Phylogenetic systematics and evolution of the spider infraorder Mygalomorphae using genomic scale data

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ABSTRACT

The Infraorder Mygalomorphae is one of the three main lineages of spiders comprising over 3,000 nominal species. This ancient group has a world-wide distribution that includes among its ranks large and charismatic taxa such as tarantulas, trapdoor spiders, and highly venomous funnel web spiders. Based on past molecular studies using Sanger-sequencing approaches, numerous mygalomorph families (e.g., Hexathelidae, Ctenizidae, Cyrtaucheniidae, Dipluridae and Nemesiidae) have been identified as non-monophyletic. However, these data were unable to sufficiently resolve the higher-level (intra- and interfamilial) relationships such that the necessary changes in classification could be made with confidence. Here we present the most comprehensive phylogenomic treatment of the spider infraorder Mygalomorphae conducted to date. We employ 472 loci obtained through Anchored Hybrid Enrichment to reconstruct relationships among all the mygalomorph spider families and estimate the timeframe of their diversification. We performed an extensive generic sampling of all currently recognized families, which has allowed us to assess their status, and as a result, propose a new classification scheme. Our generic-level sampling has also provided an evolutionary framework for revisiting questions regarding silk use in mygalomorph spiders. The first such analysis for the group within a strict phylogenetic framework shows that a sheet web is likely the plesiomorphic condition for mygalomorphs, as well as providing hints to the ancestral foraging behavior for all spiders. Our divergence time estimates, concomitant with detailed biogeographic analysis, suggest that both ancient continental-level vicariance and more recent dispersal events have played an important role in shaping modern day distributional patterns. Based on our results, we relimit the generic composition of the Ctenizidae, Cyrtaucheniidae, Dipluridae and Nemesiidae. We also elevate

five subfamilies to family rank: Anamidae (NEW RANK), Euagridae (NEW RANK), Ischnothelidae (NEW RANK), Pycnothelidae (NEW RANK), and Bemmeridae (NEW RANK). The three families Hermachidae (NEW FAMILY), Microhexuridae (NEW FAMILY), and Stasimopidae (NEW FAMILY) are newly proposed. Such a major rearrangement in classification, recognizing eight newly established family-level rank taxa, is the largest the group has seen in over three decades since Raven's (1985) taxonomic treatment.

Keywords: Biogeography, Molecular clocks, Phylogenomics, Spider web foraging, Taxonomy

Spiders placed in the infraorder Mygalomorphae are a charismatic assemblage of taxa that includes among its ranks the tarantulas, trapdoor spiders, and also some of the most venomous species, like the Sydney funnel web spider and its close relatives (Hedin et al. 2018). Today, the infraorder comprises 20 families, divided into 354 genera, with 3,006 species (World Spider Catalog 2018; Table 1). The group is ancient, known to occur in the fossil record since the Middle Triassic (Selden and Gall 1992), but with origins estimated further back into the Carboniferous, over 300 million years ago (Ayoub et al. 2007; Starrett et al. 2013; Garrison et al. 2016). Their ancient origins and concomitant sedentary nature provide a rich biogeographical context for studying geological-scale continental vicariant events (Raven 1980; Hedin and Bond 2006; Opatova et al. 2013; Opatova and Arnedo 2014a). Not surprisingly for such an ancient lineage, mygalomorph spider morphology is both interesting and complicated; they have retained a number of features considered primitive for spiders, such as a simple silk spinning system, two pairs of book lungs and paraxial cheliceral arrangement, yet are also relatively homogenous when compared to the overall morphological diversity observed among their araneomorph

71 counterparts (Hendrixson and Bond 2009). Although such striking homogeneity presents
72 significant challenges for morphology-based taxonomy, mygalomorphs have become a
73 noteworthy system for studying allopatric speciation and species crypsis (Bond et al. 2001; Bond
74 and Stockman 2008; Satler et al. 2013; Hamilton et al. 2014; Opatova and Arnedo 2014b; Hedin
75 et al. 2015; Leavitt et al. 2015; Castalanelli et al. 2017; Rix et al. 2017b; Starrett et al. 2018).
76 For less than two decades, since the first application of molecular systematics to
77 questions regarding mygalomorph phylogeny (Hedin and Bond 2006), it has been generally
78 acknowledged that the current system of classification is replete with problems that include
79 numerous instances of family-level para and/or polyphyly. In short, much of today's
80 classification scheme dates back to Raven (1985) Fig. 1a, which first applied cladistic thinking
81 towards evaluating family-level relationships on the basis of morphological character
82 argumentation. Despite the enormity of the task with respect to sheer number of taxa and
83 characters, the computational resources at the time (hardware and software) precluded a
84 "modern" analysis where characters were scored for terminal taxa and then analyzed using the
85 phylogenetic software and algorithms available today. That is, Raven relied on careful
86 examination of characters across genera followed by logic-based arguments of primary and
87 secondary homology on hand drawn trees. Post Raven (1985), a number of authors continued to
88 explore morphological characters as evidence for broad scale familial-level relationships and
89 identity (Eskov and Zonshtein 1990) further facilitated by the availability of computational
90 phylogenetic inference (Goloboff 1993; Goloboff 1995; Bond and Opell 2002; Fig. 1b). These
91 morphological studies were followed shortly thereafter by a number of early molecular
92 treatments (Hedin and Bond 2006; Ayoub et al. 2007; Fig. 2a). The first major taxonomic
93 changes to Raven's classification followed in 2012 (Bond et al. 2012; Fig. 2b) with the elevation

of the subfamily Euctenizinae to family status. A number of subsequent studies employing Sanger-sequencing based approaches continued to make progress in exploring relationships between closely related taxa (Hamilton et al. 2014; Opatova et al. 2016; Mora et al. 2017; Rix et al. 2017a; Ortiz et al. 2018; Starrett et al. 2018) and within families (Opatova et al. 2013; Opatova and Arnedo 2014a; Harrison et al. 2017; Rix et al. 2017c; Harvey et al. 2018; Lüddecke et al. 2018; Turner et al. 2018). Although Sanger approaches have been fruitful in terms of advancing our understanding of some relationships, it has been long recognized that ribosomal RNA genes (e.g., 28S and 18S), elongation factors, and mitochondrial genes, are often of limited utility in the context of deep scale divergences of mygalomorph spiders (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012).

Within the last several years, the application of phylogenomic approaches has helped to transform spider systematics (Bond et al. 2014; Fernández et al. 2014; Garrison et al. 2016; Hamilton et al. 2016b; Hedin et al. 2018; Kuntner et al. 2018; Wood et al. 2018) but more specifically has provided the data and consequently the impetus for making major changes in mygalomorph spider classification. Specifically, targeted capture approaches using anchored hybrid enrichment (AHE; Lemmon et al. 2012) and ultra-conserved elements (UCE; Faircloth et al. 2012) are now being applied to questions at multiple hierarchical levels across the spider tree of life (Hamilton et al. 2016a; Hamilton et al. 2016b; Maddison et al. 2017; Chamberland et al. 2018; Godwin et al. 2018; Hedin et al. 2018) and other arachnids (Starrett et al. 2017; Sharma et al. 2018). Implementation of genomic-based methods seemingly overcomes the perceived limitations of the traditional Sanger loci, enabling mygalomorph systematists to formally address a number of long-standing issues like the polyphyletic nature of ctenizids and hexathelids (Godwin et al. 2018; Hedin et al. 2018). In both cases, phylogenomic results stabilized the

taxonomic position of genera that were notoriously challenging to place and established new familial ranks (Halonoproctidae (Godwin et al. 2018), Atracidae, Macrothelidae and Porrhothelidae (Hedin et al. 2018)). Although these smaller scale studies have made important advances in our understanding of mygalomorph phylogeny and classification, they have all thus far lacked the broad scale phylogenetic context instrumental to understanding relationships spanning the entire infraorder.

We present here the most comprehensive phylogenomic treatment of Mygalomorphae conducted to date. Our analysis includes a broad sampling of taxa from all currently recognized families, allowing us to rigorously assess the monophyly of a number of key families (e.g., Cyrtaucheniidae, Nemesiidae, Dipluridae, Ctenizidae) and in particular formally address some long-standing taxonomic problems. Our extensive generic-level sampling also provides an evolutionary framework for revisiting questions regarding silk use in non-araneomorph spiders. We also explore questions regarding the timing and biogeographical context of mygalomorph spider diversification. We explicitly test a number of hypotheses related to the timing and origin of major mygalomorph lineages, the use of silk in mygalomorph prey capture, and the monophyly of the major mygalomorph higher-level groups and families. Past confusion in mygalomorph phylogeny and classification raises important questions regarding the utility of morphological characters in phylogenetic reconstruction, particularly in a group that was already known to be replete with homoplasy and likely suffered considerable extinction over the last 300+ million years. For example, what roles did morphological conservation, homoplasy, and shared ecology play in contributing a misleading phylogenetic signal? Although answers to some of these questions will likely remain elusive, it is our hope that the newly derived phylogenetic

139 tree presented here provides an evolutionary framework for many future questions aimed at
140 understanding this often overlooked but remarkable group of spiders.
141
142 MATERIALS & METHODS
143
144 We sampled 111 specimens representing 88 genera from all 20 currently recognized
145 mygalomorph families (Table 1). Our sampling represents approximately 25% of the infraorder's
146 known generic diversity and about 47% if the two most diverse mygalomorph families,
147 Barychelidae and Theraphosidae, are excluded from the calculation. Two representatives of the
148 suborder Mesothelae (*Liphisthius* and *Vinathela,* family Liphistiidae) were used as outgroups to
149 root phylogenies. Taxon sampling is summarized in Supplemental Table S1; all statistics related
150 to family and generic-level species diversity are extracted from the World Spider Catalog
151 (https://wsc.nmbe.ch/) accessed November 2018.
152 Whole genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen),
153 following the manufacturer's guidelines; to ensure the complete digestion of RNA, RNase A was
154 added to the mix after the tissue lysis step. Library preparation, enrichment, and sequencing were
155 performed at the Center for Anchored Phylogenomics at Florida State University
156 (http://anchoredphylogeny.com/) following the methods described in Lemmon et al. (2012),
157 Hamilton et al. (2016b) and Godwin et al. (2018).
158 Following the targeting of the 585 loci within the Spider Probe Kit v1 (Hamilton et al.
159 2016b), 472 loci were recovered and assembled. All loci were aligned with MAFFT 7.402
160 (Katoh and Standley 2013) using the L-INS-i algorithm (--localprior and --maxiterate 1000
161 flags). The alignments were scored for accuracy in ALISCORE (Misof and Misof 2009) and

162 ambiguously aligned positions were removed with ALICUT (Kück 2009). Individual loci were
163 concatenated with FASconCAT (Kück and Longo 2014) yielding a supermatrix of 93,410
164 nucleotides for 111 ingroup and two outgroup taxa (hereafter "All_taxa"); all alignments were
165 visually inspected in Geneious 10.1.3 (Biomatters 2017) prior to concatenation.

166 As a part of exploratory analyses, two additional matrixes were constructed to assess the
167 effect of taxon removal or sequence data addition on tree topology and support values. First,
168 terminals representing the family Paratropididae were removed from the "All_matrix" (hereafter
169 referred to as "No_Paratropis"). The family was recovered as a "stand-alone" lineage with
170 unresolved and unstable placement (Hedin and Bond 2006; Bond et al. 2012), potentially causing
171 conflict in topology and lowering the support of deeper nodes. In the second instance, the
172 "All_taxa" matrix was reduced to contain only one/two individuals per lineage/clade
173 (Supplemental Table S2) and combined with pre-existing transcriptomic data (Garrison et al.
174 2016) to form the "DNAAA_matrix". Mygalomorph taxa were extracted from the "BCC-75"
175 matrix (Garrison et al. 2016) and parsed into individual loci using BIOPYTHON (Hamelryck
176 and Manderick 2003; Cock et al. 2009). Partitions containing only missing data were deleted and
177 the resulting 1675 loci were blasted (tblastn command) in Geneious (Biomatters 2017) against
178 the AHE locus database in order to eliminate duplicate loci. Amino acid loci that received an e-
179 value of $< 1e^{-10}$ were excluded from the analyses. The loci that did not contain all 20 amino acids
180 were further removed from the dataset (per Stamatakis 2014).

181 Phylogenetic analyses were run on the Hopper Community Cluster at Auburn University.
182 For the DNA matrices, partition schemes under the GTR substitution model were evaluated and
183 selected based on AICc criterion in PartionFinder 2 (Lanfear et al. 2016) using the rcluster
184 algorithm (Lanfear et al. 2014) with RAxML implementation (Stamatakis 2014). Partitioning by

185 codon was not considered in the analyses because ambiguously aligned positions were removed
186 with ALICUT.

187 Maximum likelihood analyses (ML) were conducted in RAxML v 8.2.9 (Stamatakis
188 2014). Both "All_taxa" and "No_Paratropis" datasets were analyzed using the 191-partition
189 scheme and an independent GTR+G model defined for each partition. The best ML tree for each
190 matrix was selected from 1000 iterations, each starting from an independently derived
191 parsimony-based tree. Bootstrap support was inferred from 50 replicates, determined as
192 sufficient by the automatic bootstopping criterion (- N autoMRE flag) for both datasets
193 (Pattengale et al. 2010). The "DNAAA_matrix" dataset was analyzed with 1367-partition
194 scheme under the "PROTGAMMAWAG" settings. The best ML tree was selected from 1000
195 iterations and support was assessed from 100 bootstrap replicates (autoMRE).

196 Statistical significance of alternative tree topologies; that is, different, or traditional,
197 family-level arrangements (Table 2) than those recovered in our analyses, was assessed with
198 Approximately Unbiased (AU) topology test (Shimodaira 2002) implemented in CONSEL
199 (Shimodaira and Hasegawa 2001). Alternative tree topologies were obtained from 1000
200 independent constrained searches conducted in RAxML implementing the same settings,
201 partition scheme, and nucleotide substitution model as described above.

202 Bayesian Inference (BI) analyses of the "All_taxa" dataset was conducted in ExaBayes v
203 1.4.1 (Aberer et al. 2014) implementing the same partition scheme and nucleotide substitution
204 model as in the ML analyses. Two independent runs of $4x10^7$ generations with four coupled
205 chains each, starting from a parsimony tree with resampling every 1000 generations, were run
206 simultaneously. Standard deviation of split frequencies was monitored (<0.01), and the first 25%

207 were discarded as a *burn-in* for the analyses. An extended majority rule consensus tree was
208 obtained in the ExaBayes accompanying program consense (Aberer et al. 2014).

209 A species tree was inferred from 472 gene trees using ASTRAL v 4.11.2 (Mirarab and
210 Warnow 2015). Node support was assessed using ASTRAL's local posterior probabilities. Single
211 gene trees were inferred in RAxML; the best ML trees were selected from 1000 independent
212 iterations for each locus individually.

213 Divergence times were estimated in the penalized likelihood environment (Sanderson
214 2002) using the treePL algorithm (Smith and O'Meara 2012) on the tree topology obtained in
215 RAxML, and calibrated with six fossils. The calibrations are as follows: 1) Mygalomorphae:
216 Due to the lack of relevant fossil calibration points for the Mygalomorphae – Mesothelae split,
217 we used the information available in the literature from recent phylogenomic studies based on
218 more extensive outgroup fossil calibrations (Garrison et al. 2016; Fernández et al. 2018) in order
219 to constrain the age of the root node. Namely, we used the 95% confidence intervals estimated
220 for this split as the minimum (287 Ma) and maximum bounds (398 Ma); 2) Avicularioidea: The
221 age of the oldest mygalomorph fossil *Rosamygale grauvogely* (Selden and Gall 1992) from Gres-
222 a-Voltzia Formation, Vosges, France dated to Middle Triassic (Anisian) 242 Million years ago
223 (Ma) was assigned as the minimum bound for the Avicularioidea – Atypoidea split. The fossil
224 has been tentatively assigned to the family "Hexathelidae" (*sensu* Raven (Selden and Gall 1992))
225 but given the polyphyly of the family (Hedin et al. 2018), we rather interpret *Rosamygale* as an
226 Avicularioidea crown-group. The maximum bound for the Avicularioidea – Atypoidea split was
227 assigned the age of 323 Ma, corresponding to the oldest age of the Bashkirian stage
228 (Pennsylvanian, Carboniferous) that yielded currently the oldest known spider fossil
229 *Arthrolycosa* sp. putatively assigned as Mesothelae stem-group (Selden et al. 2014; Garwood et

al. 2016). This age also overlaps with the oldest boundary of the 95% confidence intervals for the Avicularioidea – Atypoidea split estimated by recent phylogenomic studies (Garrison et al. 2016; Fernández et al. 2018) based on more extensive outgroup fossil calibration; 3) "Nemesioidina" clade: The age of *Cretamygale chasei* 125 Ma (Selden 2002) from Cretaceous (Barremian, Hughes and McDougall 1990) currently classified as Nemesiidae was conservatively assigned as the minimum bound for the split between Bemmeridae/Theraphosidae and "Nemesioidina" clades. The maximum bound was assigned 242 Ma, corresponding to the age of *Rosamygale* fossil. 4) Atypoidea: Atypidae – Antrodiaetidae split was assigned minimum bound of 100 Ma representing *Ambiortiphagus ponomarenkoi* (Eskov and Zonshtein 1990) from the Lower Cretaceous (Albian) proceeding from Dund-Ula Mountain Ridge in Bayan-Khongor Province, Central Mongolia. The age of *Rosamygale* (242 Ma) was assigned as a maximum bound for the split; and 5) *Ummidia* fossils (44 Ma) from Baltic amber (Wunderlich 2011) were used to calibrate the minimum bound for the split between the North American and European species of *Ummidia*. Maximum bound of 90 Ma corresponds to the oldest boundary of the 95% confidence interval estimated for this particular split in the past (Opatova et al. 2013).

Biogeographic analyses were conducted in Reconstruct Ancestral State in Phylogenies (RASP v 3.1, Yu et al. 2015) with the dispersal-extinction-cladogenesis (DEC) model (Ree and Smith 2008) implemented in C++ version of Lagrange (Smith 2010). Analyses were run with no dispersal constraints, assigning equal probabilities to dispersal events among all defined areas, and allowing two unit areas in the ancestral distributions. The terminal taxa represented in the tree were assigned to six distribution ranges: (A) North America; (B) Europe; (C) Asia; (D) South America; (E) Africa; (F) Australia plus New Zealand (Fig. 7b). The consensus tree obtained in ExaBayes was used as an input for the analyses.

253 For ancestral state reconstruction analyses of foraging construct, each terminal taxon was
254 scored using six character states: (0) burrow with trapdoor(s); (1) brush sheet/funnel web; (2)
255 burrow with collar door; (3) purse web; (4) open burrow; (5) turret. Character codings were
256 scored from personal observations and/or the primary literature as documented in Supplemental
257 Table S3. Taxa that forage as cursorial hunters were scored as unknown or missing (Coddington
258 et al. 2018); polymorphic character states were assigned to terminals for instances where species
259 build multiple/combination of constructs (e.g., some *Cyrtauchenius* terminals build turrets as
260 juveniles but cover those turrets with trapdoors as adults, Opatova *persn. obsv*.), or closely
261 related species build more than one construct e.g., both open burrows and trapdoors (some
262 bemmerids and pycnothelids) and both turrets and collars (e.g., some *Antrodiaetus*). Ancestral
263 character state reconstructions were conducted using the R package *corHMM* (Beaulieu et al.
264 2013) on an ultrametric scaled tree with node.states=marginal; *corHMM* was preferred over
265 other methods given its capacity to accommodate missing and polymorphic characters. The R
266 package *ape* was used to scale the preferred tree topology ('chronopl') with an assigned lambda
267 value of 0.1. Character optimizations using equal (ER), symmetric (SYR), and all rates different
268 (ARD) models were explored for these categorical data using 'rayDISC' (*corHMM*). The
269 preferred model was chosen by comparing AICc values calculated using *corHMM*.
270 All relevant data, DNA and amino acid sequence alignments, phylogenetic trees, and
271 miscellaneous data matrices, were deposited in the Dryad Data Repository
272 (doi:XXXXXXXXXXX) on XX XX 2019.
273
274 *Guiding Principles for Taxonomic Changes*
275

We acknowledge that the taxonomic changes we propose are unlikely to go without criticism and will most likely require future revision and refinement. The guiding principles we used attempted to realign family-level limits as clearly as possible but remain conservative in order to minimize errors. Consequently, we followed these four guiding principles: 1) revise higher-level mygalomorph classification and familial-level taxonomy such that it better reflects the evolutionary relationships indicated by data available (i.e., the sum of collected to data, not just this analysis); 2) further refine the classification, where possible, based on previous morphological/molecular hypotheses in which subfamilial taxa are clearly defined and supported by unambiguous synapomorphies (i.e., in some instances we transferred unsampled genera to newly defined families in cases where their placement in said group appears to be well supported by other studies); 3) minimize changes in family-level classification to the extent possible that maintains a consistent phylogenetic level across the tree for the family-level rank; and 4) instances where generic-level taxon sampling and criterion 2 above preclude an unambiguous family-level assignment of a genus (for relimited families), we retain the original family assignment but denote its placement as *incertae sedis* (operationally for the purposes of this study).

RESULTS

Concatenated analyses, all taxa.—The complete dataset "All_taxa" supermatrix comprised 472 loci (93,410 nucleotides) for 113 terminals (Supplemental Table S1), whereas the reduced "No_Paratropis" dataset comprised 111 terminals. The proportion of missing data in the

299 datasets was 14.7% and 14.5%, respectively. The combined "DNAAA_matrix" included 30
300 terminals, with 14 individuals represented by both genomic (472 AHE loci) and transcriptomic
301 data (1519 loci proceeding from Garrison et al. (2016)). The total length of the
302 "DNAAA_matrix" was 305,860 characters; gaps and missing data represented 54.5% of the
303 dataset. For details see Supplemental Table S2.

304 Both ML (-ln 1728131.7475) and BI analyses performed on the "All_taxa" dataset
305 yielded the same tree topology (Fig. 3; Supplemental Fig. 1, 2). Overall, the resulting trees were
306 highly supported (bootstrap > 90, posterior probability (PP) > 0.95), with the exception of a few
307 deeper nodes. The ML and BI analyses both recovered two main clades Atypoidea and
308 Avicularioidea with strong support. The relationships within Atypoidea were fully supported; the
309 family Atypidae was recovered as a sister to Antrodiaetidae inclusive of *Hexura*
310 (Mecicobothriidae).

311 Four mygalomorph families belonging to Avicularioidea were recovered as para- or
312 polyphyletic (namely Dipluridae, Ctenizidae, Cyrtaucheniidae and Nemesiidae), prompting
313 extensive higher-level changes to mygalomorph classification (see below). Several lineages
314 formerly belonging to the families Dipluridae and Hexathelidae (see Hedin et al. (2018))
315 successively branch off near the root node of Avicularioidea as follows: Ischnothelidae (NEW
316 RANK), Microhexuridae (NEW FAMILY), Hexathelidae, Euagridae (NEW RANK),
317 Porrhothelidae and Macrothelidae. The Bipectina clade (*sensu* Goloboff (1993)) inclusive of the
318 family Atracidae was recovered in our analysis. The family Paratropididae was recovered as
319 sister to all the remaining Bipectina. The remaining taxa were placed into a clade comprising
320 Stasimopidae and the "Venom Clade" (Atracidae + Actinopodidae) and two additional clades
321 corresponding to Domiothelina and Crassitarsae (*sensu* Bond et al. (2012), but excluding

322 *Stasimopus*). The monogeneric family Stasimopidae (NEW FAMILY, formerly Ctenizidae), was
323 placed as sister to "Venom Clade", albeit with low support. Domiothelina formed a
324 monophyletic group with well resolved relationships in both ML and BI analyses. The family
325 Halonoproctidae was recovered as sister to all remaining lineages of Domiothelina. The family
326 Migidae was recovered as sister to Idiopidae, Ctenizidae (now comprising *Cteniza* and
327 *Cyrtocarenum*) and Euctenizidae. The unsupported clade corresponding to Crassitarsae (*sensu*
328 Bond et al. (2012)) was subdivided into two highly supported clades. The first one comprised the
329 family Bemmeridae (NEW RANK, formerly Cyrtaucheniidae), recovered as sister to the
330 Barychelidae and Theraphosidae. The second clade, "Nemesioidina", comprised the Nemesiidae
331 plus three lineages formerly belonging to the Nemesiidae (Pycnothelidae (NEW RANK),
332 Hermachidae (NEW FAMILY) and Anamidae (NEW FAMILY)), as well as Dipluridae,
333 Cyrtaucheniidae and Microstigmatidae. Nemesiidae was recovered as sister to all the remaining
334 families; Pycnothelidae was recovered as sister to the Dipluridae/Cyrtaucheniidae clade and the
335 Hermachidae/Microstigmatidae/Anamidae clade.

336 *No_Paratropis analysis*.—Maximum likelihood analyses of the "No_Paratropis" dataset
337 recovered a moderately different Bipectina topology contra the analyses on the full dataset (Fig.
338 4; ML best tree –ln = 1702659.2948). The discordance, however, mostly affected nodes
339 unsupported in both analyses. In the "No_Paratropis" analyses the clade comprising Bemmeridae
340 + Barychelidae + Theraphosidae was recovered with high support as sister to all the remaining
341 Bipectina families. The Stasimopidae + "Venom Clade" received moderate bootstrap support,
342 but its sister relationship to the Domiothelina clade was still unsupported, albeit with increased
343 support when compared to the analyses of the full dataset.

344 *DNAAA analysis*.—Similar to the "No_Paratropis" dataset, analyses of "DNAAA" (ML
345 best tree –ln = 2543315.0197) resulted in different topological arrangements within the Bipectina
346 clade (Fig. 5). The Domiothelina clade was recovered as sister to all the remaining diversity.
347 Stasimopidae + "Venom Clade" (moderately supported in this analysis) was recovered with low
348 support as sister to Crassitarsae. The Crassitarsae clade was highly supported and inclusive of
349 *Paratropis*.

