Evolutionary Biology of Harvestmen (Arachnida, Opiliones)

Gonzalo Giribet[1,*] and Prashant P. Sharma[2]

[1]Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138; email: ggiribet@g.harvard.edu

[2]Division of Invertebrate Zoology, American Museum of Natural History, New York, NY 10024; email: psharma@amnh.org

*Corresponding author

Abstract

Opiliones are one of the largest arachnid orders, with more than 6,500 species in 50 families. Many of these families have been erected or reorganized in the last few years since the publication of *The Biology of Opiliones*. Recent years have also seen an explosion in phylogenetic work on Opiliones, as well as in studies using Opiliones as test cases to address biogeographic and evolutionary questions more broadly. Accelerated activity in the study of Opiliones evolution has been facilitated by the discovery of several key fossils, including the oldest known Opiliones fossil, which represents a new, extinct suborder. Study of the group's biology has also benefited from rapid accrual of genomic resources, particularly with respect to transcriptomes and functional genetic tools. The rapid emergence and utility of *Phalangium opilio* as a model for evolutionary developmental biology of arthropods serve as demonstrative evidence of a new area of study in Opiliones biology, made possible through transcriptomic data.

INTRODUCTION

Opiliones (harvestmen or daddy long-legs) are the third-largest order of arachnids, after Acari (mites and ticks) and Araneae (spiders). The order encompasses more than 6,500 described extant species in about 1,500 genera, 50 families, and four suborders (58), with an additional extinct suborder recently described (36) (**Figure 1**). Harvestmen constitute an important component of many terrestrial ecosystems, especially in the soil, where they are scavengers or predators of small arthropods and worms, but also on shrubs and trees. Several harvestman species are well known in rural areas around the world, especially in temperate and tropical climates. Fossil harvestmen first appear in the Devonian Rhynie cherts (ca. 405 Ma) (28), making Opiliones one of the oldest terrestrial animal groups. The order had already diversified by the Carboniferous (Montceau-les-Mines fossils from ca. 310 Ma) (35, 36).

Broad interest in harvestman phylogenetic relationships and biogeographic history has advanced the field of opilionology greatly. The advent of transcriptomic data for all major lineages of Opiliones has precipitated the inclusion of harvestmen as models for evolutionary developmental biology (evo-devo) (94, 95, 97), only comparable with the better-studied spiders among the arachnid orders. Some harvestman groups are also model systems for behavioral ecology, and more specifically reproductive behavior, as they exhibit both indirect and direct sperm transfer, and multiple lineages have developed parental care, with both maternal and paternal, and even biparental, investment in some species (11, 61, 62, 122, 125). They thus constitute an unparalleled group of terrestrial arthropods in terms of integrating anatomical, behavioral, biogeographic, ecological, developmental, paleontological, and phylogenetic knowledge. Indeed, much of the knowledge accumulated until 2006 was compiled in a landmark book on harvestman biology (73), which triggered further interest in Opiliones. Developments since 2006 in the areas of phylogenetics, genomics, and evo-devo are substantial and constitute the foci of this review.

SYSTEMATICS AND EVOLUTIONARY HISTORY

Opiliones and Arachnid Phylogeny

The position of Opiliones in the arachnid tree of life has been hotly debated over the last decades. The most recent cladistic analyses of morphological data suggest a sister-group relationship to Scorpiones, forming the clade Stomothecata (106). Prior morphological work had suggested a sister-group relationship to a clade composed of Scorpiones, Pseudoscorpiones, and Solifugae (41, 104, 121), constituting the taxon Dromopoda, or to a clade composed of Ricinulei and Acari (120), to mention just modern cladistic studies, but many other hypotheses have been published (reviewed in 121). Molecular sequence data for arachnids have been in play for two decades, but they have not satisfactorily resolved the position of Opiliones in the arachnid tree of life (41, 67, 121), even when using up to 62 protein-encoding genes to infer their phylogenetic position (76, 77). The few combined analyses of molecules and morphology (41, 121) largely agree with the morphological data sets they encompass.

