



An Opiliones-specific ultraconserved element probe set with a near-complete family-level phylogeny

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ABSTRACT

Sequence capture of ultraconserved elements (UCEs) has transformed molecular systematics across many taxa, with arachnids being no exception. The probe set available for Arachnida has been repeatedly used across multiple arachnid lineages and taxonomic levels, however more specific probe sets for spiders have demonstrated that more UCEs can be recovered with higher probe specificity. In this study, we develop an Opiliones-specific UCE probe set targeting 1915 UCEs using a combination of probes designed from genomes and transcriptomes, as well as the most useful probes from the Arachnida probe set. We demonstrate the effectiveness of this probe set across Opiliones with the most complete family-level phylogeny made to date, including representatives from 61 of 63 currently described families. We also test UCE recovery from historical specimens with degraded DNA, examine population-level data sets, and assess “backwards compatibility” with samples hybridized with the Arachnida probe set. The resulting phylogenies – which include specimens hybridized using both the Opiliones and Arachnida probe sets, historical specimens, and transcriptomes – are largely congruent with previous multi-locus and phylogenomic analyses. The probe set is also “backwards compatible”, increasing the number of loci obtained in samples previously hybridized with the Arachnida probe set, and shows high utility down to shallow population-level divergences. This probe set has the potential to further transform Opiliones molecular systematics, resolving many long-standing taxonomic issues plaguing this lineage.

1. Introduction

Sequence capture phylogenomics, especially of ultraconserved elements (UCEs), has become a popular approach for collecting sub-genomic scale data (100s – 1000s of loci) for phylogenetic and evolutionary analyses. UCEs have been useful across a broad range of time scales, including deep time (e.g., 100s of millions of years) down to species and population level studies (e.g., Smith et al. 2014; Derkarabetian et al. 2022a). Since the initial demonstration of the UCE sequence capture approach with the Tetrapoda probe set (Faircloth et al. 2012; McCormack et al. 2012), a plethora of custom-made probe sets have been developed for many diverse lineages across the tree of life (e.g., Faircloth, 2017). This is especially true for invertebrate taxa, with these probe sets immediately and significantly contributing to recent research in these lineages, for example, Hymenoptera (Branstetter et al. 2017), Anthozoa (Quattrini et al. 2018), and Arachnida (Starrett et al. 2017). Specifically in Arachnida, the majority of the recent phylogenomic studies have used the UCE approach, targeting a diverse array of evolutionary questions across taxonomic levels (e.g., Derkarabetian

et al. 2018; Wood et al. 2018; Kulkarni et al. 2021; Azevedo et al. 2022; Chamberland et al. 2022; Derkarabetian et al. 2022a; Derkarabetian et al. 2022b).

Arachnids are an ancient lineage, with divergences between major lineages occurring at least 500 million years ago (e.g., Lozano-Fernandez et al. 2020). As such, the Arachnida probe set spans some of the deepest divergences covered in any UCE probe set to date. The Arachnida 1.1Kv1 probe set (Faircloth 2017; Starrett et al. 2017) has been extremely useful and will continue to be useful in many arachnid lineages, especially “minor” arachnid orders (e.g., Ricinulei, Palpigradi) for which developing a specific probe set may not be cost-efficient. However, it is limited in terms of the number of loci targeted (1120 loci) relative to other arthropod probe sets. Creating a probe set based on a more inclusive lineage (i.e., specific orders within Arachnida) will increase specificity of the probes and ultimately the total number of loci recovered (e.g., Gustafson et al. 2019). Within arachnids, a more specific probe set was created for the order Araneae (spiders; Kulkarni et al. 2020), which targets almost twice as many UCEs as the Arachnida probe set (2021 loci versus 1120), and even more specific probe sets were

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made for Liphistiidae targeting 3111 loci (Xu et al. 2021) and the “RTA-clade” spiders targeting 3802 loci (Zhang et al. 2023).

The order Opiliones (Fig. 1; commonly called harvesters, harvestmen, or daddy-long-legs) is the third most diverse lineage of arachnids, after Acari (mites and ticks) and Araneae, with over 6,700 species currently described (Kury et al., 2020), although estimates of true diversity are at least 10,000 species (Machado et al. 2007). The Arachnida probe set has been used heavily in Opiliones systematics, however, at the time that the Arachnida probe set was created, there were no Opiliones genomes published. Recently, Gainett et al. (2021) published the genome of *Phalangium opilio* Linnaeus, 1758, the first for Opiliones. Given this newly published genome, the deep divergences covered in the arachnid probe set, and the increasing importance of UCEs in Opiliones systematics, an Opiliones-specific UCE probe set would be useful. As seen with the spider probe sets, an Opiliones-specific probe set would increase the number of loci targeted across this lineage. Increasing the specificity and number of targeted loci will likely equate to more on-target loci sequenced from historical specimens with degraded DNA, which can be critical in systematic studies of the many rare and difficult to collect taxa.

Here, we designed and tested an Opiliones specific probe set, paying special attention to testing its efficacy on historical specimens with degraded DNA, and to “backwards compatibility” with the Arachnida probe set (i.e., ability to incorporate data from samples previously hybridized with the Arachnida probe set). Using this newly developed Opiliones-specific probe set, we reconstruct a family-level phylogeny that includes the vast majority of Opiliones families (the most complete taxon set to date), and demonstrate the utility of the probe set to species and population-level divergences.

2. Materials and methods

2.1. Genome preparation and sequencing

Limited genomic resources are available for Opiliones, and only one genome is currently published. As such, we generated genome-level sequence data for other major lineages within Opiliones for use in creating a set of genome-derived probes, including *Aoraki denticulata* (Forster, 1948) (MCZ:IZ:141172; Cyphophthalmi) and *Karamea lobata* Forster, 1954 (MCZ:IZ:152313; Laniatores).

The *Aoraki denticulata* sample was extracted and sequenced as part of a previous study (Baker et al. 2020). The sample was initially preserved in RNAlater and then flash frozen in liquid nitrogen upon arrival in the lab. The sample underwent a “double extraction” protocol (RNA and DNA), and following separation of DNA and RNA, 10 µl of DNA were isolated and purified using a Zymo-spin column (Zymo Research). From this, 2.5 µl of DNA were used in whole genome amplification using the REPLI-g WGA kit (Qiagen), then library preparation used the Kapa HyperPrep Kit (Kapa) following manufacturer’s protocol. Short reads were sequenced on an Illumina HiSeq 2500 with 150 bp paired end reads at the Bauer Core Facility at Harvard University. The *Karamea lobata* sample was sequenced with both short and long reads. For short read sequencing, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen), library preparation followed manufacturer’s protocol using the Kapa HyperPrep Kit (Kapa), and then sequenced on a NovaSeq S4 with 150 bp paired end reads at the Bauer Core Facility at Harvard University. For long reads, high molecular weight DNA was extracted via standard phenol–chloroform extraction and sequenced on a PromethION at the Bauer Core Facility at Harvard University, yielding 6.1 Gb of data.

The genome assembly pipeline for each genome varied depending on the amount and type of data. Genome sizes for the two genomes were estimated from raw Illumina reads using Jellyfish and GenomeScope 2.0 with kmer length of 21 (Vurture et al. 2017; Ranallo-Benavidez et al. 2020). The Cyphophthalmi *A. denticulata* had an estimated genome size of 457 Mb, while the Laniatores *K. lobata* had an estimated genome size

of 1.8 Gb. Raw Illumina reads for both species were trimmed using TrimGalore (Krueger 2015). For *A. denticulata*, SPAdes v 3.12.0 (Bankevich et al. 2012) was used to assemble the short reads into contigs. The *K. lobata* long reads were assembled using flye v2.8.2 (Kolmogorov et al. 2019) and then polished with short reads using HyPo (Kundu et al. 2019). Due to the large file sizes, the trimmed reads for *K. lobata* were normalized using bbnorm (<https://github.com/BioInfoTools/BBMap>), prior to polishing to reduce computational memory needed for the program to run.

The two assembly outputs were smaller than their respective estimated genome sizes due to sequencing difficulties and both had low contiguity. *K. lobata* reads were assembled into a 738 kb assembly made up of 40,735 contigs, with a N50 of 36,834. The *A. denticulata* reads were assembled into a 373 kb assembly made up of 432,375 contigs, with a N50 of 868 bp. We ran BUSCO v5 (Manni et al., 2021) using the Arthropoda OrthoDB v10 database (1013 genes) to assess completeness. In *A. denticulata* 12.1% single copy complete BUSCO genes were recovered, an additional 32.0% were present but fragmented, and 55.3% were not detected. In *K. lobata* 37.7% single copy complete BUSCO genes were recovered, an additional 19.8% were present but fragmented, and 42.5% were not detected. Further statistics on sequencing and assembly can be found in Supplementary Table 1.

2.2. Probe set design

Probes for the Opiliones-specific probe set were derived from three different sources: published transcriptomes, published and newly generated genomes, and the already published Arachnida 1.1Kv1 probe set (Faircloth, 2017). Methods for each source of probes are described separately. Probe set design in general followed the standard pipeline of Faircloth (2017) and the associated tutorial available at <https://phyluce.readthedocs.io>. For the base genome we selected the published *Phalangium opilio* genome (Gainett et al. 2021). For this, we downloaded the soft-masked “*Phalangium_opilio_v1_softmasked_NCBI.fa*” assembly file.