350 *Topology tests*.—The AU topology test (Table 2) rejected the monophyly of all
351 alternative arrangements (i.e., alternatives using existing compositions of genera) of the families
352 Ctenizidae (*sensu* Raven (1985)), Cyrtaucheniidae (*sensu* Bond et al. (2012)), Dipluridae,
353 Hexathelidae (*sensu* Raven (1985)) and Nemesiidae in favor of the results of the unconstrained
354 search.

355 *Species tree analysis*.—Coalescence-based analysis produced a species tree from the
356 individual 472 input gene trees obtained in RAxML. The resulting quartet-based supertree
357 estimated in ASTRAL (Supplemental Fig. 3) comprised 1,228,051,216 induced quartet trees
358 from the input gene trees, representing 65.2% of all quartets present in the species tree. ASTRAL
359 yielded a different topology than the concatenated approach (Fig. 6). The analyses recovered
360 both Atypoidea and Avicularioidea clades. Ischnothelidae (NEW RANK), Microhexuridae
361 (NEW FAMILY), Hexathelidae, Euagridae (NEW RANK) and Porrhothelidae, a grade of
362 lineages branching at the base of the Avicularioidea clade in the concatenated analyses, formed a
363 highly supported clade in the species tree approach. The topology of the Bipectina clade
364 (unsupported in ASTRAL) also somewhat differed from the concatenated analyses.
365 Bemmeridae/Barychelidae/Theraphosidae clade was recovered as sister to moderately supported
366 Domiothelina + Stasimopidae/"Venom Clade", rendering the Crassitarsae paraphyletic.

367 *Molecular dating*.—The resulting dated tree is depicted in Fig. 7; Supplemental Figure 4.
368 The age of the root (398 Ma) corresponds to the maximum age constraints assigned to this split.
369 The split between Atypoidea and Avicularioidea was dated at ~ 315 Ma. The early branching
370 events within Avicularioidea dated between 240 and 173 Ma (Ischnothelidae through
371 Macrothelidae divergences). The first diversification of Bipectina is dated to 166 Ma (divergence
372 of Paratropididae) and continued at 160 Ma, with diversification into Domiothelina +
373 Stasimopidae/"Venom Clade"and Crassitarsae. The divergence time estimates for individual
374 families are reported in Table 3.
375 *Historical biogeography*.—The results of DEC analyses are summarized in Fig. 8. The
376 ancestral distributional area of the mygalomorph ancestor and the early branching Avicularioidea
377 lineages remain unresolved. The distribution of the Atypoidea ancestor was inferred to have
378 originated in the Northern hemisphere continents (North America and Asia); Bipectina likely
379 originated in Africa. The ancestral ranges of several Avicularioidea families were inferred to
380 areas spanning more than one continent, for example Africa and Australia (Migidae, Idiopidae,
381 Barychelidae, Microstigmatidae); Africa and South America (Cyrtaucheniidae, Pycnothelidae).
382 On the other hand, some Avicularioidea families experienced *in situ* diversification within a
383 single area. Stasimopidae, Bemmeridae, and Hermachidae likely evolved in Africa,
384 Paratropididae and Dipluridae in South America, Anamidae in Australia, Euctenizidae in North
385 America and Ctenizidae in Europe. DEC analyses also inferred a large number of dispersal and
386 vicariance events across the mygalomorph phylogeny, however, most of the events received low
387 probabilities (<0.7), particularly those inferred for deeper nodes and nodes within families with
388 complex distribution patterns.

389 *Evolution of foraging construct*.—We scored foraging construct for 109 taxa. Taxa that
390 are cursorial hunters (three terminals), *Paratropis* and *Microstigmata*, were scored as
391 missing/inapplicable. The foraging construct of nemesiid *Mexentypesa* is unknown to us. We
392 were unable to confirm from the literature a burrow type, although we would expect it to be a
393 typical open burrow. Because bemmerids and pycnothelids construct both trapdoors and open
394 burrows they were scored as polymorphic. Based on AICc values the equal rates model (ER)
395 produced the preferred reconstruction (AICc = 168.3399). Although the ER model was preferred,
396 ancestral state reconstructions for the other models were generally very similar.

397 The ancestral state reconstruction of mygalomorph foraging construct, summarized in
398 Fig. 9, shows a rather simple and concise pattern. Towards the root of the phylogeny there
399 appears to be an equal probability of an ancestor having either a sheet/funnel web or a burrow
400 with a trapdoor; both alternative hypotheses are explored in more detail in the discussion section
401 below. Atypoids likely had a most recent common ancestor (MRCA) with a sheet web, modified
402 as a purse web in atypids or as a collar, turret, or trapdoor in antrodiaetids. As discussed below,
403 atypoids demonstrate the full scope of variability in burrow construct modifications that can
404 evolve from a sheet web ancestral form. Avicularioidea have a MRCA that optimizes as having a
405 sheet web. The MRCA for the Bipectina clade likely had either a trapdoor or sheet web
406 (equivocal). A burrow with a trapdoor more definitively optimizes as the ancestral foraging
407 construct for Crassitarsae with subsequent losses of the trapdoor covering (optimized as the open
408 burrow state) four times in nemesiids, pycnothelids, theraphosids, and the euctenizid genus
409 *Apomastus*; some pycnothelid and anamid taxa independently lose the trapdoor and instead
410 forage from burrows with distinct turret or collar door constructs. Sheet webs are regained twice,
411 once in atracids and again in diplurids.

Discussion

The systematics and evolutionary relationships among mygalomorph families have remained an unresolved problem for many years, beginning in earnest with the first molecular analyses (Hedin and Bond 2006) which identified significant issues with Raven's (1985) morphology-based classification scheme and hypothesis of relationships. Despite the long and protracted evolutionary history of mygalomorphs, dating back to the Carboniferous (Ayoub et al. 2007; Starrett et al. 2013; Garrison et al. 2016), the identification of morphological characters necessary to build a stable classification scheme has proved to be challenging and clearly suffered from a number of inconsistencies rendering many of the families para- or polyphletic. Mygalomorph taxa often display homogenous external morphology (e.g. Hendrixson and Bond 2009), or possess an array of highly specialized morphological characteristics that could be interpreted as synapomorphies (Raven 1985), or alternatively, as traits reflecting the ecology of the taxa in question (Coyle 1971; Eskov and Zonshtein 1990). The interpretation of certain morphological characters and the subsequent placement of families in higher-level groupings thus seemingly fluctuated from one study to the next (Simon 1864; Raven 1985; Eskov and Zonshtein 1990; Goloboff 1993; Bond and Opell 2002), often being a point of contention (Raven 1985; Eskov and Zonshtein 1990; Goloboff 1993). Based on these past studies, two points seem clear. First, morphological data alone are unsuitable for confidently resolving the evolutionary history of mygalomorphs. Second, if our aim is to have a classification scheme that reflects evolutionary history, other characters are needed.

The use of phylogenomics in spiders (Bond et al. 2014; Fernández et al. 2014; Garrison et al. 2016; Hamilton et al. 2016b; Godwin et al. 2018; Hedin et al. 2018; Wood et al. 2018) now seemingly provides the framework needed to robustly and confidently resolve some of the long standing taxonomic issues that have plagued mygalomorph spider systematics. We believe that the results we discuss below allow us to assess the status of nearly all mygalomorph families and confidently resolve several long-standing questions in the group's phylogeny and higher-level classification. As a consequence, we propose extensive taxonomic changes that better reflect the origins and evolutionary relationships of the taxa. We discuss in detail 1) the temporal and biogeographical context of mygalomorph family-level diversification; 2) revisit longstanding questions regarding silk use in non-araneomorph spiders; and 3) provide a detailed account regarding the taxonomic implications of our newly formed phylogenetic hypothesis (Fig. 10). The Appendix to this paper (Bond et al. 2019) outlines the formal taxonomic changes proposed herein.

Diversification and Biogeography of Mygalomorph Spiders

Taxa having disjunct distribution patterns, particularly those with poor dispersal abilities and deep evolutionary histories, represent intriguing model systems that have captivated the attention of biogeographers for decades (Cracraft 1988; Wiley 1988; Morrone and Crisci 1995). Mygalomorph spiders with nearly a cosmopolitan distribution, highly sedentary nature (Raven 1985; Bond et al. 2001) and evolutionary history dating back to Carboniferous (Ayoub et al. 2007; Starrett et al. 2013; Garrison et al. 2016), are one such group. Historically, mygalomorph distribution patterns were usually interpreted as a result of continental-level vicariance

accompanied by gradual over-land dispersal (e.g. Raven 1980). By implementing divergence time estimation methods, many distribution patterns observed in mygalomorphs could be linked to specific geologic events such as continental breakups (Opatova et al. 2013; Opatova and Arnedo 2014a), Mediterranean tectonics (Opatova et al. 2016; Mora et al. 2017) and opening of the Okinawa Trough (Su et al. 2016). However, recent findings suggest that dispersal (via rafting or ballooning) could be a plausible mechanism of dispersal for overcoming both short (Hedin et al. 2013; Opatova and Arnedo 2014b) and long distances (Harrison et al. 2017) and might have contributed to the current distribution of mygalomorphs more often than previously thought.

The results of our biogeographic analyses (Fig.8) place the ancestral area of distribution of Atypoidea within the Northern hemisphere (across North America and Asia), whereas the ancestor of Porrhothelidae, Macrothelidae and Bipectina most likely evolved in the Southern hemisphere (across Africa and Australia). The analyses infer, with high probability, the African origin of all Bipectina, minus Paratropididae. In our dating analyses, we recovered two repeated patterns of divergences occurring among taxa with present day distributions confined mostly to the Southern hemisphere. The first set of divergences was observed up to seven times independently across the mygalomorph tree of life, involving Australian taxa, as well as taxa inhabiting either Africa or South America. The second and younger set of splits, involving South American and African taxa, was observed in three instances. Although in plants and vertebrates these patterns often result from dispersal (Procheş and Ramdhani 2013; Ruhfel et al. 2016), in dispersal-limited invertebrate groups these instances have been repeatedly linked to vicariant events related to the different phases of Gondwana breakup (Wood et al. 2012; Frazão et al. 2015; Kim and Farrell 2015; Xu et al. 2015; Andújar et al. 2016; Toussaint et al. 2017).

The first repeated pattern of divergences observed in our results concordant with the East – West Gondwana breakup was dated between 103 – 90 Ma, involving splits within the families Euagridae, Migidae, Idiopidae (putatively also Barychelidae), and divergences between Atracidae – Actinopodidae and Anamidae – Hermachidae/Microstigmatidae clade. The second pattern, potentially corresponding to the South America – Africa separation dated to 77 – 61 Ma involved divergences within Theraphosidae, Pycnothelidae and Cyrtaucheniidae. The divergence times estimated in our analyses were comparatively younger than the ages estimated for other sedentary taxa with former Gondwanan distribution, such as velvet worms or bothriurid scorpions (Murienne et al. 2014; Sharma et al. 2018). The initial phase of Gondwana breakup (Fig 7), i.e., the formation of East and West Gondwana, began approximately 165 Ma with South America and Africa drifting away from India, Madagascar, Antarctica and Australia. West Gondwana then subsequently fragmented into South America and Africa between 132 – 112 Ma (Will and Frimmel 2018). The onset of continental drifting in both instances predates the divergence time estimates inferred in our analyses by approximately 50 My, however, the initial phases of different Gondwana breakup stages were often characterized by slow tectonic movement, or even quiescence (Boger 2011; Will and Frimmel 2018; Young et al. 2018). Recent tectonic plate models suggest that the initial phase of drifting between East and West Gondwana was occurring at an extremely slow rate for at least the first ten million years (Young et al. 2018). A connection between South America and Australia also likely persisted via Antarctica until late Eocene, approximately 35 Ma (Sanmartín and Ronquist 2004), which links the divergence time estimates between Neotropical and Australasian groups of Darwin's stag beetles dated to 47 and 58 Ma, respectively, to Gondwana breakup (Kim and Farrell 2015).

Conversely, the divergence time estimates inferred in our analyses could also be affected by the limited fossil record for mygalomorphs, and our conservative approach towards nod calibration. For example, due to the inferred polyphyly of the Nemesiidae, the *Cretamygale chasei* fossil, formerly interpreted as Nemesiidae, was used to set a minimum bound for the whole "Nemesioidina" clade (see methods), rather than arbitrarily assigning the fossil to the family Nemesiidae (*sensu* this study). Despite the apparent advantages in the ability of handling genomic data, the dating analyses used in this study (Smith and O'Meara 2012), does not infer confidence intervals that are often essential for connecting the age estimates and specific geological events.

Alternatively, the observed patterns could be explained by long-distance dispersal events. Many plant and vertebrate groups with cosmopolitan or pantropical distributions, formerly perceived as a result of ancient vicariance, owe their current pattern to dispersal (Procheş and Ramdhani 2013; Ruhfel et al. 2016). Long distance dispersal events have been detected even among poorly dispersing groups of invertebrates such as euedaphic beetles (Andújar et al. 2016) or short-tailed whip-scorpion order Schizomida (Clouse et al. 2017). Although recent evidence suggests that mygalomorph spiders are also capable of trans-oceanic dispersal (Harrison et al. 2017), repeated divergence patterns imply multiple long-distance dispersal events occurring independently among unrelated taxa during the same timeframe. Continental-level vicariance thus provides a more plausible explanation for the observed pattern. The East – West Gondwana vicariant origin of the taxa is also supported by the results of the ancestral range reconstruction analyses.

The inferred ranges for all the taxon groups involved (Euagridae, Atracidae and Actinopodidae, Migidae, Idiopidae, Barychelidae, Hermachidae and Anamidae) comprised

former Gondwana continents, although the ancestral range inferred for Idiopidae, Barychelidae and Migidae (Africa and Australia) could potentially represent an artefact stemming from a geographical bias in our sampling (i.e. lacking their South American representatives in our analyses). Vicariance was also inferred with high probability in the divergences between *Allothele* and Australian euagrids, *Atrophothele* and remaining barychelids, and for most of remaining divergences (occasionally with dispersal contribution), albeit with low probabilities. The West Gondwana vicariant split between Africa and South America can likely be attributed to the structure within Pycnothelidae (*Pionothele* – remaining pycnothelids) and South American and African Cyrtaucheniids which also optimizes as an ancestral distribution spanning across both continents in both groups.

Another repeated pattern observed across our tree includes taxa with currently disjunct amphi-Atlantic distributions. Such patterns of divergence were observed within *Ummidia* and *Cyclocosmia* (Halonoproctidae) and between *Sphodros* and *Atypus* (Atypidae), and were dated to 44, 34 and 30 Ma, respectively. This pattern has previously been connected with the breakup of Laurasia (Opatova et al. 2013; Godwin et al. 2018) dating to the end of Paleocene, approximately 60 – 55 Ma (Sanmartín et al. 2001). Our divergence time estimates postdate both the geological event and previous dating results (Opatova et al. 2013). The discrepancy between these time estimates and the Laurasian breakup could be explained by either an artefact of the analyses (discussed above), or alternatively by a persistent connection of North America and Europe via a North American land bridge. This connection existed to some extent until 25 Ma and is hypothesized to have played an important role in plant dispersal (Tiffney and Manchester 2001; Milne 2006), suggesting also a suitable dispersal route for ballooning spiders like *Atypus*, *Sphodros*, *Ummidia* (Opatova et al. 2013). The current distribution of *Cyclocosmia* (East Asia

548 and South East of North America) also represents a well-known biogeographic pattern linked to
549 continental vicariance, present in both plants and animals (Boufford and Spongberg 1983;
550 Bolotov et al. 2016). Alternatively, similar distribution patterns could have resulted from
551 dispersal events between Palearctic and Nearctic regions via Beringia (Li et al. 2015; Bolotov et
552 al. 2016). However, the timing of the dispersal events inferred in other organisms (Li et al. 2015;
553 Maguilla et al. 2018) postdates the divergences inferred the divergences inferred by our analyses.
554 The Laurasian origin of the taxa is further supported by their ancestral distributions projected to
555 the Northern hemisphere continents. Moreover, analyses support the vicariant origin of
556 *Cyclocosmia* and suggest that both vicariance and dispersal shaped *Atypus* – *Sphodros* and
557 *Ummidia* distribution (albeit the latter with low probability).

558 Older divergences recovered in our analyses are more challenging to explain within a
559 biogeographic context. If we follow the same line of reasoning as explained above, and assume
560 that the actual ages of the splits are older than indicated by the results, the splits between the
561 Idiopidae and Ctenizidae/Euctenizidae clade, and Nemesiidae and the remaining diversity, could
562 correspond to the disintegration of the supercontinent Pangea occurring in Lower Jurassic (190 –
563 180 Ma) (Beutel et al. 2005; Will and Frimmel 2018). The results of the biogeographic analyses
564 suggest a combination of dispersal and vicariant events, however similarly to other deeper nodes
565 in the tree, the probabilities for various event hypotheses are low. Given the long phylogenetic
566 history of mygalomorph spiders, we predict that large scale extinction events have likely shaped
567 and potentially blurred some of the older biogeographic patterns. Gondwana was formed in the
568 southern hemisphere during Cambrian approximately 520 – 510 Ma (Veevers 2004; Domeier
569 and Torsvik 2014; Will and Frimmel 2018) and large parts of the continent experienced repeated
570 glaciations into the Silurian, Devonian, and from the late Carboniferous to early Triassic. During

571 the orogenic processes prompted by the collision of Gondwana with Laurussia and subsequent
572 formation of Pangea ~320 Ma (Golonka 2007; Nance et al. 2014; Matthews et al. 2016),
573 substantial portions of both continents were glaciated as a result of continental scale orogeny,
574 and by the early Permian (approximately 300 Ma) the ice coverage reached to 30 ° S latitude
575 (Veevers 2004). Suitable habitats were thus repeatedly fragmented, likely causing taxon
576 distributional shifts and large-scale extinction events (Coccioni and Galeotti 1994; Zachos et al.
577 2001; Chen and Benton 2012). The consequences of these events could be potentially reflected
578 in the mygalomorph phylogeny by the existence of many early-branching monogeneric lineages
579 such as Microhexuridae, Porrhothelidae, Macrothelidae, Paratropididae and Stasimopidae, that
580 do not seem to have any close relatives among extant taxa. Interestingly, deep divergences far
581 preceding the breakup of Pangea have also been observed for a similarly ancient and sedentary
582 group – the velvet worms (Boyer et al. 2007; Murienne et al. 2014), indicating that paleoclimatic
583 events and habitat heterogeneity played an important role in the evolution of these ancient
584 groups.

585 We also detected potential long-distance dispersal events across the tree. For example,
586 the Australasian genus *Conothele* (Halonoproctidae), shows divergence between Australian and
587 Vietnamese species dating back to approximately 46 Ma. This event by far postdates their
588 vicariance during the East Gondwana breakup, when the Indian plate started drifting northwards
589 away from Australia and Antarctica around 135 Ma (Will and Frimmel 2018). In comparison to
590 its sister taxon *Ummidia*, ballooning has not been directly observed in *Conothele*. The genus
591 does however exhibits some behavioral traits associated with this particular manner of dispersal
592 (Main 1957), which along with its distribution from South East Asia to Australia suggest that
593 *Conothele* is also capable of aerial dispersal.

Another unusual pattern, that could potentially be attributed to long distance dispersal occurs within the family Pycnothelidae, where the closest relatives of the Australian genus *Stanwellia* have South American distributions. The split dates back to approximately 28 Ma which by far postdates the East – West Gondwana vicariance but might actually reflect a connection between South America and Australia via Antarctica that presumably persisted until 35 Ma (Sanmartín and Ronquist 2004). The same vicariant scenario has been postulated in the past to explain a similar distribution pattern recovered in Stag beetles (Kim and Farrell 2015). Interestingly, the biogeographic analyses also inferred vicariance for the *Stanwellia* – *Acanthogonatus* favoring South America – Australia connection over dispersal event. The two remaining cases of potential long-distance dispersal both involved Australian and African taxa. *Poecilomigas* (family Migidae) diverged from its Australia inhabiting sister taxon *Bertmanius* approximately 74 Ma, and the split between *Kiama* and *Microstigmata* (both Microstigmatidae) occurred around 70 Ma. Surprisingly, the biogeographic analyses inferred vicariance for both. However, even when a possible bias in our dating analyses is considered, both divergences are probably too young to reflect the East – West Gondwana split that started approximately 165 Ma, making the vicariant event challenging to explain. Trans-oceanic dispersal has previously been reported from the family Migidae (Harrison et al. 2017), which suggest that dispersal could plausibly explain the disjunct distribution between *Bertmainius* and *Poecilomigas.* Close association to tree trunks, a snugly fitting trapdoor and thick internal layer of silk, were hypothesized to enable the dispersal and survival via rafting (Harrison et al. 2017). Although microstigmatids belong among the few representatives of cursorial hunting mygalomorphs, they are often found under rocks and large logs, which could have potentially placed them in a likely position for rafting. However, South American microstigmatids were not sampled in our

analyses, therefore a potential vicariance via South America – Antarctica – Australia (Kim and Farrell 2015) should not be discarded.

The Role of Silk in Prey Capture by Non-Araneomorph Spiders Revisited

As noted by Coyle (1986), progress in our understanding of the role silk plays in non-araneomorph spiders lags far behind what is known about araneomorphs; this was an accurate statement in 1986, and largely remains true today. As we discuss below, a prevailing perception considered by us misleading, if not erroneous, is that mygalomorph spiders do not use silk directly in foraging for prey; that is, it is often perceived that they do not spin webs defined in either a restricted or broad sense. For the purposes of our discussion we consider two characteristics as fundamental to 'qualifying' silk as playing a role in prey capture: 1) silk is employed by the spider in a sensory capacity to transmit information and extend prey sensing space outward to localize prey; and/or 2) silks can also be used by the spider to slow, impede, or restrain prey items. These two criteria are not mutually exclusive, particularly in mygalomorphs where visual clues are not known to play a significant role in prey capture; simply put silk is employed to increase predator speed (fast predator and/or slower prey (Coddington et al. 2018)). Note that Vollrath and Selden (2007) had a much broader definition of a web that included any spider structure made of silk. Because the question we address here is specifically related to the use of silk in prey capture, our working definition is narrower than theirs. It is our hypothesis, forwarded herein, that mygalomorphs, and consequently all spiders, plesiomorphically used silk to both localize and impede prey for capture. Whereas not all mygalomorphs use silken

constructs to impede prey (criterion 2), nearly all employ it in some fashion to transmit vibrations to facilitate prey localization (criterion 1).

As documented in Figure 9, the root mygalomorph node shows an ambiguous optimization for sheet web versus trapdoor. Although the optimization of this character is equivocal, we would contend that the likely character state at this node, if araneomorphs and uraraneids (e.g., *Chimerarachne yingi*, (Wang et al. 2018)) were to be included in the analysis, would probably resolve as a sheet web. As noted by Vollrath and Selden (2007), there is a clear link between body structure and behavior in extant spiders, whereas preserved morphology in extinct taxa provides strong hints for inferring behavior. As such, that resolution requires some inferred links about the foraging behavior of *Chimerarachne* and its morphology, a "spider" whose proportionally long spinnerets suggest that they were sheet web builders (mygalomorph sheet web builders have proportionally longer spinnerets than their counterparts – see Ischnothelidae). Eskov and Selden (2005) hypothesized that *Permarachne* (at the time considered an extinct mesothele – now placed in the Uraraneida) constructed a sheet web but likely evolved *from* a protypical mesothele ancestor that constructed a trapdoor burrow with radiating lines. Alternatively, the uraraneid placement as the sister group to all spiders (Wang et al. 2018) likely requires the optimization that sheet webs are the plesiomorphic condition with trapdoor burrows independently derived in Liphistiidae – a lineage that is morphologically exceptionally unique among spiders (see below). Sheet webs, as most thoroughly documented by Coyle (1986), definitively meet both criteria outlined above; that is, they transmit and extend prey localization from a tubular retreat (typically) and serve to entangle prey. Although silk may not have initially evolved for this function in the group's common ancestor (that is, the common ancestor of Uraraneida + Araneae), these results strongly suggest that early silk use likely played

a role in prey capture. Some authors have hypothesized, that silk was initially used in reproduction (e.g., protecting eggsacs, sperm webs, etc. (Shultz 1987) or played a sensory role (Bond 1994)). However, these early functions may not be mutually exclusive; that is, one could easily imagine silken threads extending from an eggsac that allowed a spider to care for her young while still able to sense prey (or predators) from a distance.

Irrespective of the uraraneid sheet web hypothesis originally proposed by Eskov and Selden (2005), the sheet web optimization at the mygalomorph tree's root node inferred here may be considered by some to be surprising given that the sister group to all other spiders (Opisthothelae), Mesothelae, build subterranean burrows covered by trapdoors. As we discuss in more detail below, it is not unrealistic that a trapdoor covered burrow may have evolved from a sheet web (certainly the case among more derived mygalomorph taxa); the Mygalomorphae MRCA with high probability likely spun a sheet web from which trapdoor burrows were derived independently twice across the tree (once in the Atypoidea and a second time in the Bipectina). Nevertheless, we consider trapdoor burrow entrances to play a role in prey localization (discussed in more detail below), further enhanced in some liphistiids (as well as many other typical trapdoor spiders) by extending the prey sensing surface of their burrow opening via silken trip lines (also silken tabs, leaves, and other organic debris).