More recently, systematists have started using expressed sequence tags (ESTs) and transcriptomes to elucidate phylogenetic relationships among animals (31), including arachnids (92). This latter study re-examined arachnid relationships based on a large data set of up to 3,644 loci and identified possible sources of conflict in resolving the arachnid tree. Among these are accelerated evolutionary rates in certain lineages (e.g., a diphyletic Acari, Pseudoscorpiones); so, when the fastest genes were excluded, a more plausible set of relationships was recovered, including a clade composed of Opiliones, Ricinulei, and Solifugae, whereas Scorpiones were the sister group to the
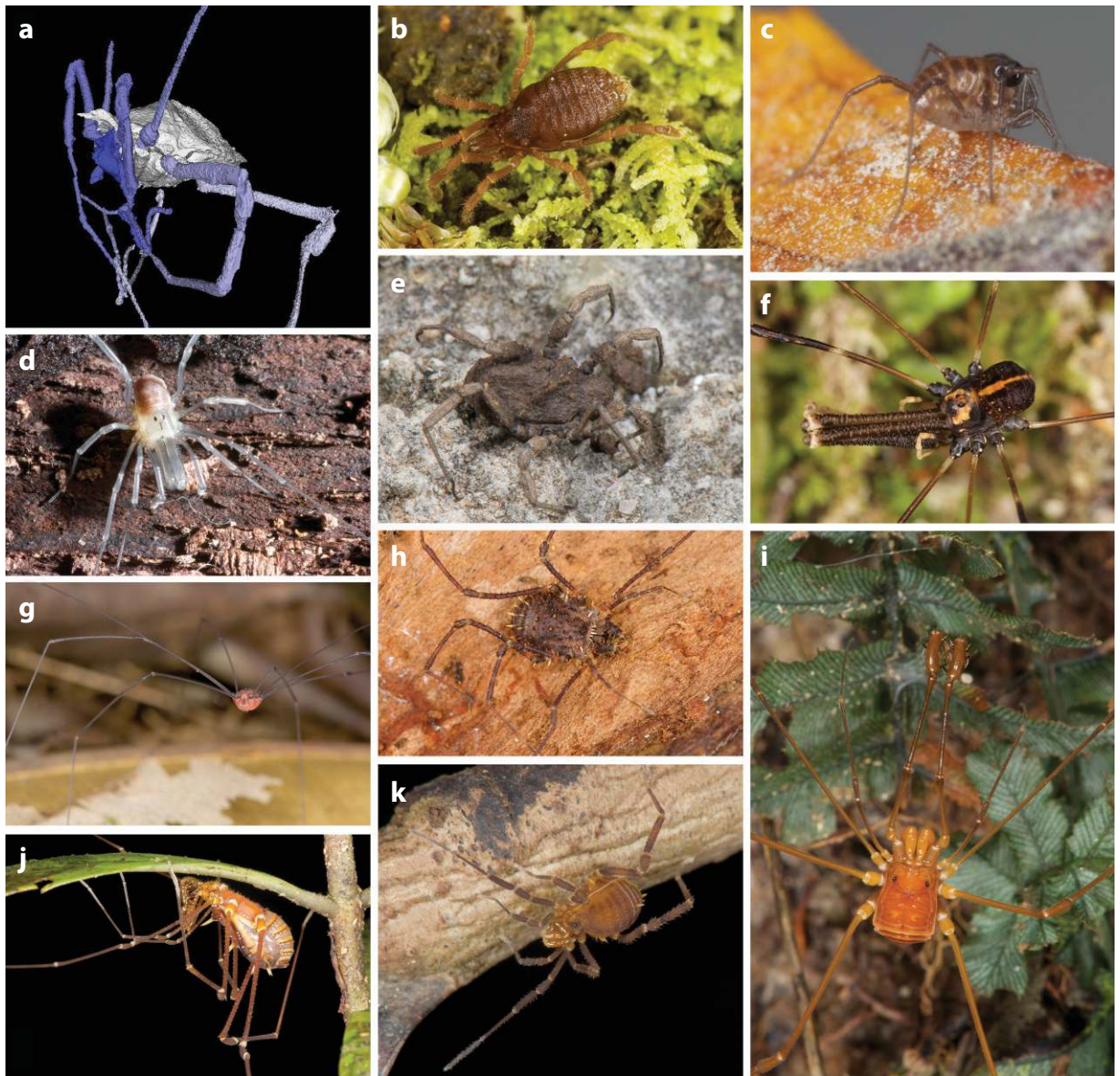
Figure 1

Exemplars of major Opiliones lineages: (*a*) 3-D reconstruction of *Hastocularis argus* (Tetrophthalmi), from the Carboniferous, (*b*) *Aoraki longitarsa* (Cyphophthalmi, Pettalidae), (*c*) *Acropsopilio neozealandiae* (Dyspnoi, Acropsopilionoidea, Acropsopilionidae), (*d*) *Taracus* sp. (Dyspnoi, Ischyropsalidoidea, Taracidae), (*e*) *Anelasmocephalus calcaneatus* (Dyspnoi, Troguloidea, Trogulidae), (*f*) *Forsteropsalis pureora* (Eupnoi, Phalangioidea, Neopilionidae), (*g*) *Caluga* n. sp. (Eupnoi, Phalangioidea, Sclerosomatidae), (*h*) *Triregia fairburni grata* (Laniatores, Triaenonychoidea, Triaenonychidae), (*i*) *Protimesius longipalpis* (Laniatores, Stygnidae), (*j*) *Phareicranaus manauara* (Laniatores, Gonyleptoidea, Gonyleptidae), and (*k*) *Fissiphallius martensi* (Laniatores, Zalmoxoidea, Fissiphalliidae).

other orders with book lungs (Tetrapulmonata, which include spiders and their allies). To our knowledge, no prior phylogeny had supported this particular position of Opiliones, and only a handful of studies proposed a similar clade, but they included Acari (124) or Acari, Palpigradi, and Pseudoscorpiones (33).

The putative clade comprising Opiliones, Ricinulei, and Solifugae is supported by the presence of highly branching tracheae, as these organisms are among the largest members of the rejected clade Apulmonata (arachnids that lack book lungs). However, this hypothesis of tracheal homology does not follow those previously suggested, and the respiratory system of Ricinulei is particularly poorly understood (33, 106). Other major characters used in arachnid phylogeny, such as the number of cheliceral segments (see discussion below), are demonstrably labile. These three orders transfer sperm or a spermatophore directly into the female's genital opening, without placing it on the ground—with leg III in Ricinulei (60), with the chelicerae in Solifugae (75), or through direct contact in the basal Opiliones suborder Cyphophthalmi (85)—but this behavior has been modified in Phalangida, which copulate. Morphological and behavioral evidence for this clade is therefore limited, and this relationship requires further testing.

Opiliones Systematics and Paleontology

Harvestman phylogeny has received broad attention in past decades (e.g., 7, 41, 43, 46, 50, 64–66, 88, 105, 107), and a consensus is forming on subordinal relationships (**Figure 2**). However, the discovery of new Carboniferous fossils has suggested the existence of a fifth and extinct suborder of Opiliones (36), and phylogenetic analysis of large molecular data sets has radically reorganized family-level relationships and composition, especially of Laniatores (72, 88, 93). Likewise, a major reorganization of Dyspnoi and Eupnoi was recently proposed (49).

The suborder Cyphophthalmi comprises three infraorders and six families whose interrelationships have been broadly studied using morphology, molecules, or both (7, 39, 43, 101). Major clades are readily recognizable in terms of anatomical synapomorphies, such as sternal opisthosomal glands defining the clade Sternophthalmi, which includes the families Troglosironidae (endemic to New Caledonia), Ogoveidae (restricted to tropical West Africa), and Neogoveidae (West Africa and the Neotropics). Internal relationships and biogeographic patterns of the constituent families have received considerable attention: Neogoveidae (5), Pettalidae (8, 9, 21, 22, 38), Sironidae (23, 44, 70), Stylocellidae (13–16), and Troglosironidae (86). Major contributions since the turn of the twenty-first century include description of 72 of the 187 known extant species (38.5%).

