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ScienceDirect
Molecular Phylogenetics and Evolution 41 (2006) 70–85
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

A total evidence assessment of the phylogeny of North American euctenizine trapdoor spiders (Araneae, Mygalomorphae, Cyrtaucheniidae) using Bayesian inference

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

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

Received 28 July 2005; revised 11 February 2006; accepted 25 April 2006
Available online 12 May 2006

Abstract

North American trapdoor spiders of the subfamily Euctenizinae (Cyrtaucheniidae) are among the most diverse mygalomorph spiders (trapdoor spiders, tarantulas, and their relatives) on the continent in terms of species numbers and ecological habits. We present a generic level phylogenetic study of the subfamily based on a total evidence approach. Our dataset comprises ~3.7 kb of molecular characters (18S and 28S rRNA gene sequences) and 71 morphological characters scored for 32 taxa. When analyzed independently, these data sets, particularly the morphology, depict very different views of mygalomorph and euctenizine relationships, albeit with weak support. However, when these data are combined we recover a tree topology that is supported by high posterior probability for most nodes. The combined data recover a phylogenetic pattern for euctenizines different than previously published and indicate the presence of a narrowly endemic new genus from central California. While euctenizine monophyly is unequivocal, the monophyly of a number of other mygalomorph groups is questionable (e.g., Cyrtaucheniidae, Mecicobothriodina, Rastelloidina). This non-monophyly is noteworthy, as our analysis represents the first employing a total evidence approach for mygalomorphs, a group known to be morphologically conservative.

Keywords: Araneae; Bayes factors; Molecular systematics; Mygalomorphae; Spider phylogeny; Total evidence

1. Introduction

The spider infraorder Mygalomorphae (tarantulas, trapdoor spiders and their relatives) comprises 2,502 species and 311 genera, currently placed into 15 families (Platnick, 2006). Despite their relative obscurity spiders belonging to this group represent an ancient lineage (Penney, 2004) with a rich evolutionary diversity. Mygalomorphs are essentially worldwide in distribution, although the tropics (worldwide) and temperate austral regions of South America, southern Africa, and Australasia are centers of generic-level diversity (Raven, 1985; Platnick, 2006). North America also has a rich diversity of mygalomorph spider species, the majority of which (>50%) remain currently undescribed [this estimate does not include the 46 described species of *Aphonopelma* Pocock, 1901 (family Theraphosidae) because the diversity in this genus is considered to be overestimated by most workers familiar with the group]. Given the relatively large body size and life history characteristics of many of these spiders (e.g., extremely long-lived, highly sedentary, etc.), the presence of such a large number of undescribed species is anomalous. This undocumented biodiversity can be largely attributed to a few species-rich genera: the ctenizid trapdoor spider genus *Ummidia* Thorell, 1875 (40–50 undescribed species, Roth, 1993; Bond and Hendrixson, 2005), the cyrtaucheniid trapdoor spider genus *Aptostichus* Simon, 1891 (~35 undescribed species, Bond and Opell, 2002; Bond, 2005), and other closely related euctenizine genera (sensu Bond and Opell, 2002). Whereas *Ummidia* is widespread throughout North

* Corresponding author. Fax: +1 252 328 4178.
E-mail address: bondja@ecu.edu (J.E. Bond).

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doi:10.1016/j.ympev.2006.04.026

America and the New World Tropics, euctenizines are restricted primarily to the American Southwest with the greatest diversity in southern California.

The North American Euctenizinae was first revised by Bond and Opell (2002), and at present comprises eight genera with 29 nominal species. Euctenizines were transferred from Ctenizidae to the family Cyrtaucheniidae by Raven (1985) and positioned as a sister group to the remaining cyrtaucheniid subfamilies, Cyrtaucheniinae and Aporoptychinae. The cosmopolitan family, at present comprises 18 genera and 126 species (Platnick, 2006). Based on a cladistic analysis of 71 morphological characters scored for 29 mygalomorph taxa, Bond and Opell (2002) found the Euctenizinae (sensu Raven, 1985) to be monophyletic with the inclusion of the South African genus *Homostola* Simon, 1892 (Fig. 1). However, these authors strongly suspected, as did Goloboff (1993a), that the family Cyrtaucheniidae is paraphyletic with respect to the Domiothelina clade. For a number of reasons, namely incomplete taxon sampling, Bond and Opell were hesitant to splinter Cyrtaucheniidae into multiple families and chose to simply relimit the Euctenizinae; however, these authors speculated that increased sampling across Raven's (1985) Rastelloidina clade would resolve a number of problematic issues related to mygalomorph classification (e.g., cyrtaucheniid monophyly).

The morphological phylogeny of Bond and Opell (2002) fully resolved the relationships among the eight described genera (including *Homostola*). As illustrated in Fig. 1, *Homostola* is hypothesized to occupy a basal position within the subfamily, a curious result given the absence of any known South American euctenizines, thus creating a noteworthy geographic break in the distribution of the group (North American–Sub Saharan Africa). The only southeastern US representative of the group, *Myrmekiaphila* Atkinson, 1886, likewise falls more basal in the phylogeny with respect to the southwestern North American taxa. Bond and Opell (2002) gave informal names to a clade comprising *Eucteniza* Ausserer, 1875 and *Neoapachella* Bond and Opell, 2002 (the Euctenizoids), and the 'California Clade' composed of largely Californian taxa (*Aptostichus*, *Promyrmekiaphila* Schenkel, 1950, and *Apomastus* Bond and Opell, 2002). *Entychides* Simon, 1888 falls to the outside of these two named clades.

The primary objective of this study is to reconstruct the phylogenetic relationships of euctenizine genera using morphological and molecular data employing an exemplar approach. For a number of reasons, both conceptual and pragmatic, euctenizine phylogeny needs revisiting. First, the paucity of morphological characters for mygalomorph phylogenetic studies echoed by Bond and Opell (2002) and elsewhere (e.g. Goloboff, 1993a) indicates that other

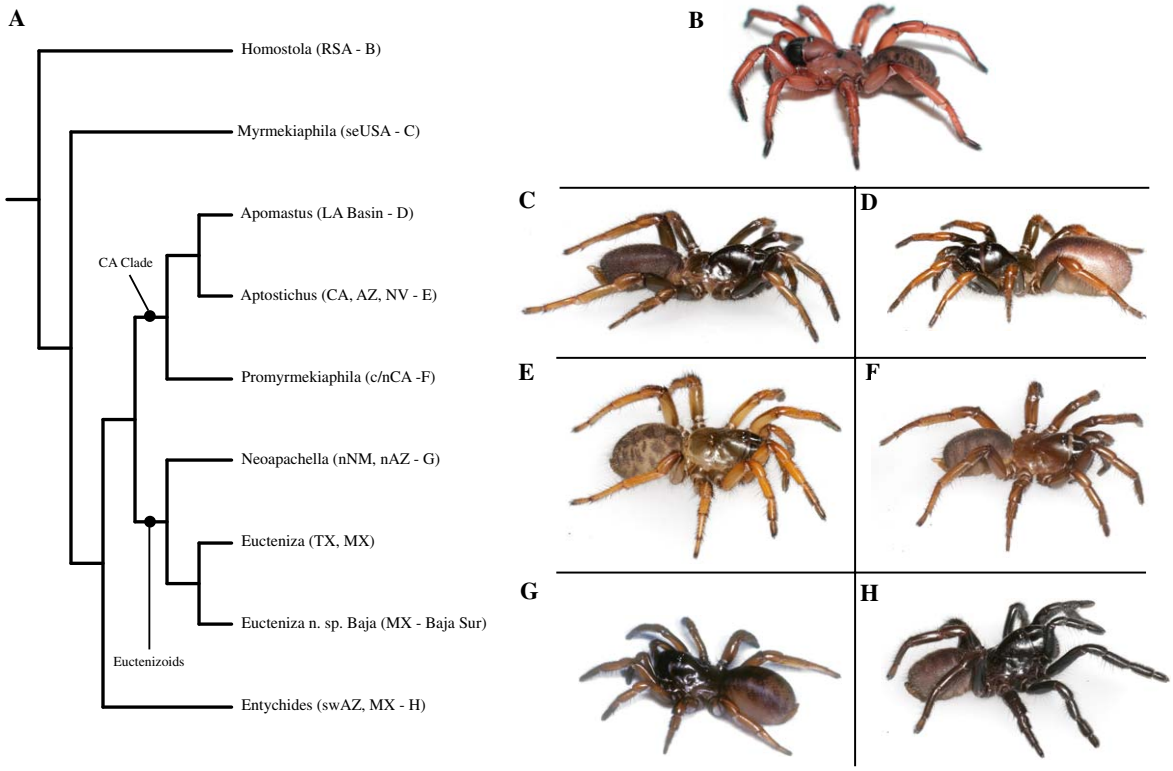

Fig. 1. (A) Phylogeny of the cyrtaucheniid subfamily Euctenizinae redrawn from Bond and Opell (2002, Fig. 6). Parenthetical notations indicate generalized distribution information and references spider images (RSA = Republic of South Africa, seUSA = southeastern United States, LA Basin = Los Angeles Basin, California, CA = California, AZ = Arizona, NV = Nevada, NM = New Mexico, c/n = central/northern, MX = Mexico, sw = southwestern; letters after hyphen refer to representative images). (B–H) Images of live euctenizine specimens. (B) *Homostola pardalina*. (C) *Myrmekiaphila fluviatilis*. (D) *Apomastus kristenae*. (E) *Aptostichus* sp. (F) *Promyrmekiaphila* sp. (G) *Neoapachella rothi*. (H) Euctenizine gen. nov., Moss Landing State Beach, California.

