by these taxa (and cited as a possible synapomorphy, Raven, 1985, p. 57), may be convergently evolved. This convergence argument is supported by the observation of similar anterolateral spines in some trapdoor-building migids and idiopines (see Goloboff, 1993; Griswold and Ledford, 2001; Raven, 1985).

Cyrtaucheniidae—The family Cyrtaucheniidae includes a diverse assemblage of taxa, often referred to as the wafer-lid trapdoor spiders (e.g., Figs. 2G, H, and M). Raven (1985) elevated the Cyrtaucheniinae to family status, and transferred to this family other genera that were then classified as diplurids and ctenizids. Raven argued that three characters supported cyrtaucheniid monophyly, including the first and second tarsi both scopulate and weakly spinose, and the presense of multilobular spermatheca. However, he also noted apparent affinities both between members of the subfamily Euctenizinae and rastelloid taxa (e.g., ctenizids), and between other cyrtaucheniids and nemesiids. Cyrtaucheniid monophyly was tentatively rejected by Goloboff (1993) with a small cyrtaucheniid sample (5 genera), and more conclusively rejected by Bond and Opell (2002), who scored morphological data for 16 cyrtaucheniid genera. In analyses of Bond and Opell (2002), sampled cyrtaucheniids form a grade of multiple lineages at the base of the Rastelloidina. Rastelloids are still recovered as monophyletic, but the authors hinted that a larger sample of non-rastelloids (e.g., additional nemesiids) might ultimately disrupt this monophyly.

We have collected molecular data for representatives of all three cyrtaucheniid subfamilies recognized by Raven (1985), including all members of the North American Euctenizinae (*Aptostichus*, *Apomastus*, *Promyrmekiaphila*, *Myrmekiaphila*, *Eucteniza*, *Entychides*, and *Neoapachella*; see Bond and Opell, 2002), one of two cyrtaucheniines

Fig. 5. Bayesian majority rule consensus tree based on analysis of combined 18S (manual alignment) and 28S (PrankJC2 alignment) matrices. Thickened black branches indicate posterior clade probabilities above 0.95; thickened grey branches indicate posterior clade probabilities above 0.50. Major clades recovered in Bayesian consensus trees of other combined matrices (PrankHKY2, Manual, 19_8, 31_14, and 33_15) are highlighted. Dark circles represent posterior clade probabilities above 0.95. Values below this cut-off, but above 0.50, are shown as numerical values. Tree statistics for all Bayesian searches are found in Table 4.

(the African *Homostola*), and a diverse assemblage of aporoptychines (*Kiama* from Australia, *Fufius* from South America, and the African *Acontius*, *Ancylotrypa* and *Spiroctenus*). Raven (1985) considered the inclusion of *Kiama* and *Ancylotrypa* into this last subfamily as *incertae sedis*. Consistent with prior morphological analyses of this family, this taxon sample is never recovered as monophyletic, and constraint analyses likewise do not support monophyly of the family (Table 5). Instead, "cyrtaucheniids" are fragmented into at least four separate lineages on rRNA trees (Figs. 4 and 5). The Australian *Kiama* is nested within a clade of mostly nemesiid taxa, and often allied with the Australian *Ixamatus*. The peculiar pustulose cuticle (Bond and Opell, 2002; Raven, 1981) and elevated, rodlike tarsal organ (Raven 1981, Fig. 63; 1985) shared by these taxa may represent morphological synapomorphies for this pairing. The aporoptychines *Fufius* and *Acontius* are also nested within a mostly nemesiid clade, but are not obviously closely related to one another. A clade of South African taxa, including *Ancylotrypa*, *Homostola*, and *Spiroctenus* (informally called the "Ancylotripine" clade), is recovered in essentially all analyses (Figs. 4 and 5). Finally, the euctenizines, as originally defined by Raven (1985), are recovered as monophyletic in essentially all analyses. Except for the placement of *Homostola*, this general phylogenetic structuring (*Kiama*, *Fufius* plus *Acontius*, *Ancylotrypa*, and euctenizines all on independent branches) is consistent with the morphological findings of Bond and Opell (2002, Fig. 6).

Theraphosidae, Barychelidae, and Paratropididae—The family Theraphosidae, including the "true" tarantulas (e.g., Fig. 2N), is the most genus-rich of all mygalomorph families (Platnick, 2006). Theraphosids share distinctive claw tufts and well-developed scopulae on all legs (Raven, 1985). As such, we have presumed monophyly of this family and have not included a large taxon sample for molecular analyses. The two genera that we have sampled (*Acanthoscurria* and *Aphonopelma*), placed into the same subfamily by Raven (1985), are always sister taxa on rRNA trees (Figs. 4 and 5). Theraphosids are clearly morphologically related to barychelids (see Raven, 1985, 1994), and this relationship is supported by rRNA trees. The placement of *Pisenor*, however, makes barychelids paraphyletic with respect to theraphosids. We note, however, that the *Pisenor* specimens that we sequenced lack teeth on the anterior booklung openings, which is a proposed synapomorphy for the Barychelidae (see Goloboff, 1993, Figs. 4 and 5). If *Pisenor* is misplaced, both Barychelidae and Theraphosidae are monophyletic.

