

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

3 **Vera Opatova<sup>1</sup>, Chris A. Hamilton<sup>2</sup>, Marshal Hedin<sup>3</sup>, Laura Montes de Oca<sup>4</sup>, Jiří Král<sup>5</sup>,**  
4 **Jason E. Bond<sup>1</sup>**

5

6 <sup>1</sup>*Department of Entomology and Nematology, University of California, Davis, CA 95616, USA*

7 <sup>2</sup>*Department of Entomology, Plant Pathology & Nematology, University of Idaho, Moscow, ID*  
8 *83844, USA*

9 <sup>3</sup>*Department of Biology, San Diego State University, San Diego, CA, 92182–4614, USA*

10 <sup>4</sup>*Departamento de Ecología y Biología Evolutiva, Instituto de Investigaciones Biológicas*  
11 *Clemente Estable, Montevideo, Uruguay.*

12 <sup>5</sup>*Department of Genetics and Microbiology, Faculty of Sciences, Charles University, Prague,*  
13 *128 44, Czech Republic*

14

15

16 Corresponding authors:

17 Vera Opatova, 1282 Academic Surge, One Shields Avenue, Davis, CA 95616

18 Telephone: +1 530-754-5805, E-mail: [vopatova@ucdavis.edu](mailto:vopatova@ucdavis.edu)

19

20 Jason E. Bond, 1282 Academic Surge, One Shields Avenue, Davis, CA 95616

21 Telephone: +1 530-754-5805, E-mail: [jbond@ucdavis.edu](mailto:jbond@ucdavis.edu)

22

23 Running Head: PHYLOGENY OF MYGALOMORPH SPIDERS

24

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

25 ABSTRACT

26

27 The Infraorder Mygalomorphae is one of the three main lineages of spiders comprising over  
28 3,000 nominal species. This ancient group has a world-wide distribution that includes among its  
29 ranks large and charismatic taxa such as tarantulas, trapdoor spiders, and highly venomous  
30 funnel web spiders. Based on past molecular studies using Sanger-sequencing approaches,  
31 numerous mygalomorph families (e.g., Hexathelidae, Ctenizidae, Cyrtaucheniidae, Dipluridae  
32 and Nemesiidae) have been identified as non-monophyletic. However, these data were unable to  
33 sufficiently resolve the higher-level (intra- and interfamilial) relationships such that the  
34 necessary changes in classification could be made with confidence. Here we present a  
35 comprehensive phylogenomic treatment of the spider infraorder Mygalomorphae. We employ  
36 472 loci obtained through Anchored Hybrid Enrichment to reconstruct relationships among all  
37 the mygalomorph spider families and estimate the timeframe of their diversification. We  
38 sampled all currently recognized families, which has allowed us to assess their status, and as a  
39 result, propose a new classification scheme. Our generic-level sampling has also provided an  
40 evolutionary framework for revisiting questions regarding silk use in mygalomorph spiders. The  
41 first such analysis for the group within a strict phylogenetic framework shows that a sheet web is  
42 likely the plesiomorphic condition for mygalomorphs, as well as providing hints to the ancestral  
43 foraging behavior for all spiders. Our divergence time estimates, concomitant with detailed  
44 biogeographic analysis, suggest that both ancient continental-level vicariance and more recent  
45 dispersal events have played an important role in shaping modern day distributional patterns.  
46 Based on our results, we relimit the generic composition of the Ctenizidae, Cyrtaucheniidae,  
47 Dipluridae and Nemesiidae. We also elevate five subfamilies to family rank: Anamidae (NEW

## PHYLOGENY OF MYGALOMORPH SPIDERS

48 RANK), Euagridae (NEW RANK), Ischnothelidae (NEW RANK), Pycnothelidae (NEW  
49 RANK), and Bemmeridae (NEW RANK). The three families Entypesidae (NEW FAMILY),  
50 Microhexuridae (NEW FAMILY), and Stasimopidae (NEW FAMILY) are newly proposed.  
51 Such a major rearrangement in classification, recognizing eight newly established family-level  
52 rank taxa, is the largest the group has seen in over three decades since Raven's (1985) taxonomic  
53 treatment.

54

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

56

57       Spiders placed in the infraorder Mygalomorphae are a charismatic assemblage of taxa  
58 that includes among its ranks the tarantulas, trapdoor spiders, and also some of the most  
59 venomous species, like the Sydney funnel web spider and its close relatives (Hedin et al. 2018).  
60 Today, the infraorder comprises 20 families, divided into 354 genera, with 3,006 species (World  
61 Spider Catalog 2018; Table 1). The group is ancient, known to occur in the fossil record since  
62 the Middle Triassic (Selden and Gall 1992), but with origins estimated further back into the  
63 Carboniferous over 300 million years ago (Ayoub et al. 2007; Starrett et al. 2013; Garrison et al.  
64 2016). Their ancient origins and concomitant sedentary nature provide a rich biogeographical  
65 context for studying geological-scale continental vicariant events (Raven 1980; Hedin and Bond  
66 2006; Opatova et al. 2013; Opatova and Arnedo 2014a). Not surprisingly for such an ancient  
67 lineage, mygalomorph spider morphology is complicated; they have retained a number of  
68 features considered primitive for spiders, such as a simple silk spinning system, two pairs of  
69 book lungs and paraxial cheliceral arrangement, yet are also relatively homogenous when  
70 compared to the overall morphological diversity observed among their araneomorph counterparts

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

71 (Hendrixson and Bond 2009). Although such striking homogeneity presents significant  
72 challenges for morphology-based taxonomy, mygalomorphs have become a noteworthy system  
73 for studying allopatric speciation and species crypsis (Bond et al. 2001; Bond and Stockman  
74 2008; Satler et al. 2013; Hamilton et al. 2014; Opatova and Arnedo 2014b; Hedin et al. 2015;  
75 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. Post Raven (1985), a number of authors continued to explore morphological  
83 characters as evidence for broad scale familial-level relationships and identity (Eskov and  
84 Zonshtein 1990), further facilitated by the availability of computational phylogenetic inference  
85 (Goloboff 1993; Goloboff 1995; Bond and Opell 2002; Fig. 1b). These morphological studies  
86 were followed shortly thereafter by a number of early molecular treatments (Hedin and Bond  
87 2006; Ayoub et al. 2007; Fig. 2a). The first major taxonomic changes to Raven's classification  
88 followed in 2012 (Bond et al. 2012; Fig. 2b) with the elevation of the subfamily Euctenizinae to  
89 family status. A number of subsequent studies employing Sanger-sequencing based approaches  
90 continued to make progress in exploring relationships between closely related taxa (Hamilton et  
91 al. 2014; Opatova et al. 2016; Mora et al. 2017; Rix et al. 2017a; Ortiz et al. 2018; Starrett et al.  
92 2018) and within families (Opatova et al. 2013; Opatova and Arnedo 2014a; Harrison et al.  
93 2017; Rix et al. 2017c; Harvey et al. 2018; Lüddecke et al. 2018; Turner et al. 2018). Although

## PHYLOGENY OF MYGALOMORPH SPIDERS

94 Sanger approaches have been fruitful in terms of advancing our understanding of some  
95 relationships, it has been long recognized that ribosomal RNA genes (e.g., 28S and 18S),  
96 elongation factors, and mitochondrial genes, are often of limited utility in the context of deep  
97 scale divergences of mygalomorph spiders (Hedin and Bond 2006; Ayoub et al. 2007; Bond et  
98 al. 2012).

99       Within the last several years, the application of phylogenomic approaches has helped to  
100 transform spider systematics (Bond et al. 2014; Fernández et al. 2014; Garrison et al. 2016;  
101 Hamilton et al. 2016b; Hedin et al. 2018; Kuntner et al. 2018; Wood et al. 2018; Hedin et al.  
102 2019), by providing the data and impetus for making major changes in mygalomorph spider  
103 classification. Specifically, targeted capture approaches using anchored hybrid enrichment  
104 (AHE; Lemmon et al. 2012) and ultra-conserved elements (UCE; Faircloth et al. 2012) are now  
105 being applied to questions at multiple hierarchical levels across the spider tree of life (Hamilton  
106 et al. 2016a; Hamilton et al. 2016b; Maddison et al. 2017; Chamberland et al. 2018; Godwin et  
107 al. 2018; Hedin et al. 2018) and other arachnids (Starrett et al. 2017; Sharma et al. 2018).  
108 Implementation of genomic-based methods seemingly overcomes the perceived limitations of the  
109 traditional Sanger loci, enabling mygalomorph systematists to formally address a number of  
110 long-standing issues like the polyphyletic nature of ctenizids and hexathelids (Godwin et al.  
111 2018; Hedin et al. 2018). In both cases, phylogenomic results stabilized the taxonomic position  
112 of genera that were notoriously challenging to place and established new familial ranks  
113 (Halonoproctidae (Godwin et al. 2018), Atracidae, Macrothelidae and Porrhothelidae (Hedin et  
114 al. 2018)). Although these smaller scale studies have made important advances in our  
115 understanding of mygalomorph phylogeny and classification, they have all thus far lacked the

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

116 broad scale phylogenetic context instrumental to understanding relationships spanning the entire  
117 infraorder.

118 We present here the most comprehensive phylogenomic treatment of Mygalomorphae  
119 conducted to date. Our analysis includes broad sampling of taxa from all currently recognized  
120 families (88 genera, 111 species), allowing us to assess the monophyly of a number of key  
121 families (e.g., Cyrtaucheniidae, Nemesiidae, Dipluridae, Ctenizidae) and address some long-  
122 standing taxonomic problems. Our generic-level sampling also provides an evolutionary  
123 framework for revisiting questions regarding silk use in non-araneomorph spiders. We explore  
124 questions regarding the timing and biogeographical context of mygalomorph spider  
125 diversification, and explicitly test a number of hypotheses related to the timing and origin of  
126 major mygalomorph lineages, the use of silk in mygalomorph prey capture, and the monophyly  
127 of the major mygalomorph higher-level groups and families.

128

## 129 MATERIALS & METHODS

130

131 We sampled a total of 113 taxa, 111 ingroup specimens representing 88 genera from all  
132 20 currently recognized mygalomorph families (Table 1) and two representatives of the suborder  
133 Mesothelae (*Liphisthius* and *Vinathela*, family Liphistiidae) as outgroups to root phylogenies.  
134 Our sampling represents approximately 25% of the infraorder's known generic diversity and  
135 about 47% if the two most diverse mygalomorph families, Barychelidae and Theraphosidae, are  
136 excluded from the calculation. Approximately 60% of AHE sequence data was newly generated  
137 for this study, additional sequences from Hamilton et al. (2016b) and Godwin et al. (2018) were  
138 included. Taxon sampling is summarized in Supplemental Table S1; all statistics related to

## PHYLOGENY OF MYGALOMORPH SPIDERS

139 family and generic-level species diversity are extracted from the World Spider Catalog  
140 (<https://wsc.nmbe.ch/>) accessed November 2018.

141 Whole genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen),  
142 following the manufacturer's guidelines; to ensure the complete digestion of RNA, RNase A was  
143 added to the mix after the tissue lysis step. Library preparation, enrichment, and sequencing were  
144 performed at the Center for Anchored Phylogenomics at Florida State University  
145 (<http://anchoredphylogeny.com/>) following the methods described in Lemmon et al. (2012),  
146 Hamilton et al. (2016b) and Godwin et al. (2018). During the development of the spider AHE  
147 loci, a mixture of spider genome sequencing and transcriptomes were utilized to build scaffolds  
148 from which probes were designed in conserved anchor regions neighboring variable flanking  
149 regions, creating a set of probes that include exons, introns, intergenic, and conserved regions of  
150 the genome. Initial probe design was based on the core arthropod orthologs and the targeted loci  
151 were then found in the spider reference genomic datasets (both spider genomes and  
152 transcriptomes). Loci that did not contain both *Aliatypus* (Antrodiaetidae) and *Aphonopelma*  
153 (Theraphosidae) were not considered for further usage. After sequencing, all reads were  
154 assembled into contigs following Prum et al. (2015), but using references derived from the  
155 *Ixodes* (a tick), *Hypochilus* (araneomorph), *Aphonopelma*, and *Aliatypus* (mygalomorphs)  
156 sequences used for probe design. Homologous nucleotide sequence sets were produced by  
157 grouping filtered consensus sequences across individuals by target locus. Orthologous groups  
158 were then determined for each target locus by a) employing a pairwise-distance measure  
159 computed for pairs of homologs; b) a neighbor-joining clustering algorithm to cluster the  
160 consensus sequences into orthologous sets; c) the identified sequences are then grouped into a  
161 cluster; d) steps A-C are repeated, treating each cluster as a sequence by using the average

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

162 distance between the cluster and other clusters/sequence when assessing distance. This approach  
163 efficiently divides the homologs into two or more orthologous sets, allowing us to identify  
164 duplications occurring before the ancestor of the taxonomic group being analyzed. For  
165 duplications occurring within the taxonomic group under investigation, this approach tends to  
166 produce a single cluster containing one sequence per taxon and a second cluster containing  
167 sequences from those taxa that are descendants from the ancestor in which the duplication  
168 occurred.

169       Following the targeting of the 585 loci within the Spider Probe Kit v1 (Hamilton et al.  
170 2016b), 472 loci were recovered and assembled. All loci were aligned with MAFFT 7.402  
171 (Kato and Standley 2013) using the L-INS-i algorithm (--localprior and --maxiterate 1000  
172 flags). The alignments were scored for accuracy in ALISCORE (Misof and Misof 2009) and  
173 ambiguously aligned positions were removed with ALICUT (Kück 2009). Individual loci were  
174 concatenated with FASconCAT (Kück and Longo 2014) yielding a supermatrix of 93,410  
175 nucleotides for 111 ingroup and two outgroup taxa (hereafter “All\_taxa”); all alignments were  
176 visually inspected in Geneious 10.1.3 (Biomatters 2017) prior to concatenation.

177       Two additional matrices were constructed to assess the effect of taxon removal or  
178 sequence data addition on tree topology and support values. First, terminals representing the  
179 family Paratropididae were removed from the “All\_taxa” (hereafter referred to as  
180 “No\_Paratropis”). The family was recovered as a “stand-alone” lineage with unresolved and  
181 unstable placement (Hedin and Bond 2006; Bond et al. 2012), potentially causing conflict in  
182 topology and lowering the support of deeper nodes. To test the effect of data addition on node  
183 support and the unresolved placement of Stasimopidae, we combined our AHE loci with pre-  
184 existing transcriptomic data (Garrison et al. 2016). To maximize the taxon sampling, we



## PHYLOGENY OF MYGALOMORPH SPIDERS

185 extracted the mygalomorph terminals from “BCC-75” matrix (Garrison et al. 2016) and  
186 combined them into the “DNAAA\_matrix” with corresponding terminals represented in our  
187 AHE dataset, in two instances, forming chimaeras at generic level (Supplemental Table S2). In  
188 order to maintain a relevant phylogenetic framework, we also retained additional individuals  
189 representing key lineages/clades that were only represented by AHE data. Terminals extracted  
190 from the “BCC-75” matrix (Garrison et al. 2016) were parsed into individual loci using  
191 BIOPYTHON (Hamelryck and Manderick 2003; Cock et al. 2009). Resulting 1675  
192 transcriptomic loci were blasted (tblastn command) in Geneious (Biomatters 2017) against a  
193 custom AHE locus database comprising nucleotide sequence data prior to ALICUT treatment.  
194 Amino acid loci that received an e-value of  $< 1e^{-10}$ ; that is, the loci already represented in AHE  
195 dataset, were excluded from the analyses. Loci that did not contain all 20 amino acids were  
196 further removed from the dataset (per Stamatakis 2014).

197       Phylogenetic analyses were run on the Hopper Community Cluster at Auburn University.  
198 For the DNA matrices, partition schemes under the GTR substitution model were evaluated and  
199 selected based on AICc criterion in PartionFinder 2 (Lanfear et al. 2016) using the rcluster  
200 algorithm (Lanfear et al. 2014) with RAxML implementation (Stamatakis 2014). Partitioning by  
201 codon was not considered in the analyses because ambiguously aligned positions were removed  
202 with ALICUT. Partition schemes and models of protein evolution for the amino acid data were  
203 inferred independently using PartionFinder 2 with rcluster algorithm prior to their concatenation  
204 with the nucleotide AHE sequences into the “DNAAA\_matrix”.

205       Maximum likelihood analyses (ML) were conducted in RAxML v 8.2.9 (Stamatakis  
206 2014). Both “All\_taxa” and “No\_Paratropis” datasets were analyzed using the 191-partition  
207 scheme and an independent GTR+G model defined for each partition. The best ML tree for each

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

208 matrix was selected from 1000 iterations, each starting from an independently derived  
209 parsimony-based tree. Bootstrap support was inferred from 50 replicates, determined as  
210 sufficient by the automatic bootstopping criterion (- N autoMRE flag) for both datasets  
211 (Pattengale et al. 2010). Additional ML analysis of “All\_taxa” dataset was also conducted in IQ-  
212 TREE (Nguyen et al. 2014), model selection for each partition was performed by with the  
213 *ModelFinder Plus*; support values were inferred via SH-aLRT test (Anisimova et al. 2011) and  
214 ultrafast bootstrapping (both 1000 replicates).

215 The “DNAAA\_matrix” mixed dataset combining both nucleotide and amino acid data  
216 was analyzed with 1367-partition scheme, applying an independent GTR+G model for each  
217 AHE partition and a corresponding protein model inferred by PartionFinder to each  
218 transcriptome partition. The best ML tree was selected from 1000 iterations and support was  
219 assessed from 100 bootstrap replicates (autoMRE).

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

226 Bayesian Inference (BI) analyses of the “All\_taxa” dataset was conducted in ExaBayes v  
227 1.4.1 (Aberer et al. 2014) implementing the same partition scheme and nucleotide substitution  
228 model as in the ML analyses. Two independent runs of  $4 \times 10^7$  generations with four coupled  
229 chains each, starting from a parsimony tree with resampling every 1000 generations, were run  
230 simultaneously. Standard deviation of split frequencies was monitored ( $<0.01$ ), and the first 25%

## PHYLOGENY OF MYGALOMORPH SPIDERS

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

233 A species tree was inferred from 472 gene trees using ASTRAL v 4.11.2 (Mirarab and  
234 Warnow 2015). Node support was assessed using ASTRAL's local posterior probabilities. Single  
235 gene trees were inferred from AHE nucleotide alignments implementing GTR+G model in  
236 RAxML; the best ML trees were selected from 1000 independent iterations for each locus  
237 individually.

238 Divergence times were estimated in the penalized likelihood environment (Sanderson  
239 2002) using the treePL algorithm (Smith and O'Meara 2012) on the tree topology obtained in  
240 RAxML, and calibrated with six fossils. The optimal settings for the thorough analysis was  
241 determined with the prime option in treePL; smoothing value = 1, determined by random  
242 subsample and replicate cross-validation method (RSRCV), was used for both dating analysis  
243 and bootstrapping (see below). The calibrations (shown in, Supplemental Fig.7) are as follows:  
244 1) Mygalomorphae: Due to the lack of relevant fossil calibration points for the Mygalomorphae –  
245 Mesothelae split, we used the information available from phylogenomic studies based on  
246 extensive outgroup fossil calibrations (Garrison et al. 2016; Fernández et al. 2018) in order to  
247 constrain the age of the root node. Namely, we used the 95% confidence intervals estimated for  
248 this split as the minimum (287 Ma) and maximum bounds (398 Ma); 2) Avicularioidea: The age  
249 of the oldest mygalomorph fossil *Rosamygale grauvogely* (Selden and Gall 1992) from Gres-a-  
250 Voltzia Formation, Vosges, France dated to Middle Triassic (Anisian) 242 Million years ago  
251 (Ma) was assigned as the minimum bound for the Avicularioidea – Atypoidea split. The fossil  
252 has been tentatively assigned to the family “Hexathelidae” (*sensu* Raven) but given the  
253 polyphyly of the family (Hedin et al. 2018), we rather interpret *Rosamygale* as an Avicularioidea

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

254 crown-group. The maximum bound for the Avicularioidea – Atypoidea split was assigned the  
255 age of 323 Ma, corresponding to the oldest age of the Bashkirian stage (Pennsylvanian,  
256 Carboniferous) that yielded the oldest spider fossil *Arthrolycosa* sp. putatively assigned as  
257 Mesothelae stem-group (Selden et al. 2014; Garwood et al. 2016). This age also overlaps with  
258 the oldest boundary of the 95% confidence intervals for the Avicularioidea – Atypoidea split  
259 estimated by recent phylogenomic studies (Garrison et al. 2016; Fernández et al. 2018); 3)  
260 “Nemesioidina” clade: The age of *Cretamygale chasei* 125 Ma (Selden 2002) from Cretaceous  
261 (Barremian, Hughes and McDougall 1990) currently classified as Nemesiidae was  
262 conservatively assigned as the minimum bound for the split between Bemmeridae/Theraphosidae  
263 and “Nemesioidina” clades. The maximum bound was assigned 242 Ma, corresponding to the  
264 age of *Rosamygale* fossil. 4) Atypoidea: Atypidae – Antrodiaetidae split was assigned minimum  
265 bound of 100 Ma representing *Ambiortiphagus ponomarenkoi* (Eskov and Zonshtein 1990) from  
266 the Lower Cretaceous (Albian) proceeding from Dund-Ula Mountain Ridge in Bayan-Khongor  
267 Province, Central Mongolia. The age of *Rosamygale* (242 Ma) was assigned as a maximum  
268 bound for the split; and 5) *Ummidia* fossils (44 Ma) from Baltic amber (Wunderlich 2011) were  
269 used to calibrate the minimum bound for the split between the North American and European  
270 species of *Ummidia*. Maximum bound of 90 Ma corresponds to the oldest boundary of the 95%  
271 confidence interval estimated for this particular split (Opatova et al. 2013). The confidence  
272 intervals for node ages were assessed by dating 100 ML bootstrap phylograms inferred in  
273 PAUP\* (Swofford 2003). The trees were dated in TreePL using the same settings as described  
274 above; TreeAnnotator v. 2.4.5 (Bouckaert et al. 2018) was used for calculating the age statistics  
275 for the nodes.

## PHYLOGENY OF MYGALOMORPH SPIDERS

276 Biogeographic analyses were conducted in Reconstruct Ancestral State in Phylogenies  
277 (RASP v 3.1, Yu et al. 2015) with the dispersal-extinction-cladogenesis (DEC) model (Ree and  
278 Smith 2008) implemented in C++ version of Lagrange (Smith 2010). Analyses were run with no  
279 dispersal constraints, assigning equal probabilities to dispersal events among all defined areas,  
280 and allowing two unit areas in the ancestral distributions. The terminal taxa represented in the  
281 tree were assigned to six distribution ranges: (A) North America; (B) Europe; (C) Asia; (D)  
282 South America; (E) Africa; (F) Australia plus New Zealand (Fig. 5b). The consensus tree  
283 obtained in ExaBayes was used as an input for the analyses.

284 For ancestral state reconstruction analyses of foraging construct, each terminal taxon was  
285 scored using six character states: (0) burrow with trapdoor(s); (1) brush sheet/funnel web; (2)  
286 burrow with collar door; (3) purse web; (4) open burrow; (5) turret. Character codings were  
287 scored from personal observations and/or the primary literature as documented in Supplemental  
288 Table S3. Taxa that forage as cursorial hunters were scored as unknown or missing (Coddington  
289 et al. 2018); polymorphic character states were assigned to terminals for instances where species  
290 build multiple/combinations of constructs (e.g., some *Cyrtachenius* terminals build turrets as  
291 juveniles but cover those turrets with trapdoors as adults, *Opatova persn. obsv.*), or closely  
292 related species build more than one construct e.g., both open burrows and trapdoors (some  
293 bemberrids and pycnothelids) and both turrets and collars (e.g., some *Antrodiaetus*). Ancestral  
294 character state reconstructions were conducted using the R package *corHMM* (Beaulieu et al.  
295 2013) on an ultrametric scaled tree with `node.states=marginal`; *corHMM* was preferred over  
296 other methods given its capacity to accommodate missing and polymorphic characters. The R  
297 package *ape* was used to scale the preferred tree topology ('chronopl') with an assigned lambda  
298 value of 0.1. Character optimizations using equal (ER), symmetric (SYR), and all rates different

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

299 (ARD) models were explored for these categorical data using ‘rayDISC’ (*corHMM*). The  
300 preferred model was chosen by comparing AICc values calculated using *corHMM*.

301 All relevant data, DNA and amino acid sequence alignments, phylogenetic trees, and  
302 miscellaneous data matrices, were deposited in the Dryad Data Repository  
303 (doi:XXXXXXXXXXXX) on XX XX 2019.

304

305 RESULTS

306

307 *Concatenated analyses, all taxa.*—The complete dataset “All\_taxa” supermatrix  
308 comprises 472 loci (93,410 nucleotides) for 113 terminals (Supplemental Table S1), whereas the  
309 reduced “No\_Paratropis” dataset comprises 111 terminals. The proportion of missing data in the  
310 datasets is 14.7% and 14.5%, respectively. The combined “DNAAA\_matrix” includes 30  
311 terminals, with 14 individuals represented by both genomic (472 AHE loci) and transcriptomic  
312 data (1519 loci proceeding from Garrison et al. (2016)). The total length of the  
313 “DNAAA\_matrix” is 305,860 characters; gaps and missing data represent 54.5% of the dataset.  
314 For details see Supplemental Table S2.

315 Both ML (-ln 1728131.7475) and BI analyses performed on the “All\_taxa” dataset  
316 yielded similar tree topologies (Fig. 3; Supplemental Fig. 1, 2, 3). Overall, the resulting trees are  
317 highly supported (bootstrap > 90, posterior probability (PP) > 0.95, SH-aLRT > 95), with the  
318 exception of a few deeper nodes. The ML and BI analyses both recover two main clades  
319 Atypoidea and Avicularioidea with strong support. The relationships within Atypoidea are fully  
320 supported; the family Atypidae is recovered as a sister to Antrodiaetidae inclusive of *Hexura*  
321 (*Mecicobothriidae*).

## PHYLOGENY OF MYGALOMORPH SPIDERS

322 Four mygalomorph families belonging to Avicularioidea are recovered as para- or  
323 polyphyletic (namely Dipluridae, Ctenizidae, Cyrtaucheniidae and Nemesiidae), prompting  
324 extensive higher-level changes to mygalomorph classification (see below). Several lineages  
325 formerly belonging to the families Dipluridae and Hexathelidae (see Hedin et al. (2018))  
326 successively branch off near the root node of Avicularioidea as follows: Ischnothelidae (NEW  
327 RANK), Microhexuridae (NEW FAMILY), Hexathelidae, Euagridae (NEW RANK),  
328 Porrhothelidae and Macrothelidae. The Bipectina clade (*sensu* Goloboff (1993)) inclusive of the  
329 family Atracidae is recovered in our analysis. The family Paratropididae is recovered as sister to  
330 all the remaining Bipectina. The remaining taxa are placed into a clade comprising Stasimopidae  
331 and the “Venom Clade” (Atracidae + Actinopodidae) and two additional clades corresponding to  
332 Domiothelina and Crassitarsae (*sensu* Bond et al. (2012), but excluding *Stasimopus*). The  
333 monogeneric family Stasimopidae (NEW FAMILY, formerly Ctenizidae), is placed as sister to  
334 “Venom Clade”, albeit with low support. The ML analysis performed in IQ-TREE resulted in  
335 different placement of Stasimopidae and the “Venom Clade”. The “Venom Clade” is supported  
336 as sister to all Bipectina minus Paratropididae, whereas the family Stasimopidae is recovered  
337 with high SH-aLRT support as sister to Domiothelina. Domiothelina form a monophyletic group  
338 with well resolved relationships in both ML and BI analyses. The family Halonoproctidae is  
339 recovered as sister to all remaining lineages of Domiothelina. The family Migidae is recovered  
340 as sister to Idiopidae, Ctenizidae (now comprising *Cteniza* and *Cyrtocarenum*) and Euctenizidae.  
341 Crassitarsae (*sensu* Bond et al. (2012)), recovered with high SH-aLRT support in IQ-TREE ML  
342 inference, is subdivided into two clades supported in all analyses. The first one comprises the  
343 family Bemmeridae (NEW RANK, formerly Cyrtaucheniidae), recovered as sister to the  
344 Barychelidae and Theraphosidae. The second clade, “Nemesioidina”, comprises the Nemesiidae

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

345 plus three lineages formerly belonging to the Nemesiidae (Pycnothelidae (NEW RANK),  
346 Entypesidae (NEW FAMILY) and Anamidae (NEW FAMILY)), as well as Dipluridae,  
347 Cyrtaucheniidae and Microstigmatidae. Nemesiidae is recovered as sister to all the remaining  
348 families; Pycnothelidae is recovered as sister to the Dipluridae/Cyrtaucheniidae clade and the  
349 Entypesidae /Microstigmatidae/Anamidae clade.

