

Current Biology

Phylogenomics, Diversification Dynamics, and Comparative Transcriptomics across the Spider Tree of Life

Highlights

- We present the largest phylogenomic analysis for spiders to date
- We show a robust backbone for spider relationships with novel phylogenetic placements
- Our analyses reject the single origin of the orb web
- Genomic differences may have contributed to differential lineage diversification

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In Brief

Fernández et al. use a synergistic approach to study spider evolution through phylogenomics, comparative transcriptomics, and lineage diversification analyses. The “ancient orb web” hypothesis was rejected. Loss of foraging webs was not strongly associated to spider diversification. Notable genomic differences were found between main spider clades.

Phylogenomics, Diversification Dynamics, and Comparative Transcriptomics across the Spider Tree of Life

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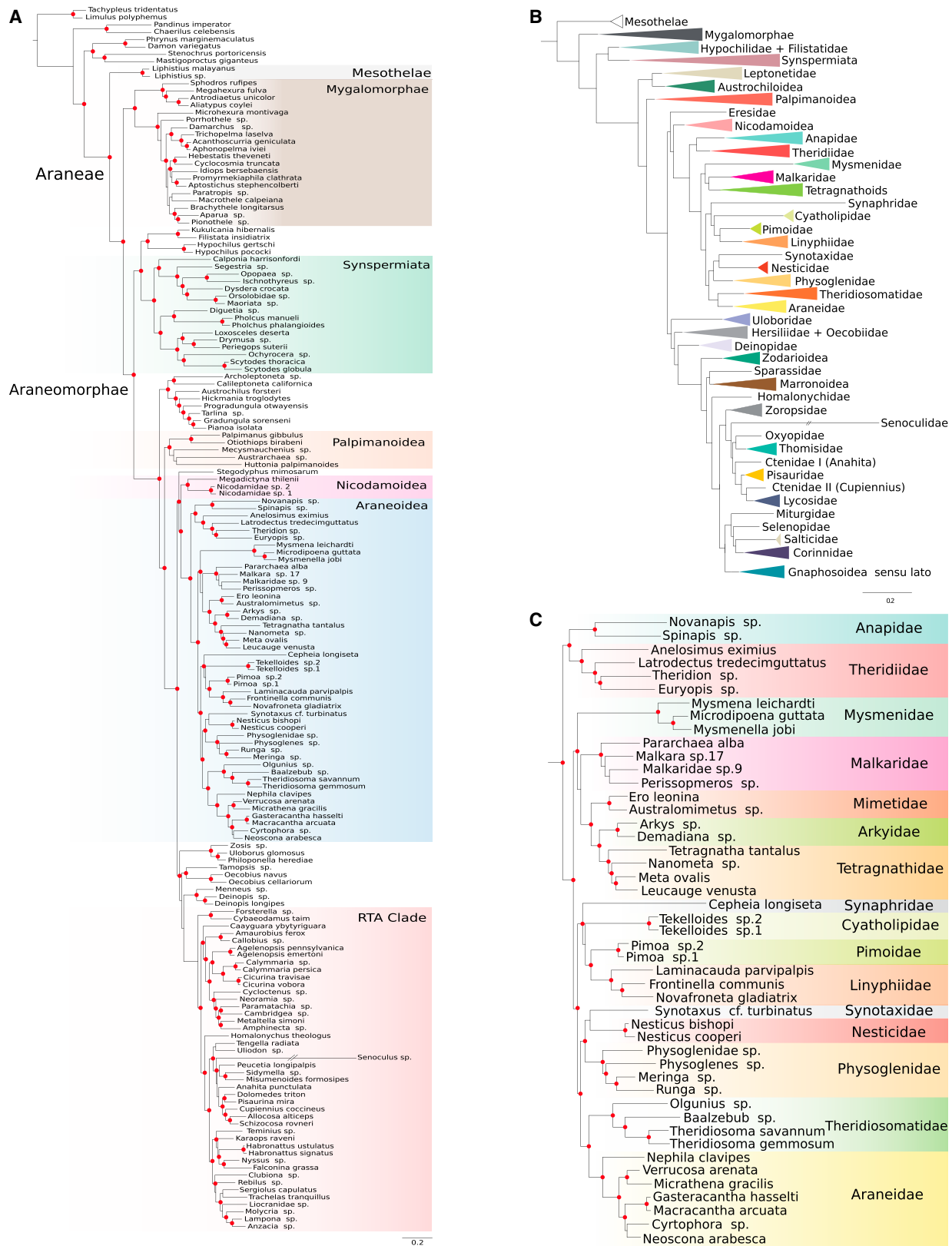
SUMMARY

Dating back to almost 400 mya, spiders are among the most diverse terrestrial predators [1]. However, despite considerable effort [1–9], their phylogenetic relationships and diversification dynamics remain poorly understood. Here, we use a synergistic approach to study spider evolution through phylogenomics, comparative transcriptomics, and lineage diversification analyses. Our analyses, based on ca. 2,500 genes from 159 spider species, reject a single origin of the orb web (the “ancient orb-web hypothesis”) and suggest that orb webs evolved multiple times since the late Triassic–Jurassic. We find no significant association between the loss of foraging webs and increases in diversification rates, suggesting that other factors (e.g., habitat heterogeneity or biotic interactions) potentially played a key role in spider diversification. Finally, we report notable genomic differences in the main spider lineages: while araneoids (ecribellate orb-weavers and their allies) reveal an enrichment in genes related to behavior and sensory reception, the retrolateral tibial apophysis (RTA) clade—the most diverse araneomorph spider lineage—shows enrichment in genes related to immune responses and polyphenic determination. This study, one of the largest invertebrate phylogenomic analyses to date, highlights the usefulness of transcriptomic data not only to build a robust backbone for the Spider Tree of Life, but also to address the genetic basis of diversification in the spider evolutionary chronicle.

RESULTS AND DISCUSSION

A Resolved Backbone of Spider Interrelationships

The main phylogenetic findings of our analyses (Figure 1) support a deep split between Mesothelae and Opisthothelae—and, within the latter, between Mygalomorphae and Araneomorphae. Due to the novelty of our sampling (see Data S4), we focus here in the results of Araneomorphae, especially around the orb-weaving clades. Nicodamoidea are corroborated as a clade for the first time using transcriptomic data, being the sister group of Araneoidea (the ecribellate orb-weavers) with strong support in all analyses. Eresidae (velvet spiders) were recovered as the sister group of the large lineage encompassing Nicodamoidea + Araneoidea in all analyses but one (see Figure S2). These nodes therefore add resolution to some of the first appearances of webs. Our study includes, for the first time, transcriptomic data for all 17 araneoid families, except Symphytognathidae, and as such, a number of novel relationships are recovered. The results refute most araneoid interfamilial relationships proposed by the extensive analyses of [1] and [2] (only 4/13 and 7/17 interfamilial araneoid nodes in common with our study, respectively). Some families, such as Synsphyridae, are resolved with robust support in new placements—in this case, as sister group to Cyatholipidae + Pimoidae + Linyphiidae. Other salient novel results relate to symphytognathoids. This putative group is essential to understand orb-web evolution [3] and includes the families Symphytognathidae, Anapidae, Theridiosomatidae, and Mysmenidae [3–5] but has never been recovered as monophyletic in any molecular analysis that includes these four families. Although we lack Symphytognathidae, our results suggest that Anapidae is sister group to Theridiidae as the earliest diverging araneoid clade (as in [2], but not in [1]), whereas Mysmenidae appears in a distantly related position, as sister group to a clade including Malkaridae, Mimetidae, Arkyidae, and Tetragnathidae, as in [2]. Theridiosomatidae is strongly supported as sister group



(legend on next page)

to Araneidae, a relationship not satisfactorily resolved in Sanger-based phylogenies [1, 2] and not addressed by previous transcriptomic analyses. These results, thus, strongly reject symphytognathoid monophyly.

Closely related to the retrolateral tibial apophysis (RTA) clade is a grade of families whose phylogenetic placement has refuted Orbiculariae (cribellate and ecribellate orb-weavers) and that has also called into question the single origin of the orb web: Uloboridae, Deinopidae, and Oecobiidae + Hersiliidae [6, 7] (the UDOH grade hereafter). This grade is also found in other recent studies of spider phylogenetics [1, 2, 8]. In agreement with previous work [e.g., 2, 6, 9], the cribellate orb-weavers (Deinopoidea: Deinopidae + Uloboridae) are not monophyletic [but see 10], as Deinopidae is the likely sister group of the RTA clade.

To further address the evolution of the RTA clade, we added numerous representatives new to genome-scale phylogenetics. Novel findings include support for Corinnidae + Salticidae and their probable sister-group relationship to Selenopidae. We also found support for Gnaphosoidea *sensu lato* + Dionycha A, following Wheeler et al. [1].

Relationships within Mygalomorphae (tarantulas and their kin) are resolved as in [8], although we have included, for the first time in a phylotranscriptomic analysis, representatives formerly classified in Hexathelidae (Sydney funnel-web spiders and relatives; now members of the families Porrhothelidae and Macrothelidae), which do not form a clade, thus refuting the monophyly of Hexathelidae, as advanced by Sanger-based studies [11, 12] and corroborated by [13]. Within Araneomorphae (“true spiders”), Synspermiata is the sister clade of Hypochilidae + Filistatidae, which together constitute the sister group to all other araneomorphs, as in [1] and [8].

