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The Evolutionary Biology of Chelicerata

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Abstract

Chelicerata constitutes an ancient, biodiverse, and ecologically significant group of Arthropoda. The study of chelicerate evolution has undergone a renaissance in the past decade, resulting in major changes to our understanding of the higher-level phylogeny and internal relationships of living orders. Included among these conceptual advances are the discoveries of multiple whole-genome duplication events in a subset of chelicerate orders, such as horseshoe crabs, spiders, and scorpions. As a result, longstanding hypotheses and textbook scenarios of chelicerate evolution, such as the monophyly of Arachnida and a single colonization of land by the common ancestor of arachnids, have come into contention. The retention of ancient, duplicated genes across this lineage also offers fertile ground for investigating the role of gene duplication in chelicerate macroevolution. This new frontier of investigation is paralleled by the timely establishment of the first gene editing protocols for arachnid models, facilitating a new generation of experimental approaches.

INTRODUCTION

Chelicerates make up an ancient subdivision of arthropods that have played prominent roles in the ecosystems of the Phanerozoic. Comprising three major extant lineages—sea spiders, horseshoe crabs, and arachnids—chelicerates are found throughout marine, limnic, and terrestrial habitats. With over 120,000 described species, this diverse clade exhibits marked asymmetry of species richness across its extant members, with some lineages harboring tens of thousands of species and other depauperate groups having only a handful of extant taxa, such as the four living species of horseshoe crabs (29, 123) (**Table 1**). Some charismatic arachnids like spiders and scorpions are universally recognizable and, unusually for most arthropods, often have unique common names in languages across the world. The charisma of these taxa is paralleled by their notoriety for toxicity and their association with human phobias. Other lineages within chelicerates are pertinent to human well-being as agricultural pests (e.g., crop and bee mites), predators of agricultural pests (e.g., spiders and predatory mites), parasites (e.g., *Sarcoptes scabiei*, the mite species that causes scabies), and disease vectors (e.g., ticks) (7, 86, 103, 104, 109).

Like all members of the phylum Arthropoda, chelicerates are characterized by a segmented exoskeleton, jointed appendages, and growth through a series of molts. However, they are readily distinguished from other arthropod groups by their segmental arrangement and the form of their appendages. Chelicerates typically bear six pairs of appendages, which consist of a pair of chelicerae (feeding appendages of the second head segment), a pair of palps (sensory and/or feeding appendages of the third head segment), and four pairs of walking legs (**Figure 1*a***). The bauplan of a chelicerate is typically divided into two groups of segments called tagmata: The anterior prosoma bears the eyes and appendages, whereas the posterior opisthosoma bears major organ systems

Table 1 Current classification of Chelicerata and estimated number of described species

Higher taxon 1	Higher taxon 2	Order	Extant	Extinct
NA	NA	Pycnogonida	1,392	13
Euchelicerata	Arachnopulmonata	Amblypygi	268	13
		Araneae	52,046	1,427
		Schizomida	372	14
		Uropygi	126	14
		Scorpiones	2,766	154
		Pseudoscorpiones	4,208	57
		Opiliones	6,686	59
		Solifugae	1,209	6
		Ricinulei	102	25
		Acariformes	>42,000	347
		Parasitiformes	>12,400	22
		Palpigradi	138	2
	Merostomata	Xiphosura	4	105
		Eurypterida	0	253
		Chasmataspidida	0	14
		Haptopoda	0	2
		Trigonotarbida	0	70
		Phalangiotarbida	0	31

Data taken from the World Arachnid Catalog (**https://wac.nmbe.ch**), accessed April 1, 2024; the World Spider Catalog (**https://wsc.nmbe.ch**), accessed April 1, 2024; the World Catalogue of Opiliones (**https://wcolite.com**), accessed April 1, 2024; and Reference 152. Abbreviation: NA, not available.