The suborder Eupnoi is divided into the superfamilies Caddoidea (2 species) and Phalangioidea (~1,800 species), the latter including four ill-defined families (Neopilionidae, Phalangiidae, Protolophidae, and Sclerosomatidae), plus the *Metopilio* group; all are in urgent need of revision. Caddoidea include 2 species in the family Caddidae, but until recently they also included Acropsopilionidae, mostly found in the southern hemisphere (as the subfamily Acropsopilioninae) (100) and now transferred to Dyspnoi (49). Another southern clade, *Hesperopilio*, was later added to Caddidae (102) but is now placed within Phalangioidea (49). The families and relationships within Phalangioidea have been only partially addressed in some recent studies (46, 49, 51, 54, 112), and only one study has provided a somewhat broad analysis of the superfamily, albeit focusing on Sclerosomatidae from East Asia and North America (51) and demonstrating nonmonophyly of traditional subfamilies. Phylogenetic relationships and higher taxonomy of Phalangioidea remain the least-understood aspects of Opiliones systematics.

Resolving the phylogenetic relationships of the suborder Dyspnoi has been a focus of attention in the last decade as well (46, 49, 82). A major emendation to the systematics of the suborder
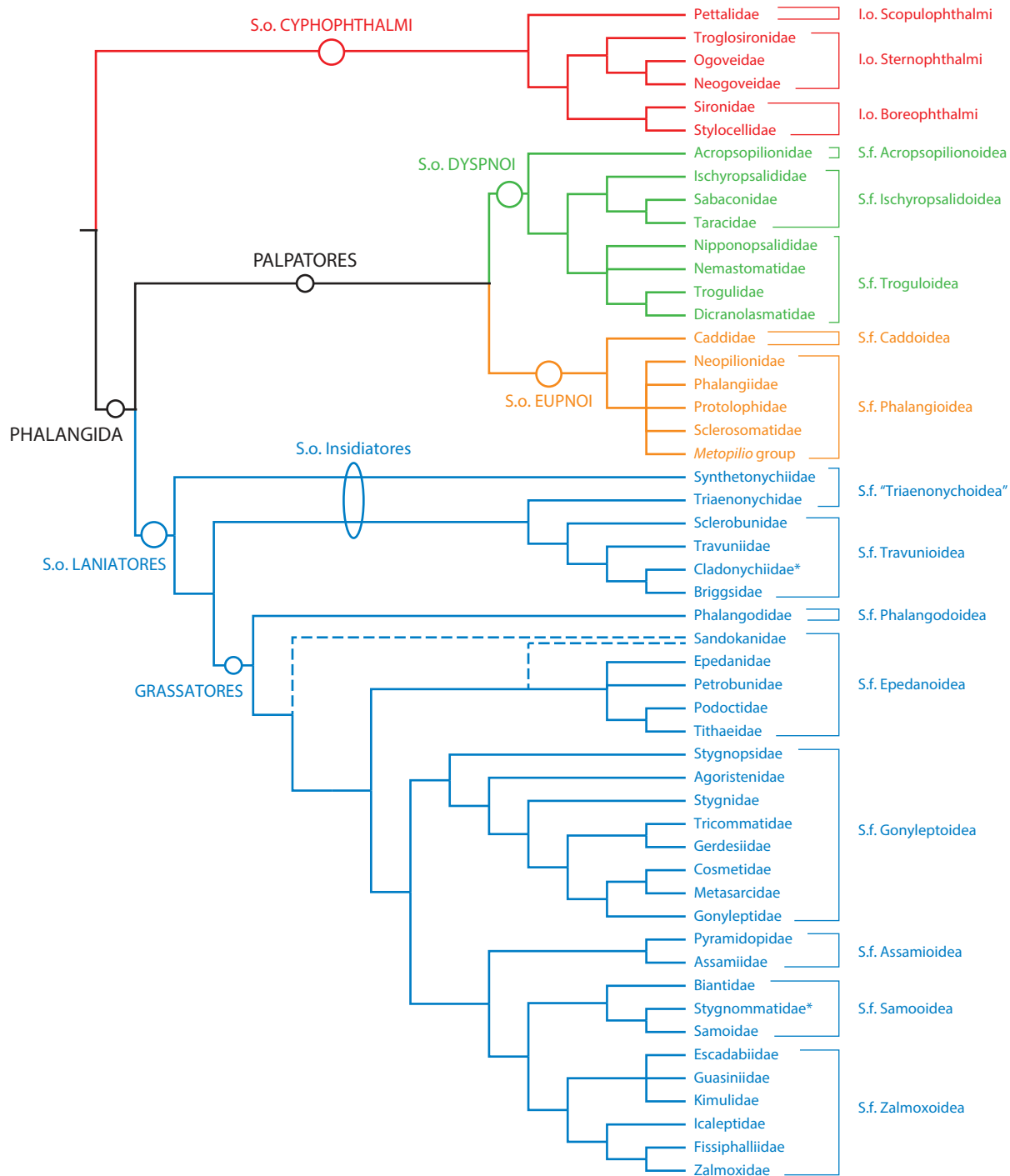
Figure 2

Summary tree of the phylogenetic relationships of the extant Opiliones families. Abbreviations: I.o., infraorder; S.f., superfamily; S.o., suborder. Unranked clades are indicated to facilitate discourse. Asterisks indicate possible paraphyletic groups.

consisted of the identification of Acropsopilionidae as the sister group to the remaining Dyspnoi (49). The suborder presently includes 305 described species in three superfamilies: Acropsopilionoidea (19 species in the single family Acropsopilionidae; 49), Ischyropsalidoidea (76 species in the families Ischyropsalididae, Sabaconidae, and Taracidae; 82), and Troguloidea (210 species in the families Dicranolasmatidae, Nemastomatidae, Nipponopsalididae, and Trogulidae; 82). Relationships within Ischyropsalidoidea are now well established despite several recent familial reassignments (46, 83). The new family Taracidae was established for *Crosbycus*, *Hesperonemastoma*, and *Taracus* in accordance with the phylogeny of these genera, whereas *Ceratolasma* was transferred to Ischyropsalididae and the family Ceratolasmatidae was dismantled (82). Within Troguloidea, the sister-group relationship of Trogulidae and Dicranolasmatidae is well established, but the relative positions of Nipponopsalididae and Nemastomatidae are less clear (46, 83, 107).