The monophyly and position of Leptonetidae has been controversial [1], but our analyses resolve Leptonetidae as monophyletic and sister group to Austrochilidae. Austrochilidae is paraphyletic with respect to Gradungulidae. These results differ from previous findings [1], which suggested Leptonetidae and Austrochilidae to be polyphyletic, with a more distant relationship between gradungulids and the latter. Palpimanoidea and Palpimanidae are both monophyletic but the relationships within the superfamily remain unstable.

Tempo and Mode of Diversification across the Spider Tree of Life: A Late Jurassic-Triassic Origin of the Orb Web

As illustrated in our chronogram (Figure 2, Data S3), the root of Araneae is estimated to be 334–397 million years old (myo), narrower than the age interval in [8], at 287–398. The origin of Mygalomorphae is dated to 203–328 million years ago (mya), overlapping with the range in the previous study [8] of 218–307. Our analyses show the origin of the orb web to be approximately 191–247 myo, assuming a single origin of the orb web, or 141–189 myo, if araneoid orbs evolved independently of cribellate orb webs. In comparison, a monophyletic

origin of the orb web was dated by [8] at 154–280 myo and by [2] at 177–236 myo. Our estimates place the origin of the orb web in the Middle Triassic to Early Jurassic (if single origin) or in the Early to Late Jurassic (if the orb web first appeared with Araneioidea). Araneomorphs appeared in the Carboniferous–Early Permian, and araneoid diversification at the “family level” occurred within an average time span of about 34 million years (my) from the early Late Jurassic to the Early Cretaceous.

Ancestral state reconstructions of web architecture, improved by the resolution of the clades highlighted above, did not support a single origin of orb webs, as inferred in previous studies [e.g., 2, 8] (Figures 3, S2, Data S1), suggesting thus that neither the orb-weavers nor their spinning work can be traced to a single origin (note that the fact that some nodes remain less supported, such as the position of Eresidae, only affects estimates of the number of origins, but should not undermine the multiple-origin hypothesis). Reconstructions based on constraints provided by our dataset (159 species with detailed scoring of web architectures, 10 states) plus the legacy data (926 species using three states to describe foraging webs) found non-monophyly of cribellate and ecribellate orbs, with some results favoring multiple origins of orbs within Araneioidea (Figures 3, S2, Data S1). When states at these nodes were explicitly forced as an orb web, comparison of the marginal likelihood of the resulting models using Bayes factors (BF) supported an orb-weaving ancestor of Araneioidea (BF −0.331226 based on our data and −1.25511 on the legacy dataset) and weak support for independent origins of cribellate and ecribellate orbs (BF 5.241716 based on our data and 6.076012 on the legacy dataset). Interestingly, the support for non-monophyly of orbs decreased significantly (from 6.076012 to 5.241716) when more states describing web architecture were considered, while the reconstructions at other nodes (e.g., at the root or at the base of Araneioidea) remained stable. We suspect that differences with previous transcriptomic analyses supporting a single origin of orbs and an orb-weaving ancestor to the RTA clade ([8]; see Table 1 and additional supplemental information at the Harvard Dataverse repository, <https://doi.org/10.7910/DVN/EJOMZP>) are mainly due to different branching patterns at the base of the RTA clade and of Araneioidea, which involve the relationships of the UDOH taxa, together with a much denser taxon sampling. The hypothesis of a single origin of the orb web (the so-called “ancient orb web hypothesis”; [2, 6, 8]) crumbles under the weight of additional transcriptomic data coupled with a significantly increased taxon sampling. None of the alternative hypotheses on the origin and evolution of the web received robust statistical support, suggesting that the evolutionary chronicle of the web is more complex and difficult to untangle than previously thought.

Our analyses, including all spider families and a better-resolved phylogenetic backbone, offer new insights on diversification dynamics of spiders. Our BAMM analyses (Figures 3 and S1) supported a significant increase in diversification in Araneioidea, particularly in the two largest families (Araneidae and Linyphiidae). When analyzing the taxonomically broad

Figure 1. Spider Interrelationships

(A) Preferred topology of 159 spider taxa using the 750-gene dataset (BUSCO-750) from ExaML. Phylogeny with all tips represented; red circles at nodes represent bootstrap support $\geq 90\%$.

(B) Diagrammatic representation of phylogeny with tips collapsed to higher taxonomic levels.

(C) Araneoid phylogeny; red circles at nodes represent bootstrap support $\geq 90\%$. See also Figure S3, Data S2, S4, and STAR Methods.

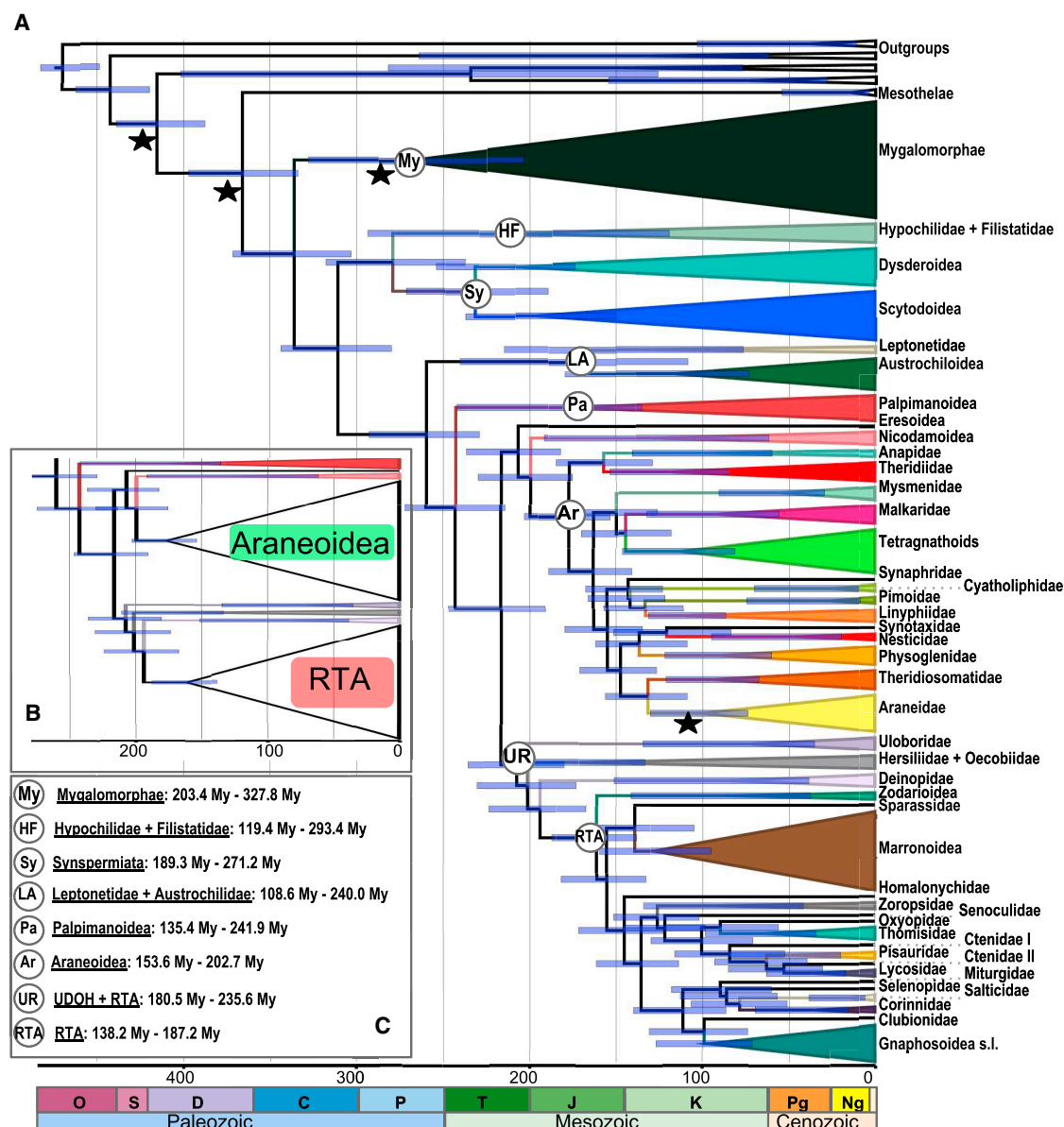


Figure 2. Chronogram of Spider Evolution

(A) Chronogram of spider evolution based on the BUSCO-255 matrix with 95% highest posterior density (HPD) bars for the dating under the uncorrelated model in PhyloBayes. Nodes that were calibrated with fossils are indicated with a star placed at the age of the fossil (some calibration points not shown since they fall within collapsed clades).

(B) Detailed chronogram highlighting the diversification ages of Araneoidea and the RTA clades.

