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Report

Phylogenomics Resolves a Spider Backbone Phylogeny and Rejects a Prevailing Paradigm for Orb Web Evolution

Jason E. Bond,[1,4,*] Nicole L. Garrison,[1,4] Chris A. Hamilton,[1] Rebecca L. Godwin,[1] Marshal Hedin,[2] and Ingi Agnarsson[3]

[1]Department of Biological Sciences and Auburn University Museum of Natural History, Auburn University, Auburn, AL 36849, USA

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

[3]Department of Biology, University of Vermont, 120A Marsh Life Science Building, 109 Carrigan Drive, Burlington, VT 05405, USA

Summary

Spiders represent an ancient predatory lineage known for their extraordinary biomaterials, including venoms and silks. These adaptations make spiders key arthropod predators in most terrestrial ecosystems. Despite ecological, biomedical, and biomaterial importance, relationships among major spider lineages remain unresolved or poorly supported [1]. Current working hypotheses for a spider "backbone" phylogeny are largely based on morphological evidence, as most molecular markers currently employed are generally inadequate for resolving deeper-level relationships. We present here a phylogenomic analysis of spiders including taxa representing all major spider lineages. Our robust phylogenetic hypothesis recovers some fundamental and uncontroversial spider clades, but rejects the prevailing paradigm of a monophyletic Orbiculariae, the most diverse lineage, containing orb-weaving spiders. Based on our results, the orb web either evolved much earlier than previously hypothesized and is ancestral for a majority of spiders or else it has multiple independent origins, as hypothesized by precladistic authors. Cribellate deinopoid orb weavers that use mechanically adhesive silk are more closely related to a diverse clade of mostly webless spiders than to the araneoid orb-weaving spiders that use adhesive droplet silks. The fundamental shift in our understanding of spider phylogeny proposed here has broad implications for interpreting the evolution of spiders, their remarkable biomaterials, and a key extended phenotype—the spider web.

Results and Discussion

With approximately 44,906 described species [2] in over 100 families, spiders represent the most species-rich lineage of generalist predators in almost every terrestrial biome. This ancient group is particularly well known for silks that can be 1,000% tougher than high-energy absorbing polymers such as Kevlar [3, 4], and most species produce a diverse array of multifunctional silks used in prey capture, burrow construction, and reproduction. A vast array of predatory venoms have evolved in spiders, and these biomolecules have tremendous biomedical [5] and agricultural [6] potential.

The consensus view of spider phylogeny summarized by Coddington [7] has changed little over the past quarter century (Figure 1); traditional morphological and published molecular systematics data sets have failed to provide a consistent view of "backbone" relationships within spiders. At present, the order is divided into clades including the Mesothelae and Opisthothelae, with Opisthothelae comprising the infraorders Mygalomorphae (e.g., trapdoor spiders, tarantulas, etc.) and Araneomorphae (e.g., jumping spiders, wolf spiders, orb-weaving spiders, etc.). The Araneomorph lineage contains the vast majority of spider diversity parceled among a number of clades (Figure 1) recognized predominantly via morphological cladistics analyses [1]. We present here a phylogenomic analysis of spider relationships based on data sets comprising 327 (d327) and 128 (d128) putatively orthologous nuclear protein coding loci (Table S1 available online). These two phylogenetic "supermatrices" were assembled for 40 spider taxa representing 33 spider families (Table S2; the bioinformatics pipeline is summarized in the Supplemental Experimental Procedures) and three outgroups (*Daphnia*, *Ixodes*, and *Hesperochernes*). Because missing data can affect parameter estimation and tree topology and inflate measures of support [9, 10], we employed both a liberal filtering of orthologs (d327) that allowed a larger number of taxa (20; 50%) to have missing loci and a more conservative approach (d128) that retains only those loci found in a majority of taxa (35; 87.5%); missing values were comparable to or less than those reported for other similar phylogenomic studies [11]. For d327, on average 73.5% of the genes were sampled per taxon, with an overall matrix completeness of 76.9%; for d128, 90.6% of the genes were sampled per taxon, with an overall matrix completeness of 91.3%. Given the topological congruence between these two data sets (reported as identical below), the smaller d128 supermatrix also afforded us the opportunity to conduct a set of more computationally intensive analyses (divergence time estimates and gene tree/species tree analyses).