Nowhere across the Mygalomorphae is the evolutionary plasticity of the ancestral sheet web better illustrated than in the Atypoidea. The antrodiaetid clade includes *Antrodiaetus* (plus *Atypoides*), *Aliatypus*, and *Hexura* (Mecicobothriidae) – all of which construct collared burrows, turreted burrows, trapdoors, and sheet webs, respectively. As demonstrated in a number of elegant experiments conducted by Coyle (1986), the trapdoor spider *Aliatypus* likely suffers from reduced foraging efficiency relative to its more open burrow (collared door) and sheet web

685 related genera. The sister group, Atypidae, builds purse webs, which are silken tubes that
686 typically extend vertically up trees or horizontally along a substrate. As discussed by Coyle
687 (1986), these spiders respond rapidly to vibrations along the tube surface; atypid purse webs
688 definitively extend the spider's prey sensory environment.

689 The Avicularioidea clade unequivocally has an MRCA that forages from a sheet web. As
690 discussed in the classification section below, diplurids are polyphyletic, with most genera
691 forming a grade of lineages that are sister to the remaining mygalomorphs. The characteristics of
692 the ischnothelid, euagrid, etc. and hexathelid funnel webs are well documented for their abilities
693 to aid in sensing/localizing prey, and in many taxa also impeding and/or entangling prey items.
694 These silken constructs play a significant role in prey capture and are by any definition a
695 "foraging web." Sheet webs are independently derived twice in atracids (formerly hexathelids)
696 and much further up the tree in the clade that includes "true" diplurids. The atracid sheet webs
697 are highly modified from silken tubes (Gray 2010) and likely not homologous to the ancestral
698 sheet web.

699 The Bipectina clade resolves as having an MRCA with a trapdoor. This clade includes
700 typical trapdoor spiders like idiopids, migids, euctenizids, and nemesiids. As already discussed
701 above, and in a number of papers (Coyle 1986; Coyle et al. 1992; Bond and Coyle 1995),
702 trapdoor spiders employ silk in prey detection at the burrow entrance and in the door directly.
703 Moreover, it is well documented that many trapdoor spider species included in this clade add
704 additional silk lines, plant material, and tabs to the burrow entrance further emphasizing the role
705 that the silken lined burrow entrance plays in detecting and localizing prey. Although trapdoor
706 covered burrows do not seemingly serve to entangle or impede prey (criterion 2, above), aspects
707 of the burrow do serve to enhance the sensory capacity of the spider. Trapdoors are

independently lost in a number of Bipectina families in favor of other burrow constructs and foraging methods – Atracidae: sheet web; Theraphosidae: open burrows (though trapdoors have reappeared ≥3 times; Hamilton *persn. obsv.*); Pycnothelidae: open burrows, turrets; Dipluridae: sheet webs; Anamidae: open burrows and collar doors; Microstigmatidae: cursorial hunters, scored as inapplicable (*sensu* paratropidids). Additionally, species of one euctenizid genus, *Apomastus*, forages from an open burrow. In a number of these taxa, personal field observations demonstrate that vibrations at the silken burrow entrance can be used to coax the spider to the surface, illustrating the functional role that silk plays in prey detection for these taxa.

As mentioned above, Coyle (1986) demonstrated that trapdoors appear to reduce foraging efficiency in some taxa. As such it begs the question as to why some mygalomorph spiders have abandoned sheet webs in favor of a foraging construct that appears to be less effective at detecting prey and clearly lacks the ability to impede or entangle prey items. Although this remains an open question, trapdoor spiders likely required additional protection from predators (e.g., parasitoid insects like pompilid wasps), desiccation, or even flooding. The reversion to an open burrow, often with an expanded collar for prey sensing (e.g., *Apomastus* burrows, (Bond 2004)), suggests that once environmental conditions or predator selective pressures change, the trapdoor can be easily lost.

In closing, we would like to emphasize two major points. First, there is evidence from both analysis of extant taxa (like the one conducted here) and the morphology of extinct taxa to strongly infer a sheet web MRCA for mygalomorphs, if not all spiders. Second, it is our supposition that most mygalomorphs build foraging webs; that is, the majority of species employ silk either in a sheet web or at a burrow entrance to detect and localize prey and that a large number of taxa use silk to impede or entangle prey (the latter considered to be the plesiomorphic

condition). We believe these points are important to make particularly in light of a recent study by Fernández et al. (2018) that scored most mygalomorph taxa as having a non-foraging burrow. Based on the results and data discussed here, not surprisingly, we question their analysis that hypothesizes mygalomorphs (and spiders as whole) to have a non-web foraging MRCA. Their interpretation stems primarily from limited taxon sampling and erroneous character state coding (Coddington et al. 2018). The latter in particular includes an interpretation that a spider foraging from a burrow is not employing silk in the process of prey capture, which is contradictory to the information available in the literature and direct observations in the field.

Mygalomorphae Systematics and Higher-level Classification

We discuss below each of the major mygalomorph groups and their constituent families. Taxonomic changes referenced herein are formally documented and new family-level taxa diagnosed in Appendix 1 (authorship to be formally attributed as Bond, Hedin, and Opatova 2019).

Atypoidea – Avicularioidea – Bipectina – Domiothelina – Crassitarsae.—With a bit of a hyperbole the composition of Atypoidea and the basal split of the mygalomorphs into the Atypoidea and Avicularioidea, now solidified by molecular data since their first implementation in mygalomorph phylogenies (Hedin and Bond 2006), could be considered the most controversial topic in mygalomorph systematics. In particular, the interpretation of the significance of similarities in spinneret morphology, constituted one of the center pieces in the debate revolving around the higher-level classification of the group (Platnick and Gertsch 1976;

754 Raven 1985; Eskov and Zonshtein 1990; Goloboff 1993) and taxon composition of some
755 families (Raven 1985). Traditionally, mygalomorph spiders were divided into two families:
756 Atypidae and Avicularíidae (=Theraphosidae; Simon 1864), later established as superfamilies
757 Atypoidea and Ctenizoidea (Chamberlin and Ivie 1946). Atypoidea, a sister group to all the
758 remaining mygalomorph families, consisted of Atypidae, Antrodiaetidae, and Mecicobothriidae.
759 However, the external similarities of mecicobothriids and diplurids motivated some authors to
760 reject the placement of Mecicobothriidae within Atypoidea (Platnick and Gertsch 1976), and
761 subsequently even the concept of Atypoidina (Atypidae + Antrodiaetidae; Raven 1985) being a
762 sister group to all remaining mygalomorphs was abandoned in favor of an alternative
763 arrangement (Fig. 1a; Raven 1985). Eskov and Zonshtein (1990) insightfully disagreed with
764 some of Raven's character assessments and alternatively supported Atypoidea (*sensu*
765 Chamberlin and Ivie (1946)), arguing that the shared traits in mecicobothriids and the diplurids,
766 for example the elongation of spinnerets, constitute an ecological convergence related to a
767 construction of a sheet web (Coyle 1986). However, their alternative hypothesis was rejected in
768 what was the first computer-based cladistic treatment of mygalomorph relationships (Goloboff
769 1993), on the basis of different interpretation of Atypoidea unifying characters presented by
770 Eskov and Zonshtein (1990).

771 Contrary to the prevailing morphology-based hypothesis (Raven 1985; Goloboff 1993),
772 the first studies using molecular data strongly supported the classic Atypoidea hypothesis (Hedin
773 and Bond 2006). Although exercising caution while interpreting the results, the authors argued in
774 favor of the placement of mecicobothriids within Atypoidea (Hedin and Bond 2006), in
775 agreement with the seemingly correct hypothesis of Eskov and Zonshtein's (1990). The root
776 node bifurcation of Mygalomorphae into Atypoidea and Avicularioidea was further established

after adding additional molecular data (Ayoub et al. 2007; Bond et al. 2012). Although with limited taxon sampling, the same arrangement has been consistently recovered in subsequent studies employing transcriptome data (Bond et al. 2014; Garrison et al. 2016) and is also supported in our analyses. We recovered the reciprocal monophyly of both Atypoidea and Avicularioidea, however, Atypoidea remains in need of a more thorough phylogenetic treatment, as some mecicobothriid genera might be phylogenetically misplaced (see further detail below).

The Avicularioidea clade was recovered in all the analyses with high support and a degree of internal resolution never achieved in past comprehensive mygalomorph phylogenetic studies. Similarly, to the results obtained in the past (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012), the concatenated analyses recovered a grade of early branching Avicularioidea lineages representing the so-called "non-diplurine diplurids" (Goloboff 1993), the Hexathelidae, Porrhothelidae and Macrothelidae. Interestingly, the species tree analysis performed in ASTRAL recovered these lineages, excluding Macrothelidae, in a single highly supported clade. We statistically explored alternative tree topologies (Table 2) to: 1) assess the monophyly of the family Dipluridae (*sensu* Raven (1985)), 2) test the species tree arrangement and 3) further test the monophyly of the family Hexathelidae (*sensu* Raven (1985)) within a wider outgroup sampling framework than in Hedin et al. (2018). The rejection of the alternative topologies by the AU topology tests, the reciprocal monophyly of the "non-diplurine diplurid" lineages correlated with a unique combination of morphological characters present in each taxon, provides strong support for taxonomic and nomenclatural changes proposed here and by other authors (Hedin et al. 2018).

As in the previous analyses (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012), all Avicularioidea families, with exception of the grade of "diplurid" and former "hexathelid"

lineages, were recovered in a single clade, close in taxon composition to the original delineation of Bipectina (*sensu* Goloboff (1993)). Interestingly, we recovered Paratropididae and Actinopodidae (removed from Bipectina by Bond et al. (2012)) alongside the Atracidae as part of that clade. The Bipectina including these three families represent the most inclusive monophyletic arrangement that is consistently recovered in all the analyses. For this reason, we choose to relimit the Bipectina clade (*sensu* Bond et al. (2012)) to include Actinopodidae, Atracidae, Stasimopidae, and Paratropididae alongside the Crassitarsae and Domiothelina.

A monophyletic group roughly consistent with Domiothelina (*sensu* Bond et al. (2012)) was recovered in all of our analyses. However, given the recent taxonomic relimitation of the family Ctenizidae (Godwin et al. 2018) and further taxonomic changes made in this paper, we formally recircumscribe the Domiothelina to include the following families: Halonoproctidae, Migidae, Idiopidae, Ctenizidae and Euctenizidae. We also relimit the family Ctenizidae to include only the genera *Cteniza* and *Cyrtocarenum* and establish a new family Stasimopidae (NEW FAMILY) (see below) to accommodate *Stasimopus* herein removed from Ctenizidae.

Albeit generally accepted as a monophyletic group (Hedin and Bond 2006; Bond et al. 2012), Crassitarsae (Raven 1985; Goloboff 1993) is an unstable clade in our analyses. The group was recovered with low support in the concatenated analyses, whereas other analyses supported an alternative placement for the Theraphosoidina clade (*sensu* Bond et al. (2012)), namely as sister to Domiothelina and the Stasimopidae/Atracinae/Actinopodidae clade (species tree analysis) or as the sister group to all Bipectina lineages (No_Paratropis dataset). The only analysis supporting the Crassitarsae was based on the combined genomic/transcriptomic dataset. Interestingly, this analysis also recovered Paratropididae within the Crassitarsae clade. Despite the uncertain status of Crassitarsae, both Theraphosoidina and the clade comprising

Cyrtaucheniidae, Dipluridae, Microstigmatidae and former "Nemesiidae" lineages (the "Nemesioidina", see below), were highly supported and mostly resolved at internal levels in all analyses. With the results at hand, we can now confidently address the long-standing taxonomic issues involving the non-monophyly of the families Dipluridae, Cyrtaucheniidae and Nemesiidae. As a result, we recircumscribe the families Cyrtaucheniidae, Dipluridae and Nemesiidae and establish the new families Anamidae (NEW RANK), Hermachidae (NEW FAMILY), Bemmeridae (NEW RANK), and Pycnothelidae (NEW RANK).

Atypidae.—The family Atypidae comprises three genera and 54 species distributed across the Holarctic, Southeastern Asia, and Africa. The family is well-defined morphologically, with enlarged maxillae being its most conspicuous synapomorphy (Raven 1985). Albeit highly supported in molecular analyses (Hedin and Bond 2006; Bond et al. 2012), the group's monophyly has never been fully assessed due to incomplete taxon sampling. In our study, we sampled all three genera and recovered the family as monophyletic with *Calommata* being sister to *Atypus* and *Sphodros*. Due to ambiguities in putatively shared characters, the relationships within the family have been challenging to decipher (Gertsch and Platnick 1980) based on morphology alone. Our results support Raven's (1985) hypothesis which unites *Atypus* and *Sphodros* based on the shape of cymbium and labidosternal suture rather than the alternative placement of *Calommata* as sister to *Sphodros* which is based largely on similarities in spermathecal morphology (*sensu* Gertsch and Platnick (1980)).

Antrodiaetidae – Mecicobothriidae.—Antrodiaetidae and Mecicobothriidae are among the smaller mygalomorph families. Antrodiaetidae comprise two genera, *Aliatypus* and *Antrodiaetus* (both included in our analysis) with predominantly Nearctic distributions. The monophyly of antrodiaetids has not been called in to question (Coyle 1971; Raven 1985; Hedin

and Bond 2006; Bond et al. 2012), however, the generic composition of the family will likely change in the future to include at least *some* mecicobothriid taxa. As already noted above, placement of the family Mecicobothriidae has been highly controversial, fluctuating from residing within the Atypoidea (Simon 1864; Chamberlin and Ivie 1946; Eskov and Zonshtein 1990), to being placed as sister to Dipluridae (Gertsch 1979), Microstigmatidae (Raven 1985), or all mygalomorphs excluding Atypidae and Antrodiaetidae (Goloboff 1993). The family comprises four genera, the North American *Hexura*, *Hexurella* and *Megahexura* and the South American *Mecicobothrium*. The debate about its phylogenetic position aside, Mecicobothriidae has received relatively little attention (Gertsch 1979). Although the placement of mecicobothriids within Atypoidea has been substantiated by numerous molecular analyses (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Bond et al. 2014; Garrison et al. 2016; Wheeler et al. 2017), the composition and monophyly remains unresolved due to these studies suffering from incomplete taxon sampling. In our analyses, we only sampled the genus *Hexura* and, similarly to previous results (Hedin and Bond 2006; Bond et al. 2012; Wheeler et al. 2017), we also recovered the genus embedded within the antrodiaetids, as opposed to *Megahexura* having close affinities to atypids (Bond et al. 2012). Future work employing extensive sampling of both Antrodiaetidae and Mecicobothriidae may thus result in relimitation of the family boundaries, or in a more extreme case, the synonym of some mecicobothriids with antrodiaetids.

"Dipluridae".—The monophyly of the diplurids was first questioned by Goloboff (1993) relatively soon after the last relimitation of the family (Raven 1985), which without a formal change being made remains still reflected in the current classification scheme. Diplurids in their original composition comprised a variety of, now in retrospect, distantly related lineages possessing to some extent widely spaced "lower" spinnerets and elongated "upper" spinnerets

869 (Simon 1889). The subsequent reassessment of morphological characters led to taxonomic
870 changes altering Dipluridae composition. Mecicobothriidae (Gertsch 1979) and Hexathelidae
871 (Raven 1980) were established as independent families and a large number of taxa was then
872 transferred to the family Nemesiidae (Raven 1985). Despite that, the elongation of posterior
873 lateral spinnerets and the wide separation of posterior median spinnerets remained among
874 characters uniting the family Dipluridae (Raven 1985). Goloboff (1993) debated the character
875 state scoring of the spinneret elongation in diplurids, hexathelids, and mecicobothriids, alluding
876 that the character definition was ambiguous, even within Dipluridae. Goloboff (1993) recovered
877 the Dipluridae (*sensu* Raven (1985)) as four independent lineages which portended the future
878 relimitation of diplurids to the subfamily Diplurinae (*sensu* Raven (1985)). The non-monophyly
879 of the diplurids was also cautiously echoed by Coyle (1995) who, while conducting a taxonomic
880 revision of the subfamily Ischnothelinae, did not find any strong synapomorphies establishing a
881 sister relationship between ischnothelines and any other diplurid taxa (Coyle 1995). Diplurids
882 have been recovered both as monophyletic (Ayoub et al. 2007; Bond et al. 2012) and
883 paraphyletic (Hedin and Bond 2006; Wheeler et al. 2017; Hedin et al. 2018). However, with
884 exception of Hedin et al. (2018), the taxon sampling was restricted to members of the subfamily
885 Euagrinae and the seemingly contradictory outcome was likely a result of a lack of strong
886 phylogenetic signal in the sequenced markers. In our analyses, we sampled representatives from
887 three diplurid subfamilies (Diplurinae, Ischnothelinae, Euagrinae) plus *Microhexura*, a genus
888 with uncertain placement (Raven 1985); Masteriinae was not included in this study. Our results
889 are congruent with Goloboff's (1993) hypothesis that Dipluridae constitutes an assemblage of
890 distantly related lineages that do not share a common ancestor. The subfamilies Ischnothelinae,
891 Euagrinae + *Microhexura* were recovered as three independent lineages at the base of the

892 Avicularioidea clade ("non-diplurine diplurids" (Goloboff 1993; Hedin et al. 2018)), whereas
893 Diplurinae taxa were placed as sister to cyrtaucheniids within proximity to nemesiids
894 (Supplemental figure Fig. 5). The presumed synapomorphy of diplurids (*sensu* Raven (1985)),
895 elongated and widely spaced spinnerets, could thus be an example of a retention of
896 plesiomorphic condition (see discussion on silk use) of the "non-diplurine diplurids" that evolved
897 convergently in Diplurinae – the genomic architecture likely already in place (see Uraraneida:
898 *Chimerarachne yingi* (Wang et al. 2018)). As already pointed out by Coyle (1971) and Eskov
899 and Zonshtein (1990), the traits related to the morphology of the spinnerets are likely correlated
900 to the type of foraging structure produced by the spiders. "Diplurids" generally build
901 conspicuous three-dimensional webs with a funnel-like retreat (Coyle 1988; Coyle and Ketner
902 1990; Coyle 1995; Eberhard and Hazzi 2013; Passanha and Brescovit 2018), a trait present in
903 many other mygalomorph taxa that have little to no evolutionary proximity to diplurids (e.g.,
904 Mecicobothriidae (Gertsch 1979), Macrothelidae (Snazell and Allison 1989; Shimojana and
905 Haupt 1998), Atracidae (Gray 2010), and is also paralleled in araneomorph funnel-web spider
906 family Agelenidae (Nentwig 1983). Interestingly, some of these groups were originally placed
907 within Dipluridae (Simon 1889), demonstrating the perceived significance of the spinneret shape
908 in spider systematics that was still prominent in subsequent studies (Gertsch 1979; Raven 1985).
909 With the results of our phylogenetic analyses, it seems safe to assume that spinneret morphology
910 is a plastic trait that has little to no informative value for higher-level classification in
911 mygalomorph spiders. This observation is further supported by the results of the topology test
912 rejecting the monophyly of Dipluridae (*sensu* Raven (1985)) as well as the monophyly of a clade
913 consisting of the "non-diplurine diplurids" (Goloboff 1993), Hexathelidae and Porrhothelidae.
914 We therefore substantially relimit the boundaries of the family Dipluridae (new circumscription).

We elevate the subfamily Ischnothelidae (NEW RANK) and Euagridae (NEW RANK) to family-level and establish the new family Microhexuridae (NEW FAMILY).

Family Dipluridae (new circumscription).—In our analyses, the family Dipluridae is represented by three South American genera *Diplura*, *Linothele* and *Harmonicon* belonging to the diplurid subfamily Diplurinae (Raven 1985). We recovered the Dipluridae as sister to Cyrtaucheniidae placed within the same clade alongside Nemesiidae. These results are roughly congruent with Goloboff (Goloboff 1993), who analyzed four genera of diplurids (*sensu* Raven (1985)) and recovered *Diplura* as the sister to the rest of the Bipectina clade. As in Raven (1985), Goloboff (1993) also noted shared characters in *Diplura* and the Crassitarsae, and hypothesized that the diplurids would be restricted to the subfamily Diplurinae in the future. Unfortunately, we did not conduct an exhaustive sampling of the "diplurid" genera and acknowledge that even after removing the Euagridae, Ischnothelidae and Microhexuridae the family likely remains non-monophyletic. Given the rough congruence between Raven's diplurid subfamilies (Raven 1985) and the lineages removed from the Dipluridae in this study, it is possible that the distinct subfamily Masteriinae (Raven 1985; Passanha and Brescovit 2018) will be elevated to family status as well.

Family Ischnothelidae (NEW RANK).—The family corresponds to the former diplurid subfamily Ischnothelinae, defined by six morphological synapomorphies (Raven 1985; Coyle 1995). In our analyses, we sampled the genera *Ischnothele* (South America) and *Thelechoris* (Africa) and recovered these together in all analyses. In previous analyses based on morphological characters, *Ischnothele* was placed as a sister group to Bipectina (Goloboff 1993), but our results suggest that ischnothelids belong among the first groups that diverged within the Avicularioidea clade. Given the morphological cohesiveness of the group, and the fact that it was

938 thoroughly revised in the past (Coyle 1995), we retain all the genera of the subfamily
939 Ischnothelinae (Raven 1985; Coyle 1995) despite our incomplete sampling. The family
940 Ischnothelidae comprises five genera (*Andethele*, *Indothele*, *Ischnothele*, *Latrothele*,
941 *Thelechoris*) with a disjunct distribution spanning across Central and South America, the
942 Antilles, Africa, Madagascar, India and Sri Lanka. All genera build conspicuous three-
943 dimensional webs and often exhibit subsocial behavioral (Coyle 1995).

944 *Family Microhexuridae (NEW FAMILY)*.—Microhexuridae is a monogeneric family
945 comprising two nominal species endemic to North America. Both species are very small (> 6
946 mm) and have allopatric distributions restricted to the high-elevation peaks in the Appalachian
947 Mountains and Pacific Northwest Cascade Range (Coyle 1981; Hedin et al. 2015). Despite being
948 recognized as a diplurid (*sensu* Raven (1985)), *Microhexura* exhibits a combination of characters
949 not present in other diplurid genera. Most notably *Microhexura* possess a longitudinal fovea and
950 spinnerets somewhat shorter than in other diplurids, which made the genus difficult to place
951 (Coyle 1981; Raven 1985). The unique position of the genus noted in the previous studies (Coyle
952 1981; Raven 1985) is further supported by our results that recovered the genus *Microhexura* as a
953 stand-alone lineage among other "non-diplurine diplurids" at the base of the Avicularioidea
954 clade. The topology test results reject both its placement within the Dipluridae (*sensu* Raven
955 (1985)), but also within the "non-diplurine diplurids"/Hexathelidae/Porrhothelidae clade
956 recovered in the species tree analyses.

957 *Family Euagridae (NEW RANK)*.—The family roughly corresponds to the former
958 subfamily Euagrinae (Raven 1985) that currently comprises 11 genera with distribution spanning
959 across Australia, Asia, Africa, and South and North America. Euagrinae differ from other
960 "diplurids" by a combined lack of cuspules and broad and short serrula. In our analyses, we only

961 sampled five Euagrinae genera: *Allothele*, *Australothele*, *Cethegus*, *Euagrus* and *Namirea*, which
962 were all recovered forming a monophyletic group towards the root node of Avicularioidea.
963 Similar results were obtained in previous analyses (Ayoub et al. 2007; Bond et al. 2012), though
964 with limited taxon sampling. The internal relationships of these taxa resemble their
965 morphological affinities (Raven 1985). The "austral genera" possess a crescent of hirsute cuticle
966 at the base of the posterior median spinnerets ("australothelinae crescent") and were recovered as
967 a monophyletic group sister to *Euagrus* that lacks this character (Raven 1985; Coyle 1988). The
968 lack of a strong synapomorphy uniting the "austral" genera and *Euagrus* + *Phyxioschema* (Raven
969 1985; Coyle 1988) alongside deep divergence dating back to nearly 170 Ma, would provide the
970 support for the division of Euagrinae in two different families. However, the decision would be
971 premature given our taxon sampling, which represents less than half of the described generic
972 diversity of Diplurinae, and in particular, lacks representatives of the subfamily Masteriinae
973 (Raven 1985) – a group that overlaps in some characters with Euagrinae. We therefore prefer to
974 elevate the subfamily Euagrinae to family rank, retaining its current generic composition – i.e.,
975 the taxa sampled in this paper along with the genera *Caledothele*, *Carrai*, *Chilehexops*,
976 *Leptothele*, *Phyxioschema*, *Stenygrocercus* and *Vilchura.*
977 *Hexathelidae – Porrhothelidae – Macrothelidae*.—The monophyly of the family
978 Hexathelidae in its original composition (*sensu* Raven (1985)) has been challenged on molecular
979 grounds on numerous occasions (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012;
980 Opatova and Arnedo 2014a; Hamilton et al. 2016b). Paralleling the situation occurring with
981 Dipluridae, Cyrtaucheniidae and Ctenizidae, families that are both widespread and notoriously
982 difficult to resolve with the traditionally Sanger-sequenced loci, formal taxonomic changes were
983 put on hold until Hedin et al. (2018). The implementation of UCEs alongside complete generic

984 sampling resulted in parceling of the family Hexathelidae (*sensu* Raven (1985)) into four distinct
985 families (Hedin et al. 2018), of which all are sampled in our analyses. Similar to the results of
986 Hedin et al. (2018), we recovered the families Hexathelidae, Porrhothelidae and Macrothelidae
987 among the early branching lineages near the Avicularioidea root; though Atracidae, was
988 recovered within the Bipectina clade sister to Actinopodidae. The family Hexathelidae now
989 comprises nine genera with a disjunct distribution (Australia, New Zealand, South America), of
990 which we only sampled the Australian *Bymainiella*. It was recovered as sister to the family
991 Euagridae with high support, which was previously indicated by EF-1g data (Ayoub et al. 2007),
992 albeit the support for this relationship was low. Both Porrhothelidae and Macrothelidae were
993 recovered as stand-alone lineages placed near the Avicularioidea root node. Both lineages are
994 monogeneric and were previously considered sister taxa (Raven 1985), but this relationship was
995 statistically rejected in the past (Opatova and Arnedo 2014a) and their unique position appears
996 stable even in light of considerable genomic data ((Hedin et al. 2018); this study). Given the
997 wide taxonomic framework of our analyses, we tested the monophyly of the family
998 Hexathelidae, in its original composition (*sensu* Raven (1985)), as well the alternative
999 arrangement recovered in the species tree analysis (Table 2); both were statistically rejected,
1000 confirming Hedin et al. (2018).