(C) Diversification times of the main spider clades. See also [Data S3](#) and [S4](#) and [STAR Methods](#).

legacy dataset, we found a wide range of diversification rates and several potential shifts within the RTA clade, where none of the lineages build foraging webs. Spider diversification has often been linked to key innovations in silk use and web architecture [14, 15]. Our findings suggest that although transition to cursorial lifestyle and different foraging web architectures (in araneoid orb-weavers) may have had significant impact on spider diversification, these may have not been the main or only drivers. This is further supported by increased diversification rates in lineages without orb-weaving ancestors (i.e., the haplogyne web

building family Pholcidae and the web-less dysderoid lineage; [Figure 3B](#)) and by the results of the RPANDA analyses.

Further evidence suggesting that web-building behavior might not be the main driver of spider diversification was found in the trait-dependent diversification analyses ([Data S1](#)). When the importance of a foraging web versus a “webless” lifestyle was tested, the best-fit model (the full HiSSE model, including transitions between the observed trait and a hidden trait) found a strong correlation between usage of web and diversification, with web-less spiders showing two times higher net diversification rates

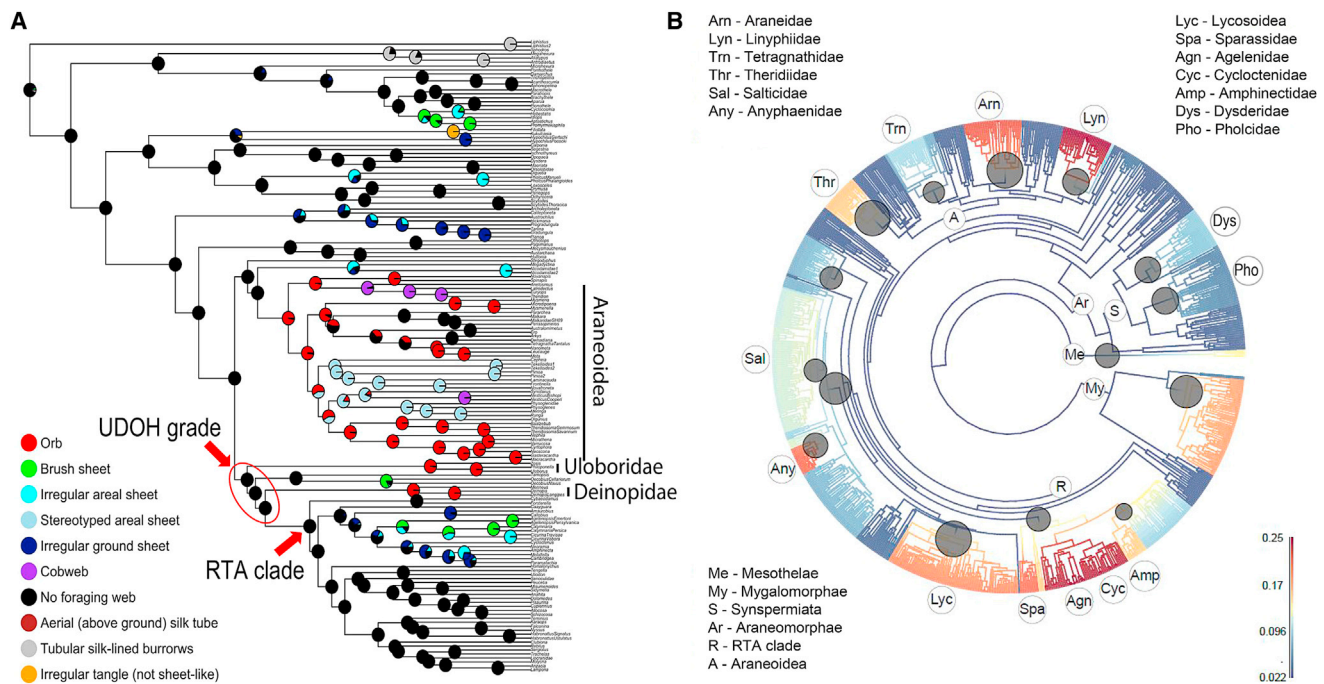


Figure 3. Ancestral State Reconstruction of Foraging Webs

(A) Ancestral state reconstruction using the phylogenomic dataset alone and the ace R function with different but symmetric rates of character transformation (SYM). This hypothesis implies three independent origins of the orb web (in Araneoidea, Uloboridae and Deinopidae; red circles) and an ancestor for the RTA clade that did not build foraging webs (black circles). See [Supplemental Information](#) for alternative reconstructions of web evolution.

(B) Rates of speciation estimated by BAMM using the legacy dataset and total sampling fraction for the backbone. Warmer colors represent higher rates. Grey circles denote estimated placement of shifts based on the single configuration that has highest posterior probability ($f = 0.011$) in the 95% credible set of distinct shifts configurations. See also [Table 1](#), [Figures S1](#) and [S2](#), and [Data S1](#) and [S2](#).

than web-building spiders (0.03895379 versus 0.01218436) and lower extinction rates (0.7556569 versus 0.9537909). Furthermore, a hidden trait was found to have a 2.5 times greater effect on diversification than web-building behavior (in spiders using webs) and an effect as significant as web loss in free-hunting spiders. When orb webs versus webless or other web architectures were tested, the four-state trait-independent HiSSE model was preferred. These results strongly suggest that webless spiders may have higher diversification rates than spiders who make webs, but loss of web building is not the main reason underlying that pattern ([Figure 3](#), [Table 1](#), [Data S1](#)). Presence and absence of orbs and potential hidden traits associated with these two phenotypes do not show strong correlation with the diversification process in orb-weavers. Thus, the shift in diversification in Araneidae, for example, is likely associated to other traits whose distribution across spiders is not necessarily linked to the presence of orbs. Previous studies suggested that both extrinsic and intrinsic factors (i.e., biotic interactions or adaptations to new environmental conditions) likely played an important role in spider diversification [9]. Our RPANDA analyses showed that, overall, higher temperatures are associated with higher diversification rates, suggesting that climatic conditions are indeed important for spider diversification. This finding is in accordance with previous studies on ectotherm diversification rates [16], indicating that a positive correlation between evolutionary rates and temperature in ectotherms may be a general pattern, as implied by the metabolic theory of ecology (MTE) [17, 18]. How-

ever, establishing the relative importance of temperature variation on spider diversity patterns remains an open question due to the lack of detailed spatial information on species distributions.

Enriched Gene Functions in the Most Diverse Spider Clades

Unlike phylogenomic methods, which use genome subsampling (e.g., ultraconserved elements or anchored hybrid enrichment), transcriptome-based approaches offer the possibility to explore protein-coding genes in an unprecedented way. When comparing gene ontology (GO) terms from the two most diverse spider lineages (Araneoidea and the RTA clade; 12,416 and 23,294 described species, respectively [19]), araneoid-enriched functions were mostly related to binding and sensory receptor activity at the molecular level, including chemokine binding, olfactory receptor binding, hormone receptor binding, and red light photoreceptor activity, among others ([Figure S3](#)). At the biological process level, numerous metabolic functions were involved, suggesting that certain types of reception are more acutely developed in the cribellate orb-weavers and their allies and that they involve a wide range of metabolic pathways. Interestingly, functions enriched in araneoids included territorial and courtship behavior. Although these functions were also retrieved in the cribellate leptonetid *Archileptoneta* sp., and in the palpi-manoids *Othiotops birabeni* and *Huttonia palpimanoides* (neither Araneoidea nor RTA clade members), our results suggest a lack

Table 1. Comparative Results of the Ancestral State Reconstruction of Web-Building Behaviors

CODING	RECONSTRUCTION	DATASET	MODEL	NO. ORB-WEB ORIGINS	CLADES WHERE ORB WEBS ORIGINATED
10 states	ACE	Phylogenomic	ER	6	Ana, Mys, Trn, Ara + Ths, Dei, Ulo
10 states	ACE	Phylogenomic	SYM	3	A, Ulo, Dei
10 states	SIMMAP	Phylogenomic	ER	6	Ana, Mys, Trn, Ara + Ths, Dei, Ulo
3 states	ACE	Legacy Sanger	ER	6	Ana + Sym, Mys, Trn, Ara + Ths, Ulo, Dei
3 states	ACE	Legacy Sanger	SYM	6	Ana + Sym, Mys, Trn, Ara, Ulo, Dei
3 states	ACE	Legacy Sanger	ARD	5	Ana + Sym, Mys + Trn, Ara + Ths, Ulo, Dei
3 states	SIMMAP	Legacy Sanger	ER	6	Ana + Sym, Mys, Trn, Ara + Ths, Ulo, Dei
3 states	SIMMAP	Legacy Sanger	SYM	6	Ana + Sym, Mys, Trn, Ara + Ths, Ulo, Dei
3 states	SIMMAP	Legacy Sanger	ARD	5	Ana + Sym, Mys + Trn, Ara + Ths, Ulo, Dei

Coding scheme (3 states or 10 states), reconstruction method (ACE or SIMMAP), dataset (our phylogenomic dataset or the legacy Sanger dataset as described in [STAR Methods](#)), number of orb-web origins, and clades where it was originated are indicated. See also [Figure 3](#) and [STAR Methods](#).

of enrichment of these functions both in the UDOH grade and in the RTA clade, as well as in Synspermiata.