Figure 2 summarizes the maximum-likelihood (ML) tree topology inferred using the d327 supermatrix comprising 110,808 aa. The data set based on the more conservative filtering of orthologs (d128; 55,447 aa) recovers an identical topology, but with slightly lower bootstrap support for some clades (Figure S1) but likewise high Bayesian posterior probabilities (1.0 for nearly all clades). Individual gene trees derived from the 128 loci set were evaluated using three species tree approaches to assess topological congruence among individual orthologs. Species tree analyses using MP-EST, NJst, and STAR are all largely in agreement with the topologies derived from both of the supermatrices (Figure S1), with the exception of the former placing *Liphistius* sister to mygalomorphs and *Stegodyphus* sister to the RTA+Deinopoidea. Given that concatenated versus species tree analyses make very different assumptions, this consistency further supports the accuracy of our phylogenomic results. Bayesian (BI) and parsimony (PA) analyses were likewise largely congruent with the ML tree topologies. We recover most lineages with strong ML bootstrap support (bs = 100%) and high BI posterior probabilities (pp = 1.00) across all analyses; as expected, PA

[4]Co-first author

*Correspondence: jbond@auburn.edu

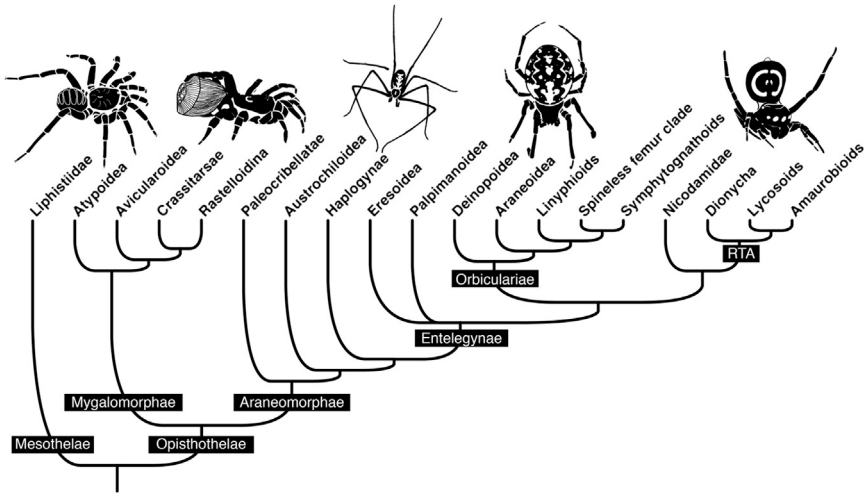

Figure 1. Summary Hypothesis of Spider Relationships

Current hypothesis of spider relationships summarized from Coddington [7]. Spiders are divided into two major infraorders, the Araneomorphae and Mygalomorphae (2,792 species). Araneomorphs comprise a majority of described diversity (41,661 of the 44,540 species), including the diverse Orbiculariae—the orb-weaving spiders. Haplogynae and Austrochiloidea exchange phylogenetic position in Griswold et al. [8]. Spider cartoons, from left to right, are as follows: *Liphistius* (Liphistiidae), *Cyclocosmia* (Ctenizidae), *Hypochilus* (Hypochilidae), *Araneus* (Araneidae), and *Maratus* (Salticidae). See also Table S3.

bs values are sometimes slightly lower, particularly for the deepest nodes (Figures 2 and S1).

A number of traditionally recognized spider clades (Figure 1) are strongly supported (Figure 2), including Mesothelae sister to monophyletic araneomorph and mygalomorph lineages. Mygalomorph relationships are generally congruent with previous hypotheses that support a monophyletic Atypoidea, Avicularioidea, Crassitarsae, and Bipectina. Domiothelina, a clade that includes the classical trapdoor spiders (Rastelloidina), is strongly supported here despite not being recovered in recent analyses [12]. Phylogenomic data do not support two competing hypotheses of mygalomorph phylogeny [12, 13] (Table S3). Among araneomorphs, a haplogyne clade (taxa with simple genitalia) is robustly supported to include *Hypochilus*—a haplogynous taxon traditionally placed in the Paleocribellate clade (Figure 1) sister to all other araneomorphs. Our results also support another key spider clade, Entelegynae, uniting spiders that share characters of the female genitalia, namely a fertilization duct connecting the spermathecae (site of sperm storage) with the oviduct (Figure S2A).

Our supermatrix and gene tree results (Figures 2 and S1) reject the current hypothesis placing all orb weavers within Orbiculariae. Instead, we find robust support for the araneoid ecribellate orbicularians (bs = 100%; pp = 1.0) and the placement of deinopoid cribellate orb weavers as the sister group to a clade consisting mostly of webless hunters (bs = 97%; pp = 1.0). Alternative hypotheses that force orbicularian monophyly and other more traditional views of araneomorph classification are all rejected by AU tests (Table S3). Only 317 out of 12,800 bootstrap replicate gene trees (d128) recovered Orbiculariae as monophyletic. Orbicularian monophyly is thus rejected by strong congruence among the 128 loci evaluated as separate gene trees and in both supermatrix "total evidence" analyses.

