



Phylogeny of entelegyne spiders: Affinities of the family Penestomidae (NEW RANK), generic phylogeny of Eresidae, and asymmetric rates of change in spinning organ evolution (Araneae, Araneoidea, Entelegynae)

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ABSTRACT

Penestomine spiders were first described from females only and placed in the family Eresidae. Discovery of the male decades later brought surprises, especially in the morphology of the male pedipalp, which features (among other things) a retrolateral tibial apophysis (RTA). The presence of an RTA is synapomorphic for a large clade of spiders exclusive of Eresidae. A molecular data matrix based on four loci was constructed to test two alternative hypotheses: (1) penestomines are eresids and the RTA is convergent, or (2) penestomines belong within the RTA clade. Taxon sampling concentrated on the Eresidae and the RTA clade, especially outside of the Dionycha and Lycosoidea. Evolution of the cribellum, conventionally characterized as a primitive araneomorph spinning organ lost multiple times, is explored. Parsimony optimization indicates repeated appearances of the cribellum. Exploration of asymmetric rates of loss and gain in both a likelihood framework and using a Sankoff matrix under parsimony reveals that cribellum homology is supported when losses are two times more likely than gains. We suggest that when complicated characters appear (under parsimony optimization) to evolve multiple times, investigators should consider alternative reconstructions featuring a relatively high rate of loss. Evolution of other morphological characters is also investigated. The results imply revised circumscription of some RTA-clade families, including Agelenidae, Amaurobiidae, Cybaeidae, Dictynidae and Hahniidae. Some nomenclatural changes are formally proposed here; others await further investigation. The family Penestomidae (NEW RANK) is established. *Tamgrinia*, not *Neoramia*, is the cribellate sister clade of the ecribellate Agelenidae. *Tamgrinia* and the subfamily Coelotinae are transferred from the family Amaurobiidae to the family Agelenidae. *Zanomys* and its relatives are not coelotines but belong to a clade tentatively identified as Macroboninae.

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1. Introduction

Morphological data has generally been successful at revealing the broad strokes of spider phylogeny, especially over the past

20 years or so (Agnarsson et al., 2006; Coddington, 1986, 1989, 1990a; Griswold et al., 1998, 1999; Platnick et al., 1991; but see Griswold et al., 2005). But there are a handful of enigmatic higher-level taxa that remain to be convincingly placed, usually due to conflicting or ambiguous morphological evidence. One of these is the Penestominae, endemic to southern Africa. Here we use a hypothesis testing approach combined with a broad taxon sampling strategy to investigate the phylogenetic affinities of the Penestominae and discuss the broad pattern of entelegyne spider evolution as suggested by our results with reference to historical literature.

We also investigate the evolution of selected morphological characters. One of these characters, cribellate silk, is conventionally

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considered a symplesiomorphic feature of araneomorph spiders that has been lost repeatedly (Coddington, 1986; Griswold et al., 2005; Lehtinen, 1967). However, phylogenetic optimization of this character under parsimony has not always recovered cribellate silk as homologous across spiders (e.g., Davies, 1998; Griswold, 1993; Silva Dávila, 2003). Morphological apparatus associated with the production of cribellate silk are complicated, consisting of a cribellum (a plate covered with tiny silk spigots located just anterior to the spinnerets; Fig. 2A) and calamistrum (a row of specialized setae on the metatarsus of leg IV). This makes it unlikely that morphologists would mistakenly identify non-homologous cribellate silk organs as the same. At the same time, cribellate silk is metabolically expensive (Opell, 1998) and performs worse in key ways than ecribellate sticky silk (Blackledge et al., 2009; Köhler and Vollrath, 1995), so we can imagine that evolutionary novelties that offer selective advantages over cribellate silk may appear relatively frequently over evolutionary time. Thus the costs of evolving or losing cribellate silk may be highly asymmetric. When it is truly much easier to lose than to gain a character, parsimony optimization may incorrectly reconstruct a character as having multiple origins when historically it has one origin and many losses totaling more than the most parsimonious number of steps (Cunningham, 1999; Swofford and Maddison, 1992). Asymmetric models of character reconstruction, which have been used to investigate the evolution of other characters considered easier to lose than to gain (e.g., Cunningham, 1999; Goldberg and Igic, 2008; Oakley and Cunningham, 2002), may be a more useful approach for understanding the evolution of cribellate silk.

Penestomines were first described from the female only and placed in the family Eresidae (Simon, 1902). Even in the original description, it was noted that these spiders are quite atypical eresids. Nevertheless, there are several morphological characters that appear to support the placement of penestomines within Eresidae including the presence of a clypeal hood (Fig. 1A and B), specialized white setae, a subrectangular carapace, and stout legs (Fig. 1C and D). Also, penestomines lack tarsal trichobothria (Fig. 2F). Eresines (all non-penestomine eresids) and most other taxa outside of the RTA clade (see below) symplesiomorphically lack trichobothria (specialized sensory setae) on the distal-most leg segment, the tarsus. Eresines and penestomines are both cribellate (some penestomines have erroneously been reported as being ecribellate, see Miller et al., in preparation); the RTA clade contains a mix of cribellate and ecribellate taxa.

Ambiguity about the proper placement of penestomines deepened with the description of the first male (Lehtinen, 1967) because the male pedipalp (intromittent organ, Fig. 1F) does not resemble those of eresines (Fig. 1E) and features a strong retrolateral tibial apophysis (RTA). The RTA (Fig. 1G) defines a major clade of spiders (more than half of spider diversity) exclusive of Eresidae (Coddington and Levi, 1991; Griswold et al., 2005).

In addition to an RTA, penestomine males also have a median apophysis (MA; Fig. 1F), a sclerite of the male pedipalp. The evolutionary history of the MA has been more homoplasious than that of the RTA and identification of this sclerite in all its homologous forms has been a major challenge in spider systematics (Agnarsson and Coddington, 2007; Agnarsson et al., 2007a; Coddington, 1990b; Griswold et al., 1998). This caveat acknowledged, the MA is commonly present in RTA-clade taxa (Fig. 1G) and orbicularians (orb-web weaving spiders and their descendants), present in penestomines (Fig. 1F), and absent from other eresids (Fig. 1E).

So the question becomes, did the RTA and MA evolve independently in penestomine eresids and RTA-clade spiders, or are penestomines misplaced in Eresidae and belong instead within the RTA-clade? To test these alternative hypotheses, we assembled a molecular data set featuring eresines, penestomines, and many RTA-clade taxa.

2. Methods

2.1. Taxon sampling

The foundation for this study came from Spagna and Gillespie (2008), which featured a broad sampling of RTA-clade taxa sequenced for fragments from four genes. To these 37 terminals we added 42 additional taxa for a total of 79 terminals. Among these are representatives of seven out of eight eresine genera. We also focused on the most problematic “core RTA clade” families (e.g., Amaurobiidae, Agelenidae, Desidae, Dictynidae, Hahniidae) with the goal of including representatives of as many subfamilies as possible (mostly following Lehtinen, 1967). Some core RTA-clade families suffer from a lack of evidence for monophyly and are awkward to diagnose. Sampling at the subfamily level provides an opportunity to reevaluate the monophyly of core RTA clade spider families while increasing the chances that a close relative of Penestominae will be included in the analysis.

Representatives of several other spider families were also included, notably some RTA-clade families that have not previously been considered in any quantitative higher-level phylogenetic analysis (Zodariidae, Homalonychidae, Chummidae), and representatives of the Oecobiidae and Hersiliidae because they are thought to be close relatives of Eresidae (Coddington and Levi, 1991). The analysis was rooted using Palpimanidae and Austrochilidae based on the most recent quantitative morphological phylogeny (Griswold et al., 2005).

The RTA clade includes two diverse and distinctive assemblages, the Dionycha and Lycosoidea. Together, these account for 83% of the RTA clade (although it is not obvious that Dionycha and Lycosoidea should form a clade together). *Penestomus* lacks traits that would associate it with this part of the RTA clade and no new Dionycha or Lycosoidea were added for this study. Similarly, the Orbicularia are also peripheral to this question and are sparsely represented here.

2.2. Extraction and sequencing

Specimens used to generate new data for this study are listed in Appendix A. In some cases, specimens were used in previous studies and we worked from extracted DNA. For new material, extraction proceeded as follows:

Whole genomic DNA was extracted from 1–4 legs with a DNEasy kit (Qiagen, Inc.) following the manufacturer's protocol for animal tissues. Specimen storage was variable with some collected into 95% ethanol or RNALater (Ambion, Austin, TX) and kept at -20°C before extraction, and others kept in 75% ethanol at room temperature for up to 10 years (typically less than 4 years).

Four gene fragments were amplified in 25 μl reactions: a ~ 540 bp region from Cytochrome Oxidase I (COI), a ~ 330 bp region of Histone H3, a ~ 770 bp region of 28S rDNA, and a region of 18S rDNA that was amplified in two fragments. One fragment (1F to 5R) was ~ 920 bp long and the other (5F to 9R) was ~ 820 bp long. Primers and annealing temperatures for each locus are in Table 1. Most reactions consisted of 2.5 μl of $10\times$ Apex buffer (Genesee Scientific, San Diego, USA), 0.42 μl of 10 mM dNTP, 2.4 μl of 25 mM MgCl_2 , 1 μl each of forward and reverse 10 mM primers, between 1.5 μl and 2.5 μl of BSA, 0.3 μl Apex Taq DNA Polymerase (Genesee Scientific, San Diego, USA), 1–4 μl of template DNA, and water to 25 μl . Reaction conditions included an initial denaturation step at 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, annealing temperatures and times as reported in Table 1 and 72°C for 1 min (H3 and COI) or 1 min 30 s (18S and 28S), followed by a final extension at 72°C for 7 min, and a hold at 4°C . The only significant exception to this protocol was for the amplifications of H3. These



Fig. 1. Morphology of Penestomidae compared with Eresidae and the RTA clade. (A and B) scanning electron micrographs; (C and D) live animals in the field; (E–G) Composite photomicrographs of male pedipalps, ventral view. (A) face of *Penestomus* sp. nov. 1 (Penestomidae), arrow indicates clypeal hood. (B) face of *Stegodyphus mimosarum* (Eresidae), arrow indicates clypeal hood (modified from Griswold et al. (2005): fig. 129A). (C) adult female *Penestomus* sp. nov. 1 with egg sac under *Eucalyptus* bark in Grahamstown, South Africa. (D) Two *Stegodyphus mimosarum* females in social colony from Ranomafana, Madagascar. (E) *Dresserus* sp. (Eresidae). (F) *Penestomus* sp. nov. 3. (G) *Callobius* sp. (Amaurobiidae). MA, median apophysis; RTA, retrolateral tibial apophysis.

reactions used 2.5 μ l of 10 \times USB PCR reaction buffer (USB Corporation, Cleveland, USA), 0.5 μ l of 10 mM dNTP, 1.5 μ l of 25 mM $MgCl_2$, 0.2 μ l each of forward and reverse 25 mM primers, 2 μ l of BSA, 0.5 μ l of HotStart-ITTM Taq DNA Polymerase (USB Corporation, Cleveland, USA), up to 4 μ l template DNA, and water to 25 μ l. Reaction conditions followed the protocol of Latiolais et al. (2006). PCR products were purified using Exonuclease 1 (Exo1) and Shrimp Alkaline Phosphatase (SAP). For every 1 μ l of PCR product, 0.01 μ l of Exo1, 0.02 μ l of SAP, and 0.11 μ l of H₂O was added. This mixture was incubated at 37 $^{\circ}C$ for 15 min and 80 $^{\circ}C$ for another 15 min.

The ABI BigDye[®] Terminator kit (version 3.1, Applied Biosystems Inc., Foster City, USA) was used to perform 10 μ l cycle sequencing reactions using 1.63 μ l 5 \times buffer, 0.5 μ l 10 mM primer, and 0.75 μ l BigDye[®] Terminator. Template DNA and water amounts were adjusted based on the concentration of DNA in each sample. Cycle sequencing parameters followed the protocol of Platt et al. (2007) with a variable annealing temperature, dependent on the melting temperature of the individual primer. Reaction sequences were obtained from an ABI 3130XL genetic analyzer

(Applied Biosystems Inc., Foster City, USA). For some 28S rDNA and 18S rDNA samples, internal primers were used in addition to external primers to provide redundant sequence coverage (see Table 1). All sequences were checked for contamination using a Blast search. Genbank accession numbers for all new sequence data generated for this study are given in Table 2.