Despite being the most diverse spider clade (and represented by 44 transcriptomes in the present study), RTA clade spiders did not show a particularly diverse set of enriched GO terms compared to araneoids (79 non-redundant GO terms in the RTA clade versus 212 in araneoids in the REVIGO analysis, biological process). At the biological process level, GO terms were mainly related to development, morphogenesis, and immune response ([Figure S3](#), see also [STAR Methods](#)). Remarkably, we found two GO terms related to environmental polyphenic determination enriched in the RTA clade, which may be related to diversification and ecological speciation in this clade, as shown in insects [20].

The comparison of GO terms enriched in ecribellate and cribellate orb weavers was similar to the previous comparison (araneoids versus RTA clade), with a long list of GO terms enriched in the ecribellates and only a handful in the cribellates ([Figure S3](#)). In ecribellates, main categories were related to anterior patterning in *Drosophila melanogaster* (e.g., compound eye morphogenesis, specification of segmental identity in thorax, cephalic furrow formation; [Figure S3](#)), sensory-related receptors, immune response, and territorial and courtship behavior. At the level of biological processes, we found functions related to trehalose catabolism ([Figure S3](#)). Trehalose is well-known to be involved in resistance to desiccation and extended longevity in nematodes [21, 22] and in dealing with abiotic stresses in insects [23]; therefore, trehalose could have been involved in ecribellate diversification by facilitating adaptation to a broader range of ecological conditions. Moreover, we also found some categories related to egg formation at the molecular function level enriched in ecribellates (i.e., structural constituent of egg coat and vitelline membrane), suggesting differences in egg architecture between both groups of orb weavers ([Figure S3](#)). Altogether, our results suggest more complex molecular genetic underpinnings in ecribellates than in other spiders and highlight the necessity of targeted studies to understand the nature of these differences with precision.

Notably, the most diverse araneomorph clades (i.e., Eresidae + Nicodamoidea + Araneoidea on one side and the UDOH grouping + the RTA clade on the other) retained one extra homologous gene in the cryptochrome gene family, cryptochrome 6 *sensu* [24], while it was lost in mygalomorphs and virtually all

basal araneomorphs. In contrast to other cryptochromes involved in internal circadian clocks, cryptochrome 6 mediates cell-autonomous external circadian clocks. We hypothesize that this cryptochrome could have helped the most diverse araneomorph lineages to acclimatize to a broader range of ecological niches (see [Data S2](#)).

Conclusions

Here, we present a synergistic approach to the study of spider evolution through phylogenomics, comparative transcriptomics, and lineage diversification analyses. The combination of an extensive transcriptomic dataset with the largest Sanger-based phylogenetic matrix of spiders to date allowed us to tackle some old-standing spider phylogenetic questions, address the dynamics of web architecture evolution and diversification, and explore the genetic singularities of the most species-rich spider groups from a comparative transcriptomics perspective. Our analyses, relying on a significantly expanded number of genes and taxa compared to previous studies, have allowed us to refine the phylogenetic history of spiders, including key lineages to understand the multiple origins of orb webs, proposing new hypotheses (e.g., the relationships of the araneoid families) and testing previously well-supported and controversial clades. However, “symphytognathoids” and their closest relatives require further phylogenetic scrutiny. Nevertheless, this study provides a robust phylogenetic scaffold based on an unprecedented amount of data, both qualitatively and quantitatively, for the Spider Tree of Life.

Our evolutionary results suggest an intricate pattern of web evolution, with multiple gains, transformations, and losses of architectures and the web itself, and reject the “ancient orb-web hypothesis,” which postulates a single origin of the orb web. We did not find a strong association between the loss of foraging webs and increases in diversification but found evidence that other traits in addition to webs (e.g., related to reception and environmental tolerance) may have had a strong impact on spider diversification dynamics. Finally, our study illustrates the role of comparative transcriptomics as a powerful hypothesis generator for evolutionary studies in non-model organisms (exemplified by the existence of an extra cryptochrome in araneomorphs or an enrichment in sensory receptor-related functions in araneoids), highlighting the usefulness of transcriptomes for understanding the spider evolutionary chronicle at a deeper level.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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 - Orthology inference
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 - Lineage diversification analyses
 - Functional annotation of transcriptomes
 - Cryptochrome gene family evolution analyses
- [DATA AND SOFTWARE AVAILABILITY](#)

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and four data files and can be found with this article online at <https://doi.org/10.1016/j.cub.2018.03.064>.

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AUTHORS CONTRIBUTIONS

Conceptualization, G.H., G.G., R.F., M.A.A., D.D.; Formal analysis, R.F., R.J.K., J.A.B., D.D.; Funding acquisition, G.H., G.G.; Project administration, R.F., G.H., G.G.; Resources, G.H., G.G., R.J.K., M.A.A.; Supervision, G.H., G.G.; Writing—Original Draft Preparation, R.F., G.H., G.G., D.D., R.J.K.; Writing—Review & Editing, R.F., G.H., G.G., D.D., R.J.K., M.A.A., J.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Spider tissue samples	This paper	See Data S4
Chemicals, Peptides, and Recombinant Proteins		
Isopropanol, molecular grade > 99%	Sigma-Aldrich	cat # I9516-500ML
Ethanol 99.5%	VWR	cat # 89125-186
TRIzol reagent	ThermoFisher Scientific	cat #15596026
1-Bromo-3-Chloropropane 99%	Sigma-Aldrich	cat #B62404-250G
Glycogen, molecular biology grade	ThermoFisher Scientific	cat #R0561
Critical Commercial Assays		
Qubit dsDNA HS Assay Kit	ThermoFisher Scientific	cat # Q32854
Bioanalyzer mRNA pico Kit	Agilent	cat #5067-1513
PrepX mRNA library construction Kit for Illumina	Wafergen	cat #400046
Dynabeads RNA purification Kit	ThermoFisher Scientific	cat #61006
KAPA library quantification Kit	KAPA Biosystems	cat #KK4835
Deposited Data		
Transcriptomes raw data	This study	see Data S4
Matrices	This study	Harvard Dataverse repository (https://doi.org/10.7910/DVN/EJOMZP).
Orthologs	This study	Harvard Dataverse repository (https://doi.org/10.7910/DVN/EJOMZP).
Additional supplemental files and figures	This study	Harvard Dataverse repository (https://doi.org/10.7910/DVN/EJOMZP).
Software and Algorithms		
BUSCO v1.1	[25]	http://busco.ezlab.org/v1/
HMMER3	[26]	http://hmmer.org/
MAFFT	[27]	https://mafft.cbrc.jp/alignment/software/
trimAl v1.2a	[28]	http://trimal.cgenomics.org/
RaxML v8.2.9	[29]	https://sco.h-its.org/exelixis/web/software/raxml/index.html
UPhO	[30]	https://github.com/ballesterus/UPhO
FastTree	[31, 32]	http://www.microbesonline.org/fasttree/
TNT	[33]	http://www.lillo.org.ar/phylogeny/
ExaML	[34]	https://sco.h-its.org/exelixis/web/software/examl/index.html
IQTree v1.5.5	[35, 36]	http://www.iqtree.org/
ASTRAL-II	[37]	https://github.com/smirarab/ASTRAL
PhyloBayes v4.1	[38]	http://www.atgc-montpellier.fr/phylobayes/
BAMM	[39, 40]	http://bamm-project.org/
BAMMtools	[39]	http://bamm-project.org/
R package coda	[41]	https://anaconda.org/r/r
R package RPANDA	[42]	https://cran.r-project.org/web/packages/RPANDA/index.html
R package ape	[43]	https://cran.r-project.org/web/packages/ape/index.html
R package phytools	[44]	https://cran.r-project.org/web/packages/phytools/index.html
BayesTraits v3.0	[45]	http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.1/BayesTraitsV3.0.1.html

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
R package GEIGER	[46]	https://cran.r-project.org/web/packages/geiger/index.html
eggNOG-mapper	[47]	https://github.com/jhcepas/eggno-mapper
BLASTp	[48]	https://blast.ncbi.nlm.nih.gov/Blast.cgi
FigTree v1.4.3	[49]	http://tree.bio.ed.ac.uk/software/figtree/
phylo.io	[50]	http://phylo.io/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Rosa Fernández (rfernandezgarcia@g.harvard.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Taxonomic sampling

Taxon name, source, Museum of Comparative Zoology (MCZ, Harvard University) voucher and SRA accession numbers are indicated in [Data S4](#).

METHOD DETAILS

Ethics statement

Spider samples were legally collected under permits 38002-RES to RF, RJK, MA, GH and GG (Department of Conservation, New Zealand), WITK13653913 to RJK and GH (Take, Use, Keep or Interfere with Culture or Natural Resources, Scientific Purpose, Australia), Resolución SINAC-SE-CUS PI-R-070-2016 to GH and GG (Costa Rica) and Autorización CONAF 031/2014 to GH (Chile).

RNA extraction, library construction and sequencing

Messenger RNA extraction and strand-specific cDNA library construction followed the protocols described in [7]. New RNA-Seq data were generated for 90 spider species using Illumina HiSeq2500 (2 × 150 bp) technologies. Available RNA-Seq data were downloaded from NCBI SRA (see [Data S4](#)). Eight chelicerates were included as outgroups. All transcriptomes were assembled *de novo* with Trinity. Previous sanitation steps, redundancy reduction, and assembly parameters are as in [7].