The "orbicularian paradigm" maintains that the basic architecture of orb web design and associated behaviors used during web construction are homologous among two distinct sister lineages, the Deinopoidea and the Araneoidea. The less diverse deinopoids (326 species) [2] use dry cribellate silk to construct sticky spirals, whereas the diverse araneoid spiders (>12,000 species) use a viscid aqueous secretion to make their webs sticky. The latter can be produced faster and more economically, and consequently has been identified as a key innovation [14, 15] promoting the success of the Orbiculariae.

Despite similarity in deinopoid and araneoid spinning morphology, web architecture, and especially its attendant behaviors, orbicularian monophyly has not been robustly supported in any modern phylogenetic analysis based solely on molecular data. As pointed out by Hormiga and Griswold [16], Orbicularian phylogeny has remained an "inherently difficult problem to resolve"; the majority of molecular phylogenetic analyses conducted thus far consistently fail to recover a clade that unites Deinopoids and Araneoids. Hausdorf [17] provided one of the first hints of orbicularian nonmonophyly followed by subsequent analyses with either mixed results [18], recovering all orb weavers as monophyletic *only* with the inclusion of morphological data [15], or as polyphyletic [1]. The latter study by Agnarsson et al. [1] strikingly portends our results; their meta-analysis placed deinopoids with members of the RTA clade, a hypothesis that was summarily rejected with the characterization that the taxa were "conspicuously misplaced." An analysis by Dimitrov et al. [19] is one of few that recovers orbicularian monophyly; however, bootstrap support was weak, and a RAxML ML analysis of their data does not differ statistically in a Shimodaira-Hasegawa test that constrains an RTA+ Deinopoid clade ($p > 0.10$). Because past analyses have relied on few genes, it is easy to understand why orbicularian nonmonophyly failed to gain acceptance. However, our results (Figure 2) are strongly supported by an array of analytical approaches, conducted to guard against bias, and thus clearly indicate that it is now time to consider the alternative hypotheses that have been "lurking" in the data for the past 15 years.

Rejection of orbicularian monophyly has important implications for the origin of the orb web and the study of silk and silk genes. Either the orb web evolved repeatedly (Figure S2B), as was hypothesized by early authors, originally based on the idea that orb webs are "near perfect" adaptations, or, as suggested by likelihood ancestral reconstruction of web types (Figures 3 and S2C; character states after Blackledge et al. [15]), it was a much earlier event in the history of spiders than hitherto recognized, and the orb web is the ancestral state for a clade containing the vast majority of extant spider diversity. Alternative web scorings we explored (data not shown) that collapse all aerial sheet webs as a single character state still recover an earlier origin of the orb web, but with slightly lower marginal probabilities. Divergence time estimates (Figure 4) based on these data (d128; Table S4) place the date of the origin of the orb web in the Lower Jurassic (187–201 million years ago). This earlier point estimate falls within the range of previous dating

