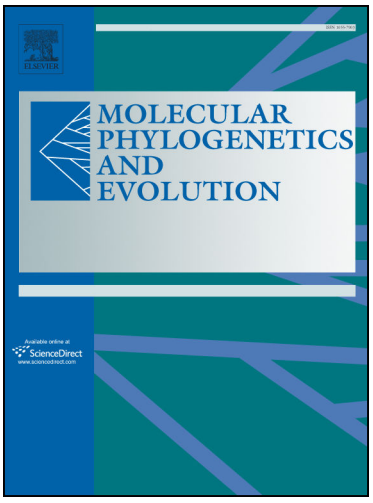

Phylogenomic data reveal three new families of poorly studied Solifugae (camel spiders)

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Phylogenomic data reveal three new families of poorly studied Solifugae (camel spiders)

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Highlights

- Solifugae families with transcontinental distributions are not monophyletic
- New family ranks proposed for *Dinorhax,* Lipophaginae and *Namibesia*
- New classification is consistent with vicariance biogeographic origin for suborders

Graphical abstract

Submitted as a separate file in the online submission system.

Abstract.

The systematics of the arachnid order Solifugae have been an enigma, owing to challenges in interpreting morphology, a paucity of molecular phylogenetic studies sampling across the group, and a dearth of taxonomic attention for many lineages. Recent work has suggested that solifuge families largely exhibit contiguous distributions and reflect patterns of vicariance, with the exception of three families: Melanoblossidae, Daesiidae and Gylippidae. Morphological studies have cast doubt on their existing circumscriptions and the present composition of these taxa renders their distributions as disjunct. We leveraged ultraconserved elements (UCEs) to test the phylogenetic placement of three key lineages of Solifugae that cause these anomalous distributions: *Dinorhax rostrumpsittaci* (putative melanoblossid), *Namibesia* (putative daesiid), and *Trichotoma* (putative gylippid). Phylogenetic placement of these three genera based on UCEs rendered the families that harbor them as para- or polyphyletic, recovering instead relationships that better accord with a biogeographic history driven by vicariance. Toward a stable and phylogenetically informed classification of Solifugae, we establish three new families, Dinorhaxidae **new rank**, Namibesiidae **new rank** and Lipophagidae **new rank**.

1. Introduction.

Over the past 15 years, molecular phylogenies of arachnids have proved paramount for settling historical debates over higher-level classification. Recent global phylogenies of spiders, scorpions, pseudoscorpions, and harvestmen using transcriptomes and target enrichment of ultraconserved elements (UCEs) have undergone iterative refinements, with ever increasing taxon sampling (Benavides et al., 2019; Bond et al., 2014; De Miranda et al., 2022; Fernandez et al., 2017; Fernández et al., 2014; Hedin et al., 2012; Kallal et al., 2021; Kulkarni et al., 2021, 2023a; Murienne et al., 2008; Santibáñez-López et al., 2022, 2019). These phylogenetic topologies revealed some disagreements with traditional taxonomic classification and paved the way for formal changes to render higher-level taxa monophyletic. Minor arachnid orders, such as Ricinulei, Schizomida, Pseudoscorpiones, Solifugae, and Amblypygi (monikered the “neglected cousins” by Harvey (2002)), have undergone similar reorganization of higher-level groupings upon influx of molecular sequence data. These advances in molecular phylogenetics facilitated the breaking of impasses engendered by plastic or inconsistent morphological characters; rarity of key lineages; difficulty of collection; and small body size. The past decade alone has witnessed the first molecular phylogenies for Ricinulei (hooded tick-spiders; Fernández and Giribet (2015)), Palpigradi (microwhip scorpions; Giribet et al. (2014)), Uropygi (vinegaroons; Clouse et al. (2017)), Schizomida (short-tailed whip scorpions; Clouse et al. (2017)), and Amblypygi (whip spiders; De Miranda et al., (2022)).