character systems need investigation if we are to achieve a well-supported subfamilial phylogeny. The phylogeny presented in Bond and Opell (2002, Fig. 6) lacks robust support for all but the most derived nodes. Second, as mentioned above, the inclusion of a South African genus (*Homostola*) in an otherwise North American group is highly suspect and requires corroboration; at the onset of this study we were doubtful of this hypothesis. Third, we are interested in the generic placement of two undescribed taxa, a new species from Moss Landing State Beach (Monterey County, California) and another from Baja California Sur (Mexico). Finally, efforts currently underway by the first author to taxonomically revise and document species relationships within genera of this diverse mygalomorph subfamily necessitate a robust phylogenetic framework to aid in generic limitations and outgroup choice.

The total evidence results presented here demonstrate that the North American Euctenizinae are a well-supported clade; however, the inclusion of the South African genus *Homostola* renders the group polyphyletic. Minor modifications are made to the taxonomic structure of clades originally proposed by Bond and Opell (2002). This analysis represents the first attempt to reconstruct higher-level relationships across the spider infraorder Mygalomorphae using morphological *and* molecular data.

2. Materials and methods

2.1. Taxon choice

Taxon choice follows the exemplar approach (Yeates, 1995; Wiens, 1998; Prendini, 2001). Terminals in our analyses are scored directly from species and more specifically those scorings can be traced directly to a single specimen or set of specimens. When possible we have tried to avoid composite taxa, terminals in combined analyses scored from multiple species (see Malia et al., 2003 for caveats regarding composite taxa). The exemplar approach, as opposed to coding higher-level taxa (summarized in Prendini, 2001), has been shown to perform well in simulation studies (Wiens, 1998) and to have a number of advantageous characteristics, namely repeatability and its functionality in simultaneous analyses of morphological and molecular data sets. This approach has been effectively employed across a disparate taxonomic range of spiders (e.g. Griswold et al., 1998; Bond and Opell, 2002) and other organismal groups (Christoffersen, 1989; Miller, 1991; Neves and Watson, 2004; Flynn et al., 2005). As a point of fact, the exemplar approach employed by Bond and Opell (2002) facilitated this study.

Appendix A lists the 32 taxa sampled for our analyses (12 euctenizines sensu lato, 20 "outgroup" taxa). We sampled all euctenizine genera, including multiple species for the more morphologically diverse genera. Although our sampling is relatively sparse outside of the Euctenizinae, we have sampled species to represent all major hypothesized lineages of the Mygalomorphae (e.g., Atypoidina, Domiothelina, Quadrathelina, Microstigmatidae, following Raven (1985)). All specimens have been assigned a unique specimen identification number and have had a label referencing this study added to their vial.

2.2. Morphological character assessment

Specimens were examined using a Leica MZ 12.5 stereomicroscope equipped with a 10× ocular and an ocular micrometer scale. Specimens examined using scanning electron microscopy were critical point dried and sputter coated with gold before viewing. Morphological characters scored are documented in detail in Bond and Opell (2002). The present analysis comprises 32 taxa and 71 unordered equally weighted characters (Appendix B). For two taxa (Nemesiid gen nov. 2 and *Homostola*), associated males are equivocal and thus male characteristics were not assessed (treated as missing in the data matrix). It should be noted that the *Ancylotrypa* Simon, 1889 male character scorings in Bond and Opell (2002) are incorrect and are correctly assessed here as a species of an undescribed genus from Ngome, South Africa. Male specimens are unavailable for the putative new genus from Moss Landing State Beach (CA) and likewise were not scored.

2.3. DNA isolation, amplification and sequencing

Genomic DNA was extracted and purified from 1 to 2 legs using the Dneasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). PCR primers and procedures used to amplify and sequence the 5′ half of the 28S rRNA as an ~2kb fragment are outlined in Mallatt and Sullivan (1998) and Winchell et al. (2002). Initial amplifications were carried out using the primer pair ZX1-AS8/OP1. Oligonucleotide sequences for 28S primers are listed in Appendix C. PCR parameters included an initial 96 °C denaturation followed by 29 cycles of 45 s at 94 °C, 45 s at 55 °C, 2 min at 72 °C, with a final 5-min extension at 72 °C. 28S PCR fragments were column purified and sequenced directly using an ABI 377 automated DNA sequencer (Applied Biosystems Inc., Forester City, CA, USA). Sequence primers included ZR2, ZR3, AS6, AS3, and AS8/OP1 (Appendix C).

Using primers published in Giribet et al. (1996), we amplified the18S rRNA gene either as a single fragment (1F-9R), or as three overlapping fragments (1F-5R, 3F-7R, 4F-9R). PCR experiments included an initial 94 °C denaturation followed by 30 cycles of 45 s at 94 °C, 45 s at 48 °C (increasing 0.2 °C per cycle), 90 s at 72 °C, with a final 10-min extension at 72 °C. All PCRs included *Ex Taq* (Takara Bio Inc.) with manufacturer provided dNTP mix and *Ex Taq* buffer (Mg^{2+}). PCR products were purified via Polyethylene Glycol (PEG) precipitation, gel verified and cycle sequenced using Big Dye Version 3 dye chemistry (ABI). Sequence primers included 1F, 3F, 4F, 5R, 7R, and 9R.

Sequence contigs for both the 28S and 18S data sets were assembled using the computer program Sequencher

(Genecodes, Madison, WI). Genbank accession numbers for sequences included in this study are listed in Appendix A.

2.4. DNA sequence alignment

Due to the paucity of insertion-deletions in the 18S data set (requiring the insertion of only three gaps), alignment of these gene sequences was trivial and thus editing and alignment for subsequent phylogenetic analysis was performed manually using the computer program Sequencher. Alignment was not a trivial issue for the 28S data set, necessitating an alternate approach. Consequently, multiple sequence alignment was performed using the new algorithm described by Löytynoja and Goldman (2005) as implemented in the Probabilistic Alignment Kit (PRANK; http://www.ebi.ac.uk/goldman/prank). This method uses a probabilistic scoring scheme and a hidden Markov model to find the most optimal alignment. It is considered to be an improvement upon previous progressive alignment programs (e.g., Clustal) because it keeps track of each gap that is introduced into a multiple sequence alignment (rather than penalizing it numerous times) and is expected to more accurately reflect the history of indel events (Higgins et al., 2005; 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, the two options available in the PRANK software package. Initial alignment progression was based on a guide tree taken from ClustalX (Thompson et al., 1997) using a pairwise gap opening and extension cost of 15-6. The raw sequence files (18S and 28S), individual alignment files, concatenated data matrices, and log files of all phylogenetic analyses are available for download at http://www.mygalomorphae.org. As discussed below total evidence phylogenetic analyses and analyses of the combined DNA data partitions did not differ for the two PRANK alignment models. For the purposes of brevity we have illustrated and discuss in detail only the HKY alignment; however, the log files and trees from the JC alignments (all permutations) are available for download at the website referenced above.

2.5. Phylogenetic analyses

The 28S alignments (see Section 3), 18S, and morphological data sets were concatenated to form a single matrix. Bayesian analyses were conducted on each separate partition—morphology, 18S and 28S (both alignments). Partitions were then sequentially added and analyzed for all possible dataset combinations (e.g., 28S + Morph, 28S + 18S, etc.) before analyzed as a total evidence (TE) matrix. The sequential addition of partitions for all possible combinations provides a framework for assessing the relative contribution of each partition to hidden branch support and the TE solution (see Gatesy et al., 1999 for summary).

2.5.1. Bayesian inference

The computer program MrModeltest ver. 2.1 (Nylander, 2004) was used to select an appropriate substitution model, by Akaike Information Criterion (AIC), for each of the molecular data partitions (18S and 28S). The morphological partition was analyzed using the Markov *k* model (Mk; Lewis, 2001; Nylander et al., 2004) with or without gamma-distributed rates (MkΓ). Final model selection for the morphological partition is based on the results of a Bayes factor analysis (see methods below).

