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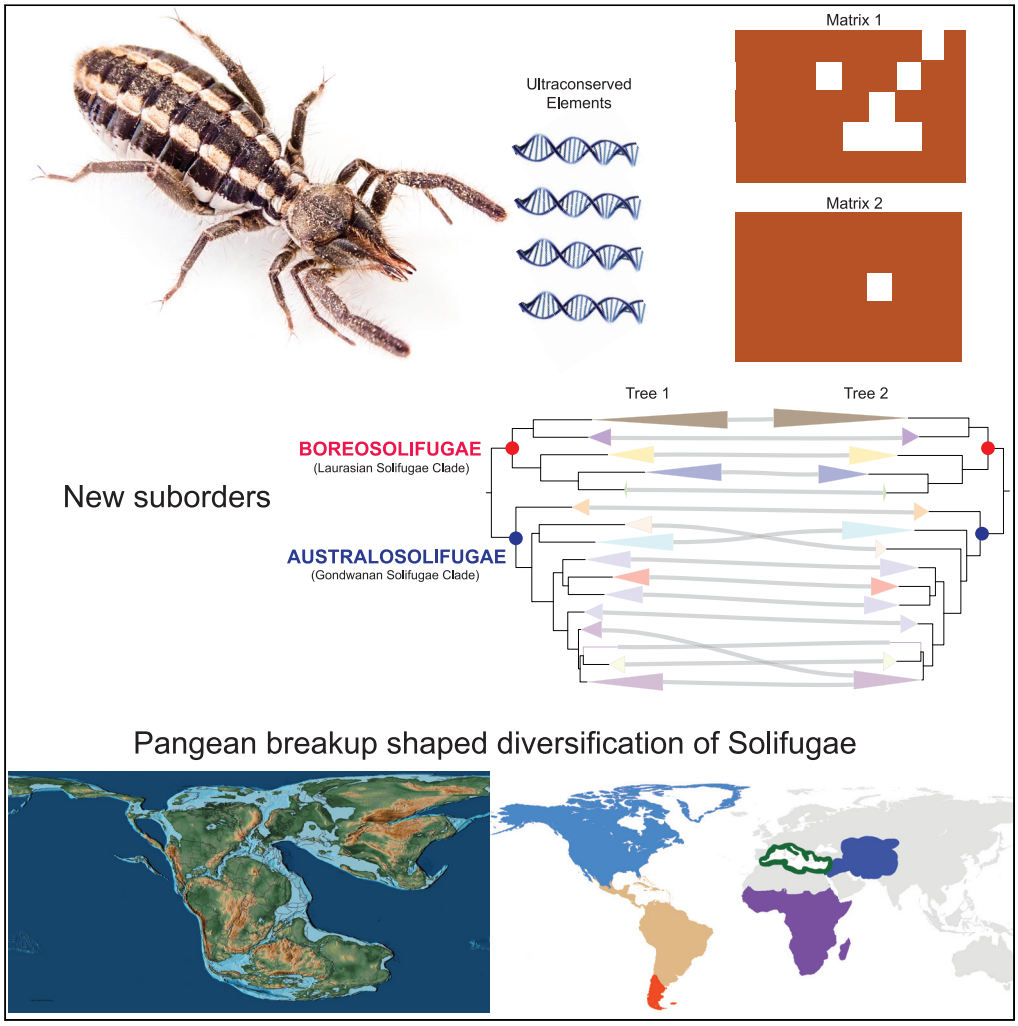

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Highlights

Ultraconserved elements unlock the first phylogeny of Solifugae (camel spiders)

A series of sensitivity analyses reconstruct robust evolutionary relationships

Ancestral areas of Solifugae suggest distributions resulted from Pangean breakup

New suborders for Laurasian (Boreosolifugae) and Gondwanan (Australosolifugae) taxa

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Neglected no longer: Phylogenomic resolution of higher-level relationships in Solifugae

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SUMMARY

Advanced sequencing technologies have expedited resolution of higher-level arthropod relationships. Yet, dark branches persist, principally among groups occurring in cryptic habitats. Among chelicerates, Solifugae ("camel spiders") is the last order lacking a higher-level phylogeny and have thus been historically characterized as "neglected [arachnid] cousins". Though renowned for aggression, remarkable running speed, and xeric adaptation, inferring solifuge relationships has been hindered by inaccessibility of diagnostic morphological characters, whereas molecular investigations have been limited to one of 12 recognized families. Our phylogenomic dataset via capture of ultraconserved elements sampling all extant families recovered a well-resolved phylogeny, with two distinct groups of New World taxa nested within a broader Paleotropical radiation. Divergence times using fossil calibrations inferred that Solifugae radiated by the Permian, and most families diverged prior to the Paleogene-Cretaceous extinction, likely driven by continental breakup. We establish Boreosolifugae new suborder uniting five Laurasian families, and Australosolifugae new suborder uniting seven Gondwanan families using morphological and biogeographic signal.

INTRODUCTION

Chelicerata is an ancient, monophyletic group of arthropods that is characterized by extensive diversity, high body plan disparity among their different orders, and inclusion of numerous charismatic taxa, such as spiders, scorpions, and horseshoe crabs. Despite their considerable diversity, nearly every group of chelicerate orders has benefitted from recent advances in molecular phylogenetics, genomics, and renewed interest in evolutionary dynamics. The past decade alone has witnessed the first molecular phylogenetic hypotheses for several orders, such as Scorpiones, Ricinulei (hooded tick-spiders), Palpigradi (microwhip scorpions), Thelyphonida (vinegaroons), Schizomida (short-tailed whip scorpions), and Amblypygi (whip spiders).[1–5] More diverse chelicerate groups, such as Acari (mites), Araneae (spiders), Opiliones (harvestmen), and Pseudoscorpiones, have witnessed a surge of evolutionary inquiry in the past ten years.[6–17] This decade is also notable for the completion of the first genomes for several chelicerate orders.[18–24] These advances have revolutionized modern views of chelicerate phylogeny and evolution, prompting reevaluations of historical paradigms of habitat transition and evolution of complex characters.[6,8,24–28]

Solifugae, variously known as "camel spiders" or "sun spiders", is a mesodiverse group (in comparison with other arachnid groups) that currently includes ca. 1,200 species classified in 12 families and 144 genera (Figure 1).[29] Notorious for their fearsome appearance, large chelicerae (relative to body size), and high bite force,[30] solifuges are one of seven chelicerate orders that are commonly referred to as "the neglected cousins" in the arachnological community, due to a dearth of systematic and phylogenetic studies.[31] Various characteristics of solifuges distinguish them from other arachnids, such as a robust pair of two-segmented chelicerae, adhesive structures on the termini of the pedipalps for prey capture, the presence of malleoli ("racquet organs", used for chemoreception and analogous to the pectines of scorpions) on the ventral surface of leg IV,[32–34] and an extensive tracheal system.[35] The last of these is understood to facilitate the rapid running speed of

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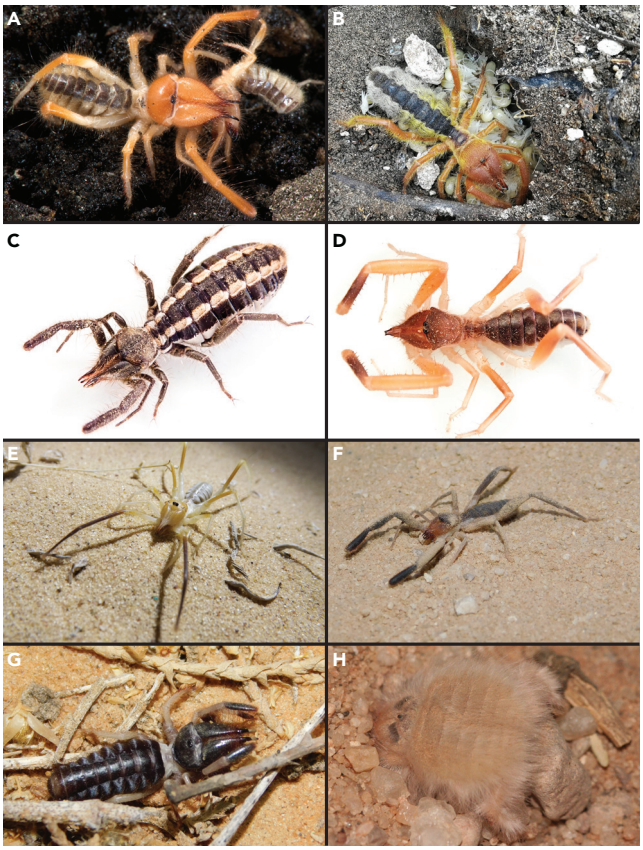


Figure 1. A sample of morphological diversity of Solifugae

(A) Adult female of *Eremobates* (Eremobatidae) from AZ, USA.

