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Sequence Capture Phylogenomics of True Spiders Reveals Convergent Evolution of Respiratory Systems

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Abstract.—The common ancestor of spiders likely used silk to line burrows or make simple webs, with specialized spinning organs and aerial webs originating with the evolution of the megadiverse "true spiders" (Araneomorphae). The base of the araneomorph tree also concentrates the greatest number of changes in respiratory structures, a character system whose evolution is still poorly understood, and that might be related to the evolution of silk glands. Emphasizing a dense sampling of multiple araneomorph lineages where tracheal systems likely originated, we gathered genomic-scale data and reconstructed a phylogeny of true spiders. This robust phylogenomic framework was used to conduct maximum likelihood and Bayesian character evolution analyses for respiratory systems, silk glands, and aerial webs, based on a combination of original and published data. Our results indicate that in true spiders, posterior book lungs were transformed into morphologically similar tracheal systems six times independently, after the evolution of novel silk gland systems and the origin of aerial webs. From these comparative data, we put forth a novel hypothesis that early-diverging web-building spiders were faced with new energetic demands for spinning, which prompted the evolution of similar tracheal systems via convergence; we also propose tests of predictions derived from this hypothesis.[Book lungs; discrete character evolution; respiratory systems; silk; spider web evolution; ultraconserved elements.]

Spiders and their webs are among the most fascinating examples of animal architecture and are at the intersection of important questions in evolutionary biology, such as the construction of their own niche, the origin of key evolutionary innovations, and the extending limits of the phenotype (Bond and Opell 1998; Blackledge et al. 2011). The common ancestor of spiders likely used silk to line burrows or make simple silken structures close to the substrate (Fernández et al. 2018; Coddington et al. 2019; Hedin et al. 2019; Opatova et al. 2019). True aerial webs, from which spiders can hang in an inverted position, originated with the "true spiders" or Araneomorphae. The key innovation that allowed for the evolution of aerial webs are specialized spinning organs; araneomorphs developed a system of ampullate silk glands, producing tough fibers, and piriform silk glands that produce a cement making highly efficient anchorages (Coddington 2005; Wolff et al. 2019). Along with the spinning organs, the posterior spider body (opisthosoma) underwent a reorganization of the respiratory organs concomitantly with the simplification of the circulatory system (Huckstorf et al. 2015).

Early-diverging spider lineages have four book lungs in consecutive pairs on the anteroventral abdomen; of the three main spider lineages, the Mesothelae (single family with ~130 known species) and Mygalomorphae (20 families, ~3000 species) retain this ancestral configuration, while most araneomorphs (96 families, ~48,500 species) have transformed the posterior book lungs into tracheae, or lost them altogether (see Ramírez 2000; Schmitz 2013; Fig. 1). When the posterior book lungs transformed into tracheae they also migrated posteriorly, close to the silk spinning structures. Only three species-poor lineages of araneomorphs retain the ancestral configuration of four book lungs (Hypochilidae, Gradungulidae, and some Austrochilidae, 29 species in total).

The origin and function of spider tracheae have long puzzled both taxonomists and physiologists (Levi 1967; Schmitz 2013). Ontogenetically, tracheae arise in various ways as modifications of the book lungs and adjacent apodemes (Purcell 1909; Ramírez 2000, 2014). Morphologically, they usually consist of four tubes that can be either short and simple or large and highly branched (Fig. 1). Physiological studies have found that extensive tracheal systems passing to the anterior body compartment (the prosoma), where locomotory functions are concentrated, contribute to aerobic metabolism during periods of high activity (Schmitz 2005), or help muscular action to monitor the web (Opell 1987). However, most spiders, including some of the most diverse spider families, spanning a broad range of body sizes and ecologies, have a small tracheal system limited to the opisthosoma (Supplementary Fig. S18 available on Dryad). The function of these small tracheal systems is still a mystery; it has been shown that blocking these tracheae has no impact on locomotory metabolism or performance, nor in CO_2 release (Schmitz and Perry 2002; Schmitz 2005).