2.3. Analyses

2.3.1. Alignment

Sequences were reconciled and initially aligned using the computer programs Sequencher 4.7 (GeneCodes Co., Ann Arbor, USA) and MUSCLE v3.7 (available via <http://www.ebi.ac.uk/Tools/muscle/>; Edgar, 2004). The COI and H3 alignments were further tested by translating the sequences into amino acids and checking for inappropriately placed stop codons in MacClade (Maddison and Maddison, 2000). Alignment of the ribosomal genes was more complicated than for the protein coding genes. We explored the sensitivity of our results to alignment parameters for the ribosomal

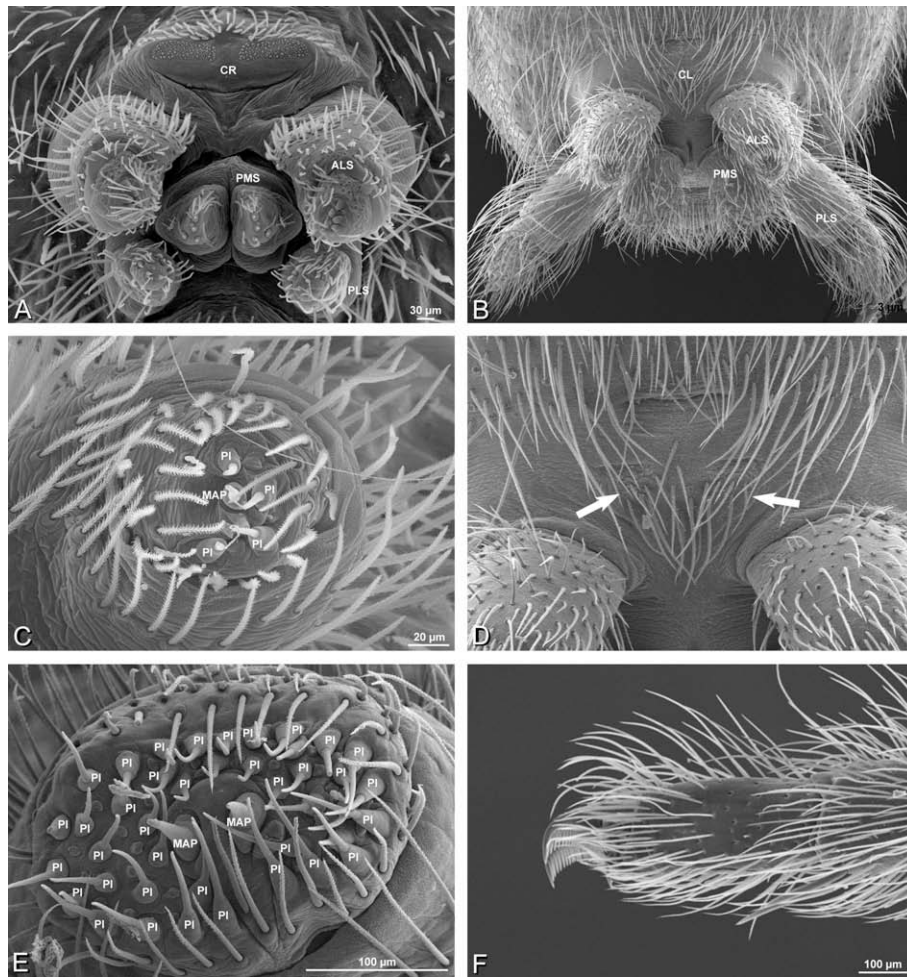


Fig. 2. Scanning electron micrographs of the spinnerets, tarsus. (A) spinnerets of female *Penestomus* sp. nov. 1. (B) spinnerets, *Coelotes atropos* (Agelenidae). (C) anterior lateral spinneret of male *Penestomus* sp. nov. 1. (D) detail showing divided colulus of *Coelotes atropos*. (E) anterior lateral spinneret of *Storenomorpha* sp. (Zodariidae). (F) tarsus of *Penestomus* sp. nov. 1 showing a lack of tarsal trichobothria. ALS, anterior lateral spinneret; CL, colulus; CR, cribellum; MAP, major ampullate gland spigot; PI, piriform gland spigot; PLS, posterior lateral spinneret; PMS, posterior median spinneret.

genes by producing four data matrices: (1) initial alignment using MUSCLE v3.7 (default settings; <http://www.ebi.ac.uk/Tools/muscle/>; Edgar, 2004) with subsequent “manual” adjustment, (2) unadjusted alignment using MUSCLE v3.7 (default settings; <http://www.ebi.ac.uk/Tools/muscle/>), (3) unadjusted alignment using MAFFT (default settings; available via <http://www.ebi.ac.uk/Tools/mafft/>; Katoh et al., 2009, 2002), (4) unadjusted alignment using ClustalW2 (default settings; available via <http://www.ebi.ac.uk/Tools/clustalw2/>; Larkin et al., 2007; Thompson et al., 1994). Alignment of the protein coding genes was held constant in all four matrices. Hereafter, alignment 1 will be referred to as the manual alignment; alignments 2–4 will be referred to as algorithmic alignments. We took this approach because we are not convinced that any existing algorithm is consistently better than a biologist at formulating hypotheses of homology. Our manual alignment approach is like that of a morphologist hypothesizing character homologies: a morphologist observes patterns of similarity and difference among organisms and formulates hypotheses of homology. However, the quality of a manual alignment may be influenced by biases, experience, and talent. To the extent that both classes of alignment yield consistent results, we can have confidence that our conclusions will be valid across a range of credible alignments. Data on the length and gappiness of each aligned locus are given in Table 3. Homogeneity of base frequency was tested using the *basefreq* command in PAUP* (Swofford, 2003; Table 4).

Unless otherwise indicated, analyses described below are based only on the manual alignment. All four alignments are available online as [supplementary documents](#).

2.3.2. Congruence

Congruent signal from among the four genes was assessed using the partition homogeneity test as implemented in PAUP*. All pairwise comparisons among the four genes (manual alignment only) were made. In all cases, homogeneity was rejected ($p < 0.05$). These results are consistent with a similar analysis performed by Spagna and Gillespie (2008). Phylogenetic analyses were run on each gene individually as well as for the entire dataset simultaneously under both Bayesian and parsimony criteria.

2.3.3. Phylogeny

Bayesian analysis was performed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). A mixed model analysis was conducted for each of the four alignments. For the two protein coding genes, each codon position was modeled independently; the two ribosomal genes were also modeled independently for a total of eight data partitions. Gaps were treated as missing, not as a fifth character state. Best fit models for each partition were determined independently according to the non-hierarchical Akaike information criterion as implemented in MrModeltest (Nylander, 2008). The SYM model was applied to

Table 1

Primer sequences, their sources, and reaction conditions used to generate data for this study.

| Locus | Annealing temperature/time | Direction | Primer | Sequence | Reference |
|----------|---|-------------------------------|----------------------|---|---|
| C01 | 50–54°/45s | Forward | C01–1628 | ATA ATG TAA TTG TTA CTG CTC ATG C | Vandergast et al. (2004) Simon et al. (1994) |
| | | Reverse | C1-N-2191 (Nancy) | CCC GGT AAA ATT AAA ATA TAA ACT TC | |
| H3 | 52°/45 s or following Latiolais et al. (2006) | Forward | LC01490-oono | CWA CAA AYC ATA RRG ATA TTG G | Modified from Folmer et al. (1994) Folmer et al. (1994) Colgan et al. (1998) |
| | | Reverse | HC02198 | TAAACTTCAGGGTGACCAAAAAATCA | |
| 28S | 50–53°/30 s | Forward | H3aF | ATG GCT CGT ACC AAG CAG ACV GC | Colgan et al. (1998) Colgan et al. (1998) Hedin and Maddison (2001) Hedin and Maddison (2001) Modified from Maddison et al. (2009) Modified from Hedin and Maddison (2001) Whiting et al. (1997) Whiting et al. (1997) |
| | | Reverse | H3aR | ATA TCC TTR GGC ATR ATR GTG AC | |
| | | Forward | H3nF | ATG GCT CGT ACC AAG CAG AC | |
| | | Forward | 28S0 | GAA ACT GCT CAA AGG TAA ACG G | |
| | | Reverse | 28S0 | GGT TCG ATT AGT CTT TCG CC | |
| | | Forward | L0264cs | CGG GTT GCT TGG GAG TGC | |
| 18S | 55°/30 s | Forward | 28S0cs | CGT GAA ACT GCT CAG AGG | Giribet et al. (1996) Giribet et al. (1996) Giribet et al. (1996) Giribet et al. (1996) Modified from Giribet et al. (1996) Modified from Giribet et al. (1996) This study This study |
| | | Internal | 28SA | GAC CCG TCT TGA AAC ACG GA | |
| | | Internal | 28SR | CCG TGT TTC AAG ACG GGT CG - modified reverse of 28SA | |
| | | Forward | 18S1F | TAC CTG GTT GAT CCT GCC AGTAG | |
| | | Reverse | 18S5R | CTT GGC AAA TGC TTT CGC | |
| | | Forward | 18S5F | GCG AAA GCA TTT GCC AAG AA | |
| Reverse | 18S9R | GAT CCT TCC GCA GGT TCA CCTAC | | | |
| Internal | 18S3FI | GTT CGA TTC CGG AGA GGG AGC | | | |
| Internal | 18S3Rs | GCT CCC TCT CCG GAA TCG AAC | | | |
| Internal | 18S_5_9_intF | ATT CCG WTA ACG ADC GAG | | | |
| Internal | 18S_5_9_intR | CTC GHT CGT TAW CGG AAT | | | |

the H3 position 2 partition, The HKY + G model was applied to the COI position 3 partition. The GTR + I + Γ model was applied to the remaining six partitions. Parameters (character state frequencies, Γ shape parameter, proportion of invariant sites, substitution rates of the GTR model, transition/transversion ratio) were estimated independently for each partition using the following command: unlink statefreq=(all) shape=(all) pinvar=(all) revmat=(all) ratio=(all). Analyses were run on the CCG (Center for Comparative Genomics) Phylocluster at the California Academy of Sciences and the CIPRES (Cyberinfrastructure for Phylogenetic Research) cluster at the San Diego Supercomputer Center. Tree search proceeded according to MrBayes defaults (two independent analyses consisting of three heated and one cold MCMC chain). Analyses proceeded at least until the deviation of split frequencies fell below 0.01. Trees were sampled every 1000 generations. Chain convergence was evaluated in Tracer (Rambaut and Drummond, 2007). At least the first 10% of each search was discarded as “burn-in”.

Parsimony analyses were performed in TNT (Goloboff et al., 2008) and PAUP*. Our search in TNT consisted of 100 iterations of random taxon addition followed by 200 iterations of TBR (tree bisection-reconnection) branch swapping on a slightly perturbed version of the matrix (the Ratchet search strategy; Nixon, 1999). The PAUP* analysis consisted of 1000 iterations of heuristic search with random taxon addition. Nonparametric bootstrap support values were calculated in PAUP* based on 1000 replicate searches each with 100 iterations of random taxon addition.

2.3.4. Hypothesis testing

No analysis recovered a monophyletic Eresidae including *Penestomus*. To test the robustness of this result, a maximum likelihood analysis constrained to recover a monophyletic Eresidae was performed in PAUP* (10 replicates of random taxon addition with TBR swapping). An unconstrained analysis was also performed. The Shimodaira–Hasegawa test (Goldman et al., 2000; Shimodaira and Hasegawa, 1999) with a fully optimized model was used to test whether the unconstrained tree is significantly more likely than the most likely tree containing a monophyletic Eresidae.

2.3.5. Character evolution

Morphological characters were mapped onto the topology resulting from the mixed model Bayesian analysis of the manual alignment of all data. The trace character function in Mesquite (Maddison and Maddison, 2008) optimizes most parsimonious character states at internodes; where multiple equally parsimonious solutions exist, the ancestral character state is equivocal (Fitch, 1971; Swofford and Maddison, 1987, 1992). Character states were determined based on specimen examination supplemented by published literature (e.g., Forster and Wilton, 1973; Griswold, 1990; Griswold et al., 2005; Jocqué, 1991; Jocqué and Dippenaar-Schoeman, 2006; Wang, 2000).

2.3.6. Asymmetric models of character state change

Oakley and Cunningham (2002) developed a method for exploring character optimization under various models of asymmetric character state change. The test can be used when character optimization under parsimony indicates that a (typically complex) character state is not homologous across the tree. The procedure allows investigators to find a minimum loss/gain ratio that preserves homology of the character of interest.