We have only included a single representative of the distinctive family Paratropididae (subfamily Paratropidinae), so cannot test the monophyly of this family. However, we can test Raven's (1985) hypothesis of a sister-taxon relationship between paratropidids and theraphosids. The *Paratropis* that we have sampled has a fairly unique rRNA sequence, and the placement of this taxon varies considerably across analyses. A grouping with theraphosids and barychelids is, however, never recovered in molecular trees. This result is perhaps not surprising, because unlike theraphosids and barychelids, paratropidines generally lack claw tufts, leg scopulae, and fine hairs clothing the legs. Glabropelmatine paratropidids are more similar to the former groups, but we have not sampled this subfamily.

Nemesiidae and Microstigmatidae—The recognized generic diversity represented in the family Nemesiidae, the tube trapdoor or wishbone spiders (e.g., Fig. 2F), is surpassed only by theraphosids and barychelids (see Table 1; Platnick, 2006). But unlike these latter two families, convincing morphological support for nemesiid monophyly is generally lacking, as it is basically impossible to cite a morphological definition that applies universally to nemesiid taxa (see Goloboff, 1995). Raven (1985) redefined the limits of this family, but even he doubted nemesiid monophyly (pp. 61, 65). These doubts were supported by analyses of Goloboff (1993, Fig. 1C). The most comprehensive analysis of nemesiid relationships (Goloboff, 1995) includes most Neotropical and several non-Neotropical "nemesiids," scored for over 100 morphological characters. Again, this analysis strongly suggests that nemesiids (*sensu* Raven, 1985) are paraphyletic with respect to other mygalomorph taxa, including Theraphosoidina, Microstigmatidae, and some cyrtaucheniids.

Our nemesiid generic sample represents five of six subfamilies recognized by Raven (1985, pp. 43, 81). These genera never form a clade exclusive of other mygalomorph taxa on molecular trees (Figs. 4 and 5). Instead, sampled nemesiids form a clade in half of the combined-data Bayesian analyses, and one-third of the parsimony analyses, but only with inclusion of *Microstigmata* and the cyrtaucheniids *Kiama*, *Acontius*, and *Fufius*. Within this larger "nemesiid" clade, several rRNA subclades with either morphological and/or biogeographical support are recovered. For example, the consistent sister pairing of *Stanwellia* (eastern Australia) and *Acanthogonatus* (South America) is supported by at least two morphological synapomorphies, including pseudosegmented tarsi in males, and a well-developed intercheliceral tumescence (see Raven, 1985, p. 48; Goloboff, 1995). An Australian clade including *Teyl*, *Pseudoteyl*, *Namea*, *Kwonkan*, and *Chenistonia* (=*Aname*) *tepperi* is consistently recovered in rRNA trees (Figs. 4 and 5). This group corresponds to part of Raven's Anamini (1985, p. 50). However, *contra* Raven (1985), other *Aname* species that we have sampled are never closely related to this clade (i.e., Anamini as a whole is not monophyletic). Another regional clade is represented by a subset of the South African nemesiids that we have sampled (*Hermacha*, undescribed genus), which together almost always form a clade. Finally, as mentioned above, a *Kiama* plus *Ixamatus* pairing (both from eastern Australia) also has morphological support.

We have sampled a single representative of the unique family Microstigmatidae (*Microstigmata*, Fig. 2E), and as suspected by both Raven (1985, p. 65) and Goloboff (1993), this taxon falls within a larger "nemesiid" clade.

This derived placement within the nemesiids suggests that many of the apomorphic characters possessed by microstigmatids (e.g., small round booklung openings) may be neotenic, as hypothesized by both Griswold (1985) and Raven (1985). Superficially, microstigmatids have a pustulose cuticle similar to *Ixamatus* and *Kiama*. However, rRNA trees do not suggest a particularly close relationship between these taxa, and SEM analyses suggest that these similarities are likely convergent (see Raven and Platnick, 1981). Although additional microstigmatids must ultimately be sampled, our molecular results are consistent with Goloboff's (1993) suggestion that "either Nemesiidae must be divided into several families, or the Microstigmatidae must become a subfamily of Nemesiidae".

