

Advances in the reconstruction of the Spider Tree of Life: a roadmap for spider systematics and comparative studies

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RUNNING TITLE: A roadmap to spider systematics

ACKNOWLEDGEMENTS

This study was funded by the following grants/fellowships: a Weintraub fellowship, Harlan fellowship, Smithsonian Predoctoral fellowship, Mortensen grant, Oscar and Jan Francke Student Research grant, two Lakeside grants and a National Geographic Society Early Career grant, Columbia College Dean’s Dissertation Completion grant to SK; National Science Foundation DEB 1457300, 1457539 grants to GH and Gonzalo Giribet, DEB 1754289 to GH, and from an Exploratory Award (year 2016) from the Global Genome Initiative (National Museum of Natural History, Smithsonian Institution) to HMW and Michael Lloyd. Authors are

grateful to Charles Griswold, Darrel Ubick, and Lauren Esposito for the curation of material and their help during our visit to the California Academy of Sciences. Fieldwork was made possible due to the generous help of Miquel Arnedo and Robert Raven in Australia and Milenko Aguilera and participation of Darko Cotoras in Chile. We also thank Charles Griswold, Bob Kallal, Chuy Ballesteros, Nicolas Hazzi, Thiago da Silva-Moreira, Dimitar Dimitrov, Miquel Arnedo, Alex Pyron, Jimmy Saw, Sarah Crews, Joseph Koh, Suresh Benjamin and Prashant Sharma for discussions that helped in improving this manuscript. Authors are grateful to the reviewers and editors for their time and effort on this manuscript. We acknowledge the computing resources, Pegasus and Hydra made available to us by The George Washington University and the Smithsonian Institution, respectively.

ABSTRACT

In the last decade and a half, advances in genetic sequencing technologies have revolutionized systematics, transforming the field as studying morphological characters; a few genetic markers have given way to genomic data sets in the phylogenomic era. A plethora of molecular phylogenetic studies on many taxonomic groups have come about, converging on, or refuting prevailing morphology or legacy-marker-based hypotheses about evolutionary affinities. Spider systematics has been no exception to this transformation and the interrelationships of several groups have now been studied using genomic data. About 50,500 extant spider species have been described so far, all with a conservative body plan, but innumerable morphological and behavioral peculiarities. Inferring the spider tree of life using morphological data has been a challenging task. Molecular data have corroborated many hypotheses of higher-level relationships, but also resulted in new groups that refute previous hypotheses. In this review, we discuss recent advances in the reconstruction of the Spider Tree of Life and highlight areas where additional effort is needed with potential solutions. We base this review on the most comprehensive spider phylogeny to date, representing 131 of the currently known 132 (99%) spider families. To achieve this sampling, we combined a legacy data set of six Sanger-based markers with newly generated and publicly available genome-scale data sets. We find that some inferred relationships between major lineages of spiders (such as Austrochiloidea, Palpimanoidea, Synspermiata, etc.) are robust across different classes of data. However, several surprising new hypotheses have emerged with different classes of molecular data. We identify and discuss the robust and controversial hypotheses and compile this blueprint to design future studies targeting systematic revisions of these problematic groups. We offer an evolutionary

framework to explore comparative questions such as evolution of venoms, silk, webs, morphological traits, and reproductive strategies.

KEYWORDS. Araneae, taxonomy, ultraconserved elements, arachnology.

INTRODUCTION

Spiders are a remarkably diverse lineage among arthropods. An apt example of their evolutionary success is that there are nearly 50,500 described species (World Spider Catalog, 2022), but with an estimated two-fold number of described species remaining undiscovered (Platnick, 1999; Agnarsson et al., 2013). This species richness is greatly asymmetrical compared to the species richness of its sister group, Pedipalpi, which contains less than 700 described species (Harvey, 2013; Ballesteros et al., 2021; Miranda et al., 2021, *in prep*). Spiders occupy all terrestrial and some aquatic habitats, and are distributed on all continents except Antarctica. The origin of spiders is estimated to be about 400 million years ago (Magalhaes et al., 2020; Kallal et al., 2021), after which, they have evolved a great diversity of shapes, sizes, behaviors, silk uses, web architectures, respiratory systems and venom compounds (Platnick et al., 2020).

Morphological and biological makeup

The synapomorphies of spiders include the production of silk from the associated spinning apparatus and the presence of venom glands opening through the cheliceral fang (Figure 1). Spigots (and their silk) originated prior to the evolution of spinnerets (Shultz 1987), a claim that is supported by the presence of spinneret-less spigots in the order Uraraneida (Selden et al., 2008). However, the hypothesis that spinnerets are exclusive to Araneae was challenged by

the discovery of *Chimerarachne yingi* Wang et al., (2018) (popularly known as “the spider with a tail”) from Burmese amber (dated 99 MYA). This fossil bears spinnerets, male pedipalps presumably modified for sperm transfer (both characters being synapomorphies of spiders) and a uropygid-like telson, and is placed as a sister group of all spiders (Wang et al., 2018; Huang et al., 2018). In extant spiders, silk is used for many tasks critical to spider biology and survival, such as constructing foraging webs (e.g., the characteristic orb web), wrapping prey, dispersal via ballooning, bonding to substrates and producing egg sacs (Figure 3). These myriad utilities of silks have been attained by the secretions of up to seven different types of glands that function individually or in combination (Kovoor, 1972, 1977). Some web building spiders bear a short transverse field of spigots (homologous to the primitive anterior median spinnerets) called the cribellum that is used to secrete a distinctive type of silk (as in net casting Deinopidae webs). The cribellum is coupled with a row of curved setae on the metatarsus of the fourth leg called the calamistrum, which is used to process and lay the cribellate silk. Webs are constructed by many lineages to capture prey, however, many other spiders (some which have secondarily lost web building, some which never had this behavior) use alternative strategies such as ambushing or active hunting. A peculiar adaptation— adhesive setae on legs such as scopulae or claw tufts are found in most of these wandering spiders (webless) and have evolved multiple times, although some web building spiders also bear adhesive setae (Wolff et al., 2013). Recently, an encyclopedic treatment of spider webs by William Eberhard revealed unparalleled diversity of webs with intricate behaviors and functions (Eberhard, 2020) forming a framework for posing many new questions about evolutionary transitions.

Spiders are generalist predators with the exception of a small proportion of specialists with a reduced diet (such as preying exclusively on terrestrial isopods, ants, moths, dipterans, or

even other spiders) (Pekár & Toft, 2015). In web-building spiders, silk is used in combination with the chelicerae to inject venom through fangs, in order to capture and immobilize the prey, whereas many hunters only rely on their legs, pedipalps and chelicerae to grasp prey with injecting venom. A large variety of venom compositions have evolved within spiders, with over 3,000 compounds recorded so far (Kuhn-Nentwig et al., 2011, Lüddecke et al., 2022). In general, venom complexity and venom gland sizes are larger in generalist spiders compared to their specialist counterparts (Pekár et al., 2018; Lüddecke et al., 2022).

In addition to these synapomorphies, another characteristic feature of spiders includes the occurrence of two types of respiratory systems –book lungs and tracheae, with most spiders having both of these types. It is hypothesized that the book lungs are the symplesiomorphic condition because they are found in the three orders of Tetrapulmonata and earliest-diverging clades of spiders, Mesothelae and Mygalomorphae and some early-diverging Araneomorphae, for example, Gradungulidae and Hypochilidae (less than 35 of about 50,500 species of araneomorph spiders) (Ramírez, 2000; Schmitz, 2013; Ramírez et al., 2021). They have two pairs of book lungs whereas most “modern” spiders (Araenomorphae) have either a combination of one pair of book lungs and tracheae (example, water spider *Argyroneta aquatica* (Clerck, 1757)) or exclusively only tracheae (for example, Symphytognathidae).

The most common (and vital) acts in spider survival are thus the result of an integration of many behaviors, for example prey capture involves prey detection, hunting behaviors, venom composition amounting to toxicity, silk (such as web or prey capture), energy demand (mitigated by respiration), in addition to other traits such as vision (except for eyeless spiders) and sensing movement and sound through vibration. All of these traits are highly diverse across Araneae and understanding their evolutionary history is essential to explore the influential factors on the

evolutionary success of different spider lineages. To understand the evolutionary history of these unique characteristics, the prerequisite is a robust phylogenetic hypothesis.

In the last three decades there have been numerous phylogenetic studies of spiders using morphological data but it has been challenging, and in some cases impossible, to satisfactorily resolve many important nodes of the spider tree of life (e.g., Griswold et al., 2005; Ramírez, 2014). The sparse genomic resources (prior to the advent of parallel sequencing) have maintained ambiguity in phylogenetic relationships of several lineages and many earlier hypotheses have been refuted with high support by these more recent genomic studies. For example, Orbiculariae, which in the past grouped cribellate and ecribellate orb weavers (e.g., Coddington 1990) has been shown not to be a natural group in multiple recent phylogenomic analyses (Bond et al., 2014; Fernández et al., 2014, 2018; Garrison et al., 2016; Kallal et al., 2021; Kulkarni et al., 2020, 2021), corroborating earlier hypotheses of non monophyly based on Sanger sequencing datasets (e.g., Blackledge et al., 2009; Dimitrov et al., 2017).

In less than a decade after Coddington & Levi's (1991) review of spider systematics, Hausdorf (1999) published the first molecular phylogeny of spiders reconstructed using 900 characters (bp) of the 28S rRNA gene. Technological developments, its reach and cost effectiveness and the number of arachnologists using nucleotide sequence data have increased substantially helping to progress our understanding of spider biology and evolution. The rapid advancement of massive parallel sequencing technology and its cost effectiveness for genomic scale data generation (Christensen et al., 2015) rapidly increased the size of molecular data sets for spiders (Figure 2). For example, some of the most recent phylogenies using genomic data were reconstructed using anchored hybrid enrichment data which included 33 taxa (19 of 114 families at the time) (Hamilton et al., 2016), transcriptomes which included 272 taxa (101 out of

128 families at the time) (Kallal et al., 2021), ultraconserved elements (UCEs) which included 248 taxa (88 out of 120 families at the time) (Kulkarni et al., 2021) and targeted 99 markers which included 303 taxa (105 out of 132 families at the time) (Shao et al., *in press*). Wheeler et al. (2017) published a densely sampled phylogeny using six genetic markers acquired via Sanger sequencing, constrained using the transcriptomes-based phylogeny of Garrison et al., (2016), which included 932 taxa (115 out of 116 families at the time). A few studies have used genomic scale data to reconstruct the evolutionary history of a specific group of spiders, such as, Mygalomorphae (Hedin et al., 2019; Opatova et al., 2020); Leptonetidae (Ledford et al., 2021), Synspermiata (Ramírez et al., 2021), Austrochiloidea (Kulkarni & Hormiga 2021), Palpimanoidea (Wood et al., 2018), Araneoidea (Fernández et al., 2018; Kallal et al., 2020; Kulkarni et al., 2020, 2021) or Salticidae (Maddison et al., 2020). The hypotheses about relationships among different lineages of spiders have been converging to some degree, however some recalcitrant nodes remain when reconstructed using different classes of data (Kulkarni et al., 2021). The need for better taxon sampling for addressing the problem about recalcitrant nodes and resolution has been echoed in the literature (Dimitrov & Hormiga 2020 and references therein).

In addition to morphology, Sanger-sequence based markers, AHEs, transcriptomes and UCE data sets, hypotheses about the phylogenetic relationships of spiders have been tested using filtering of different genomic scale data classes such as ultraconserved regions within transcriptomes, coding regions within UCEs, combination of UCEs and transcriptomes and treating the coding regions as nucleotides and amino acids (Kulkarni et al., 2021). Most phylogenetic relationships have largely converged with well-supported branches, however some relationships remain elusive. A prominent and largely explored example of recalcitrant nodes in

the spider tree of life includes the relationships between the families of the superfamily Araneoidea (ecribellate orb weavers and their relatives). Orb-weaving families, both cribellate (i.e., Deinopidae and Uloboridae) and ecribellate (e.g., Araneidae, Tetragnathidae and some “symphytognathoids”) were deemed to form a monophyletic group (Orbiculariae) based on morphological and behavioral characters (e.g., Coddington, 1990). While the monophyly of orb webs was appealing due to its simplicity, some authors had suggested that the cribellate and ecribellate orb webs have evolved convergently (reviewed in Coddington, 1986). Molecular data refuted the monophyly of Orbiculariae (e.g., Hausdorf, 1999; Blackledge et al., 2009; Bond et al., 2014; Fernández et al., 2014; Wheeler et al., 2017; Dimitrov et al., 2017). This change in the phylogenetic relationships of orb weavers affected hypotheses about the evolution the iconic orb web, with several analyses using a diversity of methods of ancestral reconstruction hypothesizing multiple origins (e.g., Fernández et al., 2018; Kallal et al., 2021) while other analyses argued for a single origin (e.g., Coddington et al., 2019; Garrison et al., 2016).