(B) Brooding female of *Paragaleodes* (Galeodidae) over a clutch of hatchlings, from Israel.

(C) Adult female of *Mummucia* (Mummuciidae) from Chile.

(D) Adult male of *Procleobis patagonicus* (Ammotrechidae) from Argentina.

(E) Adult female of unidentified Galeodidae (cf. *Galeodes*) from Israel.

(F) An unidentified Daesiidae from Israel.

(G) An unidentified Rhagodidae from Israel.

(H) An actively burrowing Hexisopodidae (cf. *Hexisopus*) from Namibia.

Photographs: G. Giribet (A); I. Armiach (B, E, F, and G); H. Iuri and A. Ojanguren-Affilastro (C and D); S. Aharon (H).

solifuges, as well as their inhabitation of some of the driest habitats on Earth. Historically, solifuges were thought to be closely related to pseudoscorpions[36,37] a hypothesis that has since been contradicted by sperm ultrastructure,[38] rare genomic changes, and the ensuing placement of pseudoscorpions as the sister group of scorpions,[24] (but see[39]). Solifugae has more recently been recovered as part of a clade with acariform mites (Poecilophysidea), and possibly also palpigrades (Cephalosomata).[27,40] A close relationship of these three orders is supported by the arrangement of the anterior body segments and the structure of the coxal glands, and is recovered by a subset of molecular analyses that have emphasized dense taxonomic sampling.[27,40–42]

By contrast to their placement in higher-level chelicerate phylogeny, the internal relationships of Solifugae remain virtually unknown.[31] A classification of the twelve extant families was proposed by Roewer[43] based on overall similarity of characters that are, in turn, highly variable across genera and species; this classification largely remains in place.[29] Beyond the incidence of poorly delimited higher-level taxa, a peculiarity of solifuge systematics is the concentration of diagnostic characters in the adult males of many lineages—in some groups, juveniles cannot be reliably assigned even to a specific family. As a result, few attempts have been made to infer phylogenetic relationships using either morphological or molecular datasets. Rhagodidae was suggested to be well-separated from the remaining solifuge families on the basis of its peculiar morphology, but this inference was not based on formal analyses and the polarity of these morphological characters is unknown.[43] Investigations of specific character systems have documented broad diversity, such as in the flagellum of the male chelicera and the histology of sperm ultrastructure, but these data have not been applied toward the goal of formal inference of evolutionary relationships.[44–46] Although molecular data offer considerable advantages over anatomical datasets for phylogenetic study of enigmatic groups like Solifugae, few works have addressed internal relationships within this order. For example, RADseq data supported the monophyly of genus *Eremocosta* and