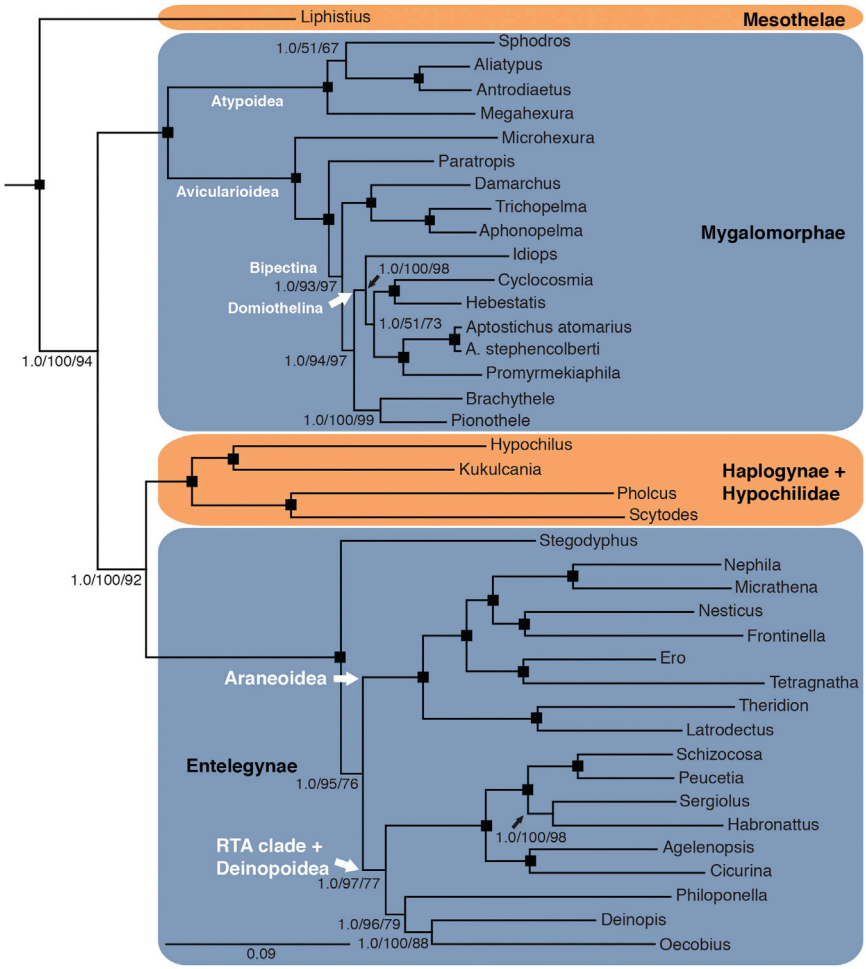

Figure 2. Phylogenomic Relationships of Major Spider Lineages

Maximum-likelihood tree topology, based on supermatrix analysis of 327 putative orthologs, showing relative support values for Bayesian, maximum-likelihood, and parsimony analyses. Filled blocks denote ML/PA bootstrap values of 100% and BI posterior probabilities of 1.0; otherwise, exact values are indicated at each node (BI-pp/ML-bs/PA-bs). The outgroups [*Daphnia* (Cladocera), *Ixodes* (Acari), and *Hesperochernes* (Pseudoscorpiones)] have been removed from the tree for illustrative purposes. See also Figure S1 and Tables S1, S2, and S3.

analyses [20] but is more precise topologically because of increased taxonomic sampling and places the origin of ecribellate orb webs considerably later (~52–113 million years ago). Both scenarios present interesting alternatives and challenges for the last three decades of orb web evolutionary research based on the orbicularian paradigm. Given that behavioral support is strong for the monophyletic origin of the orb web, a hypothesis supported in our ancestral character reconstruction (Figure 3), the single ancient origin scenario appears to be more plausible. This hypothesis also makes specific empirical predictions, for example that the morphological and genetic architecture of orb weaving should be found in RTA clade taxa that have secondarily lost orb webs. If the majority of spiders arose from orb weavers, we should find traces of this ancestry in orb-related traits, such as spinneret spigots, silk glands, and silk genes. These predictions set the stage for novel research focusing on the genetics and proteomics of "derived orb weavers" that despite their diversity remain severely understudied from a silk evolution perspective.

Our results support the classical Araneoidea, the ecribellate orb weavers and their relatives. This hypothesis is consistent with a large number of prior studies and is corroborated by morphological characters such as serrate rather than plumose setae (Figure S2D) and key character suites related to ecribellate orb webs, including gluey silk generated in aggregate silk glands and delivered through aggregate spigots (Figure S2E). The recovered phylogenetic structure within Araneoidea, however, contradicts most prior work, given the placement of cobweb spiders (Theridiidae) as sister to the remaining Araneoidea and far from their putative sister lineage Nesticidae (Figure 2). This hypothesis suggests a diphyletic origin of gumfoot webs characteristic of these two families, an important insight into web evolution (Figure 3). Conversely, these results offer a simpler evolutionary explanation for some traits. For example, it has long been argued that cobweb spiders have secondarily lost the paracymbium, a characteristic feature of the male genitalia in ecribellate orb weavers. Instead, our results suggest that the paracymbium is a potential synapomorphy for nontheridiid Araneoids (Figure S2F).

We have presented a phylogenetic hypothesis for spiders using a genomics-based data set that resolves relationships among the major spider lineages. We emphasize extensive sampling of characters, rather than taxa, because the past decade of spider molecular phylogenetics has been characterized by loci that are mostly inadequate for confidently recovering a number of the traditional relationships discussed herein. Our interpretation of the results is not intended as an attack on the prevailing state of spider systematics, but rather is meant to accentuate that generally accepted paradigms, like the monophyly of the orb-weaving spiders, may lack support; denser taxon sampling for a number of these clades will be an important next step. Our analyses recover strong support for three long-standing lineages comprising Mesothelae, Mygalomorphae, and Araneomorphae. Mygalomorph relationships do not depart topologically from recent molecular systematics studies, showing an atypoid clade sister to remaining taxa. Notably our phylogenomic analysis recovers the traditional trapdoor spider clade, the Domiothelina, a well-defined morphological group that prior molecular systematic studies have generally failed to recover.