1001 *Paratropididae*.—Paratropididae is a poorly known, and highly distinct family
1002 comprising four genera known from Central and South America. Its phylogenetic placement has
1003 been regarded as "difficult" (Bond et al. 2012), given the conflict between the morphology-based
1004 hypotheses and the results of molecular analyses. The family was originally placed within a
1005 clade "Theraphosoidina", alongside the Theraphosidae and Barychelidae (Raven 1985; Goloboff
1006 1993), however this relationship has never been recovered in any molecular analyses (Hedin and

Bond 2006; Bond et al. 2012; Hamilton et al. 2016b) and, in fact, a close relationship between Paratropididae and any other mygalomorph taxon has never been supported. Instead, the paratropidids were placed among the grade of early branching Avicularioidea (Hedin and Bond 2006; Bond et al. 2012). In our analyses, the paratropidids represent a stand-alone lineage diverging at the root of Bipectina, however, similar to previous studies (Hedin and Bond 2006; Bond et al. 2012), we only sampled representatives of the genus *Paratropis*. This not only precludes the evaluation of the family's monophyly, but may also be a mitigating factor in confidently placing it on the tree. Moreover, the stand-alone status of the lineage may also have an impact on the inferred tree topology as a whole, a fact demonstrated by the improved support values at particular nodes and the topology changes in the unsupported nodes after paratropidids were removed from the dataset (Fig. 4). Interestingly, our exploratory analysis combining AHE and transcriptomic data recovered paratropidids with high support as sister to the "Nemesioidina" clade (Fig. 5). However, the sampling in this analysis was limited both in terms of taxa and data occupancy. Despite the equivocal nature of the placement of *Paratropis* within the Bipectina, it seems clear that there is no support for its placement within Theraphosoidina (*sensu* Raven (1985)).

Atracidae – Actinopodidae (the "Venom Clade").—The family Atracidae comprises three genera endemic to eastern Australia. Formerly designated as the "hexathelid" subfamily Atracinae (Gray 2010), the group was recently elevated to family level by Hedin et al. (2018) and supported by the topology test performed on our dataset. Contrary to the hypothesis based on morphological characters (Raven 1985; Goloboff 1993), Atracidae has consistently been recovered as sister to Actinopodidae (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Hamilton et al. 2016b; Hedin et al. 2018), further corroborated by our results.

1030 Actinopodidae comprises three genera with a disjunct South American – Australian distribution
1031 and on the basis of morphological characters (eyes widely spread across the carapace), was
1032 placed as sister to Migidae (Raven 1985; Goloboff 1993). However, this relationship has never
1033 been supported in any molecular phylogenetic study. Despite the differences in the morphology
1034 of the families, the phylogenetic proximity of Atracidae/Actinopodidae is supported not only by
1035 the overwhelming amount of evidence proceeding from molecular data, but also by similarities
1036 in venom composition (for details see Hedin et al. (2018). Certain members of both families are
1037 medically important; the bites of the Sydney funnel-web spider, *Atrax robustus*, cause lethal or
1038 life-threatening conditions, and other taxa can also be dangerous to humans (Isbister et al. 2005,
1039 2015). Interestingly, the Atracidae/Actinopodidae clade was recovered within the Bipectina,
1040 which contradicts the results of a number of previous analyses (Goloboff 1993; Hedin and Bond
1041 2006; Bond et al. 2012). Unfortunately, the exact relationships of the Atracidae/Actinopodidae
1042 with other taxa remain uncertain. The group was consistently placed in all analyses as sister to
1043 the Stasimopidae, although with varying degrees of support (see below). In our analyses, both
1044 families were represented by two out of three genera; *Atrax* and *Hadronyche* for Atracidae, and
1045 *Actinopus* and *Missulena* for Actinopodidae, however, the monophyly of both families, based on
1046 complete generic sampling, appears to be strong (Hedin et al. 2018).

1047 *Family Ctenizidae (new circumscription)*.—The generic composition of the family
1048 Ctenizidae was dramatically altered by Raven's (1985) morphology-based cladistic analysis, but
1049 it became apparent that the family most likely comprised an assemblage of diverse lineages that
1050 do not share a common ancestor (Hedin and Bond 2006). This observation was repeatedly
1051 confirmed by subsequent molecular-based studies (Bond et al. 2012; Opatova et al. 2013;
1052 Wheeler et al. 2017). However, a lack of perceived phylogenetic signal, incomplete sampling, or

a combination thereof precluded previous authors from making taxonomic changes that would rectify the problem. The polyphyletic nature of the family was partially resolved by Godwin et al. (2018) when six genera were transferred to the family Halonoproctidae, circumscribing Ctenizidae to *Cteniza*, *Cyrtocarenum* and *Stasimopus*. Godwin et al. (2018) recognized that *Stasimopus* did not share a common ancestor with the remaining genera, but incomplete outgroup sampling limited their ability to definitively address the phylogenetic placement of the taxon. In our analyses, we recovered *Cteniza* and *Cyrtocarenum* as sister taxa, which agrees with previous findings (Opatova et al. 2013; Godwin et al. 2018). *Cteniza* and *Cyrtocarenum* were placed as sister to the family Euctenizidae, whereas *Stasimopus* was recovered outside of Domiothelina in a weakly supported clade as the sister lineage to Atracidae and Actinopodidae. Given the distant phylogenetic position of *Stasimopus*, with regards to the remaining ctenizids, and considering that the topology test rejected the monophyly of the three genera, we remove *Stasimopus* from the family Ctenizidae and designate it to the family Stasimopidae (NEW FAMILY). Ctenizidae is circumscribed to contain only the genera *Cteniza* and *Cyrtocarenum.* The family Ctenizidae thus now constitutes a much smaller family comprising six species with a predominantly Mediterranean distribution.

Family Stasimopidae (NEW FAMILY).—Although the placement of *Stasimopus* within the family Ctenizidae (Raven 1985) was repeatedly called into question based on earlier molecular studies (Hedin and Bond 2006; Bond et al. 2012; Opatova et al. 2013; Godwin et al. 2018), establishing its exact phylogenetic position has been difficult. In previous phylogenetic studies of the entire infraorder, *Stasimopus* was recovered as sister to Migidae (Hedin and Bond 2006), or as the sister group to the entire Domiothelina, albeit with low support (Bond et al. 2012). Importantly, when morphological characters were added to the analyses, *Stasimopus* was

recovered within the Ctenizidae (Bond et al. 2012), confirming the homoplasious nature of some morphological characters. Our results place *Stasimopus* as a stand-alone lineage forming a clade with Atracidae and Actinopodidae. This relationship was also recovered by Hamilton et al. (2016b), though in a much reduced AHE dataset, and similarly to our results, the Stasimopidae/Atracidae/Actinopodidae clade was poorly supported. Interestingly, the support values for this clade increased with the removal of the paratropidid terminals (Fig. 4) and with addition of transcriptomic data (Fig. 5), suggesting that adding more data to the matrix could be instrumental for stabilizing Stasimopidae placement. Despite the partial positional uncertainty of *Stasimopus*, the topology test soundly rejects its inclusion within Ctenizidae. Considering the results of our analyses, alongside the genus' restricted distribution to southern Africa – a biodiversity hotspot with high levels of endemicity (Myers et al. 2000; Mittermeier et al. 2011), it seems that there is sufficient evidence to support the hypothesis that *Stasimopus* represents a unique lineage within the Mygalomorphae and deserves family-level status.

Halonoproctidae.—The family currently comprises six genera and has a nearly cosmopolitan distribution. It was recently removed from Ctenizidae (Godwin et al. 2018), whose monophyly was doubted both on morphological (Raven 1985) and molecular grounds (Hedin and Bond 2006; Bond et al. 2012; Opatova et al. 2013). Similar to Godwin et al. (2018), we recovered Halonoproctidae as monophyletic in all analyses. Furthermore, the topology test rejected the monophyly of the family Ctenizidae (*sensu* Raven (1985)) as well as the monophyly of Halonoproctidae + Ctenizidae (*sensu* this study), verifying the status of the family within the context of a wider taxonomic framework. It should be noted that we did not include the genus *Bothriocyrtum* in our analyses, but given its stable position as sister to *Hebestatis* (Hedin and

1098 Bond 2006; Bond et al. 2012; Opatova et al. 2013; Godwin et al. 2018), we believe that it had
1099 little to no effect on the resulting topology and support values.

1100 *Migidae*.—The family Migidae currently comprises 11 genera with a classical
1101 Gondwanan distribution (Australia, New Zealand, Africa, Madagascar and South America). The
1102 family is well defined morphologically as taxa having longitudinal keels on cheliceral fangs and
1103 lack a rastellum (Raven 1985; Griswold and Ledford 2001). Similar to previous studies (Hedin
1104 and Bond 2006; Bond et al. 2012), our taxon sampling is rather limited. We only sampled four
1105 genera, lacking a neotropical representative; therefore, we cannot assess the monophyly of the
1106 group. The family has been recovered as sister to either *Stasimopus* (Hedin and Bond 2006) or
1107 Halonoproctidae (Bond et al. 2012), as well as in close proximity to Idiopidae and Euctenizidae
1108 (Ayoub et al. 2007; Bond et al. 2012), as we report here. In the concatenated analyses, the family
1109 was recovered with high support as sister to all the remaining Domiothelina taxa, except for
1110 Halonoproctidae. Interestingly, despite the congruent topology, this relationship was poorly
1111 supported in the species tree analysis.

1112 *Idiopidae*.—Idiopids are another family with predominantly Gondwanan distribution
1113 (South America, Asia, Africa, Australia and New Zealand), though some species are also known
1114 from the Middle East and Central America. The family is well characterized by male palpal
1115 synapomorphies (Raven 1985) and its monophyly has never been called into question by
1116 previous studies (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012).
1117 Therefore, it comes as no surprise that the family was recovered as monophyletic in our analyses.
1118 Idiopids are among the most diverse of the mygalomorphs; the family comprises 22 genera and
1119 three subfamilies: Arbanitinae, Genysinae and Idiopinae (Raven 1985; Rix et al. 2017c), of
1120 which we did not sample Genysinae. The position of Idiopidae within the Domiothelina clade

1121 was consistently recovered by both morphological (Goloboff 1993) and molecular approaches
1122 (Bond et al. 2012), but given the incomplete sampling of the family Ctenizidae (*sensu* Raven
1123 (1985)), the position within the clade varied. In our analyses, the family was placed as sister to
1124 Ctenizidae and Euctenizidae with high support.

1125 *Euctenizidae*.—The group was originally designated as a subfamily of Cyrtaucheniidae
1126 (Raven 1985), but with increasing amount of evidence for a non-monophyletic Cyrtaucheniidae
1127 (Bond and Opell 2002; Bond and Hedin 2006), Euctenizidae were elevated to family level (Bond
1128 et al. 2012). The family comprises 76 species within seven genera across a North American
1129 distribution (Bond 2017). We sampled all described genera and recovered the family as
1130 monophyletic, as well as the sister group to Ctenizidae (*sensu* this study) and Idiopidae.
1131 Although an argument could be made for collapsing Euctenizidae and Ctenizidae, aside from
1132 differences in morphology, and biogeography, the divergence depth between the two groups
1133 remains at a hierarchical level comparable to other families in the tree.

1134 *Barychelidae*.—Barychelidae is a highly diverse family (42 genera, 495 species) with a
1135 Gondwanan distribution spanning continental islands, and interestingly, also remote islands of
1136 volcanic origin (Raven 1994) indicating the ability to disperse. Like Theraphosidae,
1137 Barychelidae possess well developed scopulae and dense claw tufts, but differs in the number of
1138 cuspules, clavate trichobothria, and the shape of the maxillary anterior lobe (Raven 1985;
1139 Dippenaar-Schoeman 2002). Due to the potential ambiguity of these characters (Raven 1985;
1140 Goloboff 1993; Bond et al. 2012) some authors have considered Barychelidae, at least in part, a
1141 paraphyletic lineage of Theraphosidae (Goloboff 1993). Stemming from differences in character
1142 state interpretations, taxa tend to vacillate between the two families, with proposed membership
1143 by some authors in one family (Raven 1985; Guadanucci 2014) to be later rejected by others

(Gallon 2002; Schmidt 2002; Ríos Tamayo 2017). One example of this problem, the family placement of *Brachionopus* (Raven 1985; Gallon 2002; Schmidt 2002) was not successfully resolved until the implementation of a molecular approach (Lüddecke et al. 2018). With our limited sampling, we recovered the reciprocal monophyly of both Barychelidae and Theraphosidae. However, a thorough sampling of both families will be needed in order to assess the taxa composition of each group and stabilize the family boundaries. Barychelidae (as currently defined) is composed of two common and divergent "phenotypes" (a "naked" group that is much less setose and resemble most mygalomorph groups, and a "hairy" group that resemble small theraphosids. These two "phenotypes" have been the major drivers of taxa historically bouncing back and forth between the two families.

Theraphosidae.—The family Theraphosidae is the most diverse family of mygalomorphs with a nearly cosmopolitan distribution. Currently comprising 144 genera and over 970 species, the family is well-defined (aside from Barychelidae) by dense claw tufts and leg scopulae (Raven 1985). The monophyly is generally undoubted (Hedin and Bond 2006; Bond et al. 2012; Lüddecke et al. 2018; Turner et al. 2018), although paraphyly with respect to Barychelidae has occurred due to taxon misplacement (Goloboff 1993; Bond et al. 2012). Until recent molecular phylogenetics work (Lüddecke et al. 2018; Turner et al. 2018), attempts to reconstruct the internal relationships of Theraphosidae mostly relied on the analysis of morphological characters (Pérez-Miles 1994; Pérez-Miles et al. 1996; Pérez-Miles 2002; Guadanucci 2005, 2011; Guadanucci 2014). Molecular approaches have become more common to resolve taxonomic issues at shallow levels, such as species delimitation or uncovering species-specific phylogeographic patterns (Hamilton et al. 2011; Hendrixson et al. 2013; Hamilton et al. 2014; Graham et al. 2015; Montes de Oca et al. 2016; Ortiz et al. 2018). In our analyses, we sampled

eight genera from North and South America, Africa, and Asia, recovering the family as monophyletic in all the analyses. The subfamily Theraphosinae, here represented by the tribe Theraphosini (*Aphonopelma*, *Cyrthopholis*, *Sericopelma* and *Stichoplastoris*) (Turner et al. 2018), was also recovered with high support. On the other hand, our results substantiate the para- or polyphyletic status of the subfamily Ischnocolinae, already debated on the basis of morphological characters (Raven 1985; Guadanucci 2005; Guadanucci 2014; Guadanucci and Wendt 2014) and karyotype structure (Král et al. 2013). The genus *Catumiri* (Ischnocolinae) was recovered as sister to the rest of the theraphosid lineages, whereas *Ischnocolus*, regarded as the most "basal group" of the family (Raven 1985), appears to be related to Ornitoctoninae and Harpactirinae (here represented by *Brachionopus*). The non-monophyletic status of higher-level taxonomic units, and apparent polyphyly of some wide spread genera arises from high levels of homoplasy in the morphological characters traditionally used in Theraphosidae taxonomy (Guadanucci and Wendt 2014; Gabriel and Longhorn 2015; Hamilton et al. 2016a; Turner et al. 2018). Molecular approaches and more thorough taxonomic sampling will be essential to establishing an accurate classification scheme reflecting the evolutionary relationships within the family.

"Cyrtaucheniidae".—The family Cyrtaucheniidae, as currently defined, comprises 11 genera distributed across Africa, Asia, Europe and South America. The non-monophyletic nature of the family has been recognized for over two decades (Goloboff 1993; Bond and Opell 2002; Bond and Hedin 2006; Hedin and Bond 2006), however the incomplete taxon sampling and ambiguous placement of some cyrtaucheniid taxa, did not allow for a definitive taxonomic solution to the problem (Hedin and Bond 2006; Bond et al. 2012). During the first major relimitation of the family, the North American euctenizine lineages were raised to family level

and the taxonomically misplaced genus *Kiama* was transferred to Nemesiidae (Bond et al. 2012). Despite these rearrangements, the family remained a "taxonomic dumping ground" (Bond et al. 2012) comprising a diverse assemblage of lineages spread across the mygalomorph tree. The polyphyletic nature of the family is nearly paralleled in the status of *Cyrtauchenius*, the type genus of the family. *Cyrtauchenius*, presumably a species-rich genus inhabiting the western Mediterranean, was recently divided into two genera (Decae and Bosmans 2014). *Amblyocarenum* was removed from the synonymy of *Cyrtauchenius* based on a revision of morphological characters and its position within Cyrtaucheniidae was also questioned due to its morphological affinities to the family Nemesiidae (Decae and Bosmans 2014). As in previous analyses (Hedin and Bond 2006; Bond et al. 2012), we did not recover the family as monophyletic, instead, the five genera sampled in our study were placed within three different clades (Supplemental Fig. 5). The first group, already recognized in the past as "Ancylotripines" (Hedin and Bond 2006) or the "Homostolines" (Bond et al. 2012), was recovered within the Theraphosoidina (*sensu* Bond et al. (2012)). The second group, comprising the genus *Cyrtauchenius*, was placed as sister to Dipluridae, whereas the third group, the genus *Amblyocarenum*, was embedded within Nemesiidae. Bond et al. (2012) stressed the necessity to address the obvious polyphyly of the group, but at the same time recognized that obtaining a complete generic sampling will be a major challenge, likely hindering the future efforts to resolve Cyrtaucheniidae relationships. Conversely, our study provides crucial aspects that allows us, at least partially, to overcome the prevailing sampling limitations. Both *Cyrtauchenius* and *Amblyocarenum*, were analyzed here for the first time, enabling the identification of the cyrtaucheniid lineage carrying the type genus of the family and subsequent status assessment of the "non-cyrtaucheniid" lineages. Compared to the previous analyses (Hedin and Bond 2006;

Bond et al. 2012), our data provide a stronger phylogenetic signal resulting in high support values for the placement of the individual groups. Moreover, our dataset offers a wider taxonomic framework for testing Cyrtaucheniidae monophyly. Provided the rejection of Cyrtaucheniidae monophyly by the statistical topology test, and following the line of reasoning explained above, we therefore relimit the family Cyrtaucheniidae (new circumscription) and establish the family Bemmeridae (NEW RANK), to accommodate the African genera *Homostola* and *Spiroctenus*. We also formally propose the transfer of the genus *Amblyocarenum* to the family Nemesiidae.

Family Cyrtaucheniidae (new circumscription).—Following the results of our phylogenetic analyses, we transfer the genus *Homostola* to the newly established family Bemmeridae (NEW RANK, see below). We also formally transfer the Mediterranean genus *Amblyocarenum* to the family Nemesiidae. The family Cyrtaucheniidae is thus relimited to nine genera (three of them monotypic) distributed across Africa, Asia and South America. In this study, we analyzed two African genera: *Ancylotrypa*, *Cyrtauchenius* and one South American lineage that is morphologically proximate to *Acontius*. As a result, Cyrtaucheniidae were recovered as monophyletic in all analyses and placed as sister to Dipluridae. At present, we retain the genera *Acontius*, *Anemesia*, *Angka*, *Bolostromoides, Bolostromus*, *Fufius*, and *Rhytidicolus* (not included in this study), within Cyrtaucheniidae. We acknowledge that the family still likely remains poly- or paraphyletic, because *Fufius* is known to have affinities with the Nemesiidae (Bond et al. 2012), however, we argue that even the partial Cyrtaucheniidae taxon pruning performed in this study is preferable to retaining the high level of polyphyly. This is particularly true when obtaining fresh samples may be exceptionally difficult, especially for the African and South American representatives of the genera *Acontius* and *Bolostromus*.

Family Bemmeridae (NEW RANK).—The family Bemmeridae comprises 35 species classified in the genera *Homostola* and *Spiroctenus*, both endemic to South Africa. The group was repeatedly recovered as sister to Theraphosoidina (Hedin and Bond 2006; Bond et al. 2012), which is further substantiated by our analyses. Previous analyses also suggested the inclusion of the genus *Ancylotrypa* (Hedin and Bond 2006), an assumption that was unfortunately based on sample misidentification. The genus *Homostola* is being transferred to this family from Cyrtaucheniidae, the genus *Spiroctenus* from Nemesiidae. Both genera comprise medium-size spiders, constructing underground burrows with or without a trapdoor.

"Nemesiidae".—The cosmopolitan family Nemesiidae belongs among the most speciose mygalomorph groups. As currently defined, it comprises over 400 nominal species that are placed in 45 genera. The family was diagnosed by Raven (1985) as those taxa possessing two rows of teeth on the paired tarsal claws and having scopulate tarsi (Raven 1985); a set of characters found in other groups and thus likely homoplasious, and later reinterpreted as plesiomorphic (Goloboff 1993). Not surprisingly, the monophyly of the family Nemesiidae has never been recovered in any broad scale molecular phylogenetic analyses of mygalomorphs (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012). Moreover, even for analyses based solely on morphological characters, the family was seemingly split into five different lineages (Goloboff 1993). Paralleling the non-monophyletic nature of the family, the subfamilies established by Raven (1985) were also typically recovered as para- or polyphyletic (Goloboff 1993; Goloboff 1995; Hedin and Bond 2006; Bond et al. 2012). For this current study, we sampled five out of six nemesiid subfamilies (Raven 1985) and about 40% of the generic diversity to more thoroughly assess the composition of the family, particularly with an emphasis on resolving the position of the family Microstigmatidae and several difficult to place

1259 "cyrtaucheniid" genera (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al.
1260 2012; Wheeler et al. 2017). Like all other studies conducted to date (Goloboff 1993; Hedin and
1261 Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Garrison et al. 2016; Wheeler et al. 2017), we
1262 did not recover the Nemesiidae as monophyletic (Supplemental Fig. 5). The South African genus
1263 *Spiroctenus* was placed within Bemmeridae, whereas the remaining nemesiids were parceled
1264 among five reciprocally monophyletic lineages forming a clade with the cyrtaucheniids,
1265 diplurids, and microstigmatids (the "Nemesioidina" clade). Both microstigmatids and some
1266 "cyrtaucheniids" have been recovered as lineages embedded within Nemesiidae in the past
1267 (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Wheeler et al.
1268 2017). Goloboff (1993), suggesting that the some of the microstigmatid synapomorphies may be
1269 of neotenic nature (as proposed previously by Raven (1985) and Griswold (1985)), long ago
1270 foreshadowing the eventual synonymy of microstigmatids with the nemesiids, or alternatively,
1271 splintering Nemesiidae into multiple families. Similar alternatives were also proposed for
1272 Cyrtaucheniidae (*sensu* Raven (1985)) based on the position of *Acontius*, "*Cyrtauchenius*", and
1273 *Fufius* (Hedin and Bond 2006; Bond et al. 2012). Unfortunately, we cannot assess the position of
1274 *Acontius* and *Fufius* in this study. However, the sample representing "*Cyrtauchenius*" in Bond et
1275 al. (2012), in fact, corresponds to *Amblyocarenum*, later removed from its synonymy (Decae and
1276 Bosmans 2014). Although the phylogenetic placement of both genera could be conservatively
1277 interpreted as "embedded within Nemesiidae *sensu lato*, the placement of *Amblyocarenum* along
1278 with other genera likely has more severe taxonomic implications. *Amblyocarenum* is placed as a
1279 sole "cyrtaucheniid" within a monophyletic clade comprising the genus *Nemesia*, the type genus
1280 of the family. Interestingly, the affinities between *Amblyocarenum* and other nemesiids were also
1281 noted when the genus was removed from synonymy with *Cyrtauchenius* (Decae and Bosmans

2014). On the other hand, *Cyrtauchenius* forms a well-supported clade with two other cyrtaucheniid genera and is placed as sister to another monophyletic and morphologically distinguishable clade, the Dipluridae (*sensu* this study). The Dipluridae – Cyrtaucheniidae relationship and their placement within Nemesiidae was previously recovered in the pilot assessment of mygalomorph phylogeny with AHE data (Hamilton et al. 2016b), however, the sampling limitations of the study alluded to a different conclusion than presented in this paper. Those authors analyzed five Nemesiidae genera and, based on previous results (Hedin and Bond 2006; Bond et al. 2012), presumed *Fufius* also likely to be a nemesiid. Consequently, recovering "*Linothele*" (later identified as *Diplura*) embedded among the Nemesiidae, they understandably dismissed its placement within Dipluridae, and considered it as taxonomically misplaced nemesiid (Hamilton et al. 2016b). Given that many genera were transferred from Dipluridae to Nemesiidae in the past (Raven 1985), and the placement of some taxa remained unstable (Main 1969; Raven 1985; Main 1993), taxonomic misplacement of the sample thus seemed plausible. Arguably, the lack of broad geographic and taxonomic sampling across nemesiids, cyrtaucheniids, and diplurids, particularly considering the hypothesized polyphyly of the latter two (*sensu* Raven (1985)), would have significant impact on interpretation of the results and subsequent proposed taxonomic changes in terms of taxon/generic composition of each family. The situation is similar for Microstigmatidae, presumably another "candidate" nemesiid lineage (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012). As in our study, the sampling was limited to the genus *Microstigmata*, and the monophyly of the family was never assessed using a molecular framework (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; but see Goloboff 1993). The genus has been recovered in proximity to *Ixamatus* and another Australian nemesiid genus *Kiama* (Bond et al. 2012), which is congruent with our results. *Kiama* presents a

1305 unique combination of morphological features that, to some extent, overlaps with diagnostic
1306 characters for a handful of families, and as a result, the placement of the genus vacillated among
1307 Dipluridae (Main and Mascord 1969), Cyrtaucheniidae (Raven 1985) and Nemesiidae (Bond et
1308 al. 2012). Interestingly, both *Kiama* and *Microstigmata* possess a uniquely modified cuticle and
1309 share a similar tarsal organ morphology (Raven 1985; Bond and Opell 2002), however these
1310 similarities were dismissed as convergent or plesiomorphic by others (Raven 1985; Goloboff
1311 1993). The homology of these characters consequently needs to be reassessed in a framework
1312 using other lines of evidence other than just morphology (Bond et al. 2012; Hedin et al. 2018).
1313 The dilemma of whether to dissolve the Nemesiidae, or redefine the family boundaries to
1314 include microstigmatids and cyrtaucheniids has been repeatedly discussed in the literature
1315 (Goloboff 1993; Hedin and Bond 2006; Bond et al. 2012), with tendencies gravitating towards
1316 the later solution (e.g. (Bond et al. 2012); Fig. 1C in (Hedin et al. 2018)). However, there were
1317 several factors at play reinforcing the argument for the "inclusive" Nemesiidae. First and
1318 foremost, the limited taxon sampling in the previous analyses (Hedin and Bond 2006; Ayoub et
1319 al. 2007; Bond et al. 2012; Hamilton et al. 2016b) partially skewed the interpretation of the
1320 results. The polyphyly of the family Cyrtaucheniidae (*sensu* Raven (1985)), misplacement of
1321 *Cyrtauchenius* (see above) and lack of adequate representation of the diplurines (*sensu* Raven
1322 (1985)) presented major hurdles for accurately interpreting nemesiid relationships. Recovering
1323 *Microstigmata* or a few genera of an apparently polyphyletic family embedded within an
1324 enlarged "nemesiid" construct understandably supported the notion that a broad synonymization
1325 (lumping rather than splitting) might be the more stable and thus preferable taxonomic solution.
1326 Adding to the overall ambiguity of the results, large sections of the recovered trees often lacked
1327 support (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Wheeler et al. 2017),

adding uncertainty to the monophyly of the groups in question. We would suggest that our improved sampling and high support values brings long-needed insight and resolution to the "nemesiid" problem, which is essential for ultimately answering the question of whether or not the Nemesiidae should be fragmented into independent families or not. The family Nemesiidae (*sensu* Raven (1985)) has lacked a well-supported synapomorphy, a widely recognized fact (Goloboff 1993; Goloboff 1995; Bond et al. 2012; Harvey et al. 2018), since the family was first delimited (Raven 1985). On the other hand, some groups (e.g., subfamilies) currently recognized within the family, do possess diagnostic synapomorphic characters (Harvey et al. 2018), which is also the case for the diplurids and cyrtaucheniids (*sensu* this study) that were recovered as mutually exclusive lineages within the larger more inclusive "Nemesioidina" clade. We thus argue that the original concept of the family (*sensu* Raven (1985)) must be abandoned in favor of establishing multiple monophyletic families that can also be ultimately clearly defined morphologically. Another fact in favor of such an arrangement is the relatively deep divergences separating the lineages (Kuntner et al. 2018). Major lineages across the entire clade diversified into their respective individual groups between 116 – 83 Ma, roughly congruent with dated divergences among other families (e.g., Theraphosidae and Barychelidae split around 106 Ma and Actinopodidae diverged from Atracidae approximately 101 Ma). In agreement with the results of our analyses, we therefore relimit the family Nemesiidae (new circumscription) as outlined below.