Using the model of substitution indicated by AIC, analyses employing Bayesian inference were conducted with MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). Separate and combined analyses consisted of two simultaneous runs each with four simultaneous Markov Chain Monte Carlo (MCMC) chains run initially for 1,000,000 generations saving the current tree to file every 100 generations. Default cold and heated chain parameters were used. The separate, simultaneous runs, were compared every 1000–5000 generation to ensure convergence. Estimated parameters for each molecular partition were set to be independent, using the unlink statefreq (all), revmat = (all), shape = (all), pinvar = (all) command in MrBayes. At the end of each run we considered the sampling of the posterior distribution to be adequate if the average standard deviation of split frequencies was <0.01. MCMC runs were summarized and further investigated for convergence of all parameters, using the *sump* and *sumt* commands in MrBayes and the computer program Tracer version 3.1 (Rambaut and Drummond, 2005). Trees prior to log likelihood stabilization (burnin) and convergence were discarded before producing a majority rule consensus tree using the contype = allcompat command.

2.5.2. Bayesian hypothesis testing

We used Bayes factors to compare the posterior odds of our preferred Bayesian tree topology (see below) to Bayesian trees that forced the monophyly (see Ronquist et al., 2005) of the Euctenizinae sensu Bond and Opell (2002). We also employed Bayes factors analyses as a measure of relative congruence across data sets (see more detailed explanation below). 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 one to three million generations or more (as necessary). 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) and 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 Nylander et al., 2004; Brandley et al., 2005).

3. Results

3.1. Data characteristics and model choice

A concatenated, TE data matrix, comprising three partitions was constructed for each of the individual PRANK alignments (JC and HKY models referred to as PJC and PHKY hereafter). The 28S partition was scored for all 32 taxa. The PJC 28S alignment includes 2180 positions of which 556 were variable. The uncorrected base frequency composition across taxa appears to be homogenous ($\chi^2 = 78.91$, d.f. = 93, $P = 0.85$), and is moderately GC rich (A: 0.21, C: 0.27, G: 0.33, T: 0.19). The PHKY 28S alignment comprised 2222 positions of which 544 were variable. The 18S rRNA partition, scored for 31 taxa (data were unavailable for the new genus collected from Moss Landing), comprises 1103 characters of which 78 were variable; uncorrected base frequency composition, likewise, appears homogenous ($\chi^2 = 2.81$, d.f. = 93, $P = 1.00$) but not GC rich (A: 0.25, C: 0.23, G: 0.26, T: 0.25). Average sequence divergence (uncorrected *P*) across all taxa was 0.065 (0.001–0.165) and 0.011 (0–0.033) for the 28S and 18S partitions, respectively. The General Time Reversible model with a gamma distribution and invariants model of rate heterogeneity (GTR + Γ + I) was the best-fit for both partitions. The morphological data partition consisted of 70 parsimony informative characters, scored for all 32 taxa. A Bayes factor analysis comparing the Mk and Mk + Γ indicated that there was positive evidence in support of the latter (see Table 1). Although the evidence in support of the more parameter rich model is relatively weak, we chose to err on the side of over-parameterization (see Lemmon and Moriarty, 2004). Furthermore, subsequent TE analyses (not reported but available at http://www.mygalomorphae.org) in which we compared log likelihood values of searches using the Mk + Γ model to those without gamma showed very strong support ($2\log_e B_{10} > 100$) for the inclusion of the additional model parameter.

Table 1
Summary of results from Bayesian analyses

Analysis	ngens	ln (Ar)	ln (Hr)	asdsf	burnin	99%
Morph	2.0	−972.19	−1005.05	0.005	1.2	15730
Mk + G		−974.34	−1006.19			
Morph	2.0	−974.64	−1004.91	0.009	1.65	6826
Mk		−973.96	−1007.59			
18S	5.0	−2407.11	−2437.38	0.010	4.75	4951
		−2408.85	−2439.34			
28SJC	1.0	−10879.48	−10915.28	0.01	0.5	2102
		−10882.36	−10925.56			
28SHKY	1.0	−10777.64	−10807.42	0.007	0.6	1612
		−10779.07	−10809.58			
DNAJC	1.49	−13370.73	−13401.67	0.007	1.25	385
		−13367.68	−13400.69			
DNAHKY	2.0	−13258.33	−13289.62	0.015	1.5	2366
		−13259.36	−13289.21			
28S + Morph	1.0	−11965.63	−11999.97	0.003	0.7	355
		−11969.31	−11999.67			
18S + Morph	1.0	−3497.82	−3533.18	0.008	0.7	4805
		−3496.42	−3532.05			
TEJC	1.0	−14581.05	−14616.88	0.007	0.48	502
		−14582.38	−14612.53			
TEHKY	1.0	−14455.97	−14487.70	0.006	0.18	259
		−14456.01	−14487.99			

ngens (number of generations) and burnin are given in units of a million; Ar and Hr refer to the arithmetic and harmonic means for each of the simultaneous runs; asdsf = average standard deviation of split frequencies; 99% refers to the number of trees sampled from the 99% credible set.

3.2. Phylogenetic analyses: morphological partition

The Bayesian analysis of the morphological data partition (summarized in Table 1) resulted in a tree that recovers a Euctenizinae clade that is congruent, in terms of *taxonomic* composition only, with that of the phylogenetic hypothesis of Bond and Opell (2002, see Fig. 1). That is, the North American euctenizines group with the South African genus *Homostola*, a clade that has low support (posterior probability (*P*p) = 0.82). However, the internal relationships of the genera recovered with the Bayesian analysis are mostly incongruent with the earlier analyses. The *Homostola* exemplar (Fig. 2A) has a derived position, rather than grouping at the base of the Euctenizinae (Fig. 1) sister to all other members of the clade. Also, both the Euctenizoid and CA clades are not recovered. It is worth noting that parsimony analysis of the morphological partition conducted in PAUP* (Swofford, 2002) recover a euctenizine clade identical in composition and internal structure to that obtained from the Bayesian analysis. While these results may seem spurious when compared to those parsimony analyses reported by Bond and Opell (2002), the taxonomic composition of the previous analyses (2002) were different and a non-linear function of character to tree fit was employed (i.e., the Goloboff fit criterion; Goloboff, 1993b). Most of the deeper nodes in the morphological tree have low support (*P*p < 0.80) and placement of putative basal mygalomorph taxa (e.g., members of the Atypoidina–*Atypus* Latreille, 1804 + *Sphodros* Walckenaer, 1835) and the non-monophyly of the Cyrtaucheniidae (Euctenizinae + *Ancylotrypa* and *Kiama* Main and Mascord, 1969) are consistent with past phylogenetic hypotheses (Goloboff, 1993a; Bond and Opell, 2002). The Domiothelina, a clade that includes idopids (Idiopis Perty, 1833, *Segregara* Tucker, 1917, *Eucyrtops* Pocock, 1897), migids (*Moggridgea* Cambridge, 1875, *Poecilomigas* Simon, 1903), actinopodids (*Actinopus* Perty, 1833) and ctenizids (*Ummidia*, *Hebestatis* Simon, 1903, *Bothriocyrtum* Simon, 1891), forms a monophyletic group.

3.3. Phylogenetic analyses: DNA partitions

Bayesian analysis of the 18S data partition (Table 1 and Fig. 2B) resulted in a tree topology that lacks strong support across most of the nodes (*P*p < 0.80). North American euctenizines form a strongly supported (*P*p = 1.0) paraphyletic group with respect to the migid genera

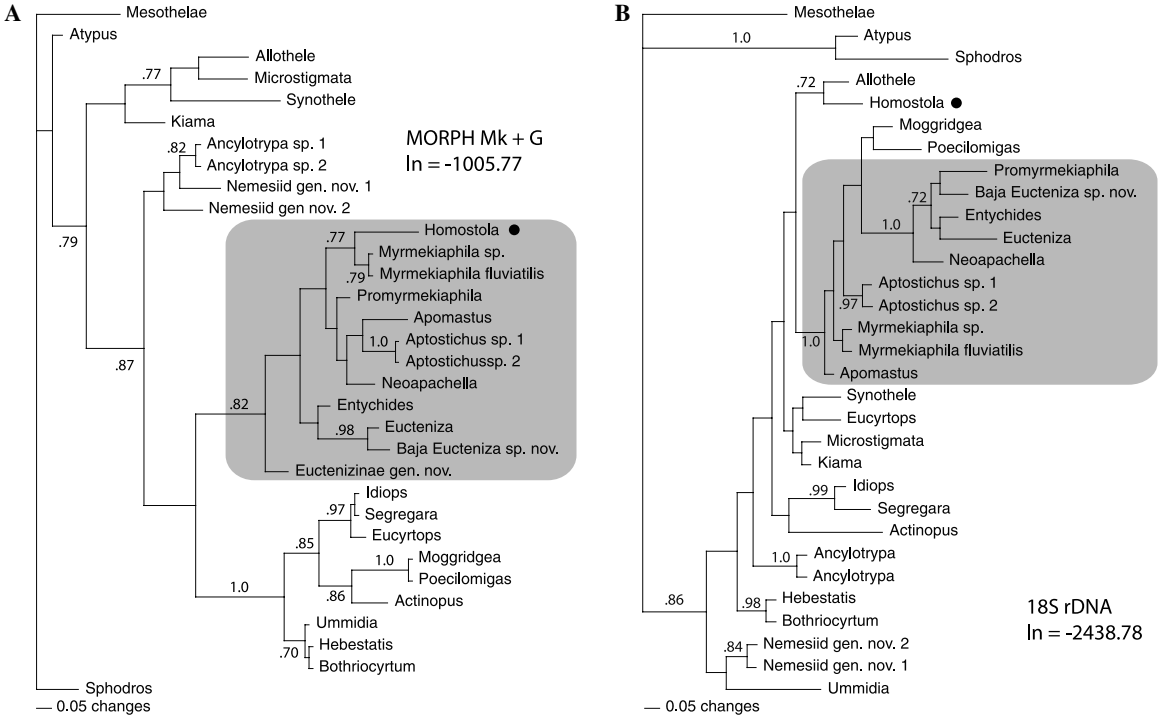

Fig. 2. Phylogenetic analysis of morphological and 18S data partitions. Gray boxes denote euctenizine taxa, solid dot denotes the South African genus *Homostola*, numbers at nodes refer to posterior probabilities; −ln values are the average for both simultaneous runs. (A) Phylogeny based on the morphological data partition. (B) Phylogeny based on the 18S rRNA partition.