Solifugae (“camel spiders” or “sun spiders”), was the last arachnid order to receive molecular phylogenetic attention (Cushing et al. 2015; Kukarni et al. 2023b). These agile, desert-adapted arachnids are equipped with robust chelicerae and are renowned for their speed and aggression. Solifuges include over 1,200 species classified in 12 families, and are distributed in all continents except Australia and Antarctica (World Solifugae Catalog, 2022). Subsequent to the first phylogenetic investigation of this group using ultraconserved elements, solifuges were divided into two suborders: Boreosolifugae unites five families predominantly distributed in Laurasian landmasses, and Australosolifugae harbors seven families predominantly distributed in Gondwanan terranes (Kulkarni et al. 2023b). Based upon ancestral area reconstruction and molecular dating, it was proposed that the phylogeny of solifuges exhibited a strong biogeographic signature of vicariance, justifying the establishment of suborders that reflected their inferred ancestral areas.

This subdivision based upon biogeography holds fairly well for the constituent members of Boreosolifugae (Eremobatidae, Galeodidae, Gylippidae, Karschiidae, and Rhagodidae) and Australosolifugae (Ammotrechidae, Ceromidae, Daesiidae, Hexisopodidae, Melanoblossidae, Mummuciidae, and Solpugidae). However, three of these families include rarely sampled lineages whose distributions are inconsistent with the previously outlined biogeographic scenario of Kulkarni et al. (2023b) (Table 1). The families Daesiidae (Australosolifugae), Melanoblossidae (Australosolifugae), and Gylippidae (Boreosolifugae) include putative members that incur transcontinental disjunct distributions. Melanoblossidae is comprised of two subfamilies: Melanoblossinae Roewer, 1933 restricted to southern Africa and Dinorhaxinae Roewer,

1933 in southeast Asia represented by a single species–*Dinorhax rostrumpsittaci* (Simon, 1877). Daesiidae includes Blossiinae Roewer, 1933, Daesiinae Kraepelin, 1899, Gluviinae Roewer, 1933, Gluviopsinae Roewer, 1933, Gnosippinae Roewer, 1933, Namibesiinae Wharton, 1981, Triditarsinae Roewer, 1933, and several genera of uncertain placement, such as *Ammotrechelis*, *Syndaesia*, and *Valdesia*. Namibesiinae consists of a single species–*Namibesia pallida* Lawrence, 1962—and was also previously regarded as part of the group of daesiid genera with uncertain subfamilial placement. Notably, several of the south American daesiids (e.g., *Ammotrechelis*, *Syndaesia*, and *Valdesia*) have been suggested to render Daesiidae paraphyletic (Kulkarni et al. 2023b), which calls into question the familial placement of the monotypic Namibesiidae. Lastly, Gylippidae includes two subfamilies: the Asian Gylippinae and the sub-Saharan Lipophaginae.

Such geographic disjunctions call into question the division of solifuges into Laurasian and Gondwanan groups. For example, the placement of *Dinorhax* (which inhabits Laurasian terranes) within the Australosolifugae, as well as the placement of the three genera of Lipophaginae (*Bdellophaga*, *Lipophaga*, and *Trichotoma*) within the Boreosolifugae, are anomalous for camel spiders. However, none of these groups were sampled in the first family-level phylogeny of Kulkarni et al. (2023b). To test the validity of a vicariant origin of the two suborders, we inferred the placement of the australosolifugid genera *Dinorhax* (Melanoblossidae, Dinorhaxinae), *Namibesia* (Daesiidae, Namibesiinae), as well as the boreosolifugid genus *Trichotoma* (Gylippidae, Lipophaginae) using a molecular phylogeny based on ultraconserved elements and leveraging museum collections for these lineages. Here, we show that the traditional placement of these three taxa is not supported by the molecular phylogeny, and that biogeography is a better predictor of relationships within Solifugae than the traditional classification.

2. Materials and methods

2.1. Taxon sampling and DNA extraction

We included 121 terminals, nine newly sequenced UCEs and others from previous studies, representing 12 Solifugae families. We integrated our sequences to the UCE dataset of Kulkarni et al. (2023b). Specimens sequenced for this study were obtained from collections of the Vietnam Academy of Science and Technology and the National Museum of Namibia. For newly sequenced specimens, 1–2 legs 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). DNA extractions were quantified using high sensitivity Qubit fluorometry (Life Technologies, Inc.).