Summing up, instability of phylogenetic relationships obscures our understanding about the evolutionary history of spiders. Here, we review recent advancements on interfamilial phylogenetic relationships across the spider tree of life. This study is designed to identify the recurring conflicting nodes with certain data classes. We discuss these relationships based on the analysis of the hitherto largest sample of spiders to date, using genomic scale data combined with traditional Sanger-sequence data set from the literature, representing 131 of currently valid 132 spider families. We review some of the history and current understanding of family groupings and their biological characteristics in a phylogenetic context. We also provide potential future directions for spider phylogenetics and systematics such as evidence for potential taxonomic changes based on grouping by monophyly.

MATERIALS AND METHODS

Taxon sampling

The ultra-conserved sequences for this study were obtained from the following sources: (1) published UCE studies: Starrett et al. (2017), Wood et al. (2018), Hedin et al. (2019), Kulkarni et al. (2020), Ramírez et al. (2020), Maddison et al. (2020), Azevedo et al. (2022); (2) transcriptome based studies: Sharma et al. (2014), Zhao et al. (2014), Fernández et al. (2014, 2018), Rix et al. (2018), Kallal et al. (2018), Shao & Li (2018), Kallal et al. (2020); (3) publicly available spider genomes on Sequence Read Archive (SRA): *Latrodectus hesperus* (Theridiidae; i5k Consortium, 2013), *Loxosceles reclusa* (Sicariidae; i5k Consortium, 2013), *Trichonephila clavipes* (Araneidae; Babb et al., 2017), *Parasteatoda tepidariorum* (Theridiidae; Schwager et al., 2017) and *Stegodyphus mimosarum* (Eresidae; Sanggaard et al., 2014); and (4) our sequencing efforts.

We analyzed 554 terminals of UCE data, representing 125 out of 132 (94.6% sampling) spider families (World Spider Catalog, 2022). The phylogenetic trees were rooted at the node containing the Xiphosura representatives, *Tachypleus tridentatus* and *Limulus polyphemus*. In addition, we combined the UCE data, with the Sanger-based six marker data set Wheeler et al., (2017), Piacentini and Ramírez (2019), additional publicly available sequences and bycatch from UCE assemblies with our UCE data set to result in a 1,362-taxon data set belonging to 131 families (99% familial representation). The details of concatenation are detailed in Table S2.

The specimens sequenced for this study come from our own fieldwork 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, Boston, Massachusetts; and the California Academy of Sciences (CAS), San Francisco, California.

For the specimens we sequenced, three to four legs were used for DNA extractions from 58 spider specimens using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA). The homogenate was incubated at 55 °C for 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.

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 seconds, 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 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 $\text{ng}/\mu\text{L}$.

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 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$ for 45 s; 18 cycles of 98 $^{\circ}\text{C}$ for 15 s, 60 $^{\circ}\text{C}$ for 30 s, 72 $^{\circ}\text{C}$ for 60 s; and a final extension of 72 $^{\circ}\text{C}$ 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.

Recovering UCEs from transcriptomes and genomes

We followed the assembly, sanitation and reading frame detection pipeline as in Fernández et al., (2018) for assembling the transcriptomes. 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`, (Kent et al., 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_lastzs_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 explore this, we computed GC content in each taxon in the concatenated UCE data set using BBMap (<https://github.com/BioInfoTools/BBMap>). We also computed missing data to map their distribution and compare if they corresponded to the inconsistent nodes across different occupancies. GC and content and missing data was mapped on the phylogeny using the `phytools` package version 0.7-70 in R Studio version 1.3.1093.

Concatenation of our UCE and legacy marker data sets

The Sanger-based data set of Wheeler et al., (2017) included the following Sanger sequenced loci: mitochondrial markers- 12S ribosomal RNA (12S), 16S ribosomal RNA (16S,) and cytochrome c oxidase subunit 1 (COI); nuclear markers- protein-coding histone H3 (H3), and small and large subunits of ribosomal RNA genes (18S and 28S, respectively). Conspecific taxa with UCE and Sanger-sequence data were concatenated. A phylogeny resulting from this data set rendered some unusual results in our preliminary analyses, such as polyphyly of Salticidae, Malkaridae, Thomisidae and Lycosidae, which has been extensively studied and is always

recovered as monophyletic. Therefore, we increased the taxon sampling for these families based on publicly available sequences, from studies such as Piacentini and Ramírez (2019) and through bycatch of sequences from the UCE assemblies. We also concatenated congeneric taxa to maximize the data completeness (see Table S2). Our goal to perform this exercise was to maximize the taxon representation and minimize the missing data class. For a more stringent tree search space within the marronoids and Dionycha clade to test if some of the polyphyletic families are rendered monophyletic, we compiled two data sets including these taxa and a few outgroups extracted from the 25% occupancy UCE data set.

Phylogenomic analyses

UCE data set: The assembly, alignment, trimming and concatenation of data were done using the PHYLUCE pipeline (publicly available at <https://phyluce.readthedocs.io/en/latest/>). We applied gene occupancies of 10%, 25% and 40% on the UCE data set. Additionally, we also analysed 1% occupancy of the UCE data set to allow inclusion of all loci in the reconstruction of the phylogeny. 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) version 2. Model selection was allowed for each unpartitioned dataset using the TEST function (Kalyaanamoorthy et al., 2018, Hoang et al., 2018). Nodal support was estimated via 1000 ultrafast bootstrap replicates (Hoang et al., 2018) with 15,000 iterations. To reduce the risk of overestimating branch support with ultrafast bootstrap due to model violations, we appended the command *-bnni*. With this command, the ultrafast bootstrap optimizes each bootstrap tree using a hill-climbing nearest neighbor interchange (NNI) search based on the corresponding bootstrap alignment (Hoang et al., 2018).

Six-marker Sanger sequencing data set: 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 and concatenation of UCE and six-marker data set used in the study.

RESULTS AND DISCUSSION

Our UCE data set included 554 taxa representing ten non-spider arachnids including two Xiphosura (*Tachyples tridentatus* and *Limulus polyphemus*), which were used to root the phylogeny. This data set included 125 of the currently known 132 (94.6 %) spider families (World Spider Catalog, 2022). Our Combined data set (UCEs+legacy marker data sets) included 1,362 taxa with 131 families (99 %) of which 381 taxa were represented by both data classes (Table S2). Model testing using the Bayesian Information Criterion (BIC) in IQ-TREE selected the GTR+I+F+G4 model for the Combined data set and all matrix occupancies (1%, 10% and 25%) UCE data sets. Statistics of captured UCE loci are listed in Table S1. All alignments files will be made available online on Figshare. The phylogenetic relationships were overall similar across except that at 1% and 10% occupancies where Araneidae was sister group to a clade including Synotaxidae plus Physoglenidae and Nesticidae whereas Araneidae was sister to Synotaxidae at occupancy 25%. Within the miniature orb-weaving spiders clade (symphytognathoids), Theridiosomatidae formed a sister group to Mysmenidae at 1% and 10% occupancy whereas Theridiosomatidae was sister group to the Anterior tracheal system (ANTS) Clade that includes the remaining symphytognathoid families. GC-content was high in only

Trogloaptor marchingtoni (Figure S1) as shown by Ramírez et al., (2021), however, omitting it from the analyses did not alter the resulting phylogenetic relationships. Missing data were calculated for the UCE data set, which was high for several taxa, particularly those that were sequenced using Arachnid probe set of Starrett et al., (2017) but matched using the Spider2Kv1 probe set of Kulkarni et al., (2020) (Figure S2).

All major clades that were obtained in the most recent transcriptomes-based study (Kallal et al., 2021) and UCEs-based study (Kulkarni et al., 2021) were recovered in this study with both UCE and Combined data set. These lineages include Araneae, Mesothelae, Opisthothelae, Mygalomorphae, Avicularioidea, Atypoidea, Araneomorphae, Hypochilidae+Filistatidae, Synspermiata, Austrochiloidea, Palpimanoidea, Nicodamoidea, Retrolateral tibial apophysis (RTA) Clade, Araneoidea, etc. A general structure of relationships between these major lineages are shown in Figure 3 and their family level relationships are shown in Figure 4.

Araneae

Platnick & Gertsch (1976) constructed the first cladogram about higher level grouping in spiders. They rejected the groupings by cheliceral orientation (Orthognatha and Labidognatha) and established two suborders Mesothelae and Opisthothelae and two infraorders within Opisthothelae — Mygalomorphae and Araneomorphae which continue to be used to date. In this section, we provide a review of the family level relationships obtained from our UCE and Combined data sets and a comparison with prevailing hypotheses compiled from the literature. We use the term “Combined phylogeny” to indicate the phylogenetic tree resulting from the

combination of the UCE-derived data set and the Wheeler et al., (2017) six Sanger-based markers (Figures 5-20).

Mesothelae

This group is an ancient lineage which includes spiders that retain many primitive characters, such as an externally segmented abdomen, four pairs of multisegmented spinnerets, two pairs of book lungs and chelicerae organized at an angle (between paraxial and diaxial). In addition, their spinnerets are situated near the middle of the abdomen and abdominal segments 12–18 are present. The extant mesotheles are classified in two families, Liphistiidae and Heptathelidae, with one and seven genera, respectively and about 150 species mainly known from China, Japan and South-East Asia (World Spider Catalog, 2022; Xu et al., 2021; Li, 2022). The oldest mesothele fossils are from the late Carboniferous period (Magalhaes et al., 2020). They construct trapdoor burrows (similar to some mygalomorphs) with radiating trip lines for prey capture (Bristowe 1976). The similarity of observable morphological characters in the spider fossils, phylogenetic placement, and age (in dated phylogenies) in extant mesotheles indicate that these spiders retain a plesiomorphic state for many characters. Haupt (2003) reconstructed a morphology-based cladogram of relationships between the Mesothelae spiders. Morphological synapomorphies of Mesothelae include presence of abdominal tergites; two pairs of book lungs; invaginations at posteromedian corners of coxae IV; trichobothrial base on the dorsal surface of distal leg segments dome-shaped with two flattened plates; flattened spurs distally on the prolateral and retrolateral sides of tibiae I-III; oval, unsclerotized areas situated proximally on the sides of metatarsi I-III (Platnick & Gertsch, 1976; Platnick & Goloboff, 1985; Haupt, 2003). The phylogenetic placement of these spiders is robust with all previous molecular data (Xu et al.,

2015; Bond et al., 2014; Fernández et al., 2014, 2018; Wheeler et al., 2017; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021), and also with our phylogenetic results (Figures 3,4,5) with strong support (100% ultrafast bootstrap (UB in remaining text)) which is sister group to the Opisthothelae clade.

Opisthothelae

In Opisthothelae the spinnerets are located close to the caudal end of the abdomen such that the 12–18 segments are inconspicuous (beyond 5th opisthosomal segments). This group consists of two major clades, Mygalomorphae and Araneomorphae. Mygalomorph spiders have paraxial chelicerae and exhibit the plesiomorphic condition of two pairs of book lungs. Araneomorphae mostly have diaxial (opposing) chelicerae. However, all Opisthothelae lack the anterior median spinnerets, although its homolog– (the cribellum, a plate-like field with numerous spigots), is present in many araneomorph spiders (see Araneomorphae section).

Our phylogenetic results (Figures 3, 4) recover a monophyletic Opisthothelae consisting of two sub-clades Mygalomorphae and Araneomorphae with strong support (100% UB). These results corroborate of other genomic-scale molecular studies supporting the monophyly of these two well-established groups (Bond et al., 2014; Fernández et al., 2014, 2018; Wheeler et al., 2017; Starrett et al., 2017; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021).

Mygalomorphae

Many mygalomorphs are large-sized spiders with two pairs of book lungs and paraxial fangs. They lack anterior median spinnerets; however, most species have both posterior median and lateral spinnerets. *Iberesia* (Nemesiidae), from Europe, has only posterior lateral spinnerets (Decae & Cardoso, 2006). A majority of these spiders construct silk-lined burrows mainly on the ground with some variations such as the open burrow of *Acanthoscurria* (Theraphosidae), tubular silk-lined burrows with trapdoor of *Actinopus* (Actinopodidae), burrow with collar door of *Antrodiaetus* (Antrodiaetidae), purse web of *Atypus* (Atypidae), and the trap-door found on tree trunks above ground (Migidae) (Opatova et al., 2021 and references therein). There are over 3,000 mygalomorph described species classified in about 30 families (Opatova et al., 2021; World Spider Catalog 2022). These spiders are poor dispersers which is reflected by their narrow geographic distributions, as illustrated by Bemmeridae (endemic to South Africa) or Anamidae (found exclusively in Australia). Some mygalomorph lineages have a Gondwanan distribution, such as the family Migidae (known from Africa, Australia, Madagascar, New Zealand and South America), making them a good system for conducting biogeographic studies. Raven (1985) reviewed the systematics, provided the first family-level cladistic hypothesis for this lineage and suggested that that loss of the anterior median spinnerets, the reduction of the anterior lateral spinnerets and the reduction of the number of sclerites in the male palp are synapomorphies of the group.