Parsimony optimization of the cribellum on the Bayesian phylogeny implies at least six independent origins of this complex silk spinning organ. Conventional thinking in arachnology assumes the cribellum is a primitive araneomorph character that has been repeatedly lost (e.g., Coddington, 1986; Forster and Wilton, 1973; Griswold et al., 2005; Ledford and Griswold, 2010; Lehtinen, 1967). When a character is actually more likely to be lost than gained, typical parsimony-based character mapping can erroneously indicate non-homology (Cunningham, 1999; Swofford and Maddison, 1992). Likelihood sensitivity analysis allows investigators to consider whether a homoplasious character distribution pattern is better explained by non-homology or by a particular loss/gain probability ratio.

We determined the minimum loss/gain ratio in a likelihood model on the Bayesian tree (manual alignment of all data) that would make homology of the cribellum significantly more likely than multiple independent origins. Calculations were performed

Table 2

GenBank accession numbers for new sequences generated for this study. Codes identify individual specimens and are kept as labels with vouchers (see also Appendix A).

| Species | Code | 28S | 18S (1st half) | 18S (2nd half) | Histone H3 | COI |
|---|-------|--------------|----------------|----------------|------------|----------|
| <i>Adonea fimbriata</i> | 14–11 | FJ948947 | FJ948864 | FJ948905 | FJ949027 | FJ948987 |
| <i>Ambohima</i> sp. | P07 | FJ948948 | FJ948865 | FJ948906 | | FJ948988 |
| cf. <i>Aschema</i> sp. | 13–15 | FJ948969 | FJ948885 | FJ948927 | FJ949046 | FJ949009 |
| <i>Cavernocymbium prentoglei</i> | 08–13 | FJ948949 | | FJ948907 | FJ949028 | FJ948989 |
| <i>Chresiona</i> sp. | 10–06 | FJ948950 | FJ948866 | FJ948908 | FJ949029 | FJ948990 |
| <i>Chumma inquieta</i> | 16–02 | FJ948951 | FJ948867 | FJ948909 | FJ949030 | FJ948991 |
| <i>Cybaeolus</i> sp. | 11–01 | FJ948952 | FJ948868 | FJ948910 | FJ949031 | FJ948992 |
| <i>Desis formidabilis</i> | 10–03 | FJ948953 | FJ948869 | FJ948911 | FJ949032 | FJ948993 |
| <i>Dorceus fastuosus</i> | 13–05 | FJ948954 | FJ948870 | FJ948912 | FJ949033 | FJ948994 |
| <i>Dresserus colsoni</i> | 13–09 | FJ948955 | FJ948871 | FJ948913 | | FJ948995 |
| <i>Dresserus kannemeyeri</i> | 09–03 | FJ948956 | FJ948872 | FJ948914 | FJ949034 | FJ948996 |
| <i>Eresus</i> cf. <i>kollari</i> | 14–04 | FJ948958 | FJ948874 | FJ948916 | FJ949036 | FJ948998 |
| <i>Eresus walckenaeri</i> | 14–05 | FJ948959 | FJ948875 | FJ948917 | FJ949037 | FJ948999 |
| <i>Eresus</i> sp. nov. | 13–06 | FJ948957 | FJ948873 | FJ948915 | FJ949035 | FJ948997 |
| <i>Gandanameno fumosa</i> | 09–05 | FJ948963 | FJ948879 | FJ948921 | FJ949041 | FJ949003 |
| <i>Gandanameno fumosa</i> | 14–06 | FJ948964 | FJ948880 | FJ948922 | FJ949042 | FJ949004 |
| <i>Gandanameno spenceri</i> | 09–02 | FJ948962 | FJ948878 | FJ948920 | FJ949040 | FJ949002 |
| <i>Gandanameno</i> sp. | 13–10 | FJ948961 | FJ948877 | FJ948919 | FJ949039 | FJ949001 |
| <i>Hahnia clathrata</i> | 11–02 | FJ948965 | FJ948881 | FJ948923 | FJ949043 | FJ949005 |
| <i>Hersilia insulana</i> | 09–09 | FJ948966 | FJ948882 | FJ948924 | FJ949044 | FJ949006 |
| <i>Hersiola macullata</i> | 14–07 | FJ948967 | FJ948883 | FJ948925 | FJ949045 | FJ949007 |
| <i>Hickmania troglodytes</i> | 13–11 | FJ948945 | FJ948862 | FJ948903 | FJ949025 | FJ948985 |
| <i>Homalonychus selenopoides</i> | 15–09 | ^a | FJ948902 | FJ948944 | FJ949062 | |
| <i>Ikuma</i> sp. | 11–06 | FJ948946 | FJ948863 | FJ948904 | FJ949026 | FJ948986 |
| <i>Mallos pallidus</i> | 16–07 | FJ948968 | FJ948884 | FJ948926 | | FJ949008 |
| <i>Oecobius</i> sp. | 16–03 | FJ948970 | FJ948886 | FJ948928 | FJ949047 | FJ949010 |
| <i>Oncodamus decipiens</i> | 10–10 | FJ948971 | FJ948887 | FJ948929 | FJ949048 | FJ949011 |
| <i>Penestomus</i> sp. nov. 1 | 08–15 | FJ948973 | FJ948889 | FJ948931 | FJ949050 | FJ949013 |
| <i>Penestomus</i> sp. nov. 2 | 11–09 | FJ948972 | FJ948888 | FJ948930 | FJ949049 | FJ949012 |
| <i>Seothyra annettae</i> | 09–04 | FJ948974 | FJ948890 | FJ948932 | FJ949051 | FJ949014 |
| " <i>Stegodyphus</i> " <i>annulipes</i> | 15–10 | FJ948960 | FJ948876 | FJ948918 | FJ949038 | FJ949000 |
| <i>Stegodyphus lineatus</i> | 14–02 | FJ948976 | FJ948892 | FJ948934 | FJ949053 | FJ949016 |
| <i>Stegodyphus mimosarum</i> | 09–06 | FJ948977 | FJ948893 | FJ948935 | FJ949054 | FJ949017 |
| <i>Stegodyphus tentoriicola</i> | 14–12 | FJ948975 | FJ948891 | FJ948933 | FJ949052 | FJ949015 |
| <i>Taira</i> sp. | 11–13 | FJ948978 | FJ948894 | FJ948936 | FJ949055 | FJ949018 |
| <i>Tamgrima alveolifera</i> | 13–13 | FJ948979 | FJ948895 | FJ948937 | FJ949056 | FJ949019 |
| <i>Tricholathys</i> sp. | 10–05 | | FJ948896 | FJ948938 | FJ949057 | FJ949020 |
| <i>Uroctea durandi</i> | 13–07 | FJ948980 | FJ948897 | FJ948939 | FJ949058 | FJ949021 |
| <i>Uroctea durandi</i> | 14–08 | FJ948981 | FJ948898 | FJ948940 | | |
| <i>Vidole capensis</i> | 10–14 | FJ948982 | FJ948899 | FJ948941 | FJ949059 | FJ949022 |
| <i>Zanomys californica</i> | 16–01 | FJ948983 | FJ948900 | FJ948942 | FJ949060 | FJ949023 |
| <i>Zodarion</i> sp. | 16–06 | FJ948984 | FJ948901 | FJ948943 | FJ949061 | FJ949024 |

^a Downloaded from Genbank (AY955557) combined with new sequence data from another specimen, both from Sonora, Mexico.**Table 3**

Aligned sequence length and percent gaps inserted into each locus during alignment. Percent gaps based on internal gaps only; incomplete 3' or 5' ends ignored. Total is the number of characters in each of the four complete alignments.

| | Aligned length | % Gaps |
|---------|----------------|--------|
| Protein | | |
| H3 | 328 | 0.00 |
| COI | 493 | 0.01 |
| Manual | | |
| 28S | 939 | 23.17 |
| 18S | 1712 | 4.45 |
| Total | 3472 | |
| Muscle | | |
| 28S | 912 | 20.93 |
| 18S | 1716 | 4.68 |
| Total | 3449 | |
| Maaft | | |
| 28S | 846 | 14.99 |
| 18S | 1710 | 4.34 |
| Total | 3377 | |
| Clustal | | |
| 28S | 881 | 15.89 |
| 18S | 1820 | 7.13 |
| Total | 3522 | |

in BayesTraits (Pagel and Meade, 2009) using the *MultiState* model. First, we estimated the rates of loss ($q_{10} = 3.799116144$) and gain**Table 4**

Chi-square test of base frequency homogeneity.

| | Base frequency | | | | Homogeneity test | | |
|-----|----------------|---------|---------|---------|------------------|-----|---------|
| | A | C | G | T | Chi-square | df | P |
| H3 | 0.26566 | 0.26420 | 0.24769 | 0.22244 | 201.20414 | 189 | 0.25823 |
| COI | 0.23267 | 0.13876 | 0.18823 | 0.44034 | 155.88765 | 204 | 0.99486 |
| 28S | 0.17799 | 0.29075 | 0.35264 | 0.17862 | 278.46039 | 228 | 0.01258 |
| 18S | 0.24451 | 0.23247 | 0.27607 | 0.24695 | 43.33203 | 234 | 1.00000 |

($q_{01} = 1.783626982$) without constraints (mean rates based on 5,050,000 iterations of MCMC analysis, burnin = 50,000). We used the *restrict* command to vary the loss/gain ratio from even (1/1) to asymmetric (2.5/1). The total rate of change ($q_{10} + q_{01}$) was kept constant (5.582743126). We used the *fossil* command to fix a particular character state (absence or presence of the cribellum) at key nodes so that the cribellum was either homologous across the tree or evolved repeatedly (Fig. 6A). We calculated the negative log likelihood and took the difference (nodes fixed present minus nodes fixed absent). A difference in negative log likelihood of >2 is conventionally interpreted as significant (Pagel, 1999).

We performed an analogous analysis using a Sankoff matrix under parsimony (Kohn et al., 1996; Maddison, 1994; Swofford and Maddison, 1992) in TNT (Goloboff et al., 2008). We explored loss/gain transformation costs ranging from even (100/100) to asym-

metric (100/1), searching for the minimum ratio that produces homology of the cribellum across the tree. For example, the following command optimizes character 0 on the tree assuming a loss/gain ratio of 100/49: *ccode(0 ; cost 0 = 0>1 100 1>0 49; piwe - ;*

3. Results

3.1. Sequence data and alignment

A chi-square test of base frequency homogeneity found that 28S is significantly CG rich. COI is moderately AT rich, but COI, H3, and 18S all have base frequencies that are statistically indistinguishable from equal (Table 4).

Alignment of protein coding genes was trivial. H3 required no gap insertions; COI required a single three nucleotide gap in one terminal. Both ribosomal genes, 28S and 18S, were aligned using four different methods. Aligned length of 28S ranged from 846

(14.99% gaps) to 939 (23.17% gaps) positions; 18S ranged from 1710 (4.34% gaps) to 1820 (7.13% gaps) positions (Table 3).

3.2. Phylogeny of Entelegynae

Bayesian analysis of the entire dataset (four loci, eight partitions, manual alignment) was allowed to proceed for 50,000,000 generations. The deviation of split frequencies remained below 0.01 after 2,402,000 generations. The tree in Fig. 3 was generated with the first 5,000,000 generations (10%) discarded as “burn-in.” Visualization of the all trees in Tracer (Rambaut and Drummond, 2007) indicated that stability had been achieved by this point in the analysis. Results from the Bayesian analyses of the algorithmic alignments shown in Figs. S1, S3, S5.