350 *No\_Paratropis analysis.*—Maximum likelihood analyses of the “No\_Paratropis” dataset  
351 recovered a moderately different Bipectina topology contra the analyses on the full dataset  
352 (Supplemental Fig. 4; ML best tree  $-\ln = 1702659.2948$ ). The discordance, however, mostly  
353 affects nodes unsupported in both analyses. In the “No\_Paratropis” analyses the clade  
354 comprising Bemmeridae + Barychelidae + Theraphosidae is recovered with high support as  
355 sister to all the remaining Bipectina families. The Stasimopidae + “Venom Clade” receive  
356 moderate bootstrap support, but its sister relationship to the Domiothelina clade remains  
357 unsupported, albeit with increased support when compared to the analyses of the full dataset.

358 *DNAAA analysis.*—Similar to the “No\_Paratropis” dataset, analyses of “DNAAA” (ML  
359 best tree  $-\ln = 2543315.0197$ ) resulted in different topological arrangements within the Bipectina  
360 clade (Fig. 4). The Domiothelina clade is recovered as sister to all the remaining diversity.  
361 Stasimopidae + “Venom Clade” (moderately supported in this analysis) is recovered with low  
362 support as sister to Crassitarsae. The Crassitarsae clade is highly supported and inclusive of  
363 *Paratropis*.

364 *Topology tests.*—The AU topology test (Table 2) rejected the monophyly of all  
365 alternative arrangements (i.e., alternatives using existing compositions of genera) of the families  
366 Ctenizidae (*sensu* Raven (1985)), Cyrtaucheniidae (*sensu* Bond et al. (2012)), Dipluridae,



## PHYLOGENY OF MYGALOMORPH SPIDERS

367 Hexathelidae (*sensu* Raven (1985)) and Nemesiidae in favor of the results of the unconstrained  
368 search.

369 *Species tree analysis.*—Coalescence-based analysis produced a species tree from the  
370 individual 472 input gene trees obtained in RAxML. The resulting quartet-based supertree  
371 estimated in ASTRAL (Supplemental Fig. 5) comprises 1,228,051,216 induced quartet trees  
372 from the input gene trees, representing 65.2% of all quartets present in the species tree. ASTRAL  
373 yielded a different topology than the concatenated approach (Supplemental Fig. 6). The analyses  
374 recover both Atypoidea and Avicularioidea clades. Ischnothelidae (NEW RANK),  
375 Microhexuridae (NEW FAMILY), Hexathelidae, Euagridae (NEW RANK) and Porrhothelidae,  
376 a grade of lineages branching at the base of the Avicularioidea clade in the concatenated  
377 analyses, form a highly supported clade in the species tree approach. The topology of the  
378 Bipectina clade (unsupported in ASTRAL) is also somewhat differed from the concatenated  
379 analyses. Bemmeridae/Barychelidae/Theraphosidae clade is recovered as sister to moderately  
380 supported Domiothelina + Stasimopidae/“Venom Clade”, rendering the Crassitarsae  
381 paraphyletic.

382 *Molecular dating.*—The resulting dated tree is depicted in Fig. 5; Supplemental Figures  
383 7, 8. The age of the root (398 Ma) corresponds to the maximum age constraints assigned to this  
384 split. The split between Atypoidea and Avicularioidea is dated at ~ 323 Ma. The early branching  
385 events within Avicularioidea dated between 254 Ma [95% confidence interval (CI): 259 – 247]  
386 and 174 Ma (179 – 162) (Ischnothelidae through Macrothelidae divergences). The first  
387 diversification of Bipectina is dated to 166 Ma (171 – 155) (divergence of Paratropididae) and  
388 continues at 158 Ma (163 – 147), with diversification into Domiothelina +

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

389 Stasimopidae/“Venom Clade” and Crassitarsae. The divergence time estimates for individual  
390 families are reported in Table 3.

391 *Historical biogeography.*—The results of DEC analyses are summarized in Fig. 6. The  
392 ancestral distributional area of the mygalomorph ancestor and the early branching Avicularioidea  
393 lineages remain unresolved. The distribution of the Atypoidea ancestor is inferred to have  
394 originated in the Northern hemisphere continents (North America and Asia); Bipectina likely  
395 originated in Africa. The ancestral ranges of several Avicularioidea families are inferred to areas  
396 spanning more than one continent, for example Africa and Australia (Migidae, Idiopidae,  
397 Barychelidae, Microstigmatidae); Africa and South America (Cyrtaucheniidae, Pycnothelidae).  
398 On the other hand, some Avicularioidea families experienced *in situ* diversification within a  
399 single area. Stasimopidae, Bemmeridae, and Entypesidae likely evolved in Africa, Paratropididae  
400 and Dipluridae in South America, Anamidae in Australia, Euctenizidae in North America and  
401 Ctenizidae in Europe. DEC analyses also infers a large number of dispersal and vicariance events  
402 across the mygalomorph phylogeny, however, most of the events receive low probabilities  
403 (<0.7).

404 *Evolution of foraging construct.*—We scored foraging construct for 109 taxa. Taxa that  
405 are cursorial hunters (three terminals), *Paratropis* and *Microstigmata*, were scored as  
406 missing/inapplicable. The foraging construct of nemesiid *Mexentypesa* is unknown to us.  
407 Because bemmerids and pycnothelids construct both trapdoors and open burrows they were  
408 scored as polymorphic. Based on AICc values the equal rates model (ER) produced the preferred  
409 reconstruction (AICc = 168.3399). Although the ER model was preferred, ancestral state  
410 reconstructions for the other models were generally very similar.

## PHYLOGENY OF MYGALOMORPH SPIDERS

411           The ancestral state reconstruction of mygalomorph foraging construct, summarized in  
412 Fig. 7, shows a rather simple and concise pattern. Towards the root of the phylogeny there  
413 appears to be an equal probability of an ancestor having either a funnel-and-sheet web or a  
414 burrow with a trapdoor. Atypoids likely had a most recent common ancestor (MRCA) with a  
415 funnel-and-sheet web, modified as a purse web in atypids or as a collar, turret, or trapdoor in  
416 antrodiaetids. As discussed below, atypoids demonstrate the full scope of variability in burrow  
417 construct modifications that can evolve from a sheet web ancestral form. Avicularioidea have a  
418 MRCA that optimizes as having a sheet web. The MRCA for the Bipectina clade likely had  
419 either a trapdoor or funnel-and-sheet web (equivocal). A burrow with a trapdoor more  
420 definitively optimizes as the ancestral foraging construct for Crassitarsae with subsequent losses  
421 of the trapdoor covering (optimized as the open burrow state) four times in nemesiids,  
422 pycnothelids, theraphosids, and the euctenizid genus *Apomastus*; some pycnothelid and anamid  
423 taxa independently lose the trapdoor and instead forage from burrows with distinct turret or  
424 collar door constructs. Funnel-and-sheet webs are regained twice, once in atracids and again in  
425 diplurids.

426

## 427 DISCUSSION

428

429           In light of the results reported herein and other past molecular based studies of  
430 mygalomorph phylogeny, two points seem clear. First, morphological data *alone* are unsuitable  
431 for resolving the evolutionary history of mygalomorphs. Second, if our aim is to have a  
432 classification scheme that reflects evolutionary history, other characters are needed. The results  
433 we discuss below allow us to assess the status of nearly all mygalomorph families and resolve

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

434 several long-standing questions in the group's phylogeny and higher-level classification. As a  
435 consequence, we propose extensive taxonomic changes that better reflect the origins and  
436 evolutionary relationships of the taxa. We discuss in detail 1) the temporal and biogeographical  
437 context of mygalomorph family-level diversification; 2) revisit longstanding questions regarding  
438 silk use in non-araneomorph spiders; and 3) provide a detailed account regarding the taxonomic  
439 implications of our newly formed phylogenetic hypothesis (Fig. 8). The Appendix to this paper  
440 (Bond et al. 2019) outlines the formal taxonomic changes proposed herein and discusses in more  
441 detail the systematics of each mygalomorph family.

442

443 *Diversification and Biogeography of Mygalomorph Spiders*

444

445 Mygalomorph spiders with nearly a cosmopolitan distribution, highly sedentary nature  
446 (Raven 1985; Bond et al. 2001) and evolutionary history dating back to Carboniferous (Ayoub et  
447 al. 2007; Starrett et al. 2013; Garrison et al. 2016), represent the type of group that has captivated  
448 the attention of biogeographers for decades (Cracraft 1988; Wiley 1988; Morrone and Crisci  
449 1995). By implementing divergence time estimation methods, many distribution patterns  
450 observed in mygalomorphs could be linked to specific geologic events such as continental  
451 breakups (Opatova et al. 2013; Opatova and Arnedo 2014a), Mediterranean tectonics (Opatova et  
452 al. 2016; Mora et al. 2017) and opening of the Okinawa Trough (Su et al. 2016). However, recent  
453 findings suggest that dispersal via rafting or could be a plausible mechanism for overcoming  
454 both short (Hedin et al. 2013; Opatova and Arnedo 2014b) and long distances (Harrison et al.  
455 2017).

## PHYLOGENY OF MYGALOMORPH SPIDERS

456           The results of our biogeographic analyses (Fig. 6) place the ancestral area of distribution  
457 of Atypoidea within the Northern hemisphere (across North America and Asia), whereas the  
458 ancestor of Porrhothelidae, Macrothelidae and Bipectina most likely evolved in the Southern  
459 hemisphere (across Africa and Australia). In our dating analyses, we recovered two repeated  
460 patterns of divergences occurring among taxa with present day distributions confined mostly to  
461 the Southern hemisphere that in dispersal-limited invertebrate groups have been repeatedly  
462 linked to vicariant events related to the different phases of Gondwana breakup (Wood et al.  
463 2012; Frazão et al. 2015; Kim and Farrell 2015; Xu et al. 2015; Andújar et al. 2016; Toussaint et  
464 al. 2017).

465           The initial phase of Gondwana breakup (Fig 5), i.e., the formation of East and West  
466 Gondwana, began approximately 165 Ma with South America and Africa drifting away from  
467 India, Madagascar, Antarctica and Australia. The pattern concordant with the East – West  
468 Gondwana breakup observed in our results involved splits within the families Euagridae,  
469 Migidae, Idiopidae (putatively also Barychelidae), and divergence between Atracidae –  
470 Actinopodidae and Anamidae – Entypesidae /Microstigmatidae clade (marked with stars on the  
471 chronogram in Fig. 5) was dated between 101 – 88 Ma. The second pattern, concordant with  
472 West Gondwana fragmentation into South America and Africa occurring between 132 – 112 Ma  
473 (Will and Frimmel 2018) involved divergences within Theraphosidae, Pycnothelidae and  
474 Cyrtaucheniidae (marked as triangles) was dated to 81 – 61 Ma. The divergence times estimated  
475 in our analyses were considerably younger than the ages estimated for other sedentary taxa with  
476 former Gondwanan distribution, such as velvet worms or bothriurid scorpions (Murienne et al.  
477 2014; Sharma et al. 2018). Our inferred divergences also postdate the onset of continental  
478 drifting in both instances by approximately 50 My, however, the initial phases of different

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

479 Gondwana breakup stages were often characterized by slow tectonic movement, or even  
480 quiescence (Boger 2011; Will and Frimmel 2018; Young et al. 2018). In case of East and West  
481 Gondwana, the drifting was occurring at an extremely slow rate for at least the first ten million  
482 years (Young et al. 2018). A connection between South America and Australia also likely  
483 persisted via Antarctica until late Eocene, approximately 35 Ma (Sanmartín and Ronquist 2004),  
484 which links the divergence time estimates between Neotropical and Australasian groups of  
485 Darwin's stag beetles dated to 47 and 58 Ma, respectively, to Gondwana breakup (Kim and  
486 Farrell 2015).

487         Conversely, the divergence time estimates inferred in our analyses could also be affected  
488 by the limited fossil record for mygalomorphs, and our conservative approach towards node  
489 calibration. For example, the *Cretamygale chasei* fossil, formerly interpreted as Nemesiidae, was  
490 used to set a minimum bound for the whole “Nemesioidina” clade (see methods), rather than  
491 arbitrarily assigning the fossil to the family Nemesiidae (*sensu* this study). Despite the apparent  
492 advantages in the ability of handling genomic data, the dating analyses used in this study (Smith  
493 and O’Meara 2012), does not allow to assign probability distribution priors to the calibration  
494 points or infer confidence intervals for the divergences. Confidence intervals are often essential  
495 for connecting the age estimates and specific geological events and while assessing them via  
496 bootstrapping potentially informs our analyses, this approach likely does not capture the full span  
497 of variation as accurately as when node calibration priors are taken into account.

498         Alternatively, the observed patterns could be explained by long-distance dispersal events  
499 as in case of other poorly dispersing groups such as euedaphic beetles (Andújar et al. 2016) or  
500 short-tailed whip-scorpion order Schizomida (Clouse et al. 2017). Although evidence suggests  
501 that mygalomorph spiders are capable of trans-oceanic dispersal (Harrison et al. 2017), repeated

## PHYLOGENY OF MYGALOMORPH SPIDERS

502 divergence patterns imply multiple long-distance dispersal events occurring independently  
503 among unrelated taxa during the same timeframe. Continental-level vicariance thus provides a  
504 more plausible explanation for the observed pattern.

505         The East – West Gondwana vicariant origin of the taxa is also supported by the results of  
506 the ancestral range reconstruction analyses. The inferred ranges for all the taxon groups  
507 displaying a distribution pattern concordant with the vicariance (Euagridae, Atracidae and  
508 Actinopodidae, Migidae, Idiopidae, Barychelidae, Entypesidae and Anamidae) comprised former  
509 Gondwana continents, although the ancestral range inferred for Idiopidae, Barychelidae and  
510 Migidae (Africa and Australia) could potentially represent an artifact stemming from a  
511 geographical bias in our sampling (i.e. lacking their South American representatives in our  
512 analyses). Vicariance was also inferred with high probability in the divergences between  
513 *Allothele* and Australian euagrids, *Atrophothele* and remaining barychelids. The West Gondwana  
514 vicariant split between Africa and South America can likely be attributed to the structure within  
515 Pycnothelidae (*Pionothele* – remaining pycnothelids) and South American and African  
516 Cyrtacheniids which also optimizes as an ancestral distribution spanning across both continents  
517 in both groups.

518         Another repeated pattern observed across our tree includes taxa with currently disjunct  
519 amphi-Atlantic distributions (marked as dots on the chronogram). Such patterns of divergence  
520 were observed within *Ummidia* and *Cyclocosmia* (Halonoproctidae) and between *Sphodros* and  
521 *Atypus* (Atypidae), and were dated to 44 Ma, 33 Ma (35 – 29) and 39 Ma (41 – 37), respectively.  
522 This pattern has previously been connected with the breakup of Laurasia (Opatova et al. 2013;  
523 Godwin et al. 2018) dating to the end of Paleocene, approximately 60 – 55 Ma (Sanmartín et al.  
524 2001) and it is supported by the ancestral distributions of the taxa projected to the Northern

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

525 hemisphere continents in our analyses. Our divergences postdate both the geological event and  
526 previous dating results (Opatova et al. 2013). The discrepancy between these time estimates and  
527 the Laurasian breakup could be explained by either an artefact of the analyses (discussed above),  
528 or alternatively by a persistent connection of North America and Europe via a North American  
529 land bridge. This connection existed to some extent until 25 Ma and is hypothesized to have  
530 played an important role in plant dispersal (Tiffney and Manchester 2001; Milne 2006),  
531 suggesting also a suitable dispersal route for ballooning spiders like *Atypus*, *Sphodros*, *Ummidia*  
532 (Opatova et al. 2013). The current distribution of *Cyclocosmia* (East Asia and South East of  
533 North America), represents a well-known biogeographic pattern linked to continental vicariance  
534 (Boufford and Spongberg 1983; Bolotov et al. 2016), which is further supported by the inferred  
535 vicariant origin of the genus. Alternatively, similar distribution patterns could have resulted from  
536 dispersal events between Palearctic and Nearctic regions via Beringia (Li et al. 2015; Bolotov et  
537 al. 2016). However, the timing of the dispersal in other organisms postdates the results of our  
538 analyses (Li et al. 2015; Maguilla et al. 2018).

539 Older divergences recovered in our analyses are more challenging to explain within a  
540 biogeographic context. If we follow the same line of reasoning as explained above, and assume  
541 that the actual ages of the splits are older than indicated by the results, the splits between the  
542 Idiopidae and Ctenizidae/Euctenizidae clade, and Nemesiidae and the remaining “Nemesioidina”  
543 diversity (marked as inverted triangles), could correspond to the disintegration of the  
544 supercontinent Pangea occurring in Lower Jurassic (190 – 180 Ma) (Beutel et al. 2005; Will and  
545 Frimmel 2018). Divergences proceeding the breakup of Pangea could be potentially linked to  
546 paleoclimatic events. Since its formation around 520 – 510 Ma (Veevers 2004; Domeier and  
547 Torsvik 2014; Will and Frimmel 2018), large parts of Gondwana experienced repeated



## PHYLOGENY OF MYGALOMORPH SPIDERS

548 glaciations in the Silurian, Devonian, late Carboniferous and early Triassic. Additional  
549 glaciations were caused by the orogenic processes prompted by the collision of Gondwana with  
550 Laurussia and subsequent formation of Pangea ~320 Ma (Golonka 2007; Nance et al. 2014;  
551 Matthews et al. 2016) in the Permian. Suitable habitats were thus repeatedly fragmented, likely  
552 causing taxon distributional shifts and large-scale extinction events (Coccioni and Galeotti 1994;  
553 Zachos et al. 2001; Chen and Benton 2012). The consequences of these events could be  
554 potentially reflected in the mygalomorph phylogeny by the existence of many early-branching  
555 monogeneric lineages such as Microhexuridae, Porrhothelidae, Macrothelidae, Paratropididae  
556 and Stasimopidae, that do not seem to have any close relatives among extant taxa. Deep  
557 divergences far preceding the breakup of Pangea have also been observed for a similarly ancient  
558 and sedentary group – the velvet worms (Boyer et al. 2007; Murienne et al. 2014), indicating that  
559 paleoclimatic events and habitat heterogeneity played an important role in the evolution of these  
560 ancient groups.

561         We also detected potential long-distance dispersal events across the tree (denoted with  
562 “\*”). The Australasian genus *Conothele* (Halonoproctidae), shows divergence between  
563 Australian and Vietnamese species dating back to approximately 46 Ma (47 – 44), which  
564 postdates their vicariance during the northward drifting of India ~ 135 Ma (Will and Frimmel  
565 2018). The genus exhibits some behavioral traits associated with ballooning (Main 1957), and it  
566 is possible that aerial dispersal played a role in *Conothele* distribution. Other cases involve  
567 Australian and African taxa. *Poecilomigas* (family Migidae) diverged from its Australia  
568 inhabiting sister taxon *Bertmainius* approximately 66 Ma (72 – 57), and *Kiama* and  
569 *Microstigmata* (both Microstigmatidae) split around 64 Ma (70 – 54). Both divergences are  
570 likely too young to reflect the East – West Gondwana vicariance (inferred by the biogeographic

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

571 analyses) that started approximately 165 Ma, even when a possible bias in our dating analyses is  
572 considered. Trans-oceanic dispersal has previously been reported from the family Migidae  
573 (Harrison et al. 2017), which suggests that dispersal could plausibly explain the disjunct  
574 distribution between *Bertmainius* and *Poecilomigas*.

575 Another unusual pattern occurs within the family Pycnothelidae, where the closest  
576 relatives of the Australian genus *Stanwellia* have South American distributions. The split dates  
577 back to approximately 28 Ma (30 - 26) which by far postdates the East – West Gondwana  
578 vicariance but might actually reflect a connection between South America and Australia via  
579 Antarctica that presumably persisted until 35 Ma (Sanmartín and Ronquist 2004). The same  
580 vicariant scenario has been postulated to explain a similar distribution pattern recovered in Stag  
581 beetles (Kim and Farrell 2015). The biogeographic analyses also inferred vicariance for the  
582 *Stanwellia* – *Acanthogonatus* favoring South America – Australia connection over dispersal  
583 event.

584

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

586

587 As noted by Coyle (1986), progress in our understanding of the role silk plays in non-  
588 araneomorph spiders lags far behind what is known about araneomorphs; this was an accurate  
589 statement in 1986, and largely remains true today. As we discuss below, a prevailing perception  
590 considered by us misleading, if not erroneous, is that mygalomorph spiders do not use silk  
591 directly in foraging for prey; that is, it has been perceived that they do not spin webs defined in  
592 either a restricted or broad sense. For the purposes of our discussion we consider two  
593 characteristics as fundamental to ‘qualifying’ silk as playing a role in prey capture: 1) silk is

## PHYLOGENY OF MYGALOMORPH SPIDERS

594 employed by the spider in a sensory capacity to transmit information and extend prey sensing  
595 space outward to localize prey; and/or 2) silks can also be used by the spider to slow, impede, or  
596 restrain prey items. These two criteria are not mutually exclusive, particularly in mygalomorphs  
597 where visual clues are not known to play a significant role in prey capture; simply put silk is  
598 employed to increase predator speed (fast predator and/or slower prey (Coddington et al. 2019)).  
599 Note that Vollrath and Selden (2007) had a much broader definition of a web that included any  
600 spider structure made of silk. Because the question we address here is specifically related to the  
601 use of silk in prey capture, our working definition is narrower than theirs. It is our hypothesis,  
602 forwarded herein, that mygalomorphs, and consequently all spiders, plesiomorphically used silk  
603 to both localize and impede prey for capture. Whereas not all mygalomorphs use silken  
604 constructs to impede prey (criterion 2), nearly all employ it in some fashion to transmit  
605 vibrations to facilitate prey localization (criterion 1).

606 As documented in Figure 7, the MRCA for all mygalomorphs shows an unambiguous  
607 optimization for a funnel-and-sheet web versus trapdoor. Although the optimization of this  
608 character is equivocal at the root node (the MRCA of liphistiids and mygalomorphs), we would  
609 contend that the likely character state at this node, if araneomorphs and uraraneids (e.g.,  
610 *Chimerarachne yingi*, (Wang et al. 2018)) were to be included in the analysis, would likely  
611 resolve as a funnel-and-sheet web. As noted by Vollrath and Selden (2007), there is a clear link  
612 between body structure and behavior in extant spiders. As such, the proportionally long  
613 spinnerets of spider sister taxon *Chimerarachne* suggest that they were funnel-and-sheet web  
614 builders (mygalomorph sheet web builders have proportionally longer spinnerets than their  
615 counterparts – see Ischnothelidae). Eskov and Selden (2005) hypothesized that *Permarachne* (at  
616 the time considered an extinct mesothele – now placed in the Uraraneida) constructed a funnel-

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

617 and-sheet web but likely evolved *from* a prototypical mesothele ancestor that constructed a  
618 trapdoor burrow with radiating lines. Alternatively, the uraraneid placement as the sister group to  
619 all spiders (Wang et al. 2018) likely requires the optimization that funnel-and-sheet webs are the  
620 plesiomorphic condition with trapdoor burrows independently derived in Liphistiidae – a lineage  
621 that is morphologically unique among spiders (see below). Funnel-and-sheet webs meet both  
622 criteria outlined above (Coyle 1986); that is, they transmit and extend prey localization from a  
623 tubular retreat (typically) and serve to entangle prey. Although silk may not have initially  
624 evolved for this function in the group’s common ancestor (that is, the common ancestor of  
625 Uraraneida + Araneae), these results strongly suggest that early silk use likely played a role in  
626 prey capture. Some authors have hypothesized that silk was initially used in reproduction (e.g.,  
627 protecting eggsacs, sperm webs, etc. (Shultz 1987) or played a sensory role (Bond 1994)).  
628 However, these early functions may not be mutually exclusive; silken threads extending from an  
629 eggsac could have allowed a spider to care for her young while still able to sense prey (or  
630 predators) from a distance.

631 Irrespective of the uraraneid sheet web hypothesis originally proposed by Eskov and  
632 Selden (2005), the funnel-and-sheet web optimization at the mygalomorph node inferred here  
633 may be considered by some to be surprising given that the sister group to all other spiders  
634 (Opisthothelae), Mesothelae, build burrows covered by trapdoors. As we discuss in more detail  
635 below, a trapdoor covered burrow may have evolved from a sheet web (certainly the case among  
636 more derived mygalomorph taxa). The Mygalomorphae MRCA with high probability spun a  
637 sheet web from which trapdoor burrows were derived independently twice across the tree (once  
638 in the Atypoidea and a second time in the Bipectina). Nevertheless, we consider trapdoor burrow  
639 entrances to play a role in prey localization (discussed in more detail below), further enhanced in

## PHYLOGENY OF MYGALOMORPH SPIDERS

640 some liphistiids and other trapdoor spiders by extending the prey sensing surface of their burrow  
641 opening via silken trip lines or leaves, and other organic debris.

642         The evolutionary plasticity of the ancestral funnel-and-sheet web is best illustrated in the  
643 Atypoidea. The antrodiaetid clade includes *Antrodiaetus* (plus *Atypoides*), *Aliatypus*, and *Hexura*  
644 – all of which construct collared burrows, turreted burrows, trapdoors, and sheet webs,  
645 respectively. As demonstrated in a number of experiments conducted by Coyle (1986), the  
646 trapdoor spider *Aliatypus* likely suffers from reduced foraging efficiency relative to its more  
647 open burrow (collared door) and sheet web related genera. The sister group, Atypidae, builds  
648 purse webs, which are silken tubes that typically extend vertically up trees or horizontally along  
649 a substrate. As discussed by Coyle (1986), these spiders respond rapidly to vibrations along the  
650 tube surface; atypid purse webs definitively extend the spider’s prey sensory environment. An  
651 optimization of web constructs across the Atypoidea conducted by Hedin et al. (2019) recovered  
652 a similar pattern.

653         The Avicularioidea clade unequivocally has an MRCA that forages from a funnel-and-  
654 sheet web. As discussed in the classification section below, diplurids are polyphyletic, with most  
655 genera forming a grade of lineages that are sister to the remaining mygalomorphs. The  
656 characteristics of the ischnothelid, euagrid, etc. and hexathelid funnel-and-sheet webs are well  
657 documented for their abilities to aid in sensing/localizing prey, and in many taxa also impeding  
658 and/or entangling prey items. These silken constructs play a significant role in prey capture and  
659 are by any definition a “foraging web.” Funnel-and-sheet webs are independently derived twice  
660 in atracids (formerly hexathelids) and much further up the tree in the clade that includes “true”  
661 diplurids. The atracid funnel-and-sheet webs are highly modified from silken tubes (Gray 2010)  
662 and likely not homologous to the ancestral funnel-and-sheet web.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

663           The Bipectina clade resolves as having an MRCA with a trapdoor. This clade includes  
664 typical trapdoor spiders like idiopids, migids, euctenizids, and nemesiids. As already discussed  
665 above, and in a number of papers (Coyle 1986; Coyle et al. 1992; Bond and Coyle 1995),  
666 trapdoor spiders employ silk in prey detection at the burrow entrance and in the door directly.  
667 Moreover, many trapdoor spider species included in this clade add additional silk lines, plant  
668 material, and tabs to the burrow entrance further emphasizing the role that the silken lined  
669 burrow entrance plays in detecting and localizing prey. Although trapdoor covered burrows do  
670 not seemingly serve to entangle or impede prey (criterion 2, above), aspects of the burrow do  
671 serve to enhance the sensory capacity of the spider. Trapdoors are independently lost in a number  
672 of Bipectina families in favor of other burrow constructs and foraging methods – Atracidae:  
673 funnel-and-sheet web; Theraphosidae: open burrows (though trapdoors have reappeared  $\geq 3$   
674 times; Hamilton *persn. obsv.*); Pycnothelidae: open burrows, turrets; Dipluridae: funnel-and-  
675 sheet webs; Anamidae: open burrows and collar doors; Microstigmatidae: cursorial hunters,  
676 scored as inapplicable (*sensu* paratropidids). Additionally, species of one euctenizid genus,  
677 *Apomastus*, forages from an open burrow. In a number of these taxa, extensive field observations  
678 demonstrate that vibrations at the silken burrow entrance can be used to coax the spider to the  
679 surface, illustrating the functional role that silk plays in prey detection for these taxa.

680           As mentioned above, Coyle (1986) demonstrated that trapdoors appear to reduce foraging  
681 efficiency in some taxa. As such it begs the question as to why some mygalomorph spiders have  
682 abandoned funnel-and-sheet webs in favor of a foraging construct that appears to be less  
683 effective at detecting prey and clearly lacks the ability to impede or entangle prey items.  
684 Although this remains an open question, trapdoor spiders likely required additional protection  
685 from predators (e.g., parasitoid insects like pompilid wasps), desiccation, or even flooding. The

## PHYLOGENY OF MYGALOMORPH SPIDERS

686 reversal to an open burrow, often with an expanded collar for prey sensing (e.g., *Apomastus*  
687 burrows, (Bond 2004)), suggests that once environmental conditions or predator selective  
688 pressures change, the trapdoor can be easily lost.