Recent advances using modern sequencing methods have resulted in radical changes to Mygalomorphae systematics. Several molecular phylogenies have recovered this group as monophyletic consisting of two sub-clades Avicularioidea and Atypoidea (Hedin and Bond, 2006; Bond et al., 2012, 2014; Garrison et al., 2016; Wheeler et al., 2017; Hedin et al., 2018; 2019; Starrett et al., 2017; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021;

Opatova et al., 2021) including our phylogenetic results (Figures 3-5) with strong support (100% UB). The most recent phylogenetic hypothesis was proposed by Opatova et al. (2021) based on a densely sampled phylogeny of mygalomorphs using anchored hybrid enrichment (AHE) data. Our UCE-based phylogeny included representatives of 23 mygalomorph families including four Atypoidea (nine terminals) and 19 Avicularioidea families (40 terminals) (Figure 4). In our analysis, the phylogenetic relationships within Atypoidea are similar to those of the UCE based phylogeny of Hedin et al. (2019) (the AHE-based phylogeny of Opatova et al., 2021 included only two atypoid families). The avicularioid family Euagridae was paraphyletic with one group representing *Allothele*, *Australothele* and *Cethegus* as a sister group of a clade of avicularioid families (including Ischnothelidae, Hexathelidae and *Euagrus*) whereas the other group representing the type genus *Euagrus* as a sister group to Ischnothelidae (Figure 4). The AHE analysis of Opatova et al. (2021) recovered Ischnothelidae as a sister group of all remaining avicularioid families and Euagridae including *Cethegus* and *Euagrus* was monophyletic with 100% UB. Aside from this conflicting hypothesis, the familial relationships between our UCE phylogeny and that of Opatova et al. (2021) are mostly congruent. The combination of Wheeler et al., (2017)'s six marker data set with our UCE data set elevated the taxon sampling of Avicularioidea to 82 terminals (24 families). However, in the resulting phylogeny of this Combined dataset, Euagridae, Hexathelidae, Ischnothelidae, Bemmeridae, Cyrtaucheniidae, Halonoproctidae, Barychelidae, Actinopodidae, Nemesiidae and Idiopidae were not monophyletic (Figure 5). In our Combined phylogeny, the taxon sampling differed from that of Opatova et al. (2021) due to multiple reasons. Only 40 of 82 avicularioid terminals were represented by both UCE and six Sanger markers dataset (Figure 5) and thus it is possible that the missing data may have influenced the phylogenetic inference. Incongruent phylogenetic

results could also be attributed to the difference in the nature of two data classes, the AHE sequences of Opatova et al., (2021) and our UCE+Sanger data set. Differences in the taxon sampling between the studies may have also caused disparities: for example, Nemesiidae polyphyly is caused by two *Calisoga* terminals (Nemesiidae) representing UCE+Sanger dataset are sister to Anamidae whereas the two other nemesiids (from Wheeler et al.'s (2017) dataset_ form a sister group of *Fufius* (Cyrtaucheniidae). Due to this limitation, we do not propose any taxonomic changes.

Araneomorphae

These so-called “modern or true spiders” represent the most speciose lineage of extant spiders with over 100 families, and about 47,000 species (World Spider Catalog, 2022). Synapomorphies of Araneomorphae (Platnick & Gertsch, 1976) include the presence of a cribellum, piriform silk glands (Coddington, 1989), diaxial (opposing) chelicerae, by having expanded palpal coxae, forming the endites that bear a distal-lateral serrula (Ramírez, 2014) and the presence of a single pair of coxal glands (mesotheles and mygalomorphs have two pairs; Millot, 1949). Additional support for the monophyly of this suborder is provided by the presence of cleistospermia (which refers to the transfer of individually encapsulated sperm cells) and the type of cytoplasmic inclusions during spermiogenesis (in the form of clusters of glycogen surrounded by membranes after the coiling process (Michalik & Ramírez, 2014 and references therein). The posterior PLS of araneomorphs have one or two segments, while mesotheles have multisegmented PLS and mygalomorphs have three or four segments (Platnick & Gertsch, 1976). The cribellum is a short plate-like field that is considered homologous to the anterior median spinnerets and occurs

intermittently throughout the Araneomorphae. The cribellum sits anterior to the three pairs of spinnerets and accommodates thousands of spigots that secrete long-lasting sticky silk (called “cribellate silk”) which is woven using a functionally co-dependent calamistrum, which is a specialized comb of setae on the fourth metatarsus. The presence of a cribellum was first used by Bertkau (1882) for classification of higher groups of spiders into Cribellata and Ecribellata. Petrunkevitch (1923) postulated that the ecribellate families are derived from cribellate spiders. In several araneomorph groups the cribellum is reduced to a nonfunctional colulus or lost altogether. Piriform silk glands, another synapomorphy of araneomorphs, secrete glue that anchors ampullate silk lines to a substrate or to stick them to each other. This glue is released through spigots (called “piriform spigots”) which are adjacent to the major ampullate spigots on the anterior lateral spinnerets (Coddington 1989).

Araneomorphae is a well-established clade, robustly supported in all morphological and molecular phylogenies (Platnick & Gertsch, 1976; Coddington & Levi, 1991; Hausdorf 1999, Bond et al., 2014, Fernández et al., 2014, 2018, Wheeler et al., 2017, Starrett et al., 2017; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021).

Hypochilidae and Filistatidae

Hypochilidae is a small family of 33 cribellate species which includes two genera—*Hypochilus* and *Ectatosticta* which are known exclusively from the United States and China respectively (World Spider Catalog, 2022). *Hypochilus* spiders construct a mesh web resembling a lampshade attached to a rock overhang and the spider rests in the middle of the web (called “lampshade web”; Forster et al., 1987). *Ectatosticta* spiders construct sheet webs among rocks or tree trunks (Lin & Li, 2020). Marx (1889) who described the first hypochilid- *Hypochilus thorelli* Marx,

1888, remarked that this spider “is so anomalous that it appears like the representative of a prototype, in which characters were united in one individual which are now distributed into widely differing genera”. It is one of the relictual groups of “modern” (Araneomorphae) spiders that retain the primitive arrangement of two pairs of book lungs, venom glands restricted to the chelicerae (Gertsch, 1958) and that lack paracribellar spigots from posterior median spinnerets (Forster et al., 1987).

The transcriptomic analysis of Bond et al., (2014) recovered Filistatidae as the sister group of Hypochilidae, an affinity grouping based on morphology first suggested by Petrunkevitch (1923). Filistatidae is a large family with 18 genera and 189 species distributed globally (World Spider Catalog, 2022). They are reclusive, cribellate spiders mostly, with most species found in subtropical arid and semiarid regions of the world (Magalhaes & Ramírez 2019). The synapomorphies of this family are a narrow metatarsus stopper (narrow socket associated to the lyriform organ) in second legs of males, an anterior row of specialized setae in the anterior lateral spinnerets, an anteriorly pronounced clypeus and a tongue-like labrum with lateral extensions (Magalhaes & Ramírez 2017). Adult members of this family possess an anterior book lung system and a posterior tracheal system. However, remnants of the primitive posterior pair of book lungs are seen in their spiderlings (Ramírez 2014, Ramírez et al., 2021). In our study, these early diverging lineages of araneomorph spiders form a clade which is a sister group to Synspermiata (Figure 6C). All high-throughput molecular data and Sanger-sequenced markers support this placement of the Hypochilidae + Filistatidae clade (Bond et al., 2014, Garrison et al., 2016, Wheeler et al., 2017, Fernández et al., 2018; Kulkarni et al., 2020, 2021, Kallal et al., 2021).

Synspermiata

The name of this group was coined by Michalik & Ramírez (2014), it includes ecribellate haplogyne spiders which have multiple spermatids fused into one synsperm (Alberti and Weinmann 1985, Burger & Michalik 2010). In general, spiders with a haplogyne condition have relatively simple male genitalia with fused sclerites and female genitalia with a single duct for both copulation and fertilization (Platnick et al., 1991), but some haplogyne spiders have complex palps (e.g., liphistiids). Several studies have shown that the internal genitalia of some haplogyne spiders are also very complex, departing from the traditional definition of the haplogyne female genitalia of Whiele (1967) (e.g., Burger et al., 2003 and references therein). At least in some Synspermiata, males insert both palps simultaneously when mating, which is a unique behavior in this group (Burger et al., 2010). Synspermiata includes three monophyletic groups: Dysderoidea, Scytodoidea, and the Lost Tracheae Clade, in addition to the families Caponiidae, Telemidae and Trogloraptoridae. The Dysderoidea families are grouped by having a unique respiratory system of tracheae placed immediately behind the book lungs and an additional posterior sperm receptacle (diverticulum) and muscle-operated valves, which allow for control of the stored sperm by the female (Burger, 2013). Caponiids have advanced tracheal spiracles (book lungs are absent) and eye reductions and are relatively larger than other spiders with only tracheal systems (Platnick, 1994; Ramírez, 2000). Trogloraptoridae is an unusual family of a single cave-dwelling species— *Trogloraptor marchingtoni* Griswold, Audisio & Ledford, 2012 with characteristic striking raptorial claws, known from caves and their surroundings in the western United States (Griswold et al., 2012). Telemidae spiders have a global distribution, with 16 genera classified in 104 species (World Spider Catalog, 2022). They produce large stacks of sperm cells (called rouleaux or spermatophores) which correspond with

the dimensions of the female reproductive tract (Wang et al., 2012; Michalik & Ramírez, 2014). A study on *Telema tenella* Simon, 1882 showed that these spiders can live up to 12 years in captivity (and produce about four egg cases containing 3-4 eggs annually) (Juberthie 1985), a lifespan that is much higher than many araneomorph spiders. The Lost Tracheae Clade includes the spider families Diguettidae, Pacullidae, Pholcidae, Plectreuridae and Tetrablemmidae. As the name suggests, these spiders have secondarily lost the posterior respiratory system (Ramírez 2000). Pholcidae is the most speciose family of the Lost Tracheae Clade, with 1,896 species classified in 97 genera distributed globally, the remaining families of this clade are relatively less speciose.

All molecular data, such as the six Sanger-sequenced markers, transcriptomes and UCEs, have supported the monophyly of Synspermiata and its sister group relationship with the Hypochilidae plus Filistatidae clade (Wheeler et al., 2017; Garrison et al., 2016; Fernández et al., 2018; Michalik et al., 2019; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021).

In our Combined phylogeny, the Dysderoidea clade (Oonopidae, Segestriidae, Orsolobidae and Dysderidae) was the sister group of Caponiidae. Trogloraptoridae and Telemidae formed a clade which was sister group Scytodoidea and the Lost Tracheae Clade. (Figure 6A). In the transcriptomic analysis of Kallal et al., (2021) and the UCE analysis of Ramírez et al., (2021) and this study, Trogloraptoridae formed a sister group of Caponiidae whereas Telemidae was a sister group of a large clade including Scytodoidea and the Lost Tracheae Clade (Figure 4). The sister group of Dysderidae in our Combined phylogeny was Orsolobidae (99% UB), similar to the results of Wheeler et al., (2017) and Ramírez et al., (2021), whereas the UCE dataset placed dysderids as a sister group of Segestriidae+Orsolobidae (Figures 4, 6A). However, within Dysderoidea, Oonopidae was a sister group to remaining families in our

study (Figure 6A) whereas Segestriidae was a sister group to the remaining dysderoids in Wheeler et al., (2017). A further study exploring the effect of data classes (such as exons, introns, UCEs) and taxon sampling on the interrelationships of Synspermiata may be useful in deriving a robust phylogeny of these spiders.

Austrochiloidea

Austrochiloid spiders form an ancient lineage in the evolution of araneomorph spiders (Platnick 1977; Forster et al., 1987; Wheeler et al., 2017; Fernández et al., 2018; Kallal et al., 2021; Kulkarni et al., 2021; Ramírez et al., 2021). This group is composed of two families, Austrochilidae and Gradungulidae, that are distributed in the Southern Hemisphere. The early divergence of these spiders, supported by their phylogenetic placement within Araneomorphae, is suggested by the retention of the plesiomorphic configuration of two pairs of book lungs in some members such as *Hickmania* (Gradungulidae, formerly placed in Austrochilidae) (Zapfe, 1955; Platnick, 1977; Forster et al., 1987; Ramírez et al., 2021; Kulkarni & Hormiga, 2021). Many austrochiloids are cribellate, but some species, such as those in *Gradungula* or *Tarlina*, are cursorial species and have lost the cribellum.

Austrochilidae includes nine species classified in two genera (*Austrochilus* and *Thaida*) which are distributed in the Andean forests of central and southern Chile and adjoining regions of Argentina. These spiders have one pair of book lungs and a posterior tracheal respiratory system (Zapfe, 1955; Platnick, 1977; Forster et al., 1987). Gradungulidae includes 17 species classified in eight genera restricted to continental Australia and New Zealand. They have retained the plesiomorphic character of two book lung pairs (Zapfe, 1955).

Our combined data set obtained a monophyletic Austrochilidae which was a sister group to Gradungulidae. This Austrochiloidea clade was a sister group to Archoleptonetidae (Figure 6D). All genome-scale data sets support the monophyly of Austrochiloidea (Fernández et al., 2018; Kulkarni et al., 2020, 2021; Ramírez et al., 2021; Kallal et al., 2021; Ledford et al., 2021; Kulkarni & Hormiga 2021).

Leptonetidae and Archoleptonetidae

Leptonetidae is a family of 22 genera grouping 370 described species distributed exclusively in the Holarctic region. Many of these species are cave-dwelling and construct small and delicate sheet webs. Cave adapted species show troglomorphic morphologies such as reduction of eyes, poor pigmentation and elongation of appendages (Ledford & Griswold, 2010; Ledford et al., 2012; Mammola & Isaia, 2017).