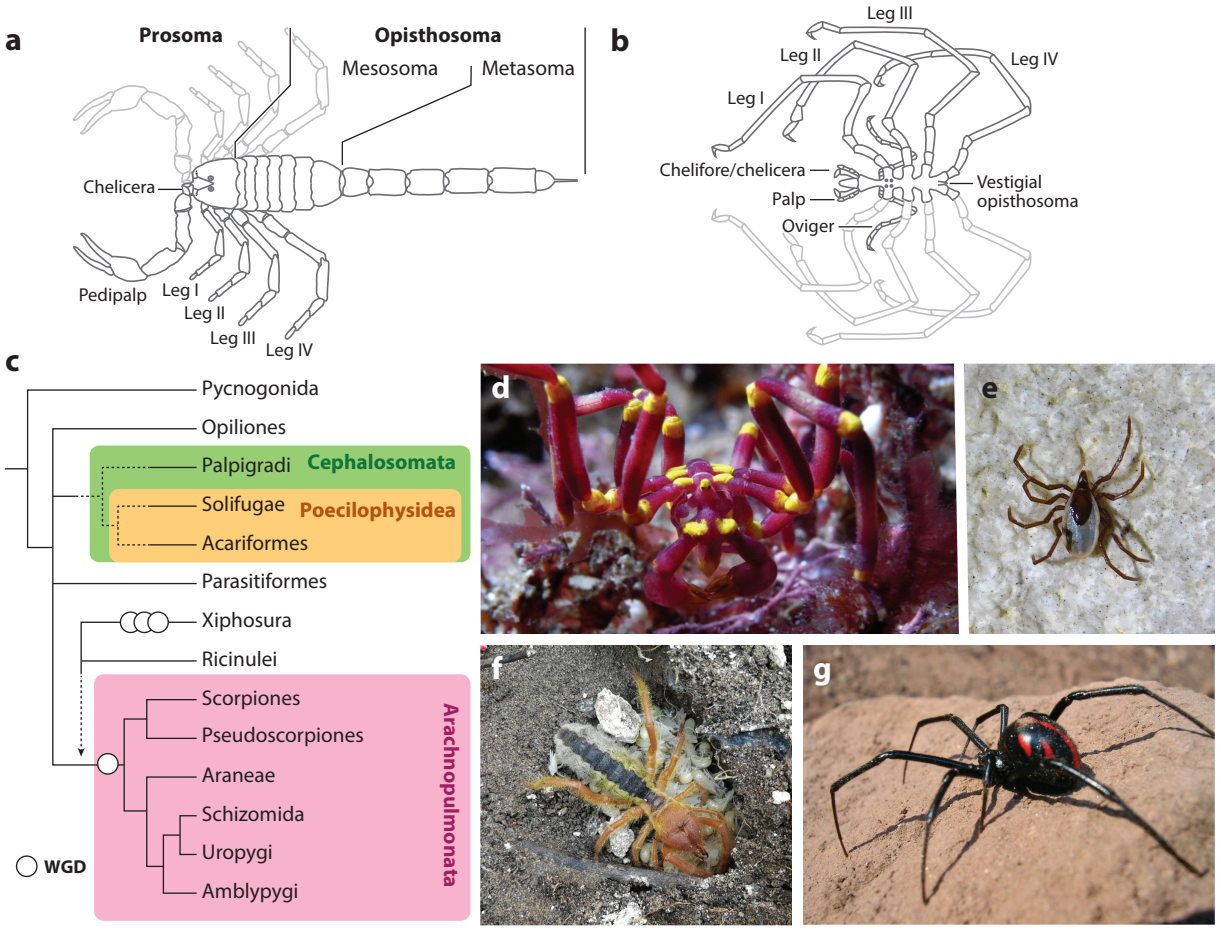

Figure 1

(*a*) Schematic of a typical chelicerate body plan, indicating appendages and tagmata. (*b*) Schematic of a sea spider body plan. Note the incidence of ovigers (egg-carrying appendages unique to this order) and the rudimentary opisthosoma. (*c*) Summary tree of the current understanding of chelicerate phylogeny, based on Reference 8. Dotted lines reflect unstable relationships. White circles indicate inferred whole-genome duplication (WGD) events. (*d–g*) Exemplars of chelicerate lineages. (*d*) *Pallenella harrisi* (Pycnogonida). Photo from Reference 10. (*e*) *Ornithodoros tholozani* (Parasitiformes, Ixodida). Photo by J.A. Ballesteros. (*f*) *Paragaleodes* sp., brooding female and offspring (Solifugae). Photo by I. Armiach. (*g*) *Latrodectus hesperus* (Araneae, Araneomorphae). Photo by J.A. Ballesteros.

and, in some lineages, modified appendage derivatives (e.g., spider spinnerets, scorpion pectines) (44).

Each of the 14 extant chelicerate orders has modified this bauplan in different ways. For example, modern sea spiders (Pycnogonida) have an additional pair of modified legs called ovigers that are typically used for egg carrying by the males and for grooming (**Figure 1*b***). Ovigers occur immediately posterior to the palps, and thus, eight-legged sea spiders have one extra appendage-bearing segment compared to arachnids. In addition, supernumerary leg-bearing segments have evolved several times in sea spiders, resulting in 10- and 12-legged genera and species (5, 10). Modern sea spiders lack a segmented opisthosoma, with the remnant of this tagma reduced to a small protrusion; a segmented opisthosoma is found only in some Paleozoic sea spider fossils (15, 99). As a result, major organ systems, such as the gut and reproductive organs, are distributed into

the appendages themselves, and the absence of gills is associated with an usual mode of respiration: peristaltic contractions of the gut (150).

Comparable, but less drastic, modifications of the antero-posterior axis occur in other groups. Acariform mites are notable in having lost segmentation posterior to the second opisthosomal segment, a modification associated with gene loss (13, 17, 60). A further derivation is found in the four-legged gall mites of the superfamily Eriophyoidea, which have additionally lost the third and fourth walking legs (25). The opposite trend occurs in other groups, like scorpions. The characteristic tail of a scorpion represents a subdivision of the opisthosoma into two tagmata (the abdomen-like mesosoma and the tail-like metasoma) and consists of five segments and a telson that houses paired venom glands (128). Other lineages still have heavily modified individual walking legs for reproduction or sensory functions. As an extreme case, Amblypygi (whip spiders) have a pair of gracile, elongate antenniform appendages that are used for sensing the environment, rather than for walking; these eight-legged arachnids are functionally hexapodous (23).

Beyond modified segments and appendages of the bauplan, chelicerates also harbor a swath of evolutionary novelties. The most compelling examples are the webs of spiders, which are composed of silk and serve an array of functions (137, 139). The silk of spiders is biomechanically remarkable, with different types of silks used for different functions and produced by glands housed in opisthosomal appendage-like structures called spinnerets (65). At the molecular level, large and internally repetitive fiber-forming structural proteins called spidroins mostly comprise the fibers; other nonspidroin genes are separately involved in silk fiber and glue production (24, 26, 27). Some spiders can produce up to seven different, task-specific fibers and adhesives, each produced by a different spigot (silk gland); each of these fibers has a unique set of biomechanical properties (adhesiveness, tensile strength, etc.). Dragline silk produced by the major ampullate glands of orb weavers has tensile strength comparable to steel, whereas silk produced by flagelliform glands can stretch up to 300% (102, 139). Spider silk has inspired many attempts at synthesis in vitro, as well as synthesis in other transgenic animals (90, 136). However, it is not a trait restricted to spiders, as silk has evolved three times in chelicerates. Tetranychoid mites (e.g., spider mites, a group of notorious agricultural and horticultural pests) produce a simpler form of silk from labial glands that is composed of fibroins and comprises the thinnest known silk fibers produced by any arthropod (4, 85). The silk of pseudoscorpions is produced by cheliceral glands and is typically used for reproduction and overwintering but remains poorly studied (68). Comparable evolutionary dynamics occur in the evolution of chelicerate venom, which has evolved independently in four lineages: spiders, scorpions, Iocheirata pseudoscorpions, and ticks (22, 64, 114). Venom is a complex cocktail that serves an array of functions spanning prey capture; predator deterrence; and, in some groups, reducing the risk of antagonistic interaction during reproduction (62, 64, 76, 112).