Family Nemesiidae (new circumscription).—The family Nemesiidae is a cosmopolitan group. Our sampling comprised five genera, including the type genus *Nemesia*, spanning species sampled from three continents (Africa, Europe and North America). These genera form a single lineage and are placed in a clade that is sister to all the former "nemesiid" lineages plus

Cyrtaucheniidae, Dipluridae and Microstigmatidae. In agreement with our results, we transfer the genus *Spiroctenus* to the newly established family Bemmeridae and transfer *Amblyocarenum* here from Cyrtaucheniidae (see discussion on Cyrtaucheniidae for justification). We remove the genera *Pionothele*, *Bayana*, *Stenoterommata*, *Stanwellia*, *Acanthogonatus, Pycnothele* and *Hermacha* and transfer them into newly erected families Pycnothelidae (NEW RANK) and Hermachidae (NEW FAMILY). We further remove the taxa included in the Anamini tribe (Harvey et al. 2018) and elevate it to the rank of family: Anamidae (NEW RANK). We also transfer the genera *Kiama, Angka,* and *Xamiatus* to the family Microstigmatidae (see details below). We acknowledge that many taxa, particularly those inhabiting South America and Asia, remain severely under-sampled in our study and therefore we cannot speak to their definitive placement at this time; as such they remain for time being assigned to Nemesiidae *incertae sedis*.

Family Anamidae (NEW RANK).—The family Anamidae corresponds to the Australian endemic tribe Anamini (Raven 1985; Harvey et al. 2018) formerly placed within the "nemesiid" subfamily Anaminae (Raven 1985). The family comprises genera *Aname*, *Chenistonia*, *Hesperonatalius*, *Kwonkan*, *Namea*, *Proshermacha*, *Teyl*, *Teyloides* and *Swolnpes*, of which we analyzed six (including *Pseudoteyl* currently synonymized with *Teyl*). The family formed a monophyletic lineage in all analyses and was recovered as sister to Hermachidae/Microstigmatidae clade. The phylogenetic relationships of the group were recently evaluated, employing both extensive taxonomic sampling and multilocus data (Harvey et al. 2018). Harvey et al. (2018) performed a complete generic sampling of the Australian Anamini and recovered the group as a well-supported clade, also including genera not sampled in our analyses. Following the results of our study and Harvey et al. (2018), we therefore transfer all the genera listed above into the family Anamidae. Morphology and biogeography do not support the

inclusion of *Longistylus* Indicatti and Lucas, 2005 in the Anamidae contra the tentative hypothesis proposed by Indicatti and Lucas (2005).

Family Hermachidae (NEW FAMILY).—We establish the family Hermachidae to accommodate the predominantly South African genus *Hermacha*, which currently comprises over 20 species. The genus was originally placed within the nemesiid subfamily Anaminae (Raven 1985), however this taxon has been shown to be polyphyletic (Goloboff 1993; Hedin and Bond 2006; Bond et al. 2012; Wheeler et al. 2017). Despite the rather undesirable monogeneric state of the family, we believe that establishing Hermachidae as a family is justified by both its reciprocal monophyly in relation to its sister taxon Microstigmatidae and the deep divergence between them, dating back to 83 Ma. We believe the monogeneric nature of the family is only temporary and other genera (particularly fellow South African genera *Lepthercus* and *Entypesa*) will eventually be transferred here following a thorough assessment of Nemesiidae phylogeny.

Family Microstigmatidae (new circumscription).—The family Microstigmatidae is well defined by morphological characters, such as small, round booklung openings and pustulose cuticle (Raven 1985; Hedin and Bond 2006). The monophyly of the family has never been assessed within a robust molecular framework because all previous studies exclusively included the South African genus *Microstigmata* (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Wheeler et al. 2017); but see (Goloboff 1993). Following the results of our analyses we relimit the family Microstigmatidae to include the genus *Kiama*. The morphological similarities in tarsal organ and cuticle structure linking *Kiama* to microstigmatids are well documented (Raven 1985; Bond and Opell 2002). Interestingly, similar characteristics are also found in the genera *Ixamatus* and *Angka* (Bond et al. 2012) suggesting their close relationships to the microstigmatids.

Family Pycnothelidae (NEW RANK).—The family Pycnothelidae is newly established here in order to accommodate taxa formerly belonging to a diverse mix of "nemesiid" subfamilies (Raven 1985). Some of the close relationships are not entirely novel. For example the sister relationship between *Stanwellia* and *Acanthogonatus* was previously recovered by Hedin and Bond (2006). Interestingly, we recovered the African genus *Pionothele*, formerly placed within the subfamily Bemerinnae, as sister to *Spiroctenus*, and sister to all pycnothelid diversity. The family distribution spans across Africa, Australia, South America and New Zealand, therefore it could be expected that more genera will be eventually transferred here following a thorough assessment, particularly of African and South American lineages.

CONCLUSIONS AND FUTURE DIRECTIONS

To some extent the results reported here serve to further emphasize that the current system for mygalomorph classification requires major restructuring. However, it is our hope that this study represents a significant leap forward in stabilizing the group's taxonomy via a robust, well-supported phylogenetic framework. Extensive taxon sampling combined with enhanced phylogenetic resolution via genomic-scale data facilitates the proposal of an alternative classification scheme that more accurately reflects the evolutionary relationships of the infraorder. Consequently, these results brought to their logical fruition require extensive changes to existing familial composition as well as the establishment of a number of new family-rank taxa. Understandably, such changes require considerable downstream scrutiny and criticism. Like Raven's (1985) classification, our newly derived framework is a hypothesis that begs further testing through additional data and increased taxon sampling.

One observation in particular from this study that seems clear is that morphological data are not uniformly suitable for reconstructing mygalomorph higher-level relationships, and to a slightly lesser degree for delimiting family rank taxa (e.g., subfamily relationships seem to be relatively stable as evidenced by the number of subfamilies elevated to family rank). Although Raven's (1985) task was heroic given its scope and lack of computational resources, the results were in no small part afflicted by the rampant homoplasy that seemingly plagues this group of spiders. It is important to acknowledge that some of Raven's hypotheses (1985) were only "wrong" in the narrow sense - discrepancies between computational analyses of genomic data and the earlier morphological work are simply a function of the temporal context. Given the data and lack of computational resources available when the analyses were conducted, the results Raven achieved were the strongest hypothesis at that time and provided a remarkably solid foundation upon which to deliver and test new hypotheses. In 1985 and shortly thereafter (Eskov and Zonshtein 1990) disagreement regarding morphological evidence for various clades and familial-level relationships and identity were in retrospect matters of subjective opinion and consequently difficult to test (likely explaining why some seemingly correct hypotheses, e.g., Atypoidea, were ignored). Alternatively though, it is important to recognize that a relatively large percentage of the new family rank taxa were once morphologically defined subfamilies – a number of which predate Raven (1985) – and thus relatively correct (i.e., morphology seemed to get it "right" some percentage of the time at a particular hierarchical level).

Issues regarding spider classification notwithstanding, phylogenomics provides an exciting new framework for considering broad scale questions related to spider evolution. Our biogeographic and dating analyses suggest that ancient vicariance and dispersal shaped the currently observed biogeographic patterns. Much of the deep phylogenetic structure across the

infraorder may be attributable to climatic heterogeneity and oscillation prior to the breakup of Gondwana/Pangea. Such deep phylogenetic and ancient divergence, coupled with low vagility, makes these spiders ideal candidates to test biogeographic hypotheses.

Furthermore, the recently held notion that most mygalomorph spiders capture prey from non-foraging burrow has critical implications for how we might understand and interpret silk across spiders as a whole (Fernández et al. 2018). Our results suggest that spiders likely foraged primitively from a silken sheet web that served classically-held web functions like increasing predator speed and enhancing prey capture success. How mygalomorph spiders build their webs and retreats, employ silk in prey capture, and the nature of their spinning apparatus remains a rich area of study that has received little attention since Coyle's (1986) treatment.

Considerable work remains in achieving final resolution of mygalomorph relationships and stable fully-resolved familial composition. Despite our best efforts, which included a combination of transcriptomic and AHE data, we are still unable to confidently resolve several of the deepest nodes; Paratropididae remains a difficult taxon to place as do the newly formed Stasimopidae. Following major relimitation, many nemesiid, diplurid, and cyrtaucheniid genera remain in an uncertain taxonomic state. Although we could speculate about placement of many taxa (e.g., Aporoptychinae cyrtaucheniids, Masteriinae diplurids, as well as numerous South American nemesiids), such questions will have to be left to future studies. At the very least, it is our hope that this study facilitates further interest and provides a solid foundation for future work in this remarkable, and ancient group of spiders.

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Figure 4. Phylogenetic tree inferred after removal of Paratropididae terminals ("No_Paratropis" dataset). Tree topology obtained in maximum likelihood (ML) analyses conducted in RAxML. Boxes near the branches denote full bootstrap support values; numbers report bootstrap support values obtained in this analysis (black) and the analysis of the full "All_taxa" dataset (red). Asterisks mark differences in topology obtained from the "All_taxa" dataset.

Figure 5. Phylogenetic tree inferred from combined AHE loci and transcriptomic data ("DNAAA" matrix). Tree topology obtained in maximum likelihood (ML) analyses conducted in RAxML. Asterisk marks placement of *Paratropis* within Crassitarsae. Boxes near the branches denote bootstrap support values; colors correspond to distinct support level categories depicted on left.

Figure 6. Comparison of resulting tree topologies obtained by concatenated analyses conducted in RAxML and ExaBayes (left) and species tree approach conducted in ASTRAL (right). Asterisks mark differences in topologies. Doted grey line denote array of lineages forming a highly supported clade in species tree analysis. Boxes on nodes denote support values obtained in each approach, color coded according to distinct support level categories depicted in bottom left corner in following order (left to right): RAxML bootstrap support, ExaBayes Bayesian posterior probabilities, ASTRAL support values.

Figure 7. Divergence time estimates inferred by treePL on a topology obtained in RAxML. The x-axis is time in million years. Geologic time scale abbreviations: N: Neogene, Pg: Paleogene, K: Cretaceous, J: Jurassic, Tr: Triassic, P: Permian, C: Carboniferous, D: Devonian. Light green block marks time frame of Gondwana breakup. Symbols on the tree nodes denote vicariant or dispersal events hypothesized for the divergences (left to right): inverted triangle=Pangea breakup, star=East-West Gondwana breakup, triangle=West Gondwana breakup, dot=Laurasia breakup, asterisk=long distance dispersal. Left corner: Map showing the position of the continents (a) prior to Gondwana breakup (adapted from (Will and Frimmel 2018), thick black lines mark the zones of continental drifting, times indicate the initiation of drifting in each zone;

(b) present day. Terminal tree taxa are color coded according to geographic areas of their sampling locations depicted in the map as follows: yellow=South America, orange=Africa, purple=Asia, red=Australia, light blue=North America, dark blue=Europe, lime green=Antarctica (no taxa assigned).

Figure 8. Ancestral areas of distribution inferred with DEC analyses implemented in RASP. Tree topology obtained in ExaBayes. Terminal tree taxa are color coded according to the biogeographic regions of their sampling locations depicted in the map (bottom left) as follows: A=North America, B=Europe, C=Asia, D=South America, E=Africa, F=Australia. Color coding of the inferred ancestral distributions corresponds to the assigned biogeographic regions, or combination of thereof depicted in the combined area legend on the left. Biogeographic events marked on the nodes: Di=dispersal, V=vicariance, Ex=extinction; black letters: events inferred with probability > 0.7.

Figure 9. Ancestral state reconstruction of mygalomorph foraging constructs. Preferred ancestral state reconstruction of foraging type using an equal rates model in the R-package *corHMM* with cursorial hunting taxa treated as inapplicable (?); tree was modified as ultrametric; AICc = 173.9083. Terminal tree taxa are color coded according the type of spinning structure depicted in the legend panel of the left.

Figure 10. Cladogram summarizing the phylogenetic relationships of mygalomorph families recovered in this paper. The topology corresponds to the arrangement obtained in the concatenated analyses (RAxML, ExaBayes). The crossed boxes on the branches represent

unsupported relationships: left maximum likelihood bootstrap support < 70%, right Bayesian inference Posterior Probability< 0.95,). Filled box=node supported only in Bayesian inference, otherwise relationships supported in both analyses.

2191

2192 Table 1. Taxon counts obtained from the World Spider Catalog (2018), undescribed and undetermined genera marked with *.

2193

Taxon	# genera	# species	# genera sampled in this study
Actinopodidae	3	69	1
Antrodiaetidae	2	35	2
Atracidae	3	35	2
Atypidae	3	54	3
Barychelidae	42	295	3
Ctenizidae	3	54	3
Cyrtaucheniidae	11	109	4 + 1*
Dipluridae	26	199	11
Euctenizidae	7	76	7 + 1*
Hexathelidae	7	45	1
Halonoproctidae	6	84	5
Idiopidae	22	374	9

Macrothelidae	1	29	1
Mecicobothriidae	4	9	1
Microstigmatidae	7	17	1
Migidae	11	97	4
Nemesiidae	45	420	18
Paratropididae	4	11	1
Porrhothelidae	1	5	1
Theraphosidae	146	989	7 + 1*
Mygalomorphae	354	3006	88

2196 Table 2. Maximum likelihood topology test results: obs, the observed log-likelihood difference to the best unconstraint topology (Fig.
2197 3), AU, p-value of the approximately unbiased test conducted in CONSEL; np, bootstrap probability calculated from the multiscale
2198 bootstrap; significant results (P<0.05) *
2199
2200

Family/Grouping	Constraint hypothesis	Included taxa	obs	AU	np
Ctenizidae	Ctenizidae_h1	*Conothele, Cteniza, Cyclocosmia, Cyrtocarenum, Hebestatis, Latouchia, Stasimopus, Ummidia*	2049.1	2e-05*	3e-05
	Ctenizidae_h2	*Conothele, Cteniza, Cyclocosmia, Cyrtocarenum, Hebestatis, Latouchia, Ummidia*	874.9	3e-44*	4e-16
	Ctenizidae_h3	*Cteniza, Cyrtocarenum, Stasimopus*	1906.6	6e-05*	1e-06
Cyrtaucheniidae	Cyrtaucheniidae_h1	*Amblyocarenum, Ancylotrypa, Cyrtaucheniidae sp., Cyrtauchenius, Homostola*	15966.1	3e-40*	6e-15
	Cyrtaucheniidae_h2	*Amblyocarenum, Ancylotrypa, Cyrtauchenius*	3487.7	6e-05*	2e-05
Dipluridae	Dipluridae_h1	*Allothele, Australothele, Cethegus, Diplura, Euagrus, Harmonicon, Ischnothele, Linothele, Microhexura, Namirea, Thelechoris*	7064.5	2e-07*	2e-07
	Dipluridae_h2	*Allothele, Australothele, Cethegus, Euagrus, Ischnothele, Microhexura, Namirea, Thelechoris*	156.8	0.007*	0.006

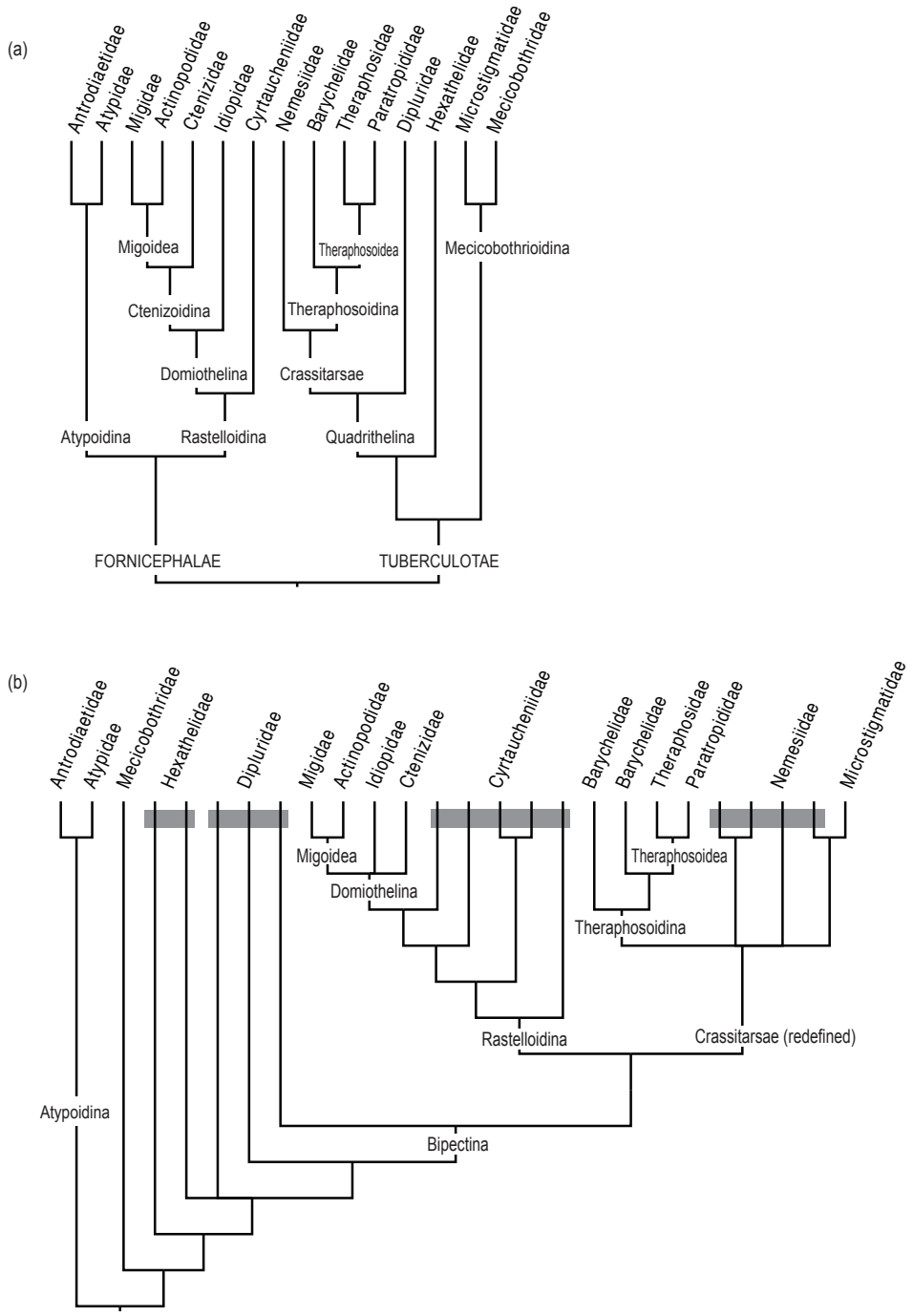
	Dipluridae_h3	*Bymainiella, Allothele, Australothele, Cethegus, Euagrus, Ischnothele, Microhexura, Namirea, Thelechoris*	157.0	0.006*	0.006
	Dipluridae_h4_astral	*Porrhothele, Bymainiella, Allothele, Australothele, Cethegus, Euagrus, Ischnothele, Microhexura, Namirea, Thelechoris*	157.1	0.007*	0.006
Hexathelidae	Hexathelidae_h1	*Atrax, Bymainiella, Hadronyche, Macrothele, Porrhothele*	4719.6	1e-58*	1e-17
	Hexathelidae_h2	*Bymainiella, Macrothele, Porrhothele*	1281.5	8e-43*	6e-16
	Hexathelidae_h3	*Macrothele, Porrhothele*	523.1	1e-40*	6e-15
Nemesiidae	Nemesiidae_h1	*Acanthogonatus, Aname, Bayana, Calisoga, Chenistonia, Hermacha, Iberesia, Ixamatus, Kiama, Kwonkan, Mexentypesa, Namea, Nemesia, Pionothele, Pseudoteyl, Spiroctenus, Stanwellia, Stenoterommata, Teyl*	18386.8	3e-73*	6e-20

2203 Table 3. Divergence time estimates obtained by treePL algorithm (Smith and O'Meara 2012) representing taxa origins (left) and the
2204 respective timeframe of their diversification (right) inferred for tree topology depicted in Fig. 7.
2205

Taxon origin	Age (Ma)	Crown Group diversification	Age (Ma)
Mygalomorphae/Mesothelae (Liphistiidae)	398	Antrodiaetidae + *Hexura*	65 – 34
Atypoidea/Avicularioidea	316	Atypidae	98 – 8
Ischnothelidae	240	Ischnothelidae	101
Microhexuridae	216	Euagridae	170 – 41
Hexathelidae/ Euagridae	189	Macrothelidae	36 – 23
Porrhothelidae	187	Atracidae	48
Macrothelidae/Bipectina	173	*Actinopus*	11
Paratropididae	167	Halonoproctidae	130 – 4
Crassitarsae	160	Migidae	94 – 74
Stasimopidae	153	Idiopidae	103 – 19
Domiothelina	158	Ctenizidae	82
Atypidae/ Antrodiaetidae + *Hexura*	146	Euctenizidae	127 – 34

Halonoproctidae	146	Bemmeridae	46 – 12
Migidae	143	Barychelidae	86 – 28
Idiopidae	137	Theraphosidae	78 – 9
Bemmeridae	136	Nemesiidae	104 – 6
Ctenizidae/ Euctenizidae	127	Pycnothelidae	80 – 28
Nemesiidae	116	Dipluridae	51 – 39
Barychelidae/ Theraphosidae	107	Cyrtaucheniidae	61 – 1
Atracidae/ Actinopodidae	101	Hermachidae	26
Pycnothelidae	103	Microstigmatidae	70
Dipluridae/ Cyrtaucheniidae	92	Anamidae	40 – 5
Anamidae	89		
Hermachidae/Microstigmatidae	83		