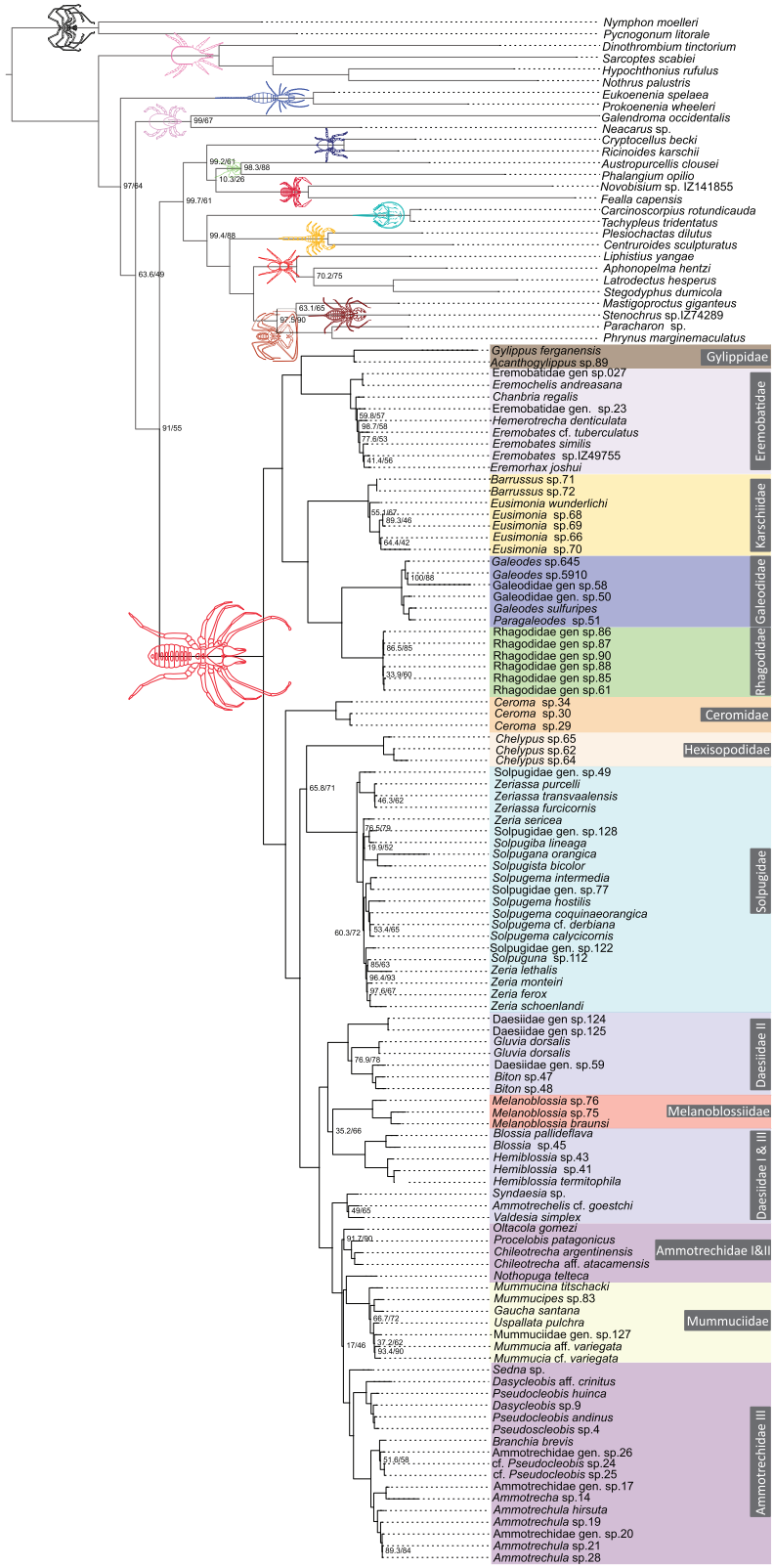

Nymphon moelleri
Pycnogonum litorale
Dinothrombium tinctorium
Sarcoptes scabiei
Hypochthonius rufulus
Nothrus palustris
Eukoenenia spelaea
Prokoenenia wheeleri
Galendroma occidentalis
Neacarus sp.
Cryptocellus becki
Ricinoides karschii
Austropurcellis clousei
Phalangium opilio
Novobisium sp. IZ141855
Fealla capensis
Carcinoscorpius rotundicauda
Tachypleus tridentatus
Plesiochactas dilutus
Centruroides sculpturatus
Liphistius yangae
Aphonopelma hentzi
Latrodectus hesperus
Stegodyphus dumicola
Mastigoproctus giganteus
Stenochrus sp.IZ74289
Paracharon sp.
Phrynus marginemaculatus
Gylippus ferganensis
Acanthogylippus sp.89
Gylippidae
Eremobatidae gen sp.027
Eremochelis andreasana
Chanbria regalis
Eremobatidae gen. sp.23
Hemerotrecha denticulata
Eremobates cf. tuberculatus
Eremobates similis
Eremobates sp.IZ49755
Eremorhax joshui
Eremobatidae
Barrussus sp.71
Barrussus sp.72
Eusimonia wunderlichi
Eusimonia sp.68
Eusimonia sp.69
Eusimonia sp.66
Eusimonia sp.70
Karschiidae
Galeodes sp.645
Galeodes sp.5910
Galeodidae gen sp.58
Galeodidae gen. sp.50
Galeodes sulfuripes
Paragaleodes sp.51
Galeodidae
Rhagodidae gen sp.86
Rhagodidae gen sp.87
Rhagodidae gen sp.90
Rhagodidae gen sp.88
Rhagodidae gen sp.85
Rhagodidae gen sp.61
Rhagodidae
Ceroma sp.34
Ceroma sp.30
Ceroma sp.29
Ceromidae
Chelypus sp.65
Chelypus sp.62
Chelypus sp.64
Hexisopodidae
Solpugidae gen. sp.49
Zeriassa purcelli
Zeriassa transvaalensis
Zeriassa furcicornis
Zeria sericea
Solpugidae gen. sp.128
Solpugiba lineaga
Solpugana orangica
Solpugista bicolor
Solpugema intermedia
Solpugidae gen. sp.77
Solpugema hostilis
Solpugema coquinaeorangica
Solpugema cf. derbiana
Solpugema calycicornis
Solpugidae gen. sp.122
Solpuguna sp.112
Zeria lethalis
Zeria monteiri
Zeria ferox
Zeria schoenlandi
Solpugidae
Daesiidae gen sp.124
Daesiidae gen sp.125
Gluvia dorsalis
Gluvia dorsalis
Daesiidae gen. sp.59
Biton sp.47
Biton sp.48
Daesiidae II
Melanoblossia sp.76
Melanoblossia sp.75
Melanoblossia braunsi
Melanoblossiidae
Blossia pallideflava
Blossia sp.45
Hemiblossia sp.43
Hemiblossia sp.41
Hemiblossia termitophila
Syndaesia sp.
Ammotrechelis cf. goestchi
Valdesia simplex
Daesiidae I & III
Oltacola gomezi
Procelobis patagonicus
Chileotrecha argentinensis
Chileotrecha aff. atacamensis
Nothopuga telteca
Ammotrechidae I&II
Mummucina titschacki
Mummucipes sp.83
Gaucha santana
Uspallata pulchra
Mummuciidae gen. sp.127
Mummucia aff. variegata
Mummucia cf. variegata
Mummuciidae
Sedna sp.
Dasycleobis aff. crinitus
Pseudocleobis huinca
Dasycleobis sp.9
Pseudocleobis andinus
Pseudoscleobis sp.4
Branchia brevis
Ammotrechidae gen. sp.26
cf. Pseudocleobis sp.24
cf. Pseudocleobis sp.25
Ammotrechidae gen. sp.17
Ammotrecha sp.14
Ammotrechula hirsuta
Ammotrechula sp.19
Ammotrechidae gen. sp.20
Ammotrechula sp.21
Ammotrechula sp.28
Ammotrechidae III

Figure 2. Phylogeny of Solifugae using ultraconserved elements

A maximum likelihood phylogeny of Chelicerata with all Solifugae family representatives using the 25% occupancy partitioned dataset of ultraconserved elements. Numbers at the nodes indicate percentage support values as Shimodaira-Hasegawa-like approximate likelihood ratio test and ultrafast bootstrap (not shown if > 95%).

suggested post glacial colonizations in North American deserts.[47] A recent analysis of Iranian solifuges based on one mitochondrial gene was able to sample seven families and the ensuing topology suggested the paraphyly of at least three families, but without significant nodal support for most interfamilial relationships.[48] The only multilocus dataset applied to solifuge relationships examined the phylogeny of the North American family Eremobatidae and this four Sanger locus-based analysis uncovered extensive non-monophyly of constituent genera, together with limited nodal support for deep nodes.[49]

Given the presumably ancient diversification of Solifugae, as inferred from the appearance of crown group lineages by the Mesozoic and multiple fossil genera,[29,50] bridging the knowledge gaps in solifuge higher-level phylogeny requires the application of phylogenomic datasets and tissue sampling from rare, often aging, tissue collections. We therefore amassed a set of 94 solifuge species drawn from natural history collections around the world and sequenced these for a chelicerate-specific suite of ultraconserved elements (UCEs), an approach notable for its demonstrable capability to accommodate even highly degraded tissues.[51] Here, we provide a robustly resolved phylogenomic tree of Solifugae, in tandem with molecular dating efforts to provide a temporal context to solifuge diversification.

RESULTS

We compiled a comprehensive dataset with high-quality ultraconserved profiles spanning all extant chelicerate orders, represented by 132 taxa. In this dataset, Solifugae was represented by 104 taxa, amounting to almost 10% of the global fauna,[29] of which we generated UCE libraries for 93 terminals (89% of taxa) and *in silico* extracted UCEs from existing UCE libraries, transcriptomes or genomes for the remainder (Table S1). Selection of outgroups prioritized the sampling of basal splits, to facilitate node calibration in molecular dating. Locus count yields through paralogy filtering with liberal (65%) and stringent (80%) probe-to-library identity and coverage mapping thresholds and data completeness across 25% and 40% occupancy thresholds are listed in Table S1. In addition, to mitigate the impact of taxa with sparsely represented UCE loci (= high missing data), we generated a reduced matrix with higher total information content.

Phylogenomic analyses

Maximum likelihood analyses based on 521 loci (25% occupancy; 65% probe-to-library identity threshold; GTR+F+I + G4 model) recovered a tree topology that divided solifuge families into two major clades, which we recognize as Boreosolifugae **new suborder** and Australosolifugae **new suborder** (Figure 6). Boreosolifugae was comprised of the Laurasian families Eremobatidae, Gylippidae, Karschiidae, Galeodidae, and Rhagodidae; these families are united by the presence of a sessile flagellum (or specialized long setae in the flagellar grove, in the case of Eremobatidae) in the chelicerae of adult males (see discussion). Australosolifugae was comprised of the Gondwanan families Ceromidae, Solpugidae, Hexisopodidae, Melanoblossiidae, Daesiidae, Mummuciidae and Ammotrechidae; these families are in turn diagnosed by the presence of a composite flagellum (see discussion). The automatic matrix reduction method (MARE) retained 267 loci and dropped the families Gylippidae, Karschiidae and Hexisopodidae, but retained the same order of interfamilial relationships and proposed suborders (Figure S1). All families except Ammotrechidae and Daesiidae were recovered as monophyletic with robust support (Figure 2). To rule out the possibility of systematic bias driven by compositional heterogeneity, GC-base proportions were mapped on the 25% occupancy phylogeny. Only two outgroup terminals, the parasitiform mite *Galendroma occidentalis* and *Cryptocellus becki* exhibited a high proportion of GC-content; excluding these taxa from the analysis did not affect the interfamilial relationships of Solifugae (Figure S2).

The backbone tree topology of Solifugae was well-resolved and the basal split between the suborders was invariably recovered across analyses (Figures 2 and 3). Mummuciidae was almost always nested within Ammotrechidae, rendering the family paraphyletic (Figures 2 and 3). Melanoblossiidae was a sister group to the Daesiidae I clade, albeit with poor support (<95%). Partitioning by UCE loci with merging of similar partitions allowed did not affect any interfamilial relationship compared with phylogenies from their unpartitioned datasets across respective occupancies (Figures S3–S5).