Chelicerates constitute the sister group to the remaining Arthropoda, which are collectively termed Mandibulata (106). The significance of this phylogenetic placement is threefold. First, inferring characteristics ancestral to all arthropods requires a sampling of extant lineages spanning the basal split of the phylum. For this reason, chelicerate exemplars feature prominently in comparative evolutionary analyses of genomics and developmental processes (31, 67, 87, 138, 141). Second, chelicerates exhibit a remarkable array of traits that have evolved convergently across the arthropod tree of life, making them potentially powerful models for understanding the developmental and genomic bases of convergent evolution. Examples of these traits include the aforementioned appendages dedicated to sensory function, acute vision, Malpighian tubules (excretory organs associated with the hindgut in terrestrial arthropods), and tubular tracheae (paired respiratory organs that open as segmentally iterated spiracles). Third, a subset of chelicerates has undergone ancient terrestrialization, resulting in successful conquest of a broad range of habitats

and ecosystems and conferring high potential for diversification in terrestrial lineages in comparison to marine counterparts. As with mandibulates, this episode of terrestrialization is thought to be ancient, as substantiated by crown-group fossils from the Devonian through the Carboniferous (122). However, as a further parallel to mandibulates, the history of chelicerate terrestrialization is complicated, with older hypotheses of a single terrestrialization event being gradually overturned by molecular phylogenetics.

Investigating these macroevolutionary phenomena requires a clear understanding of higher-level chelicerate phylogeny and the relationships within its constituent taxa, which have changed radically since a similar review of the group was published 21 years ago (29). The intention of this review is to examine transformative changes in the understanding of chelicerate phylogeny over the past decade and pinpoint challenging parts of the tree of life that compel new analytical approaches and novel data classes for resolution. We additionally highlight new investigations and areas of inquiry that are made possible by the advent of modern functional tools for emerging model systems.

THE NEW PHYLOGENY OF THE CHELICERATES

Pycnogonida: The Sister Group to the Remaining Chelicerates

The presently understood composition of Chelicerata is the product of molecular phylogenetics, with emphasis on expansion in the number and types of loci used for inferring deep relationships. Historically, chelicerates were understood to comprise arachnids, horseshoe crabs, and several extinct orders like Eurypterida (sea scorpions, which include some of the largest arthropod body fossils), Chasmataspidida (aquatic), Trigonotarbida (terrestrial), and Haptopoda (terrestrial). Uncertainty surrounded the exact placement of Pycnogonida (sea spiders), which were regarded as either the sister group to the remaining chelicerates or the sister group to all remaining arthropods (56). Early efforts to infer chelicerate relationships using morphological data typically excluded sea spiders due to their numerous derived traits, as well as the loss of many characteristics typical of marine arthropods (e.g., faceted eyes, gills, posterior segments) (132, 147). A generation of Sanger-based phylogenies also struggled to resolve sea spider placement owing to limitations in the number of informative sites and conflict between molecular and morphological partitions (57, 149). Confidence in the placement of sea spiders as the sister group of the remaining Chelicerata was achieved with the first arthropod phylogenies that exceeded 50 loci, coincident with the arrival of early short-read sequencing technology (89, 101, 105). Subsequent works with increased sampling of genes and taxa have strongly reinforced this result (**Figure 1*c***).

The internal relationships among sea spiders were previously equally challenging for a generation of Sanger-based approaches. Sea spiders are understood to be an ancient group, with crown-group body fossils appearing in the Silurian and questionable sea spider fossils in the Ordovician (107, 135). A putative larval sea spider was described from the Late Cambrian, but the interpretation of this fossil is dubious, as it lacks the characteristics associated with the archetypal four-segmented protonymphon larva (145). The gut anatomy of sea spiders (extending into all but the two distal-most segments of the legs) also made on-target Sanger sequencing with degenerate primers troublesome owing to the risk of amplifying DNA in gut contents. The combination of ancient divergences and gut content contamination may have contributed to low signal and incongruent results in Sanger-based phylogenies of sea spiders (3, 6).

This impasse was recently broken by the application of sea spider–specific target capture sequencing techniques. The first phylogenomic work spanning all sea spider families was based on a combination of mitochondrial genes, nuclear loci, and ultraconserved elements and resolved the small-bodied Austrodecidae as the sister group to the remaining sea spiders. For the first time,

almost all interfamilial relationships were resolved with high support (10). This phylogeny enabled polarization of various macroevolutionary phenomena, such as the evolution of eyes and developmental modes (20, 21). The placements of austrodecids and another miniaturized family (Rhynchothoracidae) in this study were subsequently questioned by a mitogenomic data set, which recovered these families as derived within the larger family Ammotheidae (151). However, this work in turn was suggested to have misidentified exemplars of both miniaturized families by a third study that was based on two loci (the mitogenome and 18S ribosomal RNA); this third study otherwise largely corroborated the phylogenomic study's interfamilial relationships, albeit with incomplete sampling of the families (108). Part of the disagreement between the latter two studies may have to do with the limitations of the mitogenome, a single linked locus that may lack the power to resolve deep phylogenetic relationships, especially in cases where subsets of taxa exhibit markedly dissimilar rates of evolution (134, 140). Recent progress in robust resolution of sea spider diversification has been driven by the development of genomic tools with large numbers of unlinked loci for phylogenetic inference (38, 39).

Is Arachnida Monophyletic?

The greatest upheaval in chelicerate phylogeny centers on the phylogenetic placement of horseshoe crabs. Traditionally, it was held that horseshoe crabs were the sister group to a monophyletic Arachnida (a clade of terrestrial chelicerate orders), reflecting the century-old interpretation that the arachnid radiation represented a single colonization of land by a terrestrial ancestor (132, 133, 147). This interpretation was based on the detailed fossil record of horseshoe crabs, which appear earlier than arachnid fossils, as well as the concentration of numerous plesiomorphic (ancestral) traits in horseshoe crabs, such as faceted eyes, gills, and swimming appendages (133). A notable feature of horseshoe crabs is the book gill, a lamellate, paired external respiratory organ that is thought to represent the exopod (external limb ramus) of an opisthosomal appendage. Book gills also occur in aquatic fossil orders like Eurypterida and have recently been proposed to bear support structures that facilitated subaerial respiration, as in living horseshoe crabs (41, 81). The significance of these organs in the context of terrestrialization is that they closely resemble the book lung, an internalized respiratory organ found in five orders of arachnids (scorpions and Tetrapulmonata) (116). The close similarity in architecture, function, and microanatomy of these organs prompted an elegant, stepwise scenario for the colonization of land, wherein the book gills of a marine or subaerial ancestor became internalized to form a book lung in a scorpion-like ancestor, which then diversified into the remaining arachnid orders (41, 133). The book lung was then secondarily lost or transformed into tubular tracheae in smaller-bodied arachnids, as exemplified by miniaturized spiders and the posterior pair of book lungs in Schizomida (42, 95). Consistent with this interpretation, the fossil record of Paleozoic scorpions and sea scorpions postdates the oldest horseshoe crab fossils (41, 53, 144). This scenario of terrestrialization was reinforced by a series of morphological cladistic analyses that recovered Arachnida as monophyletic, typically with scorpions branching off at the base of the arachnids, either as a single branch or with putatively related orders (16, 53, 80, 133, 147). Over time, this paradigm became entrenched in the literature and seldom challenged (40).