2.2.1. Transcriptome-derived probes

The transcriptomes selected for probe design were derived from previous studies (Hedin et al. 2012) or unpublished data, with the specific samples chosen based on the highest number of BUSCO genes found in each assembly for each of the four Opiliones suborders. Five exemplar transcriptomes were selected including: *Leptopsalis* sp. (MCZ:IZ:141287; Cyphophthalmi), *Pantopsalis cheliferoideis* (Colenso, 1883) (MCZ:IZ:133328; Eupnoi), *Trogulus martensi* Chemini, 1983 (Dyspnoi), *Algidia* sp. (MCZ:IZ:133198; Laniatores), and *Maracaynatum trinidadense* Šilhavý, 1979 (MCZ:IZ:144057; Laniatores). Two Laniatores samples were chosen as this suborder represents the majority of Opiliones diversity with over 4200 species described (~63% of Opiliones diversity). Transcriptome assembly files, after duplicates had been removed via cd-hit (Li and Godzik 2006; Fu et al. 2012), were converted to 2bit using faToTwoBit in the UCSC Genome Browser Blat package (Kent 2002). For each assembly 100 bp paired end reads were simulated using art (Huang et al., 2012). These simulated reads were then aligned to the base genome using stampy (Lunter and Goodson 2011), retaining only the reads that mapped to the base genome. Using bedtools (Quinlan and Hall 2010), the resulting BAM files were converted to BED format, sorted, and overlapping intervals were merged. Repetitive intervals were removed with phyluce_probe_strip_masked_loci_from_set and conserved loci were identified across all samples with phyluce_probe_get_multi_merge_table, both from the phyluce package (Faircloth 2016). A set of shared, conserved loci were retained if they were present in at least four of six taxa. To design a temporary probe set, fasta sequences were extracted from the base genome using phyluce_probe_get_genome_sequences_from_bed, 120 bp probes were then created using phyluce_probe_get_tiled_probes, and overlapping duplicate probes were removed using phyluce_probe_easy_lastz and phyluce_probe_remove_duplicate_hits_from_probes_using_lastz. Temporary



Fig. 1. Live photos of representative Opiliones. **A)** Cyphophthalmi: Petallidae: *Rakaia pauli*. **B)** Cyphophthalmi: Sironidae: *Siro rubens*. **C)** Cyphophthalmi: Troglodironidae: *Troglosiro sharmai*. **D)** Eupnoi: Caddidae: *Caddo pepperella*. **E)** Eupnoi: Neopilionidae: *Megalopsalis* sp. **F)** Eupnoi: Sclerosomatidae: *Leiobunum vittatum*. **G)** Dyspnoi: Acropsopilionidae: *Austropsopilio* sp. **H)** Dyspnoi: Troglidae: *Trogulus tricarinatus*. **I)** Dyspnoi: Taracidae: *Tarus pallipes*. **J)** Laniatores: Paranonychidae: *Sclerobunus* sp. **K)** Laniatores: Triaenonychidae: *Triaenobunus pectinatus*. **L)** Laniatores: Phalangodidae: *Calicina digita*. **M)** Laniatores: Epedanidae: *Metacrobunus macrochelis*. **N)** Laniatores: Podoctidae: *Hoplodino continentalis*. **O)** Laniatores: Cosmetidae: *Libitoides sayi*. **P)** Laniatores: Cranidae: *Cranidae* sp. **Q)** Laniatores: Assamiidae: *Dampetrus* sp. **R)** Laniatores: Zalmoxidae: *Zalmoxis* sp. Photo D–K,O,Q,R: Shahan Derkarabetian; A,C: Gonzalo Giribet; B: courtesy of Ludvine Lamare (iNaturalist username: ldvn); L: courtesy of Marshal Hedin; M,N,P: courtesy of Nicky Bay.

probes were then aligned to the exemplar transcriptomes with `phyluce_probe_run_multiple_lastz_sqlite` and sequences were extracted from the exemplar assemblies using `phyluce_probe_slice_sequence_from_genomes`. For the design of the final transcriptome-derived probe set, *Pantopsalis cheliferoide*s was removed given its close phylogenetic relationship to the base genome, and only probes targeting loci that were present in at least four out of five samples were included. The probe set was designed using `phyluce_probe_get_tiled_probe_from_multiple_inputs` and overlapping duplicates were removed as above.

Given the transcriptome-derived nature of these probes, and the disassociation of different exonic regions of a single gene (transcript) when simulating reads from a transcriptome assembly, there is the possibility that different sets of probes targeting distinct UCEs actually target the same gene (i.e., transcript). Preliminary assessment of the final transcriptome-derived probe set when matching contigs to probes confirmed this. As such we removed these “different exon, same gene” duplicate probes via manual curation: for each UCE, we only kept those probes that targeted loci which were found in at least 50% of the taxa in a larger dataset consisting of 16 transcriptomes plus the base genome. For each retained UCE, a “consensus transcript” was created from each of the aligned UCE matrices. Each consensus transcript was then subject to a local BLAST search in Geneious Prime 2022.1.1 (<https://www.geneious.com>) against the final transcriptome-derived probe set. For transcripts in which multiple sets of probes matched, the set of probes targeting the UCE that had the highest taxon coverage in the 16-sample data set was retained. After removing these “different exon, same gene” duplicates we ended up with the final set of deduplicated transcriptome-derived probes.

2.2.2. Genome-derived probes

In addition to the published *Phalangium opilio* genome (used as the base genome) and the two preliminary exemplar genomes newly sequenced for this study, we downloaded the low-coverage genome reads for *Mitopus morio* (Fabricius, 1779) (Eupnoi) available from Faircloth (2017), which was used in the design of the Arachnida probe set. Reads were assembled using ABySS (Simpson et al. 2009) at default settings. Probe set design followed standard procedure; the temporary probe set retained probes present in all four genomes, and the final probe set retained the loci present in at least three of four samples.

2.2.3. Arachnida-derived probes

To retain some degree of “backwards compatibility” with the many Opiliones samples that have already been hybridized and sequenced using the Arachnida probe set, we identified and retained a set of UCEs from the Arachnida probe set that performed best for Opiliones. To do this, we compiled 348 Opiliones UCE assembly files available from both published and unpublished data. The assembly files for this 348-sample data set were matched to the Arachnida probes using `phyluce_assembly_match_contigs_to_probes`. We then retained probes for all loci that were found in at least 50% of the 348 samples. From this set of probes, we filtered for any “different exon, same gene” duplicates that were previously identified in the Arachnida probe set (Hedin et al. 2018). From any identified duplicates, we retained only one set of probes, favoring the locus that was recovered in a higher number of samples.

2.2.4. Final probe set

After creation of individual probe sets from the three sources (transcriptome, genome, Arachnida), the three sets of probes were concatenated, then subjected to a local BLAST search in Geneious against the consensus transcripts database created earlier to identify any potential probes derived from different sources that match to the same gene. The set of BLAST hits for each consensus transcript was manually inspected, retaining only one set of probes for each transcript. Following this, a final round of overlapping duplicate removal was conducted using `phyluce_probe_easy_lastz` and `phyluce_probe_remove_duplicate_hits_from_probes_using_lastz`. This resulting probe set was considered the

final Opiliones-specific probe set. In the final probe set fasta file, probes derived from different sources were identified with different prefixes: transcriptome-derived probes have the prefix “7777” added to the probe number (e.g., uce-1050 in the transcriptome-derived probe set is uce-77771050 in the final probe set), “6666” was added to genome-derived probes, and “5555” was added to Arachnida-derived probes. The final probe set was sent to Daicel Arbor Biosciences (Ann Arbor, MI) for synthesis.

2.3. Probe set testing

2.3.1. Taxon sampling

As in previous studies (Derkarabetian et al. 2019b; Derkarabetian et al., 2021) we consider “fresh” specimens those preserved in 95% ethanol and kept frozen (i.e., standard preservation for genetic work), and “historical” specimens those preserved in 70–80% ethanol and kept at room temperature (i.e., with degraded DNA). Specimens were chosen with the overall goal of assessing the effectiveness of the probe set across Opiliones (Table 1), with multiple specific objectives, some of which were not mutually exclusive in terms of taxon sampling. The first objective was a preliminary attempt at reconstructing a family-level phylogeny of all Opiliones with as many families represented as possible to ensure the probe set was applicable across the Opiliones tree, although this goal will be explored in more depth in the future with a larger representation of species per family. We acquired or included specimens representing 61 / 63 extant Opiliones families described at the time of sampling for this study. Specimens of two recently described families – Otilioleptidae Acosta, 2019, a monotypic family known from a single cave system in Argentina, and Askawachidae Kury & Carvalho, 2020, a small family from the Andean Amazon – could not be obtained. Of the samples acquired, 87 (75 fresh and 12 historical) were newly hybridized with the Opiliones probe set, while 24 (16 fresh and 8 historical) were samples that had been previously hybridized with the Arachnida probes. We also included several samples as transcriptome assemblies derived from the transcriptomic study of Fernández et al. (2017).

The second objective was assessing the utility of the probe set for historical museum specimens with degraded DNA from specimens of varying ages. We hybridized and sequenced 12 historical degraded specimens ranging from 1865 to 2011, including multiple specimens that were previously hybridized with the Arachnida probe set (Derkarabetian et al. 2019b). The third objective was demonstrating the utility of the probe set at the population and species levels. Multiple samples of three different species were included from taxa that have varying levels of dispersal ability, habitat specificity, and expected population structure. These included 1) four samples of *Speleomaster lexi* Briggs, 1974 (Laniatores: Cryptomastriidae), a cave-obligate species restricted to lava tubes of southern Idaho, 2) two specimens of *Triebenobunus asper* Hickman, 1958 (Laniatores: Triebenonychidae) from across their distribution in Tasmania, plus one specimen of the very closely related *T. pectinatus* Pocock, 1903, and 3) two samples of *Megalopsalis nigricans* (Hickman, 1957) (Eupnoi: Neopilionidae), a less dispersal limited species from Tasmania, together with one specimen of the closely related *M. tasmanica* (Hogg, 1910) (see Giribet et al., 2021). For the *Megalopsalis* and *Triebenobunus* data sets, we created smilograms for each taxon set which included all three specimens. For *Speleomaster lexi* we included all loci with at least 4/5 samples.

The fourth objective was direct comparisons of specimens hybridized and sequenced using both the Arachnida and Opiliones probe sets, since the number of Opiliones samples hybridized with the Arachnida probe set is quite large (Derkarabetian et al. 2018, Derkarabetian et al., 2019a; Derkarabetian et al., 2019b; Derkarabetian et al., 2021; Derkarabetian et al., 2022a; Derkarabetian et al., 2022b; Giribet et al., 2022). Sixteen (eight fresh and eight historical) specimens were hybridized using both probe sets. For fresh specimens, the same DNA extraction was used, however, DNA for all historical specimens were re-extracted from the

Table 1
Taxon sample. UCEs column refers to the number of raw UCEs identified when matching contigs to probes. Final column refers to number of loci in the final matrix used for phylogenetic analyses. Species with asterisk indicate historical specimens (also see Table 2).