Parsimony analysis of the entire dataset (four loci, manual alignment) resulted in four equally parsimonious trees (10838 steps). Nine nodes are collapsed in the strict consensus of these four trees (Fig. 4). Both the TNT and PAUP* analyses recovered

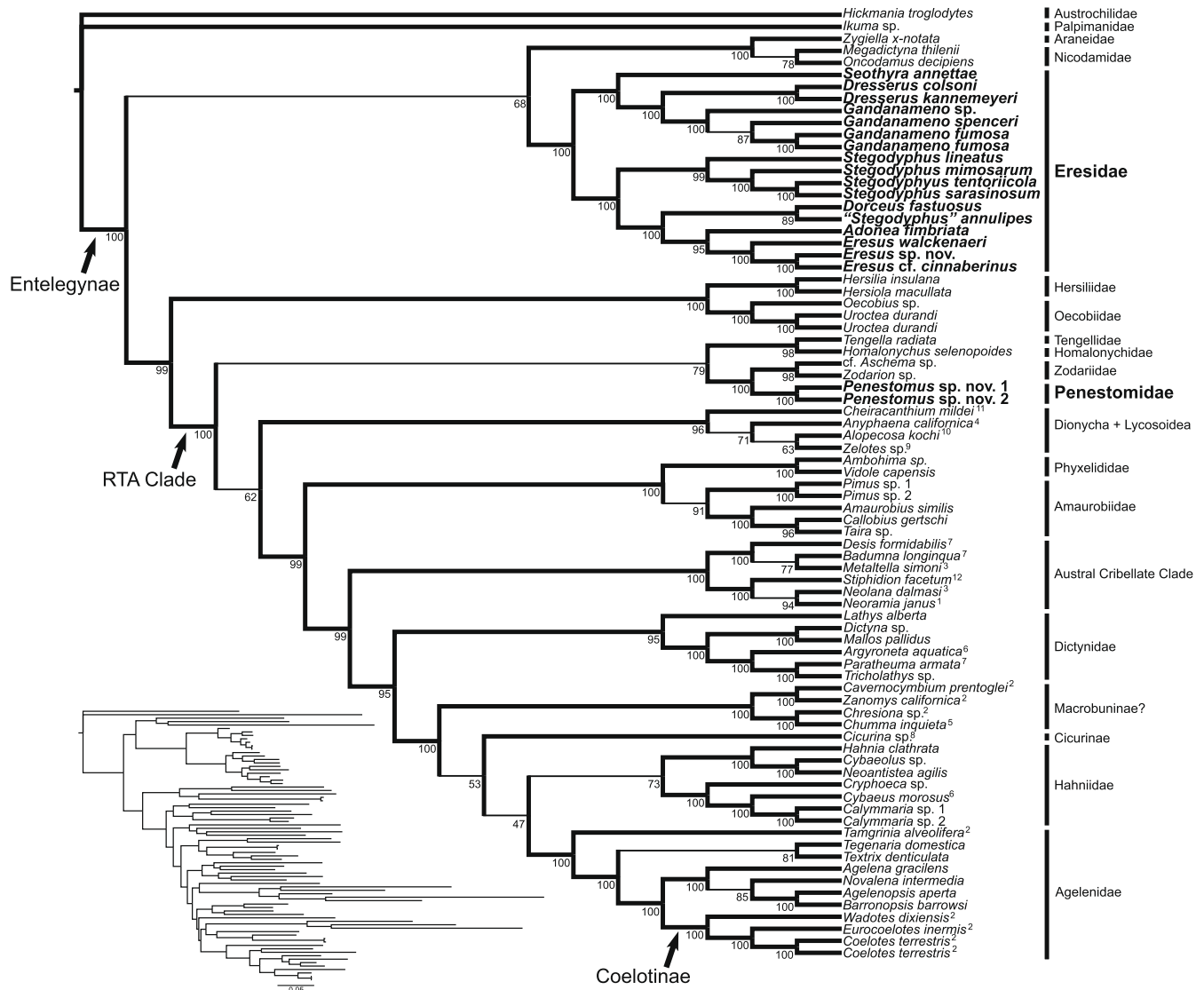


Fig. 3. Topology from Bayesian mixed model analysis of eight data partitions (28S rDNA, 18S rDNA, H3 positions 1–3, COI positions 1–3), manually adjusted alignment. Numbers at nodes are percent posterior probabilities; branches with less than 95% posterior probability drawn with fine lines. Labels spanning one or more terminals indicate clades discussed in the text. Where these labels are in conflict with or uninformative as to family circumscription in the current catalog (Platnick, 2009), superscript numbers indicate the following families: 1, Agelenidae; 2, Amaurobiidae; 3, Amphinectidae; 4, Anyphaenidae; 5, Chummiidae; 6, Cybaeidae; 7, Desidae; 8, Dictynidae; 9, Gnaphosidae; 10, Lycosidae; 11, Miturgidae; 12, Stiphididae. Eresidae and Penestomidae are indicated with large bold type; Entelegynae, the RTA clade, and subfamily Coelotinae are labeled with arrows. Inset tree has branches proportional to change.

the same four trees. The TNT analysis achieved minimum length in 81/100 replicates; PAUP* did the same in 90/1000 replicates. Given the agreement between the two software packages and especially the high success rate in the TNT analysis, it is likely that the set of most parsimonious trees was found. Results for the parsimony analyses of the algorithmic alignments shown in Figs. S2, S4, S6.

3.3. Congruence

The question of how to deal with different sources of phylogenetic data, particularly when they are not homogenous, is a perennial one in systematics (e.g., Barrett et al., 1991; Bull et al., 1993; Chippindale and Wiens, 1994; de Queiroz et al., 1995; Eernisse and Kluge, 1993). We performed combined data analysis in spite of the lack of homogeneity among the loci. We also ran analyses on each individual locus (manual alignment only). These results are summarized in Fig. 5. They indicate that no individual gene reliably anticipates the results from the entire data set but that

the ribosomal gene 28S is the most predictive locus. The protein coding genes H3 and COI predict the combined data topology only at shallow nodes.

3.4. Long branches

Bayesian trees revealed that some taxa are on conspicuously long branches. Furthermore, some points of conflict between Bayesian and parsimony trees appear consistent with the phenomenon of long-branch attraction (e.g., Felsenstein, 1978). To investigate this, we removed from the manual alignment the four most conspicuous long-branch taxa: *Paratheuma armata*, *Hahnia clathrata*, *Cybaeolus* sp., and *Neoantistea agilis*. The explanation for why these particular sequences are so anomalous (pseudogenes, paralogs, evolutionary rate variation) is beyond the scope of this investigation.

The Bayesian reanalysis of this modified matrix yields a topology that is otherwise identical to the original result. Parsimony

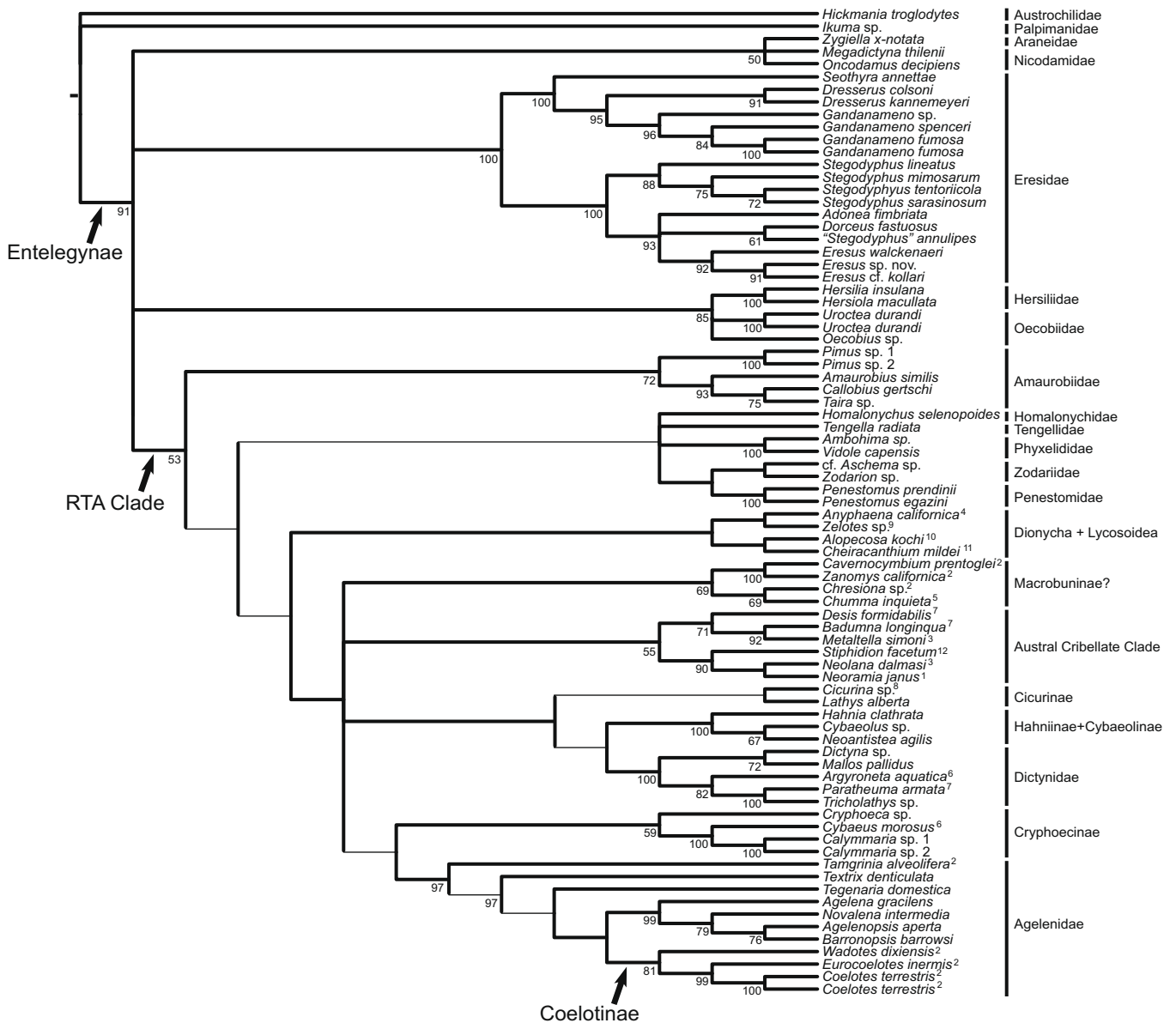


Fig. 4. Strict consensus of four most parsimonious topologies from analysis of complete molecular data set (manually adjusted alignment). Thin branches are inconsistent with the Bayesian topology. Numbers at nodes represent percent bootstrap support ≥ 50 ; other conventions as in Fig. 3.

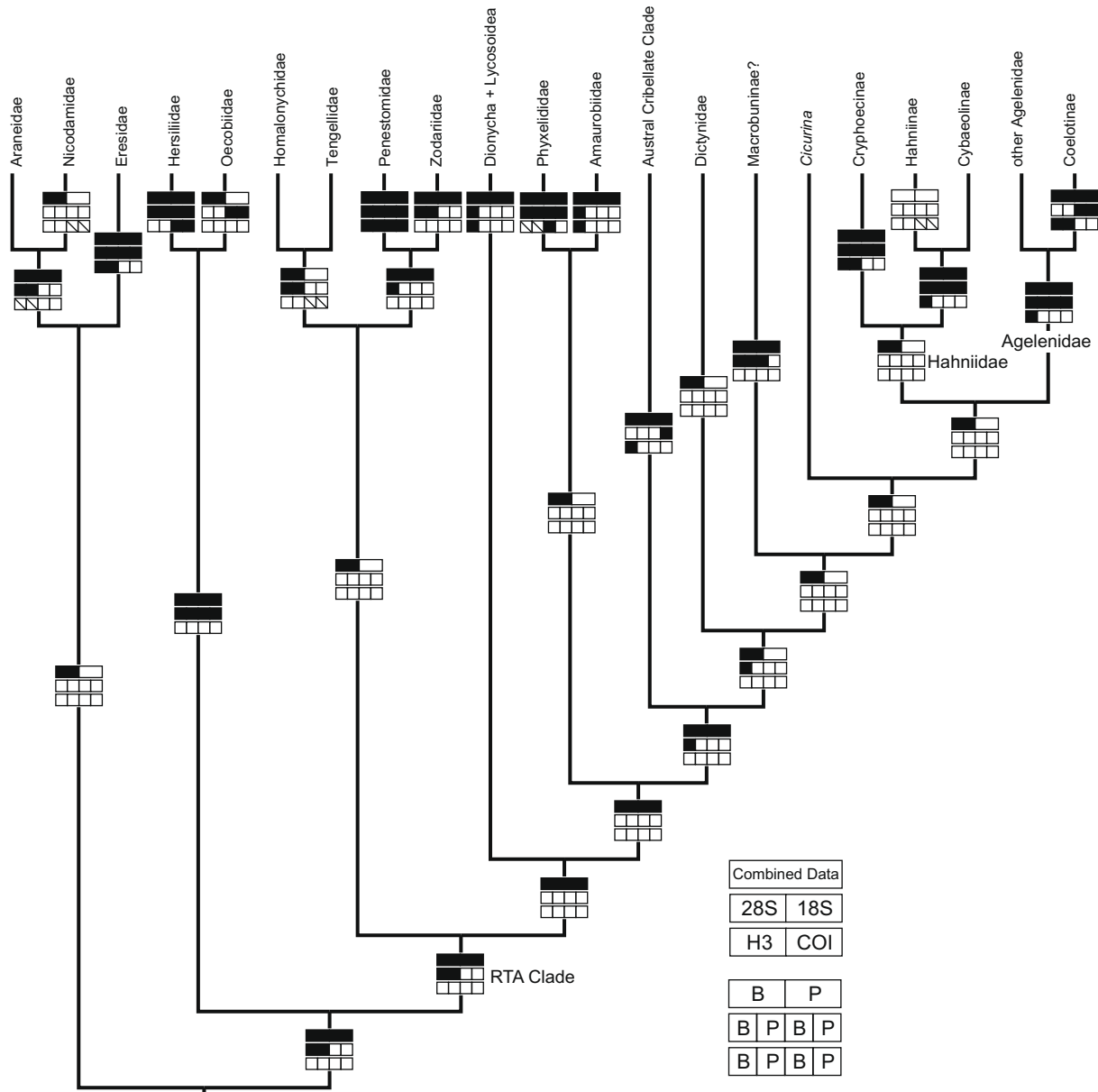


Fig. 5. Summary phylogeny of entelegyne spiders with congruence among data partitions and optimality. Topology and clade composition based on Bayesian analysis of manual alignment (see Fig. 3). Boxes at nodes indicate each data partition (combined data or individual gene) subdivided by analysis method (B, Bayesian: left side or P, parsimony: right side). Black squares indicate the clade was recovered (regardless of support) in the given analysis, white squares indicate the clade was not recovered, diagonal line indicates the clade was not tested (because one or fewer taxa from the clade were included, typically because of missing sequence data). Note that Hahniinae is a morphology-based concept that was not recovered in any molecular analysis.

reanalysis yields a unique topology (not shown). The conflict cannot be explained by long-branch attraction alone, but it would appear that the Bayesian result is less sensitive to analytical permutations than the parsimony results.