By contrast to paleontological and morphological data, molecular phylogenetics consistently struggled to recover arachnid monophyly, with early works recurrently obtaining horseshoe crabs nested inside the arachnids (19, 57, 101, 149). This result was long dismissed as an artifact of insufficient genes or taxa, and of exclusion of morphological partitions in total evidence analyses. The first phylogenomic analysis of chelicerates, constructed with short-read sequencing of messenger RNA (mRNA), recovered the nonmonophyly of arachnids in most analyses, regardless of filtering matrices by data completeness and information content (127). The exceptions were two matrices

built by concatenating subsets of slowly evolving genes, which could recover arachnid monophyly with maximal nodal support; smaller matrices with even slower partitions and larger matrices with more noisy partitions rapidly lost the ability to recover this result. While initially suggestive of a strong phylogenetic signal in slowly evolving genes, this work showed that the same dynamics of localized peaks of support could be found for mutually exclusive nodes as well, indicating that the ability to recover maximal support by tweaking criteria for evolutionary rate is no guarantor of phylogenetic accuracy.

This study was followed by a protracted debate about the significance of recovering arachnid monophyly using subsets of genes and whether morphological hypotheses should be used as litmus tests for phylogenetic accuracy in molecular studies. One school of thought regarded the recovery of arachnid monophyly as a reliable benchmark for molecular phylogenetic methods and, thus, focused on post hoc identification of desirable qualities of the methods used to recover Arachnida (66, 83, 84). Another school of thought contended that phylogenomic studies should remain agnostic of morphological hypotheses, as the goal of phylogenetics should be to test historical relationships, rather than to seek support for them (9, 11, 95). The latter group also questioned the dogma of morphological literature, highlighting cases of extensive morphological convergence in terrestrial arthropod groups and a lack of compelling evidence for arachnid monophyly in the morphological data (125). The most recent contribution to this debate was the publication of a large-scale study spanning over 500 genomes and transcriptomes and sampling all extant chelicerate orders (8). This work applied complex models of substitution processes, as well as a morphological data set of over 500 species (including fossil orders), for total evidence analysis and showed that there was no support for arachnid monophyly (**Figure 1*c***). Other molecular phylogenetic works published in the same interval with nonoverlapping sets of genes and taxa corroborated this result (12, 19, 93). Among the arguments for arachnid nonmonophyly was the observation that adding unsampled orders to data sets that had previously recovered arachnid monophyly would cause Arachnida to collapse (11, 95, 125).

The placement of aquatic groups within the arachnids is challenging to reconcile with morphological and paleontological data sets and suggests a more complex history of terrestrialization than previously thought (98, 122). This history closely parallels the case of mandibulate phylogeny, wherein the terrestrial groups Hexapoda and Myriapoda were once considered sister groups that diverged from a crustacean-like ancestor (56, 57, 100), based on uncanny morphological similarities. Also paralleling mandibulate phylogeny, the overturning of the Arachnida paradigm by molecular phylogenetics appears to be gaining gradual acceptance in the field, concomitant with the recognition that terrestrialization is a driver of morphological convergence across an array of terrestrial invertebrate groups (37, 142, 146). Most recently, paleontologists have begun to realign the interpretation of the fossil record with the new chelicerate phylogeny, emphasizing the misleading effects of large temporal gaps in the fossils of many terrestrial groups, as well as sea spiders (54, 55).

Arachnopulmonata and Rare Genomic Changes

The recovery and present composition of Arachnopulmonata constitute one of the success stories of phylogenomics (**Figure 2**). Contrary to the traditional placement of scorpions at the base of a monophyletic Arachnida, early phylogenomic works repeatedly recovered scorpions as the sister group to Tetrapulmonata (19, 101, 127). This surprising result suggested that the book lung is a derived feature of arachnids, not an ancestral one. Arachnopulmonata was cemented by the discovery of a shared whole-genome duplication in the common ancestor of spiders and scorpions, facilitated by the publication of high-quality genomes of both groups (119), as well as developmental genetic data that revealed shared suites of duplicated patterning genes in the embryos of