Suborder	Higher Lineage	Family	Species	Voucher	Probe set	SRA	Reads	UCEs	Final
Cyphophthalmi		Neogoveidae	<i>Brasilogovea microstylus</i>	MCZ143921	Opiliones	SAMN35540758	14,856,079	1217	409
Cyphophthalmi		Ogoveidae	<i>Ogovea cameroonensis</i>	MCZ132315	Arachnida	Giribet et al. (2022)		244	124
Cyphophthalmi		Pettaliidae	<i>Pettalus thwaitesi</i>	MCZ78880	Opiliones	SAMN35540800	10,143,223	1370	448
Cyphophthalmi		Pettaliidae	<i>Pettalus thwaitesi</i>	MCZ78880	Arachnida	SAMN35540801		423	177
Cyphophthalmi		Sironidae	<i>Siro exilis</i>	DAH2021122401	Opiliones	SAMN35540817	9,038,421	1287	421
Cyphophthalmi		Stylocellidae	<i>Meghalaya</i> sp.	MCZ109986	Opiliones	SAMN35540792	6,772,335	1283	433
Cyphophthalmi		Troglosironidae	<i>Troglosiro aelleni</i>	MCZ134764	Arachnida	Giribet et al. (2022)		191	90
Dyspnoi		Acropsopilionidae	<i>Acropsopilio cf. chilensis</i> Unique	MCZ138045	Transcriptome			658	317
Dyspnoi		Acropsopilionidae	<i>Acropsopilio</i> Unique		Transcriptome	Fernández et al. (2017)		967	218
Dyspnoi		Dicranolasmatidae	<i>Dicranolasma sorenseni</i> Unique		Transcriptome	Fernández et al. (2017)		647	219
Dyspnoi		Ischyropsalididae	<i>Ischyropsalis</i> sp.	MCZ60785	Opiliones	SAMN35540782	24,799,237	809	285
Dyspnoi		Nemastomatidae	<i>Mitostoma patrizii</i>	MCZ162781	Opiliones	SAMN35540794	7,832,631	1227	430
Dyspnoi		Nipponopsalididae	<i>Nipponopsalis abei</i>	MCZ134845	Opiliones	SAMN35540795	1,710,802	803	191
Dyspnoi		Sabaconidae	<i>Sabacon cavicolens</i>	MCZ71294	Opiliones	SAMN35540812	2,452,600	1177	398
Dyspnoi		Taracidae	<i>Taracus packardi</i>	SDSUTAC.OP1247	Arachnida	SAMN35540796	1,002,880	173	80
Dyspnoi		Trogulidae	<i>Trogulus tricarinatus</i>	MCZ162783	Opiliones	SAMN35540834	7,668,996	673	268
Eupnoi		Caddidae	<i>Caddo pepperella</i>	MCZ161818	Opiliones	SAMN35540760	21,702,763	937	343
Eupnoi		Globipedidae	<i>Metopilio</i> sp.	MCZ95161	Opiliones	SAMN35540793	10,196,516	1239	416
Eupnoi		Neopilionidae	<i>Megalopsalis nigricans</i>	TAS365	Opiliones	SAMN35540790	11,581,466	712	273
Eupnoi		Phalangidae	<i>Opilio canestrinii</i>	MCZ162402	Opiliones	SAMN35540797	23,090,856	260	120
Eupnoi		Protolophidae	<i>Protolophus singularis</i>	MCZ134552	Opiliones	SAMN35540808	13,378,469	936	350
Eupnoi		Sclerosomatidae	<i>Prionostemma vittatum</i>	MCZ144108	Opiliones	SAMN35540805	22,205,914	1037	371
Eupnoi		Sclerosomatidae	<i>Prionostemma vittatum</i>	MCZ144108	Arachnida	SAMN35540806	4,640,252	588	197
Laniatores	Grassatores	Phalangodidae	<i>Scotolemon</i> sp.	MM17_A296	Opiliones	SAMN35540816	7,884,747	1197	416
Laniatores	Grassatores	Sandokanidae	<i>Gnomulus latoperculum</i>	MCZ131264	Opiliones	SAMN35540775	10,369,140	1237	423
Laniatores	Grassatores	Sandokanidae	<i>Gnomulus latoperculum</i>	MCZ131264	Arachnida	SAMN35540774	2,503,330	441	203
Laniatores	Grassatores: Assamioidea	Assamiidae	<i>Dampetrus tuberculatus</i>	MCZ23368	Opiliones	SAMN35540752	14,842,966	1303	439
Laniatores	Grassatores: Assamioidea	Assamiidae	<i>Parchilon</i> sp.	MCZ127065	Opiliones	SAMN35540753	9,501,309	1131	412
Laniatores	Grassatores: Assamioidea	Pyramidopidae	<i>Pyramidopidae</i> sp.	MCZ127020	Opiliones	SAMN35540810	8,685,471	626	134
Laniatores	Grassatores: Assamioidea	Suthepiidae	<i>Suthepia inermis</i> (paratype) *	THMY10/10	Opiliones	SAMN35540826	4,637,392	1119	346
Laniatores	Grassatores: Assamioidea	Trionyxellidae	<i>Trionyxellinae</i> sp.	MCZ132482	Opiliones	SAMN35540833	11,232,853	1054	387
Laniatores	Grassatores: Epedanoidea	Beloniscidae	<i>Beloniscus</i> sp. *	MCZ37641	Arachnida	SAMN35540755	1,307,609	63	26
Laniatores	Grassatores: Epedanoidea	Epedanidae	<i>Dibunus</i> sp.	MCZ141285	Opiliones	SAMN35540765	14,811,147	1047	388
Laniatores	Grassatores: Epedanoidea	Epedanidae	<i>Dibunus</i> sp.	MCZ141285	Arachnida	SAMN35540764	7,256,277	517	197
Laniatores	Grassatores: Epedanoidea	Epedanidae	<i>Epedanidae</i> sp.	MCZ23124	Opiliones	SAMN35540766	7,862,878	1189	389
Laniatores	Grassatores: Epedanoidea	Petrobunidae	<i>Petrobunus</i> sp.	MCZ134849	Opiliones	SAMN35540799	18,686,001	1187	428
Laniatores	Grassatores: Epedanoidea	Petrobunidae	<i>Petrobunus</i> sp.	MCZ134849	Arachnida	SAMN35540798	2,155,783	132	64
Laniatores	Grassatores: Epedanoidea	Petrobunidae	<i>Zalmoxida gibbera</i>	MCZ130969	Opiliones	SAMN35540841	10,095,973	1305	453
Laniatores	Grassatores: Epedanoidea	Podoctidae	<i>Erecanana remyi</i>	MCZ151860	Opiliones	SAMN35540767	8,900,464	1297	454
Laniatores	Grassatores: Epedanoidea	Podoctidae	<i>Erecanana remyi</i>	MCZ151860	Arachnida	SAMN35540768	4,579,907	352	147
Laniatores	Grassatores: Epedanoidea	Podoctidae	<i>Podoctidae</i> sp.	MCZ18807	Opiliones	SAMN35540804	19,459,707	1264	441
Laniatores	Grassatores: Epedanoidea	Tithaeidae	<i>Tithaeus</i> sp.	MCZ131318	Opiliones	SAMN35540829	11,368,185	1272	434
Laniatores	Grassatores: Epedanoidea	Tithaeidae	<i>Tithaeus</i> sp.	MCZ131318	Arachnida	SAMN35540828	3,762,953	629	257
Laniatores	Grassatores: Gonyleptoidea	Agoristenidae	<i>Avima leiobuniformis</i>	MCZ143995	Opiliones	SAMN35540751	10,298,042	772	301
Laniatores	Grassatores: Gonyleptoidea	Ampycidae	<i>Glysterus</i> sp.	MCZ134817	Opiliones	SAMN35540773	8,516,037	823	375
Laniatores	Grassatores: Gonyleptoidea	Cosmetidae	<i>Erginulus serratipes</i> *	MCZ38069	Opiliones	SAMN35540769	9,004,415	40	3
Laniatores	Grassatores: Gonyleptoidea	Cosmetidae	<i>Erginulus clavotibialis</i>	MCZ30049	Opiliones	SAMN35540771	8,857,136	927	390
Laniatores	Grassatores: Gonyleptoidea	Cosmetidae	<i>Vonones sayi</i>	Opi1H6	Opiliones	SAMN35540840	15,333,252	872	380
Laniatores	Grassatores: Gonyleptoidea	Cosmetidae	<i>Vonones sayi</i>	Opi1H6	Arachnida	SAMN35540839	3,732,339	340	149
Laniatores	Grassatores: Gonyleptoidea	Cranidae	<i>Phareicranaus calcariferous</i>	MCZ144177	Opiliones	SAMN35540803	17,531,820	876	403
Laniatores	Grassatores: Gonyleptoidea	Cranidae	<i>Santinezia serratotibialis</i>	MCZ133913	Opiliones	SAMN35540814	5,753,674	1042	434
Laniatores	Grassatores: Gonyleptoidea	Cryptogobiidae	<i>Pseudopachylus</i> sp.	MCZ32170	Opiliones	SAMN35540809	8,540,333	952	420
Laniatores	Grassatores: Gonyleptoidea	Gerdesiidae	<i>Gonyocranaus pluto</i>	MCZ139251	Opiliones	SAMN35540776	9,783,104	767	342
Laniatores	Grassatores: Gonyleptoidea	Gonyleptidae	<i>Progonyleptoidellus striatus</i>	MCZ32030	Opiliones	SAMN35540807	12,053,022	799	367

(continued on next page)

Table 1 (continued)