The relationship of leptonetids to other groups have been tangled since the discovery of a functional cribellum in *Archoleptoneta* (the organ is absent in other Leptonetidae). Researchers have casted doubt on its placement within Leptonetidae (Ledford & Griswold, 2010; 2011) and suggested that this family could be paraphyletic, however without making any change in the classification. Later, Wheeler et al. (2017) did recover a polyphyletic Leptonetidae. An analysis of UCE-derived data suggested that the subfamily Archoleptonetinae (which includes only two genera, the cribellate *Archoleptoneta* and the ecribellate *Darkoneta*) do not nest within the clade containing other leptonetids (Ramírez et al., 2021). This subfamily was then elevated to the family rank as Archoleptonetidae by Ledford et al. (2021). This phylogenetic placement seems to be sensitive to data class and/or taxon sampling because, the transcriptomic data treated as amino acids, with three genera sampled, suggest that *Archoleptoneta* (Archoleptonetidae), *Calileptoneta*

and *Leptoneta* (both Leptonetidae) form a clade (Kallal et al., 2021). Similarly, our UCE data set representing two Archoleptonetidae and 12 Leptonetidae genera recovered a clade including the two families (Figure S3). In our Combined data set with more archoleptonetids (five terminals), Archoleptonetidae was the sister group of a clade including the Austrochiloidea+Leptonetidae clade (Figure 6D).

Palpimanoidea

Palpimanoids are a group of spiders known for their unusual chelicerae and carapace morphologies and associated predatory behaviors, many of which predominantly forage on other spiders using a variety of predatory tactics (see Wood et al., 2012). This group consists of five extant families which occur primarily in the Southern Hemisphere: Archaeidae, Huttoniidae, Mecysmaucheniidae, Palpimanidae, and Stenochilidae. Palpimanoids have peg teeth on the promargin of the paturon, a cheliceral gland mound, and most have dense scopulae on the first pair of legs (Wood et al., 2012). Prior to this, Forster & Platnick (1984) had proposed a larger grouping of Palpimanoidea which included Pararchaeidae, Holarchaeidae, Micropholcommatidae and Textricellidae (all were families at the time) and Mimetidae in addition to the present members of this superfamily. This expanded view of Palpimanoidea was based on the presence of cheliceral peg teeth and gland mounds in Mimetidae and Pararchaeidae, both characters reduced in Holarchaeidae, Micropholcommatidae and Textricellidae, chelicerae originating from a sclerotized foramen in the carapace in Pararchaeidae, a convex carapace in Micropholcommatidae and Textricellidae, with Holarchaeidae having an intermediate state between the two (Forster & Platnick, 1984). All these taxa are now placed in Araneoidea as follows: Pararchaeinae (Malkaridae), *Holarchaea*, Textricellini and Micropholcommatinae

(Anapidae) based on multiple phylogenetic studies (Schütt, 2000; Rix & Harvey, 2010a; Lopardo et al., 2011; Dimitrov et al., 2017; Kulkarni et al., in prep).

A combination of morphology and four Sanger-sequenced genetic markers strongly supported the monophyly of Palpimanoidea. However, six genetic markers (without morphology) recovered a poorly supported paraphyletic grouping for Palpimanoidea (Wheeler et al., 2017). In our UCE phylogeny, Palpimanoidea was monophyletic and formed a sister group of entelegyne spiders with strong support (Figure 3, 4). This placement of Palpimanoidea within the spider phylogeny and its interfamilial relationships were previously recovered in the transcriptome-based analyses of Fernández et al., (2018) and Kallal et al., (2021), the UCE-based analyses of Wood et al., (2018), Kulkarni et al., (2020), and Ramírez et al., (2021) and in analyses combining UCEs and transcriptomic data sets (Kulkarni et al., 2021). This relationship is of interest because the Entelegynae spiders contain the bulk of araneomorph spider species diversity, while palpimanoids contain only about 300 species (World Spider Catalog, 2022). The extensive fossil record of palpimanoids indicates that this group and Synspermiata were once dominant in the Mesozoic, with faunal turnover giving way to dominance of other Araneomorphae clades, Araneoidea and the RTA-clade, in the Cenozoic (Magalhaes et al., 2021).

Within Palpimanoidea, Palpimanidae is the sister group of the remaining four families (Figure 7), a result also found by Wood et al. (2018). However, transcriptomes analyzed as amino acids recover Palpimanidae plus Stenochilidae as a clade (Kallal et al., 2021). Huttonidae + Mecysmaucheniidae diverged next and are the sister group of a clade containing Archaeidae + Stenochilidae (Figure 7), contradicting the results of Wood et al. (2018). Our UCE data recovered Archaeidae as the sister group of Mecysmaucheniidae and Stenochilidae + Huttonidae.

Alternative placements have been obtained in other UCE and transcriptome-based studies. For example, the Huttonidae plus Stenochilidae clade was recovered using UCE data in Wood et al., (2018) and Kulkarni et al., (2021), however this branch was poorly supported in both studies. On the other hand, morphology alone recovers Archaeidae and Mecysmaucheniidae as sister groups, that are sister to Palpimanidae + Stenochilidae, and with Huttoniidae the earliest diverging total evidence analysis results in Mecysmaucheniidae as the earliest diverging and Archaeidae + Stenochilidae sister to Huttoniidae + Palpimanidae (Wood et al., 2012).

Entelegynae

The araneomorph lineages which are a sister group to the Palpimanoidea clade form the Entelegynae clade (over 80% of spider diversity). The entelegyne male genitalia, in general, is relatively more complex than haplogyne genitalia, and has distinct sclerites (sclerite morphology generally serves as synapomorphies for many entelegyne spider groups) and the female genitalia has a “flow-through” system, with separate copulatory and fertilization ducts (Griswold et al., 2005). The early diverging araneomorph groups such as Hypochilidae, Filistatidae, Synspermiata and Palpimanoidea have haplogyne genitalia. It is noteworthy that at least three reversals to the haplogyne condition are known to have occurred in the Entelegynae, i.e., in some tetragnathids, uloborids and anapids (Lopardo & Hormiga, 2015). Recently, Michalik et al., (2019) inferred that the entelegyne condition has evolved at least six times independently in the Synspermiata families- Pholcidae, Tetrablemmidae, Oonopidae, Ochyroceratidae and Trogloraptoridae.

Molecular phylogenies consistently support the monophyly of Entelegynae (Garrison et al., 2016; Wheeler et al., 2017; Fernández et al., 2018; Kulkarni et al., 2020, 2021; Kallal et al., 2021), including our study (Figures 3, 4). Our phylogeny suggests that Araneoidea is a sister

group to a clade that includes all the remaining entelegynes. In the latter clade, Nicodamoidea plus Eresidae are sister to a large lineage that includes the UDOH grade (see below) and the retrolateral tibial apophysis clade (RTA Clade). This topology is corroborated by the UCE-based phylogeny of Kulkarni et al., (2020) and the *AllUCEs* data set (nucleotide data of UCEs+transcriptomes) of Kulkarni et al. (2021). The recent transcriptomic analysis of Kallal et al. (2021) suggested that UDOH grade+RTA Clade is a sister group to the remaining entelegyne spiders. In that study, Eresidae was a sister group to Nicodamoidea+Araneoidea clade.

Araneoidea

This lineage of 17 cribellate spider families includes the largest diversity of web architectures, a few examples include: the orbicular web (Araneidae, “symphytognathoids”, Tetragnathidae), the cob web (Theridiidae, Nesticidae) and the sheet web (Cyatholipidae, Linyphiidae, Pimoidae), along with several instances of foraging web loss (these spiders are instead active hunters) (e.g., Mimetidae, Malkaridae, Arkyidae). Orbicular webs (webs with distinct radii and spiral) are also built by two other families outside Araneoidea—Deinopidae and Uloboridae (both of them are cribellate), which belong to the UDOH grade assemblage (see UDOH grade section below).

Untangling the relationships among the araneoid spiders families has been a challenging task (reviewed in Hormiga & Griswold 2014), since various data types such as morphology (Coddington, 1990), morphology and behavior (Griswold et al., 1998), Sanger sequencing based six markers (Dimitrov et al., 2012, 2017; Wheeler et al., 2017; Scharff et al., 2020), transcriptomes (Fernández et al., 2014, 2018; Kallal et al., 2021) and UCEs (Kulkarni et al., 2020, 2021) have recovered some conflicting phylogenetic relationships. For example, the placement of Araneidae varies across datasets: morphological data recover the family as sister

group to the remaining araneoids (Griswold et al., 1998), Sanger-based sequences as sister group to Theridiosomatidae + Synotaxidae (Dimitrov et al., 2017) or Synotaxidae (Scharff et al., 2020, Theridiosomatidae was not sampled) or as a sister group to Theridiosomatidae with transcriptomes (Fernández et al., 2018; Kallal et al., 2021) and UCEs sequences place araneids as the sister group of Synotaxidae (Kulkarni et al., 2020, 2021). Another example is provided by the miniature orb-weaving spiders (the "symphythognathoids", see section below): morphological data suggested that these families are grouped in a clade (Coddington, 1986b; Griswold, 1998; Schutt, 2003; Lopardo & Hormiga, 2008; Lopardo et al., 2011), a hypothesis rejected by the analyses of Sanger-based sequences and transcriptomes, but supported by UCE data (Kulkarni et al., 2020, 2021).

Araneoidea includes spiders with a characteristic configuration of spigots on the posterior lateral spinnerets— one flagelliform gland and two aggregate gland spigots, a synapomorphy of the group (Coddington, 1989; Griswold et al., 1998). The flagelliform and aggregate glands work in tandem to produce the sticky thread (Kovoor, 1977; Coddington, 1989) of the capture spiral. These araneoid triplet spigots may be reduced in some spiders such as *Cepheia longiseta* (Simon, 1881) (Synsphyridae) (Lopardo & Hormiga, 2008) or absent (Mimetidae) (Platnick & Shadab, 1993). All genome-scale based phylogenies recover Theridiidae as a sister group of a lineage that includes all remaining araneoid families (Garrison et al., 2016; Fernández et al., 2018; Shao & Li, 2018; Kulkarni et al., 2020, 2021; Kallal et al., 2021) (Figure 4). The transcriptome-based phylogeny of Fernández et al., (2018) placed Theridiidae as the sister group of Anapidae. However, the inclusion of Symphythognathidae representatives in the transcriptomic data set of Kallal et al. (2021) placed Anapidae as sister to Symphythognathidae, similar to the results of the UCE-based phylogeny of Kulkarni et al. (2021).

Theridiidae

Theridiids or cobweb spiders are the third largest family (after Linyphiidae and Araneidae) in Aranezoidea with 2,548 species grouped in 125 genera distributed worldwide (World Spider Catalog, 2022). The black widow spider genus *Latrodectus* known for its sexual cannibalism (Andrade, 1996) and potent toxicity (Clarke et al., 2014) and the common house spider *Parasteatoda tepidariorum* (C. L. Koch, 1841) are members of this family. *P. tepidariorum* has been widely studied in research on evolutionary and developmental biology and considered as a model organism (reviewed in Oda & Oda, 2020). Theridiids are of interest beyond taxonomy and systematics because of their ecological diversity, perhaps the largest among spider families, as illustrated by diversity of web architectures (e.g., Eberhard, 2008 a, b) and the independent evolution of kleptoparasitism (e.g., Vollrath, 1979), sociality (e.g., Agnarsson et al., 2006), and myrmecophagy (e.g., Líznavová & Pekár, 2019).

Theridiid spiders are also known as “comb-footed” spiders due to the presence of a row of bristled setae on their fourth tarsus used to direct and manipulate viscid sticky silk used to entangle the prey. This “comb-foot” condition is also present in another Aranezoidea family, Nesticidae, and also found in the non-araneoid Pholcidae (Huber & Fleckenstein, 2008). Lehtinen & Saaristo (1980) placed Nesticidae and Theridiidae in different superfamilies claiming that these setae are “purely adaptive”, thus suggesting convergent evolution of this trait. However, Coddington (1989) grouped Theridiidae and Nesticidae together based on the enlarged aggregate spigots and the presence of the fourth tarsal comb and its association with the behavior of prey capture. Griswold et al. (1998) proposed synapomorphies for Theridiidae plus Nesticidae (which they called “theridioids”) which included presence of a ‘theridiid tegular apophysis’ (a

sclerite of the male pedipalp), fourth tarsal comb, enlarged aggregate gland spigots on the posterior lateral spinnerets, and the construction of gumfoot webs. However, to date, no molecular analysis has ever supported the monophyly of theridioids (Dimitrov et al., 2012, 2017; Wheeler et al., 2017).

The morphological hypothesis about the internal relationships of Theridiidae recovered Hadrotarsinae (minute ant specialist theridiids with reduced/no webs) as a sister group of a clade composed of Latrodectinae (a monophyletic subfamily) plus remaining theridiids (Agnarsson, 2004). Arnedo et al. (2004) reconstructed the first molecular phylogeny of the family Theridiidae. In their molecular phylogeny, a clade including the sub-family Latrodectinae and the genera *Anelosimus* (in part *Selkirkiella*), *Pholcomma* and *Robertus* were a sister group to the remaining theridiids. An incremental taxon sampling of Liu et al. (2015) recovered Latrodectinae as a sister clade of the remaining theridiid lineages.