Influence of stringent homology filtering

Matrices based on stringent filtering (80% identity and coverage probe-to-library match) discarded many loci, resulting in a more compact and denser dataset across different occupancies (Table S1). Some terminals were dropped at 40% occupancy because the stringency of this filter did not recover any UCE locus for those taxa. Nevertheless, at least one taxon representing each currently recognized family was present in each occupancy dataset, thereby not compromising the higher-level taxon sampling. The tree topologies recovered for matrices constructed under this threshold recovered similar relationships as previously reported for the intersuborders and interfamilial relationships. Among Solifugae, most interfamilial relationships were similar to that of the 25% occupancy phylogeny with 65% identity and coverage thresholds (Figures 2 and S6–S9). Gylippidae was recovered as paraphyletic in 25% occupancy matrix, but this is likely attributable to low locus representation (12 UCE loci for *Gylippus ferganensis*; Table S1), and was among the dropouts of 40% data matrix. Noticeable differences include, Ceromidae as a sister group of remaining Australosolifugae at 25% versus sister group of Solpugidae with 40% dataset. Ammotrechidae I and II formed a clade with both occupancies. Some outgroup taxa represented by very few loci were placed inside Solifugae at 40%

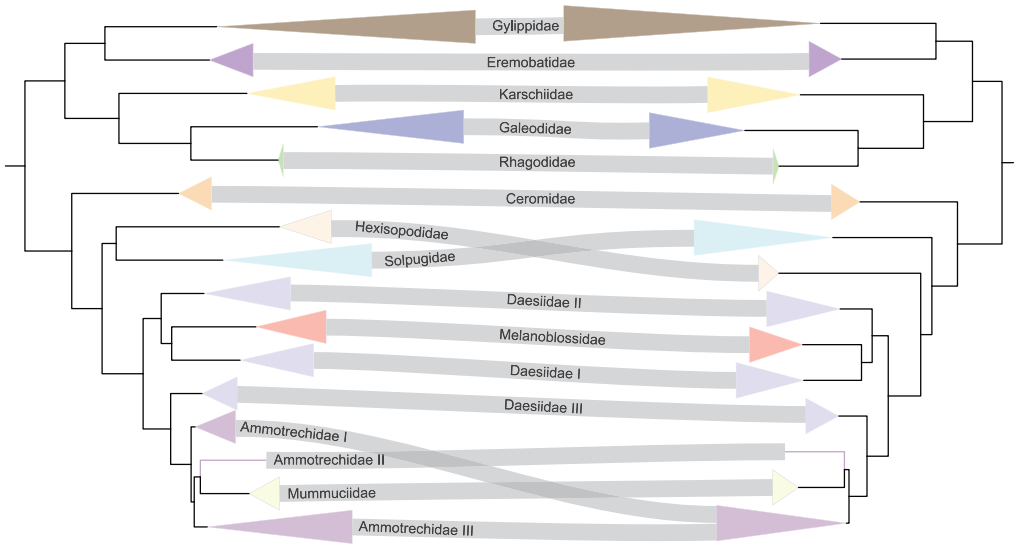


Figure 3. Comparison of Solifugae interfamilial relationships

Sankey comparison of interfamilial phylogenetic relationships of Solifugae compared between the 25% (left) and 40% (right) occupancy partitioned datasets at 65% contig to probe identity and coverage threshold.

occupancy, but with poor support. If these erroneous, poorly supported branches were to be pruned, solifuge interfamilial relationships continued to be robust even at this occupancy, which corresponds to a matrix of just 18 UCE loci (Figures S6–S9).

Divergence dating and biogeographic analyses

Divergence date optimizations calibrated with fossil age estimates in a least-squares (LSD2) and maximum likelihood (MCMCTree) frameworks, with and without ingroup fossils, recovered some non-overlapping age ranges (Figures 4 and S10–S13). Diversification of the crown group Solifugae was estimated within the Carboniferous (318–331 Ma) period. Boreosolifugae, and Australosolifugae diversified during the late diversified during the (257–278 Ma) Permian period. The MRCAs (most recent common ancestors) of most families including Gylippidae, Eremobatidae, Solpugidae, Daesiidae I, II, and Ammotrechidae I and III, originated during the Cretaceous period. The remaining families (Karschiidae, Galeodidae, Rhagodidae, Hexisopodidae, and Daesiidae III) originated post Cretaceous-Tertiary boundary.

Rhagodidae was estimated to have originated by late Miocene epoch, forming the most recently diverged family (Figure 4). LSD2 analysis recovered Carboniferous period as the origin for Crown Solifugae (Figures S7 and S13). However, for the suborders, LSD2 recovered strikingly recent divergence time estimates rendering them to have originated during the Triassic period. Exclusion of the two Solifugae fossils with LSD2 recovered more recent age range for all nodes (Figure S13).

To infer ancestral areas and reconstruct the biogeographic history of Solifugae, we analyzed the dated tree topology in tandem with five broad geographic areas using RASP (Figure 5). Historical biogeographic area optimizations were assessed by the best fitting model using RASP on our 25% occupancy dataset. The model with highest AICc weight was the dispersal-extinction-cladogenesis with jump dispersal parameter model (DEC+j; 0.58). (see Table S5; Figure S14). The DEC+*j* model recovered a combination of Turanian, African tropics, Neotropical and Mediterranean regions as the area for the most recent common ancestor (MRCA) of Solifugae (Figure 5). The ancestral area for Boreosolifugae was a combination of Turanian, Neotropical and Mediterranean (i.e., fragments of Laurasia), whereas for Australosolifugae, the Afrotropics (i.e., a constituent of Gondwana) was recovered using both models (Figure 5). Most family distributions were limited to a single biogeographic region. One group of the polyphyletic Daesiidae (clade III) is distributed within Central Chile and the Patagonian (CCP) region and the other two groups have an African origin (Figure 5). At the crown node of the CCPian and Neotropical Ammotrechidae III clade, the DEC+*j* model optimized CCP as the ancestral area, which implied a dispersal event from CCP to the Neotropics. The exclusion of the jump parameter optimized a combination of CCP and Neotropical region as the ancestral area, thus implying a vicariance event dividing the descendant lineages (Figure 5). Seven dispersal events were implied by the DEC+*j* model (marked with red arrows in Figure 5). These dispersals included three events between the Mediterranean region and African tropics; one between Jurassic to Cretaceous, two events during Paleogene; two between Neotropical and the CCP regions during the Paleogene period; one dispersal event from CCP to the Nearctic region during the Paleogene; and one African tropics to CCP regions during the Jurassic period were implied by the DEC+*j* model. Overall, the DEC+*j* model favored a single area as origin where the descendant lineages occupying different regions recovered dispersal events. Alternative coding of areas by continents for extant taxa recovered Eurasia and Africa as the ancestral areas for Boreosolifugae and Australosolifugae, respectively. A further broader area coding by supercontinent recovered Laurasia as the ancestral area for Boreosolifugae and Gondwana for Australosolifugae (Figure S11).