Hexathelidae and Dipluridae—The families Hexathelidae and Dipluridae comprise mygalomorph species typically referred to as the sheet-web, curtain-web or funnel-web spiders (e.g., Figs. 2C and D). Raven (1980, 1985) cites a single morphological synapmorphy supporting hexathelid monophyly (numerous labial cuspules), but this hypothesis was questioned by Goloboff (1993). Our hexathelid sample includes representatives of two of three subfamilies recognized by Raven (1980), including the Macrothelinae (*Macrothele*, *Porrthothele*, *Atrax*, *Hadronyche*) and the Hexathelinae (*Bymainiella*, *Paraembolides*). Although the hexathelines *Bymainiella* and *Paraembolides* of eastern Australia are consistently recovered as sister taxa, neither the macrothelines, nor the hexathelids together, form a clade on molecular trees. Instead, hexathelids are typically part of a paraphyletic grade (along with diplurids) at the base of the non-atypoid clade (Figs. 4 and 5). Raven (1985, p. 55) discusses the uncomfortable positioning of *Atrax* (*Hadronyche* was then synonymous with *Atrax*) within the Hexathelidae, requiring homoplasy in several characters, and suggests possible relationships with cyrtaucheniids. The rRNA results indicate that *Atrax* and *Hadronyche* are indeed phylogenetically distinct from other hexathelids, but are never associated with cyrtaucheniids or other rastelloids.

Raven cites three morphological synapomorphies for the Dipluridae (long posterior lateral spinnerets, widely separated posterior median spinerrets, low caput), but again, this monophyly is disputed by Goloboff (1993, Fig. 1C). Our limited sample of diplurid diversity (only Euagrinae of four recognized diplurid subfamilies) is never recovered as monophyletic on rRNA trees. With hexathelids, sampled diplurids are typically part of a paraphyletic grade at the base of the non-Atypoid clade (Figs. 4 and 5). This paraphyly and relative basal placement is consistent with the hypothesis of Goloboff (1993, Fig. 1C), who stated that "non-diplurine diplurids form a "gray area" between the four-spinnereted taxa and the more plesiomorphic, six-spinnereted hexathelids".

Higher-level clades—The molecular data consistently recover a taxon bipartition that separates a clade including the families Atypidae, Antrodiaetidae, and Mecicobothriidae, from all remaining mygalomorph taxa (Figs. 4 and 5). This primary division is consistent with one of the earliest mygalomorph classifications, as Simon (1892) placed the Antrodiaetidae, Atypidae, and Mecicobothriidae into a group separate from other mygalomorphs. This group ultimately became known as the Atypoidea (*sensu* Chamberlin and Ivie, 1945), and was later accepted by researchers such as Coyle (1971, 1974), and most recently, Eskov and Zonshtein (1990). Although Raven (1985) ultimately rejected this hypothesis (as did many others, see below), he considered Simon's hypothesis to be "comprehensive and nonregional (i.e., global)," and "highly innovative."

The Atypoidea hypothesis is one of the most controversial in mygalomorph systematics. The hypothesis was discussed at length, and ultimately rejected, by Platnick (1977); Gertsch and Platnick (1979); Raven (1985) and Goloboff (1993). These authors generally agree that antrodiaetids and atypids (=Atypoidina, *sensu* Raven, 1985, see Figs. 2A and B) are related, and Goloboff (1993) suggests that Atypoidina are sister to all remaining mygalomorphs (see Fig. 1C). At issue is the placement of the Mecicobothriidae. Mecicobothriids share several morphological features in common with antrodiaetids and atypids. Chamberlin and Ivie (1945) listed six such features (e.g., male palpus with conducter, dorsal sclerites on anterior abdominal segments, etc.), but these were later suggested to be plesiomorphies (Gertsch and Platnick, 1979; Platnick, 1977). More recently, Eskov and Zonshtein (1990) argued for Atypoidea monophyly using additional characters (e.g., shape of chelicerae, shape of male tarsi, pleurital extensions, foveal shape), but again, this hypothesis was disputed. In particular, Goloboff (1993) cites several characters (e.g., cheliceral shape, foveal shape, male palpal structure, etc.) that he scores as "*contra* Eskov and Zonshtein," indicating disagreement in interpretation of characters and character states.

Rather than provide a full discussion of the morphological evidence here, we simply note that (1) the rRNA data are consistent with an Atypoidea hypothesis, and that this hypothesis has (disputable) morphological support, and (2) it will crucial to corroborate this hypothesis using other DNA evidence, as it is possible that long branch attraction is influencing the rRNA in favor of an Atypoidea hypothesis (see below).

Another possible major grouping is the Bipectina of Goloboff (1993), a proposed clade that includes all mygalomorphs except for Atypoids, hexathelids, and non-diplurine diplurids (Fig. 1C). Bipectines share several characters, including two rows of teeth on the superior tarsal claws of both sexes, although many of these characters are reversed or modified in various derived bipectine taxa. Strictly speaking, this group is not recovered in rRNA trees, because of the relatively basal placement of the paratropidid sample (Figs. 4 and 5). Likewise, constraint analyses do not support monophyly of this group (Table 5). However, a monophyletic Bipectina was recovered in parsimony and Bayesian analyses of combined matrices with "manual" 28S alignments.