2.2. Ultraconserved element sequencing

Libraries were prepared and enriched following protocols outlined by Kulkarni et al. (2020; 2023b). All pools were enriched with the Spider2Kv1 probe set (Kulkarni et al. 2020) following the myBaits protocol 4.01 (Arbor Biosciences). Paired end sequencing (2 × 150 bp) was performed on an Illumina NovaSeq 6000 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 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. (2021). Homology screening was performed using 65% probe-to-library identity and coverage mapping thresholds.

2.3. Phylogenomic analyses

We applied gene occupancies of 1% and 25% to facilitate inclusion of the maximum number of UCEs. As a test for robustness, we also applied occupancy thresholds of 40% and 50%, though *Dinorhax* was pruned from this analysis, because of its low UCE yield (15 loci). Phylogenetic analyses were performed on the partitioned nucleotide data using IQ-TREE v.2.1.2 (Nguyen et al. 2015). Model selection was allowed for each dataset using ModelFinderPlus (Kalyaanamoorthy et al., 2018; Hoang et al. 2018) using MFP+MERGE flag for each locus partition. Nodal support was estimated via 1,000 ultrafast bootstrap (UFBoot) resampling replicates (Hoang et al. 2018) and the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010). To reduce the risk of overestimating branch support with UFBoot due to model violations, we appended the command -bnni. With this command, the UFBoot optimizes each bootstrap tree using a hill-climbing nearest neighbor interchange (NNI) search based on the corresponding bootstrap alignment (Hoang et al. 2018). Further, we also evaluated our phylogenies against two trees where the existing familial affiliation was constrained to be monophyletic for *Dinorhax* (Melanoblossidae) and *Trichotoma* (Gylippidae) using topology tests. These tests include approximately unbiased (AU), bootstrap proportion (BP), SH-aLRT, Kishino-Hasegawa (KH), and expected likelihood weight (ELW) using 1,000 resampling estimated log-likelihoods (RELL) in IQ-TREE. (Table S3).

3. Results

Our complete UCE dataset included a total of 1,237 loci, 259,261 sites and 77,716 parsimony informative sites whereas the 25% occupancy included 669 loci, 136,722 sites and 50,760 parsimony informative sites. Both datasets recovered similar phylogenetic relationships, except for the position of *Dinorhax*. Daesiidae, Melanoblossidae, Ammotrechidae and Gylippidae were recovered as non-monophyletic, whereas the remaining families formed well-supported clades.

In analyses of the 1% occupancy matrix, *Namibesia* (Daesiidae, Namibesiinae) was recovered as the sister group to a clade including Ammotrechidae, Mummuciidae, Melanoblossidae, and the remaining Daesiidae (Figure 1; BR = 99%; aLRT = 100%). *Trichotoma* (Gylippidae, Lipophaginae) was recovered as the sister group to Ceromidae within Boreosolifugae (BR = 86%; aLRT = 100%), whereas the remaining Gylippidae were recovered as sister group to Eremobatidae within Australosolifugae (Figure 1; BR = 83%; aLRT = 79.5%). These placements and the monophyly of *Namibesia* and *Trichotoma* were recovered at higher occupancies of 40% (217 loci) and 50% (36 loci) as well (Figures S2-3). *Dinorhax* (Melanoblossidae, Dinorhaxinae) formed the sister group of the clade Gylippidae + Eremobatidae within Australosolifugae (BR = 39%; aLRT = 63.4%), whereas the remaining Melanoblossidae formed a clade which was sister group of Daesiidae I (*sensu* Kulkarni et al. 2023b) within Boreosolifugae (Figure 1; BR = 88%; aLRT = 55.7%). The 25% occupancy dataset also recovered *Dinorhax* within Australosolifugae, but as the sister group to Karschiidae (Figure 2; BR = 36%; aLRT = 94.6%). Topology tests significantly excluded the placement of *Dinorhax* within Melanoblossidae and *Trichotoma* within Gylippidae (Figures S4, S5, Table S3). As a step toward a stable and phylogenetically-informed classification reflecting these results, we propose a revised familial classification for Solifugae with the three changes reflected below.