Poecilomigas and *Moggridgea*. The South African genus *Homostola* falls to the outside of the euctenizine clade as a sister group to the diplurid genus *Allothele* Tucker, 1920. Although this grouping is highly suspect, it garners weak support. What is of interest is its exclusion from the Euctenizinae. Within the "euctenizine clade" (sensu stricto) the genus *Apomastus* appears at the base of the subfamily and previously recovered groups, the euctenizoids and CA clade, are not recovered as monophyletic. At deeper phylogenetic levels the Atypoidina taxa are sister to all other mygalomorphs and cyrtaucheniids and the Domiothelina appear as polyphyletic.

As discussed earlier, alignment of the 28S data set proved problematic. We investigated the alignment of these data using both substitution models (JC and HKY) available in the software package PRANK. Results of the phylogentic analyses of both alignments are summarized in Table 1 and in Fig. 3. The tree topologies recovered from both alignments are approximately congruent differing only in their respective resolution of a few poorly supported nodes and the placement of the euctenizine genus *Apomastus*. A clade that includes all of the North American euctenizines is recovered and has strong support in both alignments ($Pp = 0.98$, 1.0). The JC alignment (Fig. 3A) places *Apomastus* as sister to all other euctenizines; however, this node has very weak support ($Pp < 0.70$). Alternatively, the HKY alignment (Fig. 3B) places *Apomastus* as sister to *Aptostichus* (low support value, $Pp = 0.82$). This sister pairing is part of a weakly supported clade that includes the southeastern genus *Myrmekiaphila* and falls to the outside of the remaining euctenizine genera. As in the separate analyses of the morphological and 18S partitions, the composition of the Euctenizoid clade, as proposed by Bond and Opell (2002), is not recovered. Instead, euctenizoids group with *Entychides* and the undescribed genus from Moss Landing (CA). Neither analysis places the Atypoidina taxa or diplurid genus *Allothele* in a more basal position, sister to all of the other mygalomorph taxa nor do either recover a monophyletic Domiothelina. However, most of these deeper internal nodes have little or no support and thus the data are not very decisive in this regard. The two alignments, while differing only slightly in resultant tree topology and relative nodal support, differ significantly in their respective average likelihood values (post burnin) for the trees obtained (−10925.09 vs. −10808.99 for JC and HKY, respectively).

Fig. 4A summarizes the combined DNA Bayesian analysis (18S + 28S). We show only the results based on the PHKY alignment as the combined data sets produced approximately identical tree topologies (both alignments) with comparable support values for all nodes and as observed earlier log likelihood values were significantly better for the PHKY alignment trees (see Table 1). The combined DNA tree (Fig. 4A) recovers a strongly supported monophyletic North American euctenizine clade; the South African genus *Homostola* forms an equally well supported grouping with the South African *Ancylotrypa* exemplars. The more derived nodes within euctenizines remained unchanged from the analysis of the 28S partition whereas the more basal nodes differ slightly in the their placement

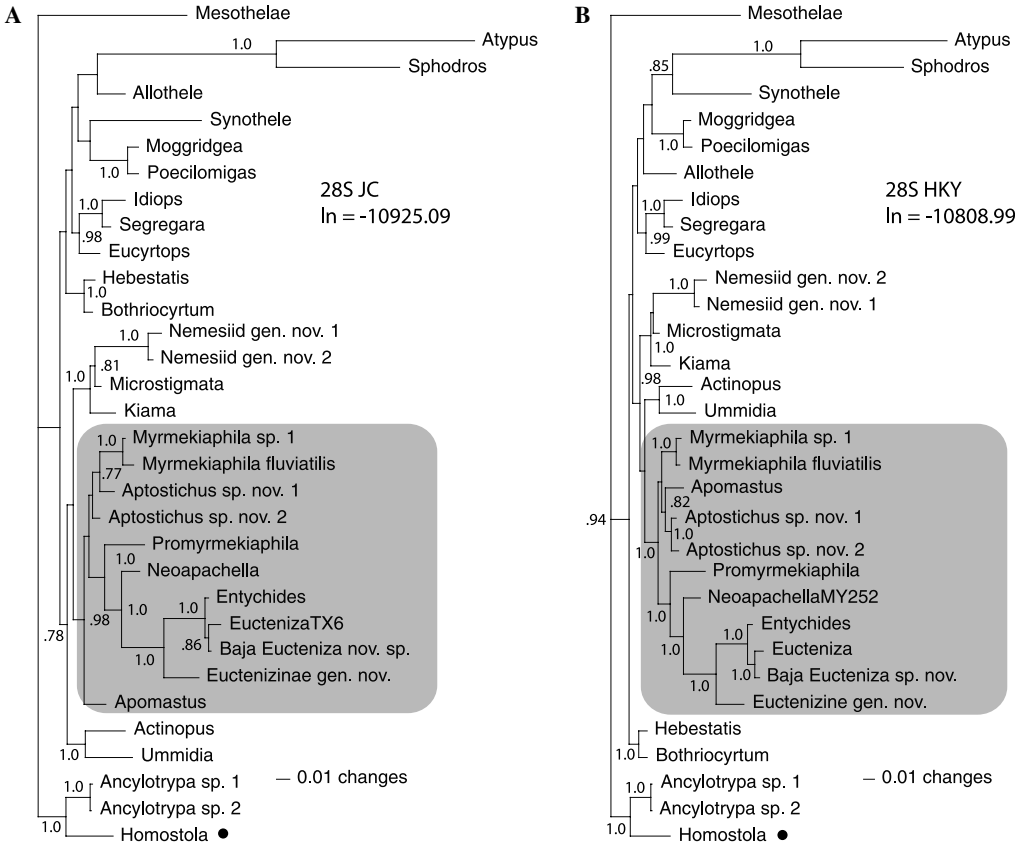

Fig. 3. Phylogenetic analysis of the 28S rRNA data partition. Gray boxes denote euctenizine taxa, solid dot denotes the South African genus *Homostola*, numbers at nodes refer to posterior probabilities; −ln values are the average for both simultaneous runs. (A) Phylogeny based on partition aligned using the PRANK JC model. (B) Phylogeny based on partition aligned using the PRANK HKY model.

of *Apomastus* and *Aptostichus* as a sister pair with respect to a more derived *Myrmekiaphila*. The Atypoidina taxa are sister to all other mygalomorphs in this analysis, but as before these deeper nodes lack credible support values.

3.4. Phylogenetic analyses: sequential combination of morphological and molecular partitions

The morphological data partition in combination with that of the 28S partition (PHKY; PJC, not shown does not differ significantly) results in a very well resolved strongly supported tree topology that is consistent, at deeper levels, with current hypotheses of mygalomorph relationships (basal Atypoidina taxa and the diplurid *Allothele*). Fig. 4B summarizes the tree topology and support values for the analysis (also see Table 1). All but two deeper level nodes that resolve the placement of problematic taxa—*Microstigmata* Strand, 1932 + *Kiama* and the undescribed South African nemesiid genera—have very strong support. The North American Euctenizinae genera form a monophyletic group (Pp = 1.0) with *Apomastus* in a basal position sister to all other euctenizines. The genus *Homostola* is considerably removed from all other "euctenizines" as part of a strongly supported sister pairing with other South African cyrtaucheniids. The node that resolves the relative positions of *Myrmekiaphila* and *Aptostichus*, as in previous analyses of the individual 28S partition, remain equivocal (Pp = 0.44, not shown on tree). Again, the CA clade is not supported and the Euctenizoid clade (Fig. 1) is paraphyletic with respect to *Entychides* and the new euctenizine genus (not included in Bond and Opell, 2002).