2206



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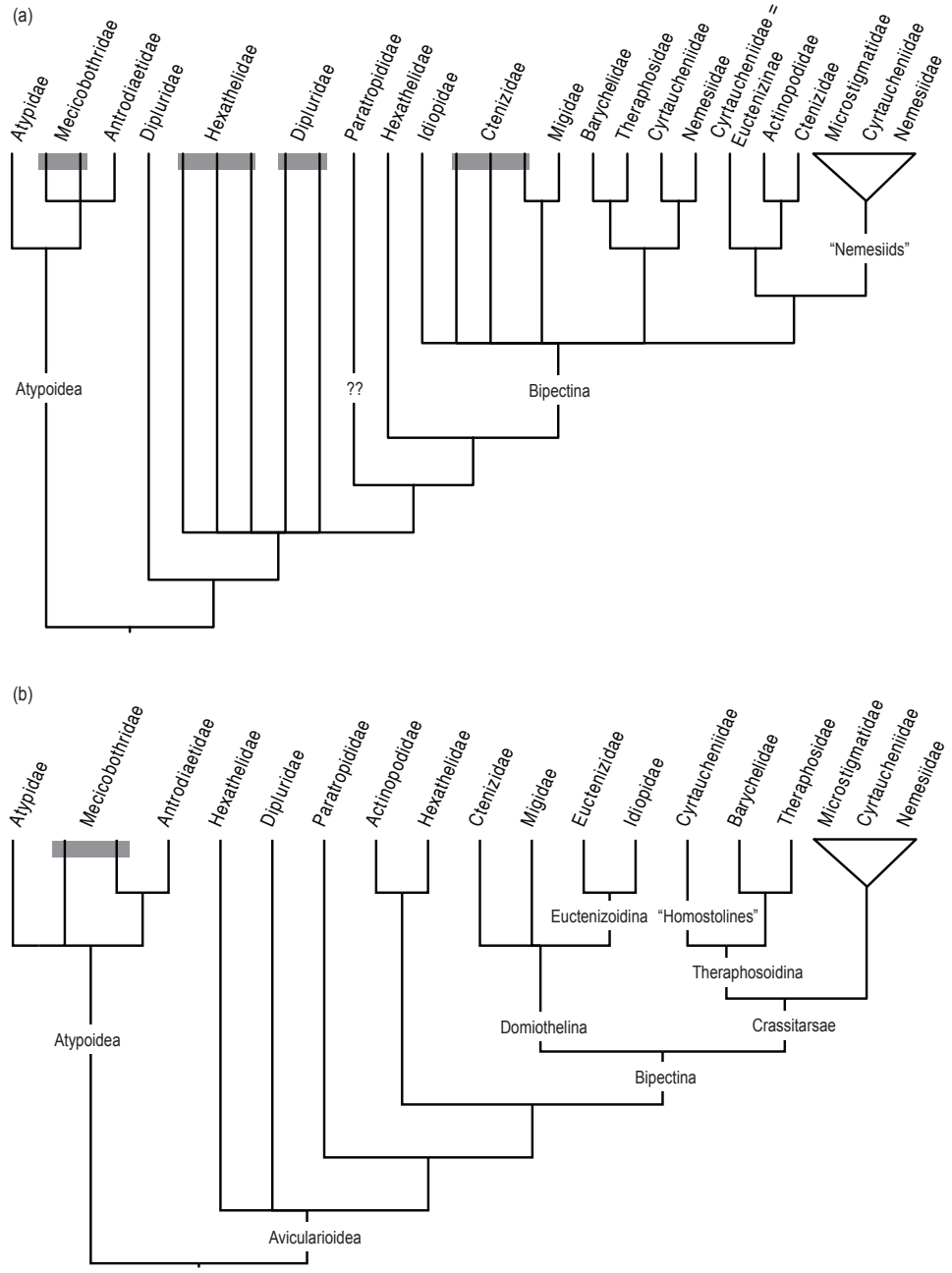
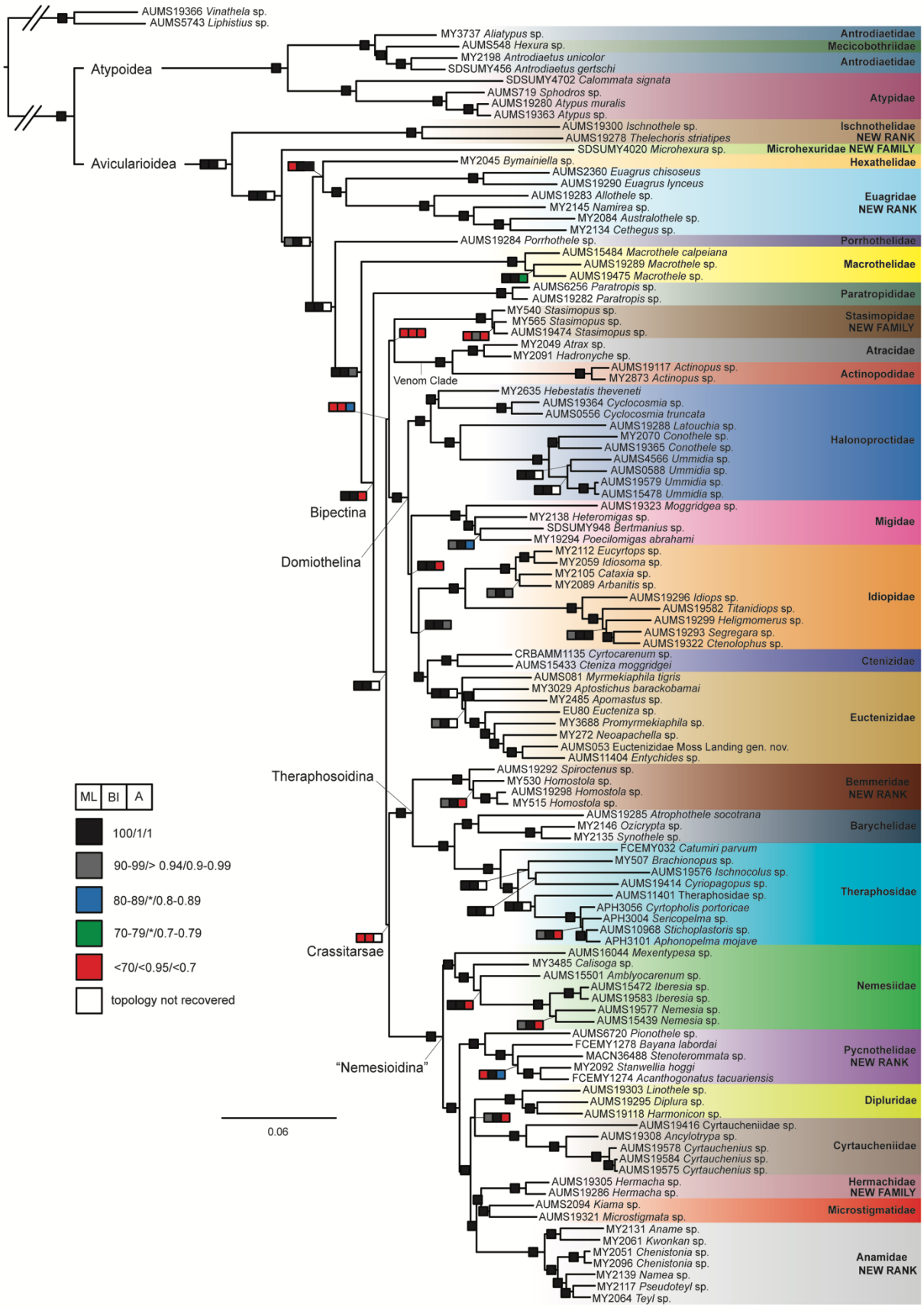


Figure 2. Phylogenetic relationships of mygalomorph families: (a) inferred from ribosomal data (*18S*, *28S*) in the first molecular analyses of the infraorder (Hedin and Bond 2006), (b) inferred in the "total evidence" approach combining three loci (*18S*, *28S*, *EF-1g*) and morphological data (Bond et al. 2012). Poly- and paraphyletic lineages are indicated by grey boxes.


AUMS19366 Vinathela sp.
AUMS5743 Liphistius sp.
Atypoidea
Avicularioidea
MY3737 Aliatypus sp.
AUMS548 Hexura sp.
MY2198 Antrodiaetus unicolor
SDSUMY456 Antrodiaetus gertschi
SDSUMY4702 Calommata signata
AUMS719 Sphodros sp.
AUMS19280 Atypus muralis
AUMS19363 Atypus sp.
AUMS19300 Ischnothele sp.
AUMS19278 Thelechoris striatipes
SDSUMY4020 Microhexura sp.
MY2045 Bymainiella sp.
AUMS2360 Euagrus chisoseus
AUMS19290 Euagrus lynceus
AUMS19283 Allothele sp.
MY2145 Namirea sp.
MY2084 Australothele sp.
MY2134 Cethegus sp.
AUMS19284 Porrhothele sp.
AUMS15484 Macrothele calpeiana
AUMS19289 Macrothele sp.
AUMS19475 Macrothele sp.
AUMS6256 Paratropis sp.
AUMS19282 Paratropis sp.
MY540 Stasimopus sp.
MY565 Stasimopus sp.
AUMS19474 Stasimopus sp.
MY2049 Atrax sp.
MY2091 Hadronyche sp.
AUMS19117 Actinopus sp.
MY2873 Actinopus sp.
Venom Clade
MY2635 Hebestatis theveneti
AUMS19364 Cyclocosmia sp.
AUMS0556 Cyclocosmia truncata
AUMS19288 Latouchia sp.
MY2070 Conothele sp.
AUMS19365 Conothele sp.
AUMS4566 Ummidia sp.
AUMS0588 Ummidia sp.
AUMS19579 Ummidia sp.
AUMS15478 Ummidia sp.
Bipectina
AUMS19323 Moggridgea sp.
MY2138 Heteromigas sp.
SDSUMY948 Bertmanius sp.
MY19294 Poecilomigas abrahami
Domiothelina
MY2112 Eucyrtops sp.
MY2059 Idiosoma sp.
MY2105 Cataxia sp.
MY2089 Arbanitis sp.
AUMS19296 Idiops sp.
AUMS19582 Titanidiops sp.
AUMS19299 Heligmomerus sp.
AUMS19293 Segregara sp.
AUMS19322 Ctenolophus sp.
CRBAMM1135 Cyrtocarenum sp.
AUMS15433 Cteniza moggridgei
AUMS081 Myrmekiaphila tigris
MY3029 Aptostichus barackobamai
MY2485 Apomastus sp.
EU80 Eucteniza sp.
MY3688 Promyrmekiaphila sp.
MY272 Neoapachella sp.
AUMS053 Euctenizidae Moss Landing gen. nov.
AUMS11404 Entychides sp.
Theraphosoidina
AUMS19292 Spiroctenus sp.
MY530 Homostola sp.
AUMS19298 Homostola sp.
MY515 Homostola sp.
AUMS19285 Atrophothele socotrana
MY2146 Ozicrypta sp.
MY2135 Synothele sp.
FCEMY032 Catumiri parvum
MY507 Brachionopus sp.
AUMS19576 Ischnocolus sp.
AUMS19414 Cyriopagopus sp.
AUMS11401 Theraphosidae sp.
APH3056 Cyrtopholis portoricae
APH3004 Sericopelma sp.
AUMS10968 Stichoplastoris sp.
APH3101 Aphonopelma mojave
Crassitarsae
AUMS16044 Mexentypesa sp.
MY3485 Calisoga sp.
AUMS15501 Amblyocarenum sp.
AUMS15472 Iberesia sp.
AUMS19583 Iberesia sp.
AUMS19577 Nemesia sp.
AUMS15439 Nemesia sp.
AUMS6720 Pionothele sp.
FCEMY1278 Bayana labordai
MACN36488 Stenoterommata sp.
MY2092 Stanwellia hoggi
FCEMY1274 Acanthogonatus tacuariensis
"Nemesioidina"
AUMS19303 Linothele sp.
AUMS19295 Diplura sp.
AUMS19118 Harmonicon sp.
AUMS19416 Cyrtaucheniidae sp.
AUMS19308 Ancylotrypa sp.
AUMS19578 Cyrtauchenius sp.
AUMS19584 Cyrtauchenius sp.
AUMS19575 Cyrtauchenius sp.
AUMS19305 Hermacha sp.
AUMS19286 Hermacha sp.
AUMS2094 Kiama sp.
AUMS19321 Microstigmata sp.
MY2131 Aname sp.
MY2061 Kwonkan sp.
MY2051 Chenistonia sp.
MY2096 Chenistonia sp.
MY2139 Namea sp.
MY2117 Pseudoteyl sp.
MY2064 Teyl sp.
Antrodiaetidae
Mecicobothriidae
Antrodiaetidae
Atypidae
Ischnothelidae NEW RANK
Microhexuridae NEW FAMILY
Hexathelidae
Euagridae NEW RANK
Porrhothelidae
Macrothelidae
Paratropididae
Stasimopidae NEW FAMILY
Atracidae
Actinopodidae
Halonoproctidae
Migidae
Idiopidae
Ctenizidae
Euctenizidae
Bemmeridae NEW RANK
Barychelidae
Theraphosidae
Nemesiidae
Pycnothelidae NEW RANK
Dipluridae
Cyrtaucheniidae
Hermachidae NEW FAMILY
Microstigmatidae
Anamidae NEW RANK
ML
BI
A
100/1/1
90-99/> 0.94/0.9-0.99
80-89/*/0.8-0.89
70-79/*/0.7-0.79
<70/<0.95/<0.7
topology not recovered
0.06

2217

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2219 Topology obtained in the Maximum Likelihood analyses. Boxes on nodes denote support values
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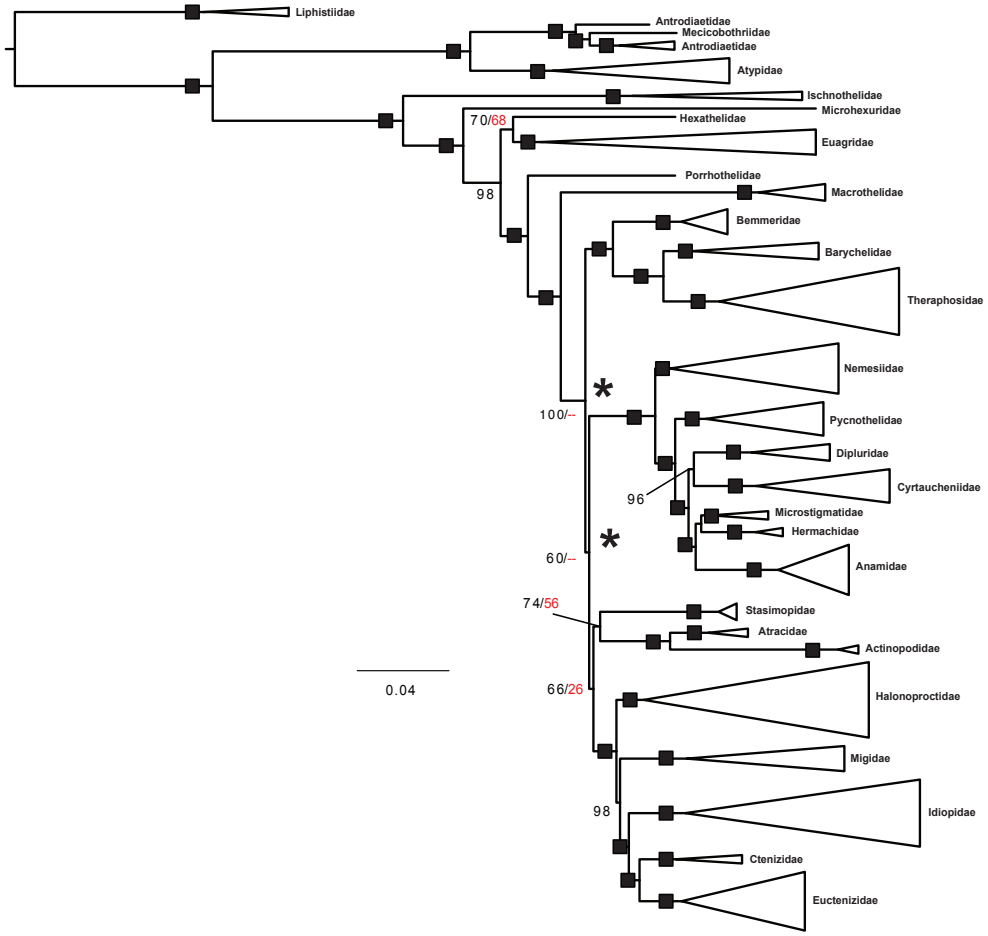
2224



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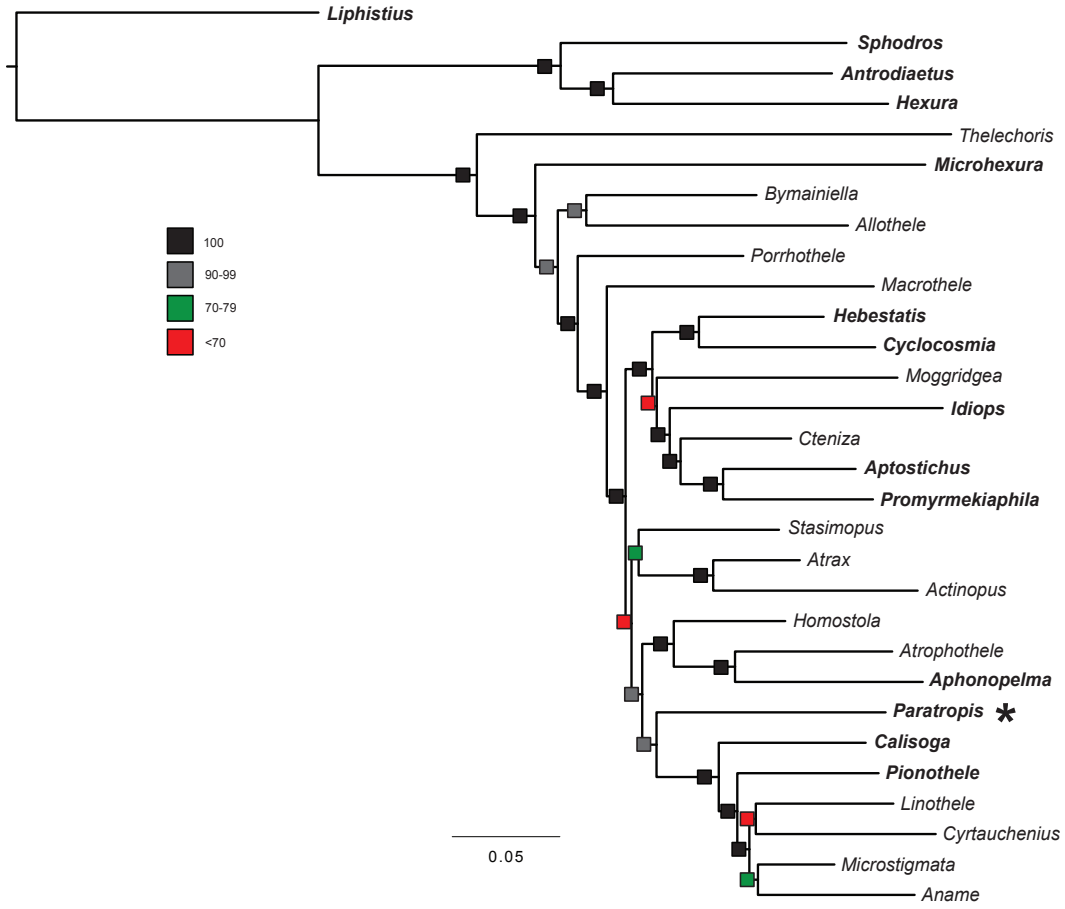

2234

2235

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2240

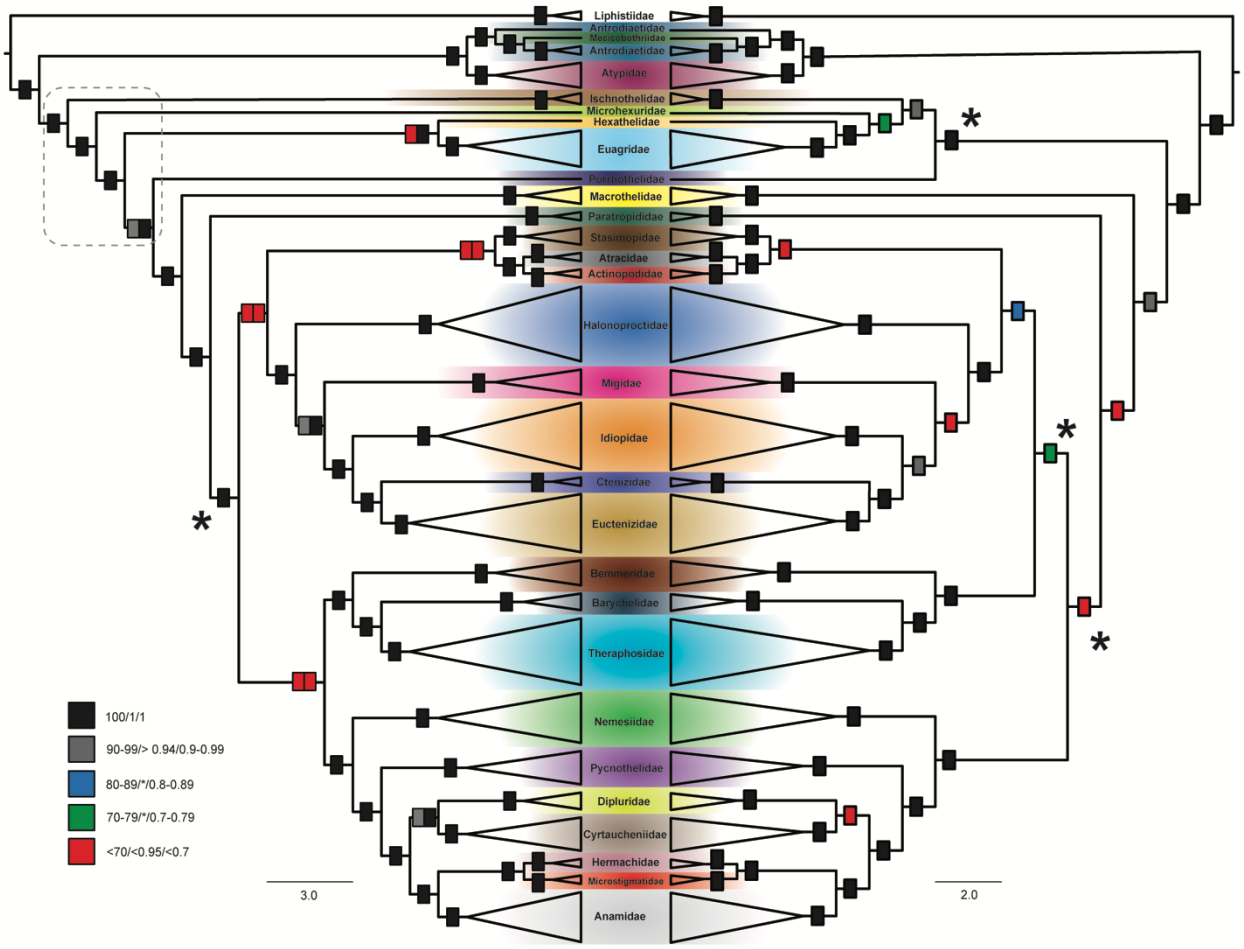
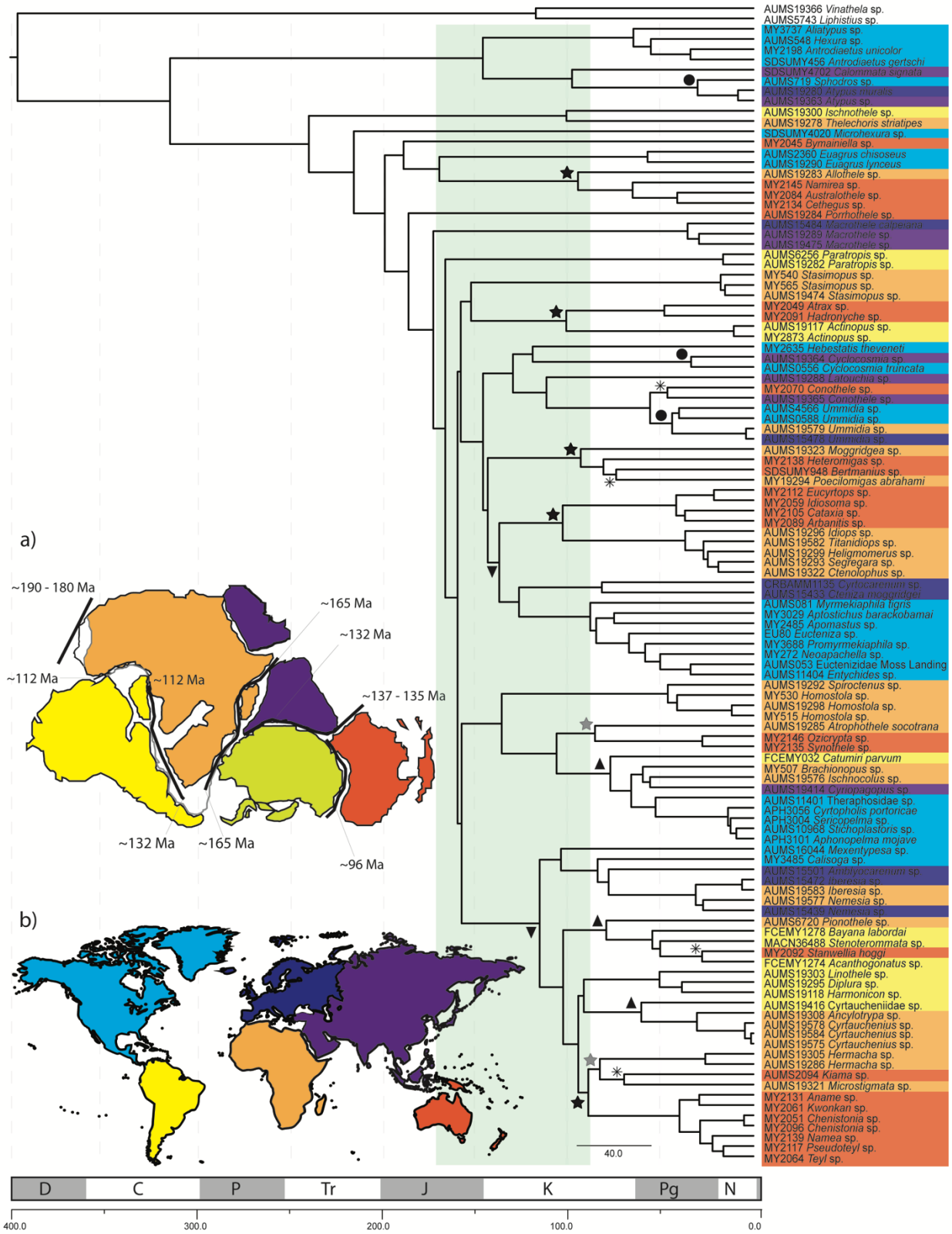
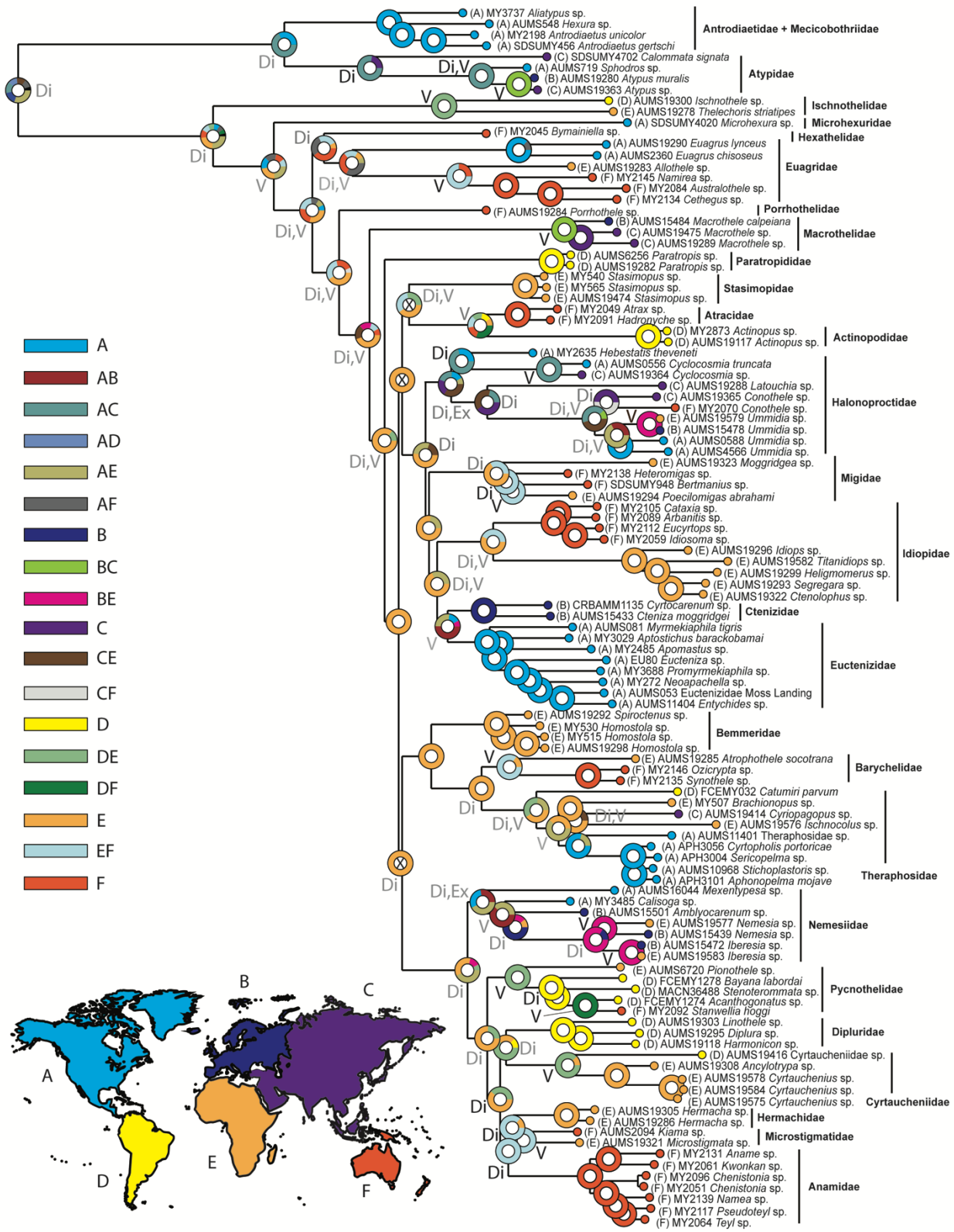