Relationships and families of Laniatores (4,138 species; 59) have changed drastically since 2007 (73), as multiple large-scale analyses of Laniatores (12, 46, 72, 88, 89) have modified our understanding of the suborder with reference to the unpublished thesis of A.B. Kury (57). Recently Kury (59) continued to divide Laniatores into two infraorders, Insidiatores (568 species; 59) and Grassatores (3,570 species; 59), the former with the superfamilies Travunioidea and Triaenonychoidea, and the latter divided into Assamioidea, Epedanoidea, Gonyleptoidea, Phalangodoidea, Samooidea, and Zalmoxoidea. However, phylogenetic analysis of molecular data suggests paraphyly of Insidiatores, as Synthetonychiidae, a family endemic to New Zealand, appeared as the sister group to all other Laniatores in two recent studies (46, 88). Similarly, Kury follows a proposal for Insidiatores from an unpublished thesis (68), including fusing Briggsidae and Cladonychiidae with Travuniidae and elevating the triaenonychid subfamilies Nipponychinae and Paranonychinae to families, transferring the latter two to Travunioidea. Little phylogenetic work has focused on Insidiatores as a whole.

Within Grassatores, the largest clade of Opiliones, the first taxonomic change since 2007 was a replacement name for Oncopodidae, now Sandokanidae (71). Additionally, phylogenetic work has resulted in other taxonomic changes. Sharma & Giribet (88) proposed the new families Petrobunidae and Tithaeidae, and Sharma et al. (93) formalized the family Pyramidopidae. Pinto-da-Rocha et al. (72) focused on the species-rich Gonyleptoidea, restoring Tricommatidae to family and providing new family status to Metasarcidae but downgrading Manaosbiidae and Cranaidae to subfamilies of Gonyleptidae. The system proposed by Kury mostly follows the phylogenetic proposal by Sharma & Giribet (88), with the exception of Zalmoxoidea and Samooidea; Kury maintains Escadabiidae and Kimulidae within Samooidea (59), although these two families appear in Zalmoxoidea, closely related to Guasiniidae, in phylogenetic analyses (88, 89). The cryptic family Sandokanidae is tentatively regarded as a member of the superfamily Epedanoidea, although its phylogenetic placement remains sensitive to algorithmic approach (46, 58, 88).

The fossil record of Opiliones is relatively rich and well preserved, with several Paleozoic representatives (27). Modern-looking members of the suborders Eupnoi and Dyspnoi are already present in the Carboniferous (35). The oldest Opiliones fossil, *Eophalangium sheari*, is from the Devonian Rhynie cherts and was previously placed in Eupnoi (28, 29). This fossil has been used in molecular dating to constrain the age of Eupnoi (46) or Palpatores (50), based on interpretation of particular characters. The recent discovery of a new Opiliones body plan in the Carboniferous Montceau-les-Mines (36) (**Figure 1a**) and of the sister-group relationship to the Devonian *Eophalangium* necessitated reevaluation of the harvestman dated phylogeny (36), specifically because of the unexpected placement of this fossil clade as sister group to Cyphophthalmi, not Eupnoi or Palpatores. Node dating of a 279-taxon data set in that study suggested a very different scenario, with Carboniferous diversification in all four suborders (36).

The Mesozoic record of Opiliones now includes Middle Jurassic Eupnoi (in the family Sclerosomatidae) from China (45, 52), in addition to Cretaceous Cyphophthalmi and Dyspnoi (40, 74) and a poorly understood Lower Carboniferous species (56). All major lineages are already represented in Cenozoic amber deposits, especially Dominican, Baltic, and Bitterfeld amber, or in the mudstones and shales from the Florissant formation in the United States (26, 27, 30). This rich fossil record, accompanied by a solid phylogenetic background based on combined morphological and molecular data, contributes to an unparalleled test case for studying diversification of ancient lineages of terrestrial arthropods (90).

Diversification and Biogeography

Opiliones have been considered extraordinary model systems for the study of historical biogeography because of their evolutionary old age (27) and limited dispersal abilities (42). The majority of Opiliones species exhibit narrow distributions, and cases of type-locality endemism are common in the group. These characteristics, together with the profusion of morphological diversity in harvestmen, facilitate the identification of constituent species and utility of many harvestman lineages for biogeographic study.

By far the best-understood lineage in this regard is Cyphophthalmi, or the so-called mite-harvestmen. The smallest suborder of harvestmen is renowned for exceptional fidelity of phylogenetic relationships to geographic distribution, with all cyphophthalmid families exclusively inhabiting formerly contiguous terranes of the supercontinents Gondwana and Laurasia (7, 16, 70). With the discovery of several key fossil harvestmen since 2007 (35, 36, 45, 52, 74), efforts to date Opiliones phylogeny with relaxed-clock approaches have revealed an unprecedented degree of correspondence between divergence times of taxa and estimated ages of landmass fragmentation (36, 43).

Illustrative of this pattern are several genera of the family Pettalidae that are endemic to New Zealand and whose distributions and clade ages bear directly on the debate over the character of the New Zealand biota, namely, whether this biota retains relicts of Gondwana or whether it comprises solely post-Oligocene colonists (8, 9, 21). Comparison of New Zealand Cyphophthalmi with other such endemic clades in this archipelago has shown that the pettalid genera are the oldest monophyletic groups in New Zealand and the only known animal groups that retain the signature of Zealandia's fragmentation from Australia in the Cretaceous (98). The contingency of distribution on geologic processes in poorly dispersing harvestmen has been further elucidated by model-based analyses of diversification rates. For example, acceleration of diversification rate in the genus *Cyphophthalmus* is correlated with the timing of the collision of two Mediterranean microplates in the Eocene (70). Similarly, short internodes at the base of the phylogeny of Stylocellidae, the oldest known clade in many fragment islands of Wallacea and Sundaland (14, 16, 110), coincide with a period of geologic dynamism and marked sea level oscillations in Southeast Asia (16).