QUANTIFICATION AND STATISTICAL ANALYSIS

Orthology inference

Orthology inference is a key task in the phylogenomic pipeline, yet little effort has been paid to assess the performance of the many available Methods and a standardized benchmarking is still elusive [51]. In order to assess the impact of different Methods, we applied two orthology inference Methods to our dataset. First, we ran the BUSCO v1.1b pipeline [25] in our taxa to detect near-universal single-copy orthologs based on evolutionarily informed expectations of gene content from the arthropod genomes included in OrthoDB v9. In brief, this method is based on the construction of hidden Markov model (HMM) profiles from amino acid alignments of single-copy genes retrieved from multiple arthropod genomes using HMMER 3 [26]. Afterward, the pipeline assesses whether BUSCO gene matches are orthologous or not and classifies positive matches as complete, fragmented, duplicated or missing [24]. The BUSCO set for arthropods include 2,675 single-copy genes. The expectations for these genes to be found in a genome and to be found only in single-copy are evolutionarily sound. After running this pipeline in each of our taxa, we retrieved complete and fragmented BUSCOs for each species (i.e., having one FASTA file per species with all the BUSCO files retrieved for that taxon), and then parsed and combined each BUSCO gene in all taxa in an individual file with custom python scripts for matrix construction (i.e., having one file per gene in FASTA format, one line per species). We constructed a first BUSCO matrix containing 2,483 genes (after removing genes represented only with 1 sequence and genes with less than 50 amino acids). Before concatenation or individual gene tree analyses, genes were aligned with MAFFT [27] (option ‘-auto’) and trimmed with trimAl 1.2a [28] (option ‘-gappyout’). This matrix (hereafter “BUSCO-2365 matrix”) resulted in a total length of 623,892 amino acids and 59.9% of missing data. To construct a second matrix with a lower amount of missing data (hereafter “BUSCO-750 matrix”), we parsed the genes present in at least 100 spiders, resulting in a matrix with 164,475 amino acids and 35.4% missing data. From the original set of 2,365 genes, we explored the phylogenetic optimality of each gene by plotting the taxon occupancy of each gene (ie, the number of taxa where that gene was retrieved) and its average bootstrap support (individual gene trees were generated with RAxML [29] for this purpose) (see Results and Discussion below). We then parsed genes with $\geq 50\%$ taxon occupancy and an average bootstrap support ≥ 50 , resulting in a matrix with 255 genes, 59,896 amino acids and 54% missing data (hereafter “BUSCO-255 matrix”).

The second orthology method explored was UPhO [30], which relies on the topology of individual gene trees to identify clades forming orthologous groups. Initial homology was performed with all-versus-all BLAST searches used a relaxed expectation value threshold of $e = 1 \times 10^{-5}$ followed by clustering in MCL with an inflation values of 2, 4, 6 and 10. Based on the number of clusters and basic cluster statistics (mcl info efficiency, $i_2 = 0.45742$, $i_4 = 0.49763$, $i_6 = 0.50947$, $i_{10} = 0.51919$), the clusters produced with i_{10} were used for downstream analyses. In order to reduce missing data, only clusters with at least 25 different species were subject to the phylogenetic pipeline to obtain putative gene-family trees (GFT) using MAFFT (option ‘-auto’), trimAl (option ‘-gappy-out’), Al2phylo (-m 50 -t 25 -p 0.25), and FastTree [31, 32] (-wag -slownni). The branches representing orthogroups, including species level in-paralogs, were identified with UPhO. The effect of relative taxon representation was explored analyzing orthogroups with at least 10 spp. and with at least 25 spp. The effect of topological support as an additional criterion for orthology was explored by comparing all orthogroups identified regardless of the support of the incident branch (-S 0) and orthogroups derived from branches with support value of at least 0.75 local support value (lsv), resulting in 2,329 orthologous genes. These genes resulted in a very sparse dataset with a high percentage of missing data (> 90%; hereafter ‘UPhO-2329’ matrix), therefore they were not concatenated in < # > order to save computational time. A smaller matrix was created by selecting only those genes for which their individual gene trees showed average bootstrap values greater than 50%, resulting in a matrix with 1,671 genes, 191,170 amino acids and 84.6% of missing data (hereafter ‘UPhO-1671’ matrix). Phylogenetic optimality was explored as described above. Genes with $\geq 25\%$ taxon occupancy and an average bootstrap support ≥ 50 were parsed, resulting in a matrix with 354 genes, 36,174 amino acids and 66.8% missing data (hereafter ‘UPhO-354’ matrix). More conservative parameters (i.e., a taxon occupancy of $\geq 50\%$ of taxa) would have resulted in a handful of genes as this method resulted to be strongly biased toward low taxon occupancy (see below), therefore this approach was not pursued. All matrices and individual gene alignments are deposited at the Harvard Dataverse repository (<https://doi.org/10.7910/DVN/EJOMZP>).

Phylogenetic inference

A first parsimony tree was inferred with TNT [33] in the BUSCO-2365 matrix to use as a starting tree in maximum likelihood (ML) exploration. The tree search strategy included sectorial searches, 4 starting Wagner trees, tree drifting for 20 cycles, ratchet for 20 cycles and tree fusing followed by TBR. ‘xmult: hits’ was set to 3. The run was stopped after the minimum length was reached three times. A similar procedure was followed with the UPhO dataset. ML inference was conducted with ExaML v3 [34] and IQ-Tree 1.5.0 [35] with the posterior mean site frequency (PMSF) model [36] as a rapid approximation to the CAT model in PhyloBayes. Models of amino acid substitution were selected using the AUTOF command in ExaML. One hundred bootstrap replicates were generated with RAxML which were then used to construct a 50% majority-rule bootstrap consensus tree. Tree certainty/internode certainty values [52] were assessed in every analyses with RAxML v8.2.9 following the considerations described in [53] for partial gene trees. A multi-species coalescent model approach was explored with ASTRAL-II [37], with individual gene trees reconstructed for each gene in each matrix with RAxML 8.2.9. Due to computational constraints, not all analyses were run in each dataset (see further details at the Harvard Dataverse repository, <https://doi.org/10.7910/DVN/EJOMZP>).

Molecular dating

Divergence time for spiders lineages was estimated through molecular dating, constrained by the position of a series of critical fossils, including Paleozoic fossils: (1) the uraraneid *Attercopus fimbriunguis* from the middle Devonian deposits of Gilboa, USA [54], (2) *Palaeothele montceauensis* from the Late Carboniferous of Montceau-les-Mines, France [55]; Mesozoic fossils: (3) *Rosamygale grau-voveli* from the Middle Triassic of Gres-a-Voltzia Formation [56], (4) *Edwa maryae* from the Late Triassic Blackstone Formation of Australia [57], (5) *Zhizhu daohugouensis* and *Z. jeholensis* from Daohugou, Middle Jurassic of Inner Mongolia, China [58], (6) an amber crown-group Linyphiidae from the Cretaceous Kdeiirji/Hammana outcrop, Lebanon [59], (7) and undescribed Palpimanidae from the Aptian, Crato Formation in Ceará, Northern Brazil, (8) *Mesozygiella dunlopi* from the Lower Cretaceous of Álava, Spain [60], and (9) two amber fossils from the Cretaceous of Myanmar, *Burmesiola cretacea* [61] and a Gamasomorphinae [62]. Justifications for taxonomic and age assignments of the fossils are described in detail in Data S3.

Divergence dates were estimated using the Bayesian relaxed molecular clock approach as implemented in PhyloBayes v.4.1 [38] with the BUSCO-255 matrix, using as constraint the ExaML topology of the BUSCO-750 matrix. An uncorrelated relaxed clock was applied to our dataset, with soft bounds [63] under a birth-death prior. Two independent MCMC chains were run for 2,500–3,000 cycles, sampling posterior rates and dates every 10 cycles with an initial burnin of 25%. Posterior estimates of divergence dates were then computed from the remaining samples of each chain.

Lineage diversification analyses

To study diversification through time, we used the program Bayesian analysis of macroevolutionary mixtures (BAMM) [39, 40]. BAMM allows to analyze highly incomplete and phylogenetically non-random datasets where different sampling fractions can be applied to each lineage and incomplete sampling of the tree backbone is also accounted for. However, when backbone topology is not completely sampled there are two different ways to account for this: by using the total sampling fraction across the whole group; or if we are certain that the missing taxa are not members of any of the sampled lineages, by calculating a sampling fraction that excludes all sampled species. Here we have used both approaches in order to ensure robustness of our results to alternative analytical parameters. Outgroup taxa were pruned prior to all BAMM analyses and proper priors and MCMC chain settings were selected using the setBAMMpriors function in the R package BAMMtools [39]. All analyses were run until satisfactory chain mixing and

effective sampling size values (ESS) were achieved. ESS were examined using the R library coda [41]. All post processing of the BAMM results including generation of graphical outputs has been carried out using functions available in BAMMtools.