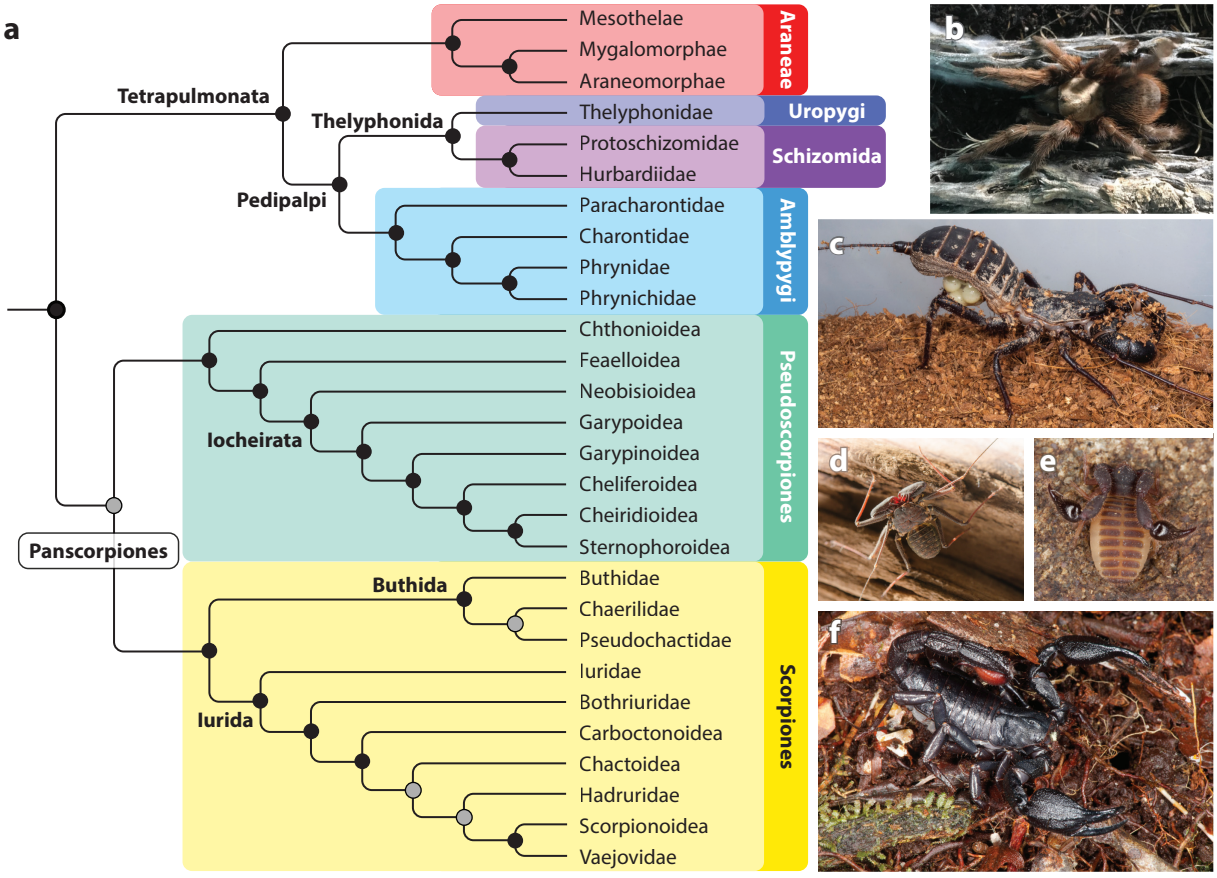

Figure 2

(*a*) Summary tree of Arachnopulmonata, indicating higher-level relationships of families, superfamilies, or suborders, based on References 8, 14, 28, 33, and 110. Solid circles on nodes indicate well-resolved relationships; gray circles indicate moderately or poorly supported nodes. (*b*) *Aphonopelma hentzi* (Araneae, Mygalomorphae). Photo by E.V.W. Setton. (*c*) Brooding female of *Mastigoproctus giganteus* (Uropygi). Photo by B.C. Klementz. (*d*) *Phrynus marginemaculatus* (Amblypygi). Photo by J.A. Ballesteros. (*e*) Chernetidae gen. sp. (Pseudoscorpiones, Cheliferoidea). Photo by G. Giribet. (*f*) *Brotheas granulatus* (Scorpiones, Chactoidea). Photo by G. Giribet.

spiders, whip spiders, and scorpions (1, 52, 63, 82, 128). The advantage of a whole-genome duplication is that such a systemic duplication event leaves a broad and testable signature across gene sequences, gene trees, microRNAs, expression patterns, and gene order (i.e., synteny), the last of which can be visualized readily using tests for self-synteny as new chromosomal-level resources become available for chelicerates (45, 91, 119, 124). These tools have been used to show that blocks of duplicated genes are broadly distributed across spider chromosomes, which is most parsimoniously explained by a whole-genome duplication event (45, 91). By contrast, an unduplicated genome occurs in apulmonate taxa, such as acariform mites, ticks, and daddy-longlegs, a condition shared with outgroups like insects and myriapods (48, 60, 61). Intriguingly, horseshoe crabs have undergone three rounds of whole-genome duplication, but these events are much more recent that the arachnopulmonate event, which predates the Silurian (94, 131).

The whole-genome duplication exhibited by arachnopulmonates has proven an invaluable vehicle for hypothesis testing. Pseudoscorpions and Solifugae have long been unstable orders in

arachnid phylogeny; they were classically regarded as sister groups based on an array of shared morphological traits but are never recovered together by molecular data sets. Instead, pseudoscorpions have often been placed within, or as a sister group to, Arachnopulmonata, particularly in analyses that densely sampled the fast-evolving pseudoscorpions (the sampling of deep splits in the order has the effect of breaking long branches, which stabilizes phylogenetic placement of fast-evolving taxa) (95). The first genomic resources for pseudoscorpions showed that they also share the whole-genome duplication found in arachnopulmonates, substantiating both their placement in this lineage and the utility of dense taxonomic sampling as a strategy for mitigating phylogenetic instability (95). The redefinition of Arachnopulmonata to include pseudoscorpions suggests that its newest member has secondarily lost the book lungs, a condition found in other small-bodied arachnopulmonates (42). Inversely, the first genomic resources for Solifugae have shown that they do not exhibit any evidence of genome duplication, suggesting that several traits shared by solifuges and pseudoscorpions represent yet more cases of morphological convergence (51).

Charismatic taxa within Arachnopulmonata have compelled significant phylogenetic attention. Spider molecular phylogeny has been scrutinized intensively, with an updated understanding of internal relationships recently reviewed elsewhere (72, 77, 148). The internal relationships of Schizomida, Uropygi, and Amblypygi have been explored through a handful of studies (28, 32, 33). The higher-level phylogeny of pseudoscorpions is well understood, with all superfamilies represented by multiple transcriptomes (14, 92). The past 10 years have also seen revitalized interest in scorpions, with the first higher-level phylogenies dramatically reorganizing familial and superfamilial relationships (111, 113, 126). A recent work sampling the venom gland transcriptomes and genomes of 100 scorpion species revealed that diversification of mammal-specific scorpion toxins occurred relatively recently, coinciding with the diversification of mammal groups that prey on scorpions. The contemporaneous diversification suggests that recent diversification of predators may have driven the cooption of toxins specialized for prey capture to serve the function of predator deterrence (110).

Outstanding Questions in the Arachnid Tree of Life

Relationships among the remaining orders of the chelicerate tree of life remain stubbornly unresolved (**Figure 3**). Among the least stable of these orders are Acariformes (mites) and Parasitiformes (mites and ticks). The recovery of this pair as sister groups (forming Acari) is consistent with similar adaptations of mouthparts in the two groups but is controversial from the perspective of molecular data, with matrices emphasizing intensive sampling failing to recover Acari (8, 83, 95). Notably, the addition of basally branching groups of these orders (e.g., Opilioacaridae, the putative sister group of Parasitiformes) to phylogenomic analyses tends to precipitate the breakup of Acari, suggesting that recovery of Acari in molecular data sets is an artifact of undersampling (96).