The correspondence between geological terranes and phylogenetic relationships is also strongly observed in the suborder Laniatores. In particular, superfamilies within the infraorder Grassatores are frequently contained within traditional zoogeographic provinces, such as the Neotropics (e.g., Gonyleptoidea and its sister group, Stygnopsidae) and the Afrotropics (e.g., Assamioidea), and one superfamily spans both of these (Samooidea) (42, 48). Within the Neotropics, much biogeographic effort has focused on the use of subfamilies of Gonyleptidae to define areas of endemism, with emphasis on endemics of the Brazilian Atlantic Rainforest (e.g., 20, 123). Related approaches in Southeast Asia have investigated the influence of geology on diversification in poorly dispersing Laniatores, such as Sandokanidae, in concert with molecular phylogenies, but limitations persist

in taxonomic sampling of small and cryptic species in this geographically complex region (87). The comparative lack of knowledge of Southeast Asian opiliofauna is highlighted by the 2011 discovery of a new Laniatores family that includes some of the smallest members (1–2 mm) of the suborder (88).

Numerous harvestman lineages with Southern Hemisphere distributions suggestive of vicariance may constitute opportune targets for future study of historical biogeography using multilocus phylogenies and fossil-calibrated molecular dating; such lineages include Triaenonychidae (excluding *Fumontana deprehendor*; 69), Neopilionidae (49, 54, 112), and Acropsopilionidae (49). Nevertheless, earlier characterizations of Opiliones as generally poor dispersers and de facto excellent models for study of historical biogeography are at odds with case studies of dispersal among harvestmen. One of the most extreme cases is the superfamily Zalmoxoidea, which occurs in the Neotropics, save for a single nested Australasian clade with high dispersal capabilities (42). In this case, phylogenetic relationships of Zalmoxoidea, together with molecular dating and model-based reconstructions of ancestral ranges, support colonization of Australasia via transoceanic dispersal out of the Neotropics (89). This pattern constitutes an uncommon biogeographic signal that contrasts with the strongly Paleotropical character of Australasian arthropod faunas (37). Other groups of temperate harvestmen, such as Sironidae, Caddidae, and Sabaconidae, exhibit classic Holarctic distributions, but intercontinental dispersal across land bridges has been inferred at least for sabaconids (83) and caddids (108). Another notable exception to the low vagility of Opiliones is the genus *Acropsopilio*, known from Chile, New Zealand, Venezuela, North America, and Japan (18, 34, 47, 103, 109, 111), although its monophyly remains untested phylogenetically.

Given the incidence of extreme opposite biogeographic signals in the group—the congruence of Cyphophthalmi phylogeny and divergence times with continental drift, juxtaposed with trans-Pacific dispersal in Zalmoxoidea—the characterization of Opiliones as a model system for study of vicariance biogeography requires greater scrutiny and possibly calls for methodical evaluation of family-level groups on a case-by-case basis, especially within Phalangioidea. Although diversification driven by geologic processes may be the predominant explanatory hypothesis for harvestman distributions, the prevalence of dispersal remains to be investigated, particularly for lineages with disjunct distributions (e.g., Acropsopilionidae; 49) and species endemic to oceanic islands (e.g., the Laniatores families Podoctidae and Samoidae; 88, 89).

GENOMICS AND TRANSCRIPTOMICS

Until recently, few genomic resources existed for Opiliones, causing major gaps in knowledge of harvestman genetics. A body of literature has addressed cytogenetics of Opiliones (118), including observation of chromosome numbers in hybrid zones (48) and correspondence with geography (119). Population genetics of harvestmen remains in its infancy, with exclusive application to phylogeography (6, 32, 116). Beyond descriptive approaches, little experimental genetic work has been conducted on the group.

Transcriptomic resources for Opiliones have grown steadily in the last few years. Presently, transcriptomes are available for 14 species in all major clades (50, 79, 92). The first published harvestman transcriptome was that of the cyphophthalmid *Metasiro americanus* (79), sequenced using Illumina short-read technology. This species and its transcriptome were instrumental in demonstrating the incidence of paralogy in genes commonly regarded as single-copy orthologs and frequently used in Sanger-based phylogenetics. Specifically, two or more copies of housekeeping genes such as those encoding methionine adenosyltransferase and elongation factor-1α were demonstrated to occur in *M. americanus*, with immediate implications for phylogenetic studies (17).

A handful of transcriptomes was subsequently used to assess subordinal relationships in Opiliones (50, 92), recovering unambiguous support for the traditional relationship of (Cyphophthalmi, (Laniatores, (Eupnoi + Dyspnoi))). However, the sampling of all four suborders to date omits key lineages that correspond to the earliest divergences in each group. Specifically, Pettalidae (sister group to the remaining Cyphophthalmi; 43), Caddidae (sister group to the remaining Eupnoi; 46), Acropsopilionidae (sister group to the remaining Dyspnoi; 49), and Synthetonychiidae (sister group to the remaining Laniatores; 88) all remain to be sampled. Although these omissions do not affect inference of subordinal relationships, they preclude certain downstream applications, such as molecular dating and inference of phylogenetic informativeness, which in turn requires ultrametric (dated) trees (117). This has not prevented some authors from attempting these approaches (50), but misapplication of fossil calibrations to ipso facto derived nodes makes the resulting inferences unreliable. Apropos, discovery of new fossils and concomitant reinterpretations of fossil placements, together with total-evidence dating, have yielded different estimates for basal divergences of some suborders, as discussed above (36, 90).