Essentially all other higher-level groups proposed by Raven (1985), including the Fornicephalae, Rastelloidina, Domiothelina, Ctenizoidina, Tuberculotae, Mecicobothrioidina, Quadrithelina, Crassitarsae, and Theraphosoidina (see Fig. 1B) are not formally supported by molecular analyses. We, however, have not conducted statistical tests to reject most of these hypotheses. Of these, we believe that both the Domiothelina (*sensu* Raven, 1985) and Crassitarsae (as redefined by Goloboff, 1993, Fig. 1C) are viable taxonomic hypotheses, although the latter clade almost certainly includes several taxa currently classified as cyrtaucheniids (e.g., *Kiama*).

4.2. Summary hypothesis and regional biogeographic patterns

Fig. 6 summarizes, in a conservative manner, mygalomorph relationships as reflected in rRNA molecular phylogenies. This summary diagram provides a graphical point-of-comparison between previous and future hypotheses, and we expect this provisional hypothesis to change and evolve as additional data and taxa are considered. Although we believe that formal changes in the classification system of Mygalomorphae are needed and are imminent, these revisions are not made here, but rather will be made after completion of an on-going study involving combined analysis of both morphological and molecular characters (Bond and Hedin, unpublished).

Many mygalomorphs are sedentary, dispersal-limited animals with apparently deep evolutionary histories. As such, these spiders have long been favored by historical biogeographers (see Raven, 1980; Platnick, 1981; Griswold and Ledford, 2001). The phylogenetic framework provided by our molecular data (Fig. 6) sheds new light on mygalomorph biogeography. First, we have evidence supporting the monophyly of some classic Gonwandaland taxa, such as Migidae and Idiopidae. Migids are found in southern South America, Africa, Madagascar, Australia, New Zealand, and New Caledonia, while essentially all idiopids are distributed in southern South America, southern Africa, Madagascar, Australia, Tasmania, New Zealand, and India (see Table 1). Although additional taxon sampling is required to confirm monophyly of these families, our results are at least consistent with a deep history for these taxa. Another piece of evidence in favor of ancient phylogenetic diversification is the observation of regional, family-level faunas that are polyphyletic. The nemesiid fauna of Australia, which includes representatives of several different lineages (e.g., *Stanwellia*, derived Anamini, *Ixamatus* plus *Kiama*), is one such example. If we view the polyphyly of this regional fauna as evidence for taxonomic and biogeographic diversification prior to continental breakup, rather than more recent dispersal, then deep history is implied. Finally, layered upon this relatively deep history is evidence for more recent, continent-limited (i.e., regional), diversification. Examples include the Australian idiopid and Anamini radiations, the North American euctenizine radiation, and the African "ancylotrypine" radiation. For each of these well-supported monophyletic groups, our taxon sample is fairly comprehensive, allowing us to reject the possibility of undetected relatives living on other continents.

4.3. Caveats of molecular results

Nuclear ribosomal genes have been used successfully and extensively in animal molecular systematics, at many different taxonomic levels (e.g., Kjer, 2004; Mallatt et al., 2004; Winchell et al., 2002). Despite this proven utility, these genes pose several well-known data collection and analytical problems (e.g., contamination, paralogy, alignment), several of which we have faced in this study. In particular, we are concerned with the issue of lineage-specific rate acceleration, or so-called "episodic change," which has been revealed in the rRNA genes of a wide variety of eukaryotic taxa (Friedrich and Tautz, 1997; Omilian and Taylor, 2001; Philippe and Germot, 2000; Stiller and Hall, 1999). Substitution rate acceleration often impacts both 28S and 18S simultaneously, and can accentuate among-site rate variation, cause biases in base composition, and lead to dramatic length variation. If rate acceleration occurs independently in multiple lineages, problems of long-branch attraction (Felsenstein, 1978) can arise. In fact, one of the most well studied examples of possible long-branch attraction involves rate acceleration of rDNA genes in the insect orders Diptera and Strepsiptera (Whiting et al., 1997; Huelsenbeck, 1997, 1998).

Signs of rate acceleration are evident in both rRNA genes of three mygalomorph lineages, including the atypids, antrodiaetids and mecicobothriids (i.e., the Atypoids). This rate acceleration is evidenced by several patterns in the data. Extensive nucleotide insertions characterize atypoid expansion segments, making these sequences longer than all other mygalomorph sequences. Unique substitutions, found in otherwise conserved regions, are evident in many atypoid sequences. There are hints of shared biases in base composition (e.g., the 28S genes of atypoids are relatively AT rich, Table 2), although this variation is not statistically significant. Finally, the rRNA genes in these lineages are variable at unexpectedly low taxonomic levels. For example, 18S is highly variable at the species level in the antrodiaetids *Antrodiaetus* and *Aliatypus* (Hendrixson and Bond, 2006; Hedin, unpublished data). These signs of shared rate acceleration force us to question the validity of the observed Atypoidea grouping, because independent rate acceleration may be causing long-branch attraction of these lineages. Alternatively, rate acceleration of rRNA genes may be a synapomorphy uniting the Atypoidea. As argued above, the morphological evidence supporting an Atypoidea hypothesis is contentious, so congruence with morphology does not strongly favor one hypothesis