Other unstable groups in this part of the tree include Palpigradi and Solifugae. Notably, recent phylogenomic efforts using site-heterogeneous models have recovered palpigrades and solifuges in a clade with Acariformes, forming Cephalosomata (8); this trio of orders is united by a subdivided prosoma and was initially proposed based on morphology and analyses of two ribosomal genes (97). Within Cephalosomata, a counterintuitive sister group relationship of the large-bodied Solifugae and the miniaturized Acariformes (Poecilophysidea) is also supported by a morphological character (a characteristic furrow on the ventral prosoma), analysis of ribosomal genes, and phylogenomic analyses using site-heterogeneous models (8, 43, 97).

The remaining unstable orders in the soft polytomy at the base of the arachnid tree are Xiphosura, Opiliones, and Ricinulei. Some phylogenomic analyses have recovered Xiphosura and

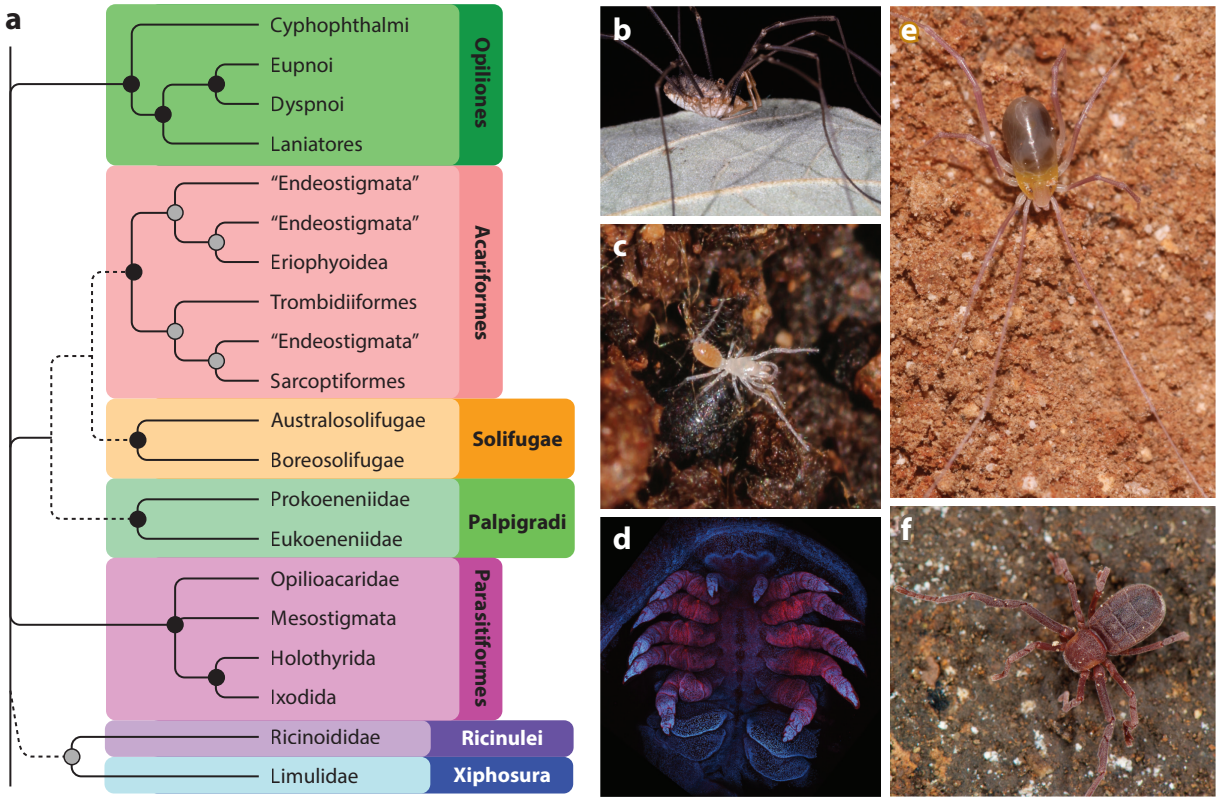

Figure 3

(*a*) Summary tree of orders forming a soft polytomy at the base of the arachnids, based on References 8, 35, 58, 74, 75, and 78. Dotted lines reflect unstable relationships. Solid circles on nodes indicate well-resolved relationships; gray circles indicate moderately or poorly supported nodes. (*b*) *Phalangium opilio* (Opiliones, Eupnoi). Photo by C.M. Baker. (*c*) *Eukoenenia* n. sp. (Palpigradi, Eukoeneniidae). Photo by S. Aharon. (*d*) Fluorescent expression of a paralog of the Hox gene *Deformed* in an embryo of *Limulus polyphemus* (Xiphosura). Photo by G. Gainett. (*e*) *Adenacarus* n. sp. (Parasitiformes, Opilioacaridae). Photo by J.A. Ballesteros. (*f*) *Pseudocellus pearsei* (Ricinulei). Photo by G. Giribet.

Ricinulei as a clade, with this group in turn being a sister group to Arachnopulmonata (8, 11, 83, 127). This clique of taxa is putatively united by the presence of a chelate palp (secondarily reduced or lost in Tetrapulmonata) but little else. Ricinulei share a condition of sperm ultrastructure (a flagellum coiled around the sperm body) with several orders of Arachnopulmonata and also superficially resemble the extinct tetrapulmonate order Trigonotarbida (95). The absence of a genome for Ricinulei precludes a direct assessment of affinity with arachnopulmonates via assaying for the signature of whole-genome duplication. By contrast, little inkling of phylogenetic placement is available for Opiliones, which clearly lack a whole-genome duplication and exhibit the aflagellate sperm condition found in solifuges, palpigrades, acariforms, and parasitiforms (48). However, a recent work on Opiliones neuroanatomy and functional genetics has shown that extant daddy-longlegs retain vestigial lateral eyes as adults, as well as a pair of rudimentary median eyes. The latter is significant because a second pair of median eyes is only known to occur in sea spiders and in embryos of horseshoe crabs (these fuse into a single eye by adulthood) and is entirely lost in groups like spiders and scorpions. This discovery suggests that the neuroanatomy of Opiliones

may be more plesiomorphic than that of arachnopulmonates and may represent a transitional state in chelicerate eye evolution (50).