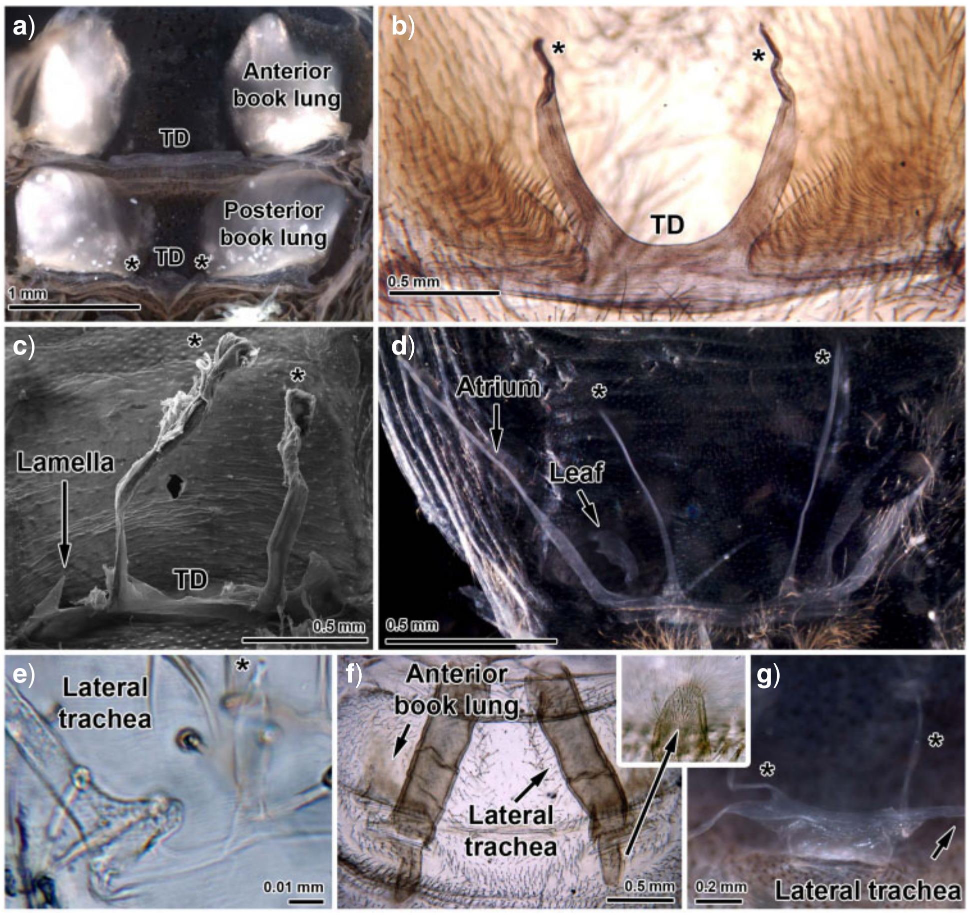

FIGURE 1. Respiratory structures of selected spider taxa. a) *Liphistius yamasakii* (Mesothelae, Liphistiidae), b) *Hickmania troglodytes* (Araneomorphae, Austrochilidae), c) *Kukulcania hibernalis* (Araneomorphae, Filistatidae), d) *Austrochilus melon* (Araneomorphae, Austrochilidae), e) *Archoleptoneta schusteri* (Araneomorphae, Leptonetidae, Archoleptonetinae), f) *Dysdera crocata* (Araneomorphae, Synspermiata, Dysderidae), inset to posterior branch of lateral trachea, g) *Polybetes pythagoricus* (Araneomorphae, Entelegynae, Sparassidae). * = apodeme; TD = transverse duct.

Recent phylogenies from genomic-scale data imply that tracheate spiders are polyphyletic (Supplementary Fig. S1 available on Dryad at https://doi.org/10.5061/dryad.3bk3j9kfd), or that some lineages reacquired book lungs from tracheae—a possibility considered unlikely by Huckstorf et al. (2015). However, these prior analyses lacked a dense sampling of early-diverging araneomorphs or were limited in sequence data. In this study, we combine a genomic-scale data set derived from sequence capture of ultraconserved elements (UCEs) with a dense sampling of araneomorph lineages where tracheal systems likely originated. We then analyze new morphological data on respiratory systems, test the origin of silk gland systems and aerial webs, and propose a novel hypothesis for the origin and diversity of respiratory structures in true spiders.