689         It is our supposition that most mygalomorphs build foraging webs; that is, the majority of  
690 species employ silk either in a funnel-and-sheet web or at a burrow entrance to detect and  
691 localize prey and that a large number of taxa use silk to impede or entangle prey (the latter  
692 considered to be the plesiomorphic condition). These points are important to make particularly in  
693 light of a recent study by Fernández et al. (2018) that scored most mygalomorph taxa as having a  
694 non-foraging burrow. Based on the results and data discussed here, not surprisingly, we would  
695 question any analysis that hypothesizes mygalomorphs (and spiders as whole) to have a non-web  
696 foraging MRCA. Such an interpretation likely stems from limited taxon sampling and major  
697 differences in character state coding (Coddington et al. 2019). The latter in particular includes an  
698 interpretation that a spider foraging from a burrow is not employing silk in the process of prey  
699 capture, which, in our opinion contradicts the information available in the literature and direct  
700 observations in the field.

701

### 702 *Mygalomorphae Systematics and Higher-level Classification*

703

704         The criterion of diagnosability, dictated by the ICZN, that all nominal taxa be  
705 diagnosable (morphologically or otherwise) presents some problems for higher level  
706 classification schemes based on these genomic data. Although one may recognize a molecularly  
707 defined lineage as a family rank taxon (with respect to other previously defined lineages at that  
708 equivalent rank), it is unreasonable to necessarily expect that some set of morphological features

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

709 will reveal themselves as diagnostic for this newly identified lineage/taxon. A process of  
710 reciprocal illumination may lead to the discovery of such diagnostic characteristics, but that there  
711 are no guarantees. One might infer (based on character homoplasy) that the overriding notion of  
712 taxic homology, that diagnostic characters are used to unite groups (Panchen 1992), was an  
713 implicit driving force in previous attempts delineate mygalomorph families and other higher-  
714 level taxa (*sensu* Raven (1985)); an approach that at times seemingly interpreted diagnosability  
715 as synapomorphy. A diagnostic character(s) for a family does not necessarily infer  
716 synapomorphy any more than characters in a taxonomic key. A lineage that is hierarchically  
717 located at the same level among other family rank taxa should potentially be viewed as a family  
718 rank taxon regardless of whether or not it is morphologically diagnosable.

719 Taxonomic changes referenced herein are formally documented and new family-level  
720 taxa diagnosed in Appendix 1 (authorship to be formally attributed as Bond, Opatova, and Hedin  
721 2019). We acknowledge that the taxonomic changes we propose are unlikely to go without  
722 criticism and will most likely require future revision and refinement. The four guiding principles  
723 we used attempted to realign family-level limits as clearly as possible but remain conservative in  
724 order to minimize errors: 1) revise higher-level mygalomorph classification and familial-level  
725 taxonomy such that it better reflects the evolutionary relationships indicated by data available  
726 (i.e., the sum of collected to data, not just this analysis); 2) further refine the classification, where  
727 possible, based on previous morphological/molecular hypotheses in which subfamilial taxa are  
728 clearly defined and supported by unambiguous synapomorphies; in some instances we  
729 transferred unsampled genera to newly defined families in cases where their placement in said  
730 group appears to be well supported in other studies; 3) minimize changes in family-level  
731 classification to the extent possible that maintains a consistent phylogenetic level across the tree



## PHYLOGENY OF MYGALOMORPH SPIDERS

732 for the family-level rank; and 4) instances where generic-level taxon sampling and criterion 2  
733 above preclude an unambiguous family-level assignment of a genus (for delimited families), we  
734 retain the original family assignment but denote its placement as *incertae sedis* (operationally for  
735 the purposes of this study). We discuss below the restructuring of the major mygalomorph  
736 groups and their constituent families with a particular emphasis on longstanding problems in the  
737 families Dipluridae, Cyrtaucheniidae, and Nemesiidae.

738

739 *Higher Level Groupings: Atypoidea – Avicularioidea – Bipectina – Domiothelina*

740 *Crassitarsae*.—The interpretation of the significance of similarities in spinneret morphology,  
741 constituted one of the center pieces in the debate revolving around the higher-level classification  
742 of the Mygalomorphae (Platnick and Gertsch 1976; Raven 1985; Eskov and Zonshtein 1990;  
743 Goloboff 1993) and taxon composition of some families (Raven 1985). Traditionally,  
744 mygalomorph spiders were divided into two families: Atypidae and Aviculariidae  
745 (=Theraphosidae; Simon 1864), later established as superfamilies Atypoidea and Ctenizoidea  
746 (Chamberlin and Ivie 1946). Atypoidea, a sister group to all the remaining mygalomorph  
747 families, consisted of Atypidae, Antrodiaetidae, and Mecicobothriidae. However, the external  
748 similarities of mecicobothriids and diplurids motivated some authors to reject the placement of  
749 Mecicobothriidae within Atypoidea (Platnick and Gertsch 1976), and subsequently even the  
750 concept of Atypoidina (Atypidae + Antrodiaetidae; Raven 1985) being a sister group to all  
751 remaining mygalomorphs was abandoned in favor of an alternative arrangement (Fig. 1a; Raven  
752 1985). Eskov and Zonshtein (1990) disagreed with some of Raven's character assessments and  
753 alternatively supported Atypoidea (*sensu* Chamberlin and Ivie (1946)), arguing that the shared  
754 traits in mecicobothriids and the diplurids, for example the elongation of spinnerets, constitute an

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

755 ecological convergence related to a construction of a funnel-and-sheet web (Coyle 1986).  
756 However, their alternative hypothesis was rejected in what was the first computer-based cladistic  
757 treatment of mygalomorph relationships (Goloboff 1993), on the basis of different interpretation  
758 of Atypoidea unifying characters presented by Eskov and Zonshtein (1990).

759         Contrary to the prevailing morphology-based hypothesis (Raven 1985; Goloboff 1993),  
760 the first studies using molecular data strongly supported the classic Atypoidea hypothesis (Hedin  
761 and Bond 2006). The root node bifurcation of Mygalomorphae into Atypoidea and  
762 Avicularioidea was further established after adding additional molecular data (Ayoub et al. 2007;  
763 Bond et al. 2012). Although with limited taxon sampling, the same arrangement has been  
764 consistently recovered in subsequent studies employing transcriptome data (Bond et al. 2014;  
765 Garrison et al. 2016) and is also supported in our analyses. A recent study by Hedin et al. (2019);  
766 published while this study was in review, provided a much needed overhaul of the Atypoidea,  
767 recognizing two additional families (Hexurellidae and Megahexuridae).

768         The Avicularioidea clade was recovered in all the analyses with high support and a  
769 degree of internal resolution never achieved in past comprehensive mygalomorph phylogenetic  
770 studies. Similarly, to the results obtained in the past (Goloboff 1993; Hedin and Bond 2006;  
771 Ayoub et al. 2007; Bond et al. 2012), the concatenated analyses recovered a grade of early  
772 branching Avicularioidea lineages representing the so-called “non-diplurine diplurids” (Goloboff  
773 1993), the Hexathelidae, Porrhothelidae and Macrothelidae. Alternatively, the species tree  
774 analysis performed in ASTRAL recovered these lineages, excluding Macrothelidae, in a single  
775 highly supported clade. We statistically explored alternative tree topologies (Table 2) to: 1)  
776 assess the monophyly of the family Dipluridae (*sensu* Raven (1985)), 2) test the species tree  
777 arrangement and 3) further test the monophyly of the family Hexathelidae (*sensu* Raven (1985))

## PHYLOGENY OF MYGALOMORPH SPIDERS

778 within a wider outgroup sampling framework than in Hedin et al. (2018). The rejection of the  
779 alternative topologies by the AU topology tests, the reciprocal monophyly of the “non-diplurine  
780 diplurid” lineages correlated with a unique combination of morphological characters present in  
781 each taxon, provides strong support for taxonomic and nomenclatural changes proposed here and  
782 by other authors (Hedin et al. 2018).

783 As in the previous analyses (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012),  
784 all Avicularioidea families, with exception of the grade of “diplurid” and former “hexathelid”  
785 lineages, were recovered in a single clade, close in taxon composition to the original delineation  
786 of Bipectina (*sensu* Goloboff (1993)). We recovered Paratropididae and Actinopodidae (removed  
787 from Bipectina by Bond et al. (2012)) alongside the Atracidae as part of that clade. The  
788 Bipectina including these three families represent the most inclusive monophyletic arrangement  
789 that is consistently recovered in all the analyses. For this reason, we choose to relimit the  
790 Bipectina clade (*sensu* Bond et al. (2012)) to include Actinopodidae, Atracidae, Stasimopidae,  
791 and Paratropididae alongside the Crassitarsae and Domiothelina.

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

799 Albeit generally accepted as a monophyletic group (Hedin and Bond 2006; Bond et al.  
800 2012), Crassitarsae (Raven 1985; Goloboff 1993) is an unstable clade in our analyses. The group

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

801 was recovered in the concatenated analyses (supported in IQ-TREE ML inference and  
802 genomic/transcriptomic dataset), whereas other analyses supported an alternative placement for  
803 the Theraphosoidina clade (*sensu* Bond et al. (2012)), namely as sister to Domiothelina and the  
804 Stasimopidae/Atracinae/Actinopodidae clade (species tree analysis) or as the sister group to all  
805 Bipectina lineages (No\_Paratropis dataset). Despite the uncertain status of Crassitarsae, both  
806 Theraphosoidina and the clade comprising Cyртаcheniidae, Dipluridae, Microstigmatidae and  
807 former “Nemesiidae” lineages (the “Nemesioidina”, see below), were highly supported and  
808 mostly resolved at internal levels in all analyses. With the results at hand, we can now address  
809 the long-standing taxonomic issues involving the non-monophyly of the families Dipluridae,  
810 Cyртаcheniidae and Nemesiidae. As a result, we recircumscribe the families Cyртаcheniidae,  
811 Dipluridae and Nemesiidae and establish the new families Anamidae (NEW RANK),  
812 Entypesidae (NEW FAMILY), Bemmeridae (NEW RANK), and Pycnothelidae (NEW RANK).

813        “*Dipluridae*”.—The monophyly of the diplurids was first questioned by Goloboff (1993)  
814 relatively soon after the last relimitation of the family (Raven 1985), which without a formal  
815 change being made remains still reflected in the current classification scheme. Diplurids in their  
816 original composition comprised a variety of, now in retrospect, *very* distantly related lineages  
817 possessing to some extent widely spaced “lower” spinnerets and elongated “upper” spinnerets  
818 (Simon 1889); which typifies the pitfalls of establishing groups of the basis of homoplastic,  
819 convergently evolved characteristics. Mecicobothriidae (Gertsch 1979) and Hexathelidae (Raven  
820 1980) were subsequently removed from Dipluridae and established as independent families and a  
821 large number of taxa was then transferred to the family Nemesiidae (Raven 1985). Despite that,  
822 the elongation of posterior lateral spinnerets and the wide separation of posterior median  
823 spinnerets remained among characters uniting the family Dipluridae (Raven 1985). Goloboff

## PHYLOGENY OF MYGALOMORPH SPIDERS

824 (1993) debated the character state scoring of the spinneret elongation in diplurids, hexathelids,  
825 and mecicobothriids, alluding that the character definition was ambiguous, even within  
826 Dipluridae. Goloboff (1993) recovered the Dipluridae (*sensu* Raven (1985)) as four independent  
827 lineages which portended the future relimitation of diplurids to the subfamily Diplurinae (*sensu*  
828 Raven (1985)). The non-monophyly of the diplurids was also cautiously echoed by Coyle (1995)  
829 who, while conducting a taxonomic revision of the subfamily Ischnothelinae, did not find any  
830 strong synapomorphies establishing a sister relationship between ischnothelines and any other  
831 diplurid taxa (Coyle 1995). Diplurids have been recovered both as monophyletic (Ayoub et al.  
832 2007; Bond et al. 2012) and paraphyletic (Hedin and Bond 2006; Wheeler et al. 2017; Hedin et  
833 al. 2018). However, with exception of Hedin et al. (2018), the taxon sampling was restricted to  
834 members of the subfamily Euagrinae and the seemingly contradictory outcome was likely a  
835 result of a lack of strong phylogenetic signal in the sequenced markers. In our analyses, we  
836 sampled representatives from three diplurid subfamilies (Diplurinae, Ischnothelinae, Euagrinae)  
837 plus *Microhexura*, a genus with uncertain placement (Raven 1985); Masteriinae was not  
838 included in this study. Our results are congruent with Goloboff's (1993) hypothesis that  
839 Dipluridae constitutes an assemblage of distantly related lineages that do not share a common  
840 ancestor. The subfamilies Ischnothelinae, Euagrinae + *Microhexura* were recovered as three  
841 independent lineages at the base of the Avicularioidea clade ("non-diplurine diplurids" (Goloboff  
842 1993; Hedin et al. 2018)), whereas Diplurinae taxa were placed as sister to cyrtaucheniids within  
843 proximity to nemesiids (Supplemental figure Fig. 9). The presumed synapomorphy of diplurids  
844 (*sensu* Raven (1985)), elongated and widely spaced spinnerets, could thus be an example of a  
845 retention of plesiomorphic condition (see discussion on silk use) of the "non-diplurine diplurids"  
846 that evolved convergently in Diplurinae – the genomic architecture likely already in place (see

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

847 Uraraneida: *Chimerarachne yingi* (Wang et al. 2018)). As already pointed out by Coyle (1971)  
848 and Eskov and Zonshtein (1990), the traits related to the morphology of the spinnerets are likely  
849 correlated to the type of foraging structure produced by the spiders. “Diplurids” generally build  
850 conspicuous three-dimensional sheet-like webs with a funnel-like retreat (Coyle 1988; Coyle and  
851 Ketner 1990; Coyle 1995; Eberhard and Hazzi 2013; Passanha and Brescovit 2018), a trait  
852 present in many other mygalomorph taxa that have little to no evolutionary proximity to  
853 diplurids (e.g., Mecicobothiidae (Gertsch 1979), Macrothelidae (Snazell and Allison 1989;  
854 Shimojana and Haupt 1998), Atracidae (Gray 2010), and is also paralleled in araneomorph  
855 funnel-web spider family Agelenidae (Nentwig 1983). With the results of our phylogenetic  
856 analyses, it seems safe to assume that spinneret morphology is a plastic trait that has little to no  
857 informative value for *higher-level* classification in mygalomorph spiders. This observation is  
858 further supported by the results of the topology test rejecting the monophyly of Dipluridae (*sensu*  
859 Raven (1985)) as well as the monophyly of a clade consisting of the “non-diplurine diplurids”  
860 (Goloboff 1993), Hexathelidae and Porrhothelidae. We therefore substantially relimit the  
861 boundaries of the family Dipluridae (new circumscription). We elevate the subfamily  
862 Ischnothelidae (NEW RANK) and Euagridae (NEW RANK) to family-level and establish the  
863 new family Microhexuridae (NEW FAMILY).

864 “*Nemesiidae*”.—The cosmopolitan family Nemesiidae belongs among the most speciose  
865 mygalomorph groups. As currently defined, it comprises over 400 nominal species that are  
866 placed in 45 genera. The family was diagnosed by Raven (1985) as those taxa possessing two  
867 rows of teeth on the paired tarsal claws and having scopulate tarsi (Raven 1985); a set of  
868 characters found in other groups and thus likely homoplasious, and later reinterpreted as  
869 plesiomorphic (Goloboff 1993). Not surprisingly, the monophyly of the family Nemesiidae has

## PHYLOGENY OF MYGALOMORPH SPIDERS

870 never been recovered in any broad scale molecular phylogenetic analyses of mygalomorphs  
871 (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012). Moreover, even for analyses based  
872 solely on morphological characters, the family was seemingly split into five different lineages  
873 (Goloboff 1993). Paralleling the non-monophyletic nature of the family, the subfamilies  
874 established by Raven (1985) were also typically recovered as para- or polyphyletic (Goloboff  
875 1993; Goloboff 1995; Hedin and Bond 2006; Bond et al. 2012). For this current study, we  
876 sampled five out of six nemesiid subfamilies (Raven 1985) and about 40% of the generic  
877 diversity to more thoroughly assess the composition of the family, particularly with an emphasis  
878 on resolving the position of the family Microstigmatidae and several difficult to place  
879 “cyrtaucheniid” genera (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al.  
880 2012; Wheeler et al. 2017). Like all other studies conducted to date (Goloboff 1993; Hedin and  
881 Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Garrison et al. 2016; Wheeler et al. 2017), we  
882 did not recover the Nemesiidae as monophyletic (Supplemental Fig. 9). The South African genus  
883 *Spiroctenus* was placed within Bemmeridae, whereas the remaining nemesiids were parceled  
884 among five reciprocally monophyletic lineages forming a clade with the cyrtaucheniids,  
885 diplurids, and microstigmatids (the “Nemesioidina” clade). Both microstigmatids and some  
886 “cyrtaucheniids” have been recovered as lineages embedded within Nemesiidae in the past  
887 (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Wheeler et al.  
888 2017). Goloboff (1993), suggested that the some of the microstigmatid synapomorphies may be  
889 of neotenic nature (as proposed previously by Raven (1985) and Griswold (1985)),  
890 foreshadowing the eventual synonymy of microstigmatids with the nemesiids, or alternatively,  
891 splintering Nemesiidae into multiple families. Similar alternatives were also proposed for  
892 Cyrtaucheniidae (*sensu* Raven (1985)) based on the position of *Acontius*, “*Cyrtauchenius*”, and

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

893 *Fufius* (Hedin and Bond 2006; Bond et al. 2012). Unfortunately, we cannot assess the position of  
894 *Acontius* and *Fufius* in this study. However, the sample representing “*Cyrtauchenius*” in Bond et  
895 al. (2012), in fact, corresponds to *Amblyocarenum*, later removed from its synonymy (Decae and  
896 Bosmans 2014). Although the phylogenetic placement of both genera could be conservatively  
897 interpreted as “embedded within Nemesiidae *sensu lato*, the placement of *Amblyocarenum* along  
898 with other genera likely has more severe taxonomic implications. *Amblyocarenum* is placed as a  
899 sole “cyrtaucheniid” within a monophyletic clade comprising the genus *Nemesia*, the type genus  
900 of the family. The affinities between *Amblyocarenum* and other nemesiids were also noted when  
901 the genus was removed from synonymy with *Cyrtauchenius* (Decae and Bosmans 2014). On the  
902 other hand, *Cyrtauchenius* forms a well-supported clade with two other cyrtaucheniid genera and  
903 is placed as sister to another monophyletic and morphologically distinguishable clade, the  
904 Dipluridae (*sensu* this study). The Dipluridae – Cyrtaucheniidae relationship and their placement  
905 within Nemesiidae was previously recovered in the pilot assessment of mygalomorph phylogeny  
906 with AHE data (Hamilton et al. 2016b), however, the sampling limitations of the study and the  
907 interpretation of *Fufius* and “*Linothele*” (later identified as *Diplura*) as taxonomically misplaced  
908 nemesiids alluded to a different conclusion than presented in this paper. The situation is similar  
909 for Microstigmatidae, presumably another “candidate” nemesiid lineage (Hedin and Bond 2006;  
910 Ayoub et al. 2007; Bond et al. 2012). The genus *Microstigmata* has been recovered in proximity  
911 to *Ixamatus* and another Australian nemesiid genus *Kiama* (Bond et al. 2012), which is  
912 congruent with our results. *Kiama* presents a unique combination of morphological features that,  
913 to some extent, overlaps with diagnostic characters for a handful of families, and as a result, the  
914 placement of the genus vacillated among Dipluridae (Main and Mascord 1969), Cyrtaucheniidae  
915 (Raven 1985) and Nemesiidae (Bond et al. 2012). Both *Kiama* and *Microstigmata* possess a



## PHYLOGENY OF MYGALOMORPH SPIDERS

916 uniquely modified cuticle and share a similar tarsal organ morphology (Raven 1985; Bond and  
917 Opell 2002), however these similarities were dismissed as convergent or plesiomorphic by others  
918 (Raven 1985; Goloboff 1993). The homology of these characters consequently needs to be  
919 reassessed in a framework using other lines of evidence other than just morphology (Bond et al.  
920 2012; Hedin et al. 2018).

921         The dilemma of whether to dissolve the Nemesiidae, or redefine the family boundaries to  
922 include microstigmatids and cyrtaucheniids has been repeatedly discussed in the literature  
923 (Goloboff 1993; Hedin and Bond 2006; Bond et al. 2012), with tendencies gravitating towards  
924 the later solution (e.g. Bond et al. 2012; Fig. 1C in Hedin et al. (2018)). However, there were  
925 several factors at play reinforcing the argument for the “inclusive” Nemesiidae. First and  
926 foremost, the limited taxon sampling in the previous analyses (Hedin and Bond 2006; Ayoub et  
927 al. 2007; Bond et al. 2012; Hamilton et al. 2016b) partially skewed the interpretation of the  
928 results. The polyphyly of the family Cyrtaucheniidae (*sensu* Raven (1985)), misplacement of  
929 *Cyrtauchenius* (see above) and lack of adequate representation of the diplurines (*sensu* Raven  
930 (1985)) presented major hurdles for accurately interpreting nemesiid relationships. Recovering  
931 *Microstigmata* or a few genera of an apparently polyphyletic family embedded within an  
932 enlarged “nemesiid” construct understandably supported the notion that a broad synonymization  
933 (lumping rather than splitting) might be the more stable and thus preferable taxonomic solution.  
934 Adding to the overall ambiguity of the results, large sections of the recovered trees often lacked  
935 support (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Wheeler et al. 2017),  
936 adding uncertainty to the monophyly of the groups in question. We would suggest that our  
937 improved sampling and high support values brings long-needed insight and resolution to the  
938 “nemesiid” problem, which is essential for ultimately answering the question of whether or not

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

939 the Nemesiidae should be fragmented into independent families or not. The family Nemesiidae  
940 (*sensu* Raven (1985)) has lacked a well-supported synapomorphy, a widely recognized fact  
941 (Goloboff 1993; Goloboff 1995; Bond et al. 2012; Harvey et al. 2018), since the family was first  
942 delimited (Raven 1985). On the other hand, some groups (e.g., subfamilies) currently recognized  
943 within the family, do possess diagnostic synapomorphic characters (Harvey et al. 2018), which is  
944 also the case for the diplurids and cyrtaucheniids (*sensu* this study) that were recovered as  
945 mutually exclusive lineages within the larger more inclusive “Nemesioidina” clade. We thus  
946 argue that the original concept of the family (*sensu* Raven (1985)) must be abandoned in favor of  
947 establishing multiple monophyletic families that can also be ultimately clearly defined  
948 morphologically. Another fact in favor of such an arrangement is the relatively deep divergences  
949 separating the lineages (Kuntner et al. 2018). Major lineages across the entire clade diversified  
950 into their respective individual groups between 115 – 79 Ma, roughly congruent with dated  
951 divergences among other families (e.g., Theraphosidae and Barychelidae split around 107 Ma  
952 and Actinopodidae diverged from Atracidae approximately 98 Ma). In agreement with the results  
953 of our analyses, we therefore relimit the family Nemesiidae (new circumscription) as outlined in  
954 the Appendix 1.

955

956 CONCLUSIONS AND FUTURE DIRECTIONS

957

958 It is our hope that this study represents a significant leap forward in stabilizing  
959 mygalomorph spider classification via a robust, well-supported phylogenetic framework.  
960 Extensive taxon sampling combined with enhanced phylogenetic resolution using genomic-scale  
961 data facilitates the proposal of an alternative classification scheme that more accurately reflects

## PHYLOGENY OF MYGALOMORPH SPIDERS

962 the evolutionary relationships of the infraorder. Consequently, these results brought to their  
963 logical fruition require extensive changes to existing familial composition as well as the  
964 establishment of a number of new family-rank taxa. Understandably, such changes require  
965 considerable downstream scrutiny and criticism. This newly derived framework is a hypothesis  
966 that begs further testing through additional data and increased taxon sampling.

967         One observation in particular from this study that seems clear is that morphological data  
968 are not uniformly suitable for reconstructing mygalomorph higher-level relationships, and to a  
969 slightly lesser degree for delimiting family rank taxa (e.g., subfamily relationships seem to be  
970 relatively stable as evidenced by the number of subfamilies elevated to family rank). Raven's  
971 (1985) results were in no small part afflicted by the rampant homoplasy that seemingly plagues  
972 this group of spiders. Given the data and lack of computational resources available when the  
973 analyses were conducted, the results Raven achieved represented the strongest prevailing  
974 hypothesis at that time and provided a framework upon which to test new hypotheses. In 1985  
975 and shortly thereafter (Eskov and Zonshtein 1990) disagreement regarding morphological  
976 evidence for various clades and familial-level relationships and identity were in retrospect  
977 matters of subjective opinion and consequently difficult to test (likely explaining why some  
978 seemingly correct hypotheses, e.g., *Atypoidea*, were ignored). Alternatively though, it is  
979 important to recognize that a relatively large percentage of the new family rank taxa were once  
980 morphologically defined subfamilies – a number of which predate Raven (1985) – and thus  
981 relatively correct.

982         Issues regarding spider classification notwithstanding, phylogenomics provides an  
983 exciting new framework for considering broad scale questions related to spider evolution. Our  
984 biogeographic and dating analyses suggest that ancient vicariance and dispersal shaped the

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

985 currently observed biogeographic patterns. Much of the deep phylogenetic structure across the  
986 infraorder may be attributable to climatic heterogeneity and oscillation prior to the breakup of  
987 Gondwana/Pangea. Such deep phylogenetic and ancient divergence, coupled with low vagility,  
988 makes these spiders ideal candidates to test biogeographic hypotheses.

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

997         Considerable work remains in achieving final resolution of mygalomorph relationships  
998 and stable fully-resolved familial composition. Despite our best efforts, which included a  
999 combination of transcriptomic and AHE data, we are still unable to resolve several of the deepest  
1000 nodes; Paratropididae remains a difficult taxon to place as do the newly formed Stasimopidae.  
1001 Despite our best efforts and major relimitation, many nemesiid, diplurid, and cyrtaucheniid  
1002 genera remain in an uncertain taxonomic state. Although we could speculate about placement of  
1003 many taxa (e.g., Aporoptychinae cyrtaucheniids, Masteriinae diplurids, as well as numerous  
1004 South American nemesiids), such questions will have to be left to future studies. At the very  
1005 least, it is our hope that this study facilitates further interest and provides a solid foundation for  
1006 future work in this intriguing, and ancient group of spiders.

1007

## PHYLOGENY OF MYGALOMORPH SPIDERS

1008 ACKNOWLEDGEMENTS

1009

1010 We thank M. Arnedo, R. Booyesen, A. Decae, M. Forman, N. Garrison, R. Godwin, J. Grismer,

1011 C. Haddad, S. Huber, V. Hula, P. Just, J. Malumbres-Olarte, T. Mazuch, JA. Neethling, J.

1012 Rodriguez, F. Štáhlavský, J. Starrett, C. Stephen, and D. Weinmann for help with field collecting

1013 and providing additional samples. We are grateful to M. Harvey, M. Kuntner, C. Griswold, S.

1014 Zonshtein, B. Wiegmann, J. Ledford, and an anonymous reviewer for comments on earlier

1015 versions of the manuscript. The computational analyses were completed in part with resources

1016 provided by the Auburn University Hopper supercomputing cluster. This project was funded in

1017 part by National Science Foundation grants DEB-0841610 to JEB, Doctoral Dissertation

1018 Improvement Grant DEB-1311494 to CAH, Czech Science Foundation grant 16-10298S to JK,

1019 Auburn University Department of Biological Sciences and College of Sciences and Mathematics

1020 (JEB), an Auburn University Cellular and Molecular Biosciences Peaks of Excellence Research

1021 Fellowship (CAH), and Evert and Marion Schlinger Foundation, University of California Davis

1022 (JEB).

1023

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1024 LITERATURE CITED

1025

1026 Aberer AJ, Kobert K, Stamatakis A. 2014. ExaBayes: massively parallel Bayesian tree inference  
1027 for the whole-genome era. *Mol Biol Evol*, 31:2553-2556.

1028

1029 Andújar C, Faille A, Pérez-González S, Zaballos JP, Vogler AP, Ribera I. 2016. Gondwanian  
1030 relicts and oceanic dispersal in a cosmopolitan radiation of euedaphic ground beetles.  
1031 *Mol. Phylogen. Evol.*, 99:235-246.

1032

1033 Anisimova M, Gil M, Dufayard J-F, Dessimoz C, Gascuel O. 2011. Survey of branch support  
1034 methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation  
1035 schemes. *Syst Biol*, 60:685-699.

1036

1037 Ayoub NA, Garb JE, Hedin M, Hayashi CY. 2007. Utility of the nuclear protein-coding gene,  
1038 elongation factor-1 gamma (EF-1g), for spider systematics, emphasizing family level  
1039 relationships of tarantulas and their kin (Araneae: Mygalomorphae). *Mol. Phylogen. Evol.*,  
1040 42:394-409.

1041

1042 Beaulieu JM, O'Meara BC, Donoghue MJ. 2013. Identifying hidden rate changes in the  
1043 evolution of a binary morphological character: the evolution of plant habit in campanulid  
1044 angiosperms. *Syst Biol*, 62:725-737.