3.2. CLASSIFICATION

Dinorhaxidae Roewer **new rank**

Dinorhaxinae Roewer, 1933

Type genus. *Dinorhax* Simon, 1879

Composition. *Dinorhax rostrumpsittaci* (Simon, 1877), monotypic.

Nomenclatorial note. The subfamily Dinorhaxinae was proposed by Roewer, 1933, and therefore, we propose the authority of Dinorhaxidae **new rank** to be Roewer, 1933 following the (ICZN, Articles 34.1, 50.3).

Type locality: Jailolo (as Gilolo), Maluku, Indonesia.

Distribution. Indonesia (Maluku), Vietnam.

Diagnosis. [Adapted from Yamasaki et al. (2018) for *Dinorhax*] Dinorhaxidae is distinguishable from Solifugae families by the combination of following characters: two or three eyespots on each anterolateral propeltidium lobe, a slit-like anus on the venter of terminal abdominal segment, three dorsal spiniform setae on metatarsus II and III, and undivided telotarsi II, III, and IV. In males, the cheliceral fixed finger possesses one sessile form flagellum extending ventrally.

Remarks. Roewer (1934) included two genera in his Dinorhaxinae, *Dinorhax* from southeast Asia and *Lawrencega* from Namibia and South Africa based on two tarsomeres on leg IV. However, Lawrence (1967) transferred *Lawrencega* to Melanoblossinae. We could not find material to include this genus in our phylogenetic analysis. Wharton (1981) doubted its placement and indicated that the insertion point of the flagellum in *Dinorhax* resembles that in Karschiidae. Bird et al. (2015) noted that *Dinorhax* resembles Rhagodidae and Hexisopodidae with respect to cheliceral morphology, Eremobatidae with respect to cheliceral dentition, and *Karschia* Walter, 1889 (Karschiidae) in terms of flagellar morphology. *Dinorhax* is unique because it is the only Solifugae from southeast Asia and the only solifuge which occurs in tropical habitats. Yamasaki et al. (2018) redescribed and barcoded fresh material of *D. rostrumpsittaci* using cytochrome *c* oxidase subunit I from Vietnam (the same material studied herein).

Namibesiidae Wharton **new rank**

Namibesiinae Wharton, 1981

Type genus. *Namibesia* Lawrence, 1962

Type locality. Farm Djab, Kuiseb River Valley, Swakopmund, Namibia.

Composition. *Namibesia pallida* Lawrence, 1962, monotypic.

Nomenclatorial note. The subfamily Namibesiinae was proposed by Wharton, 1981, therefore we propose the authority of Namibesiidae new rank to be Wharton, 1981 following the (ICZN, Articles 34.1, 50.3).

Distribution. Namibia

Diagnosis. [Adapted from Bird et al. (2015) for Namibesiinae] Namibesiidae is distinguishable from other Solifugae families by an unusually large number of secondary teeth on fixed finger: two or three subdistal and submedial in both sexes. The finger dentition on movable finger is unmodified in both sexes represented by a single movable finger submedial tooth situated between pronounced proximal and medial teeth.

Lipophagidae Wharton **new rank**

Lipophaginae Wharton 1981

Type genus. *Lipophaga* Purcell, 1903.

Type locality. St. Helena Bay, Malmesbury Division, South Africa.

Composition. *Trichotoma brunnea* Lawrence, 1968; *Trichotoma fusca* (Roewer, 1941); *Trichotoma michaelseni* (Kraepelin, 1914); *Lipophaga kraepelini* Roewer, 1933; *Lipophaga trispinosa* Purcell, 1903; *Lipophaga schultzei* (Kraepelin, 1908); *Bdellophaga angulata* Wharton, 1981.

Distribution. southern Africa

Diagnosis. [Adapted from Bird et al. (2015) for Lipophaginae] Lipophagidae is distinguishable from other Solifugae families by the absence of male flagellum. In *Lipophaga* and *Bdellophaga*, it is replaced by a cluster of modified dorsal and ventral flagellar setae, including a ventral flagellar seta on the fixed finger (type B setiform flagellar complex), undifferentiated in *Trichotoma*. Lipophagids are characterized by

absent (in *Trichotoma*) or one subdistal tooth, one submedial tooth on fixed finger and single medial tooth on the movable finger.