Figure 6. Comparison of resulting tree topologies obtained by concatenated analyses conducted in RAxML and ExaBayes (left) and species tree approach conducted in ASTRAL (right). Asterisks mark differences in topologies. Doted grey line denote array of lineages forming a highly supported clade in species tree analysis. Boxes on nodes denote support values obtained in each approach, color coded according to distinct support level categories depicted in bottom left corner in following order (left to right): RAxML bootstrap support, ExaBayes Bayesian posterior probabilities, ASTRAL support values.


AUMS19366 Vinathela sp.
AUMS5743 Liphistius sp.
MY3737 Allatypus sp.
AUMS548 Hexura sp.
MY2198 Antrodiaetus unicolor
SDSUMY456 Antrodiaetus gertschi
SDSUMY4702 Calommata signata
AUMS719 Sphodros sp.
AUMS19280 Atypus muralis
AUMS19363 Atypus sp.
AUMS19300 Ischnothele sp.
AUMS19278 Thelechoris striatipes
SDSUMY4020 Microhexura sp.
MY2045 Bymainiella sp.
AUMS2360 Euagrus chisoseus
AUMS19290 Euagrus lynceus
AUMS19283 Allothele sp.
MY2145 Namirea sp.
MY2084 Australothele sp.
MY2134 Cethegus sp.
AUMS19284 Porrhothele sp.
AUMS15484 Macrothele calpeiana
AUMS19289 Macrothele sp.
AUMS19476 Macrothele sp.
AUMS6256 Paratropis sp.
AUMS19282 Paratropis sp.
MY540 Stasimopus sp.
MY565 Stasimopus sp.
AUMS19474 Stasimopus sp.
MY2049 Atrax sp.
MY2091 Hadronyche sp.
AUMS19117 Actinopus sp.
MY2873 Actinopus sp.
MY2635 Hebestatis theveneti
AUMS19364 Cyclocosmia sp.
AUMS0556 Cyclocosmia truncata
AUMS19288 Latouchia sp.
MY2070 Conothele sp.
AUMS19365 Conothele sp.
AUMS4566 Ummidia sp.
AUMS0588 Ummidia sp.
AUMS19579 Ummidia sp.
AUMS15478 Ummidia sp.
AUMS19323 Moggridgea sp.
MY2138 Heteromigas sp.
SDSUMY948 Bertmanius sp.
MY19294 Poecilomigas abrahami
MY2112 Eucyrtops sp.
MY2059 Idiosoma sp.
MY2105 Cataxia sp.
MY2089 Arbanitis sp.
AUMS19296 Idiops sp.
AUMS19582 Titanidiops sp.
AUMS19299 Heligmomerus sp.
AUMS19293 Segregara sp.
AUMS19322 Ctenolophus sp.
CRBAMM1135 Cyrtocarenum sp.
AUMS15433 Ctenizza moggridgei
AUMS081 Myrmekiaphila tignis
MY3029 Aptostichus barackobamai
MY2485 Apomastus sp.
EU80 Euctenizza sp.
MY3688 Promyrmekiaphila sp.
MY272 Neoapachella sp.
AUMS053 Euctenizidae Moss Landing
AUMS11404 Entychides sp.
AUMS19292 Spiroctenus sp.
MY530 Homostola sp.
AUMS19298 Homostola sp.
MY515 Homostola sp.
AUMS19285 Atrophothele socotrana
MY2146 Ozicrypta sp.
MY2135 Synothele sp.
FCEMY032 Catumiri parvum
MY507 Brachionopus sp.
AUMS19576 Ischnocolus sp.
AUMS19414 Cyriopagopus sp.
AUMS11401 Theraphosidae sp.
APH3056 Cyrtopholis portoricae
APH3004 Sericopelma sp.
AUMS10968 Stichoplastoris sp.
APH3101 Aphonopelma mojave
AUMS16044 Mexentypesa sp.
MY3485 Calisoga sp.
AUMS15501 Ambylocarenum sp.
AUMS15472 Iberesia sp.
AUMS19583 Iberesia sp.
AUMS19577 Nemesia sp.
AUMS15429 Nemesia sp.
AUMS6720 Pionothele sp.
FCEMY1278 Bayana labordai
MACN36488 Stenoterommata sp.
MY2092 Stanwellia hoggi
FCEMY1274 Acanthogonatus sp.
AUMS19303 Linothele sp.
AUMS19295 Diplura sp.
AUMS19118 Harmonicon sp.
AUMS19416 Cyrtaucheniidae sp.
AUMS19308 Ancylotrypa sp.
AUMS19578 Cyrtauchenius sp.
AUMS19584 Cyrtauchenius sp.
AUMS19575 Cyrtauchenius sp.
AUMS19305 Hermacha sp.
AUMS19286 Hermacha sp.
AUMS2094 Kiama sp.
AUMS19321 Microstigmata sp.
MY2131 Aname sp.
MY2061 Kwonkan sp.
MY2051 Chenistonia sp.
MY2096 Chenistonia sp.
MY2139 Namea sp.
MY2117 Pseudoteyl sp.
MY2064 Teyl sp.
a)
~190 - 180 Ma
~165 Ma
~132 Ma
~112 Ma
~112 Ma
~137 - 135 Ma
~132 Ma
~165 Ma
~96 Ma
b)
40.0
D
C
P
Tr
J
K
Pg
N
400.0
300.0
200.0
100.0
0.0

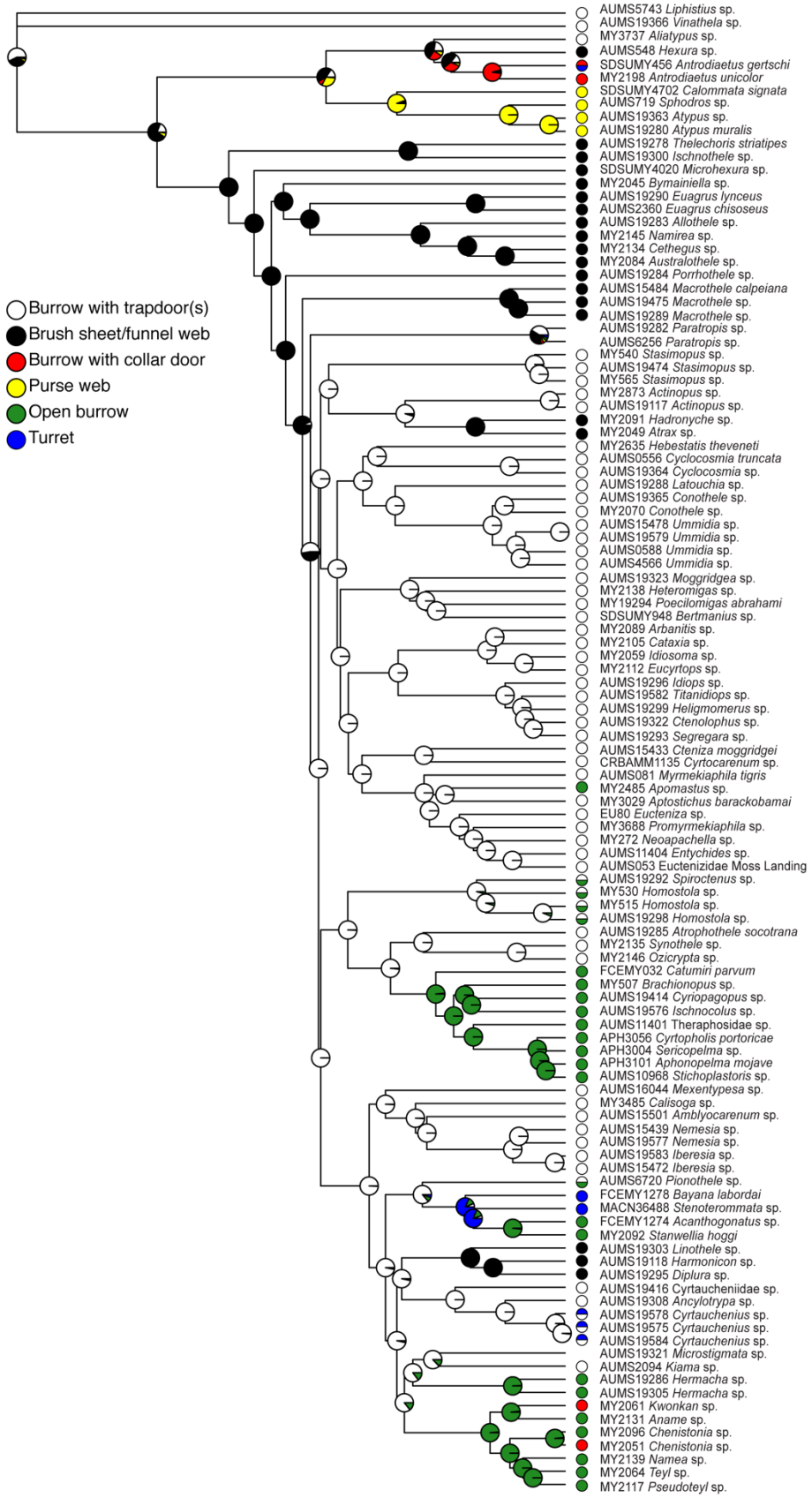
2250

Figure 7. Divergence time estimates inferred by treePL on a topology obtained in RAxML. The x-axis is time in million years. Geologic time scale abbreviations: N: Neogene, Pg: Paleogene, K: Cretaceous, J: Jurassic, Tr: Triassic, P: Permian, C: Carboniferous, D: Devonian. Light green block marks time frame of Gondwana breakup. Symbols on the tree nodes denote vicariant or dispersal events hypothesized for the divergences (left to right): inverted triangle=Pangea breakup, star=East-West Gondwana breakup, triangle=West Gondwana breakup, dot=Laurasia breakup, asterisk=long distance dispersal. Left corner: Map showing the position of the continents (a) prior to Gondwana breakup (adapted from (Will and Frimmel 2018), thick black lines mark the zones of continental drifting, times indicate the initiation of drifting in each zone; (b) present day. Terminal tree taxa are color coded according to geographic areas of their sampling locations depicted in the map as follows: yellow=South America, orange=Africa, purple=Asia, red=Australia, light blue=North America, dark blue=Europe, lime green=Antarctica (no taxa assigned).


(A) MY3737 Aliatypus sp.
(A) AUMS548 Hexura sp.
(A) MY2198 Antrodiaetus unicolor
(A) SDSUMY456 Antrodiaetus gertschi
Antrodiaetidae + Mecicobothriidae
(C) SDSUMY4702 Calommata signata
(A) AUMS719 Sphodros sp.
(B) AUMS19280 Atypus muralis
(C) AUMS19363 Atypus sp.
Atypidae
(D) AUMS19300 Ischnothele sp.
(E) AUMS19278 Thelechoris striatipes
Ischnothelidae
(A) SDSUMY4020 Microhexura sp.
Microhexuridae
(F) MY2045 Bymainiella sp.
Hexathelidae
(A) AUMS19290 Euagrus lynceus
(A) AUMS2360 Euagrus chisoseus
(E) AUMS19283 Allothele sp.
(F) MY2145 Namirea sp.
(F) MY2084 Australothele sp.
(F) MY2134 Cethegus sp.
Euagridae
(F) AUMS19284 Porrhothele sp.
Porrhothelidae
(B) AUMS15484 Macrothele calpeiana
(C) AUMS19475 Macrothele sp.
(C) AUMS19289 Macrothele sp.
Macrothelidae
(D) AUMS6256 Paratropis sp.
(D) AUMS19282 Paratropis sp.
Paratropididae
(E) MY540 Stasimopus sp.
(E) MY565 Stasimopus sp.
(E) AUMS19474 Stasimopus sp.
Stasimopidae
(F) MY2049 Atrax sp.
(F) MY2091 Hadronyche sp.
Atracidae
(D) MY2873 Actinopus sp.
(D) AUMS19117 Actinopus sp.
Actinopodidae
(A) MY2635 Hebestatis theveneti
(A) AUMS0556 Cyclocosmia truncata
(C) AUMS19364 Cyclocosmia sp.
(C) AUMS19288 Latouchia sp.
(C) AUMS19365 Conothele sp.
(F) MY2070 Conothele sp.
(E) AUMS19579 Ummidia sp.
(B) AUMS15478 Ummidia sp.
(A) AUMS0588 Ummidia sp.
(A) AUMS4566 Ummidia sp.
Halonoproctidae
(E) AUMS19323 Moggridgea sp.
(F) MY2138 Heteromigas sp.
(F) SDSUMY948 Bertmanius sp.
(E) AUMS19294 Poecilomigas abrahami
Migidae
(F) MY2105 Cataxia sp.
(F) MY2089 Arbanitis sp.
(F) MY2112 Eucyrtops sp.
(F) MY2059 Idiosoma sp.
(E) AUMS19296 Idiops sp.
(E) AUMS19582 Titanidiops sp.
(E) AUMS19299 Heligmomerus sp.
(E) AUMS19293 Segregara sp.
(E) AUMS19322 Ctenolophus sp.
Idiopidae
(B) CRBAMM1135 Cyrtocarenum sp.
(B) AUMS15433 Cteniza moggridgei
Ctenizidae
(A) AUMS081 Myrmekiaphila tigris
(A) MY3029 Aptostichus barackobamai
(A) MY2485 Apomastus sp.
(A) EU80 Eucteniza sp.
(A) MY3688 Promyrmekiaphila sp.
(A) MY272 Neoapachella sp.
(A) AUMS053 Euctenizidae Moss Landing
(A) AUMS11404 Entychides sp.
Euctenizidae
(E) AUMS19292 Spiroctenus sp.
(E) MY530 Homostola sp.
(E) MY515 Homostola sp.
(E) AUMS19298 Homostola sp.
Bemmeridae
(E) AUMS19285 Atrophothele socotrana
(F) MY2146 Ozicrypta sp.
(F) MY2135 Synothele sp.
Barychelidae
(D) FCEMY032 Catumiri parvum
(E) MY507 Brachionopus sp.
(C) AUMS19414 Cyriopagopus sp.
(E) AUMS19576 Ischnocolus sp.
(A) AUMS11401 Theraphosidae sp.
(A) APH3056 Cyrtopholis portoricae
(A) APH3004 Sericopelma sp.
(A) AUMS10968 Stichoplastoris sp.
(A) APH3101 Aphonopelma mojave
(A) AUMS16044 Mexentypesa sp.
Theraphosidae
(A) MY3485 Calisoga sp.
(B) AUMS15501 Amblyocarenum sp.
(E) AUMS19577 Nemesia sp.
(B) AUMS15439 Nemesia sp.
(B) AUMS15472 Iberesia sp.
(E) AUMS19583 Iberesia sp.
Nemesiidae
(E) AUMS6720 Pionothele sp.
(D) FCEMY1278 Bayana labordai
(D) MACN36488 Stenoterommata sp.
(D) FCEMY1274 Acanthogonatus sp.
(F) MY2092 Stanwellia hoggi
Pycnothelidae
(D) AUMS19303 Linothele sp.
(D) AUMS19295 Diplura sp.
(D) AUMS19118 Harmonicon sp.
Dipluridae
(D) AUMS19416 Cyrtaucheniidae sp.
(E) AUMS19308 Ancylotrypa sp.
(E) AUMS19578 Cyrtauchenius sp.
(E) AUMS19584 Cyrtauchenius sp.
(E) AUMS19575 Cyrtauchenius sp.
Cyrtaucheniidae
(E) AUMS19305 Hermacha sp.
(E) AUMS19286 Hermacha sp.
Hermachidae
(F) AUMS2094 Kiama sp.
(E) AUMS19321 Microstigmata sp.
Microstigmatidae
(F) MY2131 Aname sp.
(F) MY2061 Kwonkan sp.
(F) MY2096 Chenistonia sp.
(F) MY2051 Chenistonia sp.
(F) MY2139 Namea sp.
(F) MY2117 Pseudoteyl sp.
(F) MY2064 Teyl sp.
Anamidae
A
AB
AC
AD
AE
AF
B
BC
BE
C
CE
CF
D
DE
DF
E
EF
F
Di
Di,V
Di,Ex
V
A
B
C
D
E
F

2265

Figure 8. Ancestral areas of distribution inferred for mygalomorph spiders with DEC analyses implemented in RASP. Tree topology obtained in ExaBayes. Terminal tree taxa are color coded according to the biogeographic regions of their sampling locations depicted in the map (bottom left) as follows: A=North America, B=Europe, C=Asia, D=South America, E=Africa, F=Australia. Color coding of the inferred ancestral distributions corresponds to the assigned biogeographic regions, or combination of thereof depicted in the combined area legend on the left. Biogeographic events marked on the nodes: Di=dispersal, V=vicariance, Ex=extinction; black letters: events inferred with probability > 0.7.


Burrow with trapdoor(s)
Brush sheet/funnel web
Burrow with collar door
Purse web
Open burrow
Turret
AUMS5743 Liphistius sp.
AUMS19366 Vinathela sp.
MY3737 Aliatypus sp.
AUMS548 Hexura sp.
SDSUMY456 Antrodiaetus gertschi
MY2198 Antrodiaetus unicolor
SDSUMY4702 Calommata signata
AUMS719 Sphodros sp.
AUMS19363 Atypus sp.
AUMS19280 Atypus muralis
AUMS19278 Thelechoris striatipes
AUMS19300 Ischnothele sp.
SDSUMY4020 Microhexura sp.
MY2045 Bymainiella sp.
AUMS19290 Euagrus lynceus
AUMS2360 Euagrus chisoseus
AUMS19283 Allothele sp.
MY2145 Namirea sp.
MY2134 Cethegus sp.
MY2084 Australothele sp.
AUMS19284 Porrhothele sp.
AUMS15484 Macrothele calpeiana
AUMS19475 Macrothele sp.
AUMS19289 Macrothele sp.
AUMS19282 Paratropis sp.
AUMS6256 Paratropis sp.
MY540 Stasimopus sp.
AUMS19474 Stasimopus sp.
MY565 Stasimopus sp.
MY2873 Actinopus sp.
AUMS19117 Actinopus sp.
MY2091 Hadronyche sp.
MY2049 Atrax sp.
MY2635 Hebestatis theveneti
AUMS0556 Cyclocosmia truncata
AUMS19364 Cyclocosmia sp.
AUMS19288 Latouchia sp.
AUMS19365 Conothele sp.
MY2070 Conothele sp.
AUMS15478 Ummidia sp.
AUMS19579 Ummidia sp.
AUMS0588 Ummidia sp.
AUMS4566 Ummidia sp.
AUMS19323 Moggridgea sp.
MY2138 Heteromigas sp.
MY19294 Poecilomigas abrahami
SDSUMY948 Bertmanius sp.
MY2089 Arbanitis sp.
MY2105 Cataxia sp.
MY2059 Idiosoma sp.
MY2112 Eucyrtops sp.
AUMS19296 Idiops sp.
AUMS19582 Titanidiops sp.
AUMS19299 Heligmomerus sp.
AUMS19322 Ctenolophus sp.
AUMS19293 Segregara sp.
AUMS15433 Cteniza moggridgei
CRBAMM1135 Cyrtocarenum sp.
AUMS081 Myrmekiaphila tigris
MY2485 Apomastus sp.
MY3029 Aptostichus barackobamai
EU80 Eucteniza sp.
MY3688 Promyrmekiaphila sp.
MY272 Neoapachella sp.
AUMS11404 Entychides sp.
AUMS053 Euctenizidae Moss Landing
AUMS19292 Spiroctenus sp.
MY530 Homostola sp.
MY515 Homostola sp.
AUMS19298 Homostola sp.
AUMS19285 Atrophothele socotrana
MY2135 Synothele sp.
MY2146 Ozicrypta sp.
FCEMY032 Catumiri parvum
MY507 Brachionopus sp.
AUMS19414 Cyriopagopus sp.
AUMS19576 Ischnocolus sp.
AUMS11401 Theraphosidae sp.
APH3056 Cyrtopholis portoricae
APH3004 Sericopelma sp.
APH3101 Aphonopelma mojave
AUMS10968 Stichoplastoris sp.
AUMS16044 Mexentypesa sp.
MY3485 Calisoga sp.
AUMS15501 Amblyocarenum sp.
AUMS15439 Nemesia sp.
AUMS19577 Nemesia sp.
AUMS19583 Iberesia sp.
AUMS15472 Iberesia sp.
AUMS6720 Pionothele sp.
FCEMY1278 Bayana labordai
MACN36488 Stenoterommata sp.
FCEMY1274 Acanthogonatus sp.
MY2092 Stanwellia hoggi
AUMS19303 Linothele sp.
AUMS19118 Harmonicon sp.
AUMS19295 Diplura sp.
AUMS19416 Cyrtaucheniidae sp.
AUMS19308 Ancylotrypa sp.
AUMS19578 Cyrtauchenius sp.
AUMS19575 Cyrtauchenius sp.
AUMS19584 Cyrtauchenius sp.
AUMS19321 Microstigmata sp.
AUMS2094 Kiama sp.
AUMS19286 Hermacha sp.
AUMS19305 Hermacha sp.
MY2061 Kwonkan sp.
MY2131 Aname sp.
MY2096 Chenistonia sp.
MY2051 Chenistonia sp.
MY2139 Namea sp.
MY2064 Teyl sp.
MY2117 Pseudoteyl sp.

2274

Figure 9. Ancestral state reconstruction of mygalomorph foraging constructs. Preferred ancestral state reconstruction of foraging type using an equal rates model in the R-package *corHMM* with cursorial hunting taxa treated as inapplicable (?); tree was modified as ultrametric; AICc = 173.9083. Terminal tree taxa are color coded according the type of spinning structure depicted in the legend panel of the left.