1045

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1046 Beutel E, Nomade S, Fronabarger A, Renne P. 2005. Pangea's complex breakup: A new rapidly  
1047 changing stress field model. *Earth and Planetary Science Letters*, 236:471-485.  
1048
- 1049 Boger SD. 2011. Antarctica—before and after Gondwana. *Gondwana Research*, 19:335-371.  
1050
- 1051 Bolotov IN, Vikhrev IV, Bespalaya YV, Gofarov MY, Kondakov AV, Konopleva ES, Bolotov  
1052 NN, Lyubas AA. 2016. Multi-locus fossil-calibrated phylogeny, biogeography and a subgeneric  
1053 revision of the Margaritiferidae (Mollusca: Bivalvia: Unionoida). *Mol. Phylogenet. Evol.*,  
1054 103:104-121.  
1055
- 1056 Bond J, Opell BD. 2002. Phylogeny and taxonomy of the genera of south-western North  
1057 American Euctenizinae trapdoor spiders and their relatives (Araneae: Mygalomorphae,  
1058 Cyrtaucheniidae). *Zool. J. Linn. Soc.* , 136:487-534.  
1059
- 1060 Bond JE. 1994. Seta-spigot homology and silk production in first instar *Antrodiaetus unicolor*  
1061 spiderlings (Araneae: Antrodiaetidae). *J. Arachnol.*:19-22.  
1062
- 1063 Bond JE. 2004. Systematics of the Californian euctenizine spider genus *Apomastus* (Araneae :  
1064 Mygalomorphae : Cyrtaucheniidae): the relationship between molecular and morphological  
1065 taxonomy. *Invertebr Systemat*, 18:361-376.  
1066

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1067 Bond JE. 2017. Euctenizidae. In Ubick, D., Paquin, P. Cushing, P., Roth, V. (eds.) Spiders of  
1068 North American identification manual, 2nd Edition, 55-57. New Hampshire, American  
1069 Arachnological Society.  
1070
- 1071 Bond JE, Coyle FA. 1995. Observations on the natural history of an *Ummidia* trapdoor spider  
1072 from Costa Rica (Araneae, Ctenizidae). *J. Arachnol.*, 23:157-164.  
1073
- 1074 Bond JE, Garrison NL, Hamilton CA, Godwin RL, Hedin M, Agnarsson I. 2014. Phylogenomics  
1075 resolves a spider backbone phylogeny and rejects a prevailing paradigm for orb web evolution.  
1076 *Current Biology*, 24:1765-1771.  
1077
- 1078 Bond JE, Hedin M. 2006. A total evidence assessment of the phylogeny of North American  
1079 euctenizine trapdoor spiders (Araneae, Mygalomorphae, Cyrtaucheniidae) using Bayesian  
1080 inference. *Mol. Phylogenet. Evol.*, 41:70-85.  
1081
- 1082 Bond JE, Hedin MC, Ramirez MG, Opell BD. 2001. Deep molecular divergence in the absence  
1083 of morphological and ecological change in the Californian coastal dune endemic trapdoor spider  
1084 *Aptostichus simus*. *Mol Ecol*, 10:899-910.  
1085
- 1086 Bond JE, Hendrixson BE, Hamilton CA, Hedin M. 2012. A Reconsideration of the Classification  
1087 of the Spider Infraorder Mygalomorphae (Arachnida: Araneae) Based on Three Nuclear Genes  
1088 and Morphology. *PLoS ONE*, 7:e38753.  
1089



## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1090 Bond JE, Stockman AK. 2008. An integrative method for delimiting cohesion species: Finding  
1091 the population-species interface in a group of Californian trapdoor spiders with extreme genetic  
1092 divergence and geographic structuring. *Syst Biol*, 57:628 - 646.
- 1093
- 1094 Bouckaert R, Vaughan TG, Barido-Sottani J, Duchene S, Fourment M, Gavryushkina A, Heled J,  
1095 Jones G, Kuhnert D, De Maio N. 2018. BEAST 2.5: An Advanced Software Platform for  
1096 Bayesian Evolutionary Analysis. *BioRxiv*:474296.
- 1097
- 1098 Boufford DE, Spongberg SA. 1983. Eastern Asian-eastern North American phytogeographical  
1099 relationships-a history from the time of Linnaeus to the twentieth century. *Ann Mo Bot Gard*,  
1100 70:423-439.
- 1101
- 1102 Boyer SL, Clouse RM, Benavides LR, Sharma P, Schwendinger PJ, Karunarathna I, Giribet G.  
1103 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally  
1104 distributed group of arachnids. *J Biogeography*, 34:2070-2085.
- 1105
- 1106 Castalanelli MA, Huey JA, Hillyer MJ, Harvey MS. 2017. Molecular and morphological  
1107 evidence for a new genus of small trapdoor spiders from arid Western Australia (Araneae:  
1108 Mygalomorphae: Nemesiidae: Anaminae). *Invertebr Systemat*, 31:492-505.
- 1109
- 1110 Chamberland L, McHugh A, Kechejian S, Binford GJ, Bond JE, Coddington J, Dolman G,  
1111 Hamilton CA, Harvey MS, Kuntner M. 2018. From Gondwana to GAAR landia: Evolutionary  
1112 history and biogeography of ogre-faced spiders (*Deinopis*). *J Biogeography*, 45:2442-2457.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1113

1114 Chamberlin RV. 1917. New spiders of the family Aviculariidae. Bull. Mus. Comp. Zool., 61:25-

1115 75

1116

1117 Chamberlin RV, Ivie W. 1946. On several new American spiders. University of Utah.

1118

1119 Chen Z-Q, Benton MJ. 2012. The timing and pattern of biotic recovery following the end-

1120 Permian mass extinction. Nature Geoscience, 5:375.

1121

1122 Clouse RM, Branstetter MG, Buenaente P, Crowley LM, Czekanski-Moir J, General DEM,

1123 Giribet G, Harvey MS, Janies DA, Mohagan AB. 2017. First global molecular phylogeny and

1124 biogeographical analysis of two arachnid orders (Schizomida and Uropygi) supports a tropical

1125 Pangean origin and mid-Cretaceous diversification. J Biogeography, 44:2660-2672.

1126

1127 Coccioni R, Galeotti S. 1994. KT boundary extinction: Geologically instantaneous or gradual

1128 event? Evidence from deep-sea benthic foraminifera. Geology, 22:779-782.

1129

1130 Cock PJ, Antao T, Chang JT, Chapman BA, Cox CJ, Dalke A, Friedberg I, Hamelryck T, Kauff

1131 F, Wilczynski B. 2009. Biopython: freely available Python tools for computational molecular

1132 biology and bioinformatics. Bioinformatics, 25:1422-1423.

1133

1134 Coddington JA, Agnarsson I, Hamilton C, Bond JE. 2018. Spiders did not repeatedly gain, but

1135 repeatedly lost, foraging webs. PeerJ Preprints, 6:e27341v27341.

## PHYLOGENY OF MYGALOMORPH SPIDERS

1136

1137 Coyle F. 1986. The role of silk in prey capture by nonaraneomorph spiders.

1138

1139 Coyle FA. 1971. Systematics and natural history of the mygalomorph spider genus *Antrodiaetus*

1140 and related genera (Araneae: Antrodiaetidae). Harvard Univ Mus Compar Zool Bull.

1141

1142 Coyle FA. 1981. The mygalomorph spider genus *Microhexura* (Araneae, Dipluridae). Bull. Am.

1143 Mus. Nat. Hist., 170:64-75.

1144

1145 Coyle FA. 1986. *Chilehexops*, a new funnelweb mygalomorph spider genus from Chile

1146 (Araneae, Dipluridae). American Museum Novitates; no. 2860.

1147

1148 Coyle FA. 1988. A revision of the American funnel web mygalomorph spider genus *Euagrus*

1149 (Araneae, Dipluridae). Bulletin of the American Museum of Natural History., 187:203-292.

1150

1151 Coyle FA. 1995. A revision of the funnelweb mygalomorph spider subfamily Ischnothelinae

1152 (Araneae, Dipluridae). Bulletin of the AMNH; no. 226.

1153

1154 Coyle FA, Dellinger RE, Bennet R. 1992. Retreat architecture and construction behavior of an

1155 East African idiopine trapdoor spider (Araneae, Idiopidae). Bulletin of the of the British

1156 Arachnological Society, 9:99-104.

1157

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1158 Coyle FA, Ketner ND. 1990. Observations on the prey and prey capture behaviour of the  
1159 funnelweb mygalomorph spider genus *Ischnothele* (Araneae, Dipluridae). Bull Br arachnol Soc,  
1160 8:97-104.  
1161
- 1162 Cracraft J. 1988. Deep-history biogeography: retrieving the historical pattern of evolving  
1163 continental biotas. Syst Biol, 37:221-236.  
1164
- 1165 Decae AE. 1996. Systematics of the trapdoor spider genus *Cyrtocarenum* Ausserer, 1871  
1166 (Araneae, Ctenizidae). Bulletin of British arachnological Society, 10:161-170.  
1167
- 1168 Decae AE, Bosmans R. 2014. Synonymy of the Trapdoor Spider Genera *Cyrtauchenius* Thorell,  
1169 1869 and *Amblyocarenum* Simon, 1892 Reconsidered (Araneae, Mygalomorphae,  
1170 *Cyrtaucheniidae*). Arachnology, 16:182-192.  
1171
- 1172 Dippenaar-Schoeman AS. 2002. Baboon and trapdoor spiders of Southern Africa: an  
1173 identification manual. Agricultural Research Council Pretoria.  
1174
- 1175 Domeier M, Torsvik TH. 2014. Plate tectonics in the late Paleozoic. Geoscience Frontiers,  
1176 5:303-350.  
1177
- 1178 Eberhard WG, Hazzi NA. 2013. Web construction of *Linothele macrothelifera* (Araneae:  
1179 Dipluridae). The Journal of Arachnology, 41:70-75.  
1180

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1181 Engelbrecht I, Prendini L. 2012. Cryptic diversity of South African trapdoor spiders: Three new  
1182 species of *Stasimopus* Simon, 1892 (Mygalomorphae, Ctenizidae), and redescription of  
1183 *Stasimopus robertsi* Hewitt, 1910. American Museum Novitates:1-42.  
1184
- 1185 Eskov K, Zonshtein S. 1990. First Mesozoic mygalomorph spiders for the Lower Cretaceous of  
1186 Siberia and Mongolia, with notes on the infraorder Mygalomorphae (Chelicerata: Araneae).  
1187 Neue Jahrbuch für Geologie und Palaeontologie Abhandlungen, 178:325–368.  
1188
- 1189 Eskov KY, Selden PA. 2005. First record of spiders from the Permian period (Araneae:  
1190 Mesothelae). Bull Br arachnol Soc, 13:111-116.  
1191
- 1192 Faircloth BC, McCormack JE, Crawford NG, Harvey MG, Brumfield RT, Glenn TC. 2012.  
1193 Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary  
1194 timescales. Syst Biol, 61:717-726.  
1195
- 1196 Fernández R, Hormiga G, Giribet G. 2014. Phylogenomic analysis of spiders reveals  
1197 nonmonophyly of orb weavers. Current Biology, 24:1772-1777.  
1198
- 1199 Fernández R, Kallal RJ, Dimitrov D, Ballesteros JA, Arnedo MA, Giribet G, Hormiga G. 2018.  
1200 Phylogenomics, Diversification Dynamics, and Comparative Transcriptomics across the Spider  
1201 Tree of Life. Current Biology, 28:1489-1497. e1485.  
1202

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1203 Forster R. 1968. The spiders of New Zealand. Part II. Ctenizidae, Dipluridae and Migidae. Otago  
1204 Mus. Bull.  
1205
- 1206 Frazão A, da Silva HR, de Moraes Russo CA. 2015. The Gondwana breakup and the history of  
1207 the Atlantic and Indian oceans unveils two new clades for early neobatrachian diversification.  
1208 PloS one, 10:e0143926.  
1209
- 1210 Gabriel R, Longhorn SJ. 2015. Revised generic placement of *Brachypelma embrithes*  
1211 (Chamberlin & Ivie, 1936) and *Brachypelma angustum* Valerio, 1980, with definition of the  
1212 taxonomic features for identification of female *Sericopelma* Ausserer, 1875 (Araneae,  
1213 Theraphosidae). ZooKeys:75.  
1214
- 1215 Gallon RC. 2002. Revision of the African genera *Pterinochilus* and *Eucratoscelus* (Araneae,  
1216 Theraphosidae, Harpactirinae) with description of two new genera. Bull Br arachnol Soc,  
1217 12:201-232.  
1218
- 1219 Garrison NL, Rodriguez J, Agnarsson I, Coddington JA, Griswold CE, Hamilton CA, Hedin M,  
1220 Kocot KM, Ledford JM, Bond JE. 2016. Spider phylogenomics: untangling the Spider Tree of  
1221 Life. PeerJ, 4:e1719.  
1222
- 1223 Garwood RJ, Dunlop JA, Selden PA, Spencer AR, Atwood RC, Vo NT, Drakopoulos M. 2016.  
1224 Almost a spider: a 305-million-year-old fossil arachnid and spider origins. Proc. R. Soc. B,  
1225 283:20160125.

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1226
- 1227 Gertsch WJ. 1979. A revision of the spider family Mecicobothriidae (Araneae, Mygalomorphae).
- 1228 American Museum novitates; no. 2687.
- 1229
- 1230 Gertsch WJ, Platnick NI. 1980. A revision of the American spiders of the family Atypidae
- 1231 (Araneae, Mygalomorphae). American Museum novitates; no. 2704.
- 1232
- 1233 Godwin RL, Opatova V, Garrison NL, Hamilton CA, Bond JE. 2018. Phylogeny of a
- 1234 cosmopolitan family of morphologically conserved trapdoor spiders (Mygalomorphae,
- 1235 Ctenizidae) using Anchored Hybrid Enrichment, with a description of the family,
- 1236 Halonoproctidae Pocock 1901. *Mol. Phylogenet. Evol.*, 126:303-313.
- 1237
- 1238 Goloboff PA. 1993. A reanalysis of mygalomorph spider families (Araneae). American Museum
- 1239 Novitates, 3056:1–32.
- 1240
- 1241 Goloboff PA. 1995. A revision of the South American spiders of the family Nemesiidae
- 1242 (Araneae, Mygalomorphae): Part I: Species from Peru, Chile, Argentina, and Uruguay. *Bull.*
- 1243 *Am. Mus. Nat. Hist.*:1-189.
- 1244
- 1245 Golonka J. 2007. Late Triassic and Early Jurassic palaeogeography of the world.
- 1246 *Palaeogeography, Palaeoclimatology, Palaeoecology*, 244:297-307.
- 1247

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1248 Graham MR, Hendrixson BE, Hamilton CA, Bond JE. 2015. Miocene extensional tectonics  
1249 explain ancient patterns of diversification among turret-building tarantulas (*Aphonopelma*  
1250 *mojave* group) in the Mojave and Sonoran deserts. *J Biogeography*, 42:1052-1065.  
1251
- 1252 Gray M. 2010. A Revision of the Australian Funnel-web Spiders (Hexathelidae: Atracinae).  
1253 Records of the Australian Museum, 62:285-392.  
1254
- 1255 Griswold CE. 1985. A revision of the African spiders of the family Microstigmatidae (Araneae:  
1256 Mygalomorphae). *Annals of the Natal Museum*, 27:1-37.  
1257
- 1258 Griswold CE, Ledford J. 2001. A Monograph of the Migid Trap Door Spiders of Madagascar:  
1259 And a Review of the World Genera (Araneae, Mygalomorphae, Migidae). California Academy  
1260 of Sciences.  
1261
- 1262 Guadanucci JPL. 2005. Tarsal scopula significance in ischnocolinae phylogenetics (Araneae,  
1263 Mygalomorphae, Theraphosidae). *J. Arachnol.*:456-467.  
1264
- 1265 Guadanucci JPL. 2011. Cladistic analysis and biogeography of the genus *Oligoxystre* Vellard  
1266 1924 (Araneae: Mygalomorphae: Theraphosidae). *J. Arachnol.*, 39:320-326.  
1267
- 1268 Guadanucci JPL. 2014. Theraphosidae phylogeny: relationships of the ‘Ischnocolinae’ genera  
1269 (Araneae, Mygalomorphae). *Zool.Scr.*, 43:508-518.  
1270



## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1271 Guadanucci JPL, Wendt I. 2014. Revision of the spider genus *Ischnocolus* Ausserer, 1871  
1272 (Mygalomorphae: Theraphosidae: Ischnocolinae). J. Nat. Hist. , 48:387-402.  
1273
- 1274 Hamelryck T, Manderick B. 2003. PDB file parser and structure class implemented in Python.  
1275 Bioinformatics, 19:2308-2310.  
1276
- 1277 Hamilton CA, Formanowicz DR, Bond JE. 2011. Species Delimitation and Phylogeography of  
1278 *Aphonopelma hentzi* (Araneae, Mygalomorphae, Theraphosidae): Cryptic Diversity in North  
1279 American Tarantulas. PLoS ONE, 6:e26207.  
1280
- 1281 Hamilton CA, Hendrixson BE, Bond JE. 2016a. Taxonomic revision of the tarantula genus  
1282 *Aphonopelma* Pocock, 1901 (Araneae, Mygalomorphae, Theraphosidae) within the United  
1283 States. ZooKeys:1.  
1284
- 1285 Hamilton CA, Hendrixson BE, Brewer MS, Bond JE. 2014. An evaluation of sampling effects on  
1286 multiple DNA barcoding methods leads to an integrative approach for delimiting species: a case  
1287 study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae,  
1288 Theraphosidae). Mol Phylogenet Evol, 71:79-93.  
1289
- 1290 Hamilton CA, Lemmon AR, Lemmon EM, Bond JE. 2016b. Expanding anchored hybrid  
1291 enrichment to resolve both deep and shallow relationships within the spider tree of life. BMC  
1292 Evolutionary Biology, 16:212.  
1293

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1294 Harrison SE, Harvey MS, Cooper SJ, Austin AD, Rix MG. 2017. Across the Indian Ocean: A  
1295 remarkable example of trans-oceanic dispersal in an austral mygalomorph spider. PloS one,  
1296 12:e0180139.  
1297
- 1298 Harvey MS, Hillyer MJ, Main BY, Moulds TA, Raven RJ, Rix MG, Vink CJ, Huey JA. 2018.  
1299 Phylogenetic relationships of the Australasian open-holed trapdoor spiders (Araneae:  
1300 Mygalomorphae: Nemesiidae: Anaminae): multi-locus molecular analyses resolve the generic  
1301 classification of a highly diverse fauna. Zool. J. Linn. Soc., 184:407-452  
1302
- 1303 Hedin M, Bond JE. 2006. Molecular phylogenetics of the spider infraorder Mygalomorphae  
1304 using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of  
1305 classification. Mol. Phylogenet. Evol., 41:454-471.  
1306
- 1307 Hedin M, Carlson D, Coyle F. 2015. Sky island diversification meets the multispecies  
1308 coalescent–divergence in the spruce-fir moss spider (*Microhexura montivaga*, Araneae,  
1309 Mygalomorphae) on the highest peaks of southern Appalachia. Mol Ecol, 24:3467-3484.  
1310
- 1311 Hedin M, Derkarabetian S, Alfaro A, Ramírez MJ, Bond JE. 2019. Phylogenomic analysis and  
1312 revised classification of atypoid mygalomorph spiders (Araneae, Mygalomorphae), with notes on  
1313 arachnid ultraconserved element loci. PeerJ 7:e6864 <http://doi.org/10.7717/peerj.6864>.  
1314

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1315 Hedin M, Derkarabetian S, Ramírez MJ, Vink C, Bond JE. 2018. Phylogenomic reclassification  
1316 of the world's most venomous spiders (Mygalomorphae, Atracinae), with implications for  
1317 venom evolution. *Scientific reports*, 8:1636.  
1318
- 1319 Hedin M, Starrett J, Hayashi C. 2013. Crossing the uncrossable: novel trans-valley  
1320 biogeographic patterns revealed in the genetic history of low-dispersal mygalomorph spiders  
1321 (Antrodiaetidae, *Antrodiaetus*) from California. *Mol Ecol*, 22:508-526.  
1322
- 1323 Hendrixson BE, Bond JE. 2009. Evaluating the efficacy of continuous quantitative characters for  
1324 reconstructing the phylogeny of a morphologically homogeneous spider taxon (Araneae,  
1325 Mygalomorphae, Antrodiaetidae, *Antrodiaetus*). *Mol. Phylogenet. Evol.*, 53:300-313.  
1326
- 1327 Hendrixson BE, DeRussy BM, Hamilton CA, Bond JE. 2013. An exploration of species  
1328 boundaries in turret-building tarantulas of the Mojave Desert (Araneae, Mygalomorphae,  
1329 Theraphosidae, *Aphonopelma*). *Mol Phylogenet Evol*, 66:327-340.  
1330
- 1331 Hewitt J. 1915. VI. Notes on several four-lunged spiders in the collection of the Durban Museum  
1332 with descriptions of two new forms. *Durban Museum Novitates*, 1:125-133.  
1333
- 1334 Hewitt J. 1916. Descriptions of new South African spiders. *Annals of the Transvaal Museum*,  
1335 5:180-213.  
1336

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1337 Hughes N, McDougall A. 1990. New Wealden correlation for the Wessex basin. Proceedings of  
1338 the Geologists' Association, 101:85-90.  
1339
- 1340 Indicatti RP, Lucas SM. 2005. Description of a new genus of Nemesiidae (Araneae,  
1341 Mygalomorphae) from the Brazilian Cerrado. Zootaxa, 1088:11-16.  
1342
- 1343 Isbister GK, Gray MR, Balit CR, Raven RJ, Stokes BJ, Porges K, Tankel AS, Turner E, White J,  
1344 Fisher MM. 2005. Funnel-web spider bite: a systematic review of recorded clinical cases. The  
1345 Medical Journal of Australia, 182:407-411.  
1346
- 1347 Isbister GK, Sellors KV, Beckmann U, Chiew AL, Downes MA, Berling I. 2015.  
1348 Catecholamine-induced cardiomyopathy resulting from life-threatening funnel-web spider  
1349 envenoming. Med J Aust, 203:302-304.  
1350
- 1351 Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7:  
1352 improvements in performance and usability. Mol Biol Evol, 30:772-780.  
1353
- 1354 Kim SI, Farrell BD. 2015. Phylogeny of world stag beetles (Coleoptera: Lucanidae) reveals a  
1355 Gondwanan origin of Darwin's stag beetle. Mol. Phylogenet. Evol., 86:35-48.  
1356
- 1357 Král J, Kořínková T, Krkavcová L, Musilová J, Forman M, Herrera IMÁ, Haddad CR, Vítková  
1358 M, Henriques S, Vargas JGP. 2013. Evolution of karyotype, sex chromosomes, and meiosis in  
1359 mygalomorph spiders (Araneae: Mygalomorphae). Biol. J. Linn. Soc. , 109:377-408.

## PHYLOGENY OF MYGALOMORPH SPIDERS

1360

1361 Kück P. 2009. ALICUT: a Perlscript which cuts ALISCORE identified RSS. Department of  
1362 Bioinformatics, Zoologisches Forschungsmuseum A. Koenig (ZFMK), Bonn, Germany, version,  
1363 2.

1364

1365 Kück P, Longo GC. 2014. FASconCAT-G: extensive functions for multiple sequence alignment  
1366 preparations concerning phylogenetic studies. *Frontiers in Zoology*, 11:81.

1367

1368 Kuntner M, Hamilton CA, Cheng R-C, Gregoric M, Lupse N, Lokovsek T, Lemmon E, Lemmon  
1369 A, Agnarsson I, Coddington JA. 2018. Golden Orbweavers Ignore Biological Rules:  
1370 Phylogenomic and Comparative Analyses Unravel a Complex Evolution of Sexual Size  
1371 Dimorphism. *bioRxiv*:368233.

1372

1373 Lanfear R, Calcott B, Kainer D, Mayer C, Stamatakis A. 2014. Selecting optimal partitioning  
1374 schemes for phylogenomic datasets. *BMC evolutionary biology*, 14:82.

1375

1376 Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new  
1377 methods for selecting partitioned models of evolution for molecular and morphological  
1378 phylogenetic analyses. *Mol Biol Evol*, 34:772-773.

1379

1380 Leavitt DH, Starrett J, Westphal MF, Hedin M. 2015. Multilocus sequence data reveal dozens of  
1381 putative cryptic species in a radiation of endemic Californian mygalomorph spiders (Araneae,  
1382 Mygalomorphae, Nemesiidae). *Mol. Phylogenet. Evol.*, 91:56-67.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1383

1384 Lemmon AR, Emme SA, Lemmon EM. 2012. Anchored Hybrid Enrichment for Massively

1385 High-Throughput Phylogenomics. *Syst Biol*, 61:727-744.

1386

1387 Li J-T, Wang J-S, Nian H-H, Litvinchuk SN, Wang J, Li Y, Rao D-Q, Klaus S. 2015.

1388 Amphibians crossing the bering land bridge: evidence from holarctic treefrogs (*Hyla*, Hylidae,

1389 Anura). *Mol. Phylogenet. Evol.*, 87:80-90.

1390

1391 Lüddecke T, Krehenwinkel H, Canning G, Glaw F, Longhorn SJ, Taenzler R, Wendt I, Vences

1392 M. 2018. Discovering the silk road: Nuclear and mitochondrial sequence data resolve the

1393 phylogenetic relationships among theraphosid spider subfamilies. *Mol. Phylogenet. Evol.*, 119:63-

1394 70.

1395

1396 Maddison WP, Evans SC, Hamilton CA, Bond JE, Lemmon AR, Lemmon EM. 2017. A

1397 genome-wide phylogeny of jumping spiders (Araneae, Salticidae), using anchored hybrid

1398 enrichment. *ZooKeys*:89.

1399

1400 Maguilla E, Escudero M, Luceño M. 2018. Vicariance versus dispersal across Beringian land

1401 bridges to explain circumpolar distribution: A case study in plants with high dispersal potential. *J*

1402 *Biogeography*, 45:771-783.

1403

1404 Main B. 1957. Occurrence of the trap-door spider *Conothele malayana* (Doleschall) in Australia

1405 (Mygalomorphae: Ctenizidae). *Western Australian Naturalist*, 5:209-216.

## PHYLOGENY OF MYGALOMORPH SPIDERS

1406

1407 Main B. 1993. Biogeographic significance of the Nullarbor cave mygalomorph spider

1408 *Troglo diplura* and its taxonomic affinities. Journal of the Royal Society of Western Australia,

1409 76:77-85.

1410

1411 Main BY. 1969. A blind mygalomorph spider from a Nullarbor plain cave. Roy Soc West

1412 Australia J. 52:9-11

1413

1414 Main BY, Mascord R. 1969. A new genus of diplurid spider (Araneae: Mygalomorphae) from

1415 New South Wales. Journal of the Entomological Society of Australia (NSW), 6:24.

1416

1417 Matthews KJ, Maloney KT, Zahirovic S, Williams SE, Seton M, Mueller RD. 2016. Global plate

1418 boundary evolution and kinematics since the late Paleozoic. Global and Planetary Change,

1419 146:226-250.

1420

1421 Milne R. 2006. Northern hemisphere plant disjunctions: a window on Tertiary land bridges and

1422 climate change? Annals of Botany, 98:465-472.

1423

1424 Mirarab S, Warnow T. 2015. ASTRAL-II: coalescent-based species tree estimation with many

1425 hundreds of taxa and thousands of genes. Bioinformatics, 31:i44-i52.

1426

1427 Misof B, Misof K. 2009. A Monte Carlo approach successfully identifies randomness in multiple

1428 sequence alignments: a more objective means of data exclusion. Syst Biol, 58:21-34.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1429

1430 Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C. 2011. Global biodiversity  
1431 conservation: the critical role of hotspots. *Biodiversity hotspots*, Springer, p. 3-22.

1432

1433 Montes de Oca L, D'Elía G, Pérez-Miles F. 2016. An integrative approach for species  
1434 delimitation in the spider genus *Grammostola* (Theraphosidae, Mygalomorphae). *Zool.Scr.*,  
1435 45:322-333.

1436

1437 Mora E, Paspatis A, Decae AE, Arnedo MA. 2017. Rafting spiders or drifting islands? Origins  
1438 and diversification of the endemic trap-door spiders from the Balearic Islands, Western  
1439 Mediterranean. *J Biogeography*, 44:924-936.

1440

1441 Morrone JJ, Crisci JV. 1995. Historical biogeography: introduction to methods. *Annu Rev Ecol*  
1442 *Evol Systemat*, 26:373-401.

1443

1444 Murielle J, Daniels SR, Buckley TR, Mayer G, Giribet G. 2014. A living fossil tale of Pangaeon  
1445 biogeography. *Proceedings of the Royal Society of London B: Biological Sciences*,  
1446 281:20132648.

1447

1448 Myers N, Mittermeier RA, Mittermeier CC, da Fonseca GA, Kent J. 2000. Biodiversity hotspots  
1449 for conservation priorities. *Nature*, 403:853-858.