3.5. Phylogenetic affinities of Penestomidae

No analysis recovered a monophyletic Eresidae including *Penestomus*. The constrained Shimodaira–Hasegawa analysis also rejected inclusion of *Penestomus* in Eresidae ($p < 0.05$) for all alignments. Instead, all analyses based on the complete data set placed *Penestomus* sister to (rarely within) Zadariidae (Figs. 3 and 4, S1–S6) under both Bayesian and parsimony criteria. The Zadariidae are diagnosed by several characters not found in *Penestomus* (e.g., absence of serrula, lateral implantation of tarsal claw teeth,

long anterior lateral spinnerets; see Jocqué, 1991). Based on their morphological and molecular distinctiveness, Penestominae is promoted to family rank. Diagnosis and description of the family will appear in a taxonomic revision (Miller et al., in preparation).

3.6. Sensitivity of topology to analytical parameters

We analyzed our data under several alignments and optimality criteria to assess which results were sensitive to various analytical parameters. Consistent results will likely be supported by the data across a range of analyses.

Phylogenetic placement of some taxa was affected by alignment and optimality criteria. Within the Bayesian framework, the following elements were variable: placement of *Tengella radiata*, *Homalonychus selenopoides*, *Cheiracanthium mildei*, *Cicurina* sp.,

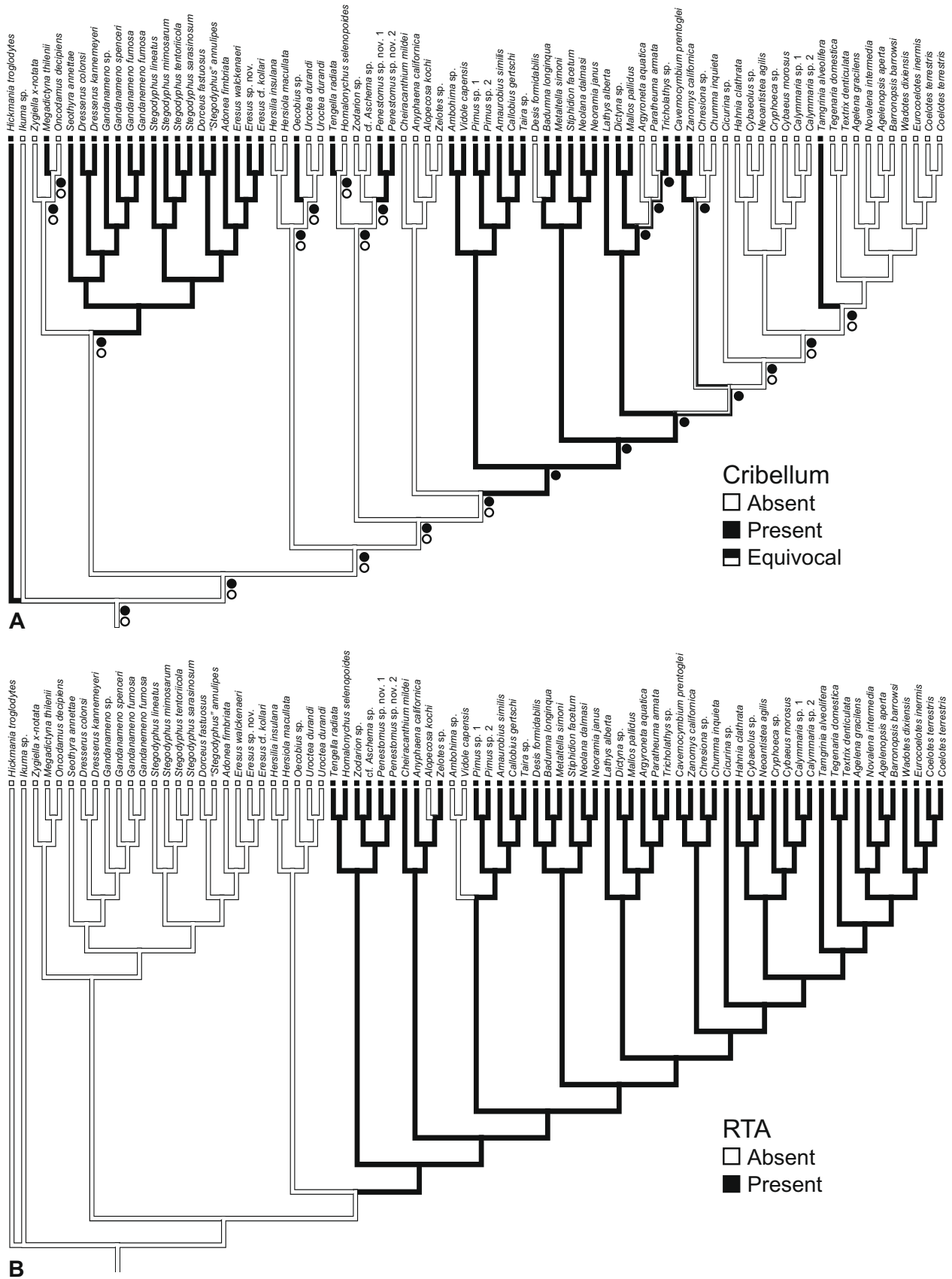


Fig. 6. Ancestral character state reconstruction of morphological characters optimized on Bayesian topology (manually adjusted alignment). (A) cribellum, black and white circles indicate nodes fixed to have cribellum present or absent, respectively, for the likelihood sensitivity analysis. (B) retrolateral tibial apophysis (RTA).

and *Megadictyna thilenii*, the relationship between Penestomidae + Zodariidae and Dionycha + Lycosoidea, the relative placement of Dictynidae and the Austral Cribellate Clade, placement of the Hahniinae + Cybaeolinae, placement of the Macrobulinae, and some internal relationships within Agelenidae (Figs. 3, S1, S3, S5).

Within a parsimony framework, strict consensus trees indicate that few relationships for major (e.g., family group) clade relationships are uncontested across the four alignments. Many of the taxa and clades (plus others) that were sensitive to alignment parameters in a Bayesian framework remain variable under parsimony (Figs. 4, S2, S4, S6).

Nevertheless, the major conclusions of this work are consistent across all analyses. *Penestomus* always groups with Zodariidae (sister to in nearly all cases, nested within in the parsimony analysis of the unadjusted Muscle alignment). The Agelenidae always include the Coelotinae with *Tamgrinia* sister to the rest of Agelenidae. The composition of several major clades (and most of their internal relationships) is consistent across all analyses (e.g., the Austral Cribellate Clade, Macrobulinae, Cryphoecinae, Amaurobiidae, Eresidae).

3.7. Cribellum evolution under asymmetric character state change

Likelihood sensitivity analysis indicates that the homology of the cribellum is supported if the rate of loss is just under two times the rate of gain (Fig. 7). Under Sankoff matrix parsimony, a loss/gain ratio of 100/49 or greater produces a reconstruction with a plesiomorphic cribellum and 15 independent losses, two more steps than under standard parsimony.

4. Discussion

4.1. Character evolution

4.1.1. Evolutionary history of the cribellum and asymmetric character evolution

Historically, araneomorph spiders were classified into two major groups based on the presence (Fig. 2A) or absence (Fig. 2B) of a cribellum. With the adoption of more phylogenetically sophisticated thinking by systematists (Forster and Wilton, 1973; Lehtinen, 1967), the cribellum came to be thought of as a primitive

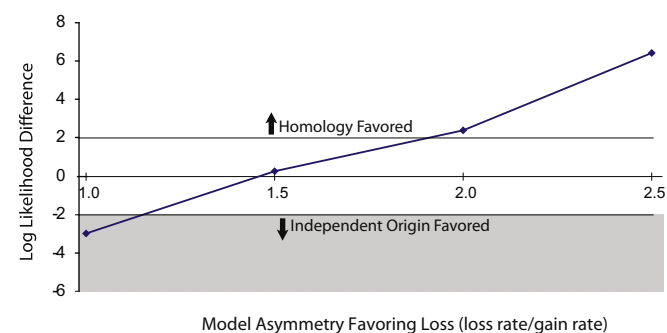


Fig. 7. Likelihood sensitivity analysis comparing models with differing amounts of rate asymmetry in cribellum evolution. Two alternative hypotheses were evaluated under these different rates: the homology hypothesis had 24 nodes fixed as cribellum present to preserve homology across the tree; the independent origin hypothesis had 16 of the same nodes fixed as cribellum absent (unconstrained when cribellum optimized under standard parsimony as unambiguously present or equivocal). The y axis represents the negative log likelihood of the homology hypothesis minus that of the independent origin hypothesis. The x axis is the magnitude of asymmetry in rates of evolution in the model. Significant support for cribellum homology (difference in negative log likelihood >2) is found when the rate of loss is just under two times the rate of gain.

araneomorph feature that has been lost multiple times. This explained the presence of a number of similar spider taxa chiefly distinguished by the presence or absence of a cribellum; these had classically been attributed to parallel evolution in cribellate and ecribellate spiders (Kaston, 1964; Kullmann, 1972). Cribellum loss is now a major theme in spider systematics (Coddington, 1986; Griswold, 1993; Griswold et al., 1999, 2005; Spagna and Gillespie, 2008).

The phylogenetic results presented here do not permit parsimonious optimization (Fitch, 1971; Swofford and Maddison, 1987, 1992) of the cribellum as a symplesiomorphic organ independently lost multiple times (Fig. 6A). But does this mean that the conventional wisdom about cribellum evolution is false? Morphological apparatus associated with cribellate silk production are complicated and unique, so it seems unlikely that investigators would conflate non-homologous structures. At the same time, cribellate silk is metabolically expensive and inferior in other ways to ecribellate sticky silk (Blackledge et al., 2009; Köhler and Vollrath, 1995). So it may be much easier to lose cribellate silk than to gain it. The question may be analogous to that of compound eyes in ostracod crustaceans (Oakley and Cunningham, 2002). The distribution of compound eyes on a well-supported phylogeny mean that one of two seemingly unlikely scenarios must be true: either very similar and complicated structures have evolved multiple times, or they have arisen once and been lost a large number of times totaling more than the most parsimonious number of steps. In the case of ostracod compound eyes, Oakley and Cunningham (2002) found that significant support for a single-origin/multiple-loss hypothesis required asymmetry in the character state change parameter >30:1. Unfortunately, it is difficult to know whether this is realistic or not. As with ostracod eyes, parsimony optimization of cribellum evolution on the tree indicates multiple origins. Some of this may be due to topological error, some to taxon sampling or extinction. For example, the likely sister group to the ecribellate genus *Cicurina* is the cribellate genus *Bromella*. While inclusion of this rare genus would have expanded the reconstructed ancestral distribution of the cribellum, it still would not have been possible (under standard parsimony) to optimize the cribellum as homologous across spiders. Phylogenetic studies with more extensive taxon sampling are currently in progress (e.g., the Assembling the Tree of Life: Phylogeny of Spiders project) and this will undoubtedly advance our understanding of these issues. But if cribellum loss is common, it might be that extinction of cribellate lineages is also common. So, even perfect sampling of extant taxa might not allow us to accurately optimize cribellum evolution under parsimony (Cunningham, 1999; Swofford and Maddison, 1992).