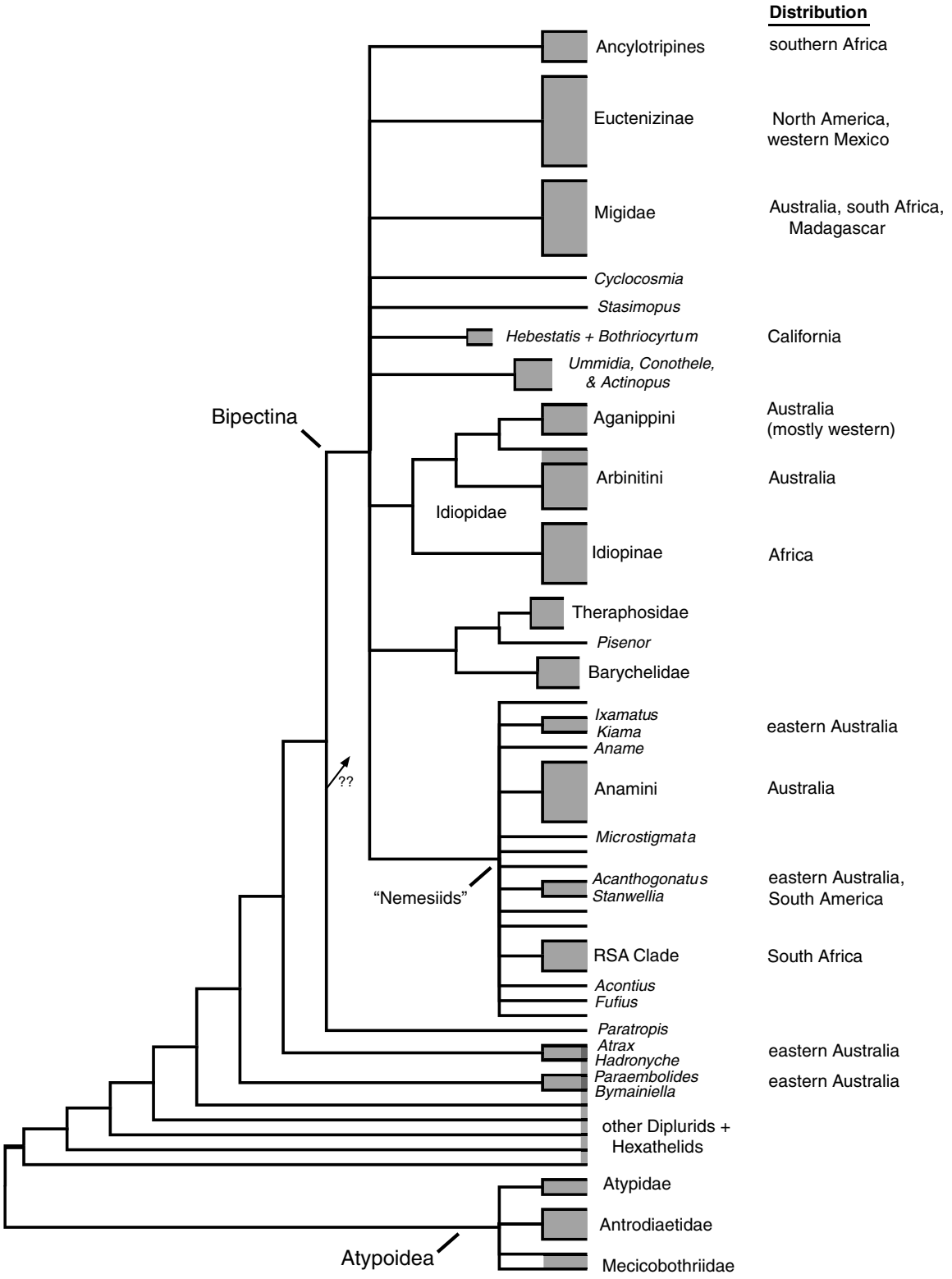

Fig. 6. Conservative summary of mygalomorph relationships based on rRNA analyses. Distributional data are included for major clades recovered in rRNA analyses; additional distributional data can be found in Table 1. The uncertain phylogenetic placement of *Paratropis* is indicated by question marks.

over another. We suggest that additional molecular data be gathered to test the Atypoidea hypothesis, and indirectly, the hypothesis of shared versus parallel rRNA rate acceleration.