Suborder	Higher Lineage	Family	Species	Voucher	Probe set	SRA	Reads	UCEs	Final
Laniatores	Grassatores: Gonyleptoidea	Gonyleptidae	<i>Sadocus polyacanthus</i>	MCZ138128	Opiliones	SAMN35540813	10,260,276	938	410
Laniatores	Grassatores: Gonyleptoidea	Manaosiidae	<i>Cranellus</i> sp.	MCZ144162	Opiliones	SAMN35540762	10,377,235	717	325
Laniatores	Grassatores: Gonyleptoidea	Manaosiidae	<i>Rhopalocranus</i> sp.	MCZ134416	Opiliones	SAMN35540811	9,691,258	862	392
Laniatores	Grassatores: Gonyleptoidea	Metasarcidae	<i>Incasarcus argenteus</i>	MCZ139250	Opiliones	SAMN35540781	5,118,296	971	414
Laniatores	Grassatores: Gonyleptoidea	Nomoclastidae	<i>Callosma abrapatricia</i> *	MZSP965	Opiliones	SAMN35540761	671,263	657	165
Laniatores	Grassatores: Gonyleptoidea	Prostyginae	<i>Cutervolus albopunctatus</i> *	MZSP950	Opiliones	SAMN35540763	955,919	351	72
Laniatores	Grassatores: Gonyleptoidea	Stygidae	<i>Stygnoplus clavotibialis</i>	MCZ144091	Opiliones	SAMN35540824	14,047,029	826	376
Laniatores	Grassatores: Gonyleptoidea	Stygnopsidae	<i>Stygnopsidae</i> sp.	MCZ143047	Opiliones	SAMN35540825	8,465,491	935	360
Laniatores	Grassatores: Samooidea	Biantidae	<i>Acrobiantes</i> sp.	MCZ151858	Opiliones	SAMN35540756	14,524,792	1300	451
Laniatores	Grassatores: Samooidea	Biantidae	<i>Biantidae</i> sp.	MCZ138024	Opiliones	SAMN35540757	10,668,468	989	375
Laniatores	Grassatores: Samooidea	Samoidae	<i>Pellobunus Unique</i>		Transcriptome	Fernández et al. (2017)		985	349
Laniatores	Grassatores: Samooidea	Stygnommatidae	<i>Stygnomma</i> sp.	MCZ144171	Opiliones	SAMN35540823	8,105,336	1249	449
Laniatores	Grassatores: Zalmoxoidea	Escadabiidae	<i>Baculigerus</i> sp.	MCZ134812	Opiliones	SAMN35540754	14,024,620	1293	448
Laniatores	Grassatores: Zalmoxoidea	Fissiphalliidae	<i>Fissiphallius martensi</i>	MCZ132625	Opiliones	SAMN35540772	8,333,134	1300	457
Laniatores	Grassatores: Zalmoxoidea	Fissiphalliidae	<i>Fissiphallius</i> sp.	MCZ130717	Opiliones	SAMN35540778	6,741,921	1329	430
Laniatores	Grassatores: Zalmoxoidea	Guasiniidae	<i>Guasinia</i> sp.	MCZ134820	Opiliones	SAMN35540777	10,773,018	1377	467
Laniatores	Grassatores: Zalmoxoidea	Icaleptidae	<i>Icaleptidae</i> sp.	MCZ141760	Opiliones	SAMN35540779	19,623,274	433	182
Laniatores	Grassatores: Zalmoxoidea	Kimulidae	<i>Kimula goodnightorum</i>	MCZ134822	Opiliones	SAMN35540783	20,121,500	984	374
Laniatores	Grassatores: Zalmoxoidea	Kimulidae	<i>Kimulidae</i> sp.	MCZ130749	Opiliones	SAMN35540784	7,896,761	1554	513
Laniatores	Grassatores: Zalmoxoidea	Zalmoxidae	<i>Ethobunus</i> cf. <i>tuberculatus</i>	MCZ143996	Opiliones	SAMN35540842	13,998,413	1009	382
Laniatores	Grassatores: Zalmoxoidea	Zalmoxidae	<i>Ethobunus</i> sp.	MCZ31589	Opiliones	SAMN35540771	14,655,636	1321	455
Laniatores	Grassatores: Zalmoxoidea	Zalmoxidae	<i>Zalmoxis</i> sp.	MCZ151614	Opiliones	SAMN35540843	16,623,471	1058	385
Laniatores	Grassatores: Zalmoxoidea	Zalmoxoidea	<i>Trypophoba</i> sp.	MCZ89431	Opiliones	SAMN35540780	12,803,386	937	361
Laniatores	Insidiatores: Travunioidea	Cladonychiidae	<i>Theromaster brunneus</i>	SDSUTAC_OP1626	Arachnida	Derkarabetian et al. (2018)		738	277
Laniatores	Insidiatores: Travunioidea	Cryptomastriidae	<i>Cryptomaster leviathan</i>	SDSUTAC_OP3794	Arachnida	Derkarabetian et al. (2018)		632	256
Laniatores	Insidiatores: Travunioidea	Cryptomastriidae	<i>Speleomaster lexi</i>	SBear	Opiliones	SAMN35540822	5,876,736	1429	478
Laniatores	Insidiatores: Travunioidea	Paranonychiidae	<i>Sclerobunus</i> sp.	Stinkpot	Opiliones	Derkarabetian et al. (2018)	22,032,356	1167	423
Laniatores	Insidiatores: Travunioidea	Travuniidae	<i>Travunia jandai</i>	SDSUTAC_OP4617	Arachnida	SAMN35540815		141	66
Laniatores	Insidiatores: Travunioidea	<i>incertae sedis</i>	<i>Yuria pulchra</i>	SDSUTAC_OP4263	Arachnida	Derkarabetian et al. (2018)		710	282
Laniatores	Insidiatores: Triaenonychoidea	Buemarinoidea	<i>Buemarinoia patrizii</i>	MCZ162780	Opiliones	SAMN35540759	8,541,302	1172	438
Laniatores	Insidiatores: Triaenonychoidea	Lomanellidae	<i>Lomanella</i> sp.	MCZ152657	Opiliones	SAMN35540788	15,543,542	1150	426
Laniatores	Insidiatores: Triaenonychoidea	Synthetonychiidae	<i>Synthetonychia minuta</i>	MCZ136018	Arachnida	SAMN35540827	57,433,186	708	251
Laniatores	Insidiatores: Triaenonychoidea	Triaenonychiidae	<i>Triaenobunus apser</i>	TAS315	Opiliones	SAMN35540830	5,185,172	1079	385
Outgroup	Araneae		<i>Hypochilus</i> sp.	SDSUTAC_H595	Arachnida	Starrett et al. (2017)		286	133
Outgroup	Acari: Trombidiformes		<i>Neomolgus</i> sp.	AR014	Arachnida	Starrett et al. (2017)		263	93
Outgroup	Scorpiones		<i>Paravaejovis</i> sp.	AR018	Arachnida	Starrett et al. (2017)		229	110

same individual used previously in Derkarabetian et al. (2019b). The fifth objective was assessing “backwards compatibility” of the Opiliones probe set by incorporating samples previously hybridized with the Arachnida probe set. Sixteen fresh Arachnida-hybridized specimens were incorporated into bioinformatic and phylogenetic analyses, where the majority of samples were hybridized with the Opiliones probe set. Most of these samples were representatives used for the first objective either from previously unpublished data or from Derkarabetian et al. (2018). We also assess “backwards compatibility” on the full data set of Derkarabetian et al. (2018).

2.3.2. UCE lab work

Fresh samples were extracted using the Qiagen DNeasy Blood and Tissue kit (Qiagen), and historical specimens were extracted using the extraction protocol described in Derkarabetian et al. (2019b), derived from Tin et al. (2014). Target capture of UCEs largely followed standard protocol as conducted in the recent UCE studies in Opiliones which used the Arachnida probe set (e.g., Derkarabetian et al. 2018; Derkarabetian et al., 2019; Derkarabetian et al., 2021), with some modifications detailed here. We used the Kapa HyperPlus Kit for library preparation across all samples. For fresh samples, an enzymatic fragmentation time of 3 min was used, based on preliminary testing of standard Qiagen DNA extractions. DNA quality of historical specimens was assessed via an Agilent TapeStation to determine if fragmentation was needed. We hybridized samples with the Opiliones-specific probes (Daicel Arbor Biosciences kit, version 5 chemistry) using a “touchdown” protocol: 62 °C for 4 h, 60 °C for 16 h, and 55 °C for 4 h. All post-hybridization washing steps were done at 60 °C. Sequencing was done on an Illumina NovaSeq 6000 S4 with 150 bp paired end reads at the Bauer Core Facility at Harvard University.

2.3.3. Phylogenetic analyses

Raw read data was processed using standard practices for Opiliones UCEs (e.g., Derkarabetian et al. 2021). Quality control was done with Illumiprocessor (Faircloth 2013) and assemblies were created using SPAdes (Bankevich et al. 2012) at default settings. Phyluce (Faircloth 2016) was used for data processing, matching contigs to probes using minimum coverage and minimum identity values of 65. Gblocks (Tala-vera and Castresana, 2007) was used at conservative settings (–b1 0.5 –b2 0.85 –b3 4 –b4 8) to only retain coding regions of the UCEs. Following this, all loci were manually inspected and edited in Geneious. Two different phylogenetic analyses were conducted on a final ~50% gene occupancy matrix. A concatenated unpartitioned RAxML v.8 (Stamatakis 2014) analysis was run with 200 rapid bootstrap replicates and search for best scoring tree. A partitioned IQTREE2 (Nguyen et al. 2015) analysis was run with 1000 ultrafast bootstrap replicates (Hoang et al. 2018) merging loci according to the model identified with ModelFinder (Kalyaanamoorthy et al. 2017). Smilograms were created using the phyluce script `phyluce_align_get_smilogram_from_alignments` for the three sets of species/population level sampling.

3. Results

3.1. Probe set

Raw read data for newly sequenced samples are available from SRA under BioProject PRJNA975553 (Table 1). The final Opiliones-specific probe set consisted of 16,513 probes targeting 1,915 loci with contributions from the three different sources as follows: transcriptomes – 9,141 probes targeting 1,109 loci; genomes – 3,958 probes targeting 564 loci; and Arachnida probe set – 3,414 probes targeting 254 loci. The probe set is available as an “Expert” panel from Arbor Biosciences.

3.2. Phylogenomic reconstruction

The final 50% gene occupancy matrix consisted of 595 UCEs with a

total length of 146,545 bp and is available from the Harvard Dataverse (<https://doi.org/10.7910/DVN/E5ZZ45>). The phylogenomic analyses are almost entirely congruent across the RAxML (Fig. 2) and IQ-TREE (Supplementary Fig. 1) analyses. Relationships among suborders and families are consistent with previous phylogenomic analyses based on transcriptomes (Fernández et al., 2017; Benavides et al. 2021) and UCEs using the Arachnida probe-set (Derkarabetian et al. 2018; Derkarabetian et al. 2021; Giribet et al., 2022), in addition to more comprehensive multi-locus Sanger-based studies (e.g., Giribet et al., 2010). Cyphophthalmi are the earliest diverging suborder of Opiliones with internal relationships identical to the most recent UCE study (Giribet et al. 2022), including the recovery of Boreophthalmi (Stylocellidae Hansen & Sørensen, 1904 + Sironidae Leach, 1816), which was not found in the transcriptomic study of Fernández et al. (2017). Relationships within Dyspnoi are identical to those obtained with transcriptomic data, recovering Acropsopilionidae Roewer, 1923 as the earliest diverging lineage and a split between the consistently recovered Ischyropsalidoidea and Troguloidea. Eupnoi are similarly recovered as in transcriptomic data, including recovering Caddidae Banks, 1893 as sister group to all other Eupnoi. We also find a sister group relationship between Phalangiidae Latrielle, 1802 and Globipedidae Kury & Cokendolpher, 2020, the latter of which is a lineage (the “*Metopilio* group”) recently elevated to family (Kury et al. 2020) that had not been included in any previous phylogenomic studies. Within Laniatores, all supra-familial lineages were recovered as expected. Insidiatores (Travunioidea + Triaenonychoidea) is recovered in UCE data as in transcriptomic studies, as opposed to previous UCE analyses with lower taxon sampling that recovered Travunioidea as the sister lineage to all other Laniatores (Derkarabetian et al., 2018; Derkarabetian et al., 2021). The ubiquitously supported Grassatores is recovered, with Phalangodidae Simon, 1879 and Sandokanidae Özdikmen & Kury, 2007 supported as early-diverging as in previous transcriptomic studies. Epedanoidea, Assamioidea (except Pyramidopidae Sharma, Prieto & Giribet, 2011, see below), Gonyleptoidea, Samooidea, and Zalmoxoidea are all recovered with high support, with internal relationships largely agreeing with transcriptomic studies (Fernández et al., 2017; Benavides et al. 2021).