MATERIALS AND METHODS

Taxon Sampling and Matrix Assembly

We used UCE sequence capture data, building upon the results of Wood et al. (2018). Including a mesothele and two mygalomorphs as outgroups, we assembled an araneomorph taxon sample that emphasized early-diverging lineages, and included many taxa never before sampled in a molecular phylogenetic analysis (see Supplementary Table S1 available on Dryad). UCE loci were obtained and processed as in Hedin et al. (2019), and multiple phylogenomic analyses were conducted to explore impacts of analysis method, data partitioning, and base composition biases (see Supplementary Methods and Results available on Dryad).

Morphology Data and Character Evolution

We scored the morphology of the respiratory system for a subset of sampled taxa (68 of 96) covering all major lineages. Our scorings were based on original dissections of 40 species, supplemented with published data (Supplementary Table S2 available on Dryad). We explored the effect of alternative coding schemes for multiple states and missing entries. We also scored 68 taxa for the presence of the major ampullate + piriform gland system, and for aerial webs (Supplementary Table S3 available on Dryad). To distinguish aerial webs from silken tubes or burrows ("substrate webs"), we defined them as foraging webs from which the spiders hang in an inverted position. Ancestral states were estimated using maximum likelihood, with the fit of different character evolution models compared with the Akaike information criterion (AIC). Correlated evolution between discrete characters was tested in a Bayesian framework using a threshold model from quantitative genetics (Felsenstein 2012; Revell 2014). Additional details for all character analyses are provided in the Supplementary Methods and Results and Tables S2–S5 available on Dryad.

RESULTS

Voucher specimen data and relevant UCE summary values (e.g., cleaned reads, number of contigs, etc.) are found in Supplementary Table S1 available on Dryad. Raw reads from our 53 original samples have been submitted to the SRA (BioProject ID: PRJNA610839); individual locus alignments and tree files are available on Dryad and TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S26343). The final primary matrix (534_25_noP) included 534 loci, with a combined alignment length of ~120,000 base pairs and 56,561 parsimony informative sites. Phylogenetic results are in general robust to alternative models of molecular evolution, data partitioning scheme, and optimality criteria, and most clades are recovered with high support (Fig. 2, Supplementary S2–S5, S13 available on Dryad). Our UCE phylogenies are also largely congruent with previous phylogenomic analyses (Fernández et al. 2018). Important taxon additions include the hypochilid *Ectatosticta*, thus recovering a monophyletic Hypochilidae, and two telemids, suggesting that this family is sister to Scytodoidea + Pholcoidea (as in Shao and Li 2018). Our dense sample of leptonetids suggests that this family is diphyletic, with Archoleptonetinae separate from Leptonetinae. This result refutes a hypothesis of leptonetid polyphyly (Wheeler et al. 2017) but is consistent with predictions made by Ledford and Griswold (2010) based on morphology. In exploratory analyses, *Trogloraptor* was recovered as sister to telemids but on a very long branch. After accounting for high GC bias (Supplementary Figs. S6–11 available on Dryad), the position of *Trogloraptor* stabilized to closely match phylotranscriptomic results (Michalik et al. 2019) and morphological evidence (Griswold et al. 2012). The placement resolved for *Huttonia* is unusual, but since it belongs to a clade homogeneous in respiratory system morphology, its placement did not impact character evolution analyses. Finally, early-diverging relationships in Entelegynae, in particular the placements of uloborids and oecobiids, are unstable across analyses (see also Garrison et al. 2016; Fernández et al. 2018); because all spiders in this clade have tracheae, this uncertainty has no impact on our main character evolution results.