4.4. Future directions in mygalomorph systematics

Although the taxon sample considered here is fairly representative of mygalomorph diversity, many important

sampling gaps remain. From a biogeographic perspective, the faunas of the Mediterranean, tropical Asia and Africa, and both temperate and tropical South America remain undersampled. The inclusion of additional taxa from temperate South America and India will be particularly important in testing biogeographic scenarios for several families with mostly Gondwanan distributions (e.g., migids, idiopids). Taxonomically, more sampling is needed for the following groups: South American microstigmatids and paratropidids; nemesiids from South America, Europe, and Asia; Asian ctenizids; diplurids and hexathelids; and genysine Idiopids (e.g., *Neocteniza*). In addition to increased taxon sampling, mygalomorph systematics still awaits the development of additional, slowly evolving, molecular phylogenetic markers (e.g., nuclear protein-coding genes), and the integration of both molecular and morphological data into a single, global analysis. Ultimately, we believe that such data will be necessary if we are to reconstruct a robust phylogenetic hypothesis of Mygalomorphae that can be used to reclassify mygalomorph diversity, interpret biogeographic history, and comprehend patterns of morphological evolution in this diverse group of spiders.

Acknowledgments

This study was supported by National Science Foundation grants DEB 0108575 (to M.H. and J.E.B.), DEB 0322650 supplement (to M.H.), and DEB 0236020 (to J.E.B.). David Beamer, Sarah Crews, Brent Hendrixson, and Steven Thomas assisted in data collection and analysis. Tom Prentice and Brent Hendrixson helped in the collection of North American taxa. Special thanks to Arthur Fong at the California Department of Parks and Recreation for arranging CA State Parks collecting permits. Collections in South Africa were made possible with help from Elizabeth Kassimatis, Jaques van Heerden, Martin Paulsen, Roland Jones and Norman Larsen. Dr. Ansie Dippenaar-Schoeman deserves special thanks for her generous assistance and hospitality in South Africa. Barbara York Main, Bert Main, Mark Harvey, Mike Gray, Graham Wishart, Fred Coyle, and Rob Raven provided assistance, housing hospitality and collections help in Australia. Australian collections were made under permit from Western Australia (License Number SF4101), New South Wales (License Number A1397), and Queensland (Permit Number WISP01242003), and exported under permit from Environment Australia (Permit No. WT2003-1002). Additional specimens were collected by Pedro Cardosa, Charles Griswold, Wayne Maddison, Lorenzo Prendini, Martín Ramírez, Robert Raven and Cor Vink. The collecting efforts of Griswold, Maddison, Prendini, and Ramírez were supported by an NSF ATOL Grant (EAR0228699) to Ward Wheeler, Jonathan Coddington, Gustavo Hormiga, Lorenzo Prendini and Petra Sierwald (http://research.amnh.org/atol/files/). Suggestions by two anonymous reviewers helped to improve the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.05.017.

References

Amenta, N., St. John, K., Klingner, J., et. al., 2005. Tree Set Visualization module for Mesquite. <http://comet.lehman.cuny.edu/treeviz/index.html/>.

Ben Ali, A., Wuyts, J., Wachter, R.D., Meyer, A., Van de Peer, Y., 1999. Construction of a variability map for eukaryotic large subunit ribosomal RNA. Nucleic Acids Res. 27, 2825–2831.

Bond, J.E., 2004. Systematics of the Californian euctenizine spider genus *Apomastus* (Araneae: Mygalomorphae: Cyrtaucheniidae): the relationship between molecular and morphological taxonomy. Invert. Syst. 18, 361–376.

Bond, J.E., Hedin, M., 2006. A total evidence assessment of the phylogeny of North American euctenizine trapdoor spiders (Araneae, Mygalomorphae, Cyrtaucheniidae) using Bayesian inference. Mol. Phylogen. Evol., in press, doi:10.1016/j.ympev.2006.04.026.

Bond, J.E., Opell, B.D., 2002. Phylogeny and taxonomy of the genera of south-western North American Euctenizine trapdoor spiders and their relatives (Araneae: Mygalomorphae, Cyrtaucheniidae). Zool. J. Linn. Soc. 136, 487–534.

Bond, J.E., Hedin, M.C., Ramirez, M.G., Opell, B.D., 2001. Deep molecular divergence in the absence of morphological and ecological change in the California coast dune endemic trapdoor spider *Aptostichus simus*. Mol. Ecol. 10, 899–910.

Brandley, M.C., Schmitz, A., Reeder, T.Ws., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.

Chamberlin, R.V., Ivie, W., 1945. On some Nearctic mygalomorph spiders. Ann. Entomol. Soc. Am. 38, 549–558.

Coddington, J.A., Giribet, G., Harvey, M.S., Prendini, L., Walter, D., 2004. Arachnida. In: Cracraft, J., Donoghue, M.J. (Eds.), Assembling the Tree of Life. Oxford University Press, pp. 296–318.

Coyle, F.A., 1971. Systematics and natural history of the mygalomorph spider genus *Antrodiaetus* and related genera (Araneae: Antrodiaetidae). Bull. Mus. Comp. Zool. 141, 269–402.