Remarks. While we trialed the sequencing of *Lipophaga* specimens in this study, the age and state of preservation of this material precluded successful capture of a high number of UCEs. Here, we follow the interpretation of Bird et al. (2015) regarding the composition of this lineage, but future efforts to study the composition of Lipophaginae must test this inference by way of sampling all three constituent genera.

4. Discussion

Solifugae diversification was hypothesized to have been shaped by the Pangean breakup (Kulkarni et al. 2023b). The signature of vicariance and supercontinental fragmentation in the Solifugae tree of life is principally represented by its suborders–the Laurasian Boreosolifugae and the Gondwanan Australosolifugae. While derived groups within both suborders have since dispersed out of their ancestral ranges (Kulkarni et al. 2023b), the disjunct distributions of the traditionally defined Gylippidae and Melanoblossidae brought into question the broad subdivision of solifuges into northern and southern groups. But based on a single Sanger-sequenced marker (28S rRNA), Kulkarni et al. (2023b) recovered the putative gylippid *Trichotoma michaelseni* to be a member of Australosolifugae, and specifically, the sister group of Ceromidae, a result confirmed by expanded taxonomic and molecular sampling in this study. The phylogenetic placements recovered herein demonstrate that *Dinorhax* is similarly no exception to the demarcations incurred by Pangean breakup at the subordinal level. The recovery of this Southeast Asian species (previously considered closely related to the southern African Melanoblossidae) within Boreosolifugae underscores the congruence between the phylogeny of solifuges and the signature of vicariance in the distributions of this group.

Two species-rich and broadly distributed families, Daesiidae and Ammotrechidae, remain non-monophyletic at present. As a step toward rendering daesiids a more cohesive group, we established Namibesiidae as a separate family. Future efforts toward resolving the non-monophyly of these groups must emphasize dense sampling, in tandem with tests of relationships using expanded molecular datasets.

CRediT authorship contribution statement

Siddharth Kulkarni: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Takeshi Yamasaki:** Resources. **Luong Thi Hong Phung**: Resources, Writing – review & editing; **Nanguei Karuaera**: Resources, Writing – review & editing, **Savel Daniels**: Resources, Writing – review & editing, **Efrat Gavish-Regev**: Funding acquisition, Resources, Writing – review & editing, **Prashant Sharma:** Conceptualization, Data curation, Funding acquisition,

Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Accessibility

All alignment files and phylogenetic trees associated with this study will be made available online on Dryad. Raw sequence reads are available from the NCBI Sequence Read Archive, under BioProject accession PRJNAXXXXXX.

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FIGURE AND TABLE LEGENDS

Figure 1. A 1% occupancy maximum likelihood phylogeny reconstructed ultraconserved elements (UCEs) of Solifugae including 1,237 loci. Ultrafast bootstrap support was 95% or greater at most nodes, unless indicated otherwise.

Figure 2. A sankey comparison of interfamilial phylogenetic relationships of Solifugae compared between the 1% (left) and 25% (right) occupancy datasets.

Table 1. Distribution of Solifugae families taken from the World Solifugae Catalog (2022). Families with transcontinental disjunct distribution are marked in red.

Family	**Distribution**
Ammotrechidae	Neotropical
Ceromidae	Africa

Daesiidae	Africa+Turanian+Neotropical
Eremobatidae	Nearctic
Karschiidae	Africa+Turanian
Galeodidae	Africa+Turanian+India
Gylippidae	Africa+Asia
Hexisopodidae	Africa
Melanoblossidae	Africa+Asia
Solpugidae	Africa+Turanian
Mummuciidae	Neotropical
Rhagodidae	Africa+Turanian+India

Supplementary Figures

Figure S1. A maximum likelihood-based phylogeny of Solifugae using 25% UCE dataset.

Figure S2. A maximum likelihood-based phylogeny of Solifugae using 40% UCE dataset, without the inclusion of *Dinorhax*.

Figure S3. A maximum likelihood-based phylogeny of Solifugae using 50% UCE dataset, without the inclusion of *Dinorhax*.