Recently [64] have criticized the BAMM analytical approach. Using simulations they identified two possible major flaws with the BAMM approach: first they claimed that the likelihood function used to estimate model parameters is incorrect; and second, they suggested that the compound Poisson process prior model is incoherent. We have considered carefully the arguments of [64] as a large part of our discussion on diversification patterns is based on results from analyses in BAMM. Some of the [64] concerns have been addressed by [65]. A detailed rebuttal by [40] identified several issues in the way [64] run their simulations showing that BAMM performs as intended and does not use wrong likelihood and incoherent prior.

To ensure that our results are robust to different analytical approaches in addition to BAMM we used an alternative approach to study diversification dynamics, RPANDA [42]. RPANDA allows to fit different models of diversification including trait and environmental models. It also accommodates incomplete taxon sampling but only as a global sampling fraction. To identify distinct modes of diversification across a topology RPANDA uses model-free approaches based on graph theory.

To investigate the diversification dynamics of spiders in relation to environmental variability we used global temperature and the *fit_env* function. We choose temperature because general trends of global temperature are available over long timescales and because according to the expectations of the Metabolic Theory of Ecology (MTE), temperature should have an effect on diversification rates [17, 18]. Recently a positive correlation between rates of evolutionary diversification of ectotherms and temperature has been shown empirically [16], yet the generality of this pattern remains unknown. Here despite the lack of distributional data (current or past), which limits the scope of these analyses, we test whether there is an indication of correlation between temperature and diversification through time and if this relationship corresponds to the expectations of MTE and previous findings. Because RPANDA comes with environmental data on average global temperatures limited to the Cenozoic we extended it using data from [66] to cover the time since the origin of spiders. Although the temporal resolution and precision of [66] data are coarser than data for recent geological periods and some of the models from which it derives have been debated [67], it is indicative for the global temperature trends during this time interval.

Foraging behavior (i.e., based on the use of foraging webs or active hunting) has been linked to diversity patterns in spiders [8, 9]. Furthermore, growing evidence that orb-weavers are not monophyletic [1, 2, 6, 68] has raised the question about the potential multiple origins of the orb web itself. To study the effects of foraging behavior on spider diversification and the evolution of orb webs, we have scored the web building behavior and web architecture of all taxa included in our analyses. We used the dated topology and the R packages ape [43] and phytools [44] to reconstruct the evolution of web building behaviors related to foraging webs in spiders. We fitted models of web evolution under equal (ER), symmetric (SYM) and all rates different (ARD) character transformation matrices using a maximum likelihood approach as implemented in the *ace* function in ape and under a Bayesian framework using *make.simmap* in phytools. In order to test if models assuming different ancestral states for Araneioidea and for the clade that includes both cribellate and ecribellate orb-weavers and the RTA clade differ significantly we used the fossilized command in BayesTraits v3.0 [45] and Bayes factors as comparison criterion. Because our web types matrix includes ten different states there are many parameters that need to be estimated and currently BayesTraits allows fitting such complex models only when using the reversible-jump Markov chain Monte Carlo [69].

Diversification analyses may be influenced by sparse taxon sampling, especially when some of the backbone lineages are not represented. To minimize such potential negative effects we also run our diversification analyses incorporating the legacy data generated by the Spider Tree of Life project [1]. This legacy dataset contains almost 1,000 terminals and most importantly it includes representatives of all but one spider families (Synsphyridae, which we include in this study with the sequencing of a transcriptome). To take advantage of the robust topology generated in the present study and of the extensive taxon coverage of the legacy dataset we implemented our transcriptomic phylogenetic results as a backbone constraint in a RAxML analysis of the original Spider Tree of Life data (a total of six markers from the mitochondrial [12S rRNA, 16S rRNA, cytochrome c oxidase subunit I] and nuclear [histone H3, 18S rRNA, 28S rRNA] genomes). The ML tree from the constrained RAxML run was then transformed into an ultrametric tree using the following procedure: first, we used the function *congruify.phylo* and our date phylogeny in the R package GEIGER [46] to generate a set of congruent nodes and their ages with the constrained legacy topology to be used as dating calibrations in the program treePL [70]; then, we used a smoothing parameter of 0.01 (selected by random cross validation in treePL) and the set of calibrations provided by *congruify.phylo* to date the legacy topology in treePL. The dated tree was then used in all diversification analyses using the legacy dataset. We also scored foraging web types for the legacy dataset but in this case we used just three states to describe the web building behaviors –non orb web present, orb web present and no foraging web. The legacy dataset was then subjected to ancestral states reconstruction and diversification analysis analyses following the methodology outlined above.

To test explicitly whether web building behavior has had a major effect on spider diversification we performed trait dependent diversification analyses using the hidden states diversification and extension framework (HiSSE) implemented in the R package hisse [71]. The HiSSE model derives from the binary state speciation and extinction (BiSSE) of [72] but unlike BiSSE it allows to test whether some unknown trait (not considered in the observed trait data) has more significant effect on diversification than the observed traits. This approach is more realistic as such scenario (namely, the existence of a causal trait yet to be identified) is not only possible but often very likely. In addition, the original BiSSE implementation has been shown to be prone to type I errors and finds significant associations between tested traits and diversification where there is none [73]. Finally, HiSSE allows to test for character independent diversification and accounts for incomplete sampling. To be able to analyze our datasets in HiSSE we rescored the web legacy matrix and produced two binary matrices: one with state web and webless and another one with states non-orb and orb web. These we

designed to test whether the transition to webless state or to orb web has a significant effect on global overall diversification patterns in spiders. Several HiSSE and BiSSE equivalent models were run (see [Data S1](#)) and AIC scores were used to select the best fitting model. Character dependent diversification analyses were run only with the legacy data as it has much broader taxonomic sampling and represent better the phylogenetic distribution of the different web building behaviors that we consider for these analyses.

Functional annotation of transcriptomes

We run a functional annotation of each spider transcriptome in our dataset with eggNOG-mapper [47]. This tool uses precomputed eggNOG-based orthology assignments [74]. The use of orthology predictions for functional annotation in this tool is considered more precise than traditional homology searches, as it avoids transferring annotations from paralogs (duplicate genes with a higher chance of being involved in functional divergence). We reduced redundancy by eliminating all duplicated Gene Ontology (GO) terms and keeping a list of unique entries per taxon. In addition, we concatenated all GO terms of all taxa belonging to a specific clade at different nested hierarchical levels (level 1: Mygalomorphae and Araneomorphae; level 2: Synspermiata and non-Synspermiata araneomorphs; level 3: Araneioidea + Nicodamoidea + Eresidae and RTA clade + UDOH grade; level 4a: Eresidae and Araneioidea + Nicodamoidea; level 4a1: Araneioidea and Nicodamoidea; level 4b: RTA clade and UDOH grade), reducing redundancy again to have a list of GO terms present in the pool of samples belonging to each clade or grade. To explore the effect of depth of sequencing or tissue diversity in the recovery of clade-specific GO terms (therefore ensuring the validity of this approach), we mapped back these clade-specific GO terms to each taxon within the clade and analyzed the relationship between the percentage of the retrieved GO terms and assembled megabases in each assembly (both considering all genes and only the longest isoforms) to account for differences in depth of sequencing or tissue heterogeneity in the samples. We tested that the list of GO terms was indeed representative of each clade (i.e., that depth of sequencing and sample heterogeneity was not affecting the GO terms retrieved for each clade excepting for a few samples with a very low number of transcripts). With these datasets, we investigated functional enrichment in certain clades/grades through a pairwise comparison of GO terms (i.e., clade-specific in the context of the pairwise comparison). We scrutinized the following pairwise comparisons: a) Araneomorphae versus Mygalomorphae; b) Araneioidea versus UDOH grade (i.e., cribellate versus cribellate orb-weavers and their relatives, respectively); c) Araneioidea versus RTA clade; and d) UDOH grade versus RTA clade. For each comparison, we contrasted the lists of GOs, represented them in Venn diagrams, removed redundancy and visualized the clade-specific, comparison-specific list of GOs in REVIGO [75]. Functional annotation results per taxon (i.e., eggNOG-mapper results) and clade-specific, comparison-specific lists of GOs are available in the Harvard Dataverse repository (<https://doi.org/10.7910/DVN/EJOMZP>).

Cryptochrome gene family evolution analyses

One of the most notable and widespread genetic differences between araneomorphs and mygalomorphs was related to magnetoreception and UV-light phototransduction (see Results and Discussion), hence we further investigated the nature and evolutionary history of the underlying genes. We traced them back to a single UPhO-inferred orthogroup (that contained the araneomorph-specific sequences with other homologs from the same gene family) and annotated all sequences with BLASTp searches [48] against the non-redundant (nr) database in NCBI (e-value $1e-20$). All sequences in this orthogroup were annotated as cryptochromes. In order to understand gene family evolution and to classify the homologs in a phylogenetic context, we retrieved all cryptochrome protein sequences from arthropods from [24]. Cryptochrome DASH proteins from several species were selected as outgroups. The sequences were aligned with MAFFT [27] (option ‘-auto’) and trimmed with trimAl 1.2a [28] (option ‘-gappout’), resulting in a matrix with 939 terminals and 484 amino acid positions. A maximum-likelihood tree was built with RAxML v. 8.2.9 [29]. One hundred bootstrap replicates were generated and were then used to construct a majority-rule bootstrap consensus tree. In addition, Bayesian inference was explored with IQ-TREE v. 1.5.5 [35] using the C60 empirical mixture model [36]. The trees were visualized with FigTree 1.4.3. (<http://tree.bio.ed.ac.uk/software/figtree/>) [49] and phylo.io [50].