Coyle, F.A., 1974. Systematics of the trapdoor spider genus *Aliatypus* (Araneae: Antrodiaetidae). Psyche 81, 431–500.

Coyle, F.A., 1986. The role of silk in prey capture by nonaraneomorph spiders. In: Shear, W.A. (Ed.), Spiders: Webs, Behavior and Evolution. Stanford University Press, Stanford, pp. 269–305.

Decae, A., Cardoso, P., 2005. *Iberesia*, a new genus of trapdoor spiders (Araneae, Nemesiidae) from Portugal and Spain. Revista Ibérica de Aracnologia 12, pp. 3–11.

Eskov, K., Zonshtein, S., 1990. First Mesozoic mygalomorph spiders from the Lower Cretaceous of Siberia and Mongolia, with notes on the system and evolution of the infraorder Mygalomorphae (Chelicerata: Araneae). Neues Jahrb. Geol. Palaontol. Abh. 178, 325–368.

Felsenstein, J., 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27, 401–410.

Friedrich, M., Tautz, D., 1997. An episodic change of rDNA nucleotide substitution rate has occurred during the emergence of the insect order Diptera. Mol. Biol. Evol. 14, 644–653.

Gertsch, W.J., Platnick, N.I., 1979. A revision of the spider family Mecicobothriidae (Araneae, Mygalomorphae). Am. Mus. Nov. 2687, 1–32.

Goloboff, P.A., 1993. A reanalysis of mygalomorph spider families (Araneae). Am. Mus. Nov. 3056, 1–32.

Goloboff, P.A., 1995. A revision of the South American spiders of the family Nemesiidae (Araneae, Mygalomorphae). Part I: Species from Peru, Chile, Argentina, and Uruguay. Bull. Am. Mus. Nat. Hist. 224, 1–189.

Goloboff, P.A., 1999. Analyzing large datasets in reasonable times: solutions for composite optima. Cladistics 15, 415–428.

Goloboff, P.A., Platnick, N.I., 1987. A review of the Chilean spiders of the superfamily Migoidea (Araneae, Mygalomorphae). Am. Mus. Nov. 2888, 1–15.

Goloboff, P.A., Farris, S., Nixon, K. 2003. T.N.T.: Tree analysis using new technology. Program and documentation, available at <http://www.zmuc.dk/public/phylogeny/tnt/>.

Griswold, C.E., 1985. A revision of the African spiders of the family Microstigmatidae (Araneae: Mygalomorphae). Ann. Natal. Mus. 27, 1–37.

Griswold, C., Ledford, J., 2001. A monograph of the migid trap-door spiders of Madagascar, with a phylogeny of world genera (Araneae, Mygalomorphae, Migidae). Occ. Pap. Calif. Acad. Sci. 151, 1–120.

Hendriks, L., Van Broeckhoven, C., Vandenberghe, A., Van de Peer, Y., De Wachter, R., 1988. Primary and secondary structure of the 18S ribosomal RNA of the bird spider *Eurypelma californica* and evolutionary relationships among eukaryotic phyla. Eur. J. Biochem. 177, 15–20.

Hendrixson, B.E., Bond, J.E., 2005. Testing species boundaries in the *Antrodiaetus unicolor* complex (Araneae: Mygalomorphae: Antrodiaetidae): "Paraphyly" and cryptic diversity. Mol. Phylogenet. Evol. 36, 405–416.

Hendrixson, B.E., Bond, J.E., 2006. Molecular phylogeny and biogeography of an ancient Holarctic lineage of mygalomorph spiders (Araneae: Antrodiaetidae: *Antrodiaetus*). Mol. Phylogenet. Evol., in revision.

Hillis, D.M., Heath, T.A., St. John, K., 2005. Analysis and visualization of tree space. Syst. Biol. 53, 471–482.

Huelsenbeck, J.P., 1997. Is the Felsenstein Zone a fly trap? Syst. Biol. 46, 69–74.

Huelsenbeck, J.P., 1998. Systematic bias in phylogenetic analysis: Is the Strepsiptera problem solved? Syst. Biol. 47, 519–537.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Kass, R.E., Rafferty, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Kjer, K.M., 2004. Aligned 18S and insect phylogeny. Syst. Biol. 53, 506–514.

Löytynoja, A., Goldman, N., 2005. An algorithm for progressive multiple alignment of sequences with insertions. Proc. Natl. Acad. Sci. USA 102, 10557–10562.

Maddison, D.R., Maddison, W.P., 2001. MacClade: analysis of phylogeny and character evolution, version 4.0. Sinauer Associates, Sunderland, MA, USA.

Maddison, W.P., Maddison, D.R., 2004. Mesquite: A Modular System for Evolutionary Analysis. Version 1.05, online at <http://mesquiteproject.org/> (last accessed 20.01.06).