Of the existing harvestman transcriptomic data sets, the most heavily utilized is a developmental transcriptome of the eupnoid *Phalangium opilio*, a tractable laboratory species recently deployed in the study of evolutionary developmental biology (discussed below) (91, 94, 95, 97). Intriguingly, many transcription factors involved in embryonic development occur as single-copy orthologs in *P. opilio*, as well as in mite and tick genomes (94, 95). By contrast, duplicates of many developmental genes, particularly Hox and appendage patterning genes, are known to occur in spiders and scorpions, with differential expression of paralog pairs indicating sub- or neofunctionalization (84, 96). The mechanism of paralogy in spiders and scorpions (whether by whole-genome duplication or duplications of specific genomic regions) is not known. Although the relative size of harvestman genomes has not been estimated to date, the absence of paralogs in harvestman transcriptomes for surveyed developmental genes suggests smaller genome size, because of either the absence of duplication events or the loss of paralogs if duplication had occurred. The case of paralogy in *M. americanus* appears to be specific to certain genomic regions (e.g., housekeeping genes), as markers duplicated in spiders and scorpions occur in single copy in this Opiliones species as well.

The transcriptomes of harvestmen have also proven to be effective tools for gene discovery and evolutionary inference. Specifically, the *M. americanus* library has revealed a single ortholog of hemocyanin, an oxygen-binding pigment in mollusks and some arthropods (e.g., horseshoe crabs, scorpions, tetrapulmonate arachnids; 78). The placement of this sequence in a phylogeny of the eight hemocyanin subunits of Chelicerata indicated a divergence prior to the divergence of the Hc2/f and Hc5A/d subunits of Scorpiones + Tetrapulmonata (99), lending credence to this proposed relationship (76, 92). No other harvestman transcriptome sequenced to date has revealed hemocyanin orthologs, although some immunochemical analyses have long suggested the existence of hemocyanin in harvestmen (63, but see 78). The discovery of the cyphophthalmid hemocyanin may be attributable to the sampling of a large number of sequenced individuals, including several postembryonic (juvenile) stages, in the transcriptome of *M. americanus* (79). However, hemocyanins were not obtained in the developmental transcriptome of the eupnoid *P. opilio*.

Many questions concerning the genomic architecture of Opiliones require the sequencing of complete genomes. Such a data source is required to observe pseudogenes and/or transcriptionally repressed paralogs (e.g., of developmental genes), facilitate gene discovery (e.g., harvestman hemocyanins), and understand gene regulation. In addition, a harvestman genome has the potential to elucidate the evolutionary history of Opiliones and their phylogenetic placement among arachnids. Analyses of gene order in cases of potential whole-genome duplication have provided effective constraints on phylogenetic tree topologies and inference of evolutionary history in other organisms (e.g., 25, 80, 81).

OPILIONES AS AN EMERGING MODEL IN EVOLUTIONARY DEVELOPMENTAL BIOLOGY

Although most work in developmental biology still focuses on model organisms, the availability of genomic and transcriptomic resources has narrowed the gap between model and emerging model systems. One discipline that has benefited greatly in this regard is evolutionary developmental biology. Inferring the evolution of developmental mechanisms requires comparisons across multiple species. Historically, comparisons of developmental gene expression in arthropods have utilized a handful of models, with many of these concentrated within insects (e.g., 53). By 2010, only three tractable species in two orders represented arachnids with respect to reliable gene expression protocols and functional approaches: the spiders *Cupiennius salei* and *Parasteatoda tepidariorum*, and the mite *Tetranychus urticae* (1, 19, 24). A second mite, *Archegozetes longisetosus*, has played a key role in the developmental literature (113–115), but functional techniques have not been achieved in the species, limiting its utility.

The first appearance of Opiliones in the evolutionary developmental literature occurred recently, with *P. opilio* emerging as a new experimental model for harvestman development (94, 95, 97). This eupnoid proved a natural choice to represent the order: It is one of the few harvestman species to have a broad distribution, and it has one of the shortest known developmental cycles among Opiliones. In contrast to many temperate members of Eupnoi, *P. opilio* lays multiple, large, synchronously developing egg clutches (50–250 per clutch) that do not undergo diapause, which greatly enhances its tractability for study of embryonic development (2). Recent success of in situ hybridization techniques with embryos of *P. opilio* has led to rapid proliferation of gene expression data for this species (**Figure 3**).

The inclusion of Opiliones among arachnid developmental models uniquely facilitates investigation of genetic mechanisms underlying morphological evolution in chelicerate arthropods because harvestmen retain key plesiomorphies, or primitive characters, that do not occur in spiders or mites. Comparison of developmental processes in these three arachnid orders, with reference to mandibulate arthropod outgroups, enables the polarization of characters, a requirement for evolutionary developmental inference.

An illustrative example of an evolutionarily significant plesiomorphy retained in Opiliones is the morphology of the chelicera. The namesake and sole synapomorphy of Chelicerata, the chelicera is typically a short appendage consisting of two to four segments that is involved in feeding, although sexually dimorphic chelicerae occur in many arachnid groups, including Opiliones (**Figure 1***f*). The number of segments and morphology of the chelicerae have played an important role in arachnid phylogeny (106), with character optimizations supporting a three-segmented, chelate (i.e., articulated) chelicera in the ancestral arachnid and a larger number of segments in fossil pycnogonids and horseshoe crabs (nonterrestrial chelicerates; 3, 10). Two-segmented chelate types are observed in such lineages as Solifugae and Pseudoscorpiones. Tetrapulmonates bear a two-segmented chelicera of jackknife shape (nonchelate). Investigating the genetic basis of evolutionary transitions in cheliceral morphology was previously not possible; of the heretofore available model systems, the spider chelicera is greatly derived and the mite chelicera (two-segmented, chelate) represents an intermediate state (**Figure 4**).