Our UCE-based phylogeny recovered Latrodectinae as the sister group of all remaining theridiids, which were represented by nine genera (15 terminals). Our Combined phylogeny however recovered a larger clade which included a monophyletic Latrodectinae as a sister group of paraphyletic Pholcommatinae (*Enoplognatha* and *Pholcomma*) and Argyrodoxinae (*Ariamnes*, *Argyrodes* and *Neospintharus*) and other theridiids (Figure 8). The ‘lost colulus’ clade which includes Theridiinae and Anelosiminae (a grouping proposed by Agnarsson (2004) based on the absence of a colulus and colular setae) was also recovered in our Combined analysis (Figure 8). In the UCE phylogeny, *Euryopsis* (Hadrotarsinae) was a sister group to the Theridiinae + Anelosiminae clade with high support (99% UB). (Figure 8).

Symphytognathoid clade

This clade includes four or five families of minute spiders (<2 mm) known as the “symphytognathoids” (an informal group name proposed by Coddington, 1986b): Anapidae, Mysmenidae, Symphytognathidae and Theridiosomatidae, most of which construct orb-webs (Eberhard 1987) with various degrees of architectural modifications. Lopardo et al. (2011) added Synsphyridae to this group based on a phylogenetic analysis combining morphological and molecular data. Symphytognathoid webs are architecturally quite diverse ranging from typical orb webs to a multitude of variation such as irregular webs and sheet webs. Some symphytognathoids are kleptoparasites that do not build any foraging webs, but instead occupy the webs of their host spider. Most mysmenids build spherical or planar orbs, symphytognathids build a two-dimensional horizontal orb web, theridiosomatids build orb webs— although some of them are highly modified (e.g., sticky lines connected to water surface), anapids build orb webs with out of plane radii, and at least some synsphyrids build sheet or irregular webs (Coddington, 1986; Coddington and Valerio, 1980; Eberhard 1987; Rix and Harvey, 2010a; Lopardo et al., 2011, Cotoras et al., 2021). 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* Coyle & Meigs, 1989 (Mysmenidae) and *Curimagua bayano* Forster & Platnick, 1977 (Symphytognathidae) live in the webs of diplurid spiders (Griswold et al., 1998; Vollrath, 1978) and *Sofanapis antillanca* Platnick & Forster, 1989 (Anapidae) live in the sheet webs of austrochilids (Ramírez & Platnick, 1999).

The genealogical relationships of the symphytognathoids themselves have an interesting history. The monophyly of “symphytognathoids” has been supported by morphological and behavioral characters (Coddington, 1986b; Eberhard, 1987; Griswold et al., 1998; Schütt, 2003; Lopardo &

Hormiga, 2008; Lopardo et al., 2011; Hormiga & Griswold, 2014), but they have appeared as either paraphyletic or polyphyletic in molecular phylogenies using the six Sanger-based markers (Dimitrov et al., 2012, 2017; Wheeler et al., 2017) or transcriptomes (Fernández 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 *Holarchaea*) as sister to Theridiidae and “Anapidae II” (represented by *Gerstchanapis*, *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 polyphyletic origins of “symphytognathoids” (Fernández et al., 2018; Kallal et al., 2021). In a parsimony analysis, Kallal et al. (2020) 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 are nested within symphytognathoids (Kulkarni et al., 2021). All prior molecular analyses, including Sanger sequencing based six markers and amino acid data from transcriptomes, rejected the monophyly of symphytognathoids. Interestingly, the polyphyly of this group received high ultrafast bootstrap support by transcriptomes. This paradox of highly supported but incongruent relationships across

phylogenomic datasets was explored through analyses of exons, ultraconserved loci, a combination of these data as amino acids and nucleotides which recovered monophyly of “symphytognathoids” (Kulkarni et al., in 2021). This discordance due to nucleotide (rendering monophyly) and amino acid data (recovering polyphyly) between the position of symphytognathoids within Araneoidea was also observed by the 99-target enrichment study of Shao et al. (*in press*). This paradox is not unique to these spiders, but has also been observed in snakes (e.g., Klein et al., 2021), birds (e.g., Cloutier et al., 2019) and arachnids (e.g., Ballesteros et al., 2021).

Our UCE and Combined data sets recovered the symphytognathoids clade (Figures 3, 4, 9). Theridiosomatidae formed a sister group to the remaining symphytognathoids, a lineage referred to as the “Anterior tracheal system clade” by Lopardo et al., (2011). Interestingly, *Trogloneta*, an unusual mysmenid with fused chelicerae (Schutt, 2003) similar to Symphytognathidae spiders, was placed as a sister group to Synaphridae + Symphytognathidae with high support (Figure 9; 95% UB). This genus has been placed within Mysmenidae (Lopardo et al., 2011, 2015). Among Anapidae, both UCEs and the Combined data set recovered micropholcommatines nested with Anapidae (Figure 9) similar to the total evidence analysis of Lopardo et al. (2011).

Linyphioids Clade

This clade was informally named by Hormiga (1994, 2000) to group the families Linyphiidae and Pimoidae. The monophyly of linyphioids is supported by the following synapomorphies: cheliceral stridulatory striae, patella-tibia autospasy, enlargement of the peripheral cylindrical spigot base on the posterior lateral spinnerets, a 9+0 axonemal pattern in the sperm and an ectal

cymbial process in the male palp (Hormiga, 1993, 1994a, b; Michalik and Hormiga, 2010; Hormiga et al., 2021).

Linyphiidae is the second largest family of spiders and the largest in Araneoidea with about 4,720 species classified in 624 genera. About 10% of all described spiders are linyphiids (World Spider Catalog, 2022). Although the ancestral web of Araneoidea probably was an orb (Fernandez et al., 2018; Kallal et al., 2021), linyphiids build sheet webs of varying degrees of complexity (Hormiga & Eberhard in review). These spiders are distributed globally, but are more abundant at higher altitudes, particularly in temperate regions (Hormiga, 1994b), contrary to the typical biological pattern of increasing species diversity towards the equator (Lomolino, 2004). These spiders have been found on most oceanic islands, far away from continental masses, such as Saint Helena, Tristan da Cunha or the Juan Fernandez islands. In the latter archipelago 15 endemic species of *Laminacauda* and ten species of *Neomaso* occur, suggesting their long dispersal abilities (Arnedo & Hormiga 2021). Linyphiidae have been classified into several subfamilies (Mynogleninae, Dubiaraneinae, Erigoninae, Linyphiinae, Micronetinae, Ipainae and Stemonyphantinae) although no comprehensive phylogenetic classification exists for the family and only some of existing subfamilies have been corroborated as clades (e.g., Stemonyphantinae and Mynogleninae) while others have never been repeatedly shown not to be monophyletic (e.g., Dubiraneinae or Micronetinae) (Hormiga, 2000; Miller & Hormiga, 2004; Frick & Scharff, 2018; Wang et al., 2015; Hormiga et al., 2021; Moreira et al., *in prep*). In our combined analysis, Stemonyphantinae and Mynogleninae were monophyletic whereas Erigoninae, and Micronetinae were polyphyletic (Figure 10A).

Wunderlich (1986) suggested that *Pimoa* was the sister group of Linyphiidae and accommodated it in a new subfamily (Pimoinae), which Hormiga (1993) elevated to family rank.

Hormiga (1994a) monographed Pimoidae, and added new species to *Pimoa*. Subsequently, the genera *Weintrauboa* (Hormiga 2003), *Nanoa* (Hormiga et al., 2005), and *Putaoa* (Hormiga & Tu 2008) were placed in Pimoidae based on morphology-based cladistic analyses. Molecular phylogenies using the six markers, transcriptomes and UCEs however, recovered a paraphyletic Pimoidae with *Weintrauboa* and *Putaoa* nesting in Linyphiidae (Dimitrov et al., 2012; Wang et al., 2015; Dimitrov et al., 2017; Wheeler et al., 2017; Fernández et al., 2018; Kallal et al., 2021). More recently, Hormiga et al., (2021) addressed the placement of *Weintrauboa* and *Putaoa* using Sanger sequencing data and formalized the transfer of *Weintrauboa* and *Putaoa* to linyphiid subfamily Stemonyphantinae. The remaining two genera, *Nanoa* and *Pimoa* were hypothesized to be sister groups based on their male genitalic morphology (Hormiga et al., 2005), which was corroborated by the molecular data (Dimitrov et al., 2012, 2017; Hormiga et al., 2021). Our study also placed *Weintrauboa* and *Putaoa* in Stemonyphantinae and *Pimoa* and *Nanoa* form the Pimoidae clade (Figure 10A). Currently, Pimoidae includes 85 species classified in two genera with *Nanoa* (with single species *N. enana*) from the United States and *Pimoa* with 84 species distributed in the Holarctic region (World Spider Catalog, 2022).

Cyatholipidae

This is a meso-diverse family with 58 species classified into 23 genera distributed in Africa, Madagascar, Australia and New Zealand where they construct sheet webs generally in moist forests (Griswold, 2001; World Spider Catalog, 2022). Griswold (2001) proposed the first phylogenetic hypothesis using morphology. In our Combined data set, *Tekella* and *Tekelloides* form a clade similar to Griswold (2001), however the genera were not monophyletic (Figure

10B). The genera *Alaranea*, *Cyatholipus*, *Scharffia* and *Ulwembua* formed a clade whereas in Griswold's (2001) analysis, these genera did not form a clade.

Malkaridae + “tetragnathoids” Clade

Malkaridae is a family of 57 species classified in 13 genera distributed in the southern hemisphere with a monotypic genus known from Chile and Argentina (*Chilenodes*) and the remaining from Australia, New Zealand and New Caledonia (World Spider Catalog, 2022). They are web-less, active hunters that live in the leaf litter and mosses of temperate and tropical wet forests (Platnick & Forster, 1987; Rix, 2006; Rix & Harvey, 2010b; Hormiga & Scharff, 2020). These spiders are relatively difficult to find leading to few specimens in natural history collections and scarce information. Further, some of their morphological features made it difficult to understand their affinities. For example, in one clade (Pararchaeinae) the presence of peg teeth on the chelicerae and the unusual shape of the carapace suggested an affinity with the palpimanoids, specifically Archaeidae and Mecysmaucheniidae (Forster and Platnick, 1984). Yet, molecular sequencing removed this lineage from the Palpimanoidea and firmly placed it with the araneoid Malkaridae (Forster, 1949; Rix, 2006; Wood et al., 2012; Dimitrov et al., 2017). Thus, Pararchaeinae is an example of convergence with Mecysmaucheniidae – both lineages have similar morphologies in order to produce “trap-jaw” predatory strikes with their highly maneuverable chelicerae (Kallal, Elias & Wood, 2021). Recently Hormiga & Scharff (2020) revised malkarids of New Zealand and proposed a phylogenetic hypothesis for the family which now includes four subfamilies- Malkarinae, Pararchaeinae, Tingotinginae and Sternoidinae.

Tetragnathidae is a relatively large family with 983 species classified in 45 genera distributed globally except Antarctica (World Spider Catalog, 2022). The majority construct typical orb webs similar to other orb weaving members of Araneoidea (e.g., Araneidae), however their webs usually have open hubs (Álvarez-Padilla & Hormiga, 2011). Some have adopted a web-less, active hunter or cursorial lifestyle (e.g. Berger et al., 2021). Some tetragnathid genera such as *Tetragnatha* are secondarily-haplogyne whereas most of them have entelegyne genitalia (Griswold et al., 1998; Álvarez-Padilla & Hormiga 2011). The taxonomy and systematics of various tetragnathid groups has a convoluted history (see Álvarez-Padilla & Hormiga, 2011), which has now settled on grouping genera into four subfamilies, namely Tetragnathinae, Nanometinae, Metainae and Leucauginae (Kallal & Hormiga, 2018; Álvarez-Padilla et al., 2020; Ballesteros & Hormiga 2021).

Arkyidae is a relatively small family with two genera and 38 species known from New Guinea, Australia and New Caledonia (World Spider Catalog, 2022). They do not construct foraging webs and instead are sit-and-wait or ambush predators. Arkyids have a field of short dense macrosetae on the prolateral surface of the first tarsus in males and have enlarged aggregate gland spigots on the posterior lateral spinnerets. This family was recently elevated from subfamily Arkyinae (Araneidae) to its own family by Dimitrov et al. (2017). Prior to being in Araneidae, arkyids were placed in Thomisidae, Mimetidae (which at the time was considered to be a palpimanoid family based on the presence of cheliceral peg teeth and gland mounds) and Tetragnathidae (Forster & Platnick, 1984; reviewed in Framenau et al., 2010).

Mimetidae is a family of araneophagic spiders which has earned them the name “pirate spiders”. They include 159 species classified in eight genera distributed globally except Antarctica (World Spider Catalog, 2022). Similar to arkyids, they do not construct any foraging

web, instead they have developed a sophisticated method of aggressive mimicry for hunting spiders in webs. They mimic the behavior of ensnared prey on the web of other spiders, or the courtship vibrations of their prey's conspecific male by plucking on the web of their prey, to lure the prey spider from their web and then attack and feed on them (Cutler 1972, Jackson & Whitehouse, 1986). Mimetids have a conspicuous line of raptorial macrosetae on the prolateral surfaces of the tibiae and metatarsi of first two legs (Platnick & Shadab, 1993), which presumably assists in prey capture (similar macrosetae are found in many malkarids). The taxonomy and systematics of this family was recently revised by Benavides et al., (2017) and Benavides & Hormiga (2020).