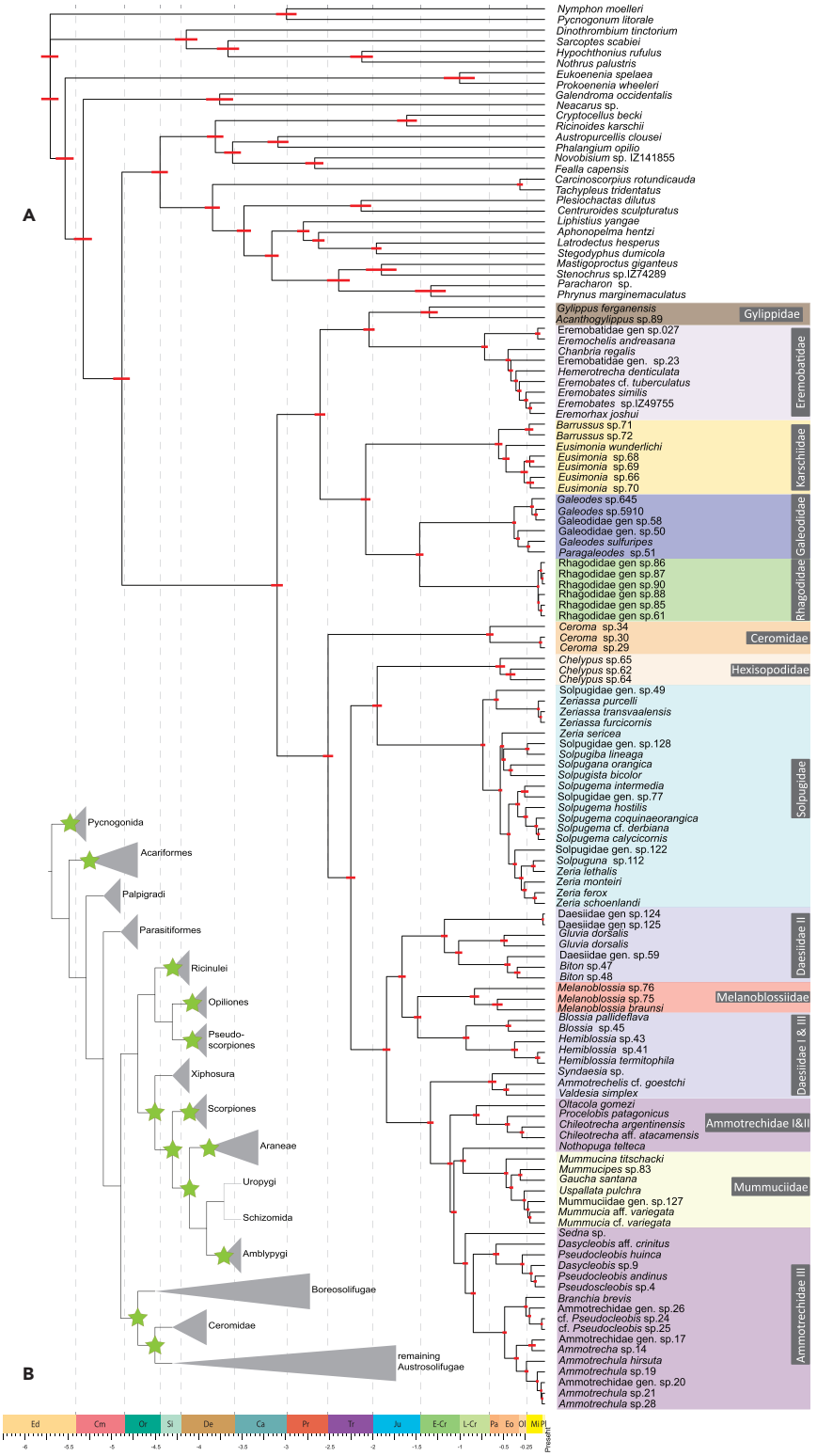
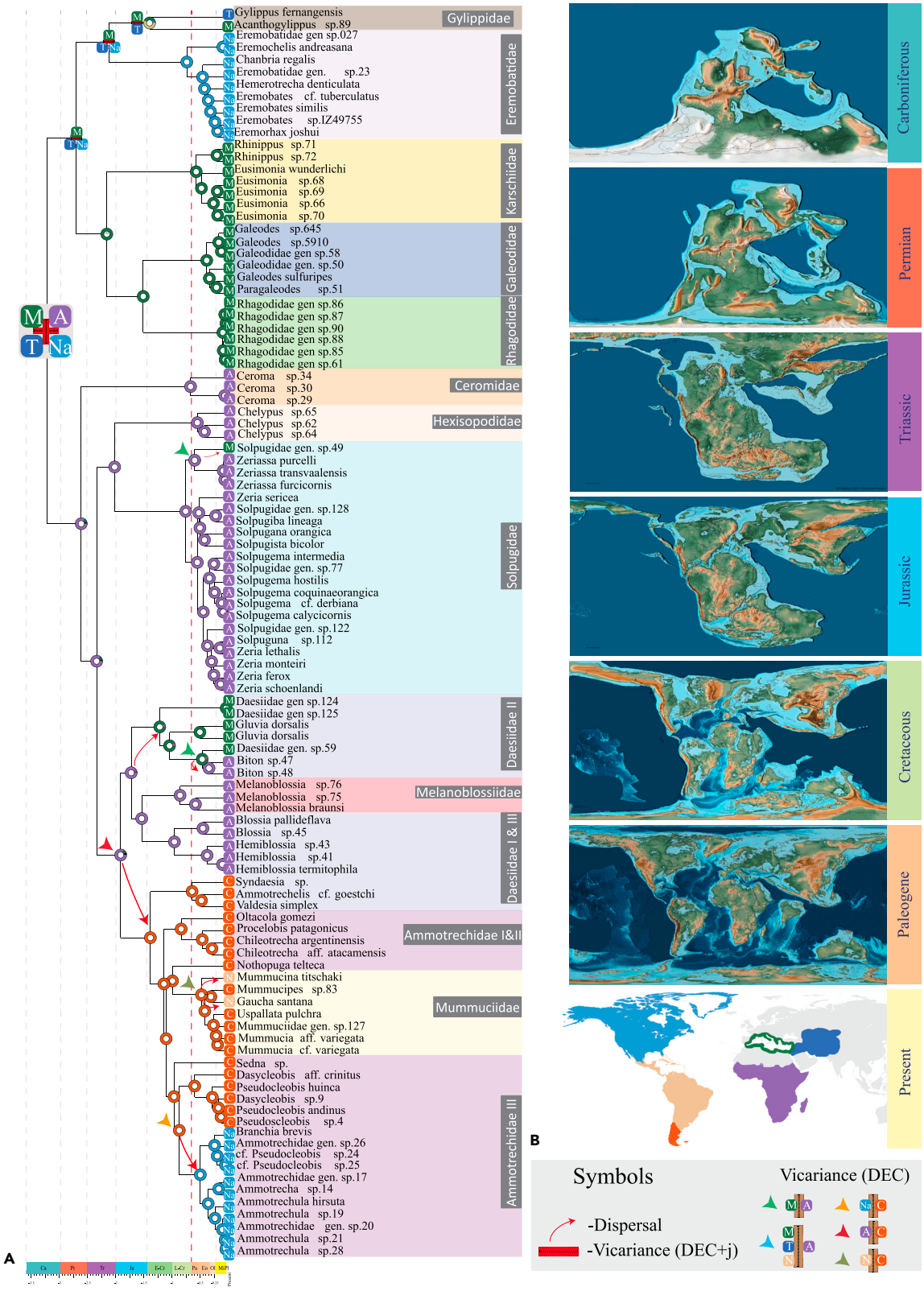


Figure 4. A time-calibrated phylogeny of Solifugae recovers Paleozoic radiation

(A) A maximum likelihood phylogeny of Chelicerata with all Solifugae families represented, time-calibrated using fossils reconstructed using the UCE dataset for occupancy 25%.

(B) Placement of fossils used as prior calibrations (marked with 'star' symbol) for the MCMCTree analysis.


Gylippus fernangensis
Acanthogylippus sp.89
Gylippidae
Eremobatidae gen sp.027
Eremochelis andreasana
Chanbria regalis
Eremobatidae gen. sp.23
Hemerotrecha denticulata
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Paragaleodes sp.51
Galeodidae
Rhagodidae gen sp.86
Rhagodidae gen sp.87
Rhagodidae gen sp.90
Rhagodidae gen sp.88
Rhagodidae gen sp.85
Rhagodidae gen sp.61
Rhagodidae
Ceroma sp.34
Ceroma sp.30
Ceroma sp.29
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Zeria lethalis
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Zeria ferox
Zeria schoenlandi
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Daesiidae gen sp.124
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Gluvia dorsalis
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Daesiidae gen. sp.59
Biton sp.47
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Sedna sp.
Dasycleobis aff. crinitus
Pseudocleobis huinca
Dasycleobis sp.9
Pseudocleobis andinus
Pseudoscleobis sp.4
Branchia brevis
Ammotrechidae gen. sp.26
cf. Pseudocleobis sp.24
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Ammotrechidae gen. sp.17
Ammotrechula sp.14
Ammotrechula hirsuta
Ammotrechula sp.19
Ammotrechidae gen. sp.20
Ammotrechula sp.21
Ammotrechula sp.28
Ammotrechidae III
A
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Paleogene
Present
B
Symbols
-Dispersal
-Vicariance (DEC+j)
Vicariance (DEC)

Figure 5. Historical biogeography of Solifugae supports Pangean diversification

(A) Biogeographic hypothesis obtained from the Dispersal-Extinction-Cladogenesis + jump (DEC+*j*) parameter model of RASP analyses on the fossil-calibrated MCMCTree analysis using the 25% occupancy dataset. The dotted line in red indicates the Cretaceous Triassic boundary.