Using two approaches to the question of asymmetric character state change, we found that a scenario where loss was about two times more likely than gain was necessary to explain the cribellum as a plesiomorphic feature independently lost multiple times. It is impossible to assess with certainty whether this threshold of character state change asymmetry is realistic in this case. But in our judgment, the auxiliary assumption of asymmetric evolutionary rates makes a more compelling hypothesis than multiple independent origins of cribellate silk.

Complicated characters that appear (under parsimony optimization) to evolve multiple times are not uncommon in systematics. We suggest that the alternative, a relatively high rate of loss, should be considered when investigating the evolution of such characters. This principal is essentially a probabilistic (rather than categorical) interpretation of Dollo's law (Simpson, 1953).

The phylogenetic pattern of character state distribution associated with asymmetrical gain:loss rates is probably best understood in cave systems, where eye loss and other stereotypical cave adaptations occur repeatedly in distantly related taxa. Weins et al. (2003) proposed a generalized method for investigating the

evolution of taxa when some share both similar adaptive zones (e.g., caves) and similar traits that could be the result of either homology or convergence. Other controversial evolutionary questions that have been or could be analyzed under alternatives to standard parsimony optimization include flower polymorphism associated with cross-pollination (Kohn et al., 1996), sexual dichromatism in ducks (Omland, 1997), symmetry in asterid flowers (Ree and Donoghue, 1999), larval feeding in echinoderms (Cunningham, 1999), coiled shells in gastropods (Collin and Cipriani, 2003), wings in walking stick insects (Stone and French, 2003; Trueman et al., 2004; Whiting et al., 2003), vivipary and salt secretion in mangroves and other plants (Shi et al., 2005), homology of venom across squamate reptiles (Fry et al., 2006), re-evolution of lost digits in lizards (Kohlsdorf and Wagner, 2006; but see Galis et al., in press), sexual reproduction in oribatid mites (Domes et al., 2007), the tadpole stage in frogs (Wiens et al., 2007), and a feeding larval stage in marine gastropods (Collin, 2004; Collin et al., 2007). Note that exploring asymmetric models of character evolution does not necessarily lead to conclusions favoring homology of complex character states over convergence (e.g., Collin et al., 2007; Goldberg and Igic, 2008; Shi et al., 2005; Wiens et al., 2007).

4.1.2. Homoplasy and the placement of the Penestomidae

Morphological characters that seemed to support placement of Penestomines with Eresidae (e.g., carapace shape, stout legs, specialized white setae, the clypeal hood, lack of tarsal trichobothria) have apparently misled previous workers. Some of these characters have not been rigorously defined or scored across spider taxa. But the clypeal hood (Fig. 1A and B) is a rare trait that made a reasonably credible putative synapomorphy for Eresidae and Penestomidae (Griswold et al., 1999, 2005), and losses of tarsal trichobothria within the RTA clade are rare (Fig. S7B). Non-RTA-clade spiders have trichobothria on various leg segments, but not the tarsi.

The first male penestomid was not described until more than 60 years after the first female (Lehtinen, 1967). Male specimens remain rare in collections (only four of 60 total adults in a new revision; Miller et al., in preparation). Yet only males carry certain characters of importance in spider phylogeny. Among these are the RTA and the MA, features of the male pedipalp (see below). This is in part why penestomids have remained an enigmatic taxon for decades. Our results show that penestomids are not eresids that have independently evolved an RTA and MA. Rather, penestomids are part of a large clade where nearly all members exhibit both an RTA and MA.

Our results consistently suggest a single origin of the RTA with losses in wolf spiders (*Alopecosa*) and Phyxelididae (Fig. 6B). Among the Phyxelididae, an RTA is present only in the genus *Vytftitia*, which is considered sister to the remaining phyxelidids; the loss of the RTA supports in part the monophyly of the remaining phyxelidids (Griswold, 1990). At least one parallel origin of a retrolateral tibial apophysis occurs within the Araneoidea (the linyphiid subfamily Erigoninae; Hormiga, 1994). Nicodamids also have a tibial apophysis but its dorsal (rather than retrolateral) origin has caused investigators to consider it non-homologous with the RTA (Griswold et al., 2005).

The MA is one of several sclerites arising from the tegulum, the central lobe of the male palpal bulb. Tegular sclerites vary in number, position, and form. This makes morphology-based homology assessment of these sclerites across spiders extremely challenging (Agnarsson and Coddington, 2008; Agnarsson et al., 2007a; Coddington, 1990b; Griswold et al., 1998). According to our results, the MA has at least two origins, once in the araneoid-nicodamid lineage, and once coincident with the RTA (Fig. S7A). The MA has been repeatedly lost.

4.2. Systematics

4.2.1. Orbiculariae

Orb-weaving spiders and their relatives (the Orbiculariae) are conventionally divided into two sister groups, the cribellate Deinopoidea and the ecribellate Araneoidea (Coddington, 1986; Griswold et al., 1998). The most recent study on this question (Blackledge et al., 2009) was based on a combination of morphological, behavioral, and molecular data and concluded with support for the monophyletic origin of the orb-web. They advance a preferred tree based on simultaneous analysis of all data that includes the traditional Deinopoidea and slightly updated versions of the Araneoidea and Orbiculariae, all as monophyletic groups (their fig. 2). However, molecular data alone never recovered Deinopoidea, and Orbiculariae is monophyletic only with the inclusion of the RTA clade (their figs. S3, S5). Thus the molecular data alone imply one of two scenarios: either the orb-web has been independently derived at least twice or RTA-clade spiders are descended from orb-weaving ancestors. While the monophyletic origin theory always imagined secondary losses of the orb-web (Coddington, 1986; Griswold et al., 1998), the idea that RTA-clade spiders are derived orb-weavers is a significant departure. Because most of the classical characters supporting the original monophyletic origin theory concern the orb-web itself, the lack of corroboration from an independent source of data (i.e., molecular sequence) cannot be considered supportive of the original hypothesis.

Whether this novel picture of orb-web evolution reflects history or an artifact of gene selection remains an open question. It is worth noting that other molecular studies, particularly those dominated by ribosomal genes (Hausdorf, 1999; Wu et al., 2002), have also failed to recover Orbiculariae. However, Ayoub et al. (2007) did recover Orbiculariae, Araneoidea, and Deinopoidea using a single nuclear protein coding gene.

4.2.2. Nicodamidae

This family from Australia/New Zealand includes both cribellate and ecribellate members. We added the ecribellate *Oncodamus* to the cribellate *Megadictyna* already sequenced by Spagna and Gillespie (2008) to test the monophyly of the family. The precise sister group of Nicodamidae is not well established but positions close to orbicularians, the RTA clade, and the family Titanocidae have all been proposed (Coddington et al., 2004; Griswold et al., 1999, 2005; Harvey, 1995). Our results support a hypothesis of orbicularian affinity, consistent with Blackledge et al. (2009). Titanocids were not included in our study so their possible affinity with nicodamids was not investigated.

4.2.3. Eresidae

Seven of the eight remaining eresid genera were included in this study. The family is divided into two major clades: *Seothyra*, *Dresserus*, and *Gandanameno* form a southern and eastern African clade. *Seothyra* is exclusively southern African, whereas the sister genera *Dresserus* and *Gandanameno* occur in southern and eastern Africa. The other major clade comprises *Stegodyphus*, *Eresus*, *Adonea*, and *Dorceus*; *Eresus*, *Adonea*, and *Dorceus* form a Palearctic/Mediterranean clade; *Stegodyphus* is found in Africa, the paleotropics, and the Amazon. The remaining genus, *Paradonea*, which was unavailable for this study, is from southern Africa.

Recent phylogenies have placed Eresidae with the Oecobiidae and Hersiliidae in the Eresoidea (Agnarsson et al., 2006; Coddington et al., 2004; Coddington and Levi, 1991; Griswold et al., 1999), which in turn is sister to a large clade dominated by the Orbiculariae and RTA clade. All Bayesian and some parsimony analyses (Figs. 3, S1, S3–S5) placed Eresidae sister to nicodamids plus (at least araneoid) orb-weavers.

Johannesen et al. (2007) used mitochondrial sequence data to investigate phylogenetic relationships in *Stegodyphus*. Included in the analysis were two other eresid genera, *Eresus* and *Gandana-meno*. Their results suggested that *Stegodyphus* is paraphyletic with respect to *Eresus* (their fig. 1). While our taxon sampling within *Stegodyphus* was more limited, we selected a representative from each of the four clades recovered by Johannesen et al. (2007) to test whether our data would support a monophyletic or paraphyletic *Stegodyphus*. In addition, we included a species of uncertain affinity described as *Eresus annulipes*, currently cataloged in *Stegodyphus* (Platnick, 2009). By contrast with Johannesen et al. (2007), we did not find *Eresus* nesting within well established *Stegodyphus* species. Only the enigmatic *Stegodyphus annulipes* appears to disrupt the monophyly of *Stegodyphus*.

New World Eresids. Eresidae is an almost exclusively Old World family. However, there are records of two species from Brazil. *Stegodyphus annulipes* was described from Brazil in 1856. We included *S. annulipes* in our study but our specimen was collected in the Negev desert of Israel and identified after rediscovery of the type of *S. annulipes* in Paris (M. Řezáč, unpublished data). Thus we believe that *S. annulipes* is in fact a Mediterranean, not a Neotropical, species. *Stegodyphus manus* was collected from Amazonian Brazil in 1973 (Kraus and Kraus, 1992). In contrast to *S. annulipes*, this seems to be a legitimate New World eresid.

4.2.4. Oecobiioidea

Oecobiidae and Hersiliidae together form a clade, the Oecobiioidea. They are united in part by their unique prey attack behavior (Coddington and Levi, 1991). A series of phylogenetic studies suggested that Oecobiidae, Hersiliidae, and Eresidae form a clade, the Eresoidea (Agnarsson et al., 2006; Coddington et al., 2004; Coddington and Levi, 1991; Griswold et al., 1999, 2005; Platnick et al., 1991) based mostly on characteristics of the spinneret spigot morphology. Some other investigators (e.g., Wunderlich, 2004) have not accepted a close relationship between Eresidae and Oecobiioidea. All Bayesian analyses place Oecobiioidea sister to the RTA clade (Figs. 3, S1, S3, S5); a close relationship between Eresidae and the Oecobiioidea is never supported.

Like the orb-weavers, the modern Oecobiidae comprises cribellate and ecribellate taxa that historically had their similarities explained as convergent evolution (Shear, 1970; Simon, 1892, 1893). It is worth noting that Wu et al. (2002) included one cribellate and one ecribellate oecobiid in an analysis based on 12S rRNA sequence data and failed to recover a monophyletic Oecobiidae. By contrast, cribellate *Oecobius* and ecribellate *Uroctea* form a well-supported clade in our analysis.

4.2.5. Zodariioidea

Zodariids and penestomids consistently formed a monophyletic group, although the close relatives of that clade were different among the various alignments and optimality criteria (Figs. 3 and 4, S1–S6). Previous investigators have suggested that Chummidae and Homalonychidae might be close relatives of zodariids (Jocqué, 2001; Jocqué and Dippenaar-Schoeman, 2006; Roth, 1984). Homalonychids were found to be close to zodariids in some analyses. Chummids were never close to zodariids. Zodariids, penestomids, chummids and homalonychids have never been included in a quantitative higher-level phylogenetic analysis.

One line of morphological evidence that could support a close relationship between zodariids and penestomids has to do with the arrangement of spigots on the anterior lateral spinnerets. Typically, the major ampullate gland spigots (MAP) are placed on the margin of the spinning field (e.g., Griswold et al., 2005: fig. 49B) but in both zodariids (Fig. 2E) and penestomids (Fig. 2C) the MAP are placed in the center of the field of piriform gland spigots.