DATA AND SOFTWARE AVAILABILITY

Raw data for all transcriptomes newly generated for this study are deposited in the NCBI SRA server, BioProject PRJNA432042. Accession numbers for each taxon are specified in [Data S1](#). The matrices used to generate all phylogenetic hypotheses as well as additional supplemental information are deposited in the Harvard Dataverse repository (<https://doi.org/10.7910/DVN/EJOMZP>).

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Supplemental Information

**Phylogenomics, Diversification Dynamics,
and Comparative Transcriptomics
across the Spider Tree of Life**

Rosa Fernández, Robert J. Kallal, Dimitar Dimitrov, Jesús A. Ballesteros, Miquel A. Arnedo, Gonzalo Giribet, and Gustavo Hormiga

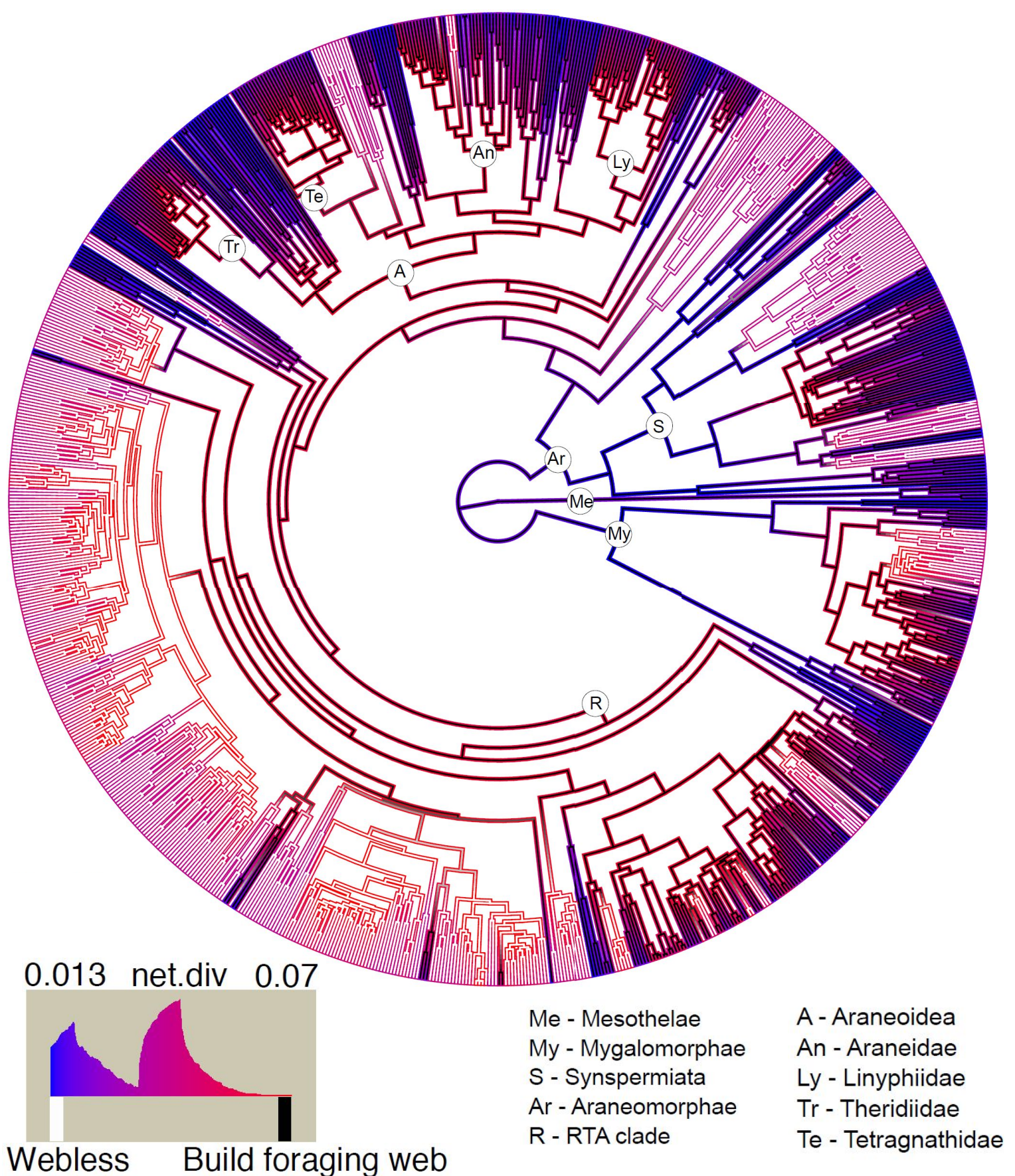


Figure S1. Lineage diversification dynamics results, Related to Figure 3 and STAR

METHODS. Result from the hidden states diversification analyses of foraging web used based on the legacy dataset. The tree summarizes the best performing model (the full model). The inside colors of the branches represent the reconstructed web evolution (webless or building foraging web, respectively) and the outside color represents the rate of net diversification. Warmer colors correspond to higher rates. The upper part of the bottom left legend shows the distribution of diversification rates at the tips and the colors correspond to the different net diversification rates values. The bottom part shows the two observed states and the corresponding branch colors.

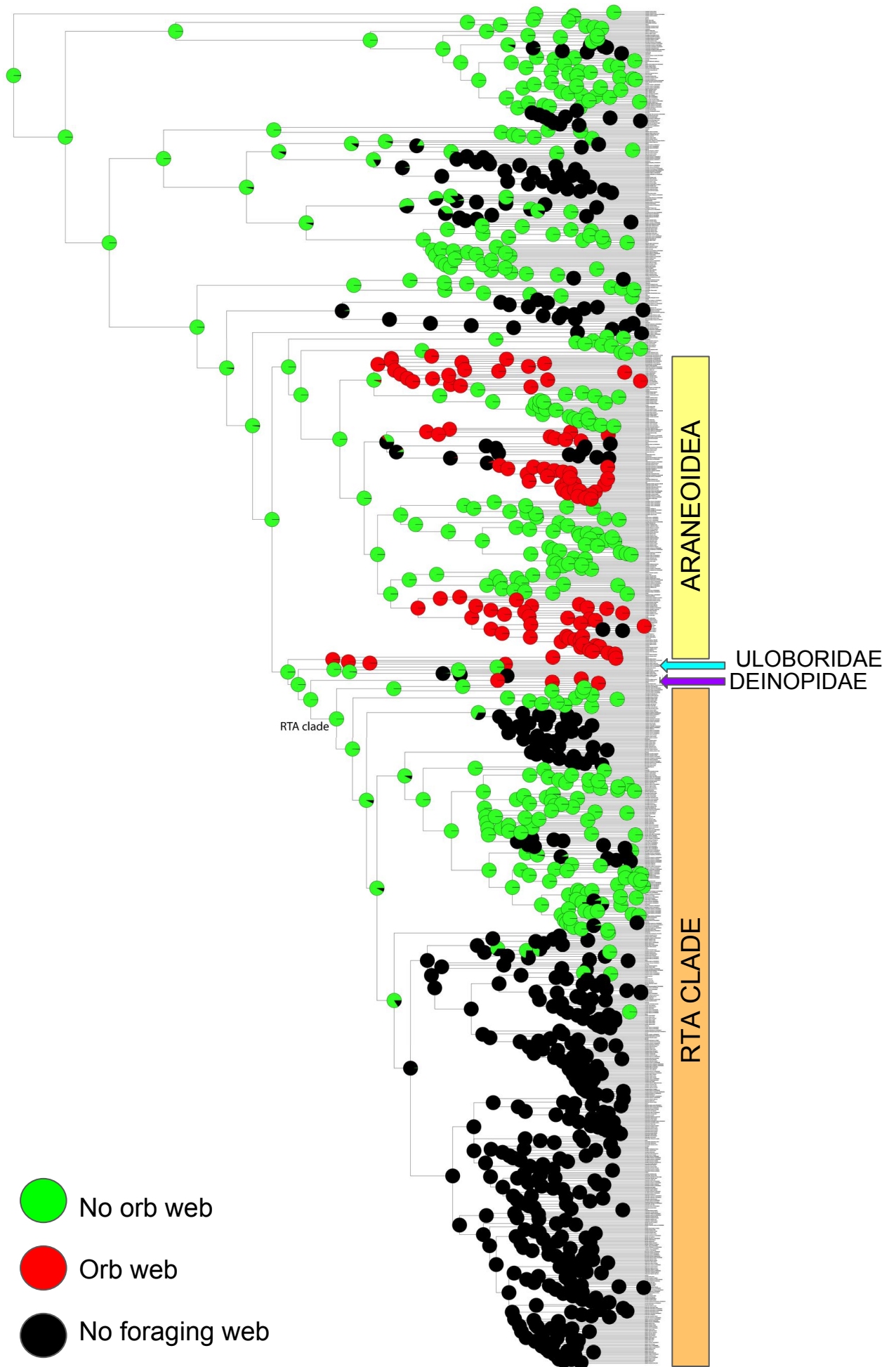
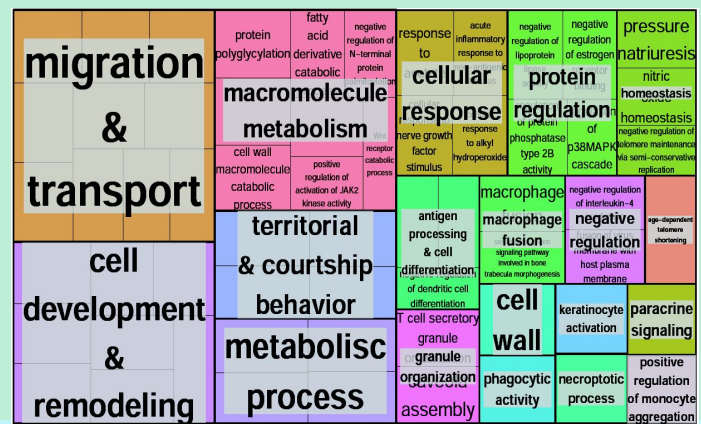
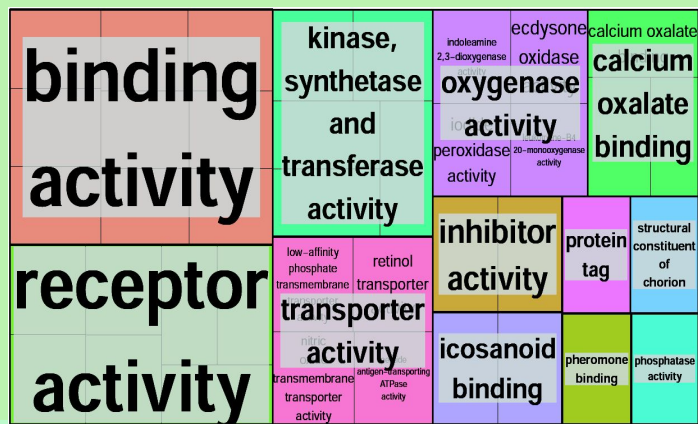