The greatest departure from current systematic thinking is the nonmonophyly of the classical orb weavers—Orbiculariae. Although previous analyses [15–17] hint that the orbicularian hypothesis lacked support or is paraphyletic with respect to the RTA clade [15], the data presented here clearly indicate that a paradigm shift may be needed, and

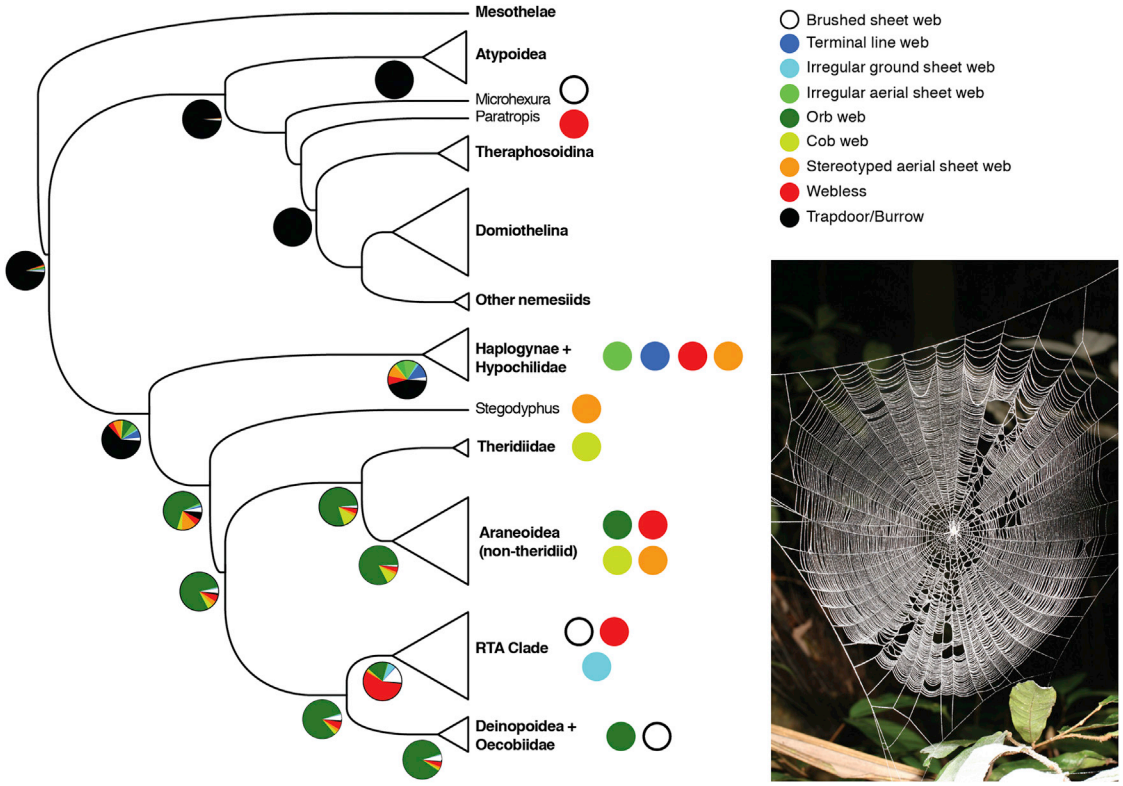

Figure 3. Ancestral State Reconstruction of Web Type

Summary tree showing the maximum-likelihood ancestral state reconstruction for web type across the major spider lineages. Pie charts denote the relative likelihood that an ancestor had a particular web type (character scorings adapted from Blackledge et al. [15]; upper inset, legend; lower inset, araneoid orb web photo). See also Figure S2 for full tree reconstruction.

it is time that these alternative hypotheses receive more attention rather than be discarded as erroneous. Our analyses show that cribellate deinopoids+Oecobiidae share a most recent common ancestor with a group that comprises webless hunting spiders. Another phylogenomic analysis [21] also failed to support Orbiculariae, although the only nonaraneoid exemplar (*Uloborus*) could not be precisely placed. These authors did not formally test the age of orb webs, but they reach similar conclusions as supported here. Consequently, the orb web may have evolved much earlier than previously hypothesized or, less plausibly, multiple times independently. The "ancient orb web hypothesis" we propose herein is consistent with the recent discovery of fossils of very large cribellate orb weavers from Jurassic deposits in China and classical behavioral data supporting orb homology [16, 22]. Such a fundamental shift in the placement of orb web origins suggests that the vast majority of extant spider diversity shared an ancestor that may have foraged for prey from an early cribellate orb web and potentially changes how we interpret and study spider-spinning morphology, behavior, and silk gene evolution.

Experimental Procedures

Taxon Sampling and Sample Preparation

Thirty-nine animals were field collected and flash frozen in liquid nitrogen or preserved in RNAlater. RNA extractions using a hybrid Trizol kit were performed on cephalothorax tissue from a single specimen or multiple small-bodied individuals. Total RNA was used for cDNA library preparation and subsequent sequencing via Illumina RNA sequencing with HiSEQ paired-end 50/100 bp chemistry; barcoded libraries were typically pooled four to a flow cell lane. The data were augmented with two additional raw transcriptome contig sets from the NCBI Trace Archive (Table S1), and previously assembled *Ixodes* and *Daphnia* expressed sequence tags were used in the bioinformatics pipeline.