4.2.6. Dionycha, Lycosoidea

Together, Dionycha and Lycosoidea represent more than half of spider diversity (Platnick, 2009). Dionycha are the two clawed spiders (spiders primitively have three claws on each tarsus; Coddington and Levi, 1991); Lycosoidea includes the wolf spiders and their relatives, characterized primarily by features of the eyes and genitalia (Griswold, 1993; Sierwald, 1990; Silva Dávila, 2003). Our study was primarily concerned with relationships among the most problematic core RTA clades, i.e., the RTA clade exclusive of the megadiverse Dionycha and Lycosoidea. Questions about interrelationships or monophyly of Dionycha and Lycosoidea are beyond the scope of this study and we added no new taxa from these lineages. Nevertheless, four of our exemplars clustered together in most analyses: *Anyphaena* (Dionycha: Anyphaenidae), *Zelotes* (Dionycha: Gnaphosidae), *Alopecosa* (Lycosoidea: Lycosidae) and (with less consistency) *Cheiracanthium* (problematically placed in Miturgidae; Deeleman-Reinhold, 2001; Jocqué and Dippenaar-Schoeman, 2006; Ramírez et al., 1997).

4.2.7. Amaurobiidae

Amaurobiidae may be a much smaller clade than the current catalog (Platnick, 2009) would indicate. We consistently found a monophyletic Amaurobiidae similar to the Amaurobiinae of Lehtinen (1967) plus the genus *Pimus*, which Lehtinen placed in the Macrobininae (see below). This suggests Amaurobiidae is a Holarctic, entirely cribellate family. Other genera currently cataloged in Amaurobiidae consistently grouped with the Agelenidae (e.g., *Coelotes*, *Tamgrinia*) or the clade we provisionally call Macrobininae (e.g., *Chresiona*, *Zanomys*).

4.2.8. Dictynidae

Recent authors have placed *Argyroneta* together with *Cybaeus* and related genera in their own family (Grothendieck and Kraus, 1994; Jocqué and Dippenaar-Schoeman, 2006; Selden, 2002; see Hahniidae, below). However, studies on molecular phylogeny (Spagna and Gillespie, 2008; this study) and karyotype analysis (Král, 1995) have failed to find support for this relationship. A close relationship between *Argyroneta* and *Paratheuma* is supported by both studies on molecular phylogeny. This is intriguing since both genera spend a substantial part of their lives under water. Placement of *Argyroneta* and *Paratheuma* (referred to by the junior synonyms *Swainsia* and *Litisides*) within Dictynidae was anticipated by Lehtinen (1967). *Paratheuma* is currently cataloged in the Desidae (Platnick, 2009).

Most Dictynidae in our analysis are on conspicuously long branches; one clade of hahniids (Hahniinae + Cybaolinae) also has long branches (Fig. 3). For some alignments including manual alignment, the long-branch hahniids and dictynids group together in the parsimony analysis (Fig. 5) but not the Bayesian analysis, consistent (in part) with the phenomenon of long-branch attraction (e.g., Felsenstein, 1978; see also Section 3.4.).

4.2.9. Macrobininae?

Lehtinen (1967) included *Zanomys*, *Chresiona*, and *Pimus* in his amaurobiid subfamily Macrobininae. As noted above, our data support *Pimus* as part of the Amaurobiidae sensu stricto, but *Zanomys* and *Chresiona* plus two other exemplars (*Cavernocymbium* and *Chumma*) consistently form a clade (Figs. 3 and 4, S1–S6). Unfortunately, we did not include *Macrobunus* in our analysis, so our name for this clade is provisional. A close relationship between *Zanomys* and *Cavernocymbium* (also *Parazanomys*) was already established (Ubick, 2005) but placement of *Chumma* (the sole genus in the family Chummidae) here instead of near Zodariidae is a novel finding. Ubick's (2005) suggestion that *Zanomys* and its close relatives are the cribellate sister group to the Coelotinae is not supported by our data. A more focused study including *Macrobunus* will be

required to determine whether our tentative name for this clade is appropriate, and whether Chummidae should be redefined. We have no evidence from morphology that our exemplar taxa share any of the characters proposed as synapomorphic for Macroboninae. Most conspicuously, zanomyines have a divided cribellum (Ubick, 2005; contra Lehtinen, 1967: 334) while most recent work on macrobunines conceives them as a clade with an entire cribellum, occasionally lost (Griswold et al., 2005; see also Compagnucci and Ramirez, 2000).

Ubick (2005) included a third genus in his zanomyine complex: *Parazanomys*. Sequencing of this genus was attempted for this study but ultimately failed. Ubick's hypothesis of a close relationship between the zanomyine complex and coelotines was based on morphological similarities that are most clearly expressed in *Parazanomys*. Future investigations could show that *Parazanomys* is a coelotine, but assuming no radical changes in the phylogeny, such a result would disrupt the monophyly of the zanomyine complex.

4.2.10. Cicurinae

Cicurina is currently cataloged in Dictynidae (Platnick, 2009). According to some analytical permutations (e.g., most Bayesian analyses, Figs. 3, S1, S3), *Cicurina* is sister to Hahniidae + Agelenidae. It is interesting to note that this clade (except for the distinctive Hahniinae) is very similar to a broad circumscription of Agelenidae accepted by a previous generation of arachnologists (e.g., Exline, 1938; Roth and Brame, 1972), but not reflected by the current catalog (Platnick, 2009).

Lathys was included in Lehtinen's Cicurinae. This is supported in some parsimony analyses but no Bayesian analyses, where it consistently groups with other Dictynidae. *Lathys* and *Bromella* are the only cribellate cicurine genera and *Bromella* (not included) is particularly similar to the ecribellate *Cicurina* (Lehtinen, 1967; see also Section 4.1.1.).

4.2.11. Hahniidae

All three hahniid subfamilies (Hahniinae, Cybaeolinae, and Cryphoecinae) were represented in our analysis. Most Bayesian (but not parsimony) analyses recovered a monophyletic Hahniidae with the addition of *Cybaeus* grouping with members of the hahniid subfamily Cryphoecinae (Fig. 3). A similar result was recovered by Spagna and Gillespie (2008). Lehtinen (1967) considered *Cybaeus* an ecribellate but otherwise unremarkable dictynid (see also Section 4.2.8).

Hahniines are typically characterized by a distinctive transverse arrangement of the spinnerets (Lehtinen, 1967). The hahniine exemplars in our analysis are *Hahnia* and *Neoantistia*. Hahniine monophyly is disrupted by our single cybaeoline exemplar (Figs. 3 and 4); *Cybaeolus* has a more typical spinneret arrangement. However, Lehtinen (1967: 362–364) reports that spinneret arrangement within hahniines can be quite variable, referencing one genus (*Muizenbergia*, currently synonymized under *Hahnia*; Platnick, 2009) that includes both unmodified and hahniine-like spinneret arrangements.

4.2.12. Agelenidae

Lehtinen (1967) limited Agelenidae to two subfamilies: Agelinae and Coelotinae, scattering other traditional agelenid taxa among various families. Noting unspecified similarity in genital structures between coelotines and amaurobiids, Wunderlich (1986: 24) synonymized Agelenidae under Amaurobiidae. Platnick (2009) did not accept the family level synonymy but did accept the transfer of coelotine genera to the Amaurobiidae. Spagna and Gillespie (2008) (see also Ke-Ran and Kai-Ya, 2005) recovered coelotines within the Agelenidae. Although they did not explicitly advocate transfer of Coelotines from Amaurobiidae back to Agelen-

idae, we do (Appendix B). In addition to consistent results across several phylogenetic studies, Coelotines feature a divided colulus (Fig. 2D), a key characteristic of Agelenidae (also found in some Hahniidae). Also, the karyotypes of *Agelena* and *Coelotes* are characterized by a pattern of secondary constrictions that is unique among entelegyne spiders studied so far. In both genera, at least two chromosome pairs contain intercalary secondary constrictions, one of which is duplicated (Král, 1994).

Forster and Wilton (1973) placed the cribellate *Neoramia* in the Agelenidae. A series of phylogenetic studies have used this genus to represent Agelenidae (Blackledge et al., 2009; Griswold et al., 1999, 2005). Griswold et al. (1999) found that *Neoramia* belonged to the "fused paracribellar clade," a group of mostly austral taxa with fused paracribellar spigots. Spagna and Gillespie (2008) found that *Neoramia* belongs within the "Austral cribellate clade" (similar in composition to the "fused paracribellar clade") but that this clade was not closely related to the classical, ecribellate Agelenidae (Lehtinen, 1967; Roth and Brame, 1972). Blackledge et al.'s (2009) analysis included a chimeric terminal with morphological data based on *Neoramia* and sequence data from the ecribellate agelenid *Agelenopsis*. We report here that the Himalayan genus *Tamgrinia*, not *Neoramia*, is the cribellate sister group to Agelenidae (Figs. 3–5). Wu et al. (2002) used the mitochondrial ribosomal gene 12S to investigate the position of *Tamgrinia*, finding it sister to coelotines, which in turn were sister to agelenines. A phylogenetic study based on morphology also recovered coelotines as closer to agelenids than to amaurobiids, and placed *Tamgrinia* sister to agelenids including coelotines (Wang and Martens, 2009).

The Coelotinae and *Tamgrinia* are transferred from Amaurobiidae to the Agelenidae (Appendix B). Cribellate spiders from Australia and New Zealand currently cataloged as Agelenidae (e.g., *Neoramia*, *Tuapoka*, *Ahua*, *Huka*; Platnick, 2009) probably belong to the Austral Cribellate clade, but we do not formally propose transfer at this time.

5. Conclusions

There is a great deal of consistency across various analytical permutations in the recovery of clades at roughly the family and subfamily level. This is complicated by a handful of taxa that do not consistently fall with a particular clade (e.g., *Tengella radiata*, *Homalonychus selenopoides*, *Cheiracanthium mildei*, *Cicurina* sp.). Relationships among family and subfamily clades are more sensitive to analytical permutations (alignment parameters, analysis method). Support tends to be low along the tree backbone, especially in parsimony analyses (but note that parsimony bootstrap and Bayesian posterior probability values cannot be directly compared; e.g., Alfaro and Holder, 2006).

The principal conclusions of this study are insensitive to analytical permutations. These include the consistent and statistically supported placement of *Penestomus* within the RTA clade and outside of Eresidae, and a revised circumscription of Agelenidae including Coelotinae and the genus *Tamgrinia*. Other consistent results may encourage more focused investigations in the future. For example, what are the true limits of the Amaurobiidae? Is it a Holarctic cribellate family or a more heterogeneous global taxon? What about the composition and nomenclature of the unique clade tentatively called here "Macroboninae?" What are the appropriate family groups within the Austral Cribellate Clade?

The low level of congruence among commonly used genes presents a problem for the question of family group relationships within the Entelegynae. Molecular phylogenetics in spiders is dominated by a small number of genes and the loci used in our study are fairly typical. Agnarsson et al. (2007b) emphasized that confidence in their results was enhanced when support came from mul-

tiple independent lines of evidence. Many key nodes in the phylogeny presented here lack corroboration from multiple loci. Questions about deep level spider relationships would clearly benefit from additional genes evolving at an appropriate rate. But despite focused efforts, technical challenges mean that new loci are introduced to spider phylogenetics at a very slow pace (Ayoub et al., 2007; Vink et al., 2008, 2005).

Our study of cribellum evolution is only the latest to explore the history of a complicated character system that appears (under parsimony optimization) to evolve multiple times. Character optimization is one of the most powerful methods in systematics for investigating the evolution of interesting biological phenomena. However, in cases where a character is really much easier to lose than to gain, standard parsimony optimization can be misleading (Cunningham, 1999; Swofford and Maddison, 1992). When faced with similar conditions, we encourage our colleagues to consider asymmetric models of character state change.

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Appendix A

Specimens used to generate new data for this study. The species name is given with the family (according to Platnick, 2009 except as newly proposed in this work) and sometimes subfamily in parentheses followed by the specimen code and collection data. For specimens that have been used in previous studies, this is indicated; specimens first sequenced for this study deposited at the California Academy of Sciences unless otherwise indicated.

Adonea fimbriata (Eresidae)

14-11. Israel: Sede Boquer, Negev desert, 30°51'13.76"N 34°46'55"E, 7 April 2004, M. Rezac.

Ambohima sp. (Phyxelididae)

P07. Madagascar: Fianarantsoa, Parc National Ranomafana, Talatekey forest 42.3 km 58°NE Fianarantsoa, 21.2578°S 47.4227°E, 1050 m, 24 January 2005 to 14 December 2006, montane rainforest, CASENT 9024485, H. Wood, J. Miller, J.J. Rafonomezantsoa, E. Rajeriarison, V. Andriamanany. *Extraction from Griswold, Wood and Carmichael, unpublished data.*

cf. *Aschema* sp. (Zodariidae)

13-15. Madagascar: Antsiranana, Montagne d'Ambre, Reserve Speciale Number Deux, Francomme, 6.8 km 35°NE Joffreville, 12.4713°S 49.2128°E, 800 m, 17 December 2005, montane rainforest, general collecting, CASENT 9023831, HW007, H. Wood, H. Raholiarisendra, J. Rabemahafaly.