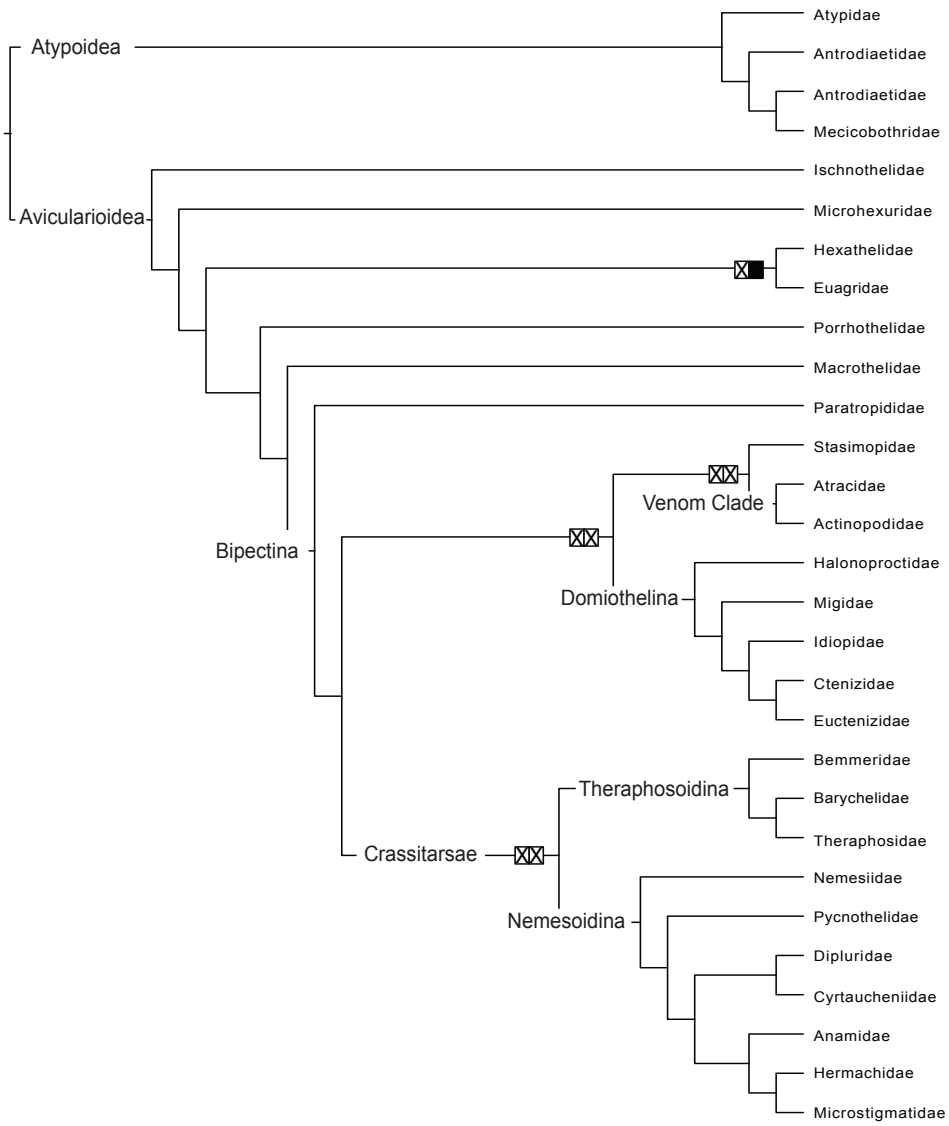


Figure 10. Cladogram summarizing the phylogenetic relationships of mygalomorph families recovered in this paper. The topology corresponds to the arrangement obtained in the concatenated analyses (RAxML, ExaBayes). The crossed boxes on the branches represent unsupported relationships: left maximum likelihood bootstrap support < 70%, right Bayesian inference Posterior Probability< 0.95,). Filled box=node supported only in Bayesian inference, otherwise relationships supported in both analyses.

APPENDIX 1: Revised Taxonomy for some of the families of the Spider Infraorder Mygalomorphae (Araneae)

SUPPLEMENTAL FILES:

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.[NNNN]

Tables and Figures

Table S1: Specimen locality data

Table S2: Specimens used in the combined analyses of genomic and transcriptomic data (DNAAA_matrix). AHE dataset: specimens sequenced in this study, AA dataset: data proceeding from Garrison et al. (2016) "BCC-75" matrix.

Table S3: Character coding for web type ancestral state reconstruction

Supplemental Figure 1. Tree topology obtained in maximum likelihood (ML) analyses conducted in RAxML. Values on the nodes correspond to bootstrap support.

Supplemental Figure 2. Tree topology obtained in Bayesian inference (BI) analyses conducted in ExaBayes. Values on the nodes correspond to the Bayesian posterior probability (PP).

Supplemental Figure 3. Tree topology obtained in species tree analysis conducted in ASTRAL. Values on the nodes correspond to the ASTRAL support values.

Supplemental Figure 4. Divergence time estimates of mygalomorph spiders inferred by treePL on a topology obtained in RAxML, values near the nodes correspond to the inferred timing of the divergences. The x-axis is time in million years.

Supplemental Figure 5. Phylogenetic tree of Mygalomorphae summarizing results from concatenated and species tree approaches within a framework of a current classification scheme (World Spider Catalog 2018). Topology obtained in the Maximum Likelihood analyses. Boxes on nodes denote support values obtained in each approach (left to right): RAxML bootstrap support, ExaBayes Bayesian posterior probabilities (PP), ASTRAL support values. Colour of the boxes corresponds to distinct support level categories depicted in bottom left corner. One filled box indicates full support in all analyses; white box=topology not recovered in species tree analysis.

Data Files

ASTRAL_all_taxa.tre

ASTRAL_all_taxa.trees

ExaBayes_all_taxa.tre

RASP_ingroup.tre_dis.csv

RAxML_DNAAA.nex

RAxML_DNAAA.tre

RAxML_DNAAA_partitions.txt

RAxML_ExaBayes_all_taxa.nex

RAxML_ExaBayes_all_taxa_partitions.txt

RAxML_No_Paratropis.nex

RAxML_No_Paratropis.tre

RAxML_No_Paratropis_partitions.txt

RAxML_all_taxa.tre

silkUseAncStateRecon.txt

treePL_dated.tre

Taxonomic Appendix: Revised Taxonomy for some of the families of the Spider Infraorder Mygalomorphae (Araneae)

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[2]*Department of Biology, San Diego State University, San Diego, CA, 92182–4614, USA*

Families remaining unchanged in their composition

Actinopodidae

Antrodiaetidae

Atracidae

Atypidae

Barychelidae

Euctenizidae

Halonoproctidae

Hexathelidae

Idiopidae

Macrothelidae

Mecicobothriidae

Migidae

Paratropididae

Porrhothelidae

Theraphosidae

**denotes genera included in analyses in the accompanying paper (Opatova et al. 2019); @ denotes that the taxon was definitively placed by another study supporting the designated placement here*

Relimitation of the family Dipluridae Simon, 1889

Family Dipluridae Simon, 1889 (new circumscription)

Type genus: *Diplura* C. L. Koch, 1850 (type species *Mygale macrura* Koch, 1841)

Remarks. The family Dipluridae is relimited here to include members of the subfamilies Diplurinae Simon, 1889 and Masteriinae Simon, 1889. Of the Diplurinae genera, *Trechona* C. L. Koch, 1850 and *Troglodiplura* Main, 1969 were not included in our analysis but are nevertheless retained, at least until such time that they are included in a molecular study; *Trechona's* inclusion appears justified based on stridulatory and palpal endite characters (Raven 1985), whereas Main (1993) justifies the placement of *Troglodiplura* in Diplurinae contra Raven (1985). The relimited family herein conservatively also includes Masteriinae which we predict is likely to be recognized as a standalone family, closely related to Microhexuridae at some point in the future; the subfamily was recently reviewed by Passanha and Brescovit (2018), however, the authors

provide no insight regarding affinities to other mygalomorph taxa. The relimited Dipluridae will require a significantly revised diagnosis at some point in the future.

List of included subfamilies and genera

Diplurinae Simon, 1889

**Diplura* C. L. Koch, 1850

**Harmonicon* F. O. Pickard-Cambridge, 1896

**Linothele* Karsch, 1879

Trechona C. L. Koch, 1850

Troglodiplura Main, 1969

Masteriinae Simon, 1889

Masteria C. L. Koch, 1873

Siremata Passanha and Brescovit, 2018

Striamea Raven, 1981

Family Ischnothelidae F.O.P Cambridge, 1897 NEW RANK

Type genus: *Ischnothele* Ausserer, 1875 (type species *Ischnothele caudata* Ausserer, 1875)

Remarks. Our sampling of ischonothelines comprised two genera that span the taxonomic breadth of the subfamily (Coyle 1995, Figures 21, 22) but omit three others. As discussed by

Coyle (1995) in his revision of the subfamily, the group is supported by a number of unique morphological characters that taken in combination can be used for diagnosis. Distinguishing characteristics for this newly elevated family are: 1) an elongate cymbial apophysis; 2) collariform trichobothrial bases; and 3) fused silk spigots.

List of included genera

**Ischnothele* Ausserer, 1875

Andethele Coyle, 1995

Indothele Coyle, 1995

Lathrothele Benoit, 1965

**Thelechoris* Karsch, 1881

Family Euagridae Raven, 1979 NEW RANK

Type genus: *Euagrus* Ausserer, 1875 (type species *Euagrus mexicanus* Ausserer, 1875)

Remarks and revised diagnosis. Unfortunately, our sampling of Euagrinae diplurids is depauperate (and should include other *Euagrus* species, see below) and thus our placement of the remaining diplurids in the subfamily Euagrinae follows authors subsequent to Raven's (1985) establishment of the family (i.e., inclusion in the subfamily is maintained per Raven (1991), Coyle (1986), Raven and Schwendinger (1995), and Ríos-Tamayo and Goloboff (2017)). Note that *Euagrus*, was considered by Coyle (1988) to be largely central/south American with the two

species, *Euagrus atropurpureus* (S. Africa) and *Euagrus formosanus* (Taiwan) as misplaced. It is important to recognize that some of these taxa may be allocated to other formerly "diplurid" families at some point in the future (e.g., *Chilehexops, Caledothele, Vilchura*). Although morphological evidence (*sensu* Coyle (1988)) seems strong that *Phyxioschema* is the sister group to *Euagrus*, the morphological cladistic analysis conducted by Ríos-Tamayo and Goloboff (2017) provides little encouragement that the subfamily is monophyletic and should indicate that any morphological diagnosis of this remaining assemblage of taxa is likely without substance. Following Raven (1985), the family can be diagnosed as those taxa having a distinct spur on the second male tibia and a centrally raised tarsal organ (also following from Ríos-Tamayo and Goloboff (2017)); Euagrinae taxa differ from australothelines by lacking an australotheline crescent (see details in the diagnosis below).

List of included Euagrinae genera

**Euagrus* Ausserer, 1875
Caledothele Raven, 1991
Chilehexops Coyle, 1986
Leptothele Raven and Schwendinger, 1995
Phyxioschema Simon, 1889
Vilchura Ríos-Tamayo and Goloboff, 2017

Australothelinae NEW SUBFAMILY Bond, Hedin, and Opatova

Type genus: ***Australothele*** Raven, 1984 (type species *Australothele maculata* Raven, 1984)

Diagnosis: Per Raven (1985), australothelines can be diagnosed from other euagrids as those taxa having an "australotheline crescent" on the posterior median spinnerets – which comprises a "crescent of hirsute cuticle isolated by pallid glabrous anterior to bases" and lacking cymbial spines (the latter character is shared by numerous other mygalomorph taxa (mentioned by Ríos-Tamayo and Goloboff (2017)). As noted by Raven (1985), a similar such crescent is also found in the putatively true diplurid genus *Masteria*. The subfamily is also supported as monophyletic in the analysis conducted by Ríos-Tamayo and Goloboff (2017) and Hedin et al. (2018).

List of genera included

**Australothele* Raven, 1984
**Allothele* Tucker, 1920
Carrai Raven, 1984
**Cethegus* Thorell, 1881
**Namirea* Raven, 1984
Stenygrocercus Simon, 1892

Microhexuridae NEW FAMILY Bond, Hedin, and Opatova

Type genus. ***Microhexura*** Crosby and Bishop, 1925 (type species *Microhexura montivaga* Crosby and Bishop, 1925)

Diagnosis: Because the family is monogeneric, characters used to diagnose Microhexuridae are those that can be attributed to the genus by Coyle (1981). Among the smallest mygalomorph spiders known, the family is diagnosed on the basis of the following unique combination of characters: 1) longitudinal fovea; 2) four spinnerets – posterior lateral spinnerets long (nearly the length of the abdomen); 3) lacking abdominal tergites; and 4) male pedipalp lacking conductor.

List of included genera

**Microhexura* Crosby and Bishop, 1925

Relimitation of the family Ctenizidae Thorell, 1887

Ctenizidae Thorell, 1887 (new circumscription)

Type genus: ***Cteniza*** Latreille, 1829 (type species *Cteniza sauvagesi* (Rossi, 1788))

Remarks and revised diagnosis. As discussed by others (e.g., Decae 1996; Godwin et al. 2018), *Cteniza* and *Cyrtocarenum* are virtually indistinguishable from each other and are very similar to other taxa like halonoproctids and stasimopids, save some questionable eye group characteristics. As outlined by Godwin et al. (2018), Ctenizidae, as delimited herein, can be distinguished from other similar taxa by lacking curved spines on the anterior legs, having a more arched and rounded caput and flatter ventral posterior carapace, and having a more oblong abdomen (rather

than ovoid); legs tend to be less stocky. Ctenizids differ from their sister group, Euctenizidae, by lacking scopulae.

List of included genera

**Cteniza* Latreille, 1829

**Cyrtocarenum* Ausserer, 1871

Stasimopidae NEW FAMILY Bond, Hedin, and Opatova

Type genus: *Stasimopus* Simon, 1892 (type species *Stasimopus caffrus* (C. L. Koch, 1842)

Diagnosis: At this time, the family Stasimopidae is monogeneric, thus characteristics used to diagnose the genus are the distinguishing characters for the family. Per Raven (1985) and summarized by Engelbrecht and Prendini (2012), stasimopids can be distinguished from other ctenizid-like taxa on the basis of the following combination of unique characters: 1) lacking a saddle-like depression on tibia III; 2) having a very wide eye group (twice as wide as it is long); and 3) palpal endites with a modified anterior lobe (described as "produced"). Based on the breadth of morphological diversity contained within the genus, which includes remarkable and somewhat bizarre carapace modifications (Engelbrecht and Prendini 2012), it is possible that *Stasimopus* could be split in to multiple genera at some point in the future.

List of included genera

**Stasimopus* Simon, 1892

Relimitation of the family Cyrtaucheniidae Simon, 1889

Cyrtaucheniidae Simon, 1889 (new circumscription)

Type genus: *Cyrtauchenius* Thorell, 1869 (type species *Cyrtauchenius terricola* (Lucas, 1846))

Remarks. Long recognized as a polyphyletic assemblage of genera, we herein relimit Cyrtaucheniidae to the Aporoptychinae minus *Kiama* and *Angka* based on Raven's (1985) original composition of the subfamily. Given the diversity of the taxa left remaining in the family, it is unlikely that a revised diagnosis will have any lasting value (if even possible to formulate, given the diverse array of taxa remaining) and as such we decline to provide one. We anticipate significant changes to the composition of the family as additional aporoptychines are added to the tree. We justify our transfer of *Angka* to Microstigmatidae based on the original description of the genus by Raven and Schwendinger (1995), where they considered it as a clear sister taxon to *Kiama* (also transferred, by us below, to the family Microstigmatidae); the transfer of *Amblyocarenum* to Nemesiidae is justified on the basis of its phylogenetic position.

List of included genera

**Cyrtauchenius* Thorell, 1869

**Ancylotrypa* Simon, 1889

Incertae sedis

Acontius Karsch, 1879

Anemesia Pocock, 1895

Bolostromoides Schiapelli and Gerschman, 1945

Bolostromus Ausserer, 1875

Fufius Simon, 1888

Rhytidicolus Simon, 1889

Transferred to other families (not included in the relimitation of Cyrtaucheniidae)

**Amblyocarenum* Simon, 1892 here transferred to Nemesiidae justified on the basis of phylogenetic placement.

[@]*Angka* Raven and Schwendinger, 1995 here transferred to Microstigmatidae on the basis of its putative sister relationship to *Kiama.*

**Homostola* Simon, 1892 here transferred to Bemmeridae justified on the basis of phylogenetic placement.

Relimitation of the family Nemesiidae Simon, 1889

Nemesiidae Simon, 1889 (new circumscription)

Type genus: ***Nemesia*** Audouin, 1826 (type species *Nemesia cellicola* Audouin, 1826)

Remarks. To a greater extent than Cyrtaucheniidae (above), what remains of the family Nemesiidae is a likely para-polyphyletic assemblage of genera. As such we consider the placement of a large number of genera in the family to be uncertain at best; many of which may prove to be pycnothelids. Consequently, much work remains, particularly if we are able to confidently place many of the South American genera.

List of included genera

**Nemesia* Audouin, 1826

**Amblyocarenum* Simon, 1892

**Calisoga* Chamberlin, 1937

**Iberesia* Decae and Cardoso, 2006

**Mexentypesa* Raven, 1987

Incertae sedis

Atmetochilus Simon, 1887

Brachythele Ausserer, 1871

Chaco Tullgren, 1905

Chilelopsis Goloboff, 1995 – most likely Pycnothelidae

Damarchilus Siliwal, Molur, and Raven, 2015

Damarchus Thorell, 1891

Diplothelopsis Tullgren, 1905

Entypesa Simon, 1902 – likely to be transferred to Hermachidae

Flamencopsis Goloboff, 1995

Gravelyia Mirza and Mondal, 2018

Hermachura Mello-Leitão, 1923

Lepthercus Purcell, 1902 – likely Hermachidae

Longistylus Indicatti and Lucas, 2005; removed from Anamidae *contra* Indicatti and Lucas (2005)

Lycinus Thorell, 1894

Neostothis Vellard, 1925

Prorachias Mello-Leitão, 1924

Psalistopoides Mello-Leitão, 1934

Pselligmus Simon, 1892

Rachias Simon, 1892; likely to be transferred to Pycnothelidae per Goloboff (1995)

Raveniola Zonstein, 1987

Sinopesa Raven and Schwendinger, 1995

Transferred to other families (not included in the relimitation of Nemesiidae)

Xamiatus Raven, 1981; here transferred to Microstigmatidae on the basis of its putative sister/close relationship with *Ixamatus* and *Kiama* (Raven 1981,1985)

Ixamatus Simon, 1887 here transferred to Microstigmatidae on the basis of its putative phylogenetic position per Harvey et al. (2018).

Anamidae Simon, 1889 NEW RANK

Type genus: ***Aname*** L. Koch, 1873 (type species *Aname pallida* L. Koch, 1873)

Remarks. A detailed diagnosis for this new family rank taxon is provided by Harvey et al. (2018).

List of included genera

**Aname* L. Koch, 1873

**Chenistonia* Hogg, 1901

@*Hesperonatalius* Castalanelli et al. 2017 per Harvey et al. (2018)

Kwonkan* Main, 1983 (=Yilgarnia* Main, 1986)

**Namea* Raven, 1984

@*Proshermacha* Simon 1908 per Harvey et al. (2018)

@*Swolnpes* Main and Framenau, 2009 per Harvey et al. (2018)

**Teyl* Main, 1975

@*Teyloides* Main, 1985 but check Bond et al. (2012) and Hedin and Bond (2006) – may be able to place elsewhere) Harvey et al. (2018)

Bemmeridae Simon, 1903 NEW RANK

Type genus: ***Spiroctenus*** Simon, 1892 (type species *Spiroctenus personatus,* Simon, 1888)

Remarks and Diagnosis. The family name is derived from the available family-level taxon Bemmereae Simon, 1903; *Bemmeris* Simon, 1903 is a junior synonym of *Spiroctenus*. The identity and consequently family-level placement of *Spiroctenus* and *Homostola* is contentious and likely to remain so. Raven (1985) considered the two genera unrelated and placed in separate families (Nemesiidae and Cyrtaucheniidae respectively), attributing Hewitt's (1915) hypothesized synonym of the two to a misidentification. To date, it would seem that a male *Homostola* specimen has never been described in detail, further complicating the situation (but see Hewitt (1916)). Based on careful specimen identification and locality data, we are confident that our sample comprises both genera. The *Spiroctenus* exemplar represents an identical male and female sequence of *Spiroctenus flavopunctatus* (Purcell, 1903); *Homostola* terminals are likely *H. pardalina affin.* (Hewitt, 1913) and *H. vulpecula* Simon, 1892. Based on our examination of specimens, Bemmeridae females can be diagnosed on the basis of the following unique combination of characters: 1) eyes on a low tubercle; 2) metatarsus and tarsus with moderate to thick scopula; 3) preening combs on leg III; 4) labium with relatively dense patch of cuspules; and 5) a rastellum comprising a row of thickened, blunt spines not borne on an

apophysis. Male *Spiroctenus* species have a very distinct mating clasper on leg I comprising a number of large spines and an apophysis at the tibia/metatarsus junction; we would predict a similar morphology for *Homostola* males.

List of included genera

**Homostola* Simon, 1892

**Spiroctenus* Simon, 1889

Pycnothelidae Chamberlin, 1917 NEW RANK

Type genus: *Pycnothele* Chamberlin, 1917 (type species *Pycnothele perdita* Chamberlin, 1917)

Remarks and Diagnosis: The genus *Pycnothele* was not included in our analysis but hypothesized morphological affinities (Goloboff 1995), have attributed it to be closely aligned with *Stanwellia*, *Acanthogonatus*, and *Stenoterommata*. Harvey et al. (2018) stated: "If a close relationship between *Stanwellia*, *Acanthogonatus*, *Stenoterommata* and *Pycnothele* is maintained, as proposed by Goloboff (1995), the subfamily-group name Pycnothelinae Chamberlin, 1917 is available for this clade (Chamberlin 1917)." In the interests of taxonomic stability, we believe the conservative decision is to transfer *Pycnothele* from Nemesiidae and include it in our newly defined clade (justified on the basis of morphological similarity). The alternative would be to propose a new family rank name (e.g., "Stanwellidae") which will be inevitably deprecated at a later time. At this time, the family Pycnothelidae can be diagnosed on

the basis of the following unique combination of characters: 1) rastellum absent; 2) aspinose tarsi; 3) preening combs absent, although reduced in some *Stanwellia* and *Acanthogonatus*; 4) labium lacks cuspules; 5) tibial spur on male leg I absent; and 6) most taxa have a reduced or absent inferior tarsal claw. We anticipate that this diagnosis might change as other nemesiids (noted as *incertae sedis* above) are added to the tree.

List of included genera

@*Pycnothele* Chamberlin, 1917

**Stanwellia* Rainbow and Pulleine, 1918

**Acanthogonatus* Karsch, 1880 (note: most likely polyphyletic)

**Bayana* Pérez-Miles, Costa and Montes de Oca, 2014

**Pionothele* Purcell, 1902

**Stenoterommata* Holmberg, 1881

Hermachidae NEW FAMILY Bond, Hedin, and Opatova

Type genus: *Hermacha* Simon, 1889 (type species *Hermacha caudata* Simon, 1889)

Diagnosis: Because the family is monogeneric, characters used to distinguish it are those that can be attributed to diagnosing the genus. Based on examination of specimens, Raven's (1985) description and Dippenaar-Shoeman (2002), Hermachidae may be diagnosed on the basis of the following unique combination of characters: 1) narrow straight thoracic groove; 2) distinctly

raised eye tubercle; 3) labium lacks cuspules; 4) weak rastellum comprising numerous short, stout bristles; 5) dense scopula on tarsi I-III; 6) leg III preening comb, present; and 7) long, digitiform spinnerets. We anticipate the inclusion of *Lepthercus* and *Entypesa* in this family at some point in the future.

Included genus

**Hermacha* Simon, 1889

Relimitation of the family Microstigmatidae Roewer, 1942

Family Microstigmatidae Roewer, 1942 (new circumscription)

Type genus: *Microstigmata* Strand, 1932 (type species *Microstigmata geophila* (Hewitt, 1916))

Remarks: The family Microstigmatidae was traditionally diagnosed as taxa have possessing, circular booklung openings. With Goloboff's (1995) inclusion of *Pseudonemesia* and *Speloctenizа* in the family, as well as *Envia* (*sensu* Ott and Höfer (2003)), that feature now only serves to diagnose a subset of these relatively unique genera. Although a scaly or pustulose cuticle may be a diagnostic character uniting all microstigmatids (Bond and Opell 2002), future SEM study of all genera will be necessary to confirm. As noted above, the transfer of *Angka* to the Microstigmatidae is based on its affinities with *Kiama* whose phylogenetic position clearly aligns here.

List of included genera

**Microstigmata* Strand, 1932

@*Angka* Raven and Schwendinger, 1995

Envia Ott and Höfer, 2003

@*Ixamatus* Simon, 1887 transferred here per Harvey et al. 2018; as noted by Raven (1985), the genus has a pustulose cuticle, a potentially diagnostic character of the family.

**Kiama* Main and Mascord, 1969

Micromygale Platnick and Forster, 1982

Ministigmata Raven and Platnick, 1981

Pseudonemesia Caporiacco, 1955

Speloctenizа Gertsch, 1982

@*Xamiatus* Raven, 1981 transferred here on the basis of morphological similarity with *Ixamatus* and its putative sister group relationship with *Kiama*; as noted by Raven (1985), the genus has a pustulose cuticle, a potentially diagnostic character of the family.

Xenonemesia Goloboff, 1989