1450



## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1451 Nance RD, Murphy JB, Santosh M. 2014. The supercontinent cycle: a retrospective essay.  
1452 Gondwana Research, 25:4-29.  
1453
- 1454 Nentwig W. 1983. The prey of web-building spiders compared with feeding experiments  
1455 (Araneae: Araneidae, Linyphiidae, Pholcidae, Agelenidae). Oecologia, 56:132-139.  
1456
- 1457 Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2014. IQ-TREE: a fast and effective  
1458 stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol, 32:268-  
1459 274.  
1460
- 1461 Opatova V, Arnedo MA. 2014a. From Gondwana to Europe: inferring the origins of  
1462 Mediterranean *Macrothele* spiders (Araneae: Hexathelidae) and the limits of the family  
1463 Hexathelidae. Invertebr Systemat, 28:361-374.  
1464
- 1465 Opatova V, Arnedo MA. 2014b. Spiders on a Hot Volcanic Roof: Colonisation Pathways and  
1466 Phylogeography of the Canary Islands Endemic Trap-Door Spider *Titanidiops canariensis*  
1467 (Araneae, Idiopidae). PLoS ONE, 9:e115078. doi:115010.111371/journal.pone.0115078.  
1468
- 1469 Opatova V, Bond JE, Arnedo MA. 2013. Ancient origins of the Mediterranean trap-door spiders  
1470 of the family Ctenizidae (Araneae, Mygalomorphae). Mol Phylogenet Evol, 69:1135-1145.  
1471

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1472 Opatova V, Bond JE, Arnedo MA. 2016. Uncovering the role of the Western Mediterranean  
1473 tectonics in shaping the diversity and distribution of the trap-door spider genus *Ummidia*  
1474 (Araneae, Ctenizidae). *J Biogeography*, 43:1955-1966.  
1475
- 1476 Ortiz D, Francke OF, Bond JE. 2018. A tangle of forms and phylogeny: Extensive morphological  
1477 homoplasy and molecular clock heterogeneity in *Bonnetina* and related tarantulas.  
1478 *Mol. Phylogenet. Evol.*, 127:55-73.  
1479
- 1480 Ott R, Höfer H. 2003. *Envia garciai*, a new genus and species of mygalomorph spiders (Araneae,  
1481 Microstigmatidae) from Brazilian Amazonia. *Iheringia. Série Zoologia*, 93:373-379.  
1482
- 1483 Panchen AL. 1992. *Classification, evolution, and the nature of biology*. Cambridge University  
1484 Press.  
1485
- 1486 Passanha V, Brescovit AD. 2018. On the Neotropical spider Subfamily Masteriinae (Araneae,  
1487 Dipluridae). *Zootaxa*, 4463:1-73.  
1488
- 1489 Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2010. How many  
1490 bootstrap replicates are necessary? *Journal of Computational Biology*, 17:337-354.  
1491
- 1492 Pérez-Miles F. 1994. Tarsal scopula division in Theraphosinae (Araneae, Theraphosidae): its  
1493 systematic significance. *J. Arachnol.*:46-53.  
1494

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1495 Pérez-Miles F. 2002. The occurrence of abdominal urticating hairs during development in  
1496 Theraphosinae (Araneae, Theraphosidae): phylogenetic implications. *J. Arachnol.*, 30:316-320.  
1497
- 1498 Pérez-Miles F, Lucas S, da Silva Jr P, Bertani R. 1996.  
1499 Prum RO, Berv JS, Dornburg A, Field DJ, Townsend JP, Lemmon EM, Lemmon AR. 2015. A  
1500 comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing.  
1501 *Nature*, 526:569.  
1502
- 1503 Raven R. 1991. A revision of the mygalomorph spider family Dipluridae in New Caledonia  
1504 (Araneae). *Mémoires du Muséum National d'Histoire Naturelle, Paris (A)*, 149:87-117.  
1505
- 1506 Raven RJ, Schwendinger P. 1995. Three new mygalomorph spider genera from Thailand and  
1507 China (Araneae). *Memoirs-Queenslad Museum*, 38:623-642.  
1508
- 1509 Raven RJ. 1980. The evolution and biogeography of the mygalomorph spider family  
1510 Hexathelidae (Araneae, Chelicerata). *J. Arachnol.*, 8:251-266.  
1511
- 1512 Raven RJ. 1981. A Review of the Australian Genera of the Mygalomorph Spider Subfamily  
1513 Diplurinae (Dipluridae: Chelicerata). *Aust. J. Zool.* , 29:321-363.  
1514
- 1515 Raven RJ. 1985. The spider infraorder Mygalomorphae (Araneae): cladistics and systematics.  
1516 *Bull. Amer. Mus. Nat. Hist.*, 182:1-180.  
1517

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 1518 Raven RJ. 1985. The spider infraorder Mygalomorphae (Araneae): cladistics and systematics.  
1519 Bull. Amer. Mus. Nat. Hist., 182:1-180.  
1520
- 1521 Raven RJ. 1994. Mygalomorph spiders of the Barychelidae in Australia and the western Pacific.  
1522 Mem. Queensl. Mus., 35:291-706.  
1523
- 1524 Ree RH, Smith SA. 2008. Maximum Likelihood Inference of Geographic Range Evolution by  
1525 Dispersal, Local Extinction, and Cladogenesis. Syst Biol, 57:4 - 14.  
1526
- 1527 Ríos Tamayo D. 2017. Re-description of *Trichopelma cubanum* (Theraphosidae: Ischnocolinae)  
1528 and comments about the familial placement of *Trichopelma*. Mun. Ent. Zool., 12:194-198.  
1529
- 1530 Ríos-Tamayo D, Goloboff P. 2017. *Vilchura calderoni*, a new genus and species of Euagrinae  
1531 (Araneae: Mygalomorphae: Dipluridae) from Chile. Arachnology, 17:183-187.  
1532
- 1533 Rix MG, Bain K, Main BY, Raven RJ, Austin AD, Cooper SJ, Harvey MS. 2017a. Systematics  
1534 of the spiny trapdoor spiders of the genus *Cataxia* (Mygalomorphae: Idiopidae) from south-  
1535 western Australia: documenting a threatened fauna in a sky-island landscape. The Journal of  
1536 Arachnology, 45:395-423.  
1537
- 1538 Rix MG, Cooper SJ, Meusemann K, Klopstein S, Harrison SE, Harvey MS, Austin AD. 2017b.  
1539 Post-Eocene climate change across continental Australia and the diversification of Australasian  
1540 spiny trapdoor spiders (Idiopidae: Arbanitinae). Mol. Phylogenet. Evol., 109:302-320.

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1541
- 1542 Rix MG, Raven RJ, Main BY, Harrison SE, Austin AD, Cooper SJ, Harvey MS. 2017c. The
- 1543 Australasian spiny trapdoor spiders of the family Idiopidae (Mygalomorphae: Arbanitinae): a
- 1544 relimitation and revision at the generic level. *Invertebr Systemat*, 31:566-634.
- 1545
- 1546 Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a
- 1547 penalized likelihood approach. *Mol Biol Evol*, 19:101-109.
- 1548
- 1549 Sanmartín I, Enghoff H, Ronquist F. 2001. Patterns of animal dispersal, vicariance and
- 1550 diversification in the Holarctic. *Biol. J. Linn. Soc.*, 73:345-390.
- 1551
- 1552 Sanmartín I, Ronquist F. 2004. Southern hemisphere biogeography inferred by event-based
- 1553 models: plant versus animal patterns. *Syst Biol*, 53:216-243.
- 1554
- 1555 Satler JD, Carstens BC, Hedin M. 2013. Multilocus Species Delimitation in a Complex of
- 1556 Morphologically Conserved Trapdoor Spiders (Mygalomorphae, Antrodiaetidae, *Aliatypus*). *Syst*
- 1557 *Biol*, 62:805-823.
- 1558
- 1559 Schmidt G. 2002. Gehören *Brachionopus* Pocock, 1897 und *Harpactirella* Purcell, 1902 zu den
- 1560 Theraphosiden. *Arthropoda*, 10:12-17.
- 1561
- 1562 Selden PA. 2002. First British Mesozoic Spider, From Cretaceous Amber Of The Isle Of Wight,
- 1563 Southern England. *Palaeontology*, 45:973-983.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1564

1565 Selden PA, Gall JC. 1992. A Triassic mygalomorph spider from the northern Vosges, France.

1566 Palaeontology, 35:211–235.

1567

1568 Selden PA, Shcherbakov DE, Dunlop JA, Eskov KY. 2014. Arachnids from the Carboniferous of

1569 Russia and Ukraine, and the Permian of Kazakhstan. Paläontologische Zeitschrift, 88:297-307.

1570

1571 Sharma PP, Baker CM, Cosgrove JG, Johnson JE, Oberski JT, Raven RJ, Harvey MS, Boyer SL,

1572 Giribet G. 2018. A revised dated phylogeny of scorpions: Phylogenomic support for ancient

1573 divergence of the temperate Gondwanan family Bothriuridae. Mol.Phylogenet.Evol., 122:37-45.

1574

1575 Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst Biol,

1576 51:492-508.

1577

1578 Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree

1579 selection. Bioinformatics, 17:1246 - 1247.

1580

1581 Shimojana M, Haupt J. 1998. Taxonomy and natural history of the funnel-web spider genus

1582 *Macrothele* (Araneae: Hexathelidae: Macrothelinae) in the Ryukyu Islands (Japan) and Taiwan.

1583 Species Diversity, 3:1-15.

1584

1585 Shultz JW. 1987. The origin of the spinning apparatus in spiders. Biological Reviews, 62:89-113.

1586

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1587 Simon E. 1864. Histoire naturelle des Araignées (Aranéides). Paris, Roret edit.
- 1588
- 1589 Simon E. 1889. Voyage de M. E. Simon au Venezuela (Decembre 1887–Avril 1888) 4e
- 1590 Mémoire. . Annales de la Société Entomologique de France, Paris, p. 169-220.
- 1591
- 1592 Smith SA. 2010. Lagrange C++ Manual.
- 1593
- 1594 Smith SA, O’Meara BC. 2012. treePL: divergence time estimation using penalized likelihood for
- 1595 large phylogenies. Bioinformatics, 28:2689-2690.
- 1596
- 1597 Snazell RG, Allison R. 1989. The genus *Macrothele* Ausserer (Araneae; Hexathelidae) in
- 1598 Europe. Bull Br arachnol Soc, 8:65-72.
- 1599
- 1600 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
- 1601 large phylogenies. Bioinformatics, 30:1312-1313.
- 1602
- 1603 Starrett J, Derkarabetian S, Hedin M, Bryson Jr RW, McCormack JE, Faircloth BC. 2017. High
- 1604 phylogenetic utility of an ultraconserved element probe set designed for Arachnida. Mol. Ecol.
- 1605 Res., 17:812-823.
- 1606
- 1607 Starrett J, Hayashi CY, Derkarabetian S, Hedin M. 2018. Cryptic elevational zonation in
- 1608 trapdoor spiders (Araneae, Antrodiaetidae, *Aliatypus janus* complex) from the California
- 1609 southern Sierra Nevada. Mol.Phylogenet.Evol., 118:403-413.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1610

1611 Starrett J, Hedin M, Ayoub N, Hayashi CY. 2013. Hemocyanin gene family evolution in spiders  
1612 (Araneae), with implications for phylogenetic relationships and divergence times in the  
1613 infraorder Mygalomorphae. *Gene*, 524:175-186.

1614

1615 Su YC, Brown RM, Chang YH, Lin CP, Tso IM. 2016. Did a Miocene–Pliocene island isolation  
1616 sequence structure diversification of funnel web spiders in the Taiwan-Ryukyu Archipelago? *J*  
1617 *Biogeography*, 43:991-1003.

1618

1619 Swofford D. 2003. PAUP\*. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*,  
1620 version 4.0b10.

1621

1622 Tiffney, Manchester. 2001. The use of geological and paleontological evidence in evaluating  
1623 plant phylogeographic hypotheses in the Northern Hemisphere tertiary. *International Journal of*  
1624 *Plant Sciences*, 162:S3-S17.

1625

1626 Toussaint EF, Bloom D, Short AE. 2017. Cretaceous West Gondwana vicariance shaped giant  
1627 water scavenger beetle biogeography. *J Biogeography*, 44:1952-1965.

1628

1629 Turner SP, Longhorn SJ, Hamilton CA, Gabriel R, Pérez-Miles F, Vogler AP. 2018. Re-  
1630 evaluating conservation priorities of New World tarantulas (Araneae: Theraphosidae) in a  
1631 molecular framework indicates non-monophyly of the genera, *Aphonopelma* and *Brachypelma*.  
1632 *Systematics and Biodiversity*, 16:89-107.



## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1633
- 1634 Veevers J. 2004. Gondwanaland from 650–500 Ma assembly through 320 Ma merger in Pangea  
1635 to 185–100 Ma breakup: supercontinental tectonics via stratigraphy and radiometric dating.  
1636 Earth-Science Reviews, 68:1-132.
- 1637
- 1638 Vollrath F, Selden P. 2007. The Role of Behavior in the Evolution of Spiders, Silks, and Webs.  
1639 Annu Rev Ecol Evol Systemat, 38:819-846.
- 1640
- 1641 Wang B, Dunlop JA, Selden PA, Garwood RJ, Shear WA, Müller P, Lei X. 2018. Cretaceous  
1642 arachnid *Chimerarachne yingi* gen. et sp. nov. illuminates spider origins. Nature ecology &  
1643 evolution, 2:614.
- 1644
- 1645 Wheeler WC, Coddington JA, Crowley LM, Dimitrov D, Goloboff PA, Griswold CE, Hormiga  
1646 G, Prendini L, Ramírez MJ, Sierwald P. 2017. The spider tree of life: phylogeny of Araneae  
1647 based on target-gene analyses from an extensive taxon sampling. Cladistics, 33:574-616.
- 1648
- 1649 Wiley EO. 1988. Vicariance biogeography. Annu Rev Ecol Evol Systemat, 19:513-542.
- 1650
- 1651 Will TM, Frimmel HE. 2018. Where does a continent prefer to break up? Some lessons from the  
1652 South Atlantic margins. Gondwana Research, 53:9-19.
- 1653

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1654 Wood HM, González VL, Lloyd M, Coddington J, Scharff N. 2018. Next-generation museum  
1655 genomics: Phylogenetic relationships among palpimanoid spiders using sequence capture  
1656 techniques (Araneae: Palpimanoidea). *Mol. Phylogenet. Evol.*, 127:907-918.

1657

1658 Wood HM, Matzke NJ, Gillespie RG, Griswold CE. 2012. Treating fossils as terminal taxa in  
1659 divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders. *Syst*  
1660 *Biol.* 62:264-284.

1661

1662 World Spider Catalog. 2018. World Spider Catalog (2018). World Spider Catalog. Version 19.5.  
1663 Natural History Museum Bern, online at <http://wsc.nmbe.ch/>, accessed on {16 November 2018}.  
1664 doi: 10.24436/2

1665

1666 Wunderlich Jh. 2011. Some fossil spiders (Araneae) in Eocene European ambers. In: Wunderlich  
1667 J editor. *Beiträge zur Araneologie*, p. 472–538.

1668

1669 Xu X, Liu F, Cheng R-C, Chen J, Xu X, Zhang Z, Ono H, Pham DS, Norma-Rashid Y, Arnedo  
1670 MA. 2015. Extant primitively segmented spiders have recently diversified from an ancient  
1671 lineage. *Proc. R. Soc. B*, 282:20142486.

1672

1673 Young A, Flament N, Maloney K, Williams S, Matthews K, Zahirovic S, Müller RD. 2018.  
1674 Global kinematics of tectonic plates and subduction zones since the late Paleozoic Era.

1675 *Geoscience Frontiers*. <https://doi.org/10.1016/j.gsf.2018.05.011>

1676

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1677 Yu Y, Harris AJ, Blair C, He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a  
1678 tool for historical biogeography. *Mol. Phylogenet. Evol.*, 87:46-49.  
1679  
1680 Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, Rhythms, and Aberrations in  
1681 Global Climate 65 Ma to Present. *Science*, 292:686-693.  
1682  
1683  
1684

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1685 Figure 1. Phylogenetic relationships of mygalomorph families reconstructed from morphological  
1686 characters: (a) Raven's classification scheme (Raven 1985), (b) results of the first cladistic  
1687 analyses performed by Goloboff (1993). Poly- and paraphyletic lineages are indicated by grey  
1688 boxes.

1689

1690 Figure 2. Phylogenetic relationships of mygalomorph families: (a) inferred from ribosomal data  
1691 (*18S*, *28S*) in the first molecular analyses of the infraorder (Hedin and Bond 2006), (b) inferred  
1692 in the "total evidence" approach combining three loci (*18S*, *28S*, *EF-1g*) and morphological data  
1693 (Bond et al. 2012). Poly- and paraphyletic lineages are indicated by grey boxes.

1694

1695 Figure 3. Phylogenetic tree summarizing results from concatenated and species tree approaches.  
1696 Topology obtained in the Maximum Likelihood analyses. Boxes on nodes denote support values  
1697 obtained in each approach (left to right): RAxML bootstrap support, ExaBayes Bayesian  
1698 posterior probabilities (PP), ASTRAL support values, IQ-Tree SH-aLRT support values. Color  
1699 coding of boxes corresponds to distinct support level categories depicted in bottom left corner.  
1700 One filled box indicates full support in all analyses; white box=topology not recovered in species  
1701 tree analysis.

1702

1703 Figure 4. Phylogenetic tree inferred from combined AHE loci and transcriptomic data  
1704 ("DNAAA" matrix). Tree topology obtained in maximum likelihood (ML) analyses conducted in  
1705 RAxML. Asterisk marks placement of *Paratropis* within Crassitarsae. Boxes near the branches  
1706 denote bootstrap support values; colors correspond to distinct support level categories depicted  
1707 on left.

## PHYLOGENY OF MYGALOMORPH SPIDERS

1708

1709 Figure 5. Divergence time estimates inferred by treePL on a topology obtained in RAxML. The  
1710 x-axis is time in million years. Geologic time scale abbreviations: N: Neogene, Pg: Paleogene,  
1711 K: Cretaceous, J: Jurassic, Tr: Triassic, P: Permian, C: Carboniferous, D: Devonian. Light green  
1712 block marks time frame of Gondwana breakup. Symbols on the tree nodes denote vicariant or  
1713 dispersal events hypothesized for the divergences (left to right): inverted triangle=Pangea  
1714 breakup, star=East-West Gondwana breakup, triangle=West Gondwana breakup, dot=Laurasia  
1715 breakup, asterisk=long distance dispersal. Left corner: Map showing the position of the  
1716 continents (a) prior to Gondwana breakup (adapted from (Will and Frimmel 2018), thick black  
1717 lines mark the zones of continental drifting, times indicate the initiation of drifting in each zone;  
1718 (b) present day. Terminal tree taxa are color coded according to geographic areas of their  
1719 sampling locations depicted in the map as follows: yellow=South America, orange=Africa,  
1720 purple=Asia, red=Australia, light blue=North America, dark blue=Europe, lime  
1721 green=Antarctica (no taxa assigned).

1722

1723 Figure 6. Ancestral areas of distribution inferred with DEC analyses implemented in RASP. Tree  
1724 topology obtained in ExaBayes. Terminal tree taxa are color coded according to the  
1725 biogeographic regions of their sampling locations depicted in the map (bottom left) as follows:  
1726 A=North America, B=Europe, C=Asia, D=South America, E=Africa, F=Australia. Color coding  
1727 of the inferred ancestral distributions corresponds to the assigned biogeographic regions, or  
1728 combination of thereof depicted in the combined area legend on the left. Biogeographic events  
1729 marked on the nodes: Di=dispersal, V=vicariance, Ex=extinction; black letters: events inferred  
1730 with probability > 0.7.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1731

1732 Figure 7. Ancestral state reconstruction of mygalomorph foraging constructs. Preferred ancestral  
1733 state reconstruction of foraging type using an equal rates model in the R-package *corHMM* with  
1734 cursorial hunting taxa treated as inapplicable (?); tree was modified as ultrametric; AICc =  
1735 173.9083. Terminal tree taxa are color coded according the type of spinning structure depicted in  
1736 the legend panel of the left.

1737

1738 Figure 8. Cladogram summarizing the phylogenetic relationships of mygalomorph families  
1739 recovered in this paper. The topology corresponds to the arrangement obtained in the  
1740 concatenated analyses (RAxML, ExaBayes, IQ-TREE). The crossed boxes on the branches  
1741 represent unsupported relationships: left ML bootstrap support < 70%, right Bayesian inference  
1742 Posterior Probability < 0.95, SH-aLRT < 95). Filled box=node supported only in Bayesian  
1743 inference, otherwise relationships supported in both analyses.

PHYLOGENY OF MYGALOMORPH SPIDERS

1744

1745 Table 1. Taxon counts obtained from the World Spider Catalog (2018), undescribed and undetermined genera marked with \*.

1746

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
Cyrtacheniidae	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

---

1747

1748



PHYLOGENY OF MYGALOMORPH SPIDERS

1749 Table 2. Maximum likelihood topology test results: obs, the observed log-likelihood difference to the best unconstraint topology (Fig.  
 1750 3), AU, p-value of the approximately unbiased test conducted in CONSEL; np, bootstrap probability calculated from the multiscale  
 1751 bootstrap; significant results (P<0.05) \*

1752

1753

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

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

1754

1755

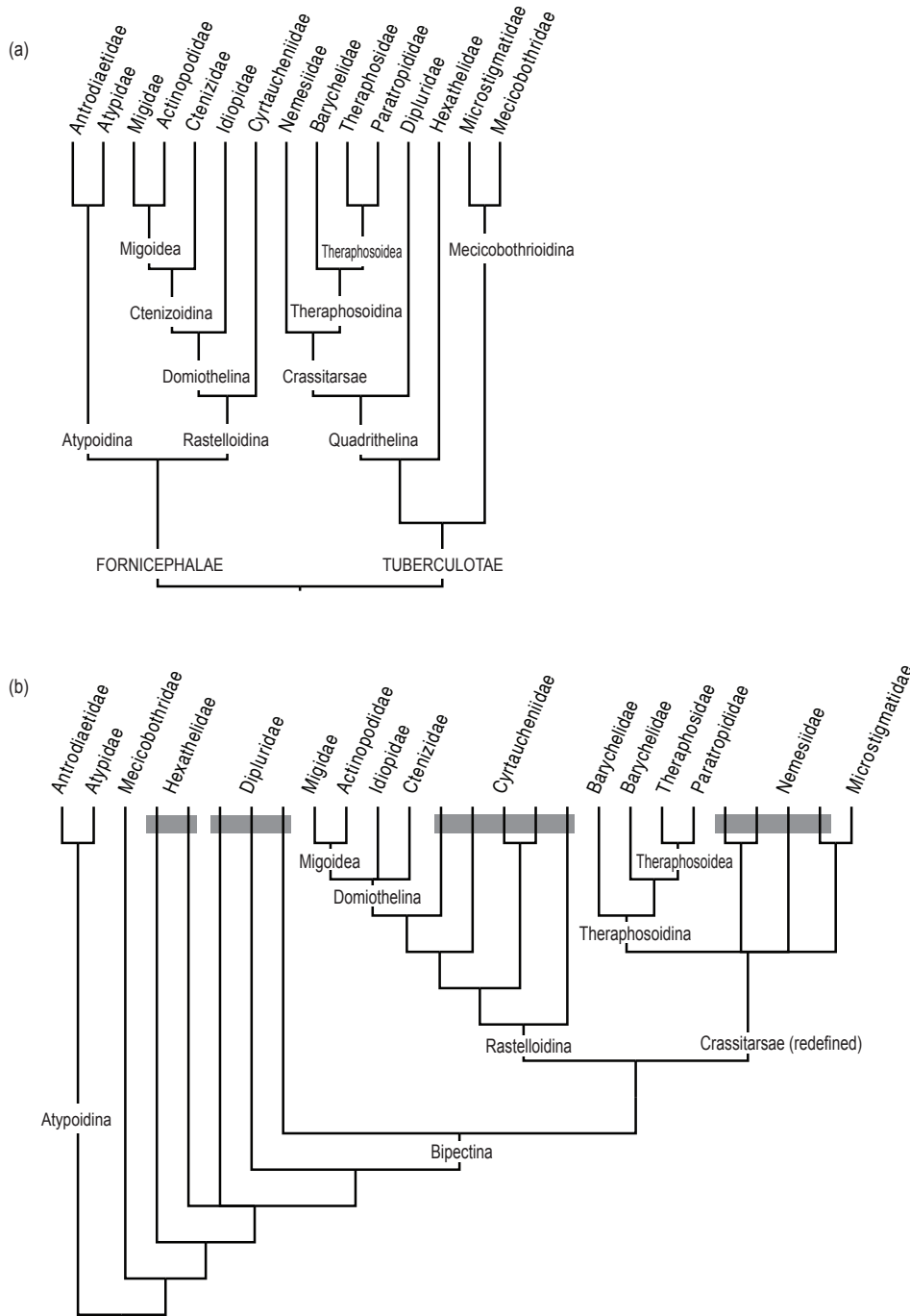
PHYLOGENY OF MYGALOMORPH SPIDERS

1756 Table 3. Divergence time estimates obtained by treePL algorithm (Smith and O’Meara 2012) representing taxa origins and the  
 1757 respective timeframe of their diversification inferred for tree topology depicted in Fig. 5. Confidence intervals (CI) for the highest  
 1758 posterior density (HDP) were inferred from 100 bootstrap replicates.

Taxon origin	Age (Ma)	HDP 95% CI (Ma)	Taxon diversification	HDP 95% CI (Ma)
Mygalomorphae/Liphistiomorphae	398	397-398	Antrodiaetidae + Mecicobothriidae	83 – 38
Atypoidea/Avicularoidea	323	323	Atypidae	123 – 10
Ischnothelidae	254	259 – 247	Ischnothelidae	125 – 118
Microhexuridae	225	232 – 216	Euagridae	174 – 41
Hexathelidae/ Euagridae	202	209 – 192	Macrothelidae	42 – 31
Porrhothelidae	187	194 – 176	Paratropididae	19 – 16
Macrothelidae/Bipectina	174	179 – 162	Atracidae	12 – 11
Paratropididae	166	171 – 155	Actinopodidae	50 – 36
Crassitarsae	158	163 – 147	Stasimopidae	20 – 12
Stasimopidae	146	152 – 135	Halonoproctidae	131– 4
Domiothelina	155	161– 145	Migidae	95 – 57

Atypidae/ Antrodiaetidae +	172	176 – 167	Idiopidae	105 – 18
Mecicobothriidae				
Halonoproctidae	143	148 – 133	Ctenizidae	83 – 64
Migidae	140	145 – 129	Euctenizidae	89 – 31
Idiopidae	133	138 – 123	Bemmeridae	49 – 9
Bemmeridae	134	139 – 126	Barychelidae	94 – 27
Ctenizidae/ Euctenizidae	121	127 – 109	Theraphosidae	95 – 9
Nemesiidae	115	120 – 107	Nemesiidae	106 – 6
Barychelidae/ Theraphosidae	107	118 – 103	Pycnothelidae	84 – 26
Atracidae/ Actinopodidae	98	102 – 89	Dipluridae	57 – 37
Pycnothelidae	102	109 – 96	Cyrtaucheniidae	70 – 1
Dipluridae/ Cyrtaucheniidae	90	98 – 85	Entypesidae	28 – 21
Anaminidae	88	92 – 81	Microstigmatidae	70 – 54
Entypesidae/Microstigmatidae	79	84 – 70	Anaminidae	47 – 5

## PHYLOGENY OF MYGALOMORPH SPIDERS



1760

1761 Figure 1. Phylogenetic relationships of mygalomorph families reconstructed from morphological

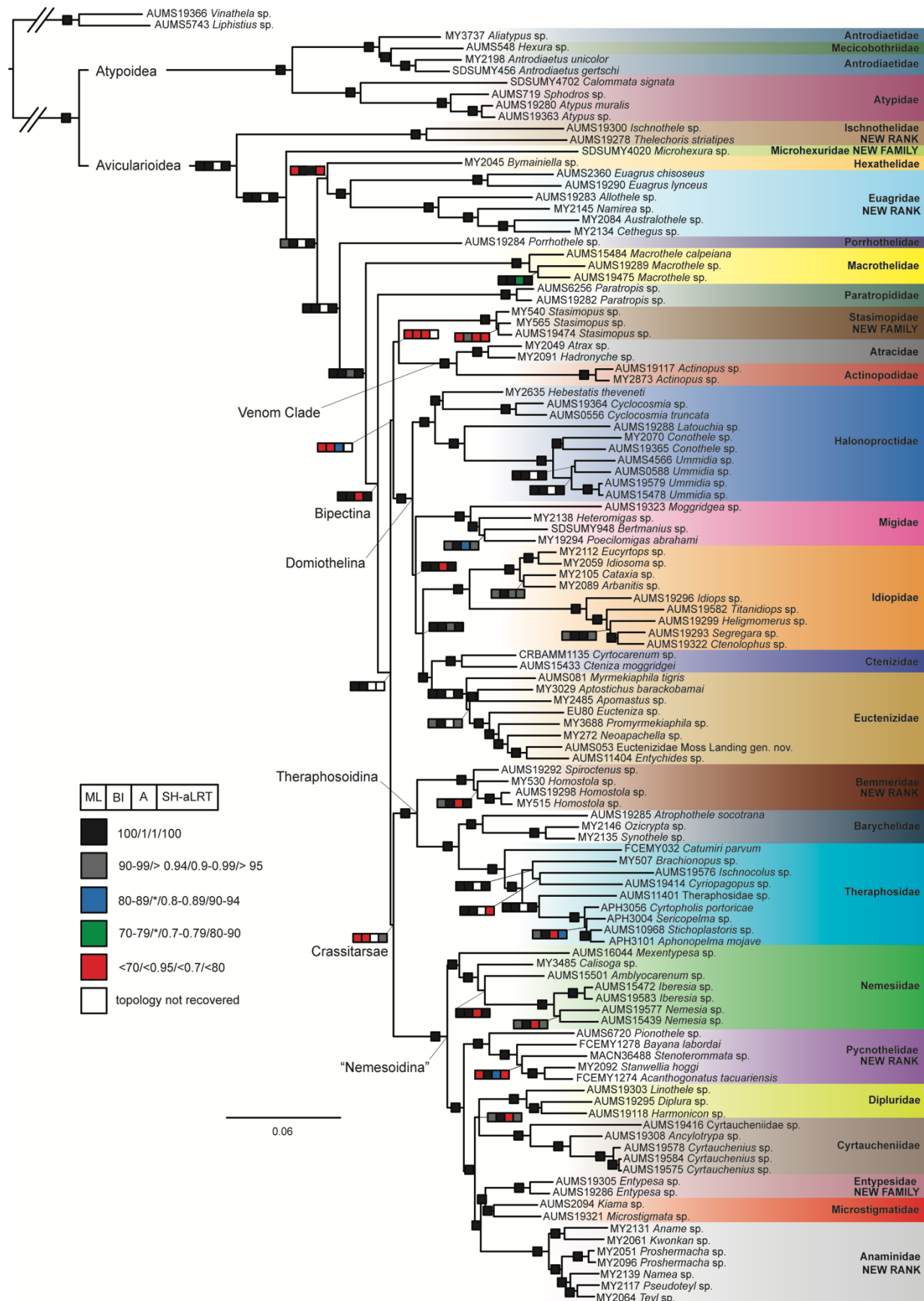
1762 characters: (a) Raven's classification scheme (Raven 1985), (b) results of the first cladistic

1763 analyses performed by Goloboff (1993). Poly- and paraphyletic lineages are indicated by grey

1764 boxes.