Cavernocymbium prentoglei (Amaurobiidae)

08-13. United States: California, Riverside Co., Kabian Co. Park, N of Quail Valley betw. Canyon Lake & Goetz Road, 33.7175°N 117.2453°E, 1687 ft., 11–18 November 2004, coastal sage scrub veg., pitfall, S. Kirschtner, T. Prentice.

Chresonia sp. (Amaurobiidae)

10-06. South Africa: Eastern Cape, Grahamstown Municipal Caravan Park, 33.3194°S 26.5238°E, 580 m, 10–19 February 2006, CASENT 9024875, J. Miller, H. Wood, L. Lotz.

Chumma inquieta (Chummidae)

16-02. South Africa: Eastern Cape, Colchester, Pearson Park Nature and Pleasure Resort, E. of Sundays River, 35 km NE Port Elizabeth, 33.698°S 25.8385°E, 5 m, 16–17 February 2006, dune vegetation, CASENT 9021953, J. Miller, H. Wood.

Cybaeolus sp. (Hahniidae: Cybaeolinae)

11-01. Chile: IX [Región de la Araucanía], Malalcahuello Way, Llongquimay, 38.4398°S 71.5102°W, 526 m, 18 January 2007, fogging *Nothofagus dombeyi*, 50 m, 29°C, 18:55PM, CASENT 9030636, E. Arias.

Desis formidabilis (Desidae)

10-03. South Africa: Western Cape, Table Mountain National Park, Kommetjie, 34.1405°S 18.3222°E, 0 m, 1 March 2006, rocky shore, under rocks and limpet shells, CASENT 9023642, J. Miller, H. Wood, N. Larsen.

Dorceus fastuosus (Eresidae)

13-05. Israel: Negev desert, Mashabim Sandy Dunes Reserve, 31°0'0.77"N 34°44'58.31"E, 13 March 2007, leg. M. Rezac.

Dresserus colsoni (Eresidae)

13-09. South Africa: KwaZulu-Natal, Ophathe Game Reserve, Ophathe River crossing, 28.39545°S 31.39405°E, 5 July 2007, active searching under logs, C. Haddad.

Dresserus kannemeyeri (Eresidae)

09-03. South Africa: Erfewis Dam Nature Reserve, 23 February 2007, under logs, C. Haddad.

Eresus cf. *kollari* (Eresidae)

14-04. Italy: Largo di Garda, Pessina, August 1999, Michael Veith. *Previously sequenced for Johannesen et al., 2007.*

Eresus walckenaeri (Eresidae)

14-05. Greece: Crete, Zenia, Michael Veith. *Previously sequenced for Johannesen et al., 2007.*

Eresus sp. nov. (Eresidae)

13-06. Israel: Sede Boquer, 27 August 2007, Jiri Kral.

Gandanameno fumosa (Eresidae)

09-05. South Africa: Eastern Cape, Middleburg, −31.5619°S 24.8038°E, 1360 m, 7 February 2006, under rocks, pine forest and fynbos, J. Miller, H. Wood, L. Lotz, M. de Jager.

Gandanameno fumosa (Eresidae)

14-06. South Africa: Free State, Bloemfontein, 29 January 2005, Milan Rezac. *Previously sequenced for Johannesen et al., 2007, identified there as Gandanameno spenceri.*

Gandanameno spenceri (Eresidae)

09-02. South Africa: Western Cape, Table Mountain National Park, Van Riebeeck Park, 33.9484°S 18.4197°E, 210 m, 28 February 2006, under rocks in pine forest and fynbos, J. Miller, H. Wood, N. Larsen.

Gandanameno sp. (Eresidae)

13-10. South Africa: KwaZulu-Natal, Ophathe Game Reserve, Ophathe River crossing, 28.39545°S 31.39405°E, 5 July 2007, active searching under logs, C. Haddad.

Hahnia clathrata (Hahniidae: Hahniinae)

11-02. South Africa: Eastern Cape, Thomas Baines Nature Reserve, 8.5 km SSW Grahamstown 33.3776°S 26.4883°E, 420 m, 9 February 2006, CASENT 9030622, J. Miller, H. Wood, L. Lotz.

Hersilia insulana (Hersiliidae)

09-09. Madagascar: Fianarantsoa, P.N. Isalo, 22.6159°S 45.3544°E, 850 m, 17 January 2006, gallery forest, H. Wood, J. Miller.

Hersiola macullata (Hersiliidae)

14-07. Israel: Sede Boqer, 30°55'N 34°46'E, under stones in wadi, Yael Lubin. *No voucher specimen.*

Hickmania troglodytes (Austrochilidae)

13-11. Australia: Tasmania, Weldborough, Weldborough Pass Rainforest Walk, 28.6km 280° WNW St Helens, 41.2166°S 147.9385°E, 470 m, 6–7 March 2006, *Nothofagus* forest, general collecting, CASENT 9023515, AU06-047, C. Griswold, D. Silva.

Homalonychus selenopoides (Homalonychidae)

15-09. Mexico: Sonora, 34.2 mi N. Caborca, 14 December 2002, G454, SC102-038, S. Crews. 28S sequence data from a different specimen (Genbank AY959908, g453, *H. selenopoides*, see Crews and Hedin 2006).

Ikuma sp. (Palpimanidae)

11-06. South Africa: Eastern Cape, Colchester, Pearson Park Nature and Pleasure Resort, E. of Sundays River, 35 km NE Port Elizabeth, 33.698°S 25.8385°E, 5 m, 16–17 February 2006, dune vegetation, CASENT 9023778, J. Miller, H. Wood.

Mallos pallidus (Dictynidae)

16-07. United States: California, Siskiyou Co., Ney Springs Creek nr. Faery Falls, 3.19 mi SSW Mt. Shasta City, 41.2626°N 122.3296°W, 3060 ft, 12–13 July 2007, CASENT 9030626, J. Ledford, J. Miller, A. Carmichael, A. Arguello, M. Mitchell.

Oecobius sp. (Oecobiidae)

16-03. Madagascar: Toliara, Forêt de Kirindy field station, 46 km NE Morondava, 20.0669°S 44.6569°E, 50 m, 20–30 January 2006, dry deciduous forest, general collecting, CASENT 9024137, HW014, H. Wood, J. Miller.

Oncodamus decipiens (Nicodamidae)

10-10. Australia: Queensland, Binna Burra, Lamington N.P., 790 m, 21–23 March 2006, CASENT 9023688, OZCG-26, C. Griswold, D. Silva, R. Raven, B. Baehr, M. Ramirez.

Penestomus sp. nov. 1 (Penestomidae)

08-15. South Africa: Eastern Cape, Grahamstown Municipal Caravan Park, 33.3194°S 26.5238°E, 580 m, 10–19 February 2006, under *Eucalyptus* bark, CASENT 9023775, J. Miller, H. Wood, L. Lotz. *See Miller et al., in preparation.*

Penestomus sp. nov. 2 (Penestomidae)

11-09. South Africa: Western Cape, Beaufort West District, Farm Spitskop 73, top of De Jager's Pass, 37.4 km NE Beaufort West, site 65, 32.0687°S 22.7561°E, 1449 m, 8 March 2007, AMNH, L. Prendini & H. Bichard. *See Miller et al., in preparation.*

Seothyra annettae (Eresidae)

09-04. Namibia, 17 km W Okahandja on Waldau River, 21.95°S 16.75°E, 14 December 1996, CASENT 9030627, W.J. Pulawski, V. Ahrens.

"Stegodyphus" annulipes (Eresidae)

15-10. Israel: Neighborhood of Nizzana, September 2007, J. Kral.

Stegodyphus lineatus (Eresidae)

14-02. Israel: Judea, in a tree along the Jerusalem-Jeriko Main Road (Highway 1), 31°49'14"N 35°22'19"E, May

2000, Jes Johannesen. *Previously sequenced for Johannesen et al., 2007.*

Stegodyphus mimosarum (Eresidae)

09-06. Madagascar: Fianarantsoa, Parc National Ranomafana, Valbio Research Station, 42.4 km 58° NE Fianarantsoa, 21.2543°S 47.4217°E, 900 m, 24 December 2005 to 15 January 2006, social web along road, CASENT 9024084, H. Wood, J. Miller, J.J. Rafonomezantsoa, E. Rajeriarison, V. Andriamanany.

Stegodyphus sarasinus (Eresidae)

All sequences from Spagna and Gillespie, 2008, identified there as Stegodyphus sp.

Stegodyphus tentoriicola (Eresidae)

14-12. South Africa: Eastern Cape, Mountain Zebra National Park, April 2000, J. Schneider. *Previously sequenced for Johannesen et al., 2007.*

Taira sp. (Amaurobiidae)

11-13. China: Yunnan, Lushui Co., Yaojiaping He at Pianma Road km 44.7, 25.97479°N 98.71027°E, 2516 m, 20 May 2005, disturbed forest, night collecting in forest and along road cuts, CASENT 9021382, CGY113, C. Griswold, D. Kavanaugh.

Tamgrinia alveolifera (Agelenidae)

13-13. China: Tibet, Longzi County, 3890 m, 8 August 2006, Mingsheng Zhu.

Tricholathys sp. (Dictynidae)

10-05. United States: California, Stanislaus Co., Del Puerto Canyon, N fork of Del Puerto Creek, 37.4883°N 121.2069°W, 70 m, 5 May 2007, CASENT 9030631, J. Miller.

Uroctea durandi (Oecobiidae)

13-07. Greece: Peloponnesus, Didyma, 23 August 2007, Jiri Kral.

Uroctea durandi (Oecobiidae)

14-08. France: St. Tropez, Axel Schönhoffer. *No voucher specimen.*

Vidole capensis (Phyxelididae)

10-14. South Africa: Eastern Cape, Grahamstown, Dassiekrans, 33.3279°S 26.5001°E, 715 m, 20 February 2006, CASENT 9023622, J. Miller, H. Wood.

Zanomys californica (Amaurobiidae)

16-01. United States: California, San Diego Co., SE junct of Mt. Laguna Hwy and I-8, road to Buckman Springs, 32.8011°N 116.5017°W, 3800 ft., 27 November 2005, Berlese funnel, oak litter, CASENT 9030625, MCH 05_111, M. Hedin.

Zodarion sp. (Zodariidae)

16-06. Madagascar: Toliara, Forêt de Kirindy field station, 46 km NE Morondava, 20.0669°S 44.6569°E, 50 m, 20–30 January 2006, dry deciduous forest, general collecting, CASENT 9024103, HW014, H. Wood, J. Miller.

Appendix B

Summary of nomenclatural changes made in this work.

The subfamily Penestominae is removed from the Eresidae and promoted to family rank:

- Penestomidae Simon, 1903 (NEW RANK)

The following genus is transferred from the Eresidae to the family Penestomidae:

- *Penestomus* Simon, 1902

The following genera comprise the Coelotinae and are transferred from the family Amaurobiidae to the family Agelenidae:

- *Alloclubionoides* Paik, 1992
- *Bifidocoelotes* Wang, 2002

- *Coelotes* Blackwall, 1841
- *Coras* Simon, 1898
- *Draconarius* Ovtchinnikov, 1999
- *Eurocoelotes* Wang, 2002
- *Femoracoelotes* Wang, 2002
- *Himalcoelotes* Wang, 2002
- *Iwogumoa* Kishida, 1955
- *Leptocoelotes* Wang, 2002
- *Lineacoelotes* Xu, Li & Wang, 2008
- *Longicoelotes* Wang, 2002
- *Notiocoelotes* Wang, Xu & Li, 2008
- *Orumckia* Koçak & Kemal, 2008
- *Pireneitega* Kishida, 1955
- *Platocoelotes* Wang, 2002
- *Robusticoelotes* Wang, 2002
- *Spiricoelotes* Wang, 2002
- *Tegeocoelotes* Ovtchinnikov, 1999
- *Tonsilla* Wang & Yin, 1992
- *Urocoras* Ovtchinnikov, 1999
- *Wadotes* Chamberlin, 1925

The following genus is transferred from the family Amaurobiidae to the family Agelenidae:

- *Tamgrinia* Lehtinen, 1967

Appendix C. Supplementary data

Supplementary data (Figs. S1–S7, four alignments in Nexus format) associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2010.02.021](https://doi.org/10.1016/j.ympev.2010.02.021).

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