Character mapping of the main architecture of the posterior respiratory system (PRS) is robust to the inclusion of "absences" as a fifth state or as missing data, pruning of terminals lacking a PRS, or coding PRS as a binary or multistate character (Supplementary Figs. S14–S17 available on Dryad). Thus, for simplicity, we discuss below the results of the multistate coding including absence as a fifth state (Fig. 3). A custom, "irreversible" evolution model that disallows regains of book lungs from tracheae or regains of respiratory structures is favored over equal rates, symmetric or all-rates-different models (AIC weight of 93%; Table 1 and Supplementary Tables S4 and S5 available on Dryad) and implies that the transformation of book lungs into tracheal structures occurred six times independently. These transformations are once to a single lamella (in Filistatidae), once to a tube plus a single leaf (in Austrochilinae), and four times to tubular tracheae (in archoleptonetines, leptonetines, Synspermiata, and Entelegynae + Palpimanoidea; Fig. 3). All branches involving morphological transitions in the respiratory system are well supported and recovered in multiple phylogenetic analyses (Fig. 2, Supplementary Fig. S13 available on Dryad), thus the ancestral state reconstruction and evolutionary model selection are robust to phylogenetic uncertainty (Supplementary Fig. S15 available on Dryad). Complex tracheal systems with tracheae supplying the prosoma are mapped as at least five independent transformations from simpler systems limited to the opisthosoma (Supplementary Fig. S18 available on Dryad). We detected four independent losses of the PRS, including one in Pholcoidea (the "lost tracheae clade"), all from simple tracheal systems limited to the opisthosoma (three from tubular tracheae and one from lamella). The ampullate + piriform gland system originated in Araneomorphae, with a single loss in our data set in the sand spider *Hexophthalma* (Supplementary Fig. S20 available on Dryad). The aerial web also originated in Araneomorphae, with several subsequent reversions to substrate web or losses (Supplementary Fig. S21 available on Dryad). We detected a significant correlation between the ampullate + piriform system and tracheae (highest posterior density of correlation 0.050–0.797, mean 0.410, $P = 0.925$, effective sample size 319) but not between aerial webs and tracheae.

DISCUSSION

We assembled a new genomic-scale data set that complements previous phylogenies reconstructed mostly using transcriptomes. The high congruence with these prior studies and strong support for both deep and shallow branches indicate that UCE-based sequence capture is a good strategy when paired with dense taxon sampling, without the stringent sampling conditions of transcriptomes (Hedin et al. 2019; Kulkarni et al. 2020). Our results indicate that the posterior book lungs of spiders were transformed six times into tracheal systems after the origin of aerial webs and the evolution