Two samples were recovered in different places across phylogenetic analyses. First, is the placement of Pyramidopidae. In the concatenated RAxML phylogeny (Fig. 2) it is found as the sister lineage to Assamiidae Sørensen, 1884 + “ZalSa” (Zalmoxoidea + Samooidea) with low support; in IQ-TREE analyses (Supplementary Fig. 1) it is the sister lineage to Gonyleptoidea + Assamiidae + “ZalSa” with high support. We suspect that including more samples of Pyramidopidae will confidently resolve the position of this poorly understood lineage. Second, Prostyginae Roewer, 1913, (sometimes referred to as Prostygnae) represented by *Cutervolus albopunctatus* Roewer, 1957, is recovered as the sister lineage to Metasarcidae Kury, 1994 + Cosmetidae Koch, 1839 (the “MECO clade” of Kury et al. 2020) in the concatenated RAxML tree with low support (Fig. 2), and as the sister lineage to Nomoclastidae Roewer, 1943 + Manaosbiidae Roewer, 1943 + “Greater Gonyleptidae” (the “GG clade” of Kury & Villareal, 2015; *sensu* Benavides et al. 2021) with low support in the IQ-TREE analysis (Supplementary Fig. 1).

Our study included several Opiliones families not previously included in any phylogenomic analyses: 1) Globipedidae, also known as the “*Metopilio* group” formerly included in Sclerosomatidae, was recovered as the sister lineage to Phalangiidae. 2) Belonsicidae Kury, Pérez-González & Proud, 2019 was recovered within Epedanoidea, as the sister lineage to Petrobunidae. 3) Suthepiidae Martens, 2020, a recently described monotypic family from Thailand (Martens 2020), was recovered within Assamiidae, suggesting the need for revision of this large family of tropical Laniatores. 4) Trionyxellidae Roewer, 1912, originally a subfamily within Assamiidae, was also recovered within Assamiidae. The status of Trionyxellidae as a family has always been uncertain, with some authors including it as a subfamily and others as a distinct family; our results suggest that family status is unwarranted. 5) Guasiniidae González-Sponga, 1997, a family with only three species,

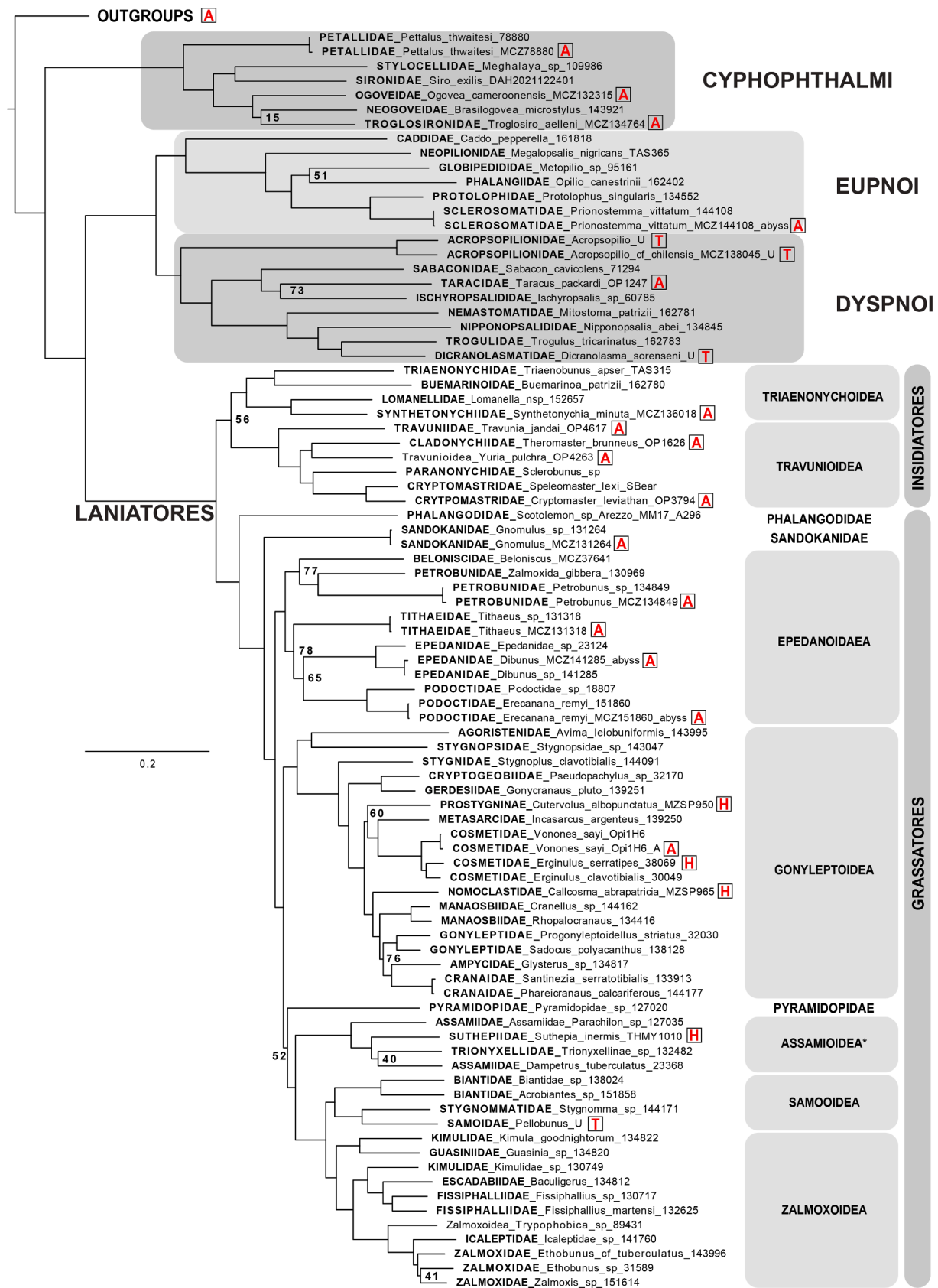


Fig. 2. Concatenated RAxML phylogeny. Bootstrap values above 80 are not shown. Boxes with “A” indicate samples hybridized with the Arachnida probe set; boxes with “T” indicate transcriptome samples, and boxes with “H” indicate historical samples.

was recovered as the sister lineage to *Kimula goodnightorum* Šilhavý, 1969 (Kimulidae Pérez-González, Kury, & Alonso-Zarazaga, 2007).

3.3. Probe set efficacy

The number of raw UCEs pulled from fresh specimens ranged from 260 to 1554 and for historical specimens the range was 12–1119 (Table 1, 2), with the lowest yield corresponding to the oldest specimen (collected in 1865) and the largest corresponding to the most recent (collected in 2011). For all samples used in phylogenomic analyses, the number of UCEs in the final matrix ranged from 3 to 346. The species- / population-level data sets recover high numbers of variable loci (Fig. 3, Table 3) across data sets: *Megalopsalis* with 411 loci, *Triaenobunus* with 609 loci, and *Speleomaster lexi* with 794 loci. For those samples hybridized using both Arachnida and Opiliones probe sets, there was an average increase of 571 loci in the number of raw loci pulled from SPAdes assemblies using the Opiliones probe set relative to the Arachnida (Table 4; range of 293–970). Regarding “backwards compatibility” of fresh samples hybridized using the Arachnida probe set (Table 5), the average number of raw Opiliones UCEs pulled from assemblies was 407 loci (range of 132–738), and the average number of UCEs in the final matrix was 166 (range of 66–277).

4. Discussion

4.1. Opiliones-specific probe set

We were deliberately much more conservative in creating our data set in this study relative to previous Opiliones UCE studies using the Arachnida probe set. For example, we used only SPAdes assemblies when matching contigs to probes, while previous studies used both SPAdes and ABySS assemblies, and we used the strictest gblocks settings used in previous Opiliones UCE studies, which are typically only used for divergence dating purposes (i.e., exon only; Hedin et al. 2019). Due to the inclusion of a significant proportion of samples (28%) that were not hybridized with the Opiliones probe set, the overall number of loci included in the final 50% gene occupancy matrix was lower than if only Opiliones-hybridized samples were included. Even with these strict data processing steps, we recover an almost completely fully supported phylogeny concordant with previous analyses (Fig. 1, Supplementary Fig. 1), where the only uncertain and weakly-supported nodes were associated with two samples of degraded DNA from historical sources.

As expected, Opiliones-specific UCEs were recovered from historical specimens, and the samples used in phylogenomic reconstruction were largely placed where they were expected. In the case of *Erginulus serratipes* (Pickard-Cambridge, 1905) MCZ:IZ:38069 (Cosmetidae), only three loci were in the final matrix, yet it was strongly supported as the sister lineage to its congener *Erginulus clavotibialis* (Pickard-Cambridge, 1905) MCZ:IZ:30049. Importantly, a paratype specimen of *Suthepia inermis* Martens, 2020, the only species in the family Suthepiidae,

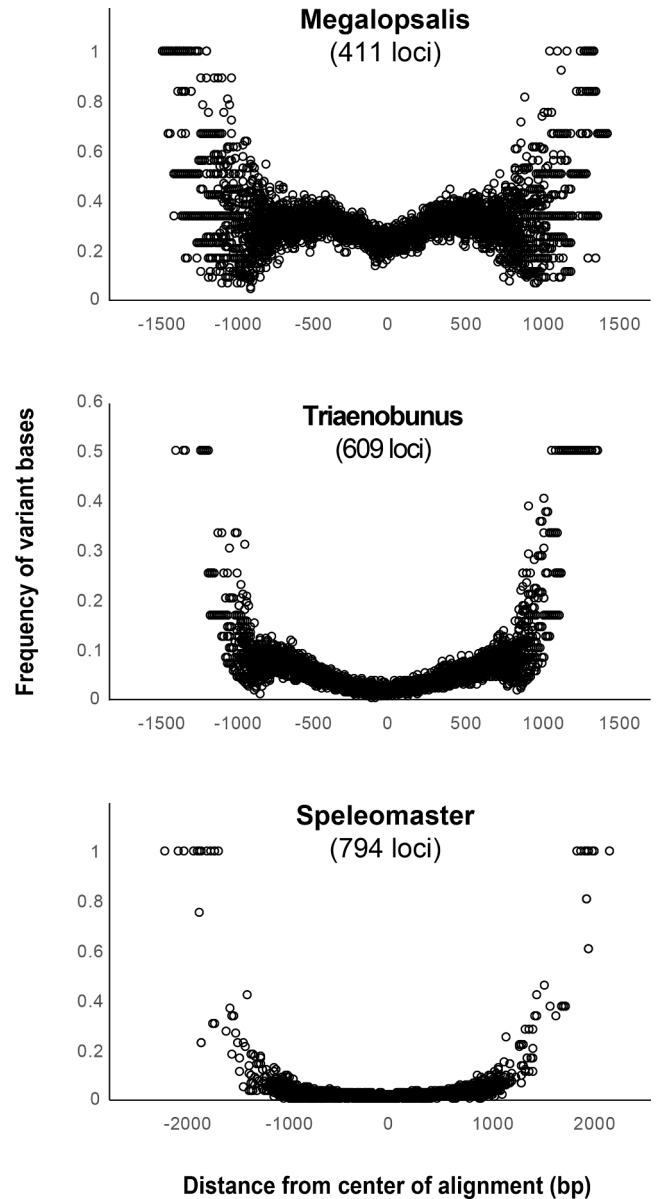


Fig. 3. “Smilograms” for species/population level data sets. Plots demonstrate an increasing probability of variability as distance from the core UCE increases. All data points with no variability are removed.