Bioinformatics and Phylogenetic Analyses

Raw Illumina data were trimmed and quality checked using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) and FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) before assembly with Trinity [23]. HaMStR [24] was used to identify orthologs for phylogenetic inference and to assemble ortholog-specific FASTA files. Individual ortholog files were aligned using MAFFT [25] followed by filtering with SCaFoS [26], Gblocks [27], Aliscore [28], and ALICUT (http://zfmk.de/web/ZFMK_Mitarbeiter/KckPatrick/Software/AliCUT/Download/index.en.html) to select and prepare alignments for phylogenetic analysis via FASconCAT [29]. These steps are combined into three bioinformatic pipelines [30]. Individual gene trees were constructed using ML and were examined visually to remove alignments containing obvious paralogs. To evaluate the effects of gene incongruence when inferring phylogeny from a large supermatrix, we estimated species trees from individual gene trees (128 gene data set only) using three partially parametric methods implemented using the programs STAR [31], MP-EST [32], and NJst [33]. Partitioned supermatrix ML trees were inferred using RAxML [34] with the PROTGAMMAWAG model, parsimony using TNT [35], and ExaBayes (http://sco.h-its.org/exelixis/web/software/exabayes/index.html) using default parameters, with no amino acid model specified and branch lengths and substitution rates linked across partitions; these analyses were conducted on the Auburn University CASIC HPC and Cyberdyne (Mollete Lab, Auburn University). Morphological character ancestral state reconstructions using ML (Mk1 model) were conducted with Mesquite 2.75 (http://mesquiteproject.org/). Alternative phylogenetic hypotheses were evaluated using the Approximately Unbiased test