Phalangium opilio represents the first tractable chelicerate developmental model with three-segmented chelicerae. By comparing the expression of genes patterning the proximodistal appendage axis in *P. opilio* and multiple spider species, Sharma et al. (94) demonstrated the presence of a *dachshund* expression domain in the proximal segment of the developing harvestman chelicera; neither this segment nor the expression domain is observed in developing spider chelicerae (**Figure 4**). An embryonic RNA interference approach to silencing this gene subsequently
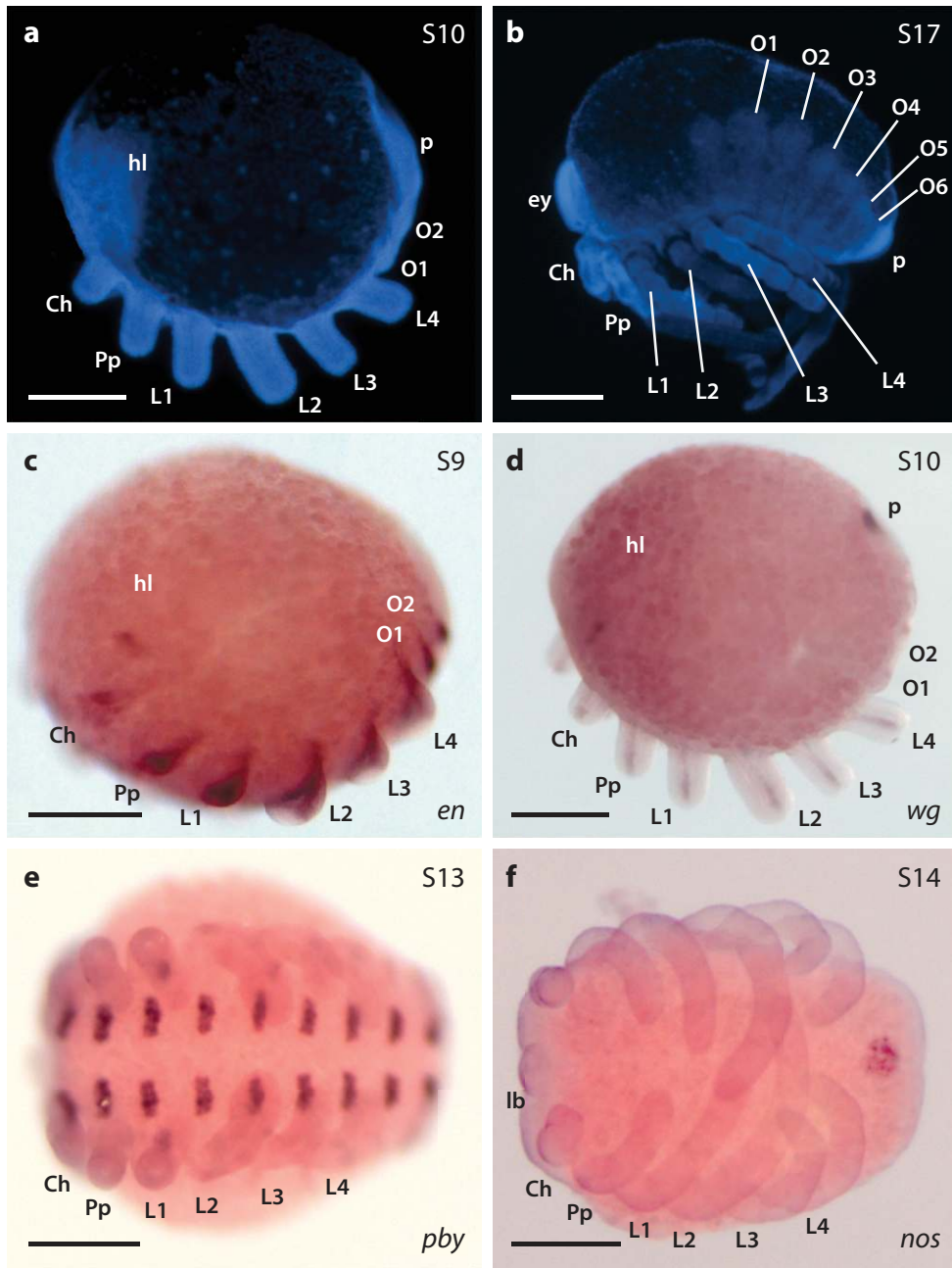
Figure 3

Embryos of *Phalangium opilio*, the model system for study of embryonic development in Opiliones.
Fluorescent staining of cell nuclei in stage 10 (*a*) and stage 17 (*b*) embryos shows progressive segmentation in
the posterior tagmata. Examples of developmental processes investigated through developmental gene
expression include segmentation, using *engrailed* (*c*), *wingless* (*d*), and *pairberry* (*e*), and embryonic germ cell
migration, using *nanos* (*f*). Abbreviations: Ch, chelicera; ey, eyefield; hl, head lobe; L, leg; lb, labrum; O,
opisthosomal segment; p, posterior region; Pp, pedipalp. All scale bars are 200 μm.