Table 2

Historical specimens. UCEs column refers to the number of raw UCEs identified when matching contigs to probes.

Suborder	Family	Species	Voucher	Year	UCEs	SRA
Eupnoi	Sclerosomatidae	<i>Leiobunum formosum</i>	MCZ36740	1865	12	SAMN35540785
Eupnoi	Sclerosomatidae	<i>Leiobunum vittatum</i>	MCZ36455.1	1950	98	SAMN35540786
Eupnoi	Sclerosomatidae	<i>Leiobunum vittatum</i>	MCZ36816	1938	81	SAMN35540787
Eupnoi	Phalangidae	<i>Phalangium opilio</i>	MCZ36220	1967	550	SAMN35540802
Laniatores	Cosmetidae	<i>Vonones ornatus</i>	MCZ37491	1919	83	SAMN35540835
Laniatores	Cosmetidae	<i>Vonones ornatus</i>	MCZ37493	1899	83	SAMN35540836
Laniatores	Cosmetidae	<i>Vonones ornatus</i>	MCZ37499	1979	94	SAMN35540837
Laniatores	Cosmetidae	<i>Vonones sayi</i>	MCZ37537	1955	55	SAMN35540838
Laniatores	Beloniscidae	<i>Beloniscus</i> sp.	MCZ37641	1981	63	SAMN35540755
Laniatores	Nomoclastidae	<i>Callcosma abrapatricia</i>	USP965	2010	657	SAMN35540761
Laniatores	Prostygninae	<i>Cutervolus albopunctatus</i>	USP950	2010	351	SAMN35540763
Laniatores	Cosmetidae	<i>Erginulus serratipes</i>	MCZ38069	1931	40	SAMN35540769
Laniatores	Suthepiidae	<i>Suthepia inermis</i>	THMY10/10	2011	1119	SAMN35540826

Table 3

Species level results used in smilograms. UCEs column refers to total number of UCEs across that taxon's data set.

Family	Species	Voucher	Reads	UCEs	SRA
Neopilionidae	<i>Megalopsalis nigricans</i>	TAS089	11,700,600	411	SAMN35540789
	<i>Megalopsalis nigricans</i>	TAS365	11,581,466		SAMN35540790
	<i>Megalopsalis tasmanica</i>	TAS233	9,145,696		SAMN35540791
Triaenonychidae	<i>Triaenobunus apser</i>	TAS315	5,185,172	609	SAMN35540830
	<i>Triaenobunus apser</i>	TAS366	20,085,329		SAMN35540831
	<i>Triaenobunus pectinatus</i>	TAS035	13,214,205		SAMN35540832
Cryptomastriidae	<i>Speleomaster lexi</i>	Arco	9,312,983	794	SAMN35540818
	<i>Speleomaster lexi</i>	Chalk	10,468,288		SAMN35540819
	<i>Speleomaster lexi</i>	CRMO11	16,291,038		SAMN35540820
	<i>Speleomaster lexi</i>	Nbear	10,634,352		SAMN35540821
	<i>Speleomaster lexi</i>	Sbear	5,876,736		SAMN35540822

Table 4

Direct comparison of effectiveness of Arachnida versus Opiliones probe sets across identical tissues. Hybridized column refers to the probe set that was used for hybridization. Matched column refers to the probe set that was used when matching contigs to probes. UCEs column refers to the number of UCEs identified when matching contigs to probes. Increase column refers to the increase in the number of UCEs for that sample from the Opiliones probe set relative to the Arachnida probe set.

Family	Species	Voucher	Hybridized	Matched	UCEs	Increase
Petrobunidae	<i>Petrobunus</i> sp.	MCZ134849	Opiliones	Opiliones	1187	
			Arachnida	Opiliones	132	
Petallidae	<i>Pettalus thwaitesi</i>	MCZ78880	Arachnida	Arachnida	217	970
			Opiliones	Opiliones	1370	
			Arachnida	Opiliones	423	
			Arachnida	Arachnida	679	691
Sclerosomatidae	<i>Prionostemma vittatum</i>	MCZ144108	Opiliones	Opiliones	1037	
			Arachnida	Opiliones	588	
			Arachnida	Arachnida	541	496
Sandokanidae	<i>Gnomulus</i> sp.	MCZ131264	Opiliones	Opiliones	1237	
			Arachnida	Opiliones	441	
			Arachnida	Arachnida	704	533
Epedanidae	<i>Dibunus</i> sp.	MCZ141285	Opiliones	Opiliones	1047	
			Arachnida	Opiliones	517	
			Arachnida	Arachnida	633	414
Podoctidae	<i>Erecanana remyi</i>	MCZ151860	Opiliones	Opiliones	1297	
			Arachnida	Opiliones	352	
			Arachnida	Arachnida	645	652
Tithaeidae	<i>Tithaeus</i> sp.	MCZ131318	Opiliones	Opiliones	1272	
			Arachnida	Opiliones	629	
			Arachnida	Arachnida	755	517
Cosmetidae	<i>Vonones sayi</i>	Opi1H6	Opiliones	Opiliones	872	
			Arachnida	Opiliones	340	
			Arachnida	Arachnida	579	293

included 1119 raw UCEs (346 in the final matrix) and was recovered within Assamiidae with high support.

Smilograms demonstrate the utility of this probe set at shallow levels (species and population) across multiple taxa with different ecological characteristics and dispersal abilities (Fig. 3). Most notably there is detectable genetic divergence among conspecific samples of a short-range endemic cave-obligate taxon. Among the three taxa tested, the proportion of variable sites decreased with decreasing dispersal ability and increasing microhabitat specificity (*Megalopsalis* having the highest dispersal ability and lowest habitat specificity and *Speleomaster lexi* with the lowest dispersal ability and highest habitat specificity).

An obvious improvement is evident in the number of recovered loci relative to the Arachnida probe set, with an average increase of 571 UCEs across samples using the more specific Opiliones probe set. There is clear “backwards compatibility” with samples hybridized using the Arachnida probe set. For example, in the study of Derkarabetian et al. (2018) an average of 518 UCEs were found across all samples using the SPAdes-only assemblies matched to the Arachnida probe set; those same SPAdes assemblies produced an average of 617 UCEs when matched to the Opiliones probe set (Supplementary Table 2). In cases of samples hybridized with both Arachnida and Opiliones probe sets, sequences were identical or nearly identical.

4.2. Opiliones phylogeny

Our recovered phylogenies are largely congruent across both analyses (with two exceptions: Pyramidopidae and Prostyginae) and are largely congruent with previous phylogenomic and Sanger-sequencing studies. This demonstrates the utility of this probe set in reconstructing Opiliones relationships across divergence times relevant to phylogenomic analyses. These analyses also represent the most complete family-level Opiliones phylogeny to date, only missing two currently described families. Below we discuss uncertainties across our analyses, placement of unsampled families, and some ongoing issues identified in this study.

Our highly-supported phylogenomic analyses, with support from previous genetic and morphological analyses for unsampled and uncertain taxa, lead to a robustly supported Opiliones family-level phylogeny. The uncertainty in placement for both Pyramidopidae and Prostyginae is not surprising given the lower quality DNA and sequencing results for both samples. The pyramidopid specimen, although fresh, was small-bodied; multiple attempts at extracting sufficient DNA from multiple other pyramidopid specimens from multiple collecting events resulted in several failures. The prostygine *Cutervolus albopunctatus* sample was a historical specimen (preserved in 80% ethanol) with degraded DNA. Despite these uncertainties, the most

Table 5

“Backwards compatibility” of the Opiliones probe set when matching contigs for samples that were previously hybridized with the Arachnida probe set. Hybridized column refers to the probe set that was used for hybridization. Matched column refers to the probe set that was used when matching contigs to probes. UCEs column refers to the number of raw UCEs identified when matching contigs to probes. Species with asterisk indicate historical specimens.

Suborder	Family	Species	Voucher	Hybridized	Matched	UCEs
Laniatores	Beloniscidae	<i>Beloniscus</i> sp. *	MCZ37641	Arachnida	Opiliones	63
				Arachnida	Arachnida	112
Cyphophthalmi	Ogoveidae	<i>Ogovea cameroonensis</i>	MCZ132315	Arachnida	Opiliones	244
				Arachnida	Arachnida	431
Laniatores	Synthetonychiidae	<i>Synthetonychia minuta</i>	MCZ136018	Arachnida	Opiliones	708
				Arachnida	Arachnida	487
Dyspnoi	Taracidae	<i>Taracus packardii</i>	SDSUTAC_OP1247	Arachnida	Opiliones	173
				Arachnida	Arachnida	232
Laniatores	Cladonychiidae	<i>Theromaster brunneus</i>	SDSUTAC_OP1626	Arachnida	Opiliones	738
				Arachnida	Arachnida	579
Laniatores	Travuniidae	<i>Travunia jandai</i>	SDSUTAC_OP4617	Arachnida	Opiliones	141
				Arachnida	Arachnida	218
Cyphophthalmi	Troglosironidae	<i>Troglosiro aelleni</i>	MCZ134764	Arachnida	Opiliones	191
				Arachnida	Arachnida	304
Laniatores		<i>Yuria pulchra</i>	SDSUTAC_OP4263	Arachnida	Opiliones	710
				Arachnida	Arachnida	536
Outgroup		<i>Hypochilus</i> sp.	SDSUTAC_H595	Arachnida	Opiliones	286
				Arachnida	Arachnida	571
Outgroup		<i>Neomolgus</i> sp.	AR014	Arachnida	Opiliones	263
				Arachnida	Arachnida	282
Outgroup		<i>Paravaejovis</i> sp.	AR018	Arachnida	Opiliones	229
				Arachnida	Arachnida	488

likely correct placement for these two taxa can be inferred from recent studies and historical morphological work. Pyramidopidae was recovered as the sister family to Assamiidae in recent molecular studies (e.g., Aharon et al., 2019); this clade is referred to as Assamioidea, although its current composition has changed since early molecular studies (e.g., Giribet et al. 2010). Prostyginae (sometimes referred to as Prostyginae) has had a difficult taxonomic history, however the most recent morphology-based analyses included this lineage in the “MECO clade” (Metasarcidae + Cosmetidae) (Kury et al. 2020; Medrano et al. 2022), which is consistent with the concatenated RAxML analyses presented here. Similarly, recent morphological analyses can be used to place the unsampled families Otilioleptidae as an early diverging lineage in the Laminata clade (Acosta, 2019) and Askawachidae in the “MECO” clade (Kury et al. 2020). Within Cyphophthalmi there are three monotypic genera currently not assigned to any family, and again, these are rare species with single collecting events in caves or remote and uncertain localities: *Ankaratra* Shear & Gruber, 1996, from Madagascar, *Marwe* Shear, 1985, from a cave in Kenya, and *Shearogovea* Giribet, 2011, from a cave in Mexico. While these samples were not included in this study, the prospect of hybridizing to degraded DNA samples seems the only way to place these specimens that have not been collected since the original specimens.