## PHYLOGENY OF MYGALOMORPH SPIDERS

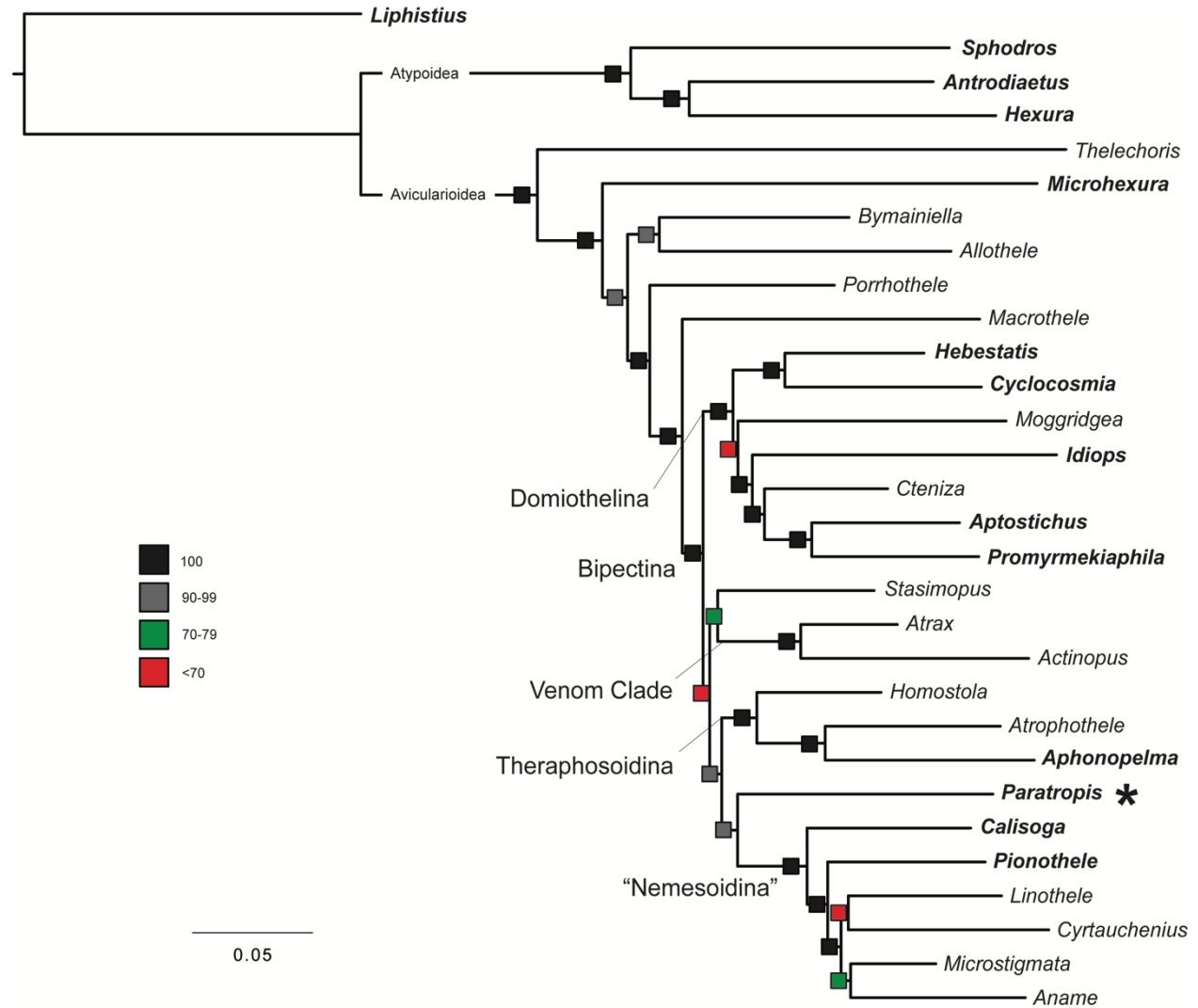


OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1771 Figure 3. Phylogenetic tree summarizing results from concatenated and species tree approaches.  
1772 Topology obtained in the Maximum Likelihood analyses. Boxes on nodes denote support values  
1773 obtained in each approach (left to right): RAxML bootstrap support, ExaBayes Bayesian  
1774 posterior probabilities (PP), ASTRAL support values, IQ-Tree SH-aLRT support values. Color  
1775 coding of the boxes corresponds to distinct support level categories depicted in bottom left  
1776 corner. One filled box indicates full support in all analyses; white box=topology not recovered in  
1777 species tree analysis.  
1778



## PHYLOGENY OF MYGALOMORPH SPIDERS



1779

1780

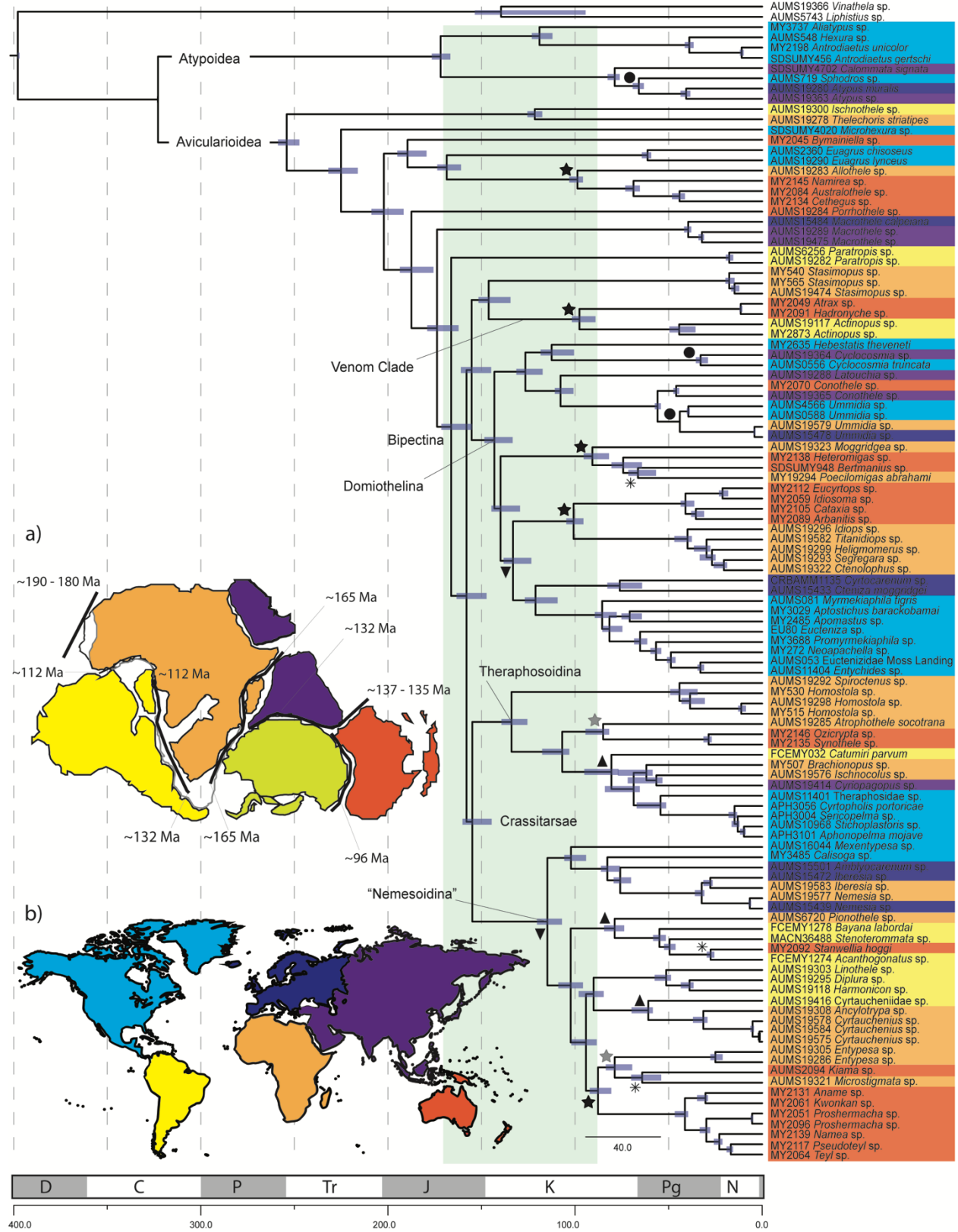
1781 Figure 4. Phylogenetic tree of inferred combined AHE loci and transcriptomic data (“DNAAA”

1782 matrix). Tree topology obtained in maximum likelihood (ML) analyses conducted in RAxML.

1783 Asterisk marks placement of *Paratropis* within Crassitarsae. Boxes near the branches denote

1784 bootstrap support values; colors correspond to distinct support level categories depicted on left.

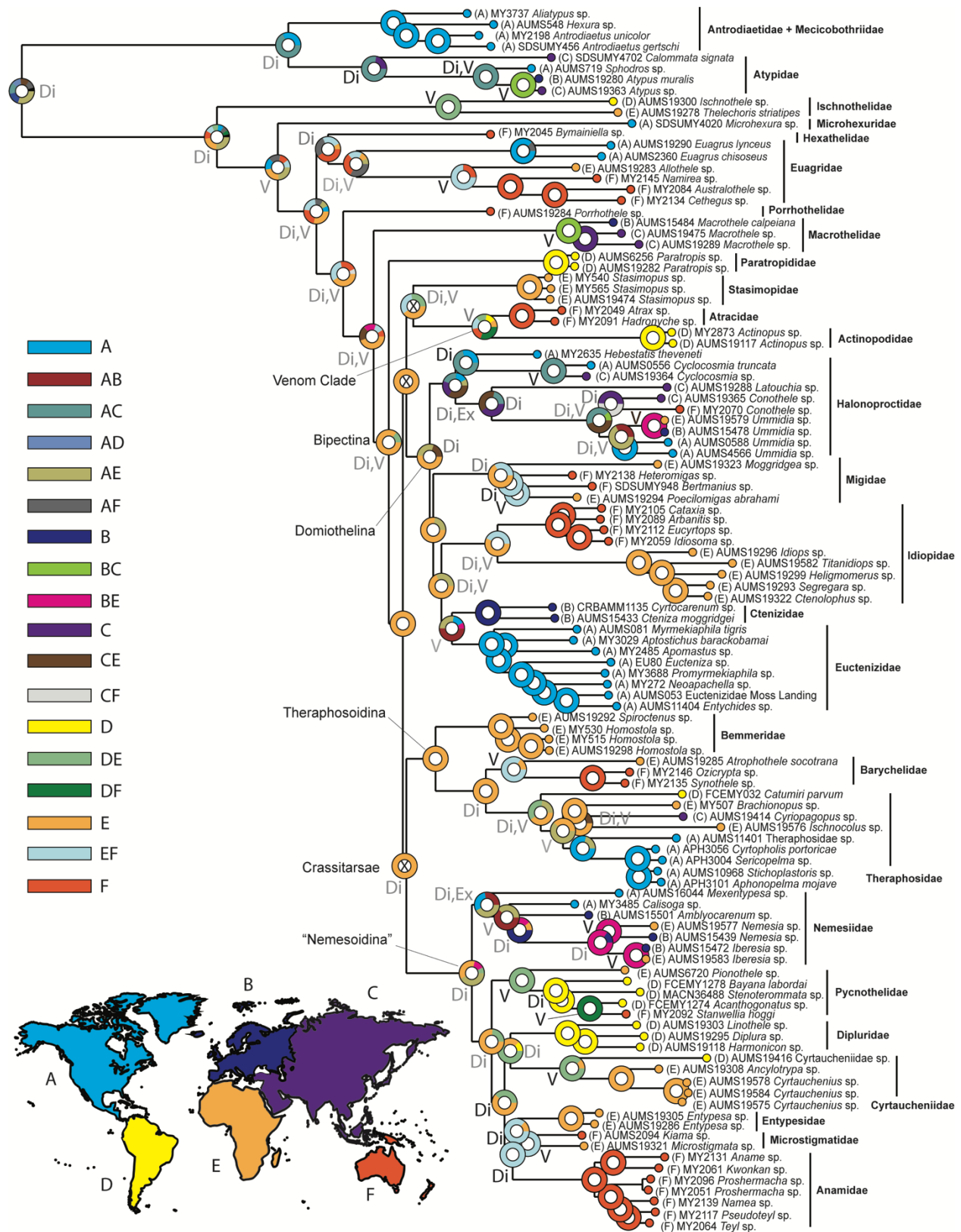
OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND



## PHYLOGENY OF MYGALOMORPH SPIDERS

1786 Figure 5. Divergence time estimates inferred by treePL on a topology obtained in RAxML. The  
1787 x-axis is time in million years. Geologic time scale abbreviations: N: Neogene, Pg: Paleogene,  
1788 K: Cretaceous, J: Jurassic, Tr: Triassic, P: Permian, C: Carboniferous, D: Devonian. Light green  
1789 block marks time frame of Gondwana breakup. Symbols on the tree nodes denote vicariant or  
1790 dispersal events hypothesized for the divergences (left to right): inverted triangle=Pangea  
1791 breakup, star=East-West Gondwana breakup, triangle=West Gondwana breakup, dot=Laurasia  
1792 breakup, asterisk=long distance dispersal. Left corner: Map showing the position of the  
1793 continents (a) prior to Gondwana breakup (adapted from (Will and Frimmel 2018), thick black  
1794 lines mark the zones of continental drifting, times indicate the initiation of drifting in each zone;  
1795 (b) present day. Terminal tree taxa are color coded according to geographic areas of their  
1796 sampling locations depicted in the map as follows: yellow=South America, orange=Africa,  
1797 purple=Asia, red=Australia, light blue=North America, dark blue=Europe, lime  
1798 green=Antarctica (no taxa assigned).  
1799

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

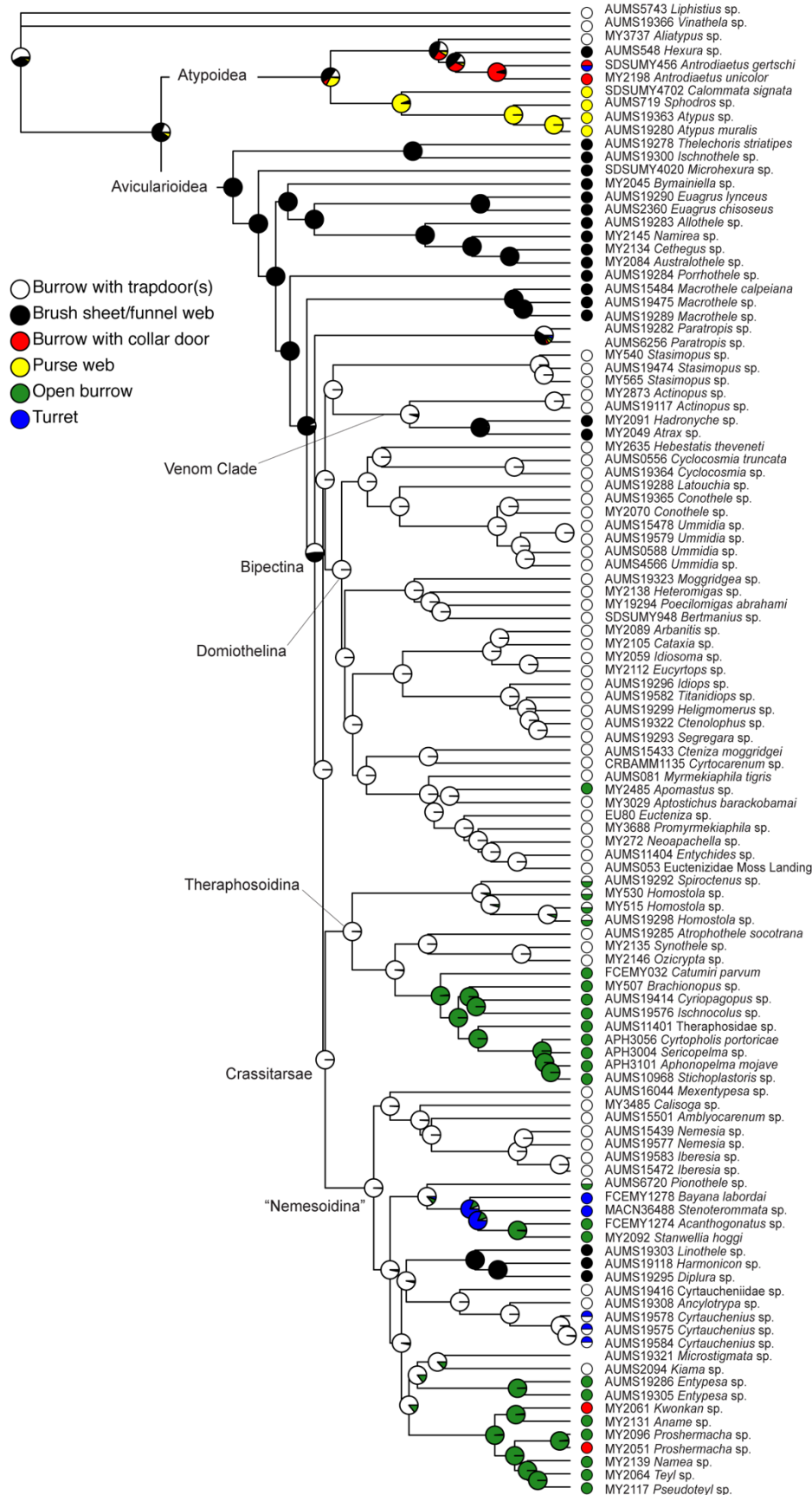


1800

## PHYLOGENY OF MYGALOMORPH SPIDERS

1801 Figure 6. Ancestral areas of distribution inferred for mygalomorph spiders with DEC analyses  
1802 implemented in RASP. Tree topology obtained in ExaBayes. Terminal tree taxa are color coded  
1803 according to the biogeographic regions of their sampling locations depicted in the map (bottom  
1804 left) as follows: A=North America, B=Europe, C=Asia, D=South America, E=Africa,  
1805 F=Australia. Color coding of the inferred ancestral distributions corresponds to the assigned  
1806 biogeographic regions, or combination of thereof depicted in the combined area legend on the  
1807 left. Biogeographic events marked on the nodes: Di=dispersal, V=vicariance, Ex=extinction;  
1808 black letters: events inferred with probability  $> 0.7$ .

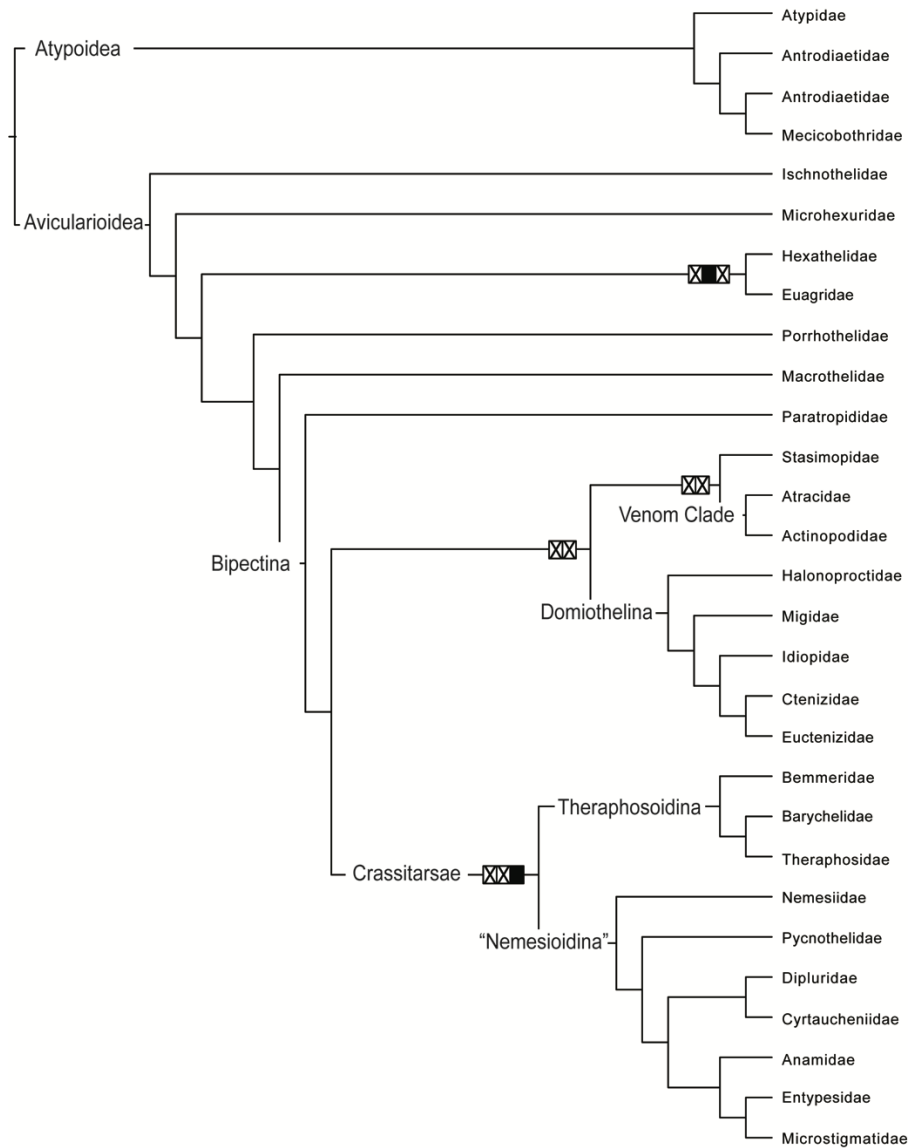
OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND



## PHYLOGENY OF MYGALOMORPH SPIDERS

1810 Figure 7. Ancestral state reconstruction of mygalomorph foraging constructs. Preferred ancestral  
1811 state reconstruction of foraging type using an equal rates model in the R-package *corHMM* with  
1812 cursorial hunting taxa treated as inapplicable (?); tree was modified as ultrametric; AICc =  
1813 173.9083. Terminal tree taxa are color coded according the type of spinning structure depicted in  
1814 the legend panel of the left.  
1815

1816



1817

1818 Figure 8. Cladogram summarizing the phylogenetic relationships of mygalomorph families  
 1819 recovered in this paper. The topology corresponds to the arrangement obtained in the  
 1820 concatenated analyses (RAxML, ExaBayes, IQ-TREE). The crossed boxes on the branches  
 1821 represent unsupported relationships: left ML bootstrap support < 70%, right Bayesian inference  
 1822 Posterior Probability < 0.95, SH-aLRT < 95). Filled box = node supported only in Bayesian  
 1823 inference, otherwise relationships supported in both analyses.

1824



## PHYLOGENY OF MYGALOMORPH SPIDERS

- 1825 APPENDIX 1: Revised Taxonomy for some of the families of the Spider Infraorder
- 1826 Mygalomorphae (Araneae)
- 1827
- 1828 SUPPLEMENTAL FILES:
- 1829
- 1830 Data available from the Dryad Digital Repository: [http://dx.doi.org/10.5061/dryad.\[NNNN\]](http://dx.doi.org/10.5061/dryad.[NNNN])
- 1831
- 1832 *Tables and Figures*
- 1833
- 1834 Table S1: Specimen locality data
- 1835
- 1836 Table S2: Specimens used in the combined analyses of genomic and transcriptomic data
- 1837 (DNAAA\_matrix). AHE dataset: specimens sequenced in this study, AA dataset: data
- 1838 proceeding from Garrison et al. (2016) “BCC-75” matrix.
- 1839
- 1840 Table S3: Character coding for web type ancestral state reconstruction
- 1841
- 1842 Supplemental Figure 1. Tree topology obtained in maximum likelihood (ML) analyses conducted
- 1843 in RAxML. Values on the nodes correspond to bootstrap support.
- 1844
- 1845 Supplemental Figure 2. Tree topology obtained in Bayesian inference (BI) analyses conducted in
- 1846 ExaBayes. Values on the nodes correspond to the Bayesian posterior probability (PP).
- 1847

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1848 Supplemental Figure 3. Tree topology obtained in maximum likelihood (ML) analyses conducted  
1849 in IQ-TREE. Values on the nodes correspond to SH-aLRT support.

1850

1851 Supplemental Figure 4. Phylogenetic tree inferred after removal of Paratropididae terminals  
1852 (“No\_Paratropis” dataset). Tree topology obtained in maximum likelihood (ML) analyses  
1853 conducted in RAxML. Boxes near the branches denote full bootstrap support values; numbers  
1854 report bootstrap support values obtained in this analysis (black) and the analysis of the full  
1855 “All\_taxa” dataset (red). Asterisks mark differences in topology obtained from the “All\_taxa”  
1856 dataset.

1857

1858 Supplemental Figure 5. Tree topology obtained in species tree analysis conducted in ASTRAL.  
1859 Values on the nodes correspond to the ASTRAL support values.

1860

1861 Supplemental Figure 6. Comparison of resulting tree topologies obtained by concatenated  
1862 analyses conducted in RAxML and ExaBayes (left) and species tree approach conducted in  
1863 ASTRAL (right). Asterisks mark differences in topologies. Dotted grey line denote array of  
1864 lineages forming a highly supported clade in species tree analysis. Boxes on nodes denote  
1865 support values obtained in each approach, color coded according to distinct support level  
1866 categories depicted in bottom left corner in following order (left to right): RAxML bootstrap  
1867 support, ExaBayes Bayesian posterior probabilities, ASTRAL support values.

1868

1869

## PHYLOGENY OF MYGALOMORPH SPIDERS

1870 Supplemental Figure 7. Divergence time estimates of mygalomorph spiders inferred by treePL  
1871 on a topology obtained in RAxML, values near the nodes correspond to the inferred timing of the  
1872 divergences. Numbered nodes represent placement of the calibrations and their minimum and  
1873 maximum ages (in brackets), 1) Mygalomorphae (Garrison et al. 2016; Fernández et al. 2018), 2)  
1874 Avicularoidea: *Rosamygale grauvogely* (Selden and Gall 1992), 3) “Nemesioidea”: *Cretamygale*  
1875 *chasei* (Selden 2002), 4) Atypoidea: *Ambiortiphagus ponomarenkoi* (Eskov and Zonshtein  
1876 1990), 5) *Ummidia* (Wunderlich 2011). The x-axis is time in million years.

1877

1878 Supplemental Figure 8. Divergence time estimates of mygalomorph spiders inferred by treePL  
1879 on a topology obtained in RAxML, values near the nodes correspond to the confidence intervals  
1880 (CI) for the highest posterior density (HDP) inferred from 100 bootstrap replicates. The x-axis is  
1881 time in million years.

1882

1883 Supplemental Figure 9. Phylogenetic tree of Mygalomorphae summarizing results from  
1884 concatenated and species tree approaches within a framework of a current classification scheme  
1885 (World Spider Catalog 2018). Topology obtained in the Maximum Likelihood analyses. Boxes  
1886 on nodes denote support values obtained in each approach (left to right): RAxML bootstrap  
1887 support, ExaBayes Bayesian posterior probabilities (PP), ASTRAL support values, IQ-Tree SH-  
1888 aLRT support values. Colour of the boxes corresponds to distinct support level categories  
1889 depicted in bottom left corner. One filled box indicates full support in all analyses; white  
1890 box=topology not recovered in species tree analysis.