(B) Graphics for Pangean breakup from Scotese, C.R., 2016. PALEOMAP Project, http://www.earthbyte.org/paleomap—paleoatlas—for—gplates/. Ancestral areas optimized for each family and higher-level nodes are marked at the respective MRCA nodes. Red arrows indicate dispersal implied by the DEC+j model whereas the colored arrowheads indicate alternative vicariance events implied by the exclusion of the *jump* parameter.

DISCUSSION

The higher-level relationships of Solifugae

A recent exploration of long branch attraction effects in chelicerate phylogeny[24] showed that taxonomic undersampling, and specifically, omitting the representation of basal nodes, exacerbated topological instability of fast-evolving arachnid orders like pseudoscorpions. Using a rare genomic change as a benchmark for phylogenetic accuracy, these analyses demonstrated that taxonomic sampling (especially of slowly evolving and basally branching groups) outperformed other approaches to mitigating long branch attraction, such as increasing matrix completeness, using coalescent-aware species tree approaches, filtering by evolutionary rate, and implementing site heterogeneous models in concatenated matrices. Ontano et al.[24] reasoned that applying this remedy to other fast-evolving orders may greatly stabilize chelicerate phylogeny, given that at least four orders exhibit a propensity for long branch attraction artifacts in Chelicerata.[26,42,52] This proposed remedy for long branch attraction is greatly potentiated by the availability of higher-level phylogenies for unstable groups, such as Acariformes[11] and Palpigradi.[2] However, representation of solifuges in phylogenomic works has historically been driven entirely by the availability of sequence-grade tissues, rather than by representation of phylogenetically significant exemplars, given that the internal phylogenetic structure of this order has never been fathomed.[25–28,53,54] It is possible that this oversight may have underlain their known predilection for topological instability in some phylogenomic datasets.[25,26,42]

To redress this basic gap in our understanding of Solifugae, we generated the first higher-level phylogeny of the group, leveraging natural history collections worldwide to sample all extant families and reconcile a dated phylogeny against the fossil record. We obtained a robust backbone tree topology, with nearly all interfamilial nodes well-supported and recovered across an array of occupancy thresholds with support. Our results show that most solifuge families represent cohesive groups, with many families exhibiting high fidelity to specific biogeographic terranes. Curiously, we recovered the North American family Eremobatidae as the sister group of Gylippidae, which is restricted to the Old World (Central Asia and South Africa), with these two taxa in turn sister group to a large clade of Old World families (Galeodidae, Karschiidae, and Rhagodidae). This result was unanticipated because gylippids were previously considered part of Karschiidae,[43] and because a North American sister group of this family implies a markedly disjunct distribution. However, this relationship is consistent with shared traits of cheliceral architecture and dentition in these two families.[46]

Paralleling this outcome, the other groups of New World solifuges were also recovered as nested within a clade of Old World taxa (Ceromidae, Solpugidae, Hexisopodidae, and Old World daesiids). Our analyses consistently recovered a clade formed by the South American family Mummuciidae, the New World family Ammotrechidae, and the South American subset of the paraphyletic group Daesiidae (the "Daesiidae III" clade). Within each, we additionally found close relationships between geographically proximate subtaxa. These results suggest a prominent phylogenetic signature in solifuge biogeographic distributions, and across varying depths of the phylogeny. This biogeographic signal, together with the stable relationships we recovered across analyses, thus serves as the namesake for the two suborders we establish herein for the Laurasian and Gondwanan solifuge clades. This definition of suborders by ancestral distribution is also upheld by alternative coding of biogeographic areas as continents or supercontinents.

Three relationships exhibited instability across analyses and invite further scrutiny. First, the fossorial group Hexisopodidae (commonly, "mole solifuges"), from southern Africa, exhibited some topological instability, being recovered as either the sister group of Solpugidae in a minority of analyses, or with a clade formed by Daesiidae + Mummuciidae + Ammotrechidae. Both placements are partially supported by available morphological data. Specifically, analyses of solifuge sperm ultrastructure previously showed that hexisopodids and solpugids share similarities in the fine structure of spermatozoa, such as a conical acrosomal vacuole that is located within the chromatin body;[45] an older work had also suggested that hexisopodids may constitute a derived subtaxon of Solpugidae.[55] But hexisopodids also exhibit digitiform protuberances of membranes and putative deposits of stored glycogen, which are similarly observed in Daesiidae and Ammotrechidae, in addition to Solpugidae.[45] These data strongly accord with the recovery of these four families in a clade across our analyses, but we add the caveat that morphological data for solifuge sperm ultrastructure remain fragmentary; characteristics of the spermatozoa have been surveyed only in seven of the 12 families to date.[44,45] In particular, data on the sperm ultrastructure of Mummuciidae, a member of this group, are not presently available.

The second relationship that exhibited instability across analyses was the placement of Mummuciidae. While mummuciids were typically recovered as nested within Ammotrechidae, we did recover this clade as the sister group of the ammotrechids in a minority of analyses (albeit without support). As with the previously discussed case, mummuciids were previously a subtaxon of Ammotrechidae, and thus a nested placement of this putative family would not be unprecedented.[43] Given the complexity of ammotrechid subfamilies (a subset of which were sampled here), we submit that future systematic revisions of the South American solifuges must consider mummuciids as potentially derived members of Ammotrechidae.

Bird et al.[46] defined three types of flagella: setiform, sessile and composite. The setiform flagellum has a uniform shape and maintains affinities with plumose setae. It is present in most Eremobatidae and Melanoblossinae. The sessile flagellum is clearly modified from its original setal form and its development does not appear to have involved a longitudinal invagination along the seta to form an alembic canal

(as occurs in the composite flagellum). A sessile flagellum occurs in Galeodidae, Rhagodidae, and Karschiidae (with the possible exception of *Karschia*). The composite flagellum comprises three sections: a stalk, a base, and a shaft. It occurs in Ammotrechidae, Ceromidae, Daesiidae, Hexisopodidae, Mummuciidae and Solpugidae. The three sections are present in Ceromidae, Hexisopodidae, Solpugidae, and some Daesiidae, whereas the shaft is considered lost in Ammotrechidae, Mummuciidae, and most Daesiidae. There are two groups where the homology of the flagellum is not clear: *Karschia* and Gylippinae. For the genus *Karschia*, three sections are recognized resembling stalk, base, and shaft; however, there is no obvious homology with the composite flagellum, and the alembic canal seems to be absent. Bird et al.[46] treated the karschiid flagellum as a sessile type. For the Gylippinae, Bird et al.[46] considered the flagellum as composite, but without a stalk and base, because it is fused to the dorsal surface of the fixed finger, similarly to Solpugidae. However, Bird et al.[46] also stated that the absence of stalk and base may suggest that the flagellum of Gylippinae is of the sessile type. In the context of our results, when mapping flagellum type only, it is equally parsimonious to consider the flagellum of Gylippinae as sessile or composite, but the loss of stalk and base implies more steps, and thus the condition of a sessile flagellum in Gylippinae becomes more parsimonious (Figures S15 and S16). Thus, Boreosolifugae is characterized by the presence of a sessile flagellum and the Australosolifugae by the presence of composite flagellum. The setiform flagellum is most likely a secondary condition that evolved independently in Eremobatidae and Melanoblossinae, as previously suggested by Bird et al.[46] If the flagellum of Gylippinae were indeed of the composite type, this would suggest that the composite flagellum is the plesiomorphic condition, and that the sessile and setiform types are derived.