We note some uncertainty and difficulty in identification of certain Zalmoxoidea taxa (Guasiniidae, Icaleptidae) due to potentially homoplastic morphological characters used as diagnostic. Guasiniidae is a microdiverse family of three species from northern South America. They are small-bodied, yellow (sometimes depigmented), and blind, typically found inside bark under leaf litter (Pinto-da-Rocha and Kury 2003). It is possible that their morphology is a result of their preferred microhabitat of deep leaf litter, a morphology that is also found in several other laniatorean taxa found in similar microhabitats, for example some Lomanellidae Mendes & Derkarabetian, 2021 (*L. parva* Forster, 1955 and *L. quasiparva* Hunt and Hickman, 1993 from Tasmania). Icaleptidae Kury & Pérez-González, 2002 includes two described species from northern South America diagnosed by a ventrally-inserted coxa IV, a character that can also be observed to some degree in other taxa like Zalmoxidae (Kury and Pérez-González 2007). It is likely that more in-depth analyses of these and related taxa will show non-monophyly of some named families. In fact, in sampling specimens for this study, three specimens morphologically identified initially as “Icaleptidae” were

included (with ventrally-inserted coxae IV), yet all were recovered as unrelated lineages within Zalmoxoidea. The included sample of *Trypophobica* Cruz-López et al., 2021 (MCZ:IZ:89431) was identified as and considered Icaleptidae in previous studies (as DNA104845 in Sharma and Giribet 2012; Cruz-López et al. 2021). However, that genus has since been removed and is now considered *incertae sedis* within Zalmoxoidea (Kury et al., 2023). The two samples identified as Kimulidae do not form a clade. Kimulidae is a Neotropical family with 28 recognized species in seven genera, but a large number of species have inadequate descriptions and lack the standard illustrations required today. Kimulidae is currently undergoing revisionary work by M.P. Pereira and A. Pérez-González, and clear delimitation of the family and its genera is needed.

5. Conclusions

Like most plant and animal taxa, molecular phylogenetics in Opiliones began with Sanger sequencing (Fig. 4). The first Opiliones sequence data, the 18S rRNA gene from *Odiellus trogluoides* (Lucas, 1846), was published in 1996 as part of a study examining relationships between Arthropoda and Tardigrada (Giribet et al. 1996). Other Opiliones Sanger data appeared afterwards as representatives of higher level arthropod phylogenetics (e.g., Regier and Shultz 1997), with the first Opiliones-centric Sanger studies appearing in 1999 (Giribet et al., 1999). The number of Sanger sequences submitted steadily increased and peaked in 2015 with 2875 sequences from multiple studies. Since 2015, the trend for Sanger data has been decreasing, largely coinciding with the increased use of transcriptomic data for phylogenomic analyses. The first short-read genome-scale data for Opiliones appeared on SRA in 2013, associated with early transcriptomic studies (Hedin et al. 2012). The first UCE data appeared in 2017, with the publication of the Arachnida probe set (Starrett et al. 2017), and has been steadily increasing since. Of note, 2022 is the first year that more sequence data were published on SRA than on GenBank, reflecting a switch by most molecular opilological research labs from multi-locus Sanger sequencing to short-read sequencing for phylogenomics or genotyping (e.g., Brown et al. 2021). The UCE approach has been transformative for molecular systematics in many taxa, arachnids being no exception. An Opiliones-specific probe set provides an opportunity to continue the ongoing molecular work in Opiliones, allowing for the recovery of more

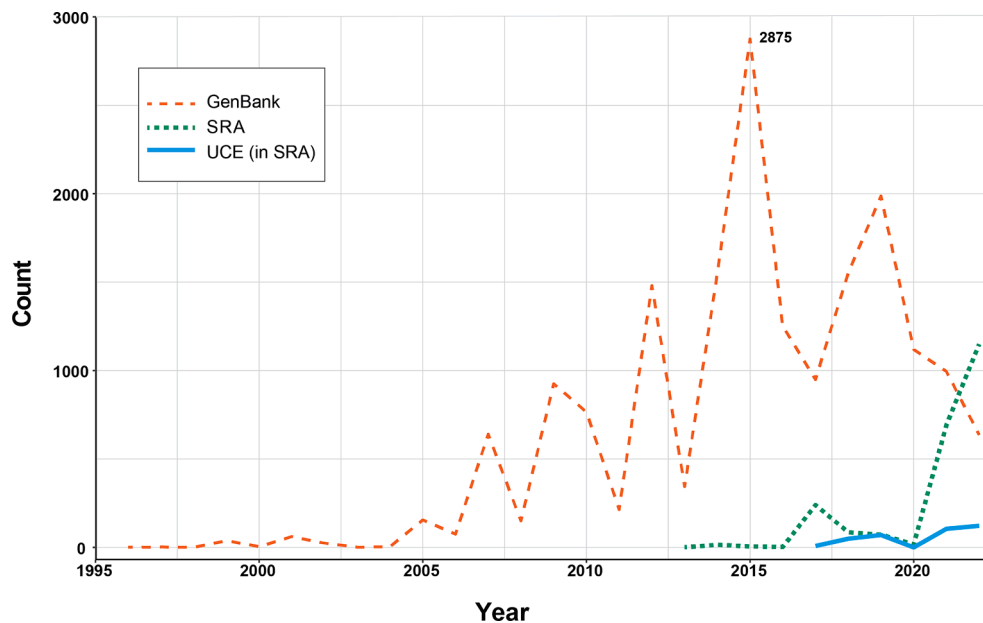


Fig. 4. Opiliones molecular phylogenetic history through sequence submissions to GenBank and SRA (Sequence Read Archive).

loci. This will be especially important for the many taxa that are rare or only known from historical specimens, especially the plethora of monotypic genera created as a result of the Roewerian system of taxonomy, and taxa with uncertain placements at the family level.

CRediT authorship contribution statement

Shahan Derkarabetian: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Data curation, Writing – original draft, Writing – review & editing. **Arianna Lord:** Investigation, Formal analysis, Visualization, Writing – review & editing. **Katherine Angier:** Investigation, Writing – review & editing. **Ella Frigyk:** Investigation, Writing – review & editing. **Gonzalo Giribet:** Conceptualization, Resources, Writing – review & editing, Funding acquisition.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107887>.

References

- Acosta, L.E., 2019. A relictual troglomorphic harvestman discovered in a volcanic cave of western Argentina: *Otilioleptes marcelae*, new genus, new species, and Otilioleptidae, new family (Arachnida, Opiliones, Gonyleptoidea). *PLoS One* 14 (10), e0223828.
- Aharon, S., Ballesteros, J.A., Crawford, A.R., Friske, K., Gainett, G., Langford, B., Aharon, S., Ballesteros, J.A., Crawford, A.R., Friske, K., Gainett, G., Langford, B., Santibáñez-López, C.E., Sharma, P.P., 2019. The anatomy of an unstable node: a Levantine relict precipitates phylogenomic dissolution of higher-level relationships of the armoured harvestmen (Arachnida: Opiliones: Laniatores). *Invertebr. Syst.* 33 (5), 697–717.
- Azevedo, G.H.F., Bougie, T., Carboni, M., Hedin, M., Ramírez, M.J., 2022. Combining genomic, phenotypic and Sanger sequencing data to elucidate the phylogeny of the two-clawed spiders (Dionycha). *Mol. Phylogenet. Evol.* 166, 107327.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19 (5), 455–477.
- Benavides, L.R., Pinto-da-Rocha, R., Giribet, G., Barrow, L., 2021. The phylogeny and evolution of the flashiest of the armored harvestmen (Arachnida: Opiliones). *Syst. Biol.* 70 (4), 648–659.
- Branstetter, M.G., Longino, J.T., Ward, P.S., Faircloth, B.C., Price, S., 2017. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods Ecol. Evol.* 8 (6), 768–776.
- Brown, T.A., Tsurusaki, N., Burns, M., Orive, M., 2021. Genomic determination of reproductive mode in facultatively parthenogenetic Opiliones. *J. Hered.* 112 (1), 34–44.
- Chamberland, L., Agnarsson, I., Quayle, I.L., Ruddy, T., Starrett, J., Bond, J.E., 2022. Biogeography and eye size evolution of the ogre-faced spiders. *Sci. Rep.* 12 (1), 17769.
- Cruz-López, J.A., Monjaraz-Ruedas, R., Colmenares, P.A., Francke, O.F., 2021. Historical biogeography of a neglected family of armoured harvestmen (Opiliones: Laniatores: Icaleptidae) with the first record and a new genus for tropical Mesoamerica. *Invertebr. Syst.* 35 (5), 493–513.
- Derkarabetian, S., Starrett, J., Tsurusaki, N., Ubick, D., Castillo, S., Hedin, M., 2018. A stable phylogenomic classification of Travunioidae (Arachnida, Opiliones, Laniatores) based on sequence capture of ultraconserved elements. *ZooKeys* 760, 1–36.
- Derkarabetian, S., Castillo, S., Koo, P.K., Ovchinnikov, S., Hedin, M., 2019a. A demonstration of unsupervised machine learning in species delimitation. *Mol. Phylogenet. Evol.* 139, 106562.
- Derkarabetian, S., Benavides, L.R., Giribet, G., 2019b. Sequence capture phylogenomics of historical ethanol-preserved museum specimens: Unlocking the rest of the vault. *Mol. Ecol. Resour.* 19 (6), 1531–1544.
- Derkarabetian, S., Baker, C.M., Hedin, M., Prieto, C.E., Giribet, G., 2021. Phylogenomic re-evaluation of Triaenonychoidea (Opiliones: Laniatores), and systematics of Triaenonychidae, including new families, genera and species. *Invertebr. Syst.* 35 (2), 133–157.
- Derkarabetian, S., Paquin, P., Reddell, J., Hedin, M., 2022a. Conservation genomics of federally endangered *Texella* harvester species (Arachnida, Opiliones, Phalangodidae) from cave and karst habitats of central Texas. *Conserv. Genet.* 23 (2), 401–416.