Figure 4

(*a*) Evolution of the chelicera superimposed on a phylogeny of Chelicerata. (*b*) Comparative gene expression of appendage patterning genes in spiders and harvestmen. Colored bars indicate gene expression domains of *Distal-less* (*red*) and *dachshund* (*green*) and coexpression of *homothorax* and *extradenticle* (*blue*). A *dachshund* domain is absent in two-segmented chelicerae of spiders but retained in the proximal segment of the harvestman three-segmented counterpart. (*c*) Hypothesized mechanism of transition from three- to two-segmented state, based on comparative gene expression and gene silencing in *Phalangium opilio*.
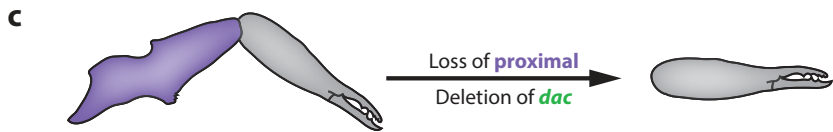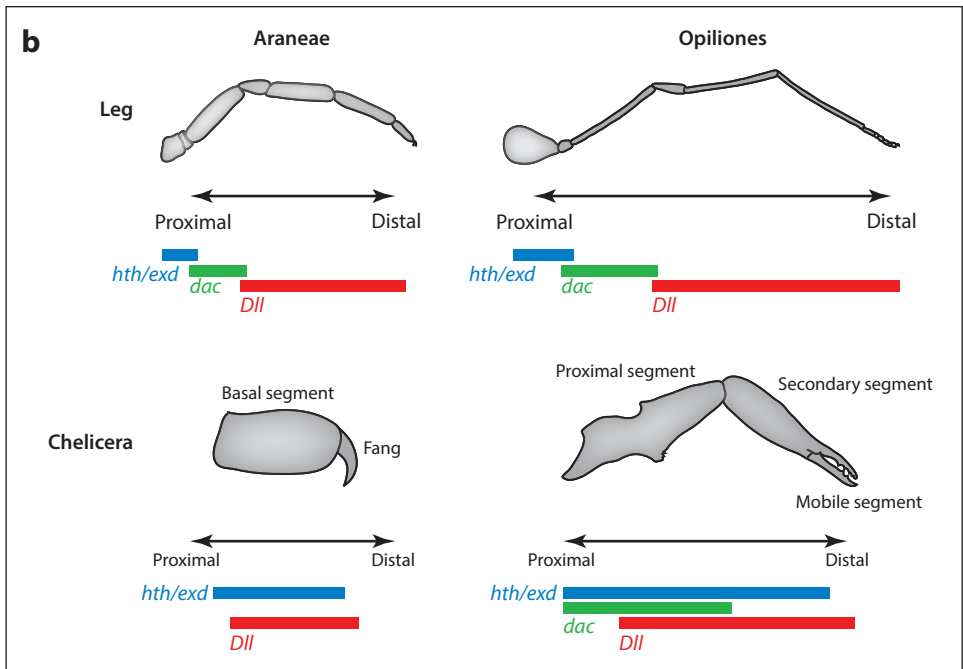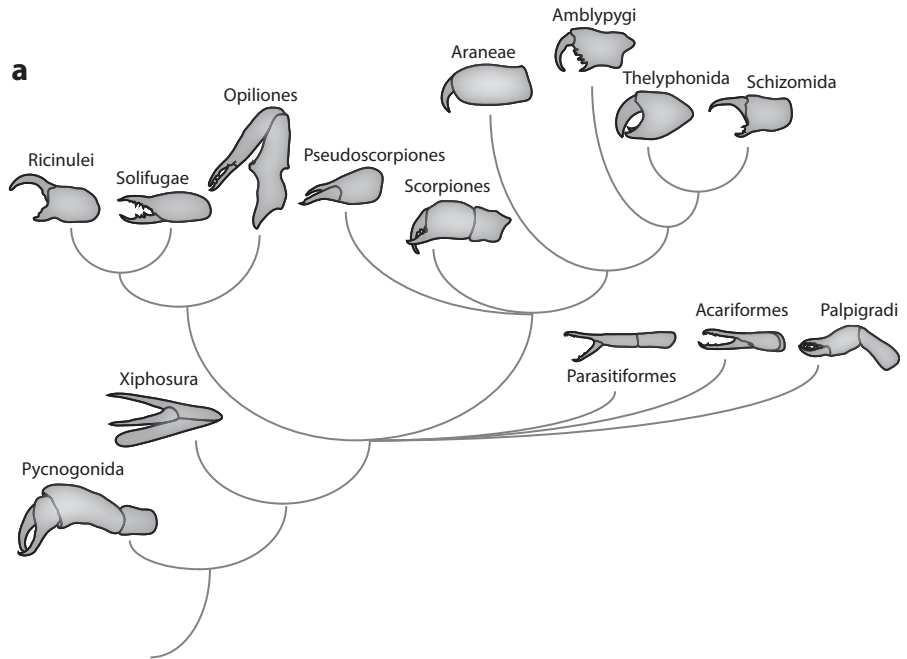
showed that loss-of-function *dachshund* phenotypes result in two-segmented chelate chelicerae, i.e., loss of the proximal segment (97). These studies suggest a key role for *dachshund* in patterning cheliceral segment number. Subsequently, this hypothesis of a genetic mechanism was extended to the mite *A. longisetosus*. Mites such as *A. longisetosus* retain a reduced third article that may constitute a segment, which is consistent with the observation of *dachshund* expression at the base of the developing mite chelicera (4).

Phalangium opilio has similarly driven recent investigation of the evolutionary basis of opisthosomal variation in arachnids, chiefly by comparisons with Hox gene expression data in spiders. It was shown that expression patterns of harvestman Hox genes are essentially identical to those of spiders, save for the expression domains of *abdominal-A* and *Abdominal-B*, whose anterior expression boundaries correspond to shifts in segmental identity, i.e., the degree of opisthosomal heteronomy (95). Although this hypothesis remains to be tested with functional approaches, these data implicate one or both of these Hox genes in patterning the diversity of arachnid segmental architecture.

Beyond arachnids, *P. opilio* has enabled inference of evolutionary patterns across arthropods. Together with data from crustaceans and myriapods, expression data from *P. opilio* and scorpion embryos demonstrated a mandibulate-specific expression pattern for *cap-n-collar*, a gene encoding a transcription factor that confers mandible identity in insects (55, 91).

Such rapid advances in research on evolutionary development of Arachnida stem in equal part from the availability of genomic and transcriptomic tools for new laboratory models, the tractability of *P. opilio*, and the evolutionary significance of harvestman morphology. Major outstanding and opportune targets for evolutionary developmental research with *P. opilio* include the mechanisms underlying (*a*) appendage elongation, characteristic of long-legged groups in Eupnoi, and (*b*) patterning of the tracheal respiratory system, whose origins are independent of hexapod and myriapod counterparts.

SUMMARY POINTS

1. Opiliones constitute an ancient lineage of terrestrial arthropods, with a fossil record dating back to the Devonian and a well-understood phylogenetic tree, facilitating the use of these arachnids for evolutionary and biogeographic studies.

2. Dense taxonomic sampling of Opiliones lineages has revealed surprising patterns, such as the identity of basally branching families and suborders (Pettalidae, Acropsopilionidae, and Synthetonychiidae), and the incidence of long-range dispersal in some groups. Molecular dating approaches to hypothesis testing are facilitated by taxonomic sampling and multiple recently discovered fossil calibration points.

3. Availability of transcriptomic resources for all extant suborders has enabled phylogenomic inference, detection of paralogy, gene discovery, and the establishment of a model system for the study of Opiliones development.

4. The tractability of *Phalangium opilio* for study of embryonic gene expression and gene silencing has accelerated representation of Opiliones in studies of evolutionary developmental biology, complementing spiders and mites among model arachnids.

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