Solifuge diversification reflects ancient vicariance across Pangaea

The chronological sequence of Pangean fragmentation is well-documented, and numerous cases of biotic distributions and divergence time estimates have been shown to retain the signature of supercontinental breakup.[56–59] Our biogeographic analysis revealed the general pattern that the distribution of most solifuge clades is delimited by single biogeographic regions. Ancestral area optimizations in the internal nodes and the distribution of extant species (e.g., near-absence on oceanic and Darwinian islands) suggest that solifuges are habitat specialists adapted to specific environments, being largely confined to xeric habitats. In previous works, the origin of Solifugae has been estimated to span the late Cambrian or Ordovician, albeit with limited sampling of ingroup taxa.[3,10,13,15,28] Like many arachnid orders, the crown group of Solifugae dates to the Carboniferous or earlier, as reflected by its fragmentary fossil record.[29,50] Many solifuge families and genera are relatively young, with diversification of many genera occurring after the Cretaceous-Paleogene extinction event. While this may reflect an artifact of limited sampling, one possibility for the observation of relatively young ages of genus-level clades is that the recent aridification and the opening of comparatively young deserts may have opened new ecological niches for Solifugae, driving the diversification of this arid-adapted arachnid group by the mid-Cenozoic.[49,60,61] Their absence on dry continental landmasses such as Australia may be attributable to the recent timing of aridification of the Sahul Shelf, as well as the mesic and cooler past conditions of the Australian interior.[62] However, assessing this hypothesis using parametric tests requires extensive sampling of extant diversity, in tandem with consideration of variance in the ages of deserts worldwide. Targeted approaches like UCE sequencing offer the promise of renewed utility of natural history collections and revitalized prospects for hypothesis testing with poorly studied taxa.

We emphasize that various subtaxa were not included in this study, which is aimed specifically at higher-level relationships between families. Given the fidelity we observed between solifuges and the terranes that they inhabit, we anticipate that future sampling efforts will identify additional cases of non-monophyletic taxa from regions not represented in this study (e.g., *Dinorhax*; the putative Daesiidae of southern Asia), which will require intensive rounds of systematic revision under a phylogenomics lens. Such genomics-driven revisionary efforts have demonstrated marked success and efficiency in modernizing the classification and evolutionary analysis of comparably diverse groups, such as scorpions and harvestmen.[4,12,63–65]

Of immediate interest in this regard are the putative "Gylippidae" of southern Africa (the genera *Bdellophaga*, *Lipophaga*, and *Trichotoma*, which collectively form the subfamily Lipophaginae), whose distribution represents a biogeographic anomaly if they were indeed nested within Boreosolifugae (Figure 6). As an initial step to testing this placement, we inferred the relationships of this group by assembling a Sanger dataset. Sequence data for four Sanger loci (one of these a nuclear ribosomal gene) were previously generated for a single species of *Trichotoma*, as an outgroup exemplar in an investigation of Eremobatidae.[49] Given that nuclear ribosomal bycatch is commonly present in both transcriptomes and UCE assemblies, we were able to generate a 22-taxon alignment sampling the major clades of Solifugae for the 421-bp region of 28S available for *Trichotoma michaelseni*. As shown in Figure S17, the gene tree of 28S recovered the basal split between Boreosolifugae and Australosolifugae, but placed *Trichotoma* within Australosolifugae, with support (as the sister group of Ceromidae). These results reinforce a basal split of solifuges that is consistent with subdivision into Laurasian and Gondwanan clades, and tentatively suggest that Lipophaginae should be elevated to family status. Extrapolating from these biogeographic trends, and based upon morphological correspondences of the chelicera,[46] we strongly suspect that the southeast Asian genus *Dinorhax* (putatively a melanoblossid) is a member of Boreosolifugae.

The phylogenetic tree topopology presented herein bookends a ca. 20-year gap of available higher-level phylogenies for the orders of Chelicerata.[31] The relationships inferred herein are anticipated to aid taxonomic revisionary efforts, and revitalize morphological comparative studies and biogeographic efforts within this understudied group.

Limitations of the study

We note that taxon sampling in this study is limited to available and sequence-grade exemplars of extant families; we were unable to obtain material for some unusual taxa, such as *Bdellophaga*, *Lipophaga*, and *Dinorhax* to test the current placement of this group. Sampling of these

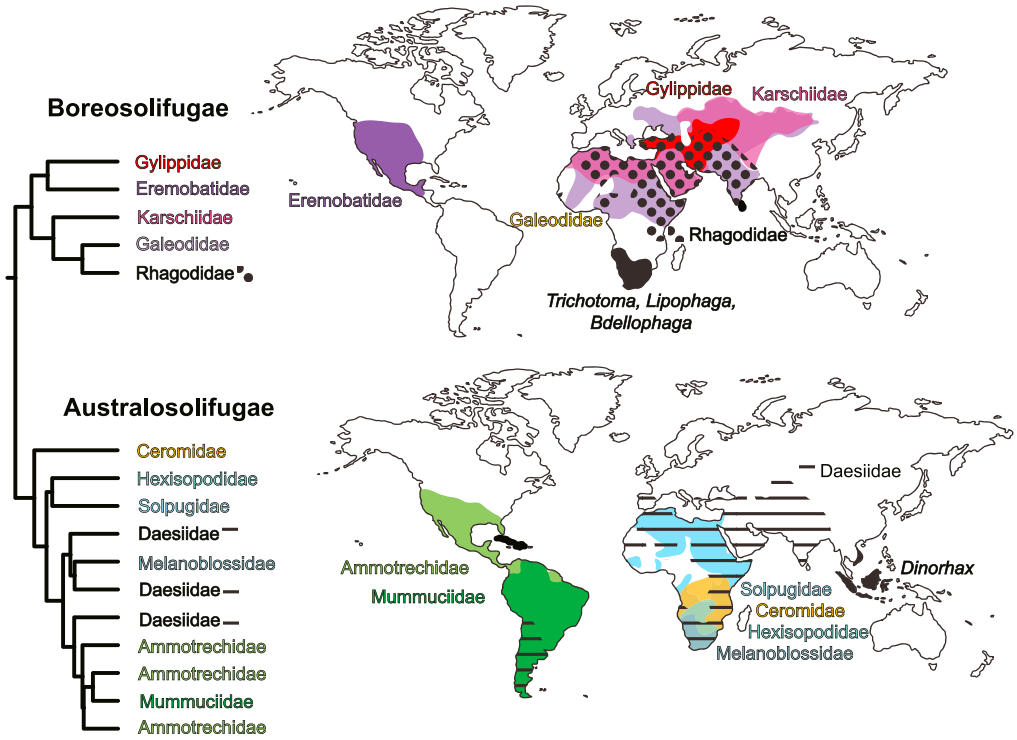

Figure 6. Distribution of Solifugae families mapped on the 25% occupancy-based UCE phylogeny and world map indicating the deep split between Laurasian and Gondwanan taxa

taxa is anticipated to test the reconstruction of the biogeographic history of the two Solifugae suborders defined herein. Future investigations of solifuge evolutionary history must emphasize the role of global perturbations of the past and the downstream effects of climatic cycles on diversification and range expansion of these cryptic, long-neglected arthropods.

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107684.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Data matrices were deposited on Mendeley	This study	https://doi.org/10.17632/r6cmspfr25.1
Raw reads are available from the NCBI Sequence Read Archive	This study	BioProject PRJNA1000606
Experimental models: Organisms/strains		
Solifugae species	This study	Table S2
Software and algorithms		
PHYLUCE pipeline	Faircloth[66]	https://phyluce.readthedocs.io/en/latest/
IQ-TREE2	Nguyen et al.[67]	http://www.iqtree.org/
BBMap	Bushnell[68]	https://github.com/BioInfoTools/BBMap
LSD2	To et al.[69]	https://github.com/tothuhien/lsd2
MCMCTREE	part of PAML v.4.8; Yang[70]; dos Reis and Yang[71]	https://github.com/abacus-gene/paml
RASP 4.0	Yu et al.[72]	https://github.com/sculab/RASP
MUSCLE v.3.2.1	Edgar[73]	https://www.ebi.ac.uk/Tools/msa/muscle/
MARE v0.1.2-rc	Meyer et al.[74]	https://bonn.leibniz-lib.de/en/research/research-centres-and-groups/mare
Other		
Spider2Kv1 probe set	Kulkarni et al.[14]	https://doi.org/10.1111/1755-0998.13099

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources can be directed to and will be fulfilled by the lead contact, Dr. Siddharth Kulkarni (sskulkarni24@wisc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw reads data have been deposited at NCBI Sequence Read Archive and are publicly available as of the date of publication. Original data matrices have been deposited on Mendeley. DOIs are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Species sampling

Specimens sequenced for this study were collected from field sites as part of our recent collecting campaigns, as well as contributed by collections of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN); The National Natural History Collections, The Hebrew University, Jerusalem, Israel (NNHC); the Denver Museum of Nature & Science, Colorado, United States (DMNS); the National Collection of Arachnida, Agricultural Research Council, South Africa (ARC-PCP), and the Zoological Museum Hamburg (ZMH). Collecting permits for study taxa were issues to different laboratories over several years; permitting data are available upon request from the authors. For *de novo* sequencing of UCEs, sampled exemplars of each of the 12 described extant families, as follows: 22 Ammotrechidae, three Ceromidae, 14 Daesiidae, two Eremobatidae, three Galeodidae, two Gylippidae, three Hexisopodidae, seven Karschiidae, three Melanoblossiidae, seven Mummuciidae, six Rhagodidae, and 21 Solpugidae. To this dataset, we added UCE loci from four published

solifuge transcriptomes and one de novo transcriptome, comprising two Eremobatidae and two Galeodidae and from four UCE assemblies comprising three Eremobatidae and one Daesiidae.