Based on the analysis combining the 18S and morphological partition it appears that the 18S data contribute minimally to the overall picture (Fig. 4C, Table 1), particularly at the shallower phylogenetic levels. This analysis definitively places the Atypoidina genera in a basal position sister to all other mygalomorph taxa included in the study (Pp = 1.0); however, most of the other internal nodes lack support. The Euctenizinae (sensu lato, Bond and Opell, 2002) is monophyletic; the genus *Homostola* is recovered as the sister group to the North American euctenizines. The intra-subfamilial relationships are similar to those depicted in Fig. 1—*Myrmekiaphila* as the sister group to all the remaining euctenizines and CA clade, *sans Promyrmekiaphila* is recovered. As in previous analyses, the Euctenizoid clade is paraphyletic.

3.5. Total evidence analysis

The results of the TE Bayesian analysis and conflict/congruence across all partitions are summarized on Fig. 5 (also see Table 1). As mentioned earlier, only the TE result

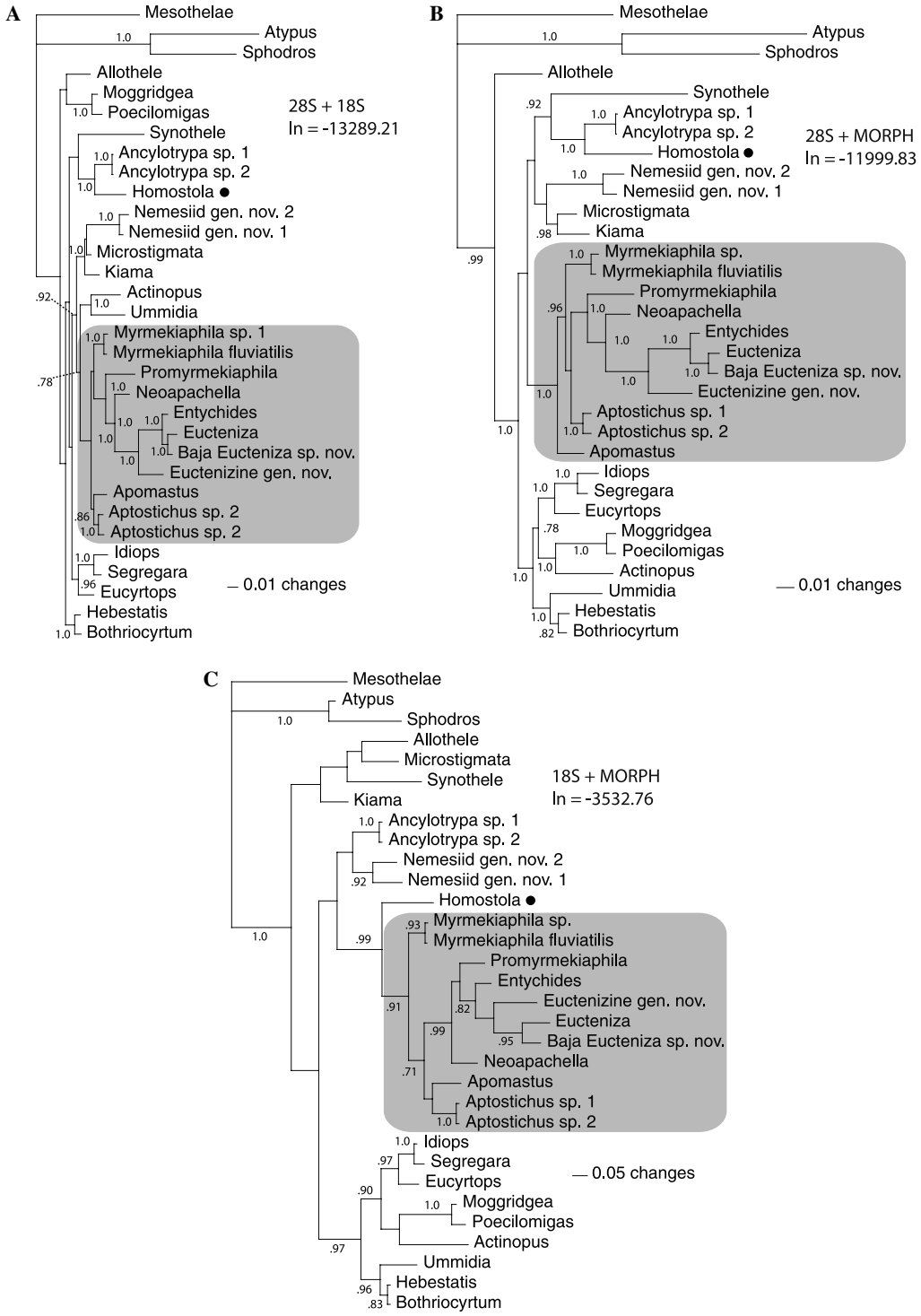

Fig. 4. Phylogenetic analyses of combined partitions. Gray boxes denote euctenizine taxa, solid dot denotes the South African genus *Homostola*, numbers at nodes refer to posterior probabilities; −ln values are the average for both simultaneous runs. (A) Analysis of DNA partitions (18S + 28S). (B) Analysis of the 28S rRNA + Morphological data partitions. (C) Analysis of the 18S rRNA + Morphological data partitions.

that includes the 28S—PHKY alignment partition is illustrated and discussed. As in most of the analyses convergence of the simultaneous analyses occurred very rapidly (Table 1); subsequent runs for additional generations (4 million post the early burnin) indicate that the chains had indeed reached stationarity in the short time period we observed. The majority of the nodes in the TE analysis have strong (posterior clade probability > 90%) to moderate support. The North American Euctenizinae genera are very strongly supported as monophyletic, as is the Domiothelina clade (Idiopidae + Actinopodidae + Migidae + Ctenizidae). As was the case in a number of the single and dual partition analyses discussed above, the South African genus *Homostola* appears far removed from other

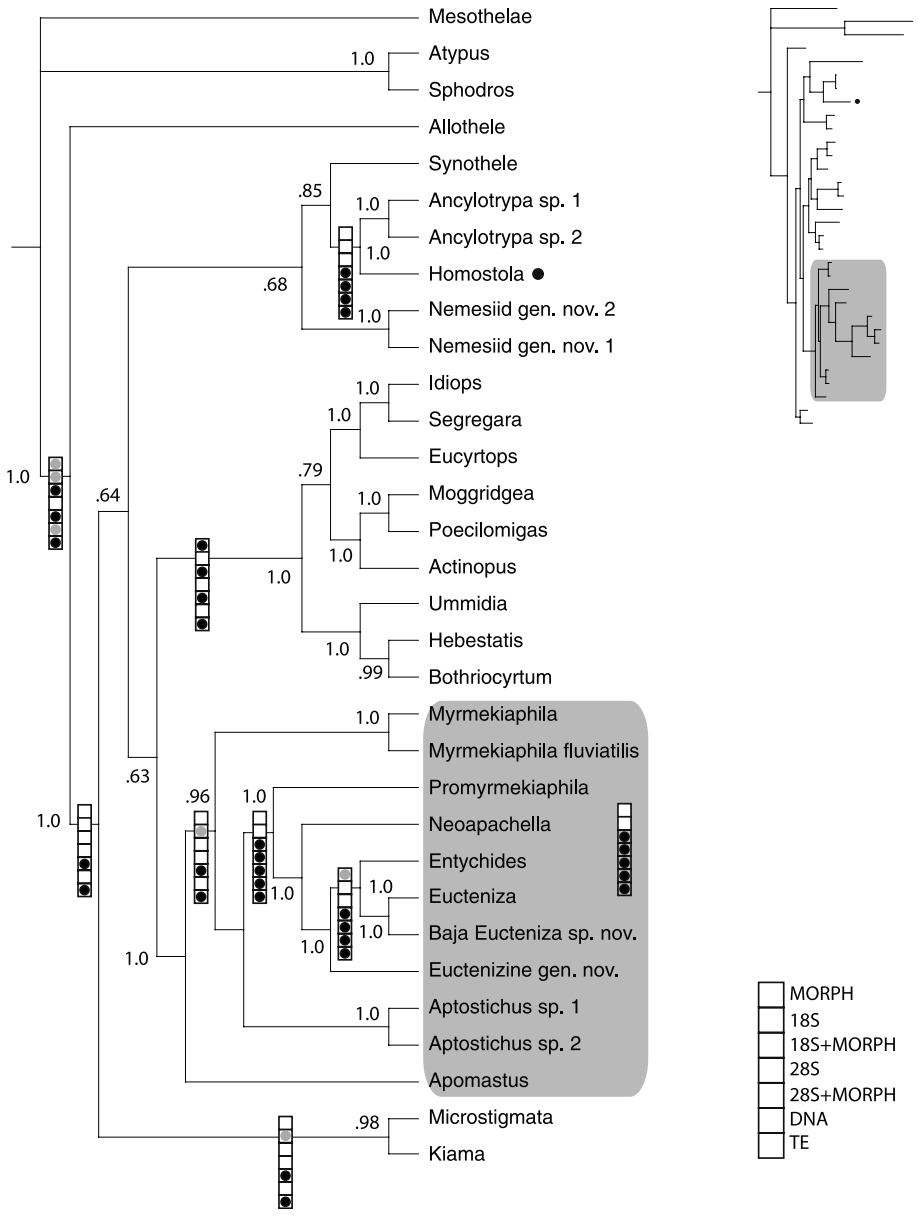

Fig. 5. Phylogenetic hypothesis based on total evidence using Bayesian inference (ln = −1488.82). Gray boxes placed on the tree denote euctenizine taxa, solid dot denotes the South African genus *Homostola*, numbers at nodes refer to posterior probabilities. Phylogenetic tree, top inset, shows branch lengths averaged from the posterior distribution of the TE analysis (28S PHKY alignment). Dot plots (legend, lower inset) indicate partition support for nodes discussed in the text; solid dots denote strong support ($Pp > 0.90$) gray dots indicate that the node appeared in analysis of that partition but was weakly supported.

euctenizines as a sister group with other South African "cyrtaucheniid" genera. Neither analysis supports a monophyletic Cyrtaucheniidae, a family whose monophyly has already been questioned (Goloboff, 1993a; Bond and Opell, 2002). Within the Euctenizinae the TE analysis places *Apomastus* as the basal sister group to all other euctenizines followed by a weakly supported node ($Pp = 0.45$) that equivocally places *Myrmekiaphila* and *Aptostichus* as successive sister groups to the remaining taxa. As in most other analyses (see dot plots, Fig. 5) the CA clade and Euctenizoid clade are not monophyletic as hypothesized previously (Bond and Opell, 2002).