1891

1892 *Data Files*

1893

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1894 ASTRAL\_all\_taxa.tre  
1895 ASTRAL\_all\_taxa.trees  
1896 ExaBayes\_all\_taxa.tre  
1897 RASP\_ingroup.tre\_dis.csv  
1898 RAxML\_DNAAA.nex  
1899 RAxML\_DNAAA.tre  
1900 RAxML\_DNAAA\_partitions.txt  
1901 RAxML\_ExaBayes\_all\_taxa.nex  
1902 RAxML\_ExaBayes\_all\_taxa\_partitions.txt  
1903 RAxML\_No\_Paratropis.nex  
1904 RAxML\_No\_Paratropis.tre  
1905 RAxML\_No\_Paratropis\_partitions.txt  
1906 RAxML\_all\_taxa.tre  
1907 silkUseAncStateRecon.txt  
1908 treePL\_dated.tre

## PHYLOGENY OF MYGALOMORPH SPIDERS

1909 **Taxonomic Appendix: Revised Taxonomy for some of the families of the Spider Infraorder**

1910 **Mygalomorphae (Araneae)**

1911

1912 **Jason E. Bond<sup>1</sup>, Vera Opatova<sup>1</sup>, and Marshal Hedin<sup>2</sup>**

1913

1914 <sup>1</sup>*Department of Entomology and Nematology, University of California, Davis, CA 95616, USA*

1915 <sup>2</sup>*Department of Biology, San Diego State University, San Diego, CA, 92182–4614, USA*

1916

1917 **Families remaining unchanged in their composition**

1918

1919 Actinopodidae – Atracidae (The Venom Clade)

1920 Remarks. The family Atracidae comprises three genera endemic to eastern Australia. Formerly

1921 designated as the “hexathelid” subfamily Atracinae (Gray 2010), the group was recently elevated

1922 to family level by Hedin et al. (2018) and supported by the topology test performed on our

1923 dataset. Contrary to the hypothesis based on morphological characters (Raven 1985; Goloboff

1924 1993), Atracidae has consistently been recovered as sister to Actinopodidae (Hedin and Bond

1925 2006; Ayoub et al. 2007; Bond et al. 2012; Hamilton et al. 2016b; Hedin et al. 2018), further

1926 corroborated by our results. Actinopodidae comprises three genera with a disjunct South

1927 American – Australian distribution and on the basis of morphological characters (eyes widely

1928 spread across the carapace), was placed as sister to Migidae (Raven 1985; Goloboff 1993).

1929 However, this relationship has never been supported in any molecular phylogenetic study.

1930 Despite the differences in the morphology of the families, the phylogenetic proximity of

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1931 Atracidae/Actinopodidae is supported not only by the overwhelming amount of evidence  
1932 proceeding from molecular data, but also by similarities in venom composition (for details see  
1933 Hedin et al. (2018). Certain members of both families are medically important; the bites of the  
1934 Sydney funnel-web spider, *Atrax robustus*, cause lethal or life-threatening conditions, and other  
1935 taxa can also be dangerous to humans (Isbister et al. 2005, 2015). The Atracidae/Actinopodidae  
1936 clade was recovered within the Bipectina, which contradicts the results of a number of previous  
1937 analyses (Goloboff 1993; Hedin and Bond 2006; Bond et al. 2012). Unfortunately, the exact  
1938 relationships of the Atracidae/Actinopodidae with other taxa remain uncertain. The group was  
1939 consistently placed in all analyses as sister to the Stasimopidae, although with varying degrees of  
1940 support (see below). In our analyses, both families were represented by two out of three genera;  
1941 *Atrax* and *Hadronyche* for Atracidae, and *Actinopus* and *Missulena* for Actinopodidae, however,  
1942 the monophyly of both families, based on complete generic sampling, appears to be strong  
1943 (Hedin et al. 2018).

1944

1945 Antrodiaetidae – Megahexuridae – Atypidae – Mecicobothriidae - Hexurellidae

1946 Remarks. Atypoid spider classification has recently undergone major revision with the  
1947 designation of new families and phylogenetic structure; see Hedin et al. (2019).

1948

1949 Barychelidae

1950 Remarks. Barychelidae is a highly diverse family (42 genera, 495 species) with a Gondwanan  
1951 distribution spanning continental islands and also remote islands of volcanic origin (Raven 1994)  
1952 indicating the ability to disperse. Like Theraphosidae, Barychelidae possess well developed  
1953 scopulae and dense claw tufts, but differs in the number of cuspules, clavate trichobothria, and

## PHYLOGENY OF MYGALOMORPH SPIDERS

1954 the shape of the maxillary anterior lobe (Raven 1985; Dippenaar-Schoeman 2002). Due to the  
1955 potential ambiguity of these characters (Raven 1985; Goloboff 1993; Bond et al. 2012) some  
1956 authors have considered Barychelidae, at least in part, a paraphyletic lineage of Theraphosidae  
1957 (Goloboff 1993). Stemming from differences in character state interpretations, taxa tend to  
1958 vacillate between the two families, with proposed membership by some authors in one family  
1959 (Raven 1985; Guadanucci 2014) to be later rejected by others (Gallon 2002; Schmidt 2002; Ríos  
1960 Tamayo 2017). One example of this problem, the family placement of *Brachionopus* (Raven  
1961 1985; Gallon 2002; Schmidt 2002) was not successfully resolved until the implementation of a  
1962 molecular approach (Lüddecke et al. 2018). With our limited sampling, we recovered the  
1963 reciprocal monophyly of both Barychelidae and Theraphosidae. However, a thorough sampling  
1964 of both families will be needed to stabilize the family boundaries. Barychelidae (as currently  
1965 defined) is composed of two common and divergent “phenotypes” (a “naked” group that is much  
1966 less setose and resemble most mygalomorph groups, and a “hairy” group that resemble small  
1967 theraphosids. These two “phenotypes” have been the major drivers of taxa historically bouncing  
1968 back and forth between the two families.

1969

1970 Euctenizidae

1971 Remarks. The group was originally designated as a subfamily of Cyrtaucheniidae (Raven 1985),  
1972 but with increasing amount of evidence for a non-monophyletic Cyrtaucheniidae (Bond and  
1973 Opell 2002; Bond and Hedin 2006), Euctenizidae were elevated to family level (Bond et al.  
1974 2012). The family comprises 76 species within seven genera across a North American  
1975 distribution (Bond 2017). We sampled all described genera and recovered the family as  
1976 monophyletic, as well as the sister group to Ctenizidae (*sensu* this study) and Idiopidae.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

1977

1978 Halonoproctidae

1979 Remarks. The family currently comprises six genera and has a nearly cosmopolitan distribution.

1980 It was recently removed from Ctenizidae (Godwin et al. 2018), whose monophyly was doubted

1981 both on morphological (Raven 1985) and molecular grounds (Hedin and Bond 2006; Bond et al.

1982 2012; Opatova et al. 2013). Similar to Godwin et al. (2018), we recovered Halonoproctidae as

1983 monophyletic in all analyses. Furthermore, the topology test rejected the monophyly of the

1984 family Ctenizidae (*sensu* Raven (1985)) as well as the monophyly of Halonoproctidae +

1985 Ctenizidae (*sensu* this study), verifying the status of the family within the context of a wider

1986 taxonomic framework. It should be noted that we did not include the genus *Bothriocyrtum* in our

1987 analyses, but given its stable position as sister to *Hebestatis* (Hedin and Bond 2006; Bond et al.

1988 2012; Opatova et al. 2013; Godwin et al. 2018), we believe that it had little to no effect on the

1989 resulting topology and support values.

1990

1991 Hexathelidae – Porrhothelidae – Macrothelidae

1992 Remarks. The monophyly of the family Hexathelidae in its original composition (*sensu* Raven

1993 (1985)) has been challenged on molecular grounds on numerous occasions (Hedin and Bond

1994 2006; Ayoub et al. 2007; Bond et al. 2012; Opatova and Arnedo 2014a; Hamilton et al. 2016b).

1995 Formal taxonomic changes were put on hold until the implementation of UCEs alongside

1996 complete generic sampling which resulted in parceling of the family Hexathelidae (*sensu* Raven

1997 (1985)) into four distinct families (Hedin et al. 2018). We sampled all the families in our

1998 analyses and similar to the results of Hedin et al. (2018), we recovered the families Hexathelidae,

1999 Porrhothelidae and Macrothelidae among the early branching lineages near the Avicularioidea



## PHYLOGENY OF MYGALOMORPH SPIDERS

2000 root; though Atracidae, was recovered within the Bipectina clade sister to Actinopodidae. The  
2001 family Hexathelidae now comprises nine genera with a disjunct distribution (Australia, New  
2002 Zealand, South America), of which we only sampled the Australian *Bymainiella*. It was  
2003 recovered as sister to the family Euagridae with high support, which was previously indicated by  
2004 EF-1g data (Ayoub et al. 2007), albeit the support for this relationship was low. Both  
2005 Porrhothelidae and Macrothelidae were recovered as stand-alone lineages placed near the  
2006 Avicularioidea root node. Both lineages are monogeneric and were previously considered sister  
2007 taxa (Raven 1985), but this relationship was statistically rejected in the past (Opatova and  
2008 Arnedo 2014a) and their unique position appears stable even in light of considerable genomic  
2009 data ((Hedin et al. 2018); this study). Given the wide taxonomic framework of our analyses, we  
2010 tested the monophyly of the family Hexathelidae, in its original composition (*sensu* Raven  
2011 (1985)), as well the alternative arrangement recovered in the species tree analysis (Table 2); both  
2012 were statistically rejected, confirming Hedin et al. (2018).  
2013 Note. Because the new family Porrhothelidae was electronically published but not registered in  
2014 ZooBank, the online registration system for the ICZN it is redscribed here.

2015

2016 Family Porrhothelidae (NEW FAMILY) Hedin and Bond

2017

2018 **Type genus:** *Porrhothele* Simon, 1892 [urn:lsid:nmbe.ch:spidergen:00021] (type species

2019 *Porrhothele antipodiana* (Walkenaer, 1837).

2020

2021 **Diagnosis:** As a consequence of its monotypy, characters used to diagnose the family

2022 Porrhothelidae are those characters attributed to the type genus. *Porrhothele* was thoroughly

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2023 diagnosed and described by Forster (1968) with additions by Raven (1980). Members of this  
2024 family can be diagnosed on the basis of the following unique combination of characters: 1) small  
2025 posterior sternal sigilla; 2) single row of promarginal cheliceral teeth; 3) male tibia swollen with  
2026 dense pattern of strong promarginal spines (illustrated in Forster (1968) e.g., p. 170, figs. 543–  
2027 548). This monotypic family is found in New Zealand.

2028

2029 Idiopidae

2030 Remarks. Idiopids are another family with predominantly Gondwanan distribution (South  
2031 America, Asia, Africa, Australia and New Zealand), though some species are also known from  
2032 the Middle East and Central America. The family is well characterized by male palpal  
2033 synapomorphies (Raven 1985) and its monophyly has never been called into question by  
2034 previous studies (Goloboff 1993; Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012).  
2035 Therefore, it comes as no surprise that the family was recovered as monophyletic in our analyses.  
2036 Idiopids are among the most diverse of the mygalomorphs; the family comprises 22 genera and  
2037 three subfamilies: Arbanitinae, Genysinae and Idiopinae (Raven 1985; Rix et al. 2017c), of  
2038 which we did not sample Genysinae. The position of Idiopidae within the Domiothelina clade  
2039 was consistently recovered by both morphological (Goloboff 1993) and molecular approaches  
2040 (Bond et al. 2012), but given the incomplete sampling of the family Ctenizidae (*sensu* Raven  
2041 (1985)), the position within the clade varied. In our analyses, the family was placed as sister to  
2042 Ctenizidae and Euctenizidae with high support.

2043

2044 Migidae

## PHYLOGENY OF MYGALOMORPH SPIDERS

2045    Remarks. The family Migidae currently comprises 11 genera with a classical Gondwanan  
2046    distribution (Australia, New Zealand, Africa, Madagascar and South America). The family is  
2047    well defined morphologically as taxa having longitudinal keels on cheliceral fangs and lack a  
2048    rastellum (Raven 1985; Griswold and Ledford 2001). Similar to previous studies (Hedin and  
2049    Bond 2006; Bond et al. 2012), our taxon sampling is rather limited. We only sampled four  
2050    genera, lacking a neotropical representative; therefore, we cannot assess the monophyly of the  
2051    group. The family has been recovered as sister to either *Stasimopus* (Hedin and Bond 2006) or  
2052    Halonoproctidae (Bond et al. 2012), as well as in close proximity to Idiopidae and Euctenizidae  
2053    (Ayoub et al. 2007; Bond et al. 2012), as we report here. In the concatenated analyses, the family  
2054    was recovered with high support as sister to all the remaining Domiothelina taxa, except for  
2055    Halonoproctidae. Despite the congruent topology, this relationship was poorly supported in the  
2056    species tree analysis.

2057

### 2058    Paratropididae

2059    Remarks. Paratropididae is a poorly known, and highly distinct family comprising four genera  
2060    known from Central and South America. Its phylogenetic placement has been regarded as  
2061    “difficult” (Bond et al. 2012), given the conflict between the morphology-based hypotheses and  
2062    the results of molecular analyses. The family was originally placed within a clade  
2063    “Theraphosoidina”, alongside the Theraphosidae and Barychelidae (Raven 1985; Goloboff  
2064    1993), however this relationship has never been recovered in any molecular analyses (Hedin and  
2065    Bond 2006; Bond et al. 2012; Hamilton et al. 2016b) and, in fact, a close relationship between  
2066    Paratropididae and any other mygalomorph taxon has never been supported. Instead, the  
2067    paratropidids were placed among the grade of early branching Avicularioidea (Hedin and Bond

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2068 2006; Bond et al. 2012). In our analyses, the paratropidids represent a stand-alone lineage  
2069 diverging at the root of Bipectina, however, similar to previous studies (Hedin and Bond 2006;  
2070 Bond et al. 2012), we only sampled representatives of the genus *Paratropis*. This not only  
2071 precludes the evaluation of the family's monophyly, but may also be a mitigating factor in  
2072 placing it on the tree. Moreover, the stand-alone status of the lineage may also have an impact on  
2073 the inferred tree topology as a whole, a fact demonstrated by the improved support values at  
2074 particular nodes and the topology changes in the unsupported nodes after paratropidids were  
2075 removed from the dataset (Supplemental Fig. 4). Our exploratory analysis combining AHE and  
2076 transcriptomic data recovered paratropidids with high support as sister to the “Nemesioidina”  
2077 clade (Fig. 5). However, the sampling in this analysis was limited both in terms of taxa and data  
2078 occupancy. Despite the equivocal nature of the placement of *Paratropis* within the Bipectina, it  
2079 seems clear that there is no support for its placement within Theraphosoidina (*sensu* Raven  
2080 (1985)).

2081

2082 Theraphosidae

2083 Remarks. The family Theraphosidae is the most diverse family of mygalomorphs with a nearly  
2084 cosmopolitan distribution. Currently comprising 144 genera and over 970 species, the family is  
2085 well-defined (aside from Barychelidae) by dense claw tufts and leg scopulae (Raven 1985). The  
2086 monophyly is generally undoubted (Hedin and Bond 2006; Bond et al. 2012; Lüddecke et al.  
2087 2018; Turner et al. 2018), although paraphyly with respect to Barychelidae has occurred due to  
2088 taxon misplacement (Goloboff 1993; Bond et al. 2012). Until recent molecular phylogenetics  
2089 work (Lüddecke et al. 2018; Turner et al. 2018), attempts to reconstruct the internal relationships  
2090 of Theraphosidae mostly relied on the analysis of morphological characters (Pérez-Miles 1994;

## PHYLOGENY OF MYGALOMORPH SPIDERS

2091 Pérez-Miles et al. 1996; Pérez-Miles 2002; Guadanucci 2005, 2011; Guadanucci 2014).  
2092 Molecular approaches have become more common to resolve taxonomic issues at shallow levels,  
2093 such as species delimitation or uncovering species-specific phylogeographic patterns (Hamilton  
2094 et al. 2011; Hendrixson et al. 2013; Hamilton et al. 2014; Graham et al. 2015; Montes de Oca et  
2095 al. 2016; Ortiz et al. 2018). In our analyses, we sampled eight genera from North and South  
2096 America, Africa, and Asia, recovering the family as monophyletic in all the analyses. The  
2097 subfamily Theraphosinae, here represented by the tribe Theraphosini (*Aphonopelma*,  
2098 *Cyrtopholis*, *Sericopelma* and *Stichoplastoris*) (Turner et al. 2018), was also recovered with  
2099 high support. On the other hand, our results substantiate the para- or polyphyletic status of the  
2100 subfamily Ischnocolinae, already debated on the basis of morphological characters (Raven 1985;  
2101 Guadanucci 2005; Guadanucci 2014; Guadanucci and Wendt 2014) and karyotype structure  
2102 (Král et al. 2013). The genus *Catumiri* (Ischnocolinae) was recovered as sister to the rest of the  
2103 theraphosid lineages, whereas *Ischnocolus*, regarded as the most “basal group” of the family  
2104 (Raven 1985), appears to be related to Ornitoctoninae and Harpactirinae (here represented by  
2105 *Brachionopus*). The non-monophyletic status of higher-level taxonomic units, and apparent  
2106 polyphyly of some wide spread genera arises from high levels of homoplasy in the  
2107 morphological characters traditionally used in Theraphosidae taxonomy (Guadanucci and Wendt  
2108 2014; Gabriel and Longhorn 2015; Hamilton et al. 2016a; Turner et al. 2018). Molecular  
2109 approaches and more thorough taxonomic sampling will be essential to establishing an accurate  
2110 classification scheme reflecting the evolutionary relationships within the family.  
2111  
2112

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2113 \*denotes genera included in analyses in the accompanying paper (Opatova et al. 2019); @

2114 denotes that the taxon was definitively placed by another study supporting the designated

2115 placement here

2116

2117 **Relimitation of the family Dipluridae Simon, 1889**

2118

2119 **Family Dipluridae Simon, 1889 (new circumscription)**

2120 In our analyses, the family Dipluridae is represented by three South American genera *Diplura*,

2121 *Linothele* and *Harmonicon* belonging to the diplurid subfamily Diplurinae (Raven 1985). We

2122 recovered the Dipluridae as sister to Cyrtoucheniidae placed within the same clade alongside

2123 Nemesiidae. These results are roughly congruent with Goloboff (Goloboff 1993), who analyzed

2124 four genera of diplurids (*sensu* Raven (1985)) and recovered *Diplura* as the sister to the rest of

2125 the Bipectina clade. As in Raven (1985), Goloboff (1993) also noted shared characters in

2126 *Diplura* and the Crassitarsae, and hypothesized that the diplurids would be restricted to the

2127 subfamily Diplurinae in the future. Unfortunately, we did not conduct an exhaustive sampling of

2128 the “diplurid” genera and acknowledge that even after removing the Euagridae, Ischnothelidae

2129 and Microhexuridae the family likely remains non-monophyletic. Given the rough congruence

2130 between Raven’s diplurid subfamilies (Raven 1985) and the lineages removed from the

2131 Dipluridae in this study, it is possible that the distinct subfamily Masteriinae (Raven 1985;

2132 Passanha and Brescovit 2018) will be elevated to family status as well.

2133

2134 **Type genus: *Diplura*** C. L. Koch, 1850 (type species *Mygale macrura* Koch, 1841)

2135

## PHYLOGENY OF MYGALOMORPH SPIDERS

2136 **Remarks.** The family Dipluridae is relimited here to include members of the subfamilies  
2137 Diplurinae Simon, 1889 and Masteriinae Simon, 1889. Of the Diplurinae genera, *Trechona* C. L.  
2138 Koch, 1850 and *Troglodiplura* Main, 1969 were not included in our analysis but are nevertheless  
2139 retained, at least until such time that they are included in a molecular study; *Trechona*'s inclusion  
2140 appears justified based on stridulatory and palpal endite characters (Raven 1985), whereas Main  
2141 (1993) justifies the placement of *Troglodiplura* in Diplurinae contra Raven (1985). The relimited  
2142 family herein conservatively also includes Masteriinae which we predict is likely to be  
2143 recognized as a standalone family, closely related to Microhexuridae at some point in the future;  
2144 the subfamily was recently reviewed by Passanha and Brescovit (2018), however, the authors  
2145 provide no insight regarding affinities to other mygalomorph taxa. The relimited Dipluridae will  
2146 require a significantly revised diagnosis at some point in the future.

2147

### 2148 **List of included subfamilies and genera**

2149

#### 2150 **Diplurinae Simon, 1889**

2151 \**Diplura* C. L. Koch, 1850

2152 \**Harmonicon* F. O. Pickard-Cambridge, 1896

2153 \**Linothele* Karsch, 1879

2154 *Trechona* C. L. Koch, 1850

2155 *Troglodiplura* Main, 1969

2156

#### 2157 **Masteriinae Simon, 1889**

2158 *Masteria* C. L. Koch, 1873

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2159 *Siremata* Passanha and Brescovit, 2018

2160 *Striamea* Raven, 1981

2161

2162 **Family Ischnothelidae F.O.P Cambridge, 1897 NEW RANK**

2163 The family corresponds to the former diplurid subfamily Ischnothelinae, defined by six

2164 morphological synapomorphies (Raven 1985; Coyle 1995). In our analyses, we sampled the

2165 genera *Ischnothele* (South America) and *Thelechoris* (Africa) and recovered these together in all

2166 analyses. In previous analyses based on morphological characters, *Ischnothele* was placed as a

2167 sister group to Bipectina (Goloboff 1993), but our results suggest that ischnothelids belong

2168 among the first groups that diverged within the Avicularioidea clade. Given the morphological

2169 cohesiveness of the group, and the fact that it was thoroughly revised in the past (Coyle 1995),

2170 we retain all the genera of the subfamily Ischnothelinae (Raven 1985; Coyle 1995) despite our

2171 incomplete sampling. The family Ischnothelidae comprises five genera (*Andethele*, *Indothele*,

2172 *Ischnothele*, *Latrothele*, *Thelechoris*) with a disjunct distribution spanning across Central and

2173 South America, the Antilles, Africa, Madagascar, India and Sri Lanka. All genera build

2174 conspicuous three-dimensional webs and often exhibit subsocial behavioral (Coyle 1995).

2175

2176 **Type genus: *Ischnothele*** Ausserer, 1875 (type species *Ischnothele caudata* Ausserer, 1875)

2177

2178 **Remarks.** Our sampling of ischnothelines comprised two genera that span the taxonomic

2179 breadth of the subfamily (Coyle 1995, Figures 21, 22) but omit three others. As discussed by

2180 Coyle (1995) in his revision of the subfamily, the group is supported by a number of unique

2181 morphological characters that taken in combination can be used for diagnosis. Distinguishing



## PHYLOGENY OF MYGALOMORPH SPIDERS

2182 characteristics for this newly elevated family are: 1) an elongate cymbial apophysis; 2)  
2183 collariform trichobothrial bases; and 3) fused silk spigots.

2184

2185 *List of included genera*

2186

2187 *\*Ischnothele* Ausserer, 1875

2188 *Andethele* Coyle, 1995

2189 *Indothele* Coyle, 1995

2190 *Lathrothele* Benoit, 1965

2191 *\*Thelechoris* Karsch, 1881

2192

2193 **Family Euagridae Raven, 1979 NEW RANK**

2194 The family roughly corresponds to the former subfamily Euagrinae (Raven 1985) that currently

2195 comprises 11 genera with distribution spanning across Australia, Asia, Africa, and South and

2196 North America. Euagrinae differ from other “diplurids” by a combined lack of cuspules and

2197 broad and short serrula. In our analyses, we only sampled five Euagrinae genera: *Allothele*,

2198 *Australothele*, *Cethegus*, *Euagrus* and *Namirea*, which were all recovered forming a

2199 monophyletic group towards the root node of Avicularioidea. Similar results were obtained in

2200 previous analyses (Ayoub et al. 2007; Bond et al. 2012), though with limited taxon sampling.

2201 The internal relationships of these taxa resemble their morphological affinities (Raven 1985).

2202 The “austral genera” possess a crescent of hirsute cuticle at the base of the posterior median

2203 spinnerets (“australothelinae crescent”) and were recovered as a monophyletic group sister to

2204 *Euagrus* that lacks this character (Raven 1985; Coyle 1988). The lack of a strong synapomorphy

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2205 uniting the “austral” genera and *Euagrus* + *Phyxioschema* (Raven 1985; Coyle 1988) alongside  
2206 deep divergence dating back to nearly 169 Ma (174 – 161), would provide the support for the  
2207 division of Euagrinae in two different families. However, the decision would be premature given  
2208 our taxon sampling, which represents less than half of the described generic diversity of  
2209 Diplurinae, and in particular, lacks representatives of the subfamily Masteriinae (Raven 1985) –  
2210 a group that overlaps in some characters with Euagrinae. We therefore prefer to elevate the  
2211 subfamily Euagrinae to family rank, retaining its current generic composition – i.e., the taxa  
2212 sampled in this paper along with the genera *Caledothele*, *Carrai*, *Chilehexops*, *Leptothele*,  
2213 *Phyxioschema*, *Stenygrocerus* and *Vilchura*.

2214

2215 **Type genus:** *Euagrus* Ausserer, 1875 (type species *Euagrus mexicanus* Ausserer, 1875)

2216

2217 **Remarks and revised diagnosis.** Unfortunately, our sampling of Euagrinae diplurids is  
2218 depauperate (and should include other *Euagrus* species, see below) and thus our placement of the  
2219 remaining diplurids in the subfamily Euagrinae follows authors subsequent to Raven’s (1985)  
2220 establishment of the family (i.e., inclusion in the subfamily is maintained per Raven (1991),  
2221 Coyle (1986), Raven and Schwendinger (1995), and Ríos-Tamayo and Goloboff (2017)). Note  
2222 that *Euagrus*, was considered by Coyle (1988) to be largely central/south American with the two  
2223 species, *Euagrus atropurpureus* (S. Africa) and *Euagrus formosanus* (Taiwan) as misplaced. It is  
2224 important to recognize that some of these taxa may be allocated to other formerly “diplurid”  
2225 families at some point in the future (e.g., *Chilehexops*, *Caledothele*, *Vilchura*). Although  
2226 morphological evidence (*sensu* Coyle (1988)) seems strong that *Phyxioschema* is the sister group  
2227 to *Euagrus*, the morphological cladistic analysis conducted by Ríos-Tamayo and Goloboff

## PHYLOGENY OF MYGALOMORPH SPIDERS

2228 (2017) provides little encouragement that the subfamily is monophyletic and should indicate that  
2229 any morphological diagnosis of this remaining assemblage of taxa is likely without substance.  
2230 Following Raven (1985), the family can be diagnosed as those taxa having a distinct spur on the  
2231 second male tibia and a centrally raised tarsal organ (also following from Ríos-Tamayo and  
2232 Goloboff (2017)); Euagrinae taxa differ from australothelines by lacking an australotheline  
2233 crescent (see details in the diagnosis below).

2234

2235 *List of included Euagrinae genera*

2236

2237 \**Euagrus* Ausserer, 1875

2238 *Caledothele* Raven, 1991

2239 *Chilehexops* Coyle, 1986

2240 *Leptothele* Raven and Schwendinger, 1995

2241 *Phyxioschema* Simon, 1889

2242 *Vilchura* Ríos-Tamayo and Goloboff, 2017

2243

2244 **Australothelinae NEW SUBFAMILY Bond, Opatova, and Hedin**

2245

2246 **Type genus:** *Australothele* Raven, 1984 (type species *Australothele maculata* Raven, 1984)

2247

2248 **Diagnosis:** Per Raven (1985), australothelines can be diagnosed from other euagrids as those  
2249 taxa having an “australotheline crescent” on the posterior median spinnerets – which comprises a  
2250 “crescent of hirsute cuticle isolated by pallid glabrous anterior to bases” and lacking cymbial

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2251 spines (the latter character is shared by numerous other mygalomorph taxa (mentioned by Ríos-  
2252 Tamayo and Goloboff (2017)). As noted by Raven (1985), a similar such crescent is also found  
2253 in the putatively true diplurid genus *Masteria*. The subfamily is also supported as monophyletic  
2254 in the analysis conducted by Ríos-Tamayo and Goloboff (2017) and Hedin et al. (2018).

2255

2256 *List of genera included*

2257

2258 \**Australothele* Raven, 1984

2259 \**Allothele* Tucker, 1920

2260 *Carrai* Raven, 1984

2261 \**Cethegus* Thorell, 1881

2262 \**Namirea* Raven, 1984

2263 *Stenygrocercus* Simon, 1892

2264

2265 **Microhexuridae NEW FAMILY Bond, Opatova, and Hedin**

2266 *Family Microhexuridae (NEW FAMILY).*—Microhexuridae is a monogeneric family comprising  
2267 two nominal species endemic to North America. Both species are very small (> 6 mm) and have  
2268 allopatric distributions restricted to the high-elevation peaks in the Appalachian Mountains and  
2269 Pacific Northwest Cascade Range (Coyle 1981; Hedin et al. 2015). Despite being recognized as a  
2270 diplurid (*sensu* Raven (1985)), *Microhexura* exhibits a combination of characters not present in  
2271 other diplurid genera. Most notably *Microhexura* possess a longitudinal fovea and spinnerets  
2272 somewhat shorter than in other diplurids, which made the genus difficult to place (Coyle 1981;  
2273 Raven 1985). The unique position of the genus noted in the previous studies (Coyle 1981; Raven

## PHYLOGENY OF MYGALOMORPH SPIDERS

2274 1985) is further supported by our results that recovered the genus *Microhexura* as a stand-alone  
2275 lineage among other “non-diplurine diplurids” at the base of the Avicularioidea clade. The  
2276 topology test results reject both its placement within the Dipluridae (*sensu* Raven (1985)), but  
2277 also within the “non-diplurine diplurids”/Hexathelidae/Porrhothelidae clade recovered in the  
2278 species tree analyses.