Outgroup sampling was influenced by previous works that have inferred various possible placements of Solifugae in the chelicerate Tree of Life, such as a sister group to Acariformes and Palpigradi, or part of a clade with Riniculei, Opiliones, and Xiphosura.[25–27,40,42] Given this instability across studies, we sampled 2–3 terminals of every extant chelicerate order, prioritizing the sampling of basal splits in each outgroup order. Outgroup datasets were drawn from published transcriptomes and from our previous UCE assemblies that were captured with the same probe set (detailed below). All tree topologies were rooted with Pycnogonida. Accession data for all specimens in this study are provided in Table S2.

Ultraconserved element sequencing and phylogenomic analyses

For newly sequenced specimens, 1–4 legs or tissue teased from a single chelicera from single specimens were used for DNA extractions using the DNeasy Blood and Tissue kit and the QIAamp DNA Mini kit (Qiagen Inc., Valencia, CA). Libraries were prepared and enriched following protocols outlined by Kulkarni et al.[14] and Miranda et al.[75] All pools were enriched with the Spider2Kv1 probe set[14] following the myBaits protocol 4.01 (Arbor Biosciences). Sequencing was performed on an Illumina NovoSeq platform. Assembly, alignment, trimming and concatenation of data were performed using the PHYLUCE pipeline (publicly available at https://phyluce.readthedocs.io/en/latest/). UCE contigs were extracted using the Spider2Kv1 probe set[14] to target 2,021 UCE loci (locus recovery listed in Table S1). To augment the UCE dataset with RNASeq datasets, we followed the assembly, sanitation, reading frame detection, and UCE retrieval pipeline outlined by Kulkarni et al.[76] Homology screening was performed by employing liberal (65%) and stringent (80%) probe-to-library identity and coverage mapping thresholds, following recent implementations.[77]

Phylogenomic analyses

The assembly, alignment, trimming and concatenation of data were done using the PHYLUCE pipeline (publicly available at https://phyluce.readthedocs.io/en/latest/). We generated a more complete matrix subset to minimize missing data by (1) filtering partitions by occupancies 25% and 40%, and (2) the automatic matrix reduction criterion using MARE v0.1.2-rc[74] with default parameters. Phylogenetic analyses were performed on the unpartitioned nucleotide data using IQ-TREE[67] version 2. Model selection was allowed for each unpartitioned dataset using the TEST function and an independent partitioned analysis using MFP+MERGE function to accommodate a best-fit partition by merging across prescribed locus partitions.[78,79] Nodal support was estimated via 2,000 ultrafast bootstrap replicates[79] with 10,000 iterations. To reduce the risk of overestimating branch support with ultrafast bootstrap due to model violations, we appended the command -bnni. With this command, the ultrafast bootstrap optimizes each bootstrap tree using a hill-climbing nearest neighbor interchange (NNI) search based on the corresponding bootstrap alignment.[79] We also trialed a multispecies coalescent analysis with Accurate Species TRee Algorithm (ASTRAL)[80,81] using gene trees, with input tree topologies reconstructed using IQ-TREE. However, the genes trees and the species tree were largely discordant. This phenomenon has been noted previously and is understood to result from the short lengths of typical UCE locus alignments,[82–84] and thus we did not proceed with this analysis.

GC-content

GC content can bias the phylogenetic inferences reconstructed using genome scale data.[85] To address this, we computed GC content for each taxon in the concatenated alignment using a custom script paired with BBMap (https://sourceforge.net/projects/bbmap/).

Phylogenomic dating

As no molecular phylogeny of the order exists and the solifuge fossil record is sparse, the timeframe of solifuge diversification is effectively unknown. To provide a temporal context to the divergences we inferred, we performed divergence time estimation using two approaches: a least-squares approach method (LSD2)[69] and a Bayesian inference approach (MCMCTree; part of PAML v.4.8[70,71]). As LSD2 requires at least one fixed date, we used an absolute calibration of 314.6 Ma for the crown Orthosterni fossil, *Compsoscorpius buthiformis*. We optimized the fossil information-based calibrations on the tree topology inferred from the 25% occupancy data set for the basis for divergence time estimation, implementing uniform node age priors to accommodate the scarcity of terrestrial chelicerate fossils. The root age was set to a range of 550–600 Mya, following Wolfe et al.[86] The crown age of Solifugae was constrained using a minimum age bound of 305 Ma, based on the Carboniferous fossil *Prosolpuga carbonaria* (Petrunkevitch, 1913). The stem age of Ceromidae was constrained using a minimum age bound of 115 Ma, based on the Cretaceous fossil *Cratosolpuga wunderlichi* (Selden and Shear 2016). The recently described Burmese amber fossil *Cushingia ellenbergeri* was not included for calibration, as characters of this species potentially accord with a melanoblossid, a gylippid, or a rhagodid identity, which precludes its use for calibrating a specific node.[87,88] Outgroup nodes were calibrated using the oldest unambiguous fossils representing those clades. Justifications and references for node calibrations are provided in Table S6.

Ancestral area reconstruction

We reconstructed ancestral areas on internal nodes of the dated preferred tree and the combined tree using the package BioGeoBEARS[89] implemented in RASP 4.0.[72] Each of the terminals was assigned to one of the following biogeographic regions: Turanian, African tropics, Neotropical, Nearctic, Central Chile-Patagonia and Mediterranean. We chose this scheme of area coding based on the distribution of the

extant solifuges and following commonly used area definitions from the literature. Some taxa such as ammotrechids and eremobatids are distributed in the United States and Mexico. For these taxa, we coded the area as Nearctic following the delimitation of this region by Escalante et al.[90] Additionally, to assess the influence of area coding, we alternatively coded areas by continents (corresponding to their geological plate); and more coarsely, as either Laurasia or Gondwana, based on the geological origin of those areas.

Assessment of *Trichotoma* placement

To infer the placement of the southern African "Gylippidae", we obtained a sequence of 28S rRNA from GenBank for *Trichotoma michaelseni* (accession no. KT276815.1). Using BLASTn, we retrieved the corresponding sequence of 28S rRNA from libraries of *Acanthogylippus* sp. (Gylippidae), *Ammotrechula* sp. 28, *Biton* sp. 47, *Blossia* sp. 45, *Ceroma* sp. 29, *Ceroma* sp. 30, *Chanbria regalis*, *Chelypus* sp. 64, *Dasycleobis* sp. 9, *Eremochelis andreasana*, *Eusimonia wunderlichi*, *Galeodes* sp., *Gluvia dorsalis*, Rhagodidae sp. 86, Rhagodidae sp. 87, *Rhinippus* sp. 71, *Uspallata pulchra*, *Zeria monteiri*, and *Zeriassa purcelli*. The tree topology was rooted with exemplars of Ricinulei (*Ricinoides feae*; JX951360.1) and Opiliones (*Troglosiro longifossa*; DQ518045.1). Alignment was performed using MUSCLE v.3.2.1[73] (Edgar 2004) and the tree topology was inferred using maximum likelihood in IQ-TREE, with automated model-fitted and 1000 ultrafast bootstrap resampling replicates (Figure S17).