3.6. Bayesian hypothesis testing

Table 2 summarizes the results of the Bayes factor analyses conducted. The analyses conducted reflect our primary interests of evaluating euctenizine monophyly and the inclusion of the South African genus *Homostola*. As mentioned above analyses of the 28S data partitions and of all partitions combined resulted in a North American euctenizine clade that excluded *Homostola*. Bayes factor analyses of these data sets were conducted to compare topologies that constrained Euctenizinae (sensu lato, see Fig. 1) to those of the optimal tree topologies. In all cases there

Table 2
Summary of Bayes factor analyses

Analysis	Model likelihood		Evidence against T_0	
	$\log_e \hat{f}(X\|T_1)$	$\log_e \hat{f}(X\|T_0)$	$2\log_e B_{10}$	
Parameter est.				
Mk G v. Mk	−1005.77	−1006.96	2.38	Positive
Euctenizines (Bond and Opell, 2002)				
DNA_HKY	−13289.21	−13357.09	135.76	V. strong
TE_HKY	−14487.86	−14543.62	111.52	V. strong
DNA_JC	−13402.03	−13479.43	154.8	V. strong
TE_JC	−14616.20	−14677.79	123.18	V. strong
Euctenizines–Homostola				
Morphology	−1005.77	−1011.46	11.38	Strong
18S	−2438.78	−2438.67	−0.22	No evidence

was very strong ($2\log_e B_{10} > 10$) evidence against the constrained topology. Alternatively, the morphological partition recovered a monophyletic Euctenizinae (sensu lato) whereas the 18S partition recovered a North American euctenizine clade (Euctenizinae sensu stricto) that was paraphyletic with respect to the migid genera included in our study. Thus we constrained these analyses to conform the narrower definition of the Euctenizinae (*sans Homostola*). In both cases the evidence against the constrained topology was relatively weak compared to all other constrained analyses. The morphological partition shows only positive evidence against the alternative whereas there was no evidence against the alternative for the 18S partition. Neither data set appears to be very decisive with respect to a North American euctenizine clade that excludes *Homostola*.

4. Discussion

The North American Euctenizinae genera form a strongly supported monophyletic group to the exclusion of the South African genus *Homostola*. This pattern of relationship is observed in the 28S partition alone and when taken in all combinations with the 18S and morphological data set partitions. Our preferred hypothesis of euctenizine phylogeny (Fig. 5) is based on total evidence. Given the amount of homoplasy and paucity of morphological characters often observed in mygalomorph taxa, it seems appropriate to favor TE over an approach that considers independent data sets separately.

The data sets when considered separate and in differential combination indicate that partitions shape tree topology in different ways and at different phylogenetic levels. The data set also appears to contain a minimal level of hidden support, that is, nodes not supported by individual partitions are recovered as strongly supported nodes in the TE analysis (Gatesy et al., 1999). Two of the more basal nodes in the phylogeny appear only in combined analysis—the node that places the diplurid genus *Allothele* outside of all non-atypoid taxa and the node that unites *Microstigmata* and the Australian cyrtaucheniid genus *Kiama*. Both of these nodes have strong support only in the TE trees and when the 28S partition is combined with morphology. Within euctenizines (sensu lato) the node that resolves *Apomastus* as sister to all of the other taxa, likewise, is strongly supported *only* in the TE analysis. Conversely the 28S and morphological data partitions play pivotal roles in the delineation of some groups and in some cases appear in conflict with other partitions (respectively). For example, the North American euctenizine clade is not supported by the morphological or 18S partitions (Fig. 4). The conflict between these two partitions and the 28S data appears to be minimal as seen by the relative weak evidence against the alternative 28S grouping confirmed by the Bayes factor analyses (Table 2). Alternatively, the 28S data partition and the TE set are relatively decisive with respect to the composition of the Euctenizinae; Bayes factor analyses all show strong evidence against a North American euctenizine + *Homostola* grouping. Finally, the morphological data appear to play a very pivotal role in the monophyly of the Domiothelina as this higher-level taxon appears only in analyses that include the morphological partition.

The TE results are manifestly different from the phylogenetic hypothesis proposed by Bond and Opell (2002) and necessitate change to the their classification scheme. First, the South African genus *Homostola* is clearly not a euctenizine despite the fact that members of this genus share a number of prominent morphological features [asymmetrical tarsal scopulae (4), spigot arrangements on the posterior lateral and posterior median spinnerets (41, 45)] with euctenizines (see further discussion of euctenizine synapomorphies below). Analyses that constrain euctenizine monophyly (sensu Bond and Opell, 2002) appear suboptimal (Table 2). This result is not surprising despite what are apparently superficial affinities with North American taxa like *Aptostichus* (Bond and Opell, 2002; Simon, 1892). Based on biogeography alone, such a hypothesis required *Homostola* to be a relict (i.e., the subfamily must have once been widespread throughout the Mediterranean and Europe or South America). At present, there is no evidence for such a distribution. Second, the Euctenizoid clade is paraphyletic with respect to *Entychides*. Bond and Opell (2002) placed *Entychides* in a more basal position in the Euctenizinae, sister to *Myrmekiaphila* and all other euctenizines. Third, the California Clade was not recovered in the TE phylogeny and is considered here to be an invalid grouping. And finally, our phylogeny indicates that the species (not included in Bond and Opell, 2002) collected from the Moss Landing locality (Appendix A, Monterey County) is a new genus. Despite the fact that this new genus will be monotypic and will likely remain so (extensive collecting efforts by us and others across California have not discovered additional related species), it fails to form a close sister pairing with any of the other nominal euctenizine genera.

As might be expected, diagnostic, morphological support for euctenizine monophyly is lacking. When character transitions are reconstructed in MacClade (Maddison and

Maddison, 2001) using ACCTRAN optimization, nine homoplasious characters (CI < 1) unambiguously optimize on the euctenizine ancestral node. The following character states are thus considered to be diagnostic, *in combination*, for the North American Euctenizinae clade (parenthetical numbers refer to characters listed in Appendix C): a fovea that is a wide and deep depression (4), female tarsal scopulae asymmetrical (34), posterior median spinnerets with two spigot types (41), apical article of posterior lateral spinnerets with a linear arrangement of 2–3 large spigots (45), preening combs on metatarsus IV (52), femur IV with a dense spine patch (54), male palpal femur with a dorsal spine row (65), and spermathecae with basal lateral extension (69).

With respect to broader issues in mygalomorph phylogeny, our results strongly suggest that the current familial and higher level classification structure of the infraorder is problematic and in need of emendation. First and foremost, the family Cyrtaucheniidae is polyphyletic. This has been discussed previously (Goloboff, 1993a, 1995; Bond and Opell, 2002) but is particularly pervasive here. The South African genera *Homostola* and *Ancylotrypa* and the Australian genus *Kiama* are more closely related to other non-Rastelloid taxa (nemesiids, barychelids, and microstigmatids) than to euctenizines, and are themselves splintered into at least two clades on most trees. As in the previous analyses our sampling is too limited to consider making formal nomenclatural changes but such changes are certainly forthcoming. The derived position of *Microstigmata longipes* Lawrence, 1938, sister to *Kiama lachrymoides* Main and Mascord, 1969 indicates that the microstigmatids are likely not sister to mecicobothriids and are not part of a basal Tuberculotae clade (the Mecicobothrioidina). The Rastelloidina as composed by Raven (1985) likely consists only of Domithelina taxa. The Domiothelina, a clade consisting of ctenizids, idiopids, actinopodids, and migids, is recovered with strong support in our TE analysis (Fig. 5).