Figure S2. Web evolution across the Spider Tree of Life, Related to Figure 3 and STAR METHODS.

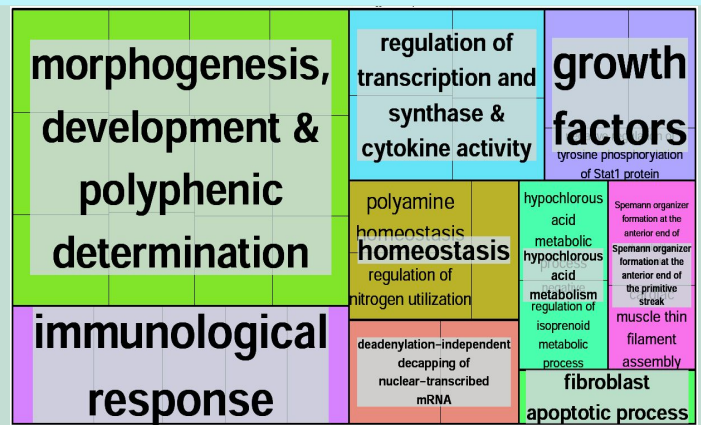
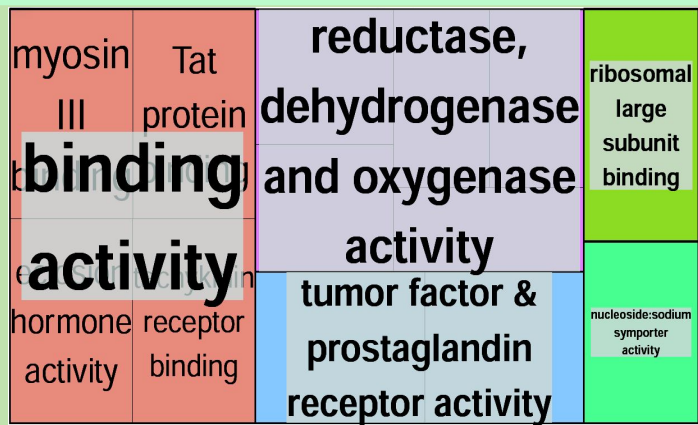
Ancestral state reconstruction of foraging webs using the legacy dataset and the ace R function with different but symmetric rates of character transformation (SYM). Three coding schemes are used: no orb web (green), orb web (red) and no foraging web (black). Main clades are indicated (Araneoidea, RTA clade, Uloboridae and Deinopidae).

BIOLOGICAL PROCESS

ARANEOIDEA



RTA CLADE

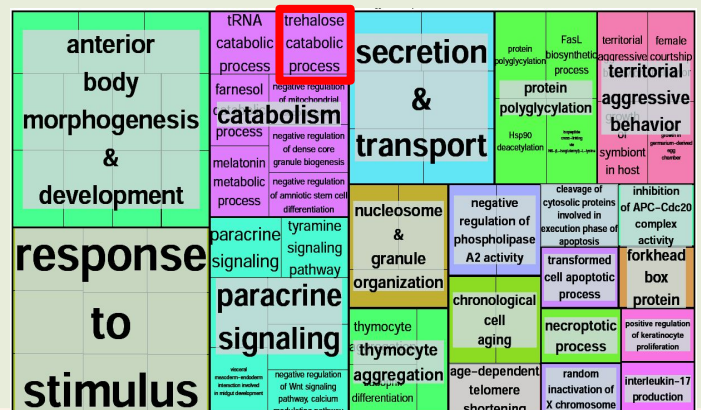
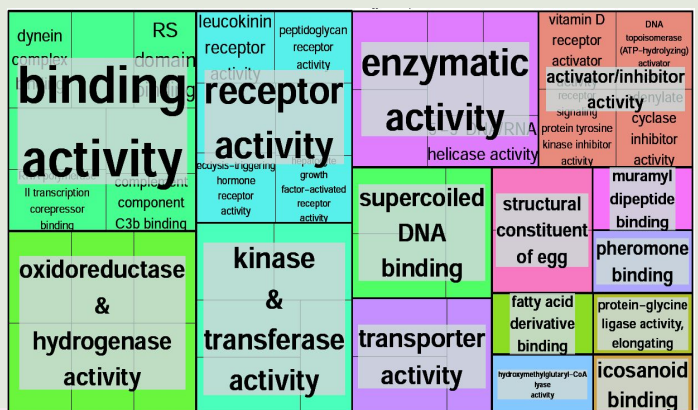


B

MOLECULAR FUNCTION

BIOLOGICAL PROCESS

Ecribellate orb weavers



Cribellate orb weavers

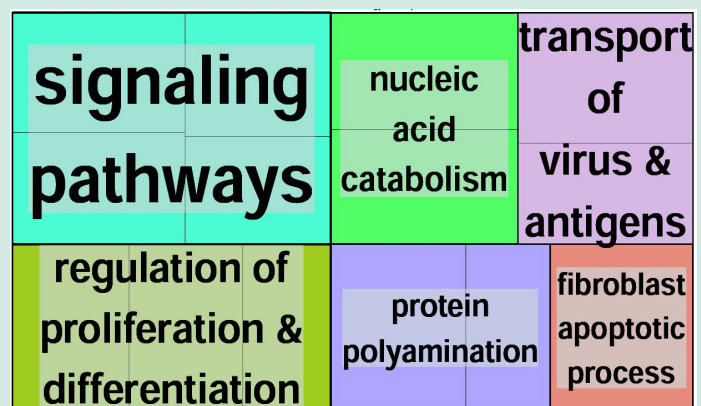
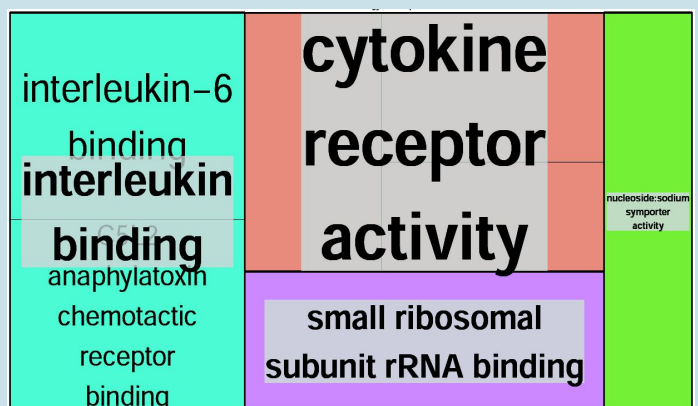


Figure S3. Comparative transcriptomics in Araneoidea and the RTA clade, Related to Figure 1 and STAR METHODS. (A) Pairwise comparison of gene ontology (GO) terms in Araneoidea and the RTA clade (molecular function and biological process). (B,C) Detail of araneoid-enriched GO terms (red-bordered, molecular function; blue-bordered, biological process). (D) Pairwise comparison of GO terms in cribellate and ecribellate orb weavers. GO terms related to trehalose catabolism are highlighted in red (ecribellate orb weavers, biological process treemap).

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