2279

2280 **Type genus.** *Microhexura* Crosby and Bishop, 1925 (type species *Microhexura montivaga*  
2281 Crosby and Bishop, 1925)

2282

2283 **Diagnosis:** Because the family is monogeneric, characters used to diagnose Microhexuridae are  
2284 those that can be attributed to the genus by Coyle (1981). Among the smallest mygalomorph  
2285 spiders known, the family is diagnosed on the basis of the following unique combination of  
2286 characters: 1) longitudinal fovea; 2) four spinnerets – posterior lateral spinnerets long (nearly the  
2287 length of the abdomen); 3) lacking abdominal tergites; and 4) male pedipalp lacking conductor.

2288

2289 *List of included genera*

2290

2291 \**Microhexura* Crosby and Bishop, 1925

2292

2293 **Relimitation of the family Ctenizidae Thorell, 1887**

2294

2295 **Ctenizidae Thorell, 1887 (new circumscription)**

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2296 The generic composition of the family Ctenizidae was dramatically altered by Raven's (1985)  
2297 morphology-based cladistic analysis, but it became apparent that the family most likely  
2298 comprised an assemblage of diverse lineages that do not share a common ancestor (Hedin and  
2299 Bond 2006). This observation was repeatedly confirmed by subsequent molecular-based studies  
2300 (Bond et al. 2012; Opatova et al. 2013; Wheeler et al. 2017). However, a lack of perceived  
2301 phylogenetic signal, incomplete sampling, or a combination thereof precluded previous authors  
2302 from making taxonomic changes that would rectify the problem. The polyphyletic nature of the  
2303 family was partially resolved by Godwin et al. (2018) when six genera were transferred to the  
2304 family Halonoproctidae, circumscribing Ctenizidae to *Cteniza*, *Cyrtocarenum* and *Stasimopus*.  
2305 Godwin et al. (2018) recognized that *Stasimopus* did not share a common ancestor with the  
2306 remaining genera, but incomplete outgroup sampling limited their ability to definitively address  
2307 the phylogenetic placement of the taxon. In our analyses, we recovered *Cteniza* and  
2308 *Cyrtocarenum* as sister taxa, which agrees with previous findings (Opatova et al. 2013; Godwin  
2309 et al. 2018). *Cteniza* and *Cyrtocarenum* were placed as sister to the family Euctenizidae, whereas  
2310 *Stasimopus* was recovered outside of Domiothelina in a weakly supported clade as the sister  
2311 lineage to Atracidae and Actinopodidae. Given the distant phylogenetic position of *Stasimopus*,  
2312 with regards to the remaining ctenizids, and considering that the topology test rejected the  
2313 monophyly of the three genera, we remove *Stasimopus* from the family Ctenizidae and designate  
2314 it to the family Stasimopidae (NEW FAMILY). Ctenizidae is circumscribed to contain only the  
2315 genera *Cteniza* and *Cyrtocarenum*. The family Ctenizidae thus now constitutes a much smaller  
2316 family comprising six species with a predominantly Mediterranean distribution.

2317

2318 **Type genus:** *Cteniza* Latreille, 1829 (type species *Cteniza sauvagesi* (Rossi, 1788))

## PHYLOGENY OF MYGALOMORPH SPIDERS

2319

2320 **Remarks and revised diagnosis.** As discussed by others (e.g., Decae 1996; Godwin et al. 2018),  
2321 *Cteniza* and *Cyrtocarenum* are virtually indistinguishable from each other and are very similar to  
2322 other taxa like halonoproctids and stasimopids, save some questionable eye group characteristics.  
2323 As outlined by Godwin et al. (2018), Ctenizidae, as delimited herein, can be distinguished from  
2324 other similar taxa by lacking curved spines on the anterior legs, having a more arched and  
2325 rounded caput and flatter ventral posterior carapace, and having a more oblong abdomen (rather  
2326 than ovoid); legs tend to be less stocky. Ctenizids differ from their sister group, Euctenizidae, by  
2327 lacking scopulae.

2328

2329 *List of included genera*

2330

2331 \**Cteniza* Latreille, 1829

2332 \**Cyrtocarenum* Ausserer, 1871

2333

2334

2335 **Stasimopidae NEW FAMILY Bond, Opatova, and Hedin**

2336 Although the placement of *Stasimopus* within the family Ctenizidae (Raven 1985) was  
2337 repeatedly called into question based on earlier molecular studies (Hedin and Bond 2006; Bond  
2338 et al. 2012; Opatova et al. 2013; Godwin et al. 2018), establishing its exact phylogenetic position  
2339 has been difficult. In previous phylogenetic studies of the entire infraorder, *Stasimopus* was  
2340 recovered as sister to Migidae (Hedin and Bond 2006), or as the sister group to the entire  
2341 Domiothelina, albeit with low support (Bond et al. 2012). Importantly, when morphological

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2342 characters were added to the analyses, *Stasimopus* was recovered within the Ctenizidae (Bond et  
2343 al. 2012), confirming the homoplasious nature of some morphological characters. Our results  
2344 place *Stasimopus* as a stand-alone lineage either forming a clade with Atracidae and  
2345 Actinopodidae or as sister to Domiothelina. The sister relationship with the “Venom Clade” was  
2346 also recovered by Hamilton et al. (2016b), though in a much reduced AHE dataset, and similarly  
2347 to our results, the clade was poorly supported. The support values for this clade increased with  
2348 the removal of the paratropidid terminals (Supplemental Fig. 4) and with addition of  
2349 transcriptomic data (Fig. 4), suggesting that adding more data to the matrix could be instrumental  
2350 for stabilizing Stasimopidae placement. Despite the partial positional uncertainty of *Stasimopus*,  
2351 the topology test rejects its inclusion within Ctenizidae. Considering the results of our analyses,  
2352 alongside the genus’ restricted distribution to southern Africa – a biodiversity hotspot with high  
2353 levels of endemism (Myers et al. 2000; Mittermeier et al. 2011), it seems that there is sufficient  
2354 evidence to support the hypothesis that *Stasimopus* represents a unique lineage within the  
2355 Mygalomorphae and deserves family-level status.

2356

2357 **Type genus:** *Stasimopus* Simon, 1892 (type species *Stasimopus caffrus* (C. L. Koch, 1842)

2358

2359 **Diagnosis:** At this time, the family Stasimopidae is monogeneric, thus characteristics used to  
2360 diagnose the genus are the distinguishing characters for the family. Per Raven (1985) and  
2361 summarized by Engelbrecht and Prendini (2012), stasimopids can be distinguished from other  
2362 ctenizid-like taxa on the basis of the following combination of unique characters: 1) lacking a  
2363 saddle-like depression on tibia III; 2) having a very wide eye group (twice as wide as it is long);  
2364 and 3) palpal endites with a modified anterior lobe (described as “produced”). Based on the



## PHYLOGENY OF MYGALOMORPH SPIDERS

2365 breadth of morphological diversity contained within the genus, which includes somewhat bizarre  
2366 carapace modifications (Engelbrecht and Prendini 2012), it is possible that *Stasimopus* could be  
2367 split in to multiple genera at some point in the future.

2368

2369 *List of included genera*

2370

2371 \**Stasimopus* Simon, 1892

2372

2373

2374 **Relimitation of the family Cyrtaucheniidae Simon, 1889**

2375

2376 **Cyrtaucheniidae Simon, 1889 (new circumscription)**

2377 Following the results of our phylogenetic analyses, we transfer the genus *Homostola* to the  
2378 newly established family Bemmeridae (NEW RANK, see below). We also formally transfer the  
2379 Mediterranean genus *Amblyocarenum* to the family Nemesiidae. The family Cyrtaucheniidae is  
2380 thus relimited to nine genera (three of them monotypic) distributed across Africa, Asia and South  
2381 America. In this study, we analyzed two African genera: *Ancylotrypa*, *Cyrtauchenius* and one  
2382 South American lineage that is morphologically proximate to *Acontius*. As a result,  
2383 Cyrtaucheniidae were recovered as monophyletic in all analyses and placed as sister to  
2384 Dipluridae. At present, we retain the genera *Acontius*, *Anemesia*, *Angka*, *Bolostromoides*,  
2385 *Bolostromus*, *Fufius*, and *Rhytidicolus* (not included in this study), within Cyrtaucheniidae. We  
2386 acknowledge that the family still likely remains poly- or paraphyletic, because *Fufius* is known  
2387 to have affinities with the Nemesiidae (Bond et al. 2012), however, we argue that even the partial

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2388 Cyrtaucheniidae taxon pruning performed in this study is preferable to retaining the high level of  
2389 polyphyly. This is particularly true when obtaining fresh samples may be exceptionally difficult,  
2390 especially for the African and South American representatives of the genera *Acontius* and  
2391 *Bolostromus*.

2392

2393

2394 **Type genus:** *Cyrtauchenius* Thorell, 1869 (type species *Cyrtauchenius terricola* (Lucas, 1846))

2395

2396 **Remarks.** Long recognized as a polyphyletic assemblage of genera, we herein relimit  
2397 Cyrtaucheniidae to the Aporoptychinae minus *Kiama* and *Angka* based on Raven's (1985)  
2398 original composition of the subfamily. Given the diversity of the taxa left remaining in the  
2399 family, it is unlikely that a revised diagnosis will have any lasting value (if even possible to  
2400 formulate, given the diverse array of taxa remaining) and as such we decline to provide one. We  
2401 anticipate significant changes to the composition of the family as additional aporoptychines are  
2402 added to the tree. We justify our transfer of *Angka* to Microstigmatidae based on the original  
2403 description of the genus by Raven and Schwendinger (1995), where they considered it as a clear  
2404 sister taxon to *Kiama* (also transferred, by us below, to the family Microstigmatidae); the transfer  
2405 of *Amblyocarenum* to Nemesiidae is justified on the basis of its phylogenetic position.

2406

2407 *List of included genera*

2408

2409 \**Cyrtauchenius* Thorell, 1869

2410 \**Ancylotrypa* Simon, 1889

## PHYLOGENY OF MYGALOMORPH SPIDERS

- 2411
- 2412 *Incertae sedis*
- 2413
- 2414 *Acontius* Karsch, 1879
- 2415 *Anemesia* Pocock, 1895
- 2416 *Bolostromoides* Schiapelli and Gerschman, 1945
- 2417 *Bolostromus* Ausserer, 1875
- 2418 *Fufius* Simon, 1888
- 2419 *Rhytidicolus* Simon, 1889
- 2420
- 2421 Transferred to other families (not included in the relimitation of Cyrtaucheniidae)
- 2422
- 2423 \**Amblyocarenum* Simon, 1892 here transferred to Nemesiidae justified on the basis of
- 2424 phylogenetic placement.
- 2425
- 2426 @*Angka* Raven and Schwendinger, 1995 here transferred to Microstigmatidae on the basis of its
- 2427 putative sister relationship to *Kiama*.
- 2428
- 2429 \**Homostola* Simon, 1892 here transferred to Bemmeridae justified on the basis of phylogenetic
- 2430 placement.
- 2431
- 2432 **Relimitation of the family Nemesiidae Simon, 1889**
- 2433

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2434 **Nemesiidae Simon, 1889 (new circumscription)**

2435

2436 The family Nemesiidae is a cosmopolitan group. Our sampling comprised five genera, including  
2437 the type genus *Nemesia*, spanning species sampled from three continents (Africa, Europe and  
2438 North America). These genera form a single lineage and are placed in a clade that is sister to all  
2439 the former “nemesiid” lineages plus Cyrtaucheniidae, Dipluridae and Microstigmatidae. In  
2440 agreement with our results, we transfer the genus *Spiroctenus* to the newly established family  
2441 Bemmeridae and transfer *Amblyocarenum* here from Cyrtaucheniidae (see discussion on  
2442 Cyrtaucheniidae for justification). We remove the genera *Pionothele*, *Bayana*, *Stenoterommata*,  
2443 *Stanwellia*, *Acanthogonatus*, *Pycnothele* and *Entypesa* and transfer them into newly erected  
2444 families Pycnothelidae (NEW RANK) and Entypesidae (NEW FAMILY). We further remove  
2445 the taxa included in the Anamini tribe (Harvey et al. 2018) and elevate it to the rank of family:  
2446 Anamidae (NEW RANK). We also transfer the genera *Kiama*, *Angka*, and *Xamiatus* to the  
2447 family Microstigmatidae (see details below). We acknowledge that many taxa, particularly those  
2448 inhabiting South America and Asia, remain severely under-sampled in our study and therefore  
2449 we cannot speak to their definitive placement at this time; as such they remain for time being  
2450 assigned to Nemesiidae *incertae sedis*.

2451

2452 **Type genus:** *Nemesia* Audouin, 1826 (type species *Nemesia cellicola* Audouin, 1826)

2453

2454 **Remarks.** To a greater extent than Cyrtaucheniidae (above), what remains of the family  
2455 Nemesiidae is a likely para-polyphyletic assemblage of genera. As such we consider the  
2456 placement of a large number of genera in the family to be uncertain at best; many of which may

## PHYLOGENY OF MYGALOMORPH SPIDERS

2457 prove to be pycnothelids. Consequently, much work remains, particularly if we are able to place

2458 many of the South American genera.

2459

2460 *List of included genera*

2461

2462 \**Nemesia* Audouin, 1826

2463 \**Amblyocarenum* Simon, 1892

2464 \**Calisoga* Chamberlin, 1937

2465 \**Iberesia* Decae and Cardoso, 2006

2466 \**Mexentypesa* Raven, 1987

2467

2468 *Incertae sedis*

2469

2470 *Atmetochilus* Simon, 1887

2471 *Brachythele* Ausserer, 1871

2472 *Chaco* Tullgren, 1905

2473 *Chilelopsis* Goloboff, 1995 – most likely Pycnothelidae

2474 *Damarchilus* Siliwal, Molur, and Raven, 2015

2475 *Damarchus* Thorell, 1891

2476 *Diplothelopsis* Tullgren, 1905

2477 *Flamencopsis* Goloboff, 1995

2478 *Gravelyia* Mirza and Mondal, 2018

2479 *Hermacha* Simon, 1902 – likely to be transferred to Entypesidae

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 2480 *Hermachura* Mello-Leitão, 1923
- 2481 *Lepthercus* Purcell, 1902 – likely Entypesidae
- 2482 *Longistylus* Indicatti and Lucas, 2005; removed from Anamidae *contra* Indicatti and Lucas
- 2483 (2005)
- 2484 *Lycinus* Thorell, 1894
- 2485 *Neostothis* Vellard, 1925
- 2486 *Prorachias* Mello-Leitão, 1924
- 2487 *Psalistopoides* Mello-Leitão, 1934
- 2488 *Pselligmus* Simon, 1892
- 2489 *Rachias* Simon, 1892; likely to be transferred to Pycnothelidae per Goloboff (1995)
- 2490 *Raveniola* Zonshtein, 1987
- 2491 *Sinopesa* Raven and Schwendinger, 1995
- 2492
- 2493 Transferred to other families (not included in the relimitation of Nemesiidae)
- 2494
- 2495 *Xamiatus* Raven, 1981; here transferred to Microstigmatidae on the basis of its putative
- 2496 sister/close relationship with *Ixamatus* and *Kiama* (Raven 1981,1985)
- 2497 *Ixamatus* Simon, 1887 here transferred to Microstigmatidae on the basis of its putative
- 2498 phylogenetic position per Harvey et al. (2018).
- 2499
- 2500 **Anamidae Simon, 1889 NEW RANK**
- 2501 The family Anamidae corresponds to the Australian endemic tribe Anamini (Raven 1985;
- 2502 Harvey et al. 2018) formerly placed within the “nemesiid” subfamily Anaminae (Raven 1985).

## PHYLOGENY OF MYGALOMORPH SPIDERS

2503 The family comprises genera *Aname*, *Chenistonia*, *Hesperonatalius*, *Kwonkan*, *Namea*,  
2504 *Proshermacha*, *Teyl*, *Teyloides* and *Swolnpes*, of which we analyzed six (including *Pseudoteyl*  
2505 currently synonymized with *Teyl*). The family formed a monophyletic lineage in all analyses and  
2506 was recovered as sister to Entypesidae/Microstigmatidae clade. The phylogenetic relationships of  
2507 the group were recently evaluated, employing both extensive taxonomic sampling and multilocus  
2508 data (Harvey et al. 2018). Harvey et al. (2018) performed a complete generic sampling of the  
2509 Australian Anamini and recovered the group as a well-supported clade, also including genera not  
2510 sampled in our analyses. Following the results of our study and Harvey et al. (2018), we  
2511 therefore transfer all the genera listed above into the family Anamidae. Morphology and  
2512 biogeography do not support the inclusion of *Longistylus* Indicatti and Lucas, 2005 in the  
2513 Anamidae contra the tentative hypothesis proposed by Indicatti and Lucas (2005).

2514

2515 **Type genus:** *Aname* L. Koch, 1873 (type species *Aname pallida* L. Koch, 1873)

2516

2517 **Remarks.** A detailed diagnosis for this new family rank taxon is provided by Harvey et al.  
2518 (2018).

2519

2520 *List of included genera*

2521

2522 \**Aname* L. Koch, 1873

2523 \**Proshermacha* Simon, 1908

2524 @*Hesperonatalius* Castalanelli et al. 2017 per Harvey et al. (2018)

2525 \**Kwonkan* Main, 1983 (= \**Yilgarnia* Main, 1986)

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2526 \**Namea* Raven, 1984

2527 @*Chenistonia* Hogg 1901 per Harvey et al. (2018)

2528 @*Swolnpes* Main and Framenau, 2009 per Harvey et al. (2018)

2529 \**Teyl* Main, 1975

2530 @*Teyloides* Main, 1985 but check Bond et al. (2012) and Hedin and Bond (2006) – may be able

2531 to place elsewhere) Harvey et al. (2018)

2532

2533 **Bemmeridae Simon, 1903 NEW RANK**

2534 The family Bemmeridae comprises 35 species classified in the genera *Homostola* and

2535 *Spiroctenus*, both endemic to South Africa. The group was repeatedly recovered as sister to

2536 Theraphosoidina (Hedin and Bond 2006; Bond et al. 2012), which is further substantiated by our

2537 analyses. Previous analyses also suggested the inclusion of the genus *Ancylotrypa* (Hedin and

2538 Bond 2006), an assumption that was unfortunately based on sample misidentification. The genus

2539 *Homostola* is being transferred to this family from Cyrtaucheniidae, the genus *Spiroctenus* from

2540 Nemesiidae. Both genera comprise medium-size spiders, constructing underground burrows with

2541 or without a trapdoor.

2542

2543 **Type genus:** *Spiroctenus* Simon, 1892 (type species *Spiroctenus personatus*, Simon, 1888)

2544

2545 **Remarks and Diagnosis.** The family name is derived from the available family-level taxon

2546 Bemmereae Simon, 1903; *Bemmeris* Simon, 1903 is a junior synonym of *Spiroctenus*. The

2547 identity and consequently family-level placement of *Spiroctenus* and *Homostola* is contentious

2548 and likely to remain so. Raven (1985) considered the two genera unrelated and placed in separate



## PHYLOGENY OF MYGALOMORPH SPIDERS

2549 families (Nemesiidae and Cyrtaucheniidae respectively), attributing Hewitt's (1915)  
2550 hypothesized synonym of the two to a misidentification. To date, it would seem that a male  
2551 *Homostola* specimen has never been described in detail, further complicating the situation (but  
2552 see Hewitt (1916)). Based on careful specimen identification and locality data, we are confident  
2553 that our sample comprises both genera. The *Spiroctenus* exemplar represents an identical male  
2554 and female sequence of *Spiroctenus flavopunctatus* (Purcell, 1903); *Homostola* terminals are  
2555 likely *H. pardalina* *affin.* (Hewitt, 1913) and *H. vulpecula* Simon, 1892. Based on our  
2556 examination of specimens, Bemmeridae females can be diagnosed on the basis of the following  
2557 unique combination of characters: 1) eyes on a low tubercle; 2) metatarsus and tarsus with  
2558 moderate to thick scopula; 3) preening combs on leg III; 4) labium with relatively dense patch of  
2559 cuspules; and 5) a rastellum comprising a row of thickened, blunt spines not borne on an  
2560 apophysis. Male *Spiroctenus* species have a very distinct mating clasper on leg I comprising a  
2561 number of large spines and an apophysis at the tibia/metatarsus junction; we would predict a  
2562 similar morphology for *Homostola* males.

2563

2564 *List of included genera*

2565

2566 \**Homostola* Simon, 1892

2567 \**Spiroctenus* Simon, 1889

2568

2569 **Pycnothelidae Chamberlin, 1917 NEW RANK**

2570 *Family Pycnothelidae (NEW RANK).*—The family Pycnothelidae is newly established here in  
2571 order to accommodate taxa formerly belonging to a diverse mix of “nemesiid” subfamilies

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2572 (Raven 1985). Some of the close relationships are not entirely novel. For example the sister  
2573 relationship between *Stanwellia* and *Acanthogonatus* was previously recovered by Hedin and  
2574 Bond (2006). We recovered the African genus *Pionothele*, formerly placed within the subfamily  
2575 Bemerinnae, as sister to *Spiroctenus*, and sister to all pycnothelid diversity. The family  
2576 distribution spans across Africa, Australia, South America and New Zealand, therefore it could  
2577 be expected that more genera will be eventually transferred here following a thorough  
2578 assessment, particularly of African and South American lineages.

2579

2580 **Type genus:** *Pycnothele* Chamberlin, 1917 (type species *Pycnothele perdita* Chamberlin, 1917)

2581

2582 **Remarks and Diagnosis:** The genus *Pycnothele* was not included in our analysis but  
2583 hypothesized morphological affinities (Goloboff 1995), have attributed it to be closely aligned  
2584 with *Stanwellia*, *Acanthogonatus*, and *Stenoterommata*. Harvey et al. (2018) stated: “If a close  
2585 relationship between *Stanwellia*, *Acanthogonatus*, *Stenoterommata* and *Pycnothele* is  
2586 maintained, as proposed by Goloboff (1995), the subfamily-group name Pycnothelinae  
2587 Chamberlin, 1917 is available for this clade (Chamberlin 1917).” In the interests of taxonomic  
2588 stability, we believe the conservative decision is to transfer *Pycnothele* from Nemesiidae and  
2589 include it in our newly defined clade (justified on the basis of morphological similarity). The  
2590 alternative would be to propose a new family rank name (e.g., “Stanwellidae”) which will be  
2591 inevitably deprecated at a later time. At this time, the family Pycnothelidae can be diagnosed on  
2592 the basis of the following unique combination of characters: 1) rastellum absent; 2) aspinose  
2593 tarsi; 3) preening combs absent, although reduced in some *Stanwellia* and *Acanthogonatus*; 4)  
2594 labium lacks cuspules; 5) tibial spur on male leg I absent; and 6) most taxa have a reduced or

## PHYLOGENY OF MYGALOMORPH SPIDERS

2595 absent inferior tarsal claw. We anticipate that this diagnosis might change as other nemesiids  
2596 (noted as *incertae sedis* above) are added to the tree.

2597

2598 *List of included genera*

2599

2600 @*Pycnothele* Chamberlin, 1917

2601 \**Stanwellia* Rainbow and Pulleine, 1918

2602 \**Acanthogonatus* Karsch, 1880 (note: most likely polyphyletic)

2603 \**Bayana* Pérez-Miles, Costa and Montes de Oca, 2014

2604 \**Pionothele* Purcell, 1902

2605 \**Stenoterommata* Holmberg, 1881

2606

2607 **Entypesidae NEW FAMILY Bond, Opatova, and Hedin**

2608

2609 We establish the family Entypesidae to accommodate the genus *Entypesa*, which currently  
2610 comprises 7 species known from Madagascar and South Africa. The genus was originally placed  
2611 within the nemesiid subfamily Anaminae (Raven 1985), however this taxon has been shown to  
2612 be polyphyletic (Bond et al. 2012). Despite the rather undesirable monogeneric state of the  
2613 family, we believe that establishing Entypesidae as a family is justified by both its reciprocal  
2614 monophyly in relation to its sister taxon Microstigmatidae and the deep divergence between  
2615 them, dating back to 79 Ma. We believe the monogeneric nature of the family is only temporary  
2616 and other genera (particularly fellow South African genera *Lepthercus* and *Hermacha*) will  
2617 eventually be transferred here following a thorough assessment of Nemesiidae phylogeny.

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

2618

2619 **Type genus:** *Entypesa* Simon, 1902 (type species *Entypesa nebulosa* Simon, 1902)

2620

2621 **Diagnosis:** Because the family is monogeneric, characters used to distinguish it are those that  
2622 can be attributed to diagnosing the genus. Based on examination of specimens, Raven's (1985)  
2623 description and Dippenaar-Shoeman (2002), Entypesidae may be diagnosed on the basis of the  
2624 following unique combination of characters: 1) narrow straight thoracic groove; 2) distinctly  
2625 raised eye tubercle; 3) labium lacks cuspules; 4) weak rastellum comprising numerous short,  
2626 stout bristles; 5) dense scopula on tarsi I-III; 6) leg III preening comb, present; and 7) long,  
2627 digitiform spinnerets. We anticipate the inclusion of *Hermacha* and *Lepthercus* in this family at  
2628 some point in the future.

2629

2630 *Included genus*

2631

2632 \* *Entypesa* Simon, 1902

2633

2634 **Relimitation of the family Microstigmatidae Roewer, 1942**

2635

2636 **Family Microstigmatidae Roewer, 1942 (new circumscription)**

2637 The family Microstigmatidae is well defined by morphological characters, such as small, round  
2638 booklung openings and pustulose cuticle (Raven 1985; Hedin and Bond 2006). The monophyly  
2639 of the family has never been assessed within a robust molecular framework because all previous  
2640 studies exclusively included the South African genus *Microstigmata* (Hedin and Bond 2006;

## PHYLOGENY OF MYGALOMORPH SPIDERS

2641 Ayoub et al. 2007; Bond et al. 2012; Wheeler et al. 2017); but see (Goloboff 1993). Following  
2642 the results of our analyses we relimit the family Microstigmatidae to include the genus *Kiama*.  
2643 The morphological similarities in tarsal organ and cuticle structure linking *Kiama* to  
2644 microstigmatids are well documented (Raven 1985; Bond and Opell 2002). Similar  
2645 characteristics are also found in the genera *Ixamatus* and *Angka* (Bond et al. 2012) suggesting  
2646 their close relationships to the microstigmatids.

2647

2648 **Type genus:** *Microstigmata* Strand, 1932 (type species *Microstigmata geophila* (Hewitt, 1916))

2649

2650 **Remarks:** The family Microstigmatidae was traditionally diagnosed as taxa have possessing,  
2651 circular booklung openings. With Goloboff's (1995) inclusion of *Pseudonemesia* and  
2652 *Spelocteniza* in the family, as well as *Envia* (*sensu* Ott and Höfer (2003)), that feature now only  
2653 serves to diagnose a subset of these relatively unique genera. Although a scaly or pustulose  
2654 cuticle may be a diagnostic character uniting all microstigmatids (Bond and Opell 2002), future  
2655 SEM study of all genera will be necessary to confirm. As noted above, the transfer of *Angka* to  
2656 the Microstigmatidae is based on its affinities with *Kiama* whose phylogenetic position clearly  
2657 aligns here.

2658

2659 *List of included genera*

2660

2661 \**Microstigmata* Strand, 1932

2662 @*Angka* Raven and Schwendinger, 1995

2663 *Envia* Ott and Höfer, 2003

OPATOVA V, HAMILTON CA, HEDIN MH, MONTES DE OCA L, KRÁL J & J BOND

- 2664 @*Ixamatus* Simon, 1887 transferred here per Harvey et al. 2018; as noted by Raven (1985), the  
2665 genus has a pustulose cuticle, a potentially diagnostic character of the family.
- 2666 \**Kiama* Main and Mascord, 1969
- 2667 *Micromygale* Platnick and Forster, 1982
- 2668 *Ministigmata* Raven and Platnick, 1981
- 2669 *Pseudonemesia* Caporiacco, 1955
- 2670 *Spelocteniza* Gertsch, 1982
- 2671 @*Xamiatus* Raven, 1981 transferred here on the basis of morphological similarity with *Ixamatus*  
2672 and its putative sister group relationship with *Kiama*; as noted by Raven (1985), the genus has a  
2673 pustulose cuticle, a potentially diagnostic character of the family.
- 2674 *Xenonemesia* Goloboff, 1989