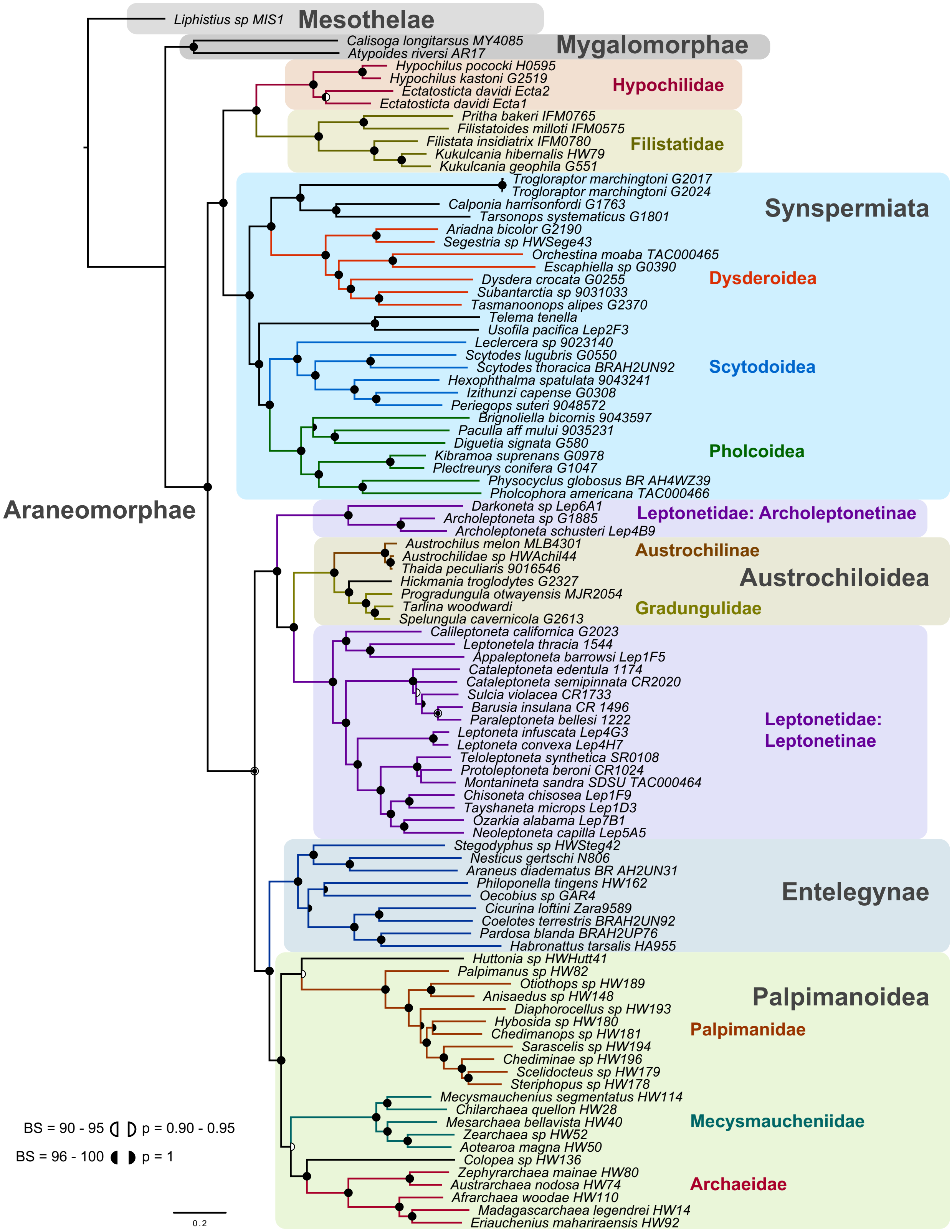

FIGURE 2. ExaBayes Bayesian inference tree of the "preferred" data set (see Supplementary Methods and Results available on Dryad), showing main spider clades and posterior probability values (P). BS = bootstrap values from ML analysis of "preferred" data set.