Based on a highly supported clade including the families Arkyidae, Mimetidae and Tetragnathidae, Hormiga (2017) named this grouping as "tetragnathoids". This clade is perhaps the only grouping within Araneoidea which is robust to Sanger sequencing (Dimitrov et al., 2017, Hormiga, 2017; Wheeler et al., 2017), transcriptomes (Garrison et al., 2016 (Arkyidae not sampled), Fernández et al., 2018; Kallal et al., 2021) and UCEs (Kulkarni et al., 2020, 2021). Malkaridae has been recovered as a sister group to the tetragnathoids using Sanger sequencing data (Dimitrov et al., 2017; Hormiga, 2017; Wheeler et al., 2017) and UCEs (Kulkarni et al., 2020, 2021), however transcriptomes suggest that Mysmenidae as a sister group to Malkaridae (Kallal et al., 2021) or Mysmenidae as a sister group to tetragnathoids (Garrison et al., 2016, Fernández et al., 2018) (Figure 11). In Tetragnathidae, the Tetragnathinae, Metainae, Nanometinae and Leucauginae subfamilies were recovered monophyletic using our combined data set (Figure 11A). In Malkaridae, Pararchaeinae and Tingotinginae were monophyletic however, Sternoidinae and Malkarinae were polyphyletic (Figure 11B).

Araneidae + Synotaxidae

Araneidae is the second most speciose family within Araneoidea (after Linyphiidae), with about 3,100 species classified in 185 genera distributed worldwide (World Spider Catalog, 2022) and the third most speciose family (after Salticidae and Linyphiidae). Some of the largest species and cosmopolitan web building spider genera such as *Nephilengys* and *Nephila* belong to this family. Most araneids construct typical orb webs, however some genera such as cyrtarachnines and mastophorines (also known as bolas spiders) dispel this phenomenon. Scharff & Coddington (1997) carried out the first large-scale cladistic analysis using morphological and behavioral characters. Several of the groups supported by that study continue to be recognized (such as gasteracanthines or cyrtophorines), while others have been placed elsewhere, such as the arkyines (“Arciinae”) which are now placed in their own family— Arkyidae. Multiple molecular data classes (six Sanger-sequenced markers, transcriptomes and UCEs) have consistently placed the lineage of *Nephila* and its close relatives in Araneidae (Dimitrov et al., 2012, 2017; Scharff et al., 2020; Kallal et al., 2021; Kulkarni et al., 2021), where it is now classified as a subfamily (Kallal et al., 2020). Synotaxidae was until recently a monogeneric family with 11 species known from South America (World Spider Catalog, 2022): *Synotaxus* species construct “chicken-wire” shaped webs (Eberhard, 1977) and are identifiable based on a stout patellar apophysis in the male palp (Exline & Levi, 1965, Santos & Rheims, 2005). Recent phylogenetic work (Ramírez et al., 2022) has expanded the circumscription of Synotaxidae to include the genera *Tekellina* Levi, 1957 (formerly in Theridiidae) and *Hamus* Lin, Ballarin & Li, *Nescina* Lin, Ballarin & Li, *Gaucelmus* Keyserling, 1884 (formerly in Nesticidae).

Our UCE phylogeny recovered Synotaxidae as the sister group of Araneidae, similar to other UCE-based studies (Kulkarni et al., 2020, 2021). Sanger-based markers recover

Theridiosomatidae+Synotaxidae (Dimitrov et al., 2017) or Synotaxidae (Scharff et al., 2020- Theridiosomatidae not sampled) whereas Theridiosomatidae is the sister group of Araneidae with transcriptomes (Fernández et al., 2018; Kallal et al., 2021). Interestingly, UCEs extracted from transcriptomes analyzed as nucleotides recover Synotaxidae as the sister group to Araneidae (Kulkarni et al., 2021). However, transcriptomic data analyzed as amino acids recover Theridiosomatidae or Synotaxidae+Theridiosomatidae as the sister group to Araneidae (Kulkarni et al., 2021: Supplementary Figures). No morphological analysis has suggested close affinities between araneids and synotaxids and we do not know of any morphological features that could be a putative synapomorphy of this clade. In the Combined phylogeny, we found that Synotaxidae (including *Gaucelmus* as recently transferred by Ramírez et al., 2022) are nested within the sister clade of Araneidae (Figure 12A).

Nicodamoidea and Eresidae

Nicodamoidea clade includes the families Megadictynidae and Nicodamidae, a superfamily rank that was established by Dimitrov et al. (2017). Megadictynidae are cribellate entelegyne spiders with two monotypic genera (*Megadictyna* and *Forstertyna*) both from New Zealand.

Nicodamidae includes ecribellate entelegyne spiders with seven genera and 27 species distributed in Australia and New Guinea (Harvey, 1995; Dimitrov et al., 2017). The sister group of Nicodamoidea in our phylogeny was Eresidae which was recovered with high support (100% UB; Figures 3,4,13). This finding is consistent with other UCE-based phylogenies (Kulkarni et al., 2020, 2021). However, this contrasts with transcriptome-based phylogeny where the data is treated as amino acids Nicodamoidea, is a sister group to Araneoidea (Fernández et al., 2018; Kallal et al., 2021).

Eresidae (velvet spiders) includes nine genera of which the genus *Stegodyphus* consists of three sub-social species— *S. sarasinorum* (South Asia), *S. dumicola* and *S. mimosarum* (Africa) (Kraus & Kraus 1988; Johannesen et al., 2007). *Stegodyphus* constructs extensive aerial cribellate sheet webs (Miller et al., 2010a). The social species share building and maintaining their webs, attack and capture prey together, and provide maternal care to the brood cooperatively (Kullman, 1972; Agnarsson et al., 2006). Interestingly, the close relatives of the social species of Eresidae are solitary species. Sociality has been estimated to have evolved independently about 18 times in spiders (Agnarsson et al., 2006) in various families such as Oxyopidae and Theridiidae. Recent studies have found out that convergent expressions of certain gene families in the social spider species (Tong et al., 2021). A stable placement of Eresidae is thus important to understand the evolution of social behavior in spiders.

A phylogeny using five Sanger sequencing markers suggested Eresidae as a sister group to the UDOH grade families Hersiliidae+Oecobiidae and the RTA Clade (Miller et al., 2010a). Eresidae is a sister group of Nicodamoidea recovered with UCE data, however it is a sister group to the Araneoidea plus Nicodamoidea clade with transcriptomes. The Sanger-based six marker phylogeny of Wheeler et al. (2017) recovered Eresidae as a sister group to the UDOH grade plus RTA Clade, similar to Miller et al., (2010a).

UDOH grade

UDOH grade is a paraphyletic assemblage (named by Fernández et al., 2018) containing the spider families Uloboridae, Deinopidae, Oecobiidae, and Hersiliidae. Uloboridae and Deinopidae are cribellate orb-weaving groups, whereas all other orb-weaving spider families are ecribellate and cluster into a monophyletic group (Araneoidea). Uloboridae includes 19 genera with 289

species with worldwide distribution. Typically, they construct an orbicular web with radii, frame threads and hub using non-sticky threads and a sticky spiral using cribellar silk. Some genera depart from this behavior, few examples of which include, only spirals in *Philoponella*, (Opell & Eberhard, 1984), a triangular orb web in *Hyptiotes* (Marples & Marples, 1937), and a single silk line of *Miagrammopes* partially covered with cribellate silk and few additional lines of support (Lubin et al., 1978). A recent study demonstrated a catapult-like mechanism used by *Hyptiotes* to capture prey. This spider stretches the web, thereby storing elastic energy, by extending an additional anchor line and releases it on sensing contact of prey with the web. The resulting jerk caused by the release of stored energy entraps and wraps the prey (Han et al., 2019). Deinopidae members are commonly called “ogre-faced” spiders due to the large posterior median eyes of some species. Deinopids have a unique behavior of waiting for prey hanging upside down with a highly modified orbicular web held in anterior legs. They cast the web towards the prey to capture it (Robinson & Robinson, 1971) which has earned them another name of “net casting” spiders. It includes 67 described species classified into three genera (*Asianopis*, *Deinopis* and *Menneus*) distributed worldwide (World Spider Catalog, 2022). Oecobiidae includes six genera represented by 120 species distributed globally, with some widely distributed synanthropic species (Santos & Gonzaga, 2003; World Spider Catalog, 2022). The small webs of *Oecobius* (used as a shelter) are commonly seen in houses. Oecobiidae includes taxa that are both cribellate (such as *Oecobius*) and ecribellate (such as *Uroctea*) (Shear, 1970). Hersiliidae includes 188 described species classified into 16 genera with global distribution (World Spider Catalog, 2022). Most hersiliids are arboreal, constructing their non-foraging webs close to tree bark or wall surface on which they move swiftly for prey capture or escaping. Oecobiidae and Hersiliidae (together called “Oecobioids” by Miller et al., 2010a) are characterized by a unique

prey attack behavior of wrapping the prey by circling around it (Crome, 1957 (op. cit. after Lehtinen, 1967: 305, Coddington and Levi, 1991).

Resolving the relationships among the UDOH families with their diverse foraging behavior (with and without web use) is crucial, as it affects the hypothesis about the evolutionary history of the web architecture and foraging behavior in spiders. In our study, all families of this group were monophyletic, including Oecobiidae (represented by the cribellate *Oecobius* and ecribellate *Uroctea*) in the combined phylogeny (Figure 13). This placement is different from the prevailing hypotheses, as described below. Morphology based cladogram recovered a monophyletic Deinopoidea which included Deinopidae and Uloboridae, however was refuted by Sanger sequencing-based phylogenies (Dimitrov et al., 2012, 2017; Wheeler et al., 2017). The close relatives of the UDOH families are the Tibial apophysis Clade, consistently recovered with the six Sanger-based markers, transcriptomes and UCEs. Transcriptomes recover Deinopidae as a sister group to the RTA+PT Clade (Garrison et al., 2016; Fernández et al., 2018; Kallal et al., 2021) with high support. In the UCE phylogeny, Deinopidae was a sister group to Hersiliidae+Oecobiidae clade. Some morphology-based phylogenetic studies (for example, Griswold et al., 1999) inferred Oecobiioidea as a sister group of Eresidae (together called Eresoidea). In our study, the Oecobiioidea + Deinopidae clade was a sister group to a clade including Uloboridae+RTA+PT Clade (Figure 3,4, 13), similar to the phylogenetic hypothesis of Wheeler et al., (2017).

The Tibial Apophysis Clade

This large clade is united by the presence of a tibial apophysis on the male pedipalp. At least two types of tibial apophysis are known— dorsal and retrolateral. Titanoecidae and Phyxelididae are

early diverging families in this clade that have a dorsal tibial apophysis (Griswold et al., 1999; 2005). Griswold et al., (1999) removed the subfamily Phyxelidinae from Amaurobiidae and elevated it to family rank, and proposed the informal name “Titanoeoidea” for grouping the families Phyxelididae and Titanoecidae clade based on their cladistic analysis of morphological data. The phyxelidid genus *Vytfutia* bears both types of tibial apophyses (TA)— a dorsal and a retrolateral apophysis on the male pedipalps, while the remaining phyxelidids only have a retrolateral tibial apophysis (Griswold et al., 2005), conjunction implies that these two tibial apophyses are not homologous. In Griswold et al. (2012), the single terminal of *Vytfutia* was sister to *Goeldia* (Titanoecidae) plus Phyxelididae. *Vytfutia* was not sampled in neither Wheeler et al. (2017) nor our UCE sampling. With our current taxon sampling, Titanoeoidea was not monophyletic, instead Titanoecidae was a sister group to the Phyxelididae plus the RTA Clade (Figure 3, 4, 14). Synapomorphies of Phyxelididae include palpal femur thorns in both sexes, modified male metatarsus I, and long, narrow, densely placed and laterally flattened paracribellar spigots on the posterior median spinnerets (Griswold et al., 1999). It is noteworthy here that a retrolateral tibial apophysis is also present in the other groups such as the linyphiid subfamily Erigoninae, suggesting convergent evolution (Araneoidea) (Hormiga, 1994).

Retrolateral Tibial Apophysis (RTA) Clade

As aforementioned, the presence of a retrolateral tibial apophysis on male pedipalp is characteristic to this large group of spiders (Coddington & Levi, 1991; Griswold et al., 2005). Our UCE phylogeny recovered a highly supported RTA Clade (100% UB) (Figure 3, 4). Two lineages the Oval calamistrum clade and Dionycha, (two-clawed spiders) make up the bulk of species richness in the RTA Clade. These are mostly cursorial spiders including the common

jumping spiders (Salticidae) which is the most speciose spider family with 6,449 species belonging to the Dionycha clade (Figure 4, 20). Most of these RTA Clade members with two-claws do not construct foraging webs, but are active hunters and their third middle claw has been replaced with clusters of specialized adhesive setae, called scopulae, that are positioned beneath the two superior claws. The third tarsal claw is used by spiders to trace silk lines on webs, but it is also present in some spiders that do not construct foraging webs (Ramírez, 2014). It has been suggested that the scopulae have evolved as a substitute for the use of silk for foraging, however some exceptions also exist (Wolff et al., 2013). For example, most salticid spiders construct silk retreats or some Lycosidae spiders that construct webs, both of which have adhesive setae.