In contrast to the interordinal relationships, our understanding of the internal phylogeny of these orders has progressed well in the past decade. Opiliones have long served as models for historical biogeography, and nearly complete family-level phylogenies are available for this group (35, 47, 59). Similarly, there has been remarkable progress in the understanding of the internal relationships in Ricinulei and Solifugae, with the first higher-level classification and a handful of new families recently proposed for the latter (46, 78, 79, 115). The least progress has been made in Palpigradi, which is limited to a single study based on three loci; the paucity of phylogenetic efforts for this group stems from the difficulty of collecting and identifying individuals of this miniaturized order (58).

The most challenging groups with regard to internal phylogeny are Acariformes and Parasitiformes, whose biodiversity and small body size pose inherent hurdles to intensive sampling. Recent works have begun to establish higher-level relationships in Acariformes, which overturn many of the classical groupings used by acarologists, such as the reciprocal monophyly of Sarcoptiformes and Trombidiiformes (29, 30, 74). Recent efforts prioritizing taxonomic sampling have proposed that Astigmata is nested within a paraphyletic Oribatida. A polyphyletic assemblage termed "Endeostigmata" spans the base of acariform phylogeny and includes several families that will prove critical for future sampling efforts to break long branches and stabilize the position of Acariformes (74). Trombidiiformes and Eriophyoidea are understood to be monophyletic (18). Nodal support for the interrelationships of these biodiverse groups is generally modest in deeper parts of the tree, partly due to the limitations of Sanger data sets and the lack of genome-scale matrices informing acariform higher-level phylogeny. Parasitiformes is somewhat better resolved, with early Sanger-based efforts recovering strong support for the order and its four constituent lineages (Opilioacariformes, Mesostigmata, Holothyrida, and Ixodida), although the placement of the fast-evolving Mesostigmata within Parasitiformes was subsequently shown to be unstable (75, 96).

Resolution of this part of the tree will likely require new phylogenetic data classes and analytical approaches. Promisingly, the influx of high-quality genomes has greatly facilitated phylogenetic resolution in other groups, such as through the discovery of rare genomic changes. Beyond genome duplications, other potential rare genomic changes awaiting discovery could include shared position of transposable elements within genes, shared tandem (segmental) duplications of blocks of genes, and shared patterns of chromosomal rearrangements. The last of these has yielded remarkable progress in resolving recalcitrant parts of the animal tree of life. Specifically, the discovery of several events of irreversible chromosomal fusion and mixing of linkage groups across animals, but to the exclusion of comb jellies (Ctenophora) and unicellular outgroups, was used to substantiate the phylogenetic placement of ctenophores as the sister group to all remaining animals, bookending 15 years of ongoing phylogenetic dispute and dueling data sets (118). The use of new analytical frameworks for the discovery of phylogenetic signals within complete genomes represents the next logical step for the resolution of higher-level chelicerate relationships.

CHELICERATES IN EVOLUTIONARY DEVELOPMENTAL BIOLOGY

Given the aforementioned issues of phylogenetic placement within their phylum, chelicerates have a long history of relevance to the evolutionary developmental biology of Arthropoda. The incidence of whole-genome duplications in the chelicerate tree of life makes this group well poised to address new questions at the fore of comparative developmental biology, namely, the role of duplicated genes in macroevolution. Whole-genome duplications represent a phenomenon where the

sudden availability of new genes can create the potential for the evolution of new gene functions and, thus, new structures. In spiders and scorpions, the duplicates of Hox genes are well known to exhibit different expression patterns and suggestive correlations with shifts in segmental identity; it has been postulated that new Hox gene copies may constitute the underlying mechanism for the evolution of novel traits of arachnopulmonates, such as the spinnerets of spiders and the pectines of scorpions (119, 124, 128). Other bioinformatic and gene expression surveys have shown widespread retention of ohnologs (gene copies resulting from whole-genome duplication) across Arachnopulmonata, as well as divergence in the expression in ohnologs of numerous transcription factors (82, 117, 143). The recent introduction of single-cell sequencing data sets has brought a new degree of precision to the characterization of gene expression, although such data sets are presently limited to spiders (2, 70, 88).

The divergence of expression patterns in pairs of paralogous genes is commonly held to reflect subfunctionalization (the subdivision of a gene's ancestral function by two daughter copies) or neofunctionalization (the acquisition of a new function by at least one daughter copy) (69, 82, 119). These processes are generally inferred on the basis of polarizing expression patterns, using data from outgroup lineages with unduplicated genomes (1, 69, 82, 124). Polarizing expression patterns of duplicated genes within arachnids are often complicated by the paucity of data sets from key groups, such as acariform mites, scorpions, and horseshoe crabs (13, 50, 63, 120). The proliferation of techniques for multiplexed, fluorescent detection of mRNAs has facilitated more precise detection of gene expression patterns in some of these challenging taxa (49). Neofunctionalization of ancient gene copies has been invoked to explain several traits of arachnids, such as the origins of leg segments and the specialization of eyes (117, 143). However, an alternative interpretation of these divergent expression patterns is developmental system drift, wherein identical homologous structures are produced despite the divergence of the underlying genetic mechanisms (**Figure 4**). It has been shown that one duplicate of a gene pair, when released from selection, often exhibits greater plasticity than its sister gene copy in its expression domain, but this lability can precede drift and eventual gene loss over time rather than reflect adaptive changes in gene function (71).

Our ability to distinguish between the competing hypotheses of neofunctionalization and developmental system drift using arachnid models is in its infancy. The glaring lack of functional data sets for many duplicated spider genes imposes a substantial impediment to testing the role of gene duplication in shaping the history of arachnopulmonate evolution (124). This gap in experimental data may be partly attributable to the limitations of available functional tools in spiders, which are currently restricted to maternal and embryonic RNA interference (124). A separate possibility is that knockdown of one duplicate induces compensatory upregulation of the other, resulting in a rescue effect, although few data sets have sought evidence of compensatory functions in duplicated spider genes. For this reason, many gaps in the understanding of chelicerate development have been bridged by arachnid models with unduplicated genomes. In particular, functional data sets in a model harvestman have proved essential for understanding the role of single-copy genes in arachnid appendage and eye evolution (50, 129, 130).