- Derkarabetian, S., Starrett, J., Hedin, M., 2022b. Using natural history to guide supervised machine learning for cryptic species delimitation with genetic data. *Front. Zool.* 19 (1), 8.
- Faircloth, B.C., 2017. Identifying conserved genomic elements and designing universal bait sets to enrich them. *Methods Ecol. Evol.* 8 (9), 1103–1112.
- Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* 61 (5), 717–726.
- Faircloth, B. C. (2013). Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming.
- Faircloth, B. C. (2016). PHYLUCe is a software package for the analysis of conserved genomic loci. *Bioinformatics*, 32(5), 786–788.
- Fernández, R., Sharma, P.P., Tourinho, A.L., Giribet, G., 2017. The Opiliones tree of life: shedding light on harvestmen relationships through transcriptomics. *P Roy Soc B: Biol Sci* 284 (1849), 20162340.
- Fu, L., Niu, B., Zhu, Z., Wu, S., Li, W., 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* 28 (23), 3150–3152.
- Gainett, G., González, V.L., Ballesteros, J.A., Setton, E.V.W., Baker, C.M., Barolo Gargiulo, L., Santibáñez-López, C.E., Coddington, J.A., Sharma, P.P., 2021. The genome of a daddy-long-legs (Opiliones) illuminates the evolution of arachnid appendages. *Proc. Roy. Soc. B* 288 (1956), 20211168.
- Giribet, G., Carranza, S., Bagunà, J., Riutort, M., Ribera, C., 1996. First molecular evidence for the existence of a Tardigrada+ Arthropoda clade. *Mol. Biol. Evol.* 13 (1), 76–84.
- Giribet, G., Sheridan, K., Baker, C.M., Painting, C.J., Holwell, G.I., Sirvid, P.J., Hormiga, G., 2021. A molecular phylogeny of the circum-Antarctic Opiliones family Neopilionidae. *Invertebr. Syst.* 35 (8), 827–849.
- Giribet, G., Vogt, L., Pérez González, A., Sharma, P., Kury, A.B., 2010. A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores. *Cladistics* 26 (4), 408–437.
- Giribet, G., Rambla, M., Carranza, S., Baguna, J., Riutort, M., Ribera, C., 1999. Phylogeny of the arachnid order Opiliones (Arthropoda) inferred from a combined approach of complete 18S and partial 28S ribosomal DNA sequences and morphology. *Mol. Phylogenet. Evol.* 11 (2), 296–307.
- Giribet, G., Shaw, M., Lord, A., Derkarabetian, S., Sharma, P., 2022. Closing a biogeographic gap: a new petalid genus from South Australia (Arachnida: Opiliones: Cyphophthalmi: Petalidae) with a UCE-based phylogeny of Cyphophthalmi. *Invertebr. Syst.* 36 (11), 1002–1016.
- Gustafson, G.T., Alexander, A., Sproul, J.S., Pflug, J.M., Maddison, D.R., Short, A.E., 2019. Ultraconserved element (UCE) probe set design: Base genome and initial design parameters critical for optimization. *Ecol. Evol.* 9 (12), 6933–6948.
- Hedin, M., Starrett, J., Akhter, S., Schönhöfer, A.L., Shultz, J.W., Moreau, C.S., 2012. Phylogenomic resolution of Paleozoic divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. *PLoS One* 7 (8), e42888.
- Hedin, M., Derkarabetian, S., Alfaro, A., Ramírez, M.J., Bond, J.E., 2019. Phylogenomic analysis and revised classification of atypoid mygalomorph spiders (Araneae, Mygalomorphae), with notes on arachnid ultraconserved element loci. *PeerJ* 7, e6864.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35 (2), 518–522.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A., Jermini, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14 (6), 587–589.
- Kent, W.J., 2002. BLAT—the BLAST-like alignment tool. *Genome Res.* 12 (4), 656–664.
- Kolmogorov, M., Yuan, J., Lin, Y., Pevzner, P.A., 2019. Assembly of long, error-prone reads using repeat graphs. *Nat. Biotechnol.* 37 (5), 540–546.
- Krueger, F. (2015). Trim Galore!: A wrapper around Cutadapt and FastQC to consistently apply adapter and quality trimming to FastQ files, with extra functionality for RRBS data. *Babraham Institute*.
- Kulkarni, S., Wood, H., Lloyd, M., Hormiga, G., 2020. Spider-specific probe set for ultraconserved elements offers new perspectives on the evolutionary history of spiders (Arachnida, Araneae). *Mol. Ecol. Resour.* 20 (1), 185–203.
- Kulkarni, S., Kallal, R.J., Wood, H., Dimitrov, D., Giribet, G., Hormiga, G., Larracuente, A., 2021. Interrogating genomic-scale data to resolve recalcitrant nodes in the Spider Tree of Life. *Mol. Biol. Evol.* 38 (3), 891–903.
- Kundu, R., Casey, J., & Sung, W. K. (2019). HyPo: super fast & accurate polisher for long read genome assemblies. *BioRxiv*, 2019-12. <https://doi.org/10.1101/2019.12.19.882506>.
- Kury, A.B., Villarreal, M., O., 2015. The prickly blade mapped: establishing homologies and a chaetotaxy for macrosetae of penis ventral plate in Gonyleptoidea (Arachnida, Opiliones, Laniatores). *Zool. J. Linn. Soc.* 174 (1), 1–46.
- Kury, A.B., García, A.F., Ahumada-C, D., 2023. A new genus of Zalmoxoidea from Colombia (Arachnida: Opiliones: Grassatores). *J. Arachnol.* 51 (1), 37–45.
- Kury, A.B., Mendes, A.C., Cardoso, L., Kury, M.S., Granado, A.A., Yoder, M.J., Kury, I.S., 2020. WCO-Lite version 1.1: an online nomenclatural catalogue of harvestmen of the world (Arachnida, Opiliones) curated in TaxonWorks. *Zootaxa* 4908 (3), 447–450.
- Kury, A.B., Pérez-González, 2007. Chapter 4. Taxonomy: Icaleptidae. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: the Biology of Opiliones*. Harvard University Press, pp. 205–207.
- Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22 (13), 1658–1659.
- Lozano-Fernandez, J., Tanner, A.R., Puttick, M.N., Vinther, J., Edgecombe, G.D., Pisani, D., 2020. A Cambrian-Ordovician terrestrialization of arachnids. *Front. Genet.* 11, 182.
- Lunter, G., Goodson, M., 2011. Stampy: a statistical algorithm for sensitive and fast mapping of Illumina sequence reads. *Genome Res.* 21 (6), 936–939.
- Machado, G., Pinto-da-Rocha, R., Giribet, G.C., 1., 2007. What are harvestmen. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: the Biology of Opiliones*. Harvard University Press, pp. 1–13.
- Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A., Zdobnov, E.M., Kelley, J., 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol. Biol. Evol.* 38 (10), 4647–4654.
- Martens, J., 2020. A mysterious dwarf: Suthepiidae nov. fam., a new harvestman family from mountains of northern Thailand (Arachnida Opiliones: Laniatores). *Rev. suisse Zool.* 127 (2), 381–391.
- McCormack, J.E., Faircloth, B.C., Crawford, N.G., Gowaty, P.A., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Res.* 22 (4), 746–754.
- Medrano, M., Kury, A. B., & Mendes, A. C. (2022). Morphology-based cladistics splinters the century-old dichotomy of the pied harvestmen (Arachnida: Gonyleptoidea: Cosmetidae). *Zool J Linn Soc*, 195(2), 585-672.
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32 (1), 268–274.
- Pinto-da-Rocha, R., Kury, A.B., 2003. Third species of Guasiniidae (Opiliones, Laniatores) with comments on familial relationships. *J. Arachnol.* 31 (3), 394–399.
- Quattrini, A.M., Faircloth, B.C., Dueñas, L.F., Bridge, T.C.L., Brugler, M.R., Calixto-Botía, I.F., DeLeo, D.M., Forêt, S., Herrera, S., Lee, S.M.Y., Miller, D.J., Prada, C., Rádis-Baptista, G., Ramírez-Portilla, C., Sánchez, J.A., Rodríguez, E., McFadden, C. S., 2018. Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: New approaches to long-standing problems. *Mol. Ecol. Resour.* 18 (2), 281–295.
- Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26 (6), 841–842.
- Ranallo-Benavidez, T.R., Jaron, K.S., Schatz, M.C., 2020. GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat. Commun.* 11 (1), 1432.
- Regier, J.C., Shultz, J.W., 1997. Molecular phylogeny of the major arthropod groups indicates polyphyly of crustaceans and a new hypothesis for the origin of hexapods. *Mol. Biol. Evol.* 14 (9), 902–913.
- Sharma, P.P., Giribet, G., 2012. Out of the Neotropics: Late Cretaceous colonization of Australasia by American arthropods. *Proc. R. Soc. B Biol. Sci.* 279 (1742), 3501–3509.
- Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J., Birol, I., 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res.* 19 (6), 1117–1123.
- Smith, B.T., Harvey, M.G., Faircloth, B.C., Glenn, T.C., Brumfield, R.T., 2014. Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Syst. Biol.* 63 (1), 83–95.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9), 1312–1313.
- Starrett, J., Derkarabetian, S., Hedin, M., Bryson Jr, R.W., McCormack, J.E., Faircloth, B. C., 2017. High phylogenetic utility of an ultraconserved element probe set designed for Arachnida. *Mol. Ecol. Resour.* 17 (4), 812–823.
- Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* 56 (4), 564–577.
- Tin, M.-Y., Economo, E.P., Mikheyev, A.S., Caramelli, D., 2014. Sequencing degraded DNA from non-destructively sampled museum specimens for RAD-tagging and low-coverage shotgun phylogenetics. *PLoS One* 9 (5), e96793.
- Vurtture, G.W., Sedlazeck, F.J., Nattestad, M., Underwood, C.J., Fang, H., Gurtowski, J., Schatz, M.C., 2017. GenomeScope: fast reference-free genome profiling from short reads. *Bioinformatics* 33 (14), 2202–2204.
- Wood, H.M., González, V.L., Lloyd, M., Coddington, J., Scharff, N., 2018. Next-generation museum genomics: Phylogenetic relationships among palpimanoid spiders using sequence capture techniques (Araneae: Palpimanoidea). *Mol. Phylogenet. Evol.* 127, 907–918.
- Xu, X., Su, Y.-C., Ho, S.Y.W., Kuntner, M., Ono, H., Liu, F., Chang, C.-C., Warrit, N., Sivayyapram, V., Aung, K.P.P., Pham, D.S., Norma-Rashid, Y., Li, D., González, V., 2021. Phylogenomic analysis of ultraconserved elements resolves the evolutionary and biogeographic history of segmented trapdoor spiders. *Syst. Biol.* 70 (6), 1110–1122.
- Zhang, J., Li, Z., Lai, J., Zhang, Z., Zhang, F., 2023. A novel probe set for the phylogenomics and evolution of RTA spiders. *Cladistics* 39 (2), 116–128.