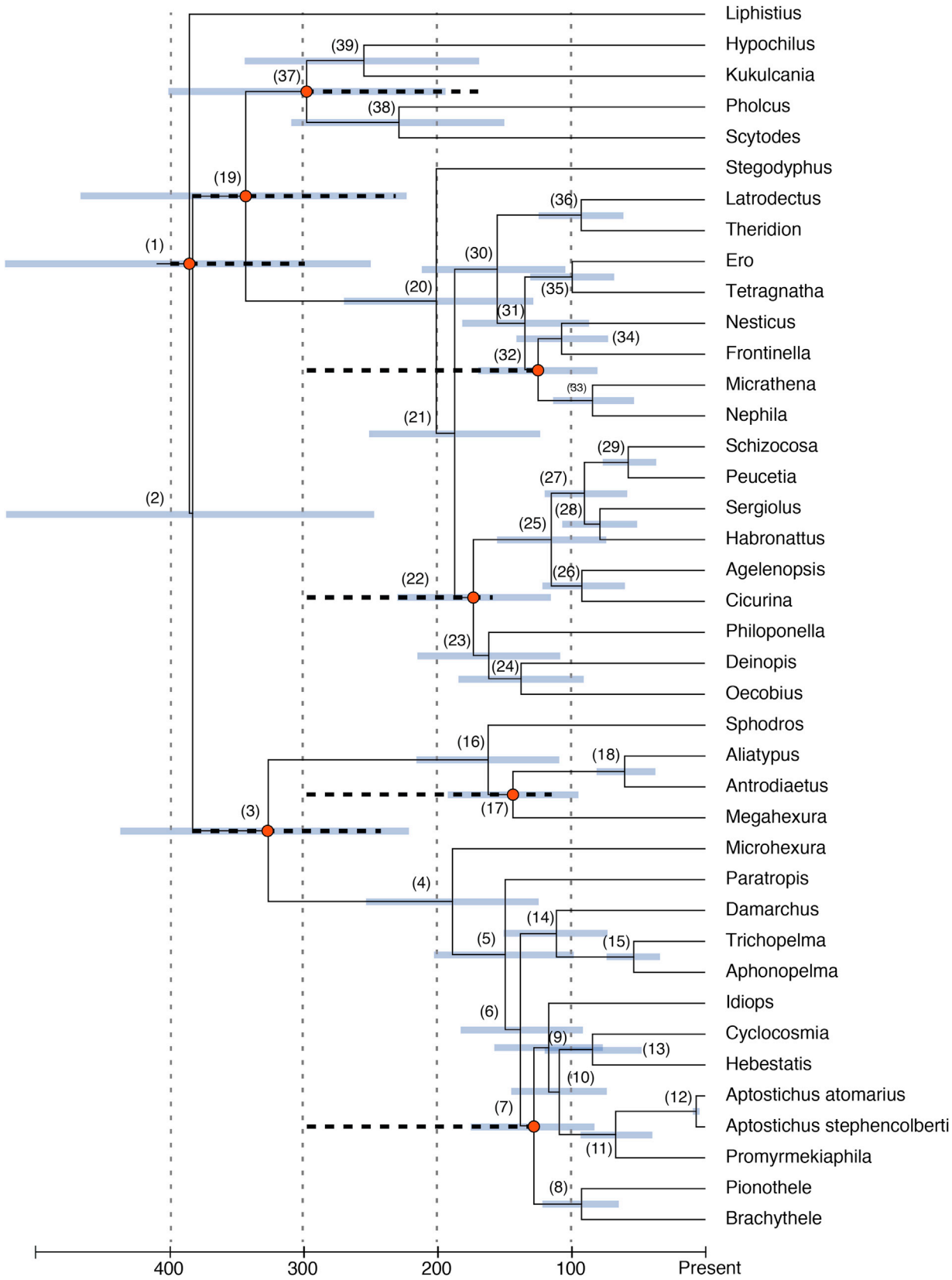

Figure 4. Chronogram Showing Estimated Divergence Times for Major Spider Lineages

RelTime estimates of lineage divergence times for each major spider lineage using the ML tree based on the d128 supermatrix. Time scale is given on the x axis. Calibrated nodes are indicated by a red dot, the maximum/minimum age boundaries are denoted with dashed lines, and blue bars at nodes reflect 95% confidence intervals of age estimates. See also Table S4 for calibration age intervals corresponding to each node (node numbers in parentheses).

implemented in CONSEL [36]. Times of divergence for the 128 gene data set were estimated with the computer program RelTime [37].

Accession Numbers

GenBank accession numbers for published sequences are PRJNA215735 and SRS471950 (*Latrodectus*) and PRJNA81585 and ERX048739 (*Stegodyphus*). Illumina transcriptome sequence data are available from the NCBI SRA database under accession numbers SAMN02836945–SAMN02836950, SAMN02837036–SAMN02837041, SAMN02837043–SAMN02837052, and SAMN02837054–SAMN02837070. Phylogenomics data matrices (d327 and d128) and corresponding partition files were deposited on June 26, 2014, in the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.6dt17.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, two figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.06.034.

Author Contributions

J.E.B. designed the study, analyzed data, and wrote the paper with N.L.G, I.A., and M.H.; N.L.G. was bioinformatics lead and shares lead authorship with J.E.B.; C.A.H. and M.H. assisted in data analysis and data analysis design; R.L.G. collected and processed specimens and assisted in data analysis. All authors discussed results and contributed to manuscript preparation.