Main, B.Y., 1985. Further studies on the systematics of ctenized trapdoor spiders: a review of the Australian genera (Araneae: Mygalomorphae: Ctenizidae). Aust. J. Zool. Suppl. Ser. 108, 1–84.

Main, B.Y., 1991. Occurrence of the trapdoor spider genus *Moggridgea* in Australia with descriptions of two new species (Araneae: Mygalomorphae: Migidae). J. Nat. Hist. 25, 383–397.

Mallatt, J.M., Garey, J.R., Schultz, R., 2004. Ecdysozoan phylogeny and Bayesian inference: First use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. Mol. Phylogenet. Evol. 31, 178–191.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldre, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Omilian, A.R., Taylor, D.J., 2001. Rate acceleration and long-branch attraction in a conserved gene of cryptic daphniid (Crustacea) species. Mol. Biol. Evol. 18, 2201–2212.

Penney, D., Wheater, C.P., Selden, P.A., 2003. Resistance of spiders to Cretaceous-Tertiary extinction events. Evolution 57, 2599–2607.

Philippe, H., Germot, A., 2000. Phylogeny of eukaryotes based on ribosomal RNA: Long-branch attraction and models of sequence evolution. Mol. Biol. Evol. 17, 830–834.

Platnick, N.I., 1977. The hypochiloid spiders: A cladistic analysis, with notes of the Atypoidea (Arachnida, Araneae). Am. Mus. Nov. 2627, 1–23.

Platnick, N.I., 1981. Spider biogeography: past, present, and future. Rev. Arachnol. 3, 85–96.

Platnick, N.I., 2006. The world spider catalog, version 6.5. American Museum of Natural History, online at <http://research.amnh.org/entomology/spiders/catalog/index.html/> (last accessed 20.01.06).

Platnick, N.I., Gertsch, W.J., 1976. The suborders of spiders: a cladistic analysis (Arachnida, Araneae). Am. Mus. Nov. 2607, 1–15.

Platnick, N.I., Shadab, M.U., 1976. A revision of the mygalomorph spider genus *Neocteniza* (Araneae, Actinopodidae). Am. Mus. Nov. 2603, 1–19.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio rests. Syst. Biol. 53, 793–808.

Rambaut, A., Drummond, A., 2005. Tracer, A program for analyzing results from Bayesian MCMC programs such as BEAST & MrBayes, version 1.3.Oxford, online at <http://evolve.zoo.ox.ac.uk/software.html?id=tracer/> (last accessed 12.02.06).

Raven, R.J., 1980. The evolution and biogeography of the mygalomorph spider family Hexathelidae (Araneae, Chelicerata). J. Arachnol. 8, 251–266.

Raven, R.J., 1981. A review of the Australian genera of the mygalomorph spider subfamily Diplurinae (Dipluridae: Chelicerata). Aust. J. Zool. 29, 321–363.

Raven, R.J., 1985. The spider infraorder Mygalomorphae (Araneae): cladistics and systematics. Bull. Am. Mus. Nat. Hist. 182, 1–180.

Raven, R.J., 1994. Mygalomorph spiders of the Barychelidae in Australia and the western Pacific. Mem. Qd. Mus. 35, 291–706.

Raven, R.J., Platnick, N.I., 1981. A revision of the American spiders of the family Microstigmatidae (Araneae, Mygalomorphae). Am. Mus. Nov. 2707, 1–20.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ronquist, F., Huelsenbeck, J.P., van der Mark, P., 2005. MrBayes 3.1 Manual, Draft 5/26/2005, online at <http://mrbayes.csit.fsu.edu/manual.php/> (last accessed 20.01.06).

Sanmartín, I., Ronquist, F., 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216–243.

Selden, P.A., 2002. First British Mesozoic spider, from Cretaceous amber of the Isle of Wight, southern England. Palaeontology 45, 973–983.

Selden, P.A., Gall, J.-C., 1992. A Triassic Mygalomorph spider from the northern Vosges, France. Paleontology 35, 211–235.

Selden, P.A., Casado, F. da C., Mesquita, M.V., 2002. Funnel-web spiders (Araneae: Dipluridae) from the Lower Cretaceous of Brazil. Boletim do 6° Simpósio sobre o Cretácio do Brasil/2do Simposio sobre el Cretácico de América del Sur (2002), 89–91.

Simon, E., 1892. Histoire Naturelle des Araignées. 1, 1-256. Paris.

Stiller, J., Hall, B., 1999. Long branch attraction and the rDNA model of early eukaryotic evolution. Mol. Biol. Evol. 16, 1270–1279.

Swofford, D.L., 2002. PAUP*: phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Sunderland, MA, USA.

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

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Winchell, C.J., Sullivan, J., Cameron, C.B., Swalla, B.J., Mallatt, J., 2002. Evaluating hypotheses of deuterostome phylogeny and chordate evolution with new LSU and SSU ribosomal DNA data. Mol. Biol. Evol. 19, 762–776.