Among the most promising avenues for investigation in functional genomics of chelicerates is the advent of gene editing tools in acariform mite and tick models. It was challenging to translate traditional methods of CRISPR-Cas9-mediated gene editing in insects to arachnid models owing to peculiarities of arachnid development, such as the first cell cleavages occurring deep inside the egg and cellularization of micromeres by the 16-cell stage (73). These limitations and technical challenges have prevented gene editing via microinjection at the one-cell stage. Recently, multiple laboratories have begun optimizing methods for delivery of Cas9 and guide RNAs for gene knockout and knockin via maternal injection into the hemolymph of females during vitellogenesis. Molecular cargo is transported from the hemolymph into the developing oocytes, priming

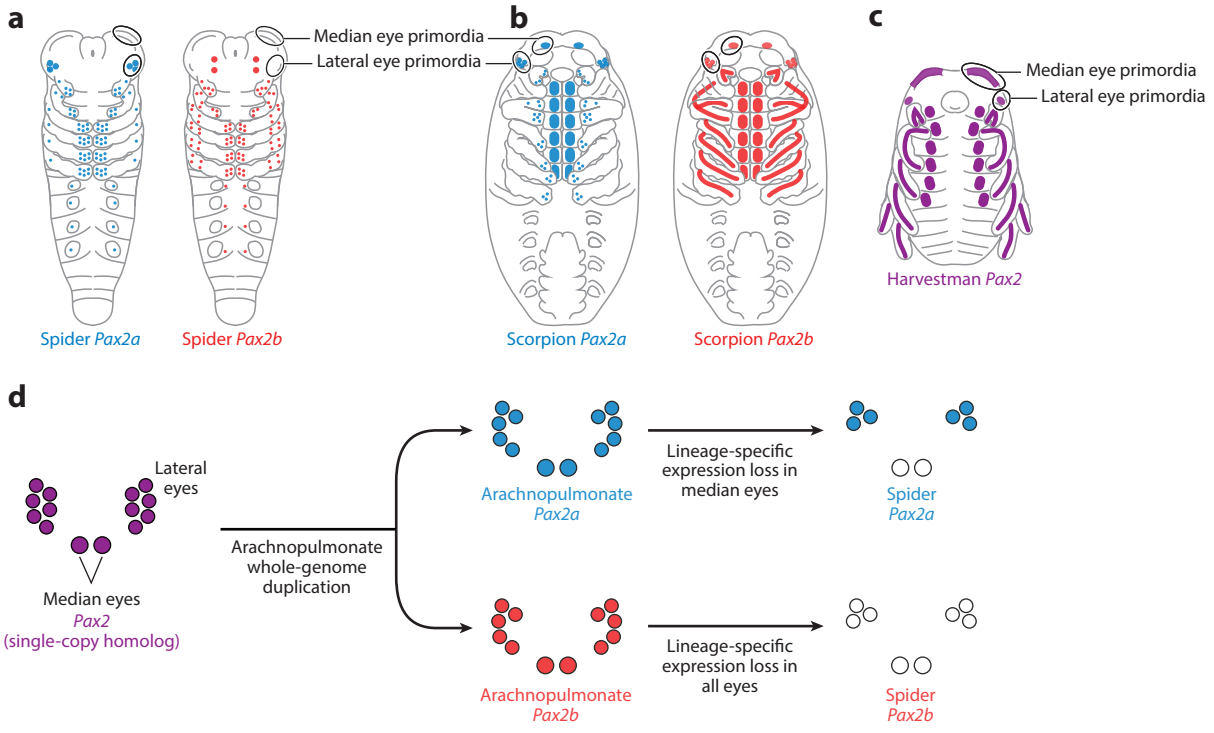

Figure 4

A case study of developmental system drift in arachnid embryogenesis, highlighting the importance of broad taxonomic sampling. (*a*) Simplified schematics of expression of *Pax2* duplicates in spiders. Early localization of *Pax2a* transcripts in the lateral eye (but not the median eye) primordia of spider embryos was initially held to reflect a prominent role for *Pax2a* in patterning chelicerate lateral eyes (69). (*b*) Simplified schematics of expression of *Pax2* duplicates in embryos of the bark scorpion *Centruroides sculpturatus*. These subsequent surveys showed that both *Pax2a* and *Pax2b* copies are expressed in both the median and lateral eye primordia of scorpions (50). (*c*) Simplified schematics of expression of the *Pax2* single-copy homolog in embryos of the harvestman *Phalangium opilio* (50). The expression of *Pax2* in both median and lateral eyes closely parallels the expression of both *Pax2* duplicates in the scorpion. (*d*) Evolutionary reconstruction of *Pax2* dynamics in chelicerates. A single-copy *Pax2* homolog is inferred to have retained expression in both eye types of nonarachnopulmonates, as well as in some arachnopulmonates after gene duplication (e.g., scorpions). The expression domains of *Pax2a* and *Pax2b* observed in spider eyes suggest lineage-specific dynamics, rather than a chelicerate-wide mechanism specific to lateral eye patterning. The exact timing of the divergence of gene expression (i.e., spider specific versus tetrapulmonate specific) is presently unknown.

them for gene editing. CRISPR-mediated gene editing has been implemented in both the tick *Ixodes scapularis* and the acariform mite *Tetranychus urticae*, with subsequent optimization of gene editing efficiency using different reagents (34, 36, 121). The application of these techniques to arachnopulmonate models may facilitate precise interrogations of gene function, with an emphasis on understanding to what degree duplicated genes have shaped the evolutionary history of groups like spiders and scorpions.

CONCLUSION

The publication of this review coincides with the twenty-first anniversary of a similar synthesis on chelicerate evolutionary biology (29). A comparison of tree topologies, available genomic resources, and the power of functional tools between then and now underscores the dramatic rate of progress in this discipline. Recent and radical shifts in phylogenetic paradigms are today

accompanied by new avenues of investigation using chromosomal-level genomes, single-cell resolution of gene expression, and advanced tools for testing hypotheses related to genetic mechanisms.

SUMMARY POINTS

1. Sea spiders are strongly supported as the sister group of Chelicerata. Horseshoe crabs are nested within the arachnids, suggesting a more complex history of terrestrialization than was previously thought.
2. The most stable part of the chelicerate tree of life is Arachnopulmonata, a group of six arachnid orders united by a shared genome duplication. Studies of the relationships among other orders are deeply conflicted, but future studies may benefit from the use of chromosomal-level genomes as sources of rare genomic changes.
3. Substantial progress has been made on the higher-level relationships within every chelicerate order, with molecular phylogenies now available for all groups.
4. The role of whole-genome duplication in arachnopulmonate evolution is not fully understood. The establishment of the first gene editing tools for chelicerate models is anticipated to improve our understanding of the functional dynamics of duplicated gene pairs.

DISCLOSURE STATEMENT

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