5. Conclusions

Foremost, this study provides the phylogenetic framework to begin considering detailed questions about euctenizine systematics and evolution. The amount of undescribed diversity, particularly within the biodiversity hotspot of the Californian Floristic Province makes this group of particular conservation importance. Our analysis, based on total evidence, indicates that the morphological phylogeny of Euctenizine taxa proposed by Bond and Opell (2002) is inaccurate. We do not find this result surprising as Bond and Opell (2002) advocated the use of both morphology and molecules for reconstructing relationships of the morphologically uniform mygalomorphs. Given the age of the mygalomorph lineage, at least late Triassic (Penney, 2004), and the fossorial lifestyle of many of its members, despite ample time, morphology (and ecology to an extent) is constrained, suggesting a strong role for selection. The molecular data are however, likewise, not without problems. Rates of molecular evolution in the 28S rRNA gene are accelerated for some mygalomorph taxa, making DNA sequence alignment problematic (e.g., see Hendrixson and Bond, 2005) and long branch attraction a likely problem.

Acknowledgments

This study was supported by National Science Foundation grants DEB 0108575 (to J.E.B.. and M.H.) and DEB 0236020 (to J.E.B.). SEM work was supported by National Science Foundation Grant DBI 0320010. David Beamer and Brent Hendrixson assisted with DNA sequencing. Tom Prentice, Wendell Icenogle, 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 assistance and hospitality in South Africa. Barbara York Main, Bert Main, Mark Harvey, Mike Gray, Graham Wishart, Fred Coyle and Rob Raven provided assistance, 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).

Appendix A

List of exemplar taxa (detailed locality data referenced by MY# available online at http://www.mygalomorphae.org)

Taxon	MY #	Locality place name	Latitude–longitude	GenBank Accession #'s
Mesothelae	MY1028	Malaysia	Not available	DQ639767, DQ639851
Liphistius malayanus Abraham, 1923		Malayasia, Selangor	Not available	dep. AMNH
Atypoidina (Atypidae)				
Atypus snetsingeri Sarno, 1973	MY2282, MY2283	Pennsylvania, USA	Not available	DQ639769, DQ639853
Atypus snetsingeri (m and f)		Pennsylvania, USA	Not available	dep. AMNH
Sphodros atlanticus Gertsch & Platnick, 1980	MY26, MY643	South Carolina, USA	N 34.75603 W82.85633	DQ639768, DQ639852
Sphodros rufipes (Latreille, 1829) (m and f)		Arkansas, USA	Not available	dep. AMNH
Dipluridae				
Allothele australis (Purcell, 1903) (m) characters scored using descriptions by Coyle (1984)	**MY575**	Eastern Cape Province, South Africa	S 33.12755 E 26.67287	DQ639784, DQ639870
Cyrtaucheniidae				
Ancylotrypa sp. 1	**MY515**	Guateng Province, South Africa	S25.72685 E 28.23768	DQ639791, DQ639878
Ancylotrypa sp. 2	**MY502**	Guateng Province, South Africa	S25.65255 E 28.34947	DQ639792, DQ639879
Homostola pardalina (Hewitt, 1913)	**MY530**	Mpumalanaga Province, South Africa	S 26.1774 E 31.21694	DQ639790, DQ639877
Nemesiidae + Microstigmatidae + *Kiama*				
Nemesiid gen. nov. 2	**MY556**	Eastern Cape Province, South Africa	S 33.12755 E 26.67287	DQ639848, DQ639944
Nemesiid gen. nov. 1	**MY546**	Kwa-Zulu Natal	S 27.82000	DQ639849, DQ639945
Nemesiid gen. nov. 1 (m)		Province, South Africa	E 31.41750	dep. PPRI
Microstimata longipes (Lawrence, 1938)	**MY543**, MY165	Kwa-Zulu Natal Province, South Africa	S 27.82000	DQ639850, DQ639946
Microstimata longipes (m)			E31.41750	dep. PPRI
Kiama lachrymoides Main & Mascord, 1969 Bond and Opell 2002	MY2094	New South Wales, Australia	S 34.69928 E 150.80639	DQ639796, DQ639884
North American Euctenizines				
Myrmekiaphila sp. 1 Bond and Opell 2002	MY2034	Alabama, USA	N 34.30959 W 87.397433	DQ639799, DQ639888
Myrmekiaphila fluviatilis (Hentz, 1850) Bond and Opell 2002	MY2234	Virginia, USA	N 37.35383 W 80.59988	DQ639800, DQ639889
Apomastus kristenae Bond, 2004 Bond and Opell 2002	MY720	California, USA	N 33.55295 W 117.76783	DQ639798, DQ639887
Aptostichus sp. 1 Bond and Opell 2002	MY264	California, USA	N 32.7118 W 116.11602	DQ639797, DQ639885
Aptostichus sp. 2 Bond and Opell 2002	MY2595	California, USA	N 33.67712 W 117.11578	DQ639797, DQ639886
Promyrmekiaphila sp. Bond and Opell 2002	MY736	California, USA	N 39.61555 W 122.51330	DQ639802, DQ639891
Neoapachella rothi Bond & Opell, 2002 Bond and Opell 2002	MY252, MY79	Arizona, USA	N 33.99555 W 109.46725	DQ639801, DQ639890
Entychides arizonicus Gertsch & Wallace 1936 Bond and Opell 2002	MY2281	Arizona, USA	N 31.93302 W 109.27203	DQ639803, DQ639892
Eucteniza rex (Chamberlin, 1940) Bond and Opell 2002	MYTX6	Texas, USA	N 27.7889 W99.45583	DQ639804, DQ639893
Eucteniza n. sp. Bond and Opell 2002	MY2698	Baja California Sur, Mexico	N 24.10028 W 110.26917	DQ639805, DQ639892
New genus, Moss Landing	**MY3072**	California, USA	N36.8115 121.7909W	—, DQ672620

(*continued on next page*)

Appendix A (*continued*)

Taxon	MY #	Locality place name	Latitude–longitude	GenBank Accession #'s
Domiothelina taxa (Idiopidae, Migidae, Actinopodidae, Ctenizidae)				
Idiops sp.	MY189	Northern Province, South Africa	S 24.78953 E 28.40.416	DQ639826, DQ639920
Bond and Opell 2002				
Segregara sp.	MY604	Western Cape Province, South Africa	S 32.33475 E 22.47475	DQ639828, DQ639922
Bond and Opell 2002				
Eucyrtops sp.	MY2071	Western Australia, Australia	S 31.28875 E 119.68583	DQ639825, DQ639919
Bond and Opell 2002				
Moggridgea *sp.*	MY623, **MY2147**	Northern Cape	S 30.64556	DQ639808, DQ639898
Bond and Opell 2002		Province, South Africa	E 18.05889	
Poecilomigas abrahami (Cambridge, 1889)	MY598	Western Cape Province, South Africa	S 33.97027 E 23.5389	DQ639809, DQ639899
Bond and Opell 2002				
Actinopus sp.	MY2873	Buenos Aires, Argentina	Not available	DQ639817, DQ639910
Bond and Opell 2002				
Ummidia *sp.*	MY2042,	Kentucky, USA	N 37.53250	DQ639815, DQ639907
Bond and Opell 2002	MY149		W 86.72960	
Hebestatis theveneti (Simon, 1891)	MY278	California, USA	N 37.50387 W 119.99405	DQ639813, DQ639905
Bond and Opell 2002				
Bothriocyrtum californicum (Cambridge, 1874)	MY66	California, USA	N 32.46720 W 117.04360	DQ639814, DQ639906
Bond and Opell 2002				

MY number in bold type indicates specimen scored for morphology; specimen data in each row below (when applicable) references specimens scored for morphology when different from those used in generating the molecular data set (museum collecting deposition given in GenBank Accession column); lower cells referencing Bond and Opell (2002) indicate character scorings carried over from that analysis. (m and f) indicates male and female specimen (respectively) scored for morphology. 18S accession numbers are listed first. AMNH = American Museum of Natural History, NY; PPRI = Plant Protection Research Institute, Pretoria, South Africa.