Zodariidae and Penestomidae

Penestomidae is a small family including one genus (*Penestomus*) with nine species known from South Africa, one of which is also recorded from Lesotho (Miller et al., 2010b). Miller et al. (2010a) inferred that Penestomidae nested within the RTA Clade and based on this placement they elevated this group to family rank by removing it from a subfamily within Eresidae. Prior to this, Lehtinen (1967) had shown that male penestomids have an RTA which is typical of the RTA Clade member and not found in any eresid spider. Zodariids are mostly nocturnal, ground-dwelling, wandering spiders, many of which feed on ants. The synapomorphies of this family are absence of serrula on the endites and a rounded prolateral tibial process fitting in a metatarsal pouch (Jocqué & Henrard, 2015).

In the UCE phylogeny, the Zodariidae + Penestomidae clade is the sister group of the remaining RTA Clade families with high support (100% UB). The monophyly of Zodariidae (two terminals) and Penestomidae (one terminal) was also highly supported in this UCE tree

(Figure 4) as well as in the Combined phylogeny with one terminal of Penestomidae and 27 terminals of Zodariidae. In Miller et al. (2010a), Penestomidae (two *Penestomus* species) is the sister group of Zodariidae (*Zodarion* and cf. *Aschema*).

A formal grouping called Zodarioidea, proposed by Miller et al., (2010a), includes the families Homalonychidae, Penestomidae and Zodariidae was. However, the Sanger-based phylogeny of Wheeler et al. (2017) found this group to be polyphyletic. In their phylogeny, Homalonychidae was a sister group of the Oval calamistrum + Dionycha Clade. Wheeler et al., (2017) point out that this grouping may be imposed by the constraints of the backbone transcriptomic phylogeny of Garrison et al., (2016) that they used. However, multiple transcriptomic phylogenies (Fernández et al., 2018; Shao & Li, 2018; Kallal et al., 2021) and various other genomic data classes (UCEs, transcriptomes as nucleotides, amino acids) (Kulkarni et al., 2021) have placed Homalonychidae as sister group to the Oval calamistrum + Dionycha Clade with high support (UB >95%). This suggests that Zodarioidea may need to be recircumscribed to only include Zodariidae + Penestomidae, however we do not formally make any nomenclatural changes in this study.

Sparassidae

The members of this family with 1,338 species classified in 95 genera includes spiders with laterigrade legs (positioned similar to the legs of a crab) (Jaeger, 2001; World Spider Catalog 2022) and fleshy, trilobate membranes at the distal region of the metatarsi, an indented tip of the claw tuft setae, membranous extensions of tarsi on the side of claw tuft plates, and the trichobothrial setae lacking the bumps on their bases (Jaeger, 1998; Ramírez, 2014). These spiders are cursorial hunters and some species can be quite large (up to 40 mm in body size),

with very long legs. Our UCE phylogeny placed Sparassidae as a sister group to the marronoid clade with high support (100% UB; Figures 4, 14) similar to the results of the previous transcriptomic (Fernández et al., 2018; Shao & Li 2018; Kallal et al., 2021) and UCE (Kulkarni et al., 2021) phylogenies. Morphological data suggest the placement of Sparassidae within the Dionycha clade (Ramírez, 2014), whereas Sanger sequencing data suggest multiple alternative placements (see Moradmand et al., 2014; Wheeler et al., 2017). The subfamilies Sparianthinae, Heteropodinae, Polybetinae and Delninae were monophyletic whereas Eusparassinae was paraphyletic (Figure 14). A more recent and more comprehensive study reconstructed a sparassid phylogeny using four Sanger sequenced markers (Gorneau et al., 2022) also recovered similar relationships (including paraphyly of Eusparassinae) for these subfamilies.

Marronoid clade

The Marronoid clade groups several spider families that are mostly brown colored, without any prominent color pattern (Hormiga coined the informal name of this clade which was first introduced in print by Wheeler et al. (2017)). Marronoids are one of the major taxonomic problems in spider classification because, as Lehtinen (1967) notes, there are many closely related groups with and without a cribellum, making it difficult to group them and define diagnoses. Marronoids include the families Amaurobiidae, Agelenidae, Hahniidae, Cybaeidae, Dictynidae, Toxopidae, Cycloctenidae and Stiphiididae (sensu Wheeler et al., 2017). Our UCE phylogeny recovered a monophyletic assemblage of all the marronoid families with high support (100% UB). All these families except Hahniidae were monophyletic (Figure 4). In the combined phylogeny, Amaurobiidae, Cycloctenidae, Dictynidae, Desidae, Hahniidae, Toxopidae were either paraphyletic or polyphyletic (Figures 15, 16), however, some alternative relationships

were recovered using the marronoid data set (Figure S5). We attempt to delve further into the reasons for each of these relationships below.

Hahniidae

These small-sized spiders have a distinctly transverse arrangement of the spinnerets in one row and an advanced position of the tracheal spiracle (Lehtinen, 1967). Hahniids are represented by 353 described species classified into 24 genera distributed worldwide except Antarctica and Madagascar (World Spider Catalog, 2022). In an unpublished dissertation, Catley (1996) suggested that the position of tracheal spiracles is highly variable among species, but instead loss of true lateral tracheae may be a synapomorphy of the family. Their linearly arranged spinnerets resemble a comb, therefore they are also called “comb-tailed spiders”. They live in the leaf litter or under bark, where they construct small sheet webs. Lehtinen (1967) placed Hahniidae in his superfamily Amaurobioidea (Miturgidae, Amaurobiidae, Liocranidae, Agelenidae and Dictynidae) whereas Forster (1970) considered it to be a member of the superfamily Dictynoidea (Dictynidae, Neolanidae, Desidae, Cybaeidae, Argynnetidae and Anyphaenidae).

Our UCE phylogeny included three Hahniidae terminals, two *Cicurina* and one *Mastigusa* species. The inclusion of the six-marker data set added another four hahniid genera—*Antistea*, *Cybaeolus*, *Hahnia* (the type genus) and *Neoantistea*. In both data sets, Hahniidae was as polyphyletic. The *Cicurina* clade was recovered as a sister group to *Mastigusa*+Cybaeidae II clade whereas the remaining hahniids including *Hahnia* formed a sister group to a larger clade including Toxopidae, Dictynidae (excluding *Lathys*), Cybaeidae I & II, *Mastigusa* and *Cicurina*. The monophyly of Hahniidae I was strongly supported (100% UB; Figure 15). Interestingly, the marronoid data set recovered a monophyletic Hahniidae except *Mastigusa* which was a sister

group to Cybaeidae (35% UB; Figure S5). Cybaeidae I and II formed a clade, however, a poorly supported branch of *Ethobuella* (Agelenidae) nested with this clade (61% UB; Figure S5).

In Wheeler et al., (2017), one terminal of *Cicurina* was the sister group of Hahniidae (albeit with a moderate support of 67% UB) and was formally moved from Dictynidae to Hahniidae based on this phylogenetic placement. It should be noted that *Cicurina* in the phylogeny of Spagna & Gillespie (2008) was a sister group to *Lathys* (Dictynidae). In the phylogeny of entelegyne spiders using Sanger sequenced markers, *Cicurina* was recovered as a sister group to Hahniidae (including *Hahnia*) +Agelenidae clade (Miller et al., 2010). The three Sanger sequencing-based marker phylogeny of Crews et al. (2020) also recovered *Cicurina* not nested within Hahniidae. The placement of remaining Hahniidae is also poorly studied and is awaiting revision.

Amaurobiidae

In these spiders, the median apophysis of the male palp is a sclerotised plate-like structure (Paquin et al., 2010). The monophyly and affinities of amaurobiids have a long and controversial history (see Lehtinen, 1967; Miller et al., 2010a for details). In its current circumscription, both cribellate (such as *Amaurobius*) and ecribellate taxa (such as *Macrobunus*) are included. In our UCE phylogeny, Amaurobiidae was polyphyletic. *Amarobius* and *Callobius* formed a clade which was a sister group to the remaining marronoid families whereas *Rubrius antracticus* was a sister group to a clade including Toxopidae+Dictynidae+Hahniidae+Cybaeidae families (Figure 4). In our Combined phylogeny, a clade comprising *Amaurobius*, *Callobius* and *Pimus* (Amaurobiidae I in Figure 15) was a sister group to the remaining marronoid families which

included the clade of other amaurobiid genera. The latter clade (Amaurobiidae II in Figures 15 and S5) received high support (96% UB) which represents the subfamily Macrobuninae.

Cycloctenidae

This family includes eight genera— six from New Zealand, one both New Zealand and Australia, and one from Indonesia, totalling 80 described species (World Spider Catalog, 2022). Forster (1979) extensively treated the taxonomy of cycloctenids and provided a long list of diagnostic characters, perhaps the most prominent being the absence of claw tufts and scopulae.

In the UCE phylogeny, *Cycloctenus* single cycloctenid terminal included, was the sister group to the clade including Stiphiididae+Desidae (Figure 4). Our Combined data set included five cycloctenid genera, each with one species of *Orepukia*, *Pakeha*, *Paravoca*, *Toxopsiella* and five species of *Cycloctenus*. In the resulting phylogeny from this data set, *Orepukia* and *Pakeha* formed a clade which was a sister group to *Aorangia* (Stiphiididae) (Figure 16). The remaining Cycloctenidae terminals formed a sister group to a clade which included the *Aorangia* (Stiphiididae) + *Orepukia* and *Pakeha* clade (Figure 16). In the phylogeny of Wheeler et al., (2017), the *Orepukia*+*Pakeha* clade was a sister group to the remaining cycloctenids with poor support (61% UB). Based on this phylogenetic placement both *Orepukia* and *Pakeha* were transferred, from Agelenidae and Amaurobiidae respectively, to Cycloctenidae by Wheeler et al., (2017). This placement of a monophyletic Cycloctenidae was recovered by our marronoid data set (Figure S5).

Dictynidae

Dictynidae includes spiders occupying diverse habitats such as dry, arid and even aquatic, semi-aquatic, seashore, freshwater and salt-flat (Spagna et al., 2010). It includes the aquatic spider *Argyroneta aquatica* which constructs a silk-tube (called “diving bell”) among aquatic vegetation and resurfaces periodically to capture an air bubble around its opisthosoma.

Dictynidae includes about 475 described species classified in 53 genera distributed worldwide except Antarctica (World Spider Catalog, 2022). In its current circumscription, both cribellate and ecribellate species are included in Dictynidae. The cribellate dictynids formed a clade in some analyses (Griswold et al., 2005), however the family is rendered polyphyletic when the ecribellate members are included (Spagna et al., 2010).

Lathys, which is currently placed in Dictynidae, was recovered as a sister group to Agelenidae, however it received poor support (15% UB) and the other dictynids formed a clade which was sister group of Toxopidae (Figure 15). Our marronoid data set recovered *Lathys* as a sister group of Toxopidae + Dictynidae clade (Figure S5). Multiple alternative placements of *Lathys*, such as a sister group to *Cheiracanthium* (Cheiracanthiidae), were recovered using single and combined Sanger-sequence-based trees (see Spagna et al., 2010). In Wheeler et al., (2017), *Lathys* was a sister to the remaining Dictynidae with low support (11% UB). The Sanger-sequence-based phylogenies of Spagna & Gillespie (2008) and Miller et al., (2010a) recovered a polyphyletic Dictynidae with *Lathys* as a sister group to *Cicurina* (currently placed in Hahniidae) and the remaining Dictynidae formed a clade. Lehtinen (1967) had already stated that Cybaeidae and Cicurinae (both were subfamilies within Dictynidae at the time) “perhaps they could be united in a single, monophyletic family”.

Desidae

Desidae includes 296 species classified in 60 genera with most species in Australia, New Zealand, and New Caledonia, and some species in south-east Asia and Africa (World Spider Catalog, 2022). Desidae has both cribellate and ecribellate species. Some desids (whose natural history is known), such as *Cambridgea* from New Zealand, construct a large sheet web with a tube-like retreat (Forster & Forster, 1999). *Desis* live inside silken retreats and inhabit intertidal zones, e.g., hiding inside barnacles or among kelp, for that reason are also known as “intertidal spiders” (Baehr et al., 2017). Desidae was monophyletic in all data sets including the UCE, Combined and the marronoid data sets (Figures 4, 15, S5).

Toxopidae

Toxopidae includes 82 species classified in 14 genera, distributed in New Zealand, Australia and some islands in the Southern Hemisphere such as Crozet Islands and Kerguelen Islands. In our UCE phylogeny, the single terminal of this family (*Midgee* sp.) formed a sister group of Dictynidae (Figure 4). In the Combined and marronoid phylogeny, with nine genera (ten terminals), Toxopidae was monophyletic and formed a sister group to Dictynidae (excluding *Lathys*) (Figure 15, S5).