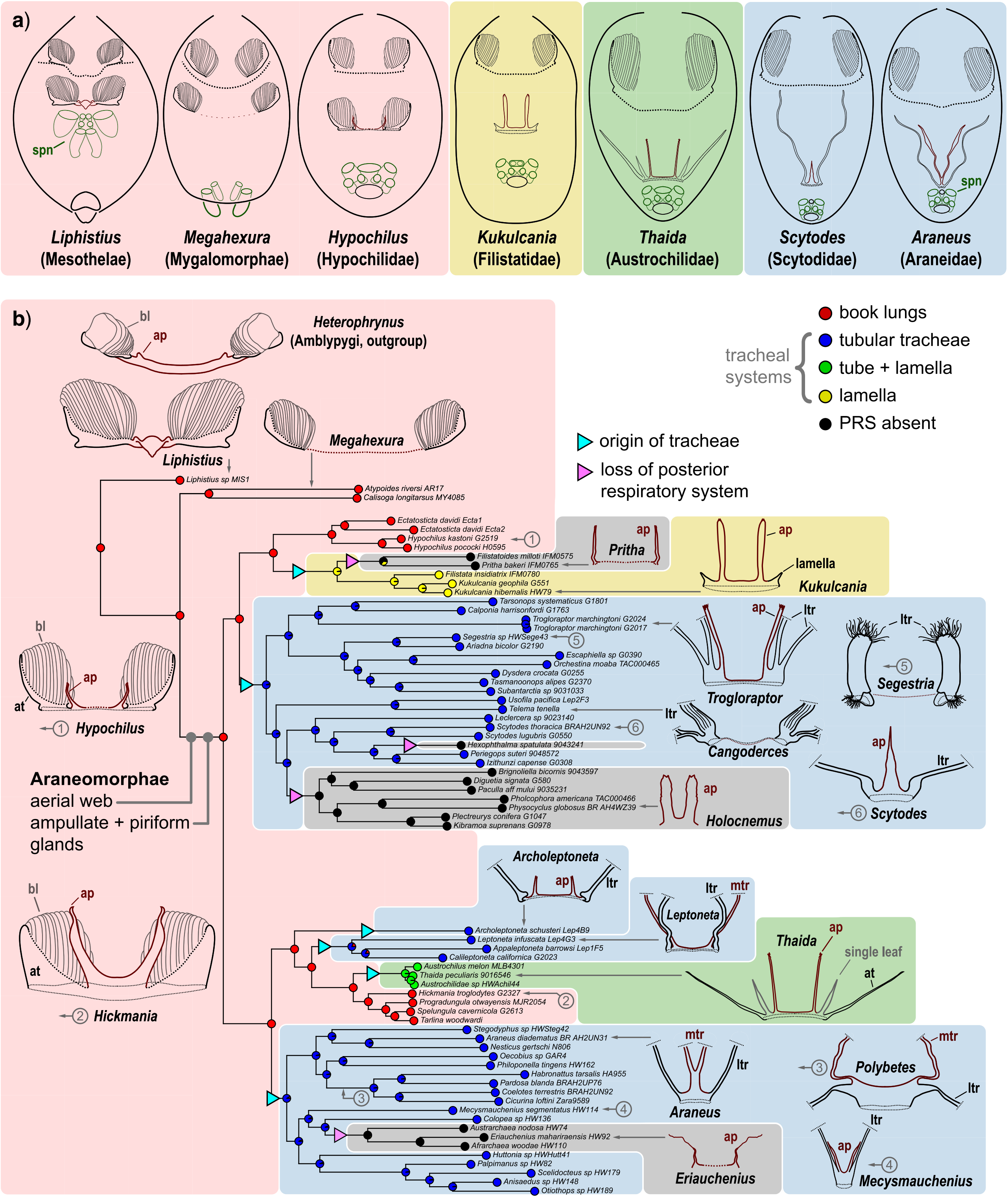

FIGURE 3. Evolution of the posterior respiratory system in araneomorph spiders. a) Schematic view of opisthosoma of exemplar taxa, showing the respiratory system in relation to spinnerets. b) Posterior respiratory system mapped by maximum likelihood using multistate coding for the 68 terminals with available morphological data. Morphology schemes are grouped by main configurations using shaded areas. ap = apodeme; at = atrium of book lung; bl = book lung; ltr = lateral trachea; mtr = median trachea, derived from apodeme; spn = spinnerets.

TABLE 1. Comparison among the different models used for character reconstruction

Trait	Model	ER	SYM	IRV	ARD
Posterior respiratory system, multistate coding	**Log likelihood**	−44.66457	−40.30172	**−36.12254**	−34.88677
	Parameters	1	10	**7**	20
	AIC	91.32913	100.60344	**86.24507**	109.77355
	AIC weights	0.07291166	0.00070617	**0.92637496**	0.00000721
Web, multistate coding (absence coded as an additional state)	**Log likelihood**	**−50.99055**	−50.26976	−48.80268	−48.51729
	Parameters	**1**	3	4	6
	AIC	**103.9811**	106.5395	105.6054	109.0346
	AIC weights	**0.55491286**	0.15440849	0.24633075	0.04434791

See Supplementary material available on Dryad for details. Preferred models according to the AIC are highlighted in bold. ER = equal rates; SYM = symmetrical rates; IRV = irreversible model; ARD = allratesdifferent model.

of the ampullate + piriform gland system of true spiders. Alternative reconstructions inferring one or two reacquisitions of book lungs were not favored by our AIC-based model selection (Supplementary Fig. S15, Tables S4 and S5 available on Dryad); furthermore, we regard the *de novo* evolution of book lungs as less parsimonious, as their morphologically complex structure is identical across all spiders possessing them (Fig. 3).

Six clades converged to a similar conformation of few tracheal tubes limited to the opisthosoma, usually close to the spinnerets: two leptonetid clades, the austrochilines, the common ancestor of palpimanoids and entelegynes, the filistatines, and the common ancestor of Synspermiata. Why have tracheae evolved so many times within araneomorph spiders? Based on anatomical proximity of tracheae to the anterior lateral spinnerets and the ampullate silk glands, we hypothesize that tracheae originated to supply the demands of the spinning system, ultimately also linked to the evolution of aerial webs. The outlets of the ampullate and piriform silk glands are strategically placed in the anterior lateral spinnerets, which are operated by a complex musculature (Eberhard 2010), and the anchorages of silk to substrate are made through a precise choreography that determines their resistance (Wolff et al. 2019). The location of silk glands, particularly the ampullate glands, and the muscles operating the spinnerets are positioned immediately dorsal to where the tracheae are located. This suggests that early-diverging web-building spiders were faced with new energetic demands for spinning, which resulted in the evolution of similar tracheal systems via convergence. A prediction of this hypothesis is that blocking a simple PRS should be detrimental to spinning performance. We are aware that the correlation is not perfect; for example, the Pholcoidea lost their tracheae but still build aerial webs using the ampullate + piriform gland system.

Our analyses also reveal at least five independent origins of extensive tracheal systems reaching the prosoma, all derived from systems limited to the opisthosoma (Supplementary Fig. S18 available on Dryad); probably many more convergences occurred within the Entelegynae. This indicates that oxygen supplementation for muscular activity in the prosoma was a later development rather than the original function of spider tracheae.

SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3bk3j9kfd.

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