



Phylogenomics illuminates the evolution of orb webs, respiratory systems and the biogeographic history of the world's smallest orb-weaving spiders (Araneae, Araneoidea, Symphytognathoids)

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ABSTRACT

The miniature orb weaving spiders (symphytognathoids) are a group of small spiders (<2 mm), including the smallest adult spider *Patu digua* (0.37 mm in body length), that have been classified into five families. The species of one of its constituent lineages, the family Anapidae, build a remarkable diversity of webs (ranging from orbs to sheet webs and irregular tangles) and even include a webless kleptoparasitic species. Anapids are also exceptional because of the extraordinary diversity of their respiratory systems. The phylogenetic relationships of symphytognathoid families have been recalcitrant with different classes of data, such as, monophyletic with morphology and its concatenation with Sanger-based six markers, paraphyletic (including a paraphyletic Anapidae) with solely Sanger-based six markers, and polyphyletic with transcriptomes. In this study, we capitalized on a large taxonomic sampling of symphytognathoids, focusing on Anapidae, and using *de novo* sequenced ultraconserved elements (UCEs) combined with UCEs recovered from available transcriptomes and genomes. We evaluated the conflicting relationships using a variety of support metrics and topology tests. We found support for the phylogenetic hypothesis proposed using morphology to obtain the “symphytognathoids” clade, Anterior Tracheal System (ANTS) Clade and monophyly of the family Anapidae. Anapidae can be divided into three major lineages, the Vichitra Clade (including *Teutoniella*, *Holarchaea*, *Sofanapis* and *Acrobleps*), the subfamily Micropholcommatinae and the Orb-weaving anapids (Owa) Clade. Biogeographic analyses reconstructed a hypothesis of multiple long-distance transoceanic dispersal events, potentially influenced by the Antarctic Circumpolar Current and West Wind Drift. In symphytognathoids, the ancestral anterior tracheal system transformed to book lungs four times and reduced book lungs five times. The posterior tracheal system was lost six times. The orb web structure was lost four times independently and transformed into sheet web once.

1. Introduction

Spiders are an ancient clade of terrestrial predators with over 50,000 described species (World Spider Catalog, 2023), occupying most habitats globally as a result of a multitude of biogeographic events (such as Pangean breakup, founder events, dispersals). They date back to the Carboniferous era (359–299 million years ago; Fernandez et al., 2018; Kallal et al., 2021) after which, they have evolved into an enormous diversity of sizes, morphologies, behaviors, silk uses, and web architectures. The smallest known adult spider is the orb weaver *Patu digua* (Symphytognathidae), with a body length of 0.37 mm (Forster and Platnick, 1977). Symphytognathidae belongs to a group of five

miniature orb weaving spider families (with adult spider sizes <2 mm) called the “symphytognathoids” (an informal name proposed by Codrington, 1986). This group includes the families Anapidae, Mysmenidae, Synsphyridae, Symphytognathidae and Theridionomidae (Hormiga and Griswold, 2014). Symphytognathoid spiders are cryptozoic, usually living in habitats with high humidity such as mosses, leaf litter, exfoliating tree bark and tree buttresses (Platnick and Forster, 1989; Rix et al., 2010; our observations). Over 625 species classified in over 100 genera of symphytognathoids have been described so far (World Spider Catalog, 2023), but their small size, cryptic habits and the understudied regions where they live suggest that a large fraction of their species diversity remains to be discovered. One study estimated the

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extant diversity of symphytognathoids to be 1,456 species (meaning 829 undiscovered) using an extrapolation of known species richness and the opinions of taxonomic experts of various groups (Agnarsson et al., 2013), but we think that future work will show that this figure is significantly higher.

The synapomorphies of symphytognathoids include a posteriorly truncated sternum, loss of the claw on the female pedipalp, greatly elongated fourth tarsal median claw, absence of the carapace fovea, a domed sternum (or at least convex) in lateral view, colulus with three or fewer setae, and the position of the tarsal organ in the basal third of all leg tarsi (Grissold et al., 1998; Schutt, 2003; Lopardo et al., 2011). In addition to these morphological synapomorphies, other peculiar traits of this group include reduction and loss of the pedipalp in adult females and a diversity of web architectures and respiratory systems (discussed below).

Pedipalps in adult male spiders are modified to serve as an intermittent secondary sexual organ. In adult females and juveniles of both sexes, the pedipalp appears as a tactile, sensory appendage which is also used for prey manipulation. Generally, the adult female pedipalp diameter is about one-third of the diameter of the first leg. However, two symphytognathoid families form an exception to this proportion. In some Anapidae species, the adult female pedipalp is reduced in diameter and number of segments, whereas it is absent in remaining female anapids and in all symphytognathids (Fig. 1) (Platnick and Forster, 1989; Rix and Harvey, 2010).

1.1. Web diversity

Silk capture webs is a feature unique to spiders, although not all spiders build webs to capture prey. Web building spiders have evolved

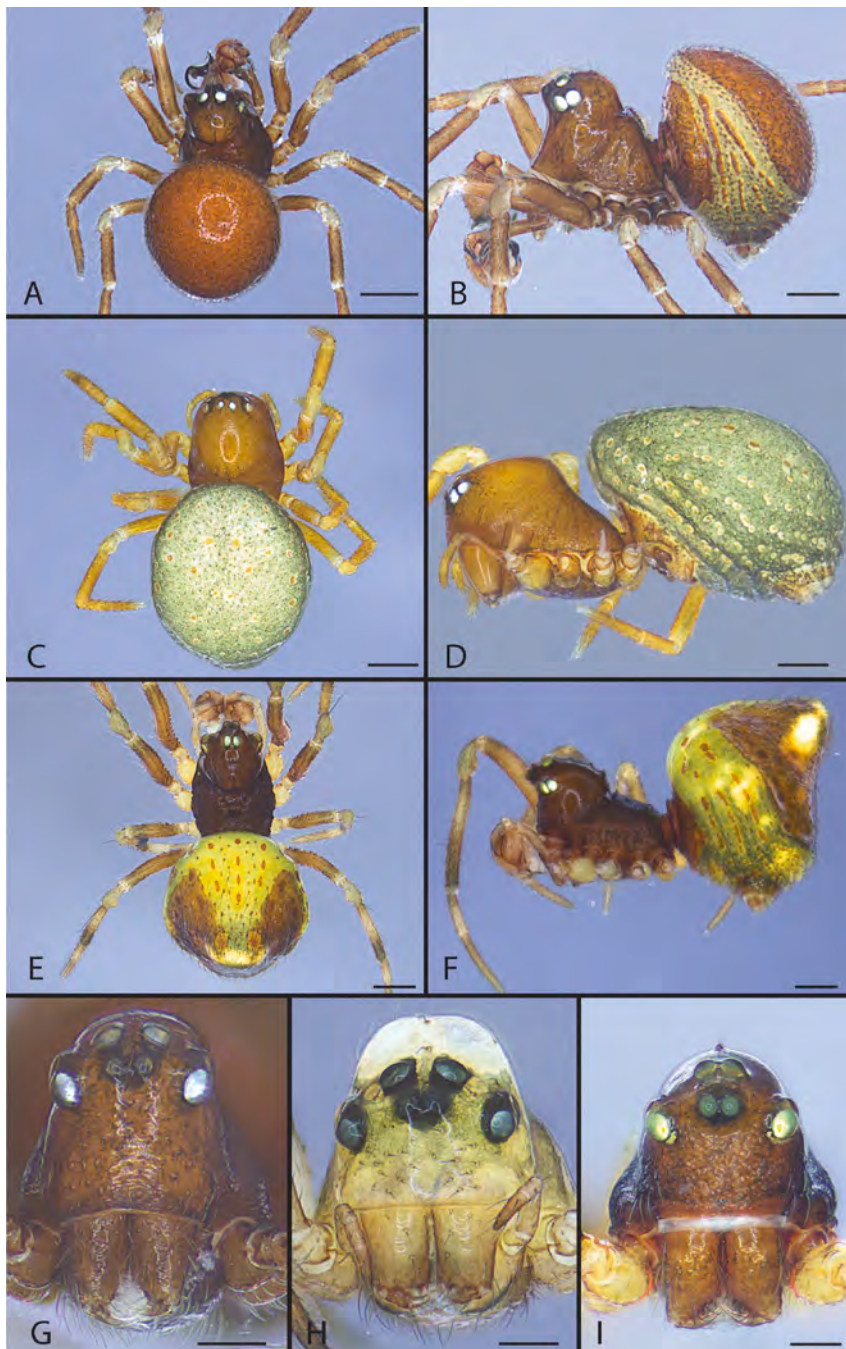


Fig. 1. A sample of morphological diversity of Anapidae spiders and pedipalps in adult females. A. Male of *Minanapis palena* from Chile (GH2905), dorsal view. B. Same specimen as in A, lateral view. C. Female of *Eterosonycha* sp. from Australia (GH2849), dorsal view. D. Same specimen as in C, lateral view. E. Male of *Pecanapis* sp. from Chile (GH2900), dorsal view. F. Same specimen as E, lateral view. G. Female of *Minanapis palena* from Chile (GH2905), frontal view. H. Female of *Elanapis aisen* from Chile (GH2892), frontal view. I. Female of *Pecanapis* sp. from Chile (GH2900), frontal view. Note the reduced pedipalps in H and their absence in G and I. Scale (A-F) 200 μ m, (G-I) 100 μ m.

an enormous diversity of architectures such as cob webs, sheet webs, funnel-shaped webs, trapdoor webs and orb webs. Some of these web forms have evolved multiple times across the spider tree of life (Kallal et al., 2021). Symphytognathoid webs are architecturally diverse and include orb webs (with a diversity of variations of the typical orb), cob webs and sheet webs, some species of symphytognathoids are kleptoparasites that do not build any foraging webs, and instead live in the web of their host (Ramírez and Platnick, 1999; Lopardo and Hormiga, 2008; Rix and Harvey, 2010). Most mysmenids build spherical or planar orbs, symphytognathids build a two-dimensional horizontal orb web, at least some synsphyrids build sheet or irregular webs, and theridiosomatids build orb webs, some of them highly modified (e.g., sticky lines connected to water surface) (Coddington and Valerio, 1980; Eberhard, 1987; Rix and Harvey, 2010; Lopardo et al., 2011; Cotoras et al., 2021; Eberhard, 2022). But due to their cryptic lifestyle, the webs and biology of most symphytognathoids remain unknown. In each of these symphytognathoid families (except Synsphyridae), there is at least one genus with a kleptoparasitic lifestyle accompanied by loss of the foraging web in all its constituent species. For example, *Mysmenopsis furtiva* (Mysmenidae) and *Curimagua bayano* (Symphytognathidae) live in the webs of diplurid spiders (Griswold et al., 1998; Ramírez and Platnick, 1999).

Among symphytognathoids, the largest diversity of web architectures is found in its most speciose family, Anapidae. In his seminal paper,

Eberhard (1987) suggested that the stereotypical web building behavior of *Anapis* and *Anapisona* could be homologized to that of other orb-weaving araneoid spiders, such as araneids and tetragnathids. Anapids construct a myriad of variations of the typical orb web such as webs with secondary tent-like radii attached over or under the orb or orb webs sharing frames (Ramírez and Platnick, 1999; Lopardo and Hormiga, 2008; Ramírez et al., 2004; Kropf, 1990; Lopardo et al., 2011; Rix and Harvey, 2010; Lopardo and Hormiga, 2015; Eberhard, 2020). For example, *Crassanapis* species build horizontal orb webs in tree cavities and *Sheranapis* spin their webs on the water surface or in tree cavities (both genera from Chile) or *Conculus lyugadinus* on water surface (from northeast Asia) Shinkai and Shinkai, 1988; Platnick and Forster, 1989). Their webs include additional radii above the sticky spiral (termed as “above-plane-radii”), forming a tent-like structure (Eberhard, 1987; Shinkai and Shinkai, 1988; Platnick and Forster, 1989; Ramírez and Platnick 1999). The above-plane-radii of some *Anapis*, *Anapisona* and *Chasmocephalon* have few silk lines between them termed as “tent lines” (Platnick and Shadab, 1978a; Coddington, 1986). Additionally, horizontal orb webs of *Anapis* and *Anapisona* constructed in tree cavities contain supplementary radii (radiating silk lines not connected to the hub) (Eberhard, 1987). *Tasmanapis strahan*, from Tasmania, constructs densely placed sticky spirals and radii similar to symphytognathid planar orb webs and adjacent webs can share web frames (Lopardo et al.,



Fig. 2. A sample of the diversity of modified orbicular webs of Anapidae spiders. A. *Anapis* sp. from Trinidad & Tobago (GH2582). B. *Risdonius* sp. from Australia (GH2847). C. *Sheranapis quellon* from Chile. D. *Maxanapis* sp. from Australia. E. *Elanapis aisen* from Chile (GH2903).

2011). *Elanapis aisen*, from Chile, builds a planar orb web that contains several supplementary radii (Ramírez and Platnick 1999). *Borneanapis belong*, from the rainforests of Brunei, is known to construct small, horizontal, triangular, sheet webs (Snazell, 2009). A sample of the anapid orb webs is depicted in Fig. 2. A sub-family of Anapidae, the Micropholcommatidae, includes species known mainly from Australia and instead construct non-orbicular webs. *Epigastrina* and *Olgania* species from Tasmania construct sheet webs in wall cracks or depressions (Rix and Harvey, 2010). *Comaroma simoni*, from Europe, builds an irregular web with long threads running downwards similar to the typical cobweb of theridiid spiders (Kropf, 1990). *Sofanapis antillanca*, from Chile, is a webless kleptoparasite found on the webs of austrochilid spiders (Ramírez and Platnick, 1999). Such a remarkable diversity of web architectures in a single family of spiders is unusual and only Theridiidae comes close to such diversity. However, the transitions of their web architecture across an evolutionary timescale remains unknown.