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References

1. Agnarsson, I., Coddington, J.A., and Kuntner, M. (2013). Systematics: progress in the study of spider diversity and evolution. In Spider Research in the 21st Century, D. Penney, ed. (Manchester: Siri Scientific Press), pp. 58–111.
2. Platnick, N.I. (2014). The World Spider Catalog v 14.5. The World Spider Catalog. http://research.amnh.org/iz/spiders/catalog/INTRO1.html.
3. Agnarsson, I., Kuntner, M., and Blackledge, T.A. (2010). Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. PLoS ONE *5*, e11234.
4. Hayashi, C.Y., Blackledge, T.A., and Lewis, R.V. (2004). Molecular and mechanical characterization of aciniform silk: uniformity of iterated sequence modules in a novel member of the spider silk fibroin gene family. Mol. Biol. Evol. *21*, 1950–1959.
5. Gui, J., Liu, B., Cao, G., Lipchik, A.M., Perez, M., Dekan, Z., Mobli, M., Daly, N.L., Alewood, P.F., Parker, L.L., et al. (2014). A tarantula-venom peptide antagonizes the TRPA1 nociceptor ion channel by binding to the S1-S4 gating domain. Curr. Biol. *24*, 473–483.
6. King, G.F., and Hardy, M.C. (2013). Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. Annu. Rev. Entomol. *58*, 475–496.
7. Coddington, J.A. (2005). Phylogeny and classification of spiders. In Spiders of North America an Identification Manual, D. Ubick, P. Paquin, P.E. Cushing, and V. Roth, eds. (Poughkeepsie: American Arachnological Society), pp. 18–24.
8. Griswold, C.E., Ramirez, M.J., Coddington, J.A., and Platnick, N.I. (2005). Atlas of phylogenetic data for entelegyne spiders (Araneae: Araneomorphae: Entelegynae) with comments on their phylogeny. Proc. Cal. Acad. Sci. *56* (*Suppl 2*), 1–324.
9. Roure, B., Baurain, D., and Philippe, H. (2013). Impact of missing data on phylogenies inferred from empirical phylogenomic data sets. Mol. Biol. Evol. *30*, 197–214.
10. Lemmon, A.R., Brown, J.M., Stanger-Hall, K., and Lemmon, E.M. (2009). The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. *58*, 130–145.
11. Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., and Halanych, K.M. (2011). Phylogenomics reveals deep molluscan relationships. Nature *477*, 452–456.
12. Bond, J.E., Hendrixson, B.E., Hamilton, C.A., and Hedin, M. (2012). A reconsideration of the classification of the spider infraorder Mygalomorphae (Arachnida: Araneae) based on three nuclear genes and morphology. PLoS ONE *7*, e38753.
13. Raven, R.J. (1985). The spider infraorder mygalomorphae (Araneae): cladistics and systematics. Bull. Am. Mus. Nat. Hist. *182*, 1–180.
14. Bond, J.E., and Opell, B.D. (1998). Testing adaptive radiation and key innovation hypotheses in spiders. Evolution *52*, 403–414.
15. Blackledge, T.A., Scharff, N., Coddington, J.A., Szüts, T., Wenzel, J.W., Hayashi, C.Y., and Agnarsson, I. (2009). Reconstructing web evolution and spider diversification in the molecular era. Proc. Natl. Acad. Sci. USA *106*, 5229–5234.
16. Hormiga, G., and Griswold, C.E. (2014). Systematics, phylogeny, and evolution of orb-weaving spiders. Annu. Rev. Entomol. *59*, 487–512.
17. Hausdorf, B. (1999). Molecular phylogeny of araneomorph spiders. J. Evol. Biol. *12*, 980–985.
18. Ayoub, N.A., Garb, J.E., Hedin, M., and Hayashi, C.Y. (2007). Utility of the nuclear protein-coding gene, elongation factor-1 gamma (EF-1gamma), for spider systematics, emphasizing family level relationships of tarantulas and their kin (Araneae: Mygalomorphae). Mol. Phylogenet. Evol. *42*, 394–409.
19. Dimitrov, D., Lopardo, L., Giribet, G., Arnedo, M.A., Alvarez-Padilla, F., and Hormiga, G. (2012). Tangled in a sparse spider web: single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. Proc. Biol. Sci. *279*, 1341–1350.
20. Starrett, J., Hedin, M., Ayoub, N., and Hayashi, C.Y. (2013). Hemocyanin gene family evolution in spiders (Araneae), with implications for phylogenetic relationships and divergence times in the infraorder Mygalomorphae. Gene *524*, 175–186.
21. Fernández, R.M., Hormiga, G., and Giribet, G. (2014). Phylogenomic analysis of spiders reveals nonmonophyly of orb weavers. Curr. Biol. Published online July 17, 2014. http://dx.doi.org/10.1016/j.cub.2014.06.035.
22. Selden, P.A., Shih, C., and Ren, D. (2013). A giant spider from the Jurassic of China reveals greater diversity of the orbicularian stem group. Naturwissenschaften *100*, 1171–1181.
23. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. *29*, 644–652.
24. Ebersberger, I., Strauss, S., and von Haeseler, A. (2009). HaMStR: profile hidden markov model based search for orthologs in ESTs. BMC Evol. Biol. *9*, 157.
25. Katoh, K., Misawa, K., Kuma, K.I., and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. *30*, 3059–3066.
26. Roure, B., Rodriguez-Ezpeleta, N., and Philippe, H. (2007). SCaFoS: a tool for selection, concatenation and fusion of sequences for phylogenomics. BMC Evol. Biol. *7* (*Suppl 1*), S2.
27. Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. *17*, 540–552.
28. Kück, P., Meusemann, K., Dambach, J., Thormann, B., von Reumont, B.M., Wägele, J.W., and Misof, B. (2010). Parametric and non-parametric masking of randomness in sequence alignments can be improved and leads to better resolved trees. Front. Zool. *7*, 10.
29. Kück, P., and Meusemann, K. (2010). FASconCAT: Convenient handling of data matrices. Mol. Phylogenet. Evol. *56*, 1115–1118.

30. Brewer, M.S., and Bond, J.E. (2013). Ordinal-level phylogenomics of the arthropod class Diplopoda (millipedes) based on an analysis of 221 nuclear protein-coding loci generated using next-generation sequence analyses. PLoS ONE *8*, e79935.
31. Liu, L., Yu, L., Pearl, D.K., and Edwards, S.V. (2009). Estimating species phylogenies using coalescence times among sequences. Syst. Biol. *58*, 468–477.
32. Liu, L., Yu, L., and Edwards, S.V. (2010). A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. BMC Evol. Biol. *10*, 302.
33. Liu, L., and Yu, L. (2011). Estimating species trees from unrooted gene trees. Syst. Biol. *60*, 661–667.
34. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics *30*, 1312–1313.
35. Goloboff, P.A., Farris, J.S., and Nixon, K.C. (2008). TNT, a free program for phylogenetic analysis. Cladistics *24*, 774–786.
36. Shimodaira, H., and Hasegawa, M. (2001). CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics *17*, 1246–1247.
37. Tamura, K., Battistuzzi, F.U., Billing-Ross, P., Murillo, O., Filipski, A., and Kumar, S. (2012). Estimating divergence times in large molecular phylogenies. Proc. Natl. Acad. Sci. USA *109*, 19333–19338.