Homalonychidae

Homalonychus, the single genus of this family, includes only two species, both known from the southern United States and northern Mexico. Homalonychids are wandering spiders that live in the desert where they can throw sand on their body to bury themselves, which is hypothesized to be a defensive behavior (Domínguez & Jiménez 2005). This family was monophyletic (both species of *Homalonychus* sampled in the Combined data set) and was recovered as a sister group

to the clade including Oval Calamistrum and Dionycha clades (Figures 3, 4, 17), similar to the findings of Wheeler et al., (2017), Fernández et al., (2018), Kulkarni et al., (2021) and Kallal et al., (2021).

Oval calamistrum (OC) clade

The Oval calamistrum (OC) clade was described by Polotow et al., (2015) and includes spiders with a calamistrum with several rows of setae. In our UCE phylogeny, the *Uliodon* (Zoropsidae) + Udubidae clade formed a sister lineage to the remaining OC clade taxa. The other zoropsid in our UCE analysis, *Tengella*, was a sister group to the lycosoid families (Figure 4). In the Combined phylogeny, two groupings of Zoropsidae were recovered (polyphyletic), one of which was a sister group of Udubidae) and the other one was a sister group to the lycosoid families similar to the UCE phylogeny (Figure 17). As Wheeler et al. (2017) stated, the placement of Zoropsidae is unstable and requires further attention.

Ctenidae

This family includes about 580 species classified in 49 genera distributed in all continents except Antarctica (World Spider Catalog, 2022). They are nocturnal, wandering spiders and are mostly ground dwelling, with a few arboreal species (Polotow & Brescovit, 2008). Members of this family have a typical “ctenid eye pattern” of 2-4-2 eyes arranged in three rows of which anterior lateral eyes are smallest, evolved convergently seven times in the RTA Clade (Griswold 1993, Hazzi & Hormiga 2022). Most ctenids are ecribellate, but some genera such as *Acanthoctenus* have retained the cribellum (Griswold et al., 2005). Additional diagnostic characters of the family are eyes with a grate-shaped tapetum, teeth on the fang furrow and chelicerae with a boss

(Griswold et al., 2005). The highly venomous and medically important spiders of the genus *Phoneutria* belong to this family (Lucas, 1988; Foelix, 2010).

In the morphological phylogeny of Silva-Dávila (2003), Ctenidae was monophyletic and a sister group of Miturgidae. In a more recent morphological study, Polotow & Brescovit (2014) recovered a monophyletic Ctenidae. However, only two outgroups *Zoropsis* and *Tengella* (both Zoropsidae) were used in the latter study, so its close relatives in the RTA Clade could not be identified. Recently, Hazzi & Hormiga (2022) published the most comprehensive phylogeny of Ctenidae representing 28 of the current 49 described genera, using nine Sanger sequenced markers where the family was monophyletic. In our UCE phylogeny, Ctenidae was monophyletic (Figure 4) and was a sister group to Psechridae similar to the transcriptomic phylogeny of Cheng & Piel (2018). In the combined phylogeny, however, *Ancylometes* (not sampled in UCE phylogeny) was recovered as a sister group to all lycosoid families with moderate support (93% UB; Figure 17A), a finding which is similar to Wheeler et al., (2017). In the phylogeny of Piacentini and Ramírez (2019), *Ancylometes* was a sister terminal to Oxyopidae with poor support (39% posterior probability, see supplementary tree of Piacentini and Ramírez, 2019). The placement of *Ancylometes* varied across analyses in Hazzi & Hormiga (2022) including a placement as sister to Oxyopidae. The current taxonomic placement of *Ancylometes* within Ctenidae is unusual because it is the only group within ctenids that constructs a nursery web (Merrett, 1988; Santos, 2007), a behavior that is primarily found in Pisauridae. Another nursery web building spider outside Pisauridae is *Cupiennius*. It was recently transferred from Ctenidae to Trechaleidae by Piacentini & Ramírez (2019) based on its highly supported phylogenetic placement. Our UCE phylogeny also recovered *Cupiennius* nested within Trechaleidae (Figure 4). Interestingly, a phylogenetic analysis based solely on the CO1 marker

recovered both non-pisaurid nursery web building spiders *Anyclometes* and *Cupiennius* formed a clade (Gámez Vargas et al., 2019).

Thomisidae

Spiders of this family are called “crab spiders” due to the laterigrade orientation of their legs, which resemble a crab. Thomisidae includes 2,168 species classified in 171 genera distributed globally (World Spider Catalog, 2022). They are sit-and-wait predators and do not construct foraging webs. Many species have cryptic body coloration and can even change the body color (Weigel, 1941). Some thomisids can mimic twigs (e.g., *Tmarus*), ants (e.g., *Aphantochilus*), bird-dung (e.g., *Phrynarachne*) (Benjamin et al., 2008; Benjamin 2011; Teixeira et al., 2013; Ileperuma Arachchi & Benjamin, 2019).

Thomisidae was recovered as polyphyletic with one clade of most thomisid representatives (45 terminals), including the type genus *Thomisus*, as a sister group to Oxyopidae in our Combined phylogeny (Figure 17A). The other clade included *Borboropactus* which was the sister group of Psecridae, however with moderate support (56% UB; Figure 17B).

Borboropactus is unusual because it has a canoe-shaped tapetum, whereas all other thomisid genera have a grate-shaped tapetum (Homman, 1934; Benjamin, 2011). This latter genus is one of the few thomisid genera found fossilized in the amber (Wunderlich, 2004). *Borboropactus* has a characteristic behavior of digging and covering itself with soil particles. A similar behavior is also found in *Stephanophis* (Thomisidae), *Cryptothele* (Zodariidae), *Sicarius* (Sicariidae) and even some mygalomorphs such as *Paratropis* (Paratropidae). Based on this unusual behavior in addition to some morphological characters, Wunderlich (2004) erected a new family (Borboropactidae) to accommodate extant and fossil *Borboropactus* species. Benjamin et al.,

(2008) used three Sanger sequence-based markers and found that *Borboropactus* is sister group to the remaining Thomisidae and thus, rejected Borboropactidae which was synonymized with Thomisidae. Morphology recovered *Borboropactus* nested within the *Stephanopsis* clade (Benjamin, 2011). In Wheeler et al., (2017), *Borboropactus* was the sister group of remaining thomisids with poor support (35% UB). In some of the Wheeler et al., (2016) analyses *Borboropactus* did not nest within Thomisidae, and the authors preferred “to keep the more traditional Thomisidae sensu lato with weak support” noting that their results were “also compatible with the split of a robust Thomisidae sensu stricto and a separate Borboropactidae as proposed by Wunderlich (2004).”

Pisauridae and *Dolomedes*

Pisauridae includes 353 species classified in 51 genera distributed globally (World Spider Catalog, 2022). Many pisaurids show a peculiar courtship behavior which involves a “nuptial gift” consisting of a prey wrapped in silk as studied in *Pisaurina mirabilis* (Clerck, 1757). If the female accepts the prey, it means that female is receptive for mating (van Hasselt, 1884; Stålhandske, 2001). A similar behavior has been observed in some spiders of the family Trechaleidae (Costa-Schmidt et al., 2008). Female pisaurids construct a tent-like silk structure when the spiderlings are about to emerge from the egg sacs. This web is called “nursery web” and is a synapomorphy of Pisauridae (Piacentini & Ramírez, 2019). Similar nursery webs have convergently evolved in other spiders such as *Peucetia* (Oxyopidae), *Cupiennius* (Trechaleidae) and *Ancylometes* (Ctenidae) (Merrett, 1988; Sierwald, 1997; Piacentini & Ramírez, 2019).

In our UCE phylogeny, Pisauridae was paraphyletic, with most of Pisauridae (in part) as sister group to a clade that includes *Dolomedes* (Pisauridae) and the Trechaleidae + Lycosidae

clade (Figure 4). In the combined phylogeny, *Dolomedes* (four terminals) + *Bradystichus* were the sister group of a clade that included Trechaleidae and Lycosidae (Figures 17, 18). Wheeler et al., (2017) and Piacentini & Ramírez, (2019) recovered the *Dolomedes* + *Bradystichus* clade as the sister of the remaining Pisauridae clade. Similarly, the eight-marker phylogeny of Albo et al., (2017) also recovered *Dolomedes* not nesting within Pisauridae. However, the transcriptomic analysis of Fernández et al. (2018), Cheng & Piel (2018) and Kallal et al. (2021) recovered a monophyletic Pisauridae with *Dolomedes* as a sister group to the remaining Pisauridae.

Lycosidae and Trechaleidae

Lycosidae are a large family including 2,453 species classified in 127 genera and distributed globally (World Spider Catalog, 2022). Lycosids are wandering, agile hunters that chase their prey, earning them the vernacular name of “wolf spiders”. Most lycosids do not construct foraging webs and some, such as *Geolycosa*, dig and live in burrows (Marshall, 1995). Lycosid females carry their egg sac attached to their spinnerets and on hatching, the spiderlings move to the mother’s abdomen and are carried by her, where they cling to modified abdominal setae. The lycosid genus *Schizocosa* has been extensively studied for visual and vibratory signaling during courtship. Male *Schizocosa* use their tibial bristles and dark pigmentation on first legs for visual display. They also use vibrational signals by stridulating, drumming of pedipalps or even bouncing their body (Hebets, 1996; Stratton, 2005). Although the family Lycosidae is nested in the RTA Clade, lycosid males lack a retrolateral tibial apophysis (Polotow et al., 2015; Poy et al., 2019).

Trechaleidae is a relatively small family with 132 species classified in 17 genera, distributed in Central and South America and one species in Japan (World Spider Catalog, 2022). Most

trechaleids live close to water bodies and have long and flexible tarsi, a character which is hypothesized to be an adaptation for walking on the water surface (Silva et al., 2008).

Lycosidae was recovered as the sister group of Trechaleidae in the UCE phylogeny (Figure 4), similar to the results from Sanger sequencing analyses (Albo et al., 2017; Wheeler et al., 2017; Piacentini & Ramírez, 2019), however, both families were polyphyletic in the Combined phylogeny (Figure 18). We integrated Piacentini & Ramírez (2019)'s and Wheeler et al. (2017)'s Lycosidae sequences to assess if increased taxon sampling rendered monophyly of these families. However, the trechaleid *Trechalea* (one terminal) formed a sister group to a clade that included *Cupiennius* (two terminals), *Arctosa kwangreungensis* Paik & Tanaka, 1986 and *Hygrolycosa rubrofasciata* (Ohlert, 1865) (Lycosidae I) and the remaining Lycosidae. The Lycosidae I branch was moderately supported (79% UB) in its placement as a sister group to the *Cupiennius* clade (Figure 18). The remaining Lycosidae (Lycosidae II) were placed sister clade to this includes Lycosidae 98 terminals. In the Sanger sequence-based phylogeny of Piacentini & Ramírez (2019), *A. kwangreungensis* did not nest with other *Arctosa* species, but instead it was a sister group to *Hygrolycosa rubrofasciata* and *Melocosa fumosa* (latter nesting within Lycosidae in this study, see Figure 18).

Dolejš (2013) suggested that *Arctosa fujii* Tanaka, 1985, *Arctosa hikosanensis* Tanaka, 1985 (two species closely related to *A. kwangreungensis*) and *H. rubrofasciata* use an empty egg sac to carry their spiderlings. This behavior is characteristic of Trechaleidae, whereas most lycosids carry spiderlings on their abdomen. Furthermore, Dolejš (2013) suggests that *A. kwangreungensis* and *Arctosa ebicha* Yaginuma, 1960 (both from China and Korea) do not belong to *Arctosa*, but may be an undescribed genus. Interestingly, *A. ebicha* nested within

Lycosidae in our Combined phylogeny (Figure 18). The inclusion of *A. fujii* and *A. hikosanensis* to our Combined data set will be useful to further investigate the placement of this group.

Dionycha

Dionychans are characterized by having a reduced or secondarily lost third claw in their leg tarsi (Coddington & Levi, 1991; Ramírez, 2014). They represent about 30% of all described spider species classified into 19 families (World Spider Catalog, 2022). There are however other spider families such as, some Dysderidae, Palpimanidae and Ctenidae, which also have convergently evolved the two-claw condition (Ramírez, 2014). Dionychans were monophyletic with high support in both the UCE phylogeny and the Combined phylogeny (both with 100% UB). The Dionycha Clade is divided into three sub-clades: Prodidomidae, Dionycha A and Dionycha B. Dionycha A clade is supported by one unambiguous synapomorphy: the cylindrical gland spigots (Cy) on the posterior median spinnerets are clustered posteriorly and isolated from the other spigots (Azevedo et al., 2022).

Prodidomidae

This family was recently restored from a subfamily within Gnaphosidae by Azevedo et al., (2022) and currently includes 192 species classified in 23 genera (World Spider Catalog, 2022). This family is united by the shaft of the minor ampullate gland spigots is reduced to a needle-like extension of the base (Platnick 1990). A cladistic analysis by Rodrigues and Rheims (2020) recovered Prodidominae (*sensu* Rodrigues and Rheims, 2020) as a sister group of Molycriinae (Gnaphosidae). However, our UCE and Combined phylogeny recovered Prodidomidae as a sister group of remaining Dionycha (Figures 4, 19, S6), similar to the results of Azevedo et al. (2022).

Trachycosmidae

This family was recently elevated by