1.2. Respiratory system

Spiders have two functional respiratory systems, book lungs and tracheae. Early diverging lineages of spiders have two pairs of book lungs, found in the second and third segments of the opisthosoma. Fossil records of Mesothelae and Mygalomorphae and of the order Uraraneida have similar arrangements (Selden et al., 2008), thus indicating that two pairs of book lungs is the plesiomorphic arrangement for Araneae. Some early diverging lineages of the so-called “modern” spiders (Araneomorphae) of the families Gradungulidae and Hypochilidae also have two pairs of book lungs. The majority of all the remaining Araneomorphae have a combination of anterior book lungs and a posterior tracheal system, with the exception of some spiders such as symphytognathoids (Anapidae, Symphytognathidae) or Telemidae that have exclusively tracheae. It is hypothesized that the posterior tracheae originated from the primitive posterior book lungs (e.g., Lamy, 1902; Purcell, 1909, 1910; Ramírez, 2000; Schmitz, 2013). The respiratory systems of the miniature orb weaving spiders can be grossly classified into three types: (i) with one pair of anterior book lungs and posterior tracheal system, (ii) with an anterior and a posterior tracheal system and (iii) with anterior tracheae only. In the latter type, the posterior respiratory system is absent from the second abdominal segment. The available literature shows a remarkable diversity of the variations of these gross types such as leaf numbers, reduced size of book lungs and branching of tracheae (e.g., Lamy, 1902; Hickman, 1943; Forster, 1959; 1980; Gertsch, 1960; Levi, 1967; Ramírez, 2000; Rix and Harvey, 2010; Lopardo and Hormiga, 2015). Lopardo et al. (2011) established the anterior tracheal system (ANTS) clade composed of all symphytognathoid families except Theridiosomatidae (see phylogenetic history section). As the name suggests, the anterior tracheal system is present in the ANTS clade members whereas anterior book lungs are present in Theridiosomatidae (Lopardo et al. 2011). In a recent study, Lopardo et al. (2022) explored the evolutionary transformations of respiratory systems in symphytognathoids focusing on mysmenids. They found that the anterior tracheal system in symphytognathoids evolved from fully developed book lungs whereas reduced book lungs have evolved independently twice from anterior tracheae. Another interesting feature of some symphytognathoid respiratory systems is that the tracheoles of the anterior tracheal system, have expanded into the prosoma and the appendages (listed in Lopardo et al. (2022)).

Some anapids, such as *Crassanapis* or *Sheranapis*, have anterior book lungs and posterior tracheae whereas *Gertschanapis* or *Caledanapis* or *Maxanapis* have an anterior tracheal system only (Forster, 1958, 1959; Ramírez, 2000). Interestingly, *Tasmanapis* has reduced anterior book lungs plus a tracheal system and a posterior tracheal system (Lopardo and Hormiga, 2015; Lopardo et al., 2022). Some micropholcommatines such as *Epigastrina* or *Eterosonycha* have anterior tracheal system only whereas *Taphiassa* has both anterior and posterior tracheal systems. The

configuration of respiratory systems of most members of this subfamily remains unknown. In sum, as illustrated by these examples and in Fig. 3, the respiratory system of anapid spiders is extremely diverse.

1.3. Phylogenetic relationships

Understanding the evolution of the remarkable diversity of webs and respiratory systems of anapids requires a robust evolutionary framework. The study of the genealogical relationships of symphytognathoids have an interesting and convoluted history (Fig. 4). Hickman (1931) erected the family Symphytognathidae in his description of *Symphytognatha globosa* from Tasmania. Forster (1959) synonymized the families Anapidae, Micropholcommatidae, Tetricellidae and Mysmeninae (at the time in family Theridiidae) with Symphytognathidae. This was a major change of grouping minute spiders with diverse morphological structures (such as reduction and loss of adult female pedipalps) and web architecture into one family. Brignoli (1970) called this change to be “insuffisante”, Levi and Randolph (1975) as “probably monophyletic” whereas Lehtinen (1975) expressed it as “surely a polyphyletic dump heap of minute Araneioidea”. Later, Forster and Platnick (1977) delimited the family Symphytognathidae to include spiders with fused chelicerae at the base. Soon, Platnick and Shadab (1978a,b) revalidated Anapidae and Mysmenidae. And then, as already mentioned, Coddington (1986) proposed the informal name symphytognathoids for the group that Forster (1970) referred to as Symphytognathidae.

The monophyly of symphytognathoids has been supported by morphological and behavioral characters (Griswold et al., 1998; Schutt, 2003; Lopardo and Hormiga, 2008; Lopardo et al., 2011; Hormiga and Griswold, 2014), but they have appeared as either paraphyletic or polyphyletic in molecular phylogenies using the six Sanger-based markers (Dimitrov et al., 2017; Wheeler et al., 2017) or transcriptomes (Fernandez et al., 2018; Kallal et al., 2021). Dimitrov et al. (2017) obtained Anapidae as paraphyletic with “Anapidae I” (represented by *Anapis*, one micropholcommatine genus (*Taphiassa*) and *Holarachaea*) as sister to Theridiidae and “Anapidae II” (represented by *Gertschanapis*, *Maxanapis* and *Chasmocephalon*) as sister to Cyatholipidae. The “Anapidae II” plus Cyatholipidae clade was sister to the Symphytognathidae lineage. Lopardo et al.’s (2011) extensive Sanger-based dataset supported symphytognathoid monophyly only when the nucleotide data were analyzed in combination with phenotypic data. It is noteworthy that transcriptomic data, analyzed as amino acids in a maximum likelihood framework, recovered symphytognathoids as polyphyletic (Fernández et al., 2018; Kallal et al., 2021). In a parsimony analysis of transcriptomic data Kallal et al. (2021) recovered Theridiosomatidae as sister to Araneidae while the other “symphytognathoid” families formed a monophyletic group. An analysis of ultraconserved elements (UCEs) using a small sample of symphytognathoids (16 species in all families except Synsphyridae and representatives of all other araneoid families) provided the first empirical support for symphytognathoid monophyly using molecular data alone, with the analyzed low occupancy datasets (Kulkarni et al. 2020). A further integrated sampling obtained by extracting UCEs from transcriptomes found that Synsphyridae too nested within symphytognathoids (Kulkarni et al. 2021). All prior molecular analyses, including phylogenomic data sets, (such as transcriptomes treated as amino acid data), have rejected the monophyly of symphytognathoids. The polyphyly of this group received high bootstrap support by transcriptomes. This paradox of highly supported but incongruent relationships across phylogenomic datasets was resolved through analyses of exons, ultraconserved loci, a combination of these data as amino acids and nucleotides which recovered monophyly of “symphytognathoids” (Kulkarni et al. 2020) at low occupancies. This latter study focused on the higher-level relationships of Araneae and therefore included only a handful of anapid representatives (10 out of the 58 valid genera).

Little is known about the biogeographic history of anapids. The most recent phylogenetic hypothesis about the families of spiders (Kallal et al.

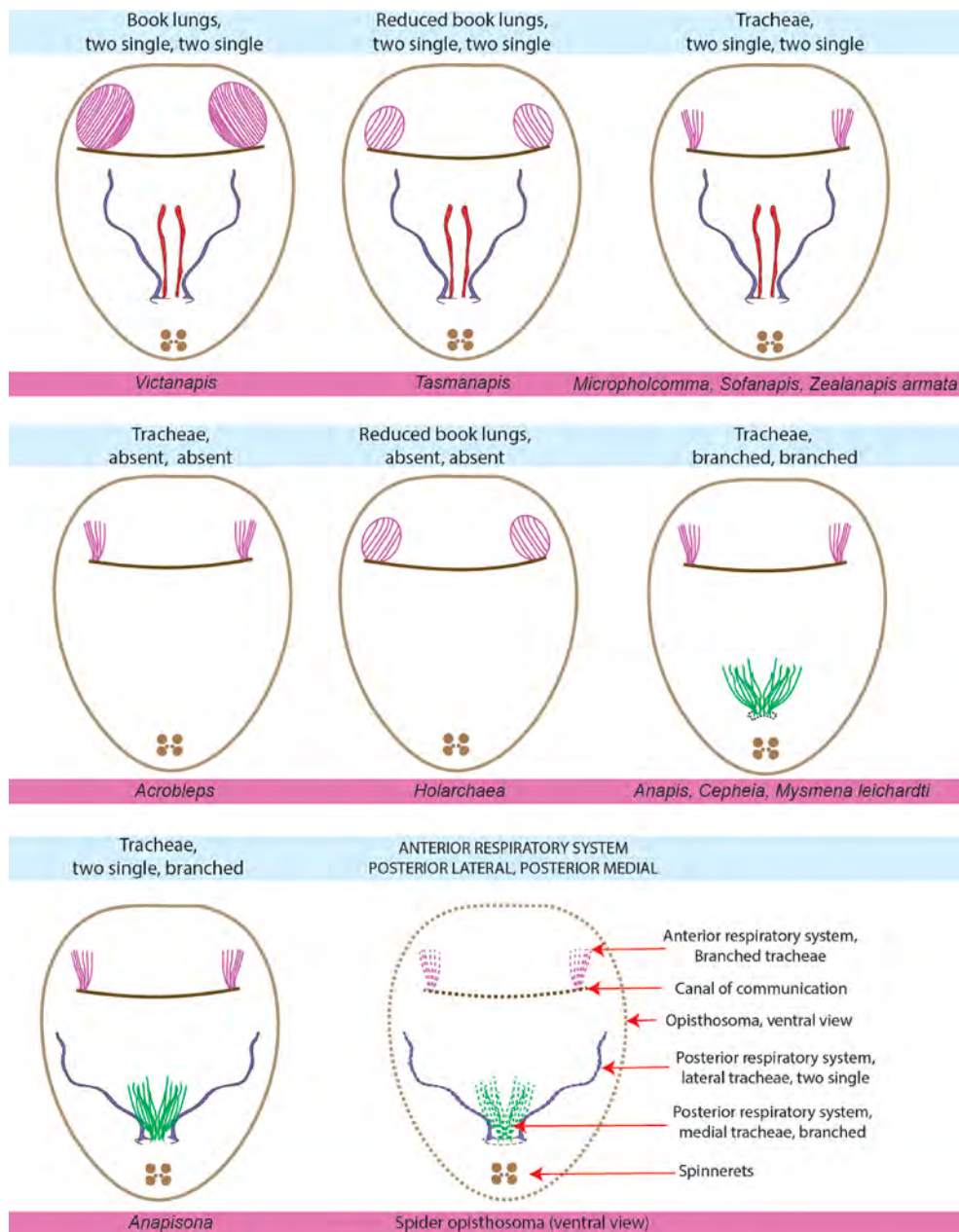


Fig. 3. Diversity of the configurations of respiratory systems in symphytognathoid spiders.

2021) suggests that Anapidae I evolved (Anapidae II representatives were not sampled) around 180 Ma (million years ago). During this time, anapids have occupied all continents except Antarctica (from where no extant spider is known) and a wide range of cryptic habitats such as in mosses and leaf litter, caves, wall cracks and cavities, under logs, in tree holes, and the water surface (Platnick and Forster, 1989; Ramírez et al., 2004; Lopardo and Hormiga, 2008; Rix and Harvey, 2010). As aforementioned, several monotypic genera have been described from distant areas such as *Acroleps* from Tasmania, *Algidiella* from New Zealand, *Borneanapis* from Brunei, *Dippenaaria* from South Africa or *Elanapis* from Chile (Hickman, 1979; Platnick and Forster, 1989; Wunderlich, 1995; Snazell, 2009; Rix & Harvey, 2010). Interestingly, three micropholcommatine genera, *Eperiella*, *Gigiella* and *Normplatnicka* are known from Australia and Chile, separated by the Pacific Ocean, a prime example of disjunct distribution (Rix & Harvey, 2010).

This diversity of web architectures, respiratory systems and disjunct biogeography of anapids makes it tempting to study their evolutionary

journey. However, unstable and conflicting “symphytognathoid” familial relationships have hindered addressing these interesting questions which demand a critical assessment of their phylogenetic relationships. In this study, we address four questions: Do symphytognathoids, the ANTS Clade and the family Anapidae represent monophyletic groups? How did various types of respiratory systems transform within Anapidae? We hypothesize that there were transformations from book lungs to reduced book lungs to tracheae. How did web architecture diversify in Anapidae? What biogeographic processes can explain the distribution of the extant Anapidae lineages? We hypothesize that a series of vicariance events following the breakup of Gondwana have shaped the distribution of extant anapids. We use newly generated ultraconserved (UCE) sequence data gathered from museum specimens and our fieldwork combined with all the available data about the respiratory systems and web architecture, including our own observations, to address these questions.

We found that the symphytognathoids, the ANTS Clade and the

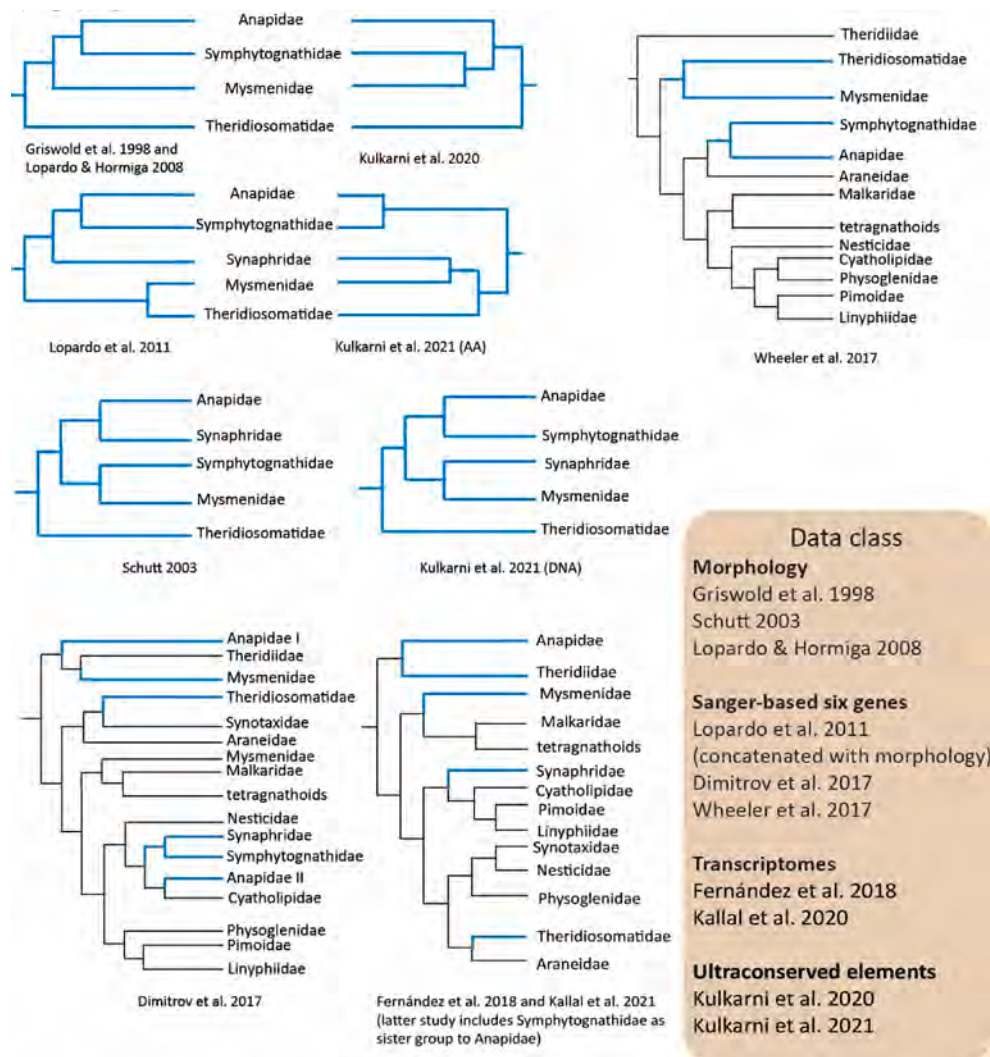


Fig. 4. Conflicting phylogenetic hypotheses about the relationships among the symphytognathoid families (marked blue) obtained from a variety of data classes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

family Anapidae are monophyletic groups; the latter lineage is represented by three major clades. Anterior tracheae in ancestral Anapidae were transformed to book lungs in a clade containing some Anapidae from the central Chilean and Patagonian sub-regions (abbreviated as CCP in the remaining text). The posterior tracheal system was present ancestrally in Anapidae and was lost multiple times. The ancestral orbicular web architecture was transformed to non-orb architecture three times. The ancestral state of the Micropholcommatinae Clade was optimized as an irregular ground sheet web. The ancestral range of Anapidae was Gondwana and multiple rounds of dispersal events explain the current distribution of its lineages.

2. Materials and methods

2.1. Taxon sampling and DNA extraction

We included 95 terminals, some newly sequenced UCEs and others from previous studies, representing 17 Araneioidea families, two nicodamoids- *Megadictyna thilenii* and a Nicodamidae undetermined species, a deinopid (*Deinopsis* sp.) and an eresid (*Stegodyphus mimosarum*), which was used to root the tree. In addition to our sequencing efforts, we included additional taxa from the data sets of Fernández et al. (2018), Kulkarni et al. (2020), and Kallal et al. (2021). We compiled another data set which included 129 terminals generated by combining the UCE

data set with 65 taxa represented by six publicly available Sanger sequenced loci- 12S ribosomal RNA (12S), 16S ribosomal RNA (16S) and cytochrome c oxidase subunit 1 (COI) genes, three nuclear genes- the protein-coding histone H3 (H3), and small and large subunits of ribosomal RNA genes (18S and 28S, respectively). COI and H3 markers were aligned using MACSE (Ranwez et al. 2011) with the invertebrate mitochondrial code followed for COI. The remaining markers (12S, 16S, 18S and 28S) were aligned using MAFFT version 7 (Kato and Standley 2013). Trimming was performed on all alignments using trimAL (Capella-Gutiérrez et al., 2009) with -gappyout setting. See Table S2 for a complete list of taxa used in the study.

The specimens sequenced for this study come from our own field-work or from the collections of the National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C.; the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts and The California Academy of Sciences (CAS), San Francisco. For the 58 sequenced specimens, three to four legs were used for DNA extractions using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA). The homogenate was incubated at 55 °C overnight and then purified following the manufacturer's protocol. The DNA extractions were quantified using high sensitivity Qubit fluorometry (Life Technologies, Inc.) and quality checked using gel electrophoresis on a 1.5% agarose gel.

2.2. Library preparation, enrichment and sequencing

Libraries were prepared and enriched following protocols in Faircloth et al. (2015), but following the modifications detailed below. Depending on prior degradation and quality of the DNA, between 7 and 100 ng of DNA were sheared between 0 and 60 s (amp = 25%, pulse = 10–10, to a target size of approximately 250–600 bp) by sonication (Q800R, Qsonica Inc.). Sheared DNA was dried completely and rehydrated to the required input volume (13 µL) and used as input for DNA library preparation (Kapa Hyper Prep Library kit, Kapa Biosystems). After ligation of universal stubs (Faircloth and Glenn, 2012), a 0.8 × SPRI bead clean was done (Kapa Pure Beads, Kapa Biosystems) on a Wafergen Apollo liquid handler (Wafergen Biosystems), resulting in 30 µL of post-ligation library. For adapter ligation, we used TruSeq-style adapters (Faircloth and Glenn, 2012). PCR conditions were as follows: 15 µL post ligation library, 25 µL HiFi HotStart polymerase (Kapa Biosystems), 2.5 µL each of Illumina TruSeq-style i5 and i7 primers, and 5 µL double-distilled water (ddH₂O). We used the following thermal protocol (Kapa Biosystems): 98 °C for 45 s; 13 cycles of 98 °C for 15 s, 65 °C for 30 s, 72 °C for 60 s, and final extension at 72 °C for 5 m. PCR cleanup was done with a 0.8 X SPRI bead clean (Kapa Pure Beads, Kapa Biosystems) on a Wafergen Apollo (TaKaRa Bio Inc. USA) with a final library volume of 20 µL. Following clean-up, libraries were divided into enrichment pools containing eight libraries combined at equimolar ratios with final concentrations of 137–184 ng/µL.

All pools were enriched with the Spider2Kv1 probes (Kulkarni et al. 2020) following the myBaits protocol 4.01 (Arbor Biosciences). Hybridization reactions were incubated for 24 h at 65 °C, subsequently all pools were bound to streptavidin beads (MyOne C1; Life Technologies), and washed. We combined 15 µL of streptavidin bead-bound, washed, enriched library with 25 µL HiFi HotStart Taq (Kapa Biosystems), 5 µL of Illumina TruSeq primer mix (5 µM forward and reverse primers) and 5 µL of ddH₂O. Post-enrichment PCR used the following thermal profile: 98 °C for 45 s; 18 cycles of 98 °C for 15 s, 60 °C for 30 s, 72 °C for 60 s; and a final extension of 72 °C for 5 m. We purified the resulting reactions using 1X bead clean using Kapa Pure Beads (Kapa Biosystems), and resuspended the enriched pools to total 22 µL.

We then quantified pools using qPCR library quantification (Kapa Biosystems) with two serial dilutions of each pool (1:100,000, 1:1,000,000), assuming an average library fragment length of 600 bp. Based on the size-adjusted concentrations estimated by qPCR, we combined all pools at an equimolar concentration of 30 nM, and size selected for 250–600 bp with a BluePippin (SageScience). We sequenced the pooled libraries in a single lane of a paired-end run on an Illumina HiSeq 2500 (2x150bp rapid run) at the University of Utah Huntsman Cancer Institute.

2.3. Recovering UCEs from transcriptomes and genomes

We followed the transcriptome assembly, sanitation and reading frame detection pipeline described in Fernández et al. (2018). Additionally, we ran the perl script for Rcorrector (Song and Florea, 2015) for error correction and downstream efficiency prior to assembly. The FASTA files of transcriptomes resulting from CD-HIT-EST were converted to 2bit format using faToTwoBit (Fu et al., 2012; Kent, 2002). Then, in the PHYLUCE environment (publicly available at <https://phyluce.readthedocs.io/en/latest/tutorial-three.html>), we created a temporary relational database to summarize probe to assembly match using: `phyluce_probe_run_multiple_lastz_sqlite` function on the 2bit files. The ultraconserved loci were recovered by the `phyluce_probe_slice_sequence_from_genomes` command. The resulting FASTA files were treated as contigs and used to match the reads to the Spider2Kv1 probes.

GC content can influence the phylogenetic relationships reconstructed using genome scale data (Benjamini and Speed 2012). To address this, we computed GC content in each taxon in the concatenated alignment using BBMap (<https://github.com/BioInfoTools/BBMap>).

2.4. Phylogenomic analyses

The assembly, alignment, trimming and concatenation of data were done using the PHYLUCE pipeline (publicly available at <https://phyluce.readthedocs.io/en/latest/tutorial-one.html>). We applied gene occupancies of 10%, 25%, 50%, 60% and 75%. We screened for orthologous and duplicate loci with the minimum identity and coverage of 65 and 65 matches. Phylogenetic analyses were performed on the unpartitioned nucleotide data using IQ-TREE (Nguyen et al., 2015) 1.7-beta version. Model selection was allowed for each dataset using the TEST function (Kalyaanamoorthy et al., 2017; Hoang et al. 2018).

Nodal support was estimated via 1,000 UFBoot replicates (Hoang et al., 2018; Minh et al., 2013) and 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). We also used concordance factors, a metric focusing on whether the best tree represents the signal well. Gene concordance factor indicates the percentage of gene trees containing a given branch in the maximum likelihood tree and site concordance factor (sCF) indicates the percentage of decisive alignment sites supporting a branch (Minh et al., 2020) and it provides insights into incomplete lineage sorting which may be a cause for discordance between the sites and the resulting trees (Zhang et al., 2019). Since UCEs are known to comprise both exons and introns spanning different genes, we refer the gene concordance factor as locus concordance factor (lcf) below. We mapped the lcf against sCF with respect to UFBoot using R version 3.6.0 (R Core Team, 2019). Locus trees for calculating concordance factors were reconstructed using the 1,000 bootstrap and model testing in IQ-TREE. UCE loci in spiders include both exonic and intronic regions and multiple UCE loci may correspond to a single gene (Hedin et al., 2019; Kulkarni et al., 2020). Therefore, we refer to the support metric of gene concordance factor as locus concordance factor in the Results and Discussion. We also reconstructed a parsimony tree using TNT version 1.5 (Goloboff et al., 2003; Goloboff and Catalano, 2016) with gaps treated as missing data.

We tested for incomplete lineage sorting (ILS) in our preferred tree data set by measuring the proportion of locus trees supporting discordant relationships. We calculated the locus internode certainty which compares the support for an internal branch in the locus tree to the support for the most prevalent conflicting resolution at that branch (Salichos et al., 2014). We also calculated the site entropy certainty using the proportion of sites supporting the most and second most prevalent discordant relationships and compared them with the UB and concordance factors. These certainty measures were calculated and visualized using a R script available from https://www.robertlanfear.com/blog/files/concordance_factors.html.

We discuss our results using a tree chosen based on 95% confidence sets recovered from topology tests, namely, approximately unbiased (AU), bootstrap proportion (BP), SH-aLRT, Kishino-Hasegawa (KH), and expected likelihood weight (ELW) using 10,000 resampling estimated log-likelihoods (RELL) in IQ-TREE. This tree is hereafter referred to as “UCE tree” [preferred by the topology tests] (Fig. 5). The Sanger sequenced six marker data set was combined with the “preferred tree” data set with a constrained “preferred tree”, hereafter referred to as “combined tree” (Fig. 6). The combined tree was used for biogeography and character evolution analyses.

2.5. Tree-calibration

We used fossil-based dates from the literature (see Table S4) to calibrate our phylogeny using two strategies: 1. treePL which is a nonparametric rate-smoothing penalized likelihood method (Smith and O’Meara, 2012) and, 2. Least-squares dating version 2 (LSD2) which

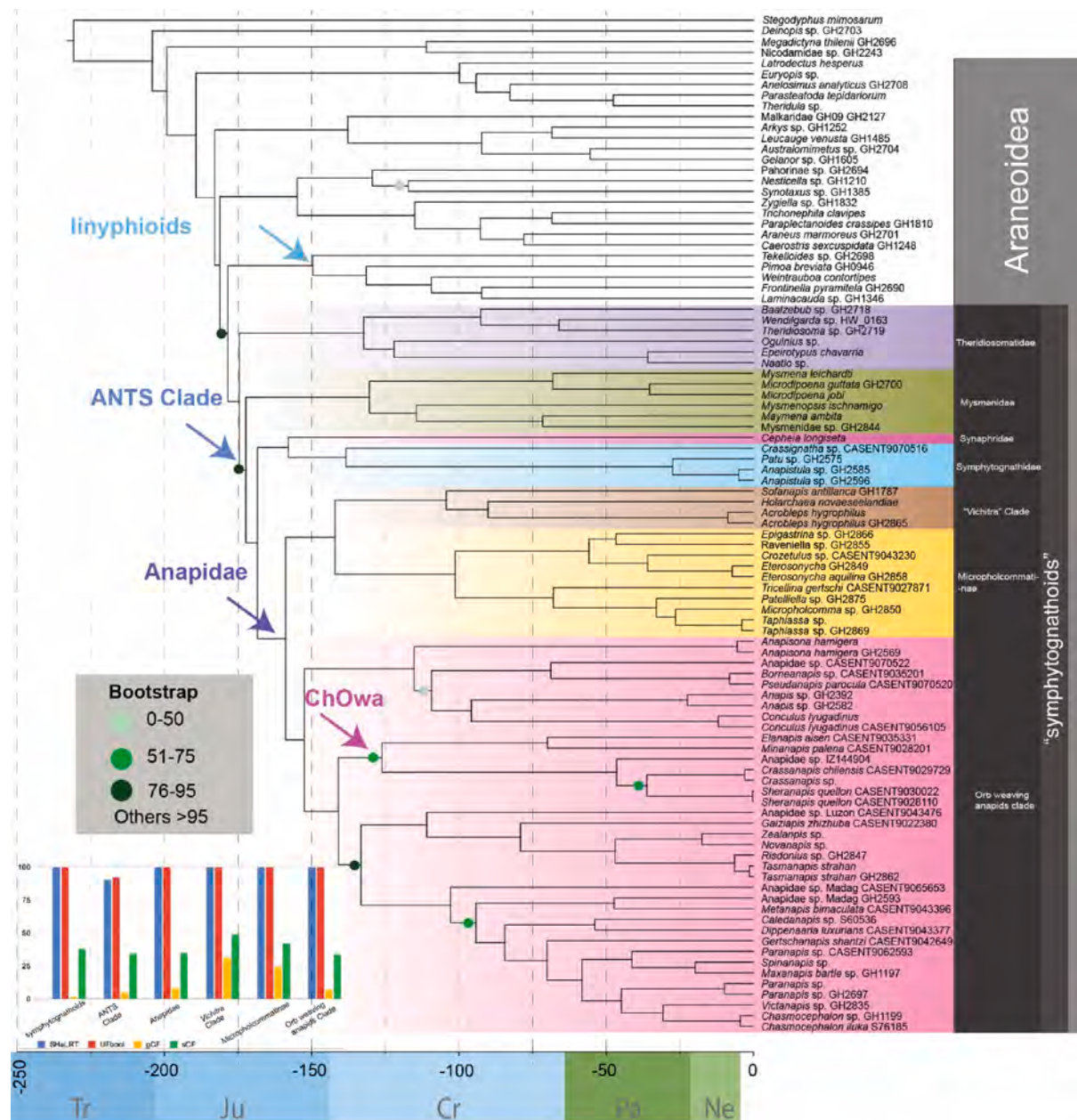


Fig. 5. A 10% occupancy maximum likelihood phylogeny reconstructed ultraconserved elements (UCEs) of symphytognathoids, time-calibrated using spider fossils (referred as “UCE phylogeny” in the text). Ultrafast bootstrap support was 95% or greater at all nodes except those marked in a gradient of green dots (dark green = 95–76%; green = 75–51%; light green = 50–0%). The bar graph at the bottom left shows various support metrics for the clades comprising symphytognathoids and major clades within Anapidae. Abbreviations: SHaLRT- Shimodaira-Hasegawa-like approximate likelihood ratio test; UFB- Ultrafast bootstrap; ICF- gene concordance factors; sCF- site concordance factors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

uses a least-squares approach based on a Gaussian model and is robust to uncorrelated violations of the molecular clock (To et al. 2016). The root age was set to a maximum of 250 Ma based on the estimated age for the node containing Eresidae by Kallal et al. (2021) because we rooted the phylogeny to *Stegodyphus mimosarum* in this study. We used information from nine fossils to constrain the time calibration (see Table S4) following the suggestion of Magalhaes et al. (2020) and Kallal et al. (2021). We used *prime* and *thorough* commands to optimize the treePL analyses, and cross validation was used to select the optimal smoothing parameter. Following Eberle et al. (2018), penalized likelihood optimization iterations were increased from the default of 2 to 5, and the number of penalized likelihood simulated annealing was doubled from 5,000 to 10,000. LSD2 requires at least one fixed date, so we used 130

Ma as fixed date for the Eocene fossil taxon Linyphiinae which was treated as stem Linyphiidae. This date (130 Ma) was calculated by averaging the minimum and maximum date for this fossil as suggested by Magalhaes et al. (2020).

2.6. Ancestral area reconstruction

We reconstructed ancestral areas on internal nodes of the dated preferred tree and the combined tree using the package BioGeoBEARS (Matze, 2013) implemented in RASP 4.0 (Yu et al., 2020). Each of the terminals was assigned to one of the following biogeographic regions: Afrotropics, Australia, Central Chile and Patagonia, Madagascar, Nearctic, Neotropics, New Caledonia, New Zealand and Oriental. The

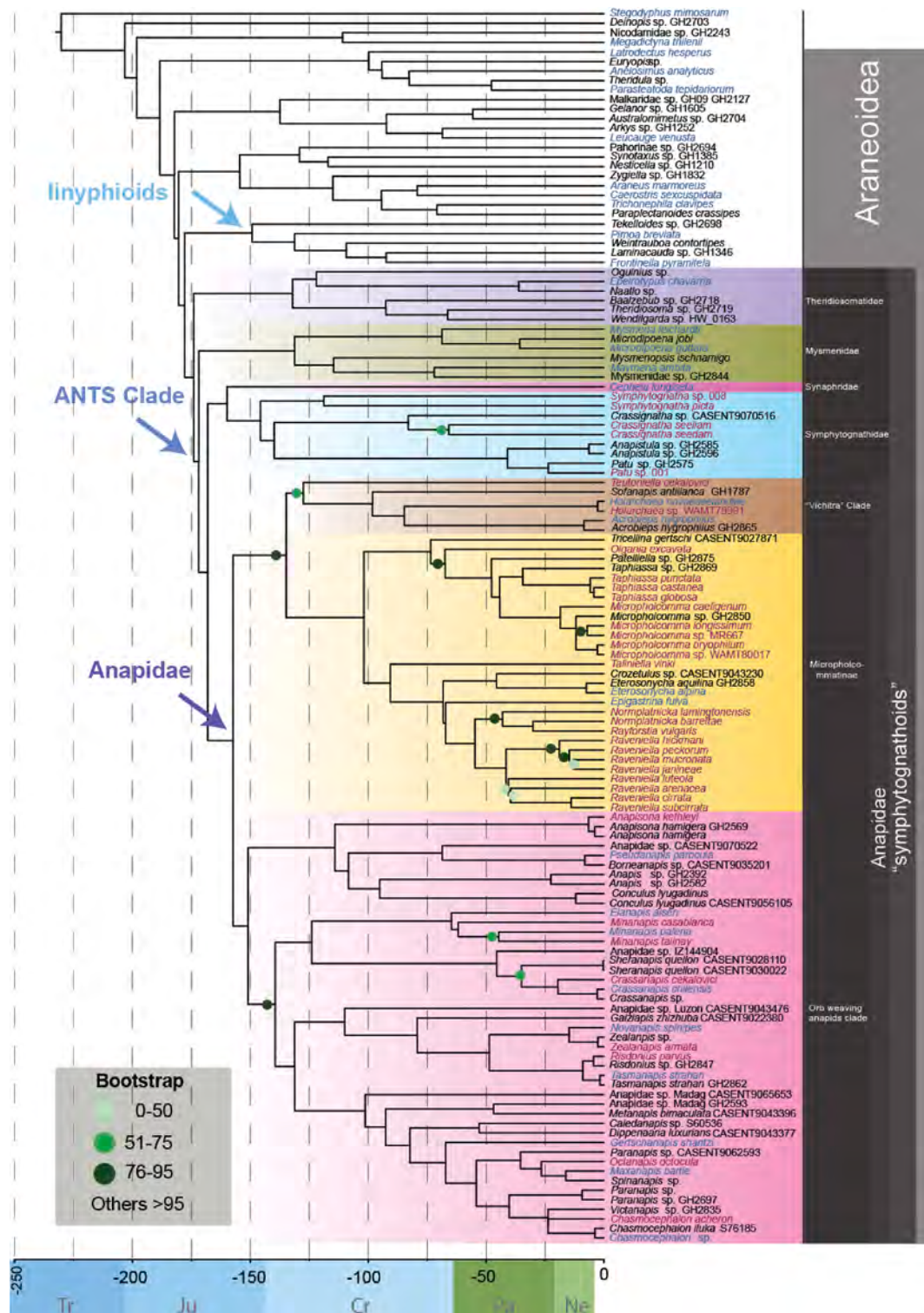


Fig. 6. A maximum likelihood phylogeny of symphytognathoids time-calibrated using spider fossils reconstructed using the UCE data set for occupancy 10% and six Sanger-sequenced markers (referred as “combined phylogeny” in the text). Ultrafast bootstrap support was mostly 100 at all nodes except those marked in a gradient of green dots. Terminal names in black represent taxa with UCE data only, red with six markers only and blue with combination of the two data classes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rationale for coding the biogeographic areas is provided in the supplementary text.

We retained only the taxa of interest (Anapidae) and the other taxa were removed, following the recommendation to remove outgroup taxa by the authors of RASP (Yu et al., 2020). Dispersals were allowed between all regions at any time. Maximum range size was constrained to

nine areas (allowing all areas) to reduce computation time. We evaluated the fit of our data to three distinct biogeographic models (DEC (Ree and Smith, 2008), DIVALIKE (Matzke, 2013) and BAYAREALIKE using likelihood ratio tests based on Akaike information criterion corrected for small sample sizes (AICc).

2.7. Diversification

We assessed whether there were any shifts in the diversification rates associated with any geological period or continental drifts. We conducted a diversification analysis using BAMM v.2.5.0 (Rabosky et al., 2013). Priors for BAMM were calculated using the *setBAMMpriors* function. Outgroups taxa (all taxa except Anapidae) were pruned prior to the analysis. We conducted multiple runs of BAMM until the effective sample size was achieved and was examined using R library coda (Plummer et al., 2006). The BAMM results were plotted using the BAMMtools package (Rabosky et al., 2014).

Agnarsson et al. (2013) estimated the total species richness of various groups of spiders. We relied on their estimates which suggested six species for *Holarchaea* (Holarchaeidae in Agnarsson et al., 2013) and 189 species for Micropholcommatinae (Micropholcommatidae in Agnarsson et al., 2013). Further, we added the estimated number of species for *Holarchaea* and Micropholcommatinae with estimates of Anapidae (429 species) since the prior two groups are currently placed with the latter family (World Spider Catalog, 2023). We used this sum of 624 species for calculating the sampling fraction for the BAMM analyses.

2.8. Respiratory system

We conducted ancestral state maximum likelihood estimates for the respiratory system in Anapidae using the *ace* function in the R package ape (Paradis and Schliep, 2019) on the dated combined tree. We generated 1,000 stochastic character histories from our dataset and calculated posterior probabilities of character state at each node using the R package phytools (Revell, 2012). Two characters were coded: Anterior respiratory system: book lungs (0), reduced book lungs (1), tracheae (2). Posterior respiratory system: tracheae present (0), tracheae absent (1).

The data for character coding were obtained from the literature listed in Table S10. We carried out optimizations using two models of character evolution: equal rates (ER; a single global rate of transition between states) and all rates different (ARD; a different transition rate estimated for all possible transformations). Further, we tested a hypothesis where book lungs can transform and reverse to reduced book lungs, the reduced book lungs can transform and reverse to the tracheal system, but the tracheal system cannot transform to book lungs (IRV1 model). We also tested a model where book lungs were forced to be ancestral anterior respiratory system, using an “irreversible” model (IRV2) built by Ramírez et al. (2021) which prohibited transitions from any state to book lungs and transitions between reduced book lungs and tracheae (Figure S7). The fit of the different reconstructions was compared using the Akaike information criterion (AIC). We pruned taxa with missing data because this analysis requires a complete data set. Model statistics are provided in Table S9.

2.9. Web architecture

We conducted ancestral state estimates for web architecture in Anapidae using the *ace* function in the R package ape (Paradis and Schliep, 2019) on the combined dated tree. We used three coding strategies as follows- I. Web type (“Web 6-state analysis”): (0) irregular aerial sheet, (1) orb, (2) three-dimensional tangle web, (3) stereotyped aerial sheet, (4) irregular ground sheet and (5) webless; II. Foraging web (“Web 2-state analysis”): (0) absent, (1) present; III. Orb web (“Orb 3-state analysis”): (0) absent, (1) present, (2) webless. Orb web is defined as a web with a frame holding radii that support a spirally arranged sticky thread. Sheet web is defined as a planar or nearly planar array where silk lines are relatively dense compared with other portions of the web (Eberhard et al., 2008). Three-dimensional tangle web indicates an irregular three-dimensional web without a distinctly identifiable unit (e.g., sector of orb) or shape (e.g., sheet). Several studies have coded the state “cobweb” for Theridiidae spiders (e.g., Blackledge et al.,

2009; Fernández et al., 2018; Kallal et al., 2021), perhaps because of their common name “cobweb spiders”. We could not find in the scientific literature an explicit definition of cobweb and therefore use alternative states for coding the theridiid webs in our taxon sample. We carried out ancestral state estimates using the equal rates (ER) model and all rates different (ARD) models. We also tested a customized irreversible (IRV) model where orb to non-orb structure and orb structure to webless state were allowed, but reverse transformations were disallowed (Figure S8). The fit of the different reconstructions were compared using the Akaike information criterion (AIC). We pruned taxa with missing data because this analysis requires a complete data set. Model statistics are provided in Table S11.

3. Results

Our UCE phylogeny has representatives of 34 of the currently 58 described anapid genera, and five new hitherto undescribed anapid genera (also implied by our results) (Fig. 5). The combined data set (UCEs plus Sanger-sequenced six markers) placed 46 genera (including five undescribed taxa) (Fig. 6). We found that in the combined data set of UCE and UCEs recovered from transcriptomes and genomes contained a total of 1,551 UCE loci out of 2,021 (77%) total UCE loci that are targeted using the Spider2Kv1 probe set. Quality-trimmed sequence reads are available from the NCBI Sequence Read Archive (BioProject PRJNA991600) (Table S1).

Topology tests were conducted between data sets of occupancies 10%, 25%, 50% and 60% which significantly supported all datasets except the 60% occupancy data set which was significantly rejected (Table S5). The 10% occupancy data set represents the largest amount of data (1,382 loci), therefore out of the three data sets within 95% confidence sets, we use this dataset for discussing the results below. Ultrafast bootstrap (UB) support values above 95 are referred to as high support and the remaining branches with low support are marked in Fig. 5. GC-content in the preferred data set (10% occupancy) varied between 41 and 50% (see Figure S5). Statistics of UCE loci and datasets are summarized in Table S1 and S3 respectively. Details about the combination of UCE contigs and the Sanger-based six markers are provided in Table S2.

A total of 938 single-loci phylogenies out of 1,381 total phylogenies contained the symphytognathoid branch and 827 single-loci phylogenies out of 1,381 total phylogenies contained the ANTS Clade branch. Symphytognathoid monophyly was supported by (locus concordance factor (ICF) 1.71% of 938 = 16 locus trees) followed by an equal number of locus trees supporting the most prevalent discordant relationship (IDF1 in Table S6). About 38% of sites supported the symphytognathoid monophyly followed by about 30% supporting the alternative relationship (sDF1 in Table S6). The monophyly of the ANTS group of families was supported by (ICF 1.81% of 827 = 14 locus trees) followed by a larger proportion of locus trees (IDF1 1.93%) supporting the most prevalent discordant relationship. About 34% sites support the ANTS group monophyly whereas the most prevalent alternative relationship is supported by about 30% sites. (see Table S6). The locus and site concordance factors were correlated, however UB was mostly above 95% (Figure S4).

The internode certainty values were highly correlated with the locus and site concordance factors (see Figure S6). The 10% occupancy UCE data set was then combined with the Sanger-sequenced six marker data set which included 129 taxa. The phylogeny resulting from the combined data set was constrained with the UCE phylogeny which added five Symphytognathidae and 31 Anapidae terminals. The concordance factors resulting from the UCE only data set are mentioned in the parentheses below as “ICF” for locus concordance factors and “sCF” for site concordance factors.

3.1. Composition of symphytognathoids

The family Theridiidae is the sister group to a clade including the remaining araneoid families (Fig. 5). The symphytognathoid taxa included in this study form a clade and all symphytognathoid families (Anapidae, Mysmenidae, Symphytognathidae and Theridiosomatidae) are monophyletic (ICF 1.71%; sCF 37.9%). Theridiosomatidae is the earliest-diverging, and sister group to the remaining symphytognathoid families, the so-called ANTS Clade (ICF 4.91%; sCF 34.3%). The only synaphrid included, *Cepheia longiseta*, is the sister group of the Symphytognathidae lineage, and together these two families are the sister group of the Anapidae clade (Fig. 5).

All symphytognathoid families were recovered monophyletic with all occupancy data sets. The family placements obtained from 25% and 50% occupancy data sets were congruent with the preferred data set (Figures S1,S2), however at occupancy 60% Mysmenidae was sister group to a clade comprising the representatives of Araneidae, Theridiosomatidae, Malkaridae, Synotaxidae, Nesticidae, and the linyphioid and tetragnathoid families. Interestingly, only at 60% occupancy, Theridiosomatidae was the sister group of Araneidae and, Cyatholipidae was the sister group to Linyphiidae (File S3) whereas in all remaining data sets, Theridiosomatidae was a sister group to the ANTS Clade and Cyatholipidae was sister group to Linyphiidae plus Pimoidae (the linyphioids).

3.2. Anapidae

The family Anapidae is composed of three major clades: the “Vichitra” Clade, Micropholcommatinae and the Orb-weaving anapids clade (Owa Clade). The “Vichitra” Clade (the name is taken from the Sanskrit word for bizarre or odd) due to the unusual traits of each genus in this clade, as detailed in the Discussion section) includes *Teutoniella cekalovici*, *Sofanapis antillanca*, *Holarchaea* species and *Acroleps hygrophilus*. The “Vichitra” Clade (ICF 31.2%; sCF 48.9%) is sister to the Micropholcommatinae (ICF 24.1%; sCF 42.1%). All orb weaving anapids from Chile formed a sub-clade within the Owa Clade which we call here as Chilean Orb weaving anapids (ChOwa) Clade. In the Micropholcommatinae Clade, Textricellini (as defined by Rix and Harvey, 2010) was monophyletic and included the genera *Taliniella* and *Rayforstia* from New Zealand, *Normplatnicka*, *Epigastrina*, *Eterosonycha* and *Raveniella* from Australia, and *Crozetulus* from Africa. Textricellini was the sister group of a clade including the genera *Olgania*, *Micropholcomma*, *Patelliella*, *Taphiassa* from Australia and New Zealand and *Tricellina* from Chile. All the remaining anapid genera were monophyletic (ICF 7.21%; sCF 34%) and formed a sister group to the clade of micropholcommatines plus the Vichitra lineage. We call this group the Orb-weaving anapids Clade because as far as we know, all species in this clade construct orbicular foraging webs (See Web evolution section and Discussion; Figs. 5, 6).

3.3. Divergence dating

treePL inferred divergence dates optimized using fossil calibrations suggested that the last common ancestor of symphytognathoids and linyphioids + Cyatholipidae occurred around 178 Ma (Fig. 5). The last common ancestor of Anapidae and Synaphridae plus Symphytognathidae occurred around 168 Ma. Within the symphytognathoid clade, families diverged from their sister lineages between 174 and 160 Ma (Fig. 5). LSD2 inferred confidence intervals suggested a range of 188–160 Ma for the divergence of the last common ancestor of symphytognathoids and linyphioids + Cyatholipidae. A range of 187–157 Ma was inferred for the occurrence of the symphytognathoid most recent common ancestor. A range of 184–149 Ma was inferred for the occurrence of the Anapidae most recent common ancestor by LSD2 and 157 Ma by treePL.

3.4. Biogeography

The best fitting model for the biogeographic analysis using RASP for our combined data set was the DIVALIKE + j (AICc = 205.6, AICc weight = 0.57) (Fig. 7), closely followed by DEC + j (AICc = 206.3, AICc weight = 0.41) (Table S8, Figure S11). Both models recovered ambiguity for the area of the most recent common ancestor (MRCA) of Anapidae, however some other internal nodes were strongly supported with unambiguous areas. The optimizations on the internal nodes for DIVA + j model are explained here. The most likely ancestral area for Vichitra + Micropholcommatinae Clade was optimized to be Australia (37%) followed by a combination of Australia and Chilean and Patagonian range (CCP) (33%). Considering the most likely ancestral area implies a dispersal from Australia to CCP (ca. 134.8 Ma) whereas the second likely scenario of Australia + CCP implies a vicariance event which diverged the MRCA of the Vichitra Clade and the Micropholcommatinae Clades along with the separation of Australia and CCP respectively. The CCP (81%) was optimized as the most likely ancestral area for the Vichitra Clade (Fig. 7). Australia was optimized to be the most likely (77%) ancestral area for Micropholcommatinae. Based on the most likely reconstructions, the implied dispersal/vicariance events are listed in Table S9. The ancestral area of the Owa Clade was retained ambiguous, some less likely areas included Oriental (17.6%) followed by Oriental + Africa (15.7%). The CCP area was unequivocally optimized as the ancestral area for the ChOwa Clade. A total of 14 dispersal events are implied from the most likely reconstructions in the Owa Clade (Table S9). The origin of the only New Caledonian representative in our phylogeny, *Caledanapis* species was inferred to be the result of a dispersal from Africa to New Caledonia (ca. 52.8 Ma).

3.5. Evolution of respiratory system

The AI criterion favored the equal rates over the all-rates different and custom irreversible models for mapping of anterior and posterior respiratory systems (Fig. 8). See Table S9 for model statistics. This preferred model implied that the ancestral respiratory system of symphytognathoids consisted of anterior book lungs and a posterior tracheal system. The ancestral book lungs transformed into tracheae once at the node of the ANTS Clade, with two reversal events of tracheae into book lungs in *Victanapis* (Anapidae) and *Maymena* (Mysmenidae). The reduced book lungs in *Teutoniella* and *Holarchaea* (Vichitra Clade), ChOwa Clade (except *Sheranapis*) and *Risdonius* + *Tasmanapis* clade are implied to have evolved from tracheae. The book lungs in *Sheranapis* (ChOwa Clade) were optimized as a reversal from reduced book lungs (Fig. 8A, B). Overall, within the ANTS Clade, the frequency of the ancestral tracheal system transforming to reduced book lungs was higher than the tracheal system transforming to book lungs and other transformations between respiratory systems (Fig. 8B).

The symphytognathoid ancestral posterior tracheal system was lost eleven times and this loss was reversed four times. Tracheae were lost in all Vichitra and Micropholcommatinae clades (both Anapidae) and in all Symphytognathidae members except *Anapistula*. The posterior tracheae were retained in *Teutoniella* and *Sofanapis* of Vichitra Clade. In Micropholcommatinae, a sub-clade including *Raveniella*, *Rayforstia*, *Eterosonycha*, *Epigastrina* and *Taliniella* have lost the tracheal system whereas the other sub-clade of remaining micropholcommatines retain this system (with the exception of loss in *Olgania*) (Fig. 8C).

3.6. Web architecture

The AICc criterion favored the equal rates model over other models for the analyses of all three character codings. See Table S11 for model statistics. The preferred model implied an ancestral orb web architecture for the symphytognathoids, ANTS clade, Anapidae and the Orb weaving anapids clade (Fig. 9). Foraging web was lost three times (Fig. 9, S13-foraging web present/absent) in the ANTS Clade as seen in *Sofanapis* and

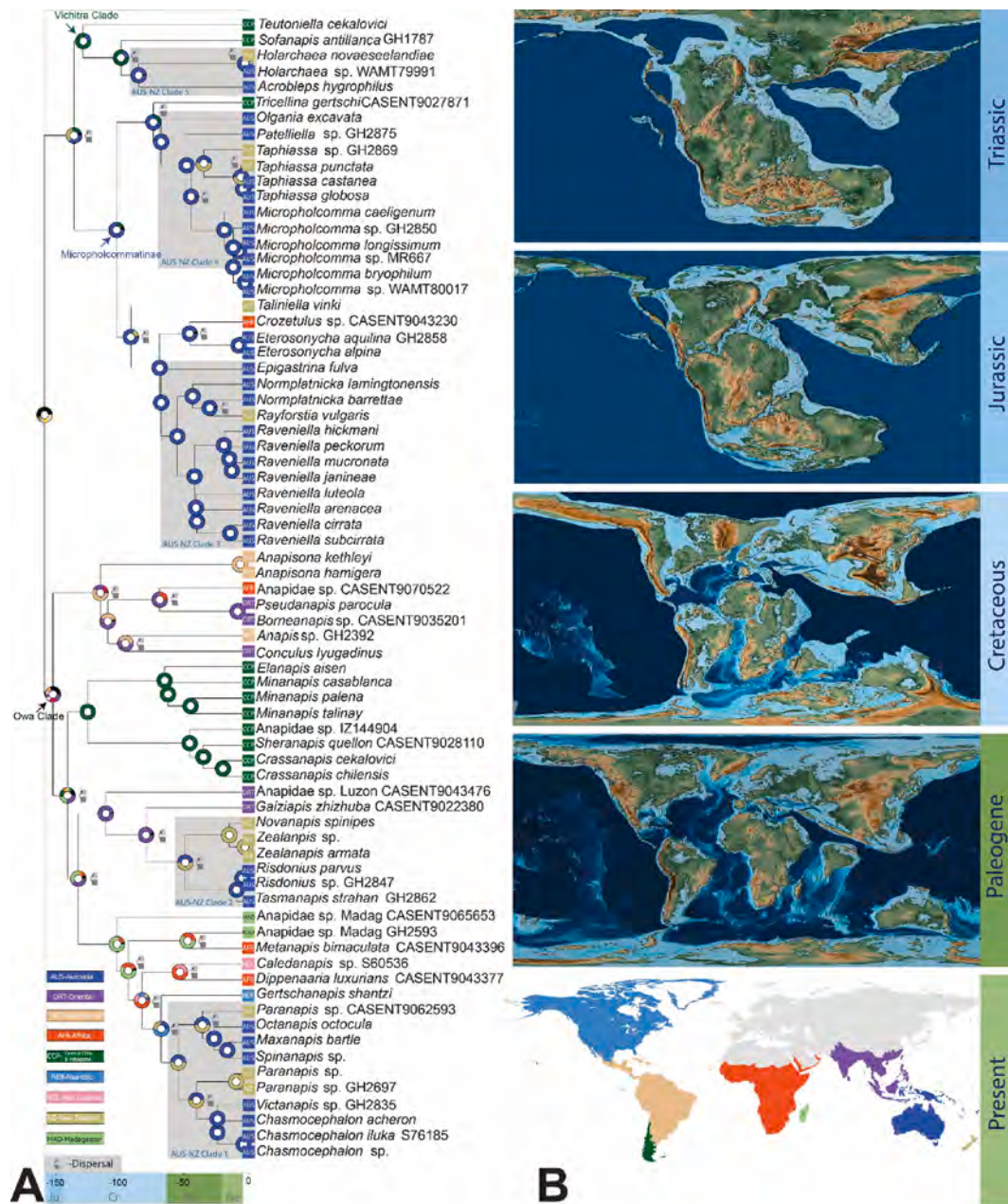


Fig. 7. A. Biogeographic hypothesis obtained from the Dispersal-Vicariance + jump parameter model of RASP analyses. B. Graphics for Pangean breakup were taken from <https://pubs.usgs.gov/gip/dynamic/historical.html>. C. Color codes indicating the biogeographic regions used for the biogeographic analyses. Note that New Caledonia is enlarged to depict that it was coded as a separate region. Pie charts at the nodes indicate optimizations of the most likely ancestral areas.

Holarchaea (Vichitra Clade) and *Mysmenopsis* (Mysmenidae) (Fig. 9, S13). The orbicular web architecture was transformed to non-orb architecture three times as seen in all the members of the subfamily Micropholcommatinae, *Cepheia* (Synaphridae) and *Borneanapis* (Owa Clade, Anapidae). In the “Web type” analysis with six character states, the ancestral state of the Micropholcommatinae Clade was optimized as an irregular ground sheet web. In the ANTS Clade, the orb web transformed to irregular ground sheet web three times (*Cepheia*, *Borneanapis* and Micropholcommatinae Clade). The irregular ground sheet web transformed to a 3-D tangle web once in the clade that included *Micropholcomma* species (Fig. 9). *Borneanapis* is the only member of the Owa Clade without an orb web (see Discussion).

The optimizations of web architecture at nodes outside symphytognathoids implied conflicting hypotheses between the “Orb 3-state” and “Web 6-state” analyses. A non-orb web architecture was optimized at the

symphytognathoids + linyphioids clade with the “Orb 3-state” analysis, whereas the “Web 6-state” analysis optimized an orb web architecture at this node. Similarly, the “Orb 3-state” optimized a non-orb web at the base of *Deinopis* (Deinopidae) whereas the “Web 6-state” analysis optimized the orb web state at this node (Fig. 9, S12) (See Discussion).

3.7. Web of *Minanapis palena*

During our fieldwork in Isla Grande de Chiloé (Chile), we collected four *Minanapis palena* specimens from individual orb webs. We observed three webs constructed in the mosses along natural slopes, and webs were photographed and radii counted. These relatively small anapids construct orb webs an average of 4 cm width, with ca. 77 densely placed spiral turns. The webs include ca. 20 true radii (those connected to the hub), ca. 60 supplementary radii and ca. 14 above-plane radii. The host

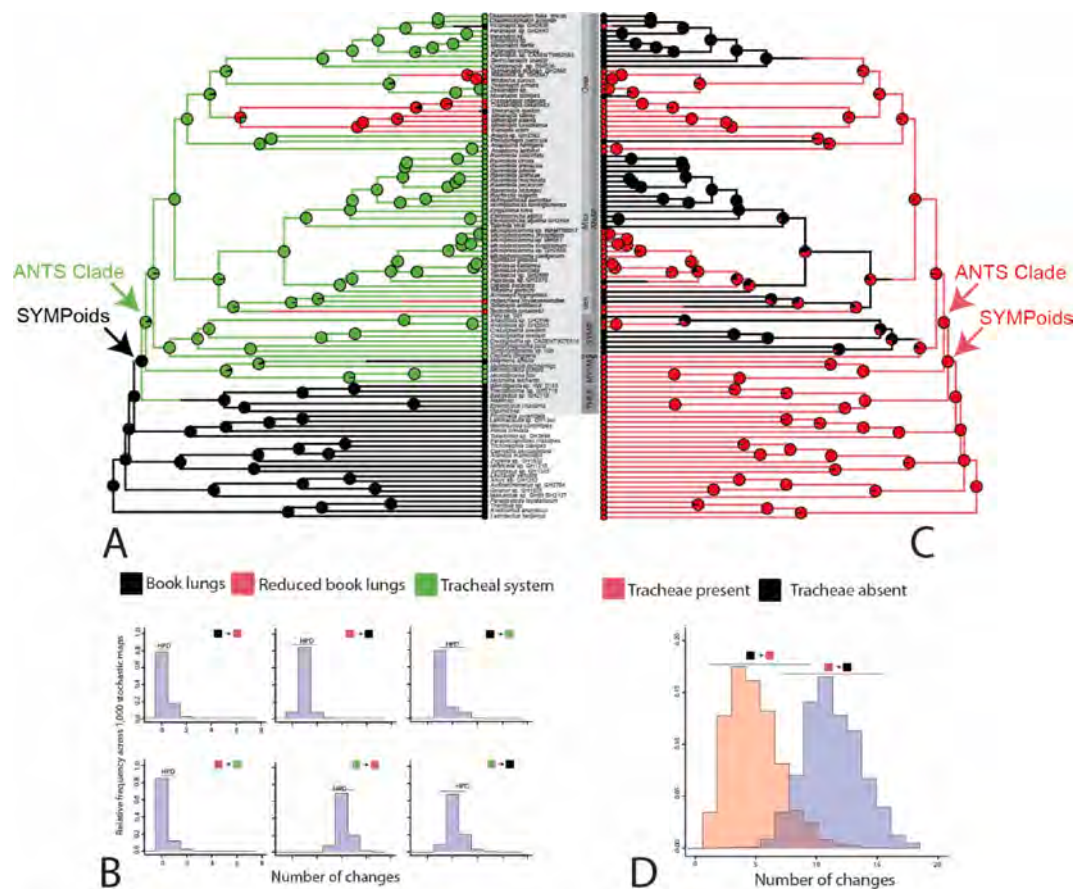


Fig. 8. Evolutionary history of the anterior (A, B) and posterior (C, D) respiratory systems in Anapidae. Characters were optimized on the combined data set phylogeny using an equal rates model in a maximum likelihood framework. Anterior respiratory system (A, B) included three states- tracheae, book lungs and reduced book lungs. Posterior respiratory system (C, D) includes two character states- tracheae-present and tracheae-absent. Branch colors on A, C were generated through a sample of 1000 stochastic characters maps with equal rates model. B, D indicate a posterior density map of the number of changes between states for the character across our sample of 1000 stochastic character maps. Abbreviations- ANAP- Anapidae; HPD- 95% high probability density; Micr- Micropholcommatinae; Owa- Orb weaving anapids Clade; SYMP- Symphytognathidae; SYMPoids- symphytognathoids; SYNA- Synaphridae; MYSM- Mysmenidae; THER- Theridiosomatidae; Vich- Vichitra Clade.

spiders were seen hanging upside down at the hub (Figure S9). This is the first report of the web architecture of any *Minanapis* species.

3.8. Diversification rates

In our combined phylogeny, we sampled two species of *Holarchaea* and 29 species of micropholcommatine spiders. Agnarsson et al. (2013) estimated that there could be a total of six species for *Holarchaea* and 189 species for Micropholcommatinae. Considering these numbers, our sampling included 33% of *Holarchaea*, 15.34% of Micropholcommatinae and 11.6% of all Anapidae described species. The BAMM analysis to model speciation-extinction rates did not reveal any shifts across the phylogeny of Anapidae (Figure S10).

4. Discussion

Rix et al. (2008) had recovered a diphyletic Anapidae (with multiple unresolved branches) and rejected the inclusion of Micropholcommatidae within Anapidae in a phylogeny reconstructed using target sequencing of two markers, as suggested by Schütt's (2000) morphological cladogram. Later, Rix and Harvey's (2010) micropholcommatine monograph included only three anapids in their molecular analysis (*Novanapis*, *Teutoniella* and an undescribed species placed as a sister group to *Teutoniella*), as Micropholcommatidae was considered a family at the time, and thus the possibility of

micropholcommatines nesting within Anapidae was not tested. Lopardo et al. (2011) used a total-evidence approach (combination of morphology, behavior, and six molecular markers) and inferred that the clade comprising "micropholcommatid" spiders was nested within Anapidae and formally ranked the group as a sub-family of Anapidae. Our study corroborated the placement of micropholcommatines within Anapidae using genome scale data (see also Wheeler et al., 2017). Rix and Harvey (2010) inferred that micropholcommatine monophyly is supported by three unambiguous morphological synapomorphies: the presence of a ligulate retrolateral patellar apophysis on the male pedipalp, the absence of a conductor and the presence of only a single anterior seta on the posterior median spinnerets.

Rix and Harvey (2010) established the "enlarged basal cylindrical gland (EbCy)" clade which included families Symphytognathidae and Anapidae (including Micropholcommatinae) which have an enlarged posterior cylindrical gland spigot on the posterior lateral spinnerets (PLS). In our results, Synaphridae, which has lost one of two cylindrical gland spigots on PLS, is nested within the Symphytognathidae plus Anapidae clade, so the grouping based on EbCy is rendered paraphyletic.

4.1. Support

Incomplete Lineage Sorting (ILS) can result in a common gene tree topology not concordant with the species tree, and thus ILS can produce erroneous relationships sampled from the tree space from the "anomaly

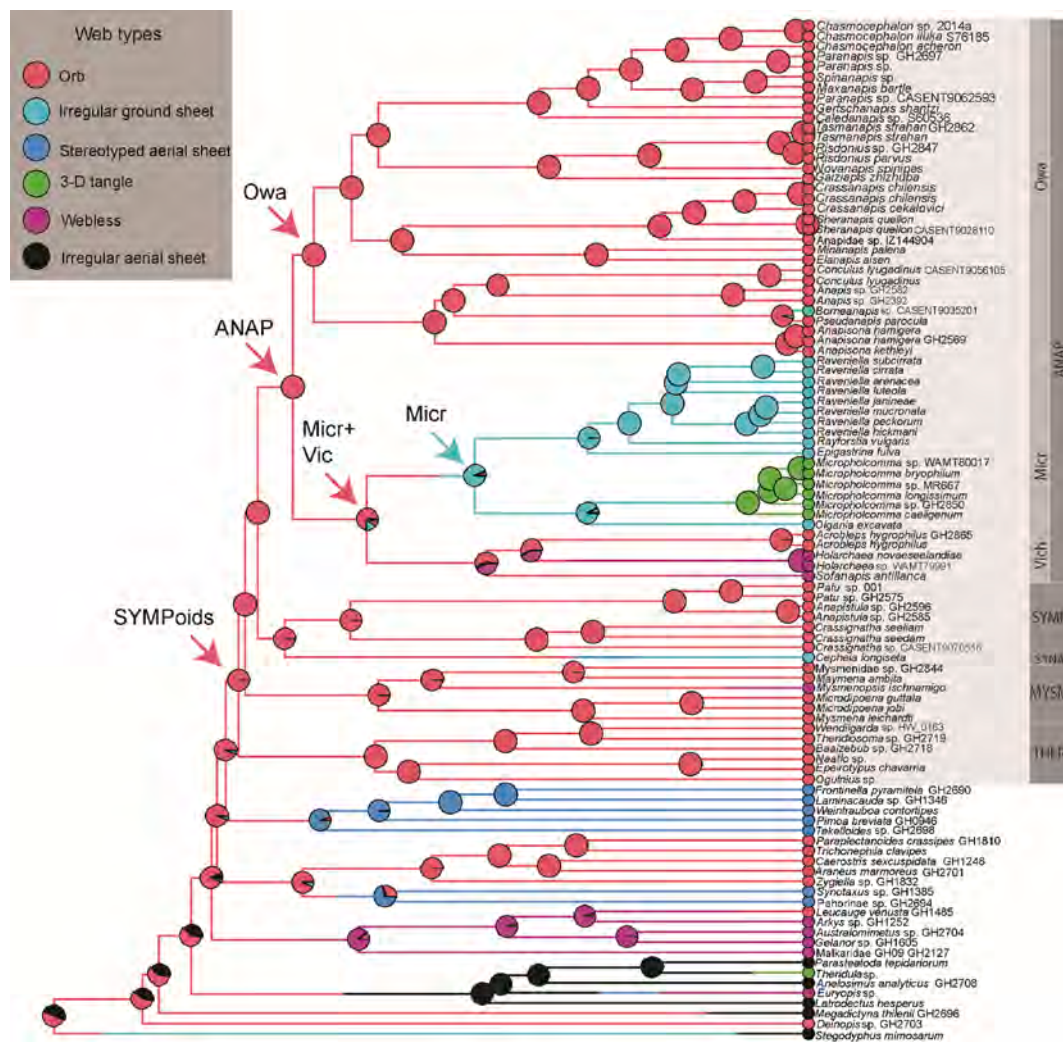


Fig. 9. Evolutionary history of the web structure in symphytognathoid spiders from the “Web 6-state analysis” optimized using an equal rates model in a maximum likelihood framework. Pie charts represent the most likely state of 1,000 simulated stochastic character maps. Branch length colors indicate one simulation. For spiders that do not construct web, an additional character “webless” was coded.

zone” (Degnan and Rosenberg, 2006). The monophyly of symphytognathoids and the ANTS Clade was supported by locus concordance factors of 1.71% and 1.81% respectively, followed by the most prevalent discordant relationships supported by 1.71% (IDF1) and 1.93% (sDF1), respectively. These numbers indicate that the most common topology among the single-locus phylogenies (in case of the ANTS Clade) was not concordant with the tree from the concatenated data set. There are two possible explanations for this: either we might be in the anomaly zone (Degnan and Rosenberg, 2006) or the informative sites for the species phylogeny from the concatenated data set are scattered across loci. Furthermore, the low proportion of single-loci phylogenies that support the symphytognathoid clade could be due to ILS since an equal number of loci support both the concordant (to that of tree from concatenated data set) and the most prevalent discordant tree. However, the sCF and sDF1 values fit the second explanation above because about 38% and 34% sites support the symphytognathoid and ANTS group monophyly respectively followed by about 30% sites supporting the most prevalent alternative relationship. The remaining loci and sites supported alternative relationships. A chi-square test on frequency of loci supporting discordant tree topologies was significantly different, thus rejecting the possibility of ILS (see IEF_p in Table S6). Further to this, the significant correlation between the internode certainty, which is a comparison of support between the concordant and the most prevalent discordant tree (Salichos et al. 2014), and the concordance factors indicate the rejection

of ILS obtained from the site concordance factors. GC content was uniform across all taxa (see Figure S5) therefore we did not use strategies to test GC content bias on the phylogenetic inference (such as omitting taxa with high GC content).

4.2. Phylogenetic relationships

Among the three higher-level lineages within Anapidae, the Micropholcommatidae clade is treated as a subfamily and diagnosed based on the synapomorphies provided by Rix & Harvey (2010) (see Section 4.2.2). A definition for the remaining two clades and assignment to formal taxonomic ranks (*sensu* the International Code of Zoological Nomenclature) would require a detailed morphological study to describe and diagnose the groups and to infer their synapomorphies, which is beyond the scope of the present work. For this reason, in this study, we use the informal names, ‘Vichitra clade’ and ‘Orb-weaving anapids clade’ for these two lineages (Fig. 5).

4.2.1. The Vichitra Clade

This grouping is rather unusual morphologically, displaying some characteristics which are absent from other anapids. For example, *Acrobleps hygrophilus* builds orb webs that are laid on the water surface (Hickman, 1979; Lopardo and Hormiga, 2008), not seen in other anapids. *Sofanapis antillanca* is the only anapid known to have a

kleptoparasitic lifestyle (Forster and Platnick, 1984; Ramírez and Platnick, 1999). Both genera are monotypic. It was conjectured that *Holarchaea* lacks venom glands (which is extremely rare in spiders), but recent exploration using micro-computed tomography revealed a venom gland (Forster and Platnick, 1984; Kallal and Wood, 2022). From the arrangement of spinnerets (with only three spigots on each spinneret) it is plausible that they do not construct any foraging web (Forster and Platnick, 1984). Only two *Holarchaea* species have been described—*H. globosa* (Hickman, 1981) from Australia and *H. novaeseelandiae* (Forster, 1949) from New Zealand. Rix (2005) observed that in captivity, *H. globosa* walks on silk lines and mostly rests on it hanging upside down, however it is not known whether those lines are used for foraging or not. Another and perhaps more prominent feature of *Holarchaea* is its long chelicerae which originates from a partial foramen in the carapace, with a raised caput and a thick clypeus that projects anteriorly (Forster and Platnick, 1984; Rix, 2005), and so somewhat similar to the morphology of Mecysmauchenidae and Archaeidae (members of the Palpimanoidea, and thus a distant relative) and Pararchaeinae (Malkaridae, Araneioidea). *Holarchaea* chelicerae also bear a gland mound which is present in all palpimanoids (Forster and Platnick, 1984; Wood et al., 2012), a character not known in symphytognathoid spiders. Given these unique characteristics, *Holarchaea* was formerly classified in its own family (Holarchaeidae) which was diagnosed by its elongate chelicerae, entelegyne female genitalia, absence of peg teeth and anteriorly projecting clypeus, and placed in the superfamily Palpimanoidea because of modifications of the carapace and chelicerae (Forster and Platnick, 1984). However, this placement has since been refuted by many phylogenetic studies which place *Holarchaea* within the Araneioidea (e.g., Rix et al., 2008; Wood et al., 2012). The molecular analysis of Dimitrov et al. (2017) resulted in a diphyletic Anapidae, but because *Holarchaea* nested within the clade that included the type genus *Anapis* (of Anapidae), Holarchaeidae was synonymized with Anapidae. Our study found that Anapidae was monophyletic with *Holarchaea* nested within. The only web building lineage in the Vichitra Clade is the monotypic genus *Acrobleps* which was formerly placed in Mysmenidae because it bears a *Trogloneta* like cone-shaped raised carapace with closely placed eyes on it (Platnick and Forster 1989). Unlike other anapid genera, *Acrobleps* lacks a labral spur (Lopardo and Hormiga 2008).

The placement of *Teutoniella* has been a challenging task for morphologists in the past. Two species of *Teutoniella* are known, *T. cekalovici* from Chile and *T. plaumanni* from Brazil. Brignoli (1981) described this genus as a member of the family Anapidae. Platnick and Forster (1986) transferred *Teutoniella* to the family Micropholcommatidae (ranked since 2015 as an anapid subfamily) based on the morphology of the Chilean species. Platnick and Forster (1986) and Rix and Harvey (2010) suggested that this peculiar lineage could be proposed as a family, however it was not formally done. Rix et al. (2008) and Rix and Harvey (2010) used a cladistic approach to infer that *Teutoniella* is a sister group to all Micropholcommatinae. The only taxonomic representative of Anapidae (as delimited at the time) in Rix and Harvey's (2010) analysis was a species of *Novanapis*. Lopardo and Hormiga (2015) used an expanded taxon sample of all symphytognathoid families which recovered the placement of *Teutoniella* within the Micropholcommatinae clade. In our study, *Teutoniella* is placed within the Vichitra Clade as a sister group to a clade which is composed of *Sofanapis* from Chile, *Acrobleps* from Tasmania and *Holarchaea* from New Zealand and Australia.

Rix and Harvey (2010) hypothesized that the absence of the posterior minor ampullate gland spigot on posterior median spinnerets was the only unambiguous synapomorphy for *Teutoniella* plus the Micropholcommatinae clade. The absence of this character in *Teutoniella* was hypothesized to be Rix and Harvey (2010) a synapomorphy for their “teutoniellid” clade, which includes *Teutoniella* and an undescribed genus from Australia. We were not able to include this undescribed genus in our analysis, so the monophyly of the teutoniellid clade could

not be tested. In other members of the Vichitra Clade, *Acrobleps* has the posterior minor ampullate spigot on the posterior median spinnerets (Lopardo and Hormiga, 2008), however, the spinneret configuration of *Sofanapis* and *Holarchaea* is poorly understood. Forster and Platnick (1984) provided a scanning electron micrograph of *Holarchaea novaeseelandiae* however, the spigot morphology cannot be clearly seen. Therefore, for now the morphological synapomorphies for the Vichitra clade remain unknown.

4.2.2. Micropholcommatinae

The presence of a ligulate apophysis on the pedipalpal patella indicates that *Crozetulus* (Schütt 2002: Figure 10) is a micropholcommatine. This finding of Schütt (2002) was significant because it showed the first record of a micropholcommatine spider outside Australasia. *Crozetulus* includes four species distributed in sub-equatorial Africa and Crozet Islands (World Spider Catalog, 2023). The position of *Crozetulus* in our study requires further exploration (i.e., resequencing from fresh specimens, currently unavailable to us), because of the low number of UCE loci recovered. One of the analyses of Lopardo and Hormiga (2015) inferred placement of the European cobweb building anapid *Comaroma simoni* as a member of micropholcommatines or as sister group of *Minanapis* or unresolved. Further sampling on non-Australasian micropholcommatines is required to illuminate the relationships of this sub-family.

4.2.3. Orb weaving anapids (Owa) Clade

This clade includes the type genus *Anapis* Simon, 1895. As the name suggests, all anapids in this lineage (whose webs are known) construct modified orb webs (except *Borneanapis*, see Web architecture section). Similar to our finding, Rix and Harvey (2010) also recovered the Chilean Owa (ChOwa) clade which included *Elanapis*, *Crassanapis* and *Sheranapis* (although *Minanapis* was not included in that study) (Fig. 5,6). This grouping was polyphyletic in the morphological cladogram of Lopardo and Hormiga (2015: Fig. 159). Interestingly, in that study, *Teutoniella* (a Vichitra Clade member in this study) and *Taphiassa* (a micropholcommatine) nested within a clade including all orb weaving anapids (Lopardo and Hormiga, 2015). The inclusion of *Teutoniella* within Micropholcommatinae renders the subfamily paraphyletic, and corroborates Rix and Harvey's (2010) exclusion of *Teutoniella* from Micropholcommatinae.

4.3. Biogeography

Diversification of several groups of organisms distributed in the southern hemisphere has been attributed to the breakup of Gondwanan (for example, marsupials (Reguero and Goin, 2021)). The Gondwanan fragmentation is well-documented, and concordant with geographic distributions of many taxa (reviewed in Sanmartín and Ronquist (2004)). Some spiders are considered to be good dispersers, as illustrated by presence of widely distributed species, and one of the mechanisms of their dispersal (ballooning) has been well documented (Duffey, 1956; Bell et al. 2005; Morley and Robert, 2018). However, some other families of spiders, such as Anamidae, extant Archaeidae, extant Cyatholipidae, and Orsolobidae, are exclusively restricted to one or more areas in the Southern Hemisphere (World Spider Catalog, 2023). It has been hypothesized that these spiders may be poor-dispersers and their distribution has been shaped by the vicariance events caused by Gondwanan breakup (Griswold 1991; Wood et al. 2013; Chousou-Polydouri et al., 2019).

The majority of anapids are described from the Southern Hemisphere (World Spider Catalog, 2023) mostly in the monographic works of Platnick and Forster (1989) and Rix and Harvey (2010). A few anapids from the Northern Hemisphere include *Gertschanapis* (United States), *Borneanapis* (Borneo), *Conculus* (China, Korea, Japan also in Indonesia), *Gaiziapis* (China), *Pseudanapis* (Japan to Australia, Mauritius, Réunion, assumed to be an introduced species in Europe (Snazell and Smithers,

2007)), *Enielkenie* (Taiwan, not sampled), *Sinanapis* (Orient) and *Zan-gherella* (southern Europe, northern Africa, not sampled). All remaining groups of the Owa Clade and all members of Micropholcommatinae (except *Comaroma* from Europe and Orient, not sampled) and Vichitra Clade are distributed in the Southern Hemisphere (World Spider Catalog, 2023). All remaining extant Anapidae spiders have very narrow distributions. For example, *Micropholcomma junee* is known only from two caves in the Junee-Florentine karst of Tasmania or *Acrobleps hygrophilus* from Tasmania (Platnick and Forster, 1989; Lopardo and Hormiga, 2008; Rix and Harvey, 2010).

We expected that the ancestral area for Anapidae would be a combination of different areas and vicariance scenarios concordant with the Gondwana breakup and would explain the distribution of the extant anapids. However, most ancestral reconstructions (such as for Vichitra or Micropholcommatinae Clades) were optimized as a singular biogeographic area and implied multiple dispersal events to the descendant clades.

We believe that the interpretations of these results have the following limitations. It is possible that additional sampling of trans-Pacific Micropholcommatinae genera, such as *Gigiella* and *Eperiella* from Australia and Chile, may provide more resolution to the biogeography of Micropholcommatinae. Our biogeographic analysis was solely based on only a sample of extant anapids and no fossil lineages were included as terminal tips. The general pattern implied by our present taxon sampling was as follows (listed in Table S9).

The CCP area (ancestral area of Vichitra Clade) was colonized and established by the dispersal of ancestral population(s) of the MRCA of Vichitra + Micropholcommatinae from Australia to CCP. Most dispersal events range from the Upper Cretaceous Period to the Pliocene epoch. The CCP and AUS regions were contiguous with Antarctica and then separated during this period, therefore a vicariant separation could be an alternative explanation. ~~However, absence of any spider extant or fossil anapid from Antarctica prevented us to test this hypothesis.~~ We interpret our results from the current taxon sample as below.

4.3.1. Australia and New Zealand

The opening of the Tasman Sea during the Upper Cretaceous (ca. 80 Ma) separated New Zealand from Australia (Molnar et al., 1975). Our biogeographic analysis implied the seven rounds of dispersal events from Australia to New Zealand, after separation, during the Eocene, Miocene and Pliocene epochs. Similar eastward dispersal (Australia to New Zealand) from the Eocene onwards is recorded in several organisms such as *Pomaderris* plants (Nge et al. 2021) where the West Wind Drift (WWD) is considered to have influenced the trans-Tasman Sea dispersals. Therefore, considering the concordant time periods, it is plausible that the WWD was the driver of the dispersal of the micropholcommatine ancestral population(s).

Interestingly, our biogeographic analysis implied another dispersal event during the Upper Cretaceous (ca. 90 Ma), prior to the known separation of Australia and New Zealand. A plausible explanation to this finding could be that an ancestral population was narrowly distributed in the present-day Australia and locally dispersed to the contiguous New Zealand area. One dispersal event from Australia to Africa is implied during the Eocene epoch. Although this seems to be a very long trans-Oceanic dispersal, it is possible that intermediate stops or lineages existed on Antarctica when it was habitable for spiders (e.g., *Stay in Antarctica* scenario). However, we could not test this conjecture in this study due to lack of two trans-Pacific taxa, *Gigiella* and *Eperiella*.

4.3.1.1. No activity during the Oligocene. The popular hypothesis about Oligocene (33.9–23.03 Ma) submersion (Cooper and Cooper, 1995) suggests that the Zealandia landmass experienced a period of marine incursion during the Oligocene epoch. Our biogeographic analysis indicates multiple dispersal events between Australia and New Zealand during this period (Fig. 7A) which predates the Oligocene epoch. This is

important because, it is in contrast with the hypothesized complete submersion hypothesis of New Zealand and New Caledonia during most of the Paleogene (Waters and Craw, 2006). According to this hypothesis, the present biota of New Zealand (including *Sphenodon* (tuatara), over 228 Ma extant reptile lineage) is a result of oceanic dispersal. Our results support the alternative and now more widely accepted hypothesis of partial submergence (Cooper and Millener, 1993; Giribet and Boyer, 2010; Brothers and Lillie, 1988) which suggests that small portions remained above the water (McLoughlin, 2001). Recent arachnid biogeographic studies, such as that of Trienonychidae opiliones and Orsolobidae spiders have inferred clades that predate the marine incursion period (Chousou-Polydouri et al., 2019; Baker et al., 2020). Our biogeographic results thus form an additional source in support of the hypothesis about partial submergence.

4.3.2. Africa and Madagascar

A dispersal event from Africa to New Caledonia occurred towards the end of the Eocene (ca. 52.88 Ma). There has been a debate about whether NC was totally submerged (implying extinction of the continental biota, so that all current biota is a result of dispersal) or whether some areas remained above water (reviewed in Heads 2019). The scenario of complete submergence would suggest an alternate possibility of some intermediate islands that facilitated the arrival of the Africa anapid ancestor to NC. There are multiple studies that have recovered dates of origin which are older than the presumed total submergence of NC. For example, the NC Clade of dung beetles (Scarabaeidae) (55 Ma) (Gunter et al., 2019), geckos (from NC are monophyletic) (48.3 Ma) (Skipwith et al., 2016) and Myrtaceae (*Myrtastrum*, 65 Ma) (Vasconcelos et al., 2017).

Two dispersal events from Madagascar to Africa occurred, each during the Upper Cretaceous period (ca. 92.5 Ma) and Eocene (ca. 46.8 Ma) epoch. Madagascar was already separated from Africa by the Upper Cretaceous period (Seward et al., 2004), so this indicates the dispersal was transoceanic. The general pattern for this whole clade (the clade including two Anapidae sp. Madag), suggests the MRCA occurred in Madagascar, although there are also affinities to Australia, suggestive that the entire clade might have an eastern Gondwanan origin, followed by multiple instances of dispersal out of eastern Gondwana to New Zealand, Africa, New Caledonia, etc.

4.3.3. Central Chile and Patagonia (CCP)

Among all Anapidae taxa, the anapids from the CCP such as ChOwa Clade and the ancestral area of Vichitra Clade (both Lower Cretaceous) have relatively deep divergences, even older than the anapids from Australia where most extant species are found. This indicates the older origins and persistently long presence of the ancestral anapid populations in that region. It is therefore plausible to be the ancestral area of Anapidae, however, this conjecture could not be tested with our current sampling.

All Chilean anapids except *Tricellina*, *Sofanapis* and *Teutoniella* are grouped in the ChOwa Clade. The most likely hypothesis suggests this clade diverged from its sister group due to dispersal from the Oriental region to the CCP region in the early Cretaceous period. *Tricellina* is a micropholcommatine which diverged from its common ancestor with Taphiassini, Patelliellini and Micropholcommatini due to a dispersal event from Australia to CCP around 73.5 Ma. Both *Teutoniella* and *Sofanapis* retained the ancestral area (which was CCP) of the MRCA of the Vichitra Clade.

Morrone (2015) proposed a biogeographic region composed of the South American transition zone and central Chilean and Patagonian sub-regions. This regionalization was based on the distribution of plants and animals. The orb weaving anapids representing the CCP clade are known only from the Southern CCP region. Symphytognathoids spiders from this region have not been found in other parts of South America despite several surveys. Their absence outside CCP may be indicative of endemism in the CCP region, and supporting the regionalization of the CCP

region proposed by Morrone (2015).

4.4. Respiratory system

Our results imply an anterior book lungs and posterior tracheal configuration for the MRCA of the symphytognathoids which is the symplesiomorphic configuration of Araneioidea. Lopardo et al.'s (2011) morphological study included the characters of the respiratory system which optimized an anterior tracheal system at the node subtending all symphytognathoid families except Theridiosomatidae. Their study proposed the name "Anterior Tracheal System (ANTS)" for this clade. Our results recovered an ancestral anterior tracheal system to the ANTS Clade, thus corroborating their inference.

Lopardo et al. (2022) thoroughly reviewed and analyzed the available and newly generated data about the respiratory system diversity in symphytognathoid spiders. They proposed two main hypotheses for the evolution of the respiratory system of symphytognathoids: 1) the anterior tracheal system evolved from fully developed book lungs and, 2) reduced book lungs originated at least twice from the tracheal system. Our results corroborate Lopardo et al. (2022) inference and further suggest that ancestral book lungs transformed into tracheae at least four times independently and that tracheae transformed back to book lungs a total of seven times (four times to reduced book lungs, and three times to unmodified book lungs). The anterior tracheal system involves some diverse structures whose evolutionary history requires a detailed morphological study and analytical treatment. This involves the extension of the tracheal system into the prosoma and their branching into tracheoles, the location of tracheal spiracles and the presence of a transverse duct.

The posterior tracheal spiracle involves another challenge which also requires generation of additional morphological data and analytical treatment. As shown in the present study, many symphytognathoids have independently lost the posterior tracheae. The species that have posterior tracheae however show some diversity, such as having some tracheae extend into the prosoma (as in *Anapis*) or having two single tracheae (as in *Micropholcomma*), branched (as in *Microdipoena*) or singular lateral tracheae (as in *Zealanapis*).

The transformation (or reduction) of book lungs into tracheae has been one of the crucial questions in spider biology because the majority of spiders have a posterior tracheal system whereas early-diverging lineages have anterior and posterior book lungs, perhaps suggesting that a posterior tracheal system may have some adaptive value. Several hypotheses have been postulated for book lung to tracheal transformations, such as increase in the demand of oxygen, or because trachea reduce water loss (which happens due to diffusion in book lungs) (Anderson and Prestwich, 1975; Levi, 1967, 1976; Schmitz, 2013; Ramírez et al. 2021). Functionally, tracheae are better at aerobic metabolism than book lungs due to the reach of branched tracheoles to tissues as opposed to book lungs which diffuse oxygen in the hemolymph (Schmitz, 2013). Experiments of spiders with a dense network of tracheae (*Marpissa muscosa*, Salticidae) and poorly tracheated (*Pardosa lugubris*, Lycosidae) on treadmills show that spiders with branched tracheae (with experimentally sealed book lungs) are better at meeting the oxygen needs than those with book lungs (with experimentally sealed tracheae) (Schmitz, 2005; 2013). Further, aerial web building is an energy demanding task, therefore having a more efficient respiratory system like tracheae is adaptive. It is thus not surprising that most aerial web building spiders have a posterior tracheal respiratory system with anterior book lungs system, however, many webless spiders also have a posterior tracheal system, adding to the mystery. Although much smaller in body size than other web building spiders, many symphytognathoids construct aerial webs. Therefore, for spiders as small as anapids, considering the smaller size of tracheae and their increased reach to tissues, having a tracheal system seems to be a cost-effective measure to maximize the respiratory surface area, minimize water loss and direct oxygen supply to tissues. This hypothesis is supported by a

general pattern as follows: the Owa Clade (Anapidae), which includes spiders that construct a two or three dimensional aerial orb web, has an anterior tracheal system with loss of the posterior tracheae altogether and, those with reduced book lungs have retained the posterior tracheal system (with the exception of *Sheranapis*). Micropholcommatines (whose web architecture is known), construct sheet webs or three-dimensional tangle-webs (Hickman, 1943, 1945; Rix and Harvey, 2010), all of which have an anterior tracheal system, however the posterior tracheae are lost in several of them (Fig. 8). Symphytognathidae spiders, which are further smaller than most Anapidae, have the configuration of anterior tracheae and loss of posterior respiratory system. Thus, a general tendency of presence of the tracheal system is seen in small-bodied spiders.

An exception to this pattern is seen in Caponiidae (adult body size of *Calponia harrisonfordi* is ca. 5.1 mm (Platnick, 1993)) which have a larger body size and have anterior tracheae. This is a distantly related family nested within the Synspermiata clade which has an anterior sieve-like tracheal system (Lamy, 1902; Ramírez, 2000). Synspermiata are one of the early diverging groups of spiders with an ecibellate haplogyne condition characterized by multiple spermatids fused into one synsperm (Alberti and Weinmann, 1985; Burger and Michalik, 2010). The loss of posterior tracheae as seen in several Symphytognathidae and Anapidae is not peculiar to these spiders. The Synspermiata Clade includes a sub-clade called the Lost Tracheae Clade. This sub-clade includes the spider families Diguettidae, Pacullidae, Pholcidae, Plectreuridae and Tetrablemmidae, all of which have secondarily lost the posterior respiratory system (Ramírez, 2000).

4.5. Web architecture

Of all anapids, only two genera, *Holarchaea* and *Sofanapis*, are known to be webless, both of which belong to the *Vichitra* Clade. *Holarchaea novaeseelandiae* bears only three spigots on each spinneret which is perhaps an indication that they do not construct foraging webs (Forster and Platnick, 1984). A similar pattern of loss of spigots with loss of foraging webs has been shown in some *Tetragnatha* (Tetragnathidae) species from Hawaii (Berger et al., 2021). *Sofanapis antillanca* is a kleptoparasite that lives on the webs of their Austrochilidae hosts in Chile (Ramírez and Platnick, 1999). Our results imply that two losses of foraging web occurred in the *Vichitra* Clade. We did not include *Teutoniella* in this analysis because the web architecture of this genus is not known. The inclusion of other kleptoparasitic genera such as *Curimagua* (Symphytognathidae) (Vollrath, 1978) can potentially change the number of losses of the foraging web, however, we were unable to generate sequences for it. Similarly, we pruned several terminals from the combined data set since data for web architecture was not available for those taxa.

In the Owa clade, all taxa (whose webs are known) construct a three-dimensional orb web, except *Elanapis aisen* and *Gaiziapis* and possibly *Borneanapis*. The latter (*Borneanapis*) was the only lineage that constructs a non-orb web that was included in our study. In the description of this monotypic genus, Snazell (2009) mentioned that *B. belalong* were collected from "very small, triangular, horizontal sheet webs built into various corners and depressions in the smooth bark of the tree". Snazell also mentioned that the web was covered with detritus which perhaps would make it difficult to closely observe the silk lines. This is problematic because the small and tightly woven orb web of some symphytognathoids can be confused with a sheet web. For example, in the original description of the genus *Patu* (Symphytognathidae), Marples (1951) mentioned that *Patu vitiensis* and *Patu samoensis* construct sheet webs. Later on, Marples (1955) mentioned that the webs of these spiders are actually orbicular on a closer observation and only appear as sheet webs due to densely placed radii and spirals. We therefore think that a closer observation of the web of *Borneanapis belalong* is required to corroborate the presence of sheet webs in this species. Another anapid placed in the Owa Clade, an undescribed species from Madagascar

("Anapidae sp. Madag GH2593" in Figs. 5, 6), was observed to construct a structure similar to the sheet webs of Cyatholipidae (GH pers obs.). The inclusion of this latter species coded as sheet web did not affect the ancestral state of the Owa Clade.

The coding scheme in our "Orb 3-state" and "Web 6-state" analyses recovered conflicting character optimizations at nodes outside symphytognathoids, although we should note that the taxon sampling of our study is optimized for ancestral character reconstructions within symphytognathoids and further taxa should be included in the analyses to study web evolution beyond this group. For example, at the base of Deinopidae, an orb web architecture was optimized with "Web 6-state" (Fig. 9) whereas a non-orb structure was optimized with "Orb 3-state" (Figure. S12). We think that this conflict could be a result of aforementioned insufficient sampling of the taxonomic groups outside symphytognathoids (the "outgroups"). Our study was not designed to explore the web architecture towards the base of our phylogeny which would require inclusion of more taxa outside symphytognathoids for a reliable optimization. Although the orb web optimized at the base of the tree, implying a monophyletic origin of orb web in our results, we believe that this particular result is an artefact of the limited and biased taxon sampling (Fig. 9).

The evolution of the orb web architecture has fascinated arachnologists for more than a century (for example, McCook, 1889; Comstock, 1912; Robinson and Robinson, 1974; Coddington, 1986; Griswold et al., 1998; Bond et al., 2014; Fernandez et al., 2014, 2018; Kallal et al., 2021). Three groups of spiders have members that construct orb webs: Deinopidae, Uloboridae and Araneioidea. The first two families are cribellate. Araneioidea are ecribellate and include 17 families of which Anapidae, Araneidae, Mysmenidae, Symphytognathidae, Tetragnathidae and Theridiosomatidae construct a diversity of orb webs. The remaining Araneioidea families build a wide variety of non-orbicular webs (sheet webs, ladder webs, irregular tangles, etc.) and some even use non-web depending strategies for foraging such as kleptoparasitism (e.g., some theridiids) or aggressive mimicry to prey on other spiders (e.g., mimetids).

The similarities between the orb webs found in Deinopidae, Uloboridae and Araneioidea, such as the presence of sticky spiral threads placed on radii converging at the center hub (Eberhard 1982, Coddington 1986), suggest that the orb web could be a result of a single origin. Initial phylogenetic studies using morphology and behavior suggested a monophyletic origin of the orb web, however ancestral state reconstructions based on molecular data refuted this hypothesis (some references include Coddington, 1986; Griswold et al., 1998; Bond et al., 2014; Fernandez et al., 2014, 2018). More recently, (Fernandez et al., 2014, 2018a, b) and Coddington et al. (2019) have debated over the origin(s) of orb webs, with the latter study hypothesizing an orb web in the common ancestor of Entelegynae, with secondarily losses in numerous lineages (reviewed in Eberhard 2022). Using an expanded taxon sample and more sophisticated methods (nested hidden states and structured Markov models), Kallal et al. (2021: 312) inferred multiple origins of the orb in entelegynes and multiple losses within Araneioidea. Our results suggest that there are multiple losses of orb webs within symphytognathoids, and thus form an additional line of evidence in this regard.

The orb web is a finished product resulting from the integration of and interactions between various nested factors such as the type of silk (further implying presence and functioning of particular glands and spigots), calamistrum-cribellum (in Deinopidae and Uloboridae), web plane orientation (ranging from vertical to horizontal), prey choice and availability, time of the day (e.g., *Deinopis* (Deinopidae) are nocturnal, *Leucauge* (Tetragnathidae) are primarily diurnal). A similar set of factors also apply to the construction of other architectures of foraging webs. The challenge therefore is to hypothesize the homologies between the stereotypical behaviors involved in web building. For example, homologizing spigots across araneoid species is relatively straightforward (e.g., Griswold et al. 1998), however, what is the homolog of an orb web

radius in a sheet web? A recent study by Kallal et al. (2021) used structured Markov modelling (SMM) (Tarasov, 2019) to account for hierarchically nested traits. Kallal et al. (2021) inferred that the orb web evolved twice in the UDOH Grade (independently in Deinopidae and Uloboridae) and either one or three times in Araneioidea (depending on their coding scheme). The types of web architecture in Anapidae, including the modifications of orb web, are just a snapshot of a larger series of transitions in web architecture in Araneioidea and deserve further research aimed at studying web building behavior and documenting the foraging webs of more anapids.

Inferring the evolution of web architectures (as a foraging strategy) is fundamental to further uncovering the diversification of spiders. Although Araneae is the only arachnid group that builds foraging webs, the most speciose lineage of spiders, the retrolateral tibial apophysis clade (RTA Clade; see Wheeler et al. 2017), includes mainly cursorial or sit-and-wait predators (thus foraging webs are secondarily absent in this clade). There are only a few webless species in Anapidae and among symphytognathoids, most of which are kleptoparasites living on the webs of other spiders.

5. Conclusions

With the most comprehensive phylogeny of symphytognathoids, we bookend the questions about the relationships of the miniature orb weaving spiders, placement of Synsphyridae within symphytognathoids and Micropholcommatinae within Anapidae. Transformations of the web architecture through symphytognathoid evolution was optimized using a simplified characterization of the very complex phenomenon of web building behavior, final architecture and function. Future investigations of symphytognathoid evolutionary history must emphasize the role of spinneret configuration in the web architecture and the causal factors of loss resulting into kleptoparasitism. A detailed morphological and behavioral study to determine the synapomorphies of placement of the Vichitra Clade is warranted. Our phylogenetic framework from this study will be useful for exploring trait evolution such as how reduction and loss of pedipalps in adult females have occurred. Another trend that needs to be researched is whether body size or habitat (or both) are correlated with the configuration of respiratory systems in these spiders. Through our divergence dating analysis, we found that Anapidae is an ancient family whose extant distribution was shaped by multiple dispersal events, primarily in the southern hemisphere. This inference makes a case to study whether the miniature orb weaving spiders disperse by ballooning or not. The record of *Crozetulus* from the oceanic Crozet Islands is a good example suggestive of a dispersal mechanism in these spiders. Summing up, symphytognathoid systematics has clearly advanced after Lehtinen's (1975) critique of "surely a polyphyletic dump heap of minute Araneioidea", which are now seen as a monophyletic group that shows remarkable diversity of morphologies, behaviors and ecologies that have arisen possibly due to miniaturization.

CRediT authorship contribution statement

Siddharth Kulkarni: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Hannah M. Wood:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Gustavo Hormiga:** 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.

Data availability

Data will be made available on request.

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Data Accessibility

All alignment files, character coding data 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 PRJNA991600.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107855>.

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