Figure S4. Topology constraint used to test inclusion of *Dinorhax* within Melanoblossidae.

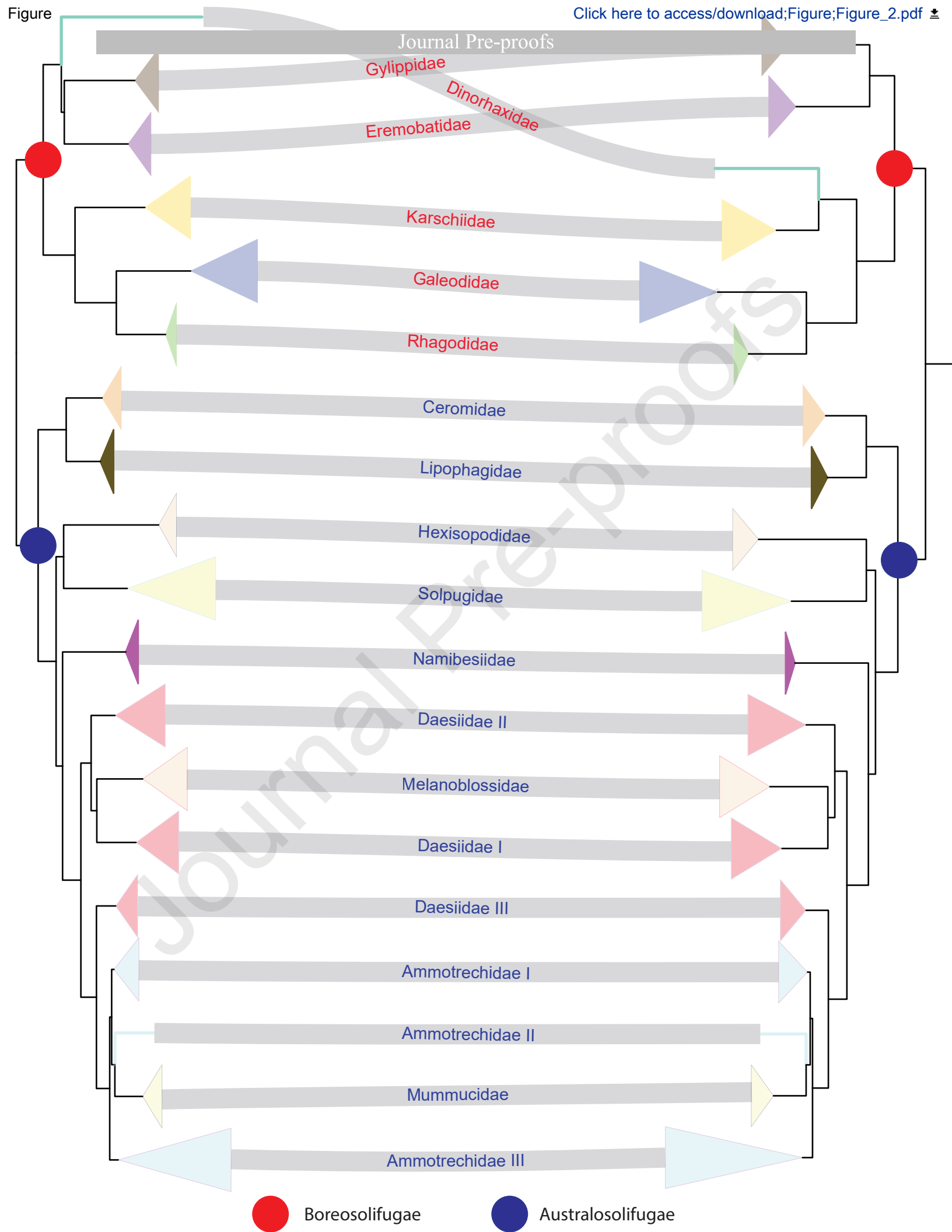
Figure S5. Topology constraint used to test inclusion of *Trichotoma* within Gylippidae.