Appendix B

Morphological character matrix and list of characters

Mesothelae	1020420001123100000000001000000000030001 0?0200100100000000000?00110?02
Atypus	1110200002011100000000000010001000121001011?0000010000000000000100110003
Sphodros	0?1130000201110300000001110100100002100101120001010000000000000100110003
Allothele	1010400012200102100000010001010000130100010000010000000000000000101100004
Synothele	1010301010200102111000000020004021010002???00???020101000000000?100113
Ancylotrypa 1	1101200000211002 01?00100??00013011?0?00101100000010000100???????????103
Ancylotrypa 2	1101200000211002 01?00100??00013011?0?00101100000010000100???????????103
Homostola	0112200000201121 01?00100??00001011?1?1011010100012010100???????????102
Nemesiid g. n. 2	1001300000002100 01?00100??00003011?0000101100???0100?0110???????????10?
Microstigmata	1020300002200112100000000000012000101012???10100000010000000001010000003
Kiama	0101210012000102000000000010003000111010011000000000000000100001000000003
Nemesiid g.n. 1	1001220001211002011001000010003022001001011001000100001101000010010003
Idiops	1101210000210013020001100000102000010102011000111100100010000010001110?
Segregara	1101210000210013020001100000102000010102011000111100100010000010001110?
Eucyrtops	1101210000210013020001100000102022010002011000111100100010000010001110?
Moggridgea	1000100100210023000011100000102000111021002001110001000100000101000101
Poecilomigas	1000100100210023000011100000102000111021002001110001000100000101000101
Actinopus	0102200100211213020011110000102000110102101200111110000100000000000010?
Ummidia	1111200000011112021001100000102000010102001000110100000111000010000101
Hebestatis	1111200000011112021001100000102000010102001000110100000111000010000101
Bothriocyrtum	1111200000011112021001100000102000010102001000110100000111000010000101
Myrmekiaphila 1	0102200000211111021001100000001012100101101010111101010100000101100202
Myrmekiaphila 2	0102200000211111021001100000001012100101101010111101010100000101100202
Promyrmek.	0102200010211113021001100000011022100001011011001101010100000101000202
Apomastus	1102300000211101011001101100011022100001111011001101010000000101000213
Aptostichus sp1	1112201010211112011001100000001022100001111011001101110101000010110021 2
Aptostichus sp2	1112201010211112011001100000001022100001111011001101110101000010110021 2

Appendix B (*continued*)

Neoapachella	0102300000211113011001100000001022100001011011001100010100110011100000202
Entychides	0102200000212113021101100000001122000001111010011100010101000010000020?
New genus	1002200000212113012?10100??00001022?0?001111?1?001100?0100???????????102
Eucteniza sp1	0102200000212123020101100000001122010101111011001110010100111110000102
Eucteniza sp2	010220000021212302010110100000112211000111?1?001110001100111110100010?

For brevity only genus name is given in the list below, for full exemplar species names see Appendix A.

Characters and character states (Bond and Opell, 2002).

1. Thorax: flat (0); sloping (1).
2. Caput: low (0); high (1).
3. Eye tubercle: absent (0); present, low (1); present, high (2).
4. Fovea: narrow (0); intermediate width and shallow (1); wide and deep (2).
5. Fovea: longitudinal (0); recurved (1); procurved (2); transverse (3).
6. Eyes: AME and PME subequal in diameter (0); AME diameter much larger than PME (1); PME much greater in diameter than AME.
7. Abdomen: without mottled striping (0); with mottled striping (1).
8. Ocular area: normal (1); occupies at least 2/3's of the cephalic region of carapace (1).
9. Female carapace: not hirsute (0); hirsute (1).
10. Sternum shape: widest at coxae III and narrowing anteriorly; sides roughly parallel (1); rounded (2).
11. Sternum: wide, almost round (0); long and slender (1); normal (2).
12. Posterior sternal sigilla: positioned in lateral margins (0); positioned medially (1).
13. Posterior sternal sigilla: small and concentric (0); large and concentric (1); large with anterior margin distorted.
14. Labium: subquadrate (0); wider than long (1); longer than wide (2).
15. Labium: setae normal, not modified as cuspules (0); a few setae modified as cuspules (1); many setae modified as cuspules.
16. Palpal endite cuspules: absent (0); large patch restricted to proximal inner margin (1); distributed uniformly across face of endite.
17. Serrula: absent (0); present (1).
18. Rastellum: absent (0); consisting of large spines, not on a mound (1); spines on a distinct process (2).
19. Posterior edge of male carapace: aspinose (0); with a distinct fringe of heavy spines (1).
20. Posterior margin of cephalothorax: sclerotization normal (0); sclerotization light (1).
21. Fangs: long and slender (0); short and thick (1).
22. Anterior legs: subequal to posterior legs in length and circumference (0); shorter and more slender than posterior legs (1).
23. Tarsi: normal (0); stout, swollen (1).
24. Palpal endites: longer than wide (0); subquadrate (1).
25. Male tarsus IV: straight (0); slightly curved (1).
26. Male tarsus I: integral (0); pseudosegmented (1).
27. Inferior tarsal claw (ITS): present, normal in size (0); reduced in size (1); absent (2).
28. ITS: edentate (0); dentate (1).
29. Tarsus: normal length (0); very short (1).
30. Superior tarsal claw (STC) IV dentition: few teeth (0); many teeth, more than four (1).
31. STC I and palp: males and females with a single row of teeth, prolateral displacement of female palpal tooth row minimal (0); male and females with a single row of teeth, evident prolateral displacement of palpal row distally, basal teeth on medial keel (1); male and female with one strong basal tooth, sometimes with a few minute teeth (2); male and female with two rows of teeth (3); male and female claws edentate (4).
32. STC I basal tooth: normal, unmodified (0); elongate and bifid (1).
33. Scopulae: absent (0); light (1); dense (2).
34. Scopulae: absent (0); present, symmetrical (1); present, asymmetrical (2).
35. Male scopulae: present on leg IV (0); absent on leg IV (1).
36. Tarsal trichobothria: single zigzag row (0); wide band (1); reduced (2); single narrow row.
37. Tarsal organ: low, usually with concentric ridges (0); elevated (1).
38. Chelicerae: single tooth row with denticles (0); two rows of equally large teeth, lacking denticles (1).
39. Small cuticular projections on legs and spinnerets: absent (0); present (1).
40. Posterior lateral spinnerets (PLS) apical article: digitiform, long (0); digitiform, short (1); domed (2).
41. Posterior median spinnerets (PMS) spigot sizes: one size (0); two or more spigot sizes (1).
42. PMS spigot density: less than on PLS (0); subequal to PLS (1).
43. PMS: slender (0); stout (1).
44. Spigot shaft sculpturation: overlapping scale-like folds (0); upturned spines (1); smooth (2).
45. Apical article of PLS: one common spigot size (0); common spigot size with linear arrangement of 2–3 very stout spigots on apical-most aspect of the distal article (1).
46. Pumpkiniform spigots: absent (0); present (1).
47. Fused spigots: absent (0); present (1).
48. Spigot bases: with invaginations (0); without (1).
49. Posterior leg spines: both dorsal and ventral (0); mostly dorsal (1).
50. Prolateral spine patch on female patella III: absent (0); large patch, more than three spines (1); small patch, 2–3 spines (2).
51. Prolateral spine patch on female patella IV: absent (0); present (1).
52. Preening combs on metatarsus IV: absent (0); present (1).
53. Spines on male cymbium: absent (0); present (1).

(*continued on next page*)

Appendix B (*continued*)

54. Patch of long, dense spines on dorsal distal-most aspect of femur IV: absent (0); present (1).
55. Sparse patch of short stout spines on dorsal distal-most aspect of femur IV: absent (0); present (1).
56. Distal ventral spine patch on tarsus IV: absent (0); present (1).
57. Digging spines on anterior walking legs and pedipalps: absent (0); present (1).
58. Male mating clasper: without proximal, ventral excavation (0); with proximal, ventral excavation (1).
59. Male mating clasper tibia I: without distinct patch of short prolateral, distal spines (0); with a distinct patch of short prolateral, distal spines (1).
60. Male mating clasper tibia I: without mid-ventral megaspine (0); with a mid-ventral megaspine (1).
61. Male tibia II: without mid-ventral megaspine (0); with a mid-ventral megaspine (1).
62. Palpal bulb: normal (0); unique conformation (1).
63. Male palpal tibia: long and slender (0); short and stout (1).
64. Male palpal tibia: without a prolateral spine patch (0); with a prolateral spine patch (1).
65. Palpal femur: dorsal spine row absent (0); dorsal spine row present (1).
66. Embous: with serrations (0); without serrations (1). 67. Male palpal bulb: distal sclerite closed (0); distal sclerite open (1).
68. Excavation of prolateral palpal tibia with short thorn-like spines: absent (0); present (1).
69. Spermathecae: multilobular (0); single lobe (1); single lobe with laterally extended base (2).
70. Lateral base of spermathecae: not enlarged or absent (0); enlarged (1).
71. Burrow entrance: collar (0); thickened (''cork'') trapdoor (1); thin trapdoor (2); open burrow or exposed tube (3); funnel web (4).

Note: Unless otherwise stated characters were scored from female specimens.

Appendix C

PCR primers used to amplify and sequence the 28S rRNA gene (from Mallatt pers. comm.; Mallatt and Sullivan, 1998; Winchell et al., 2002)

Primer	Primer sequence	Position	Direction
ZX1	ACC CGC TGA ATT TAA GCA TAT	−35	5′ → 3′
ZR3	GAA AAG AAC TTT GAA GAG AGA GTT CA	325	5′ → 3′
ZR2	GCT ATC CTG AGG GAA ACT TCG G	1157	3′ ← 5′
AS3	CCG AAG TTT CCC TCA GGA TAG C	1157	5′ → 3′
AS6	TCT TAG GAC CGA CTG ACC	1750	3′ ← 5′
AS8/OP1	AGA GCC AAT CCT TGT CCC GA	2500	3′ ← 5′

Position refers to *Onchorhynchus* 28S sequence, Genbank Accession U34341.

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