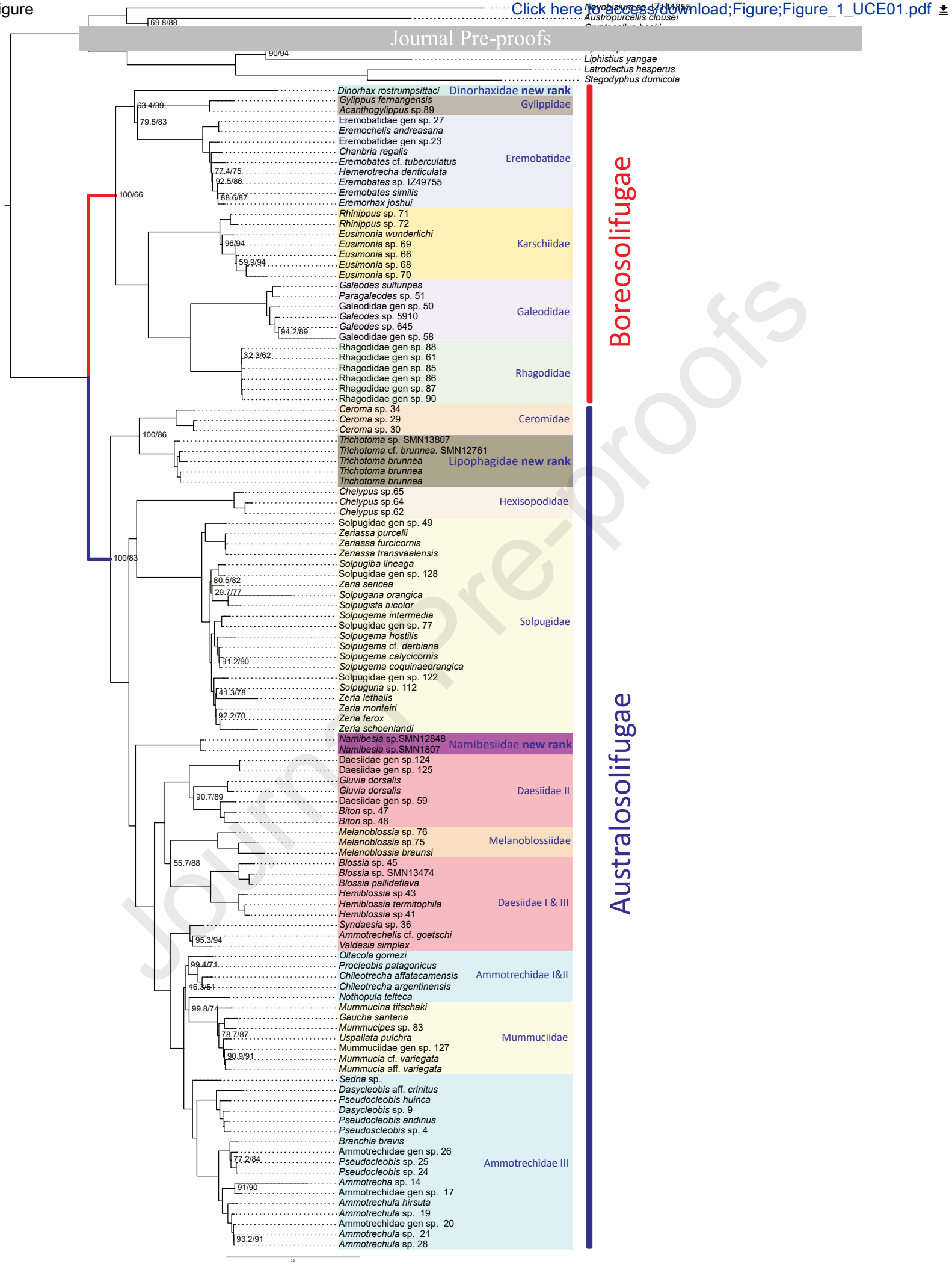
Table S1. Statistics of UCE loci recovered for each sequence and all datasets at 65% identity and coverage thresholds for probe-library match.

Table S2. Locality and collection metadata for newly UCE sequenced materials in this study.

Table S3. Statistics of the topology test conducted across different occupancies of UCE data sets with *Dinorhax* constrained within Melanoblossidae and *Trichotoma* constrained within Gylippidae, in two independent analyses.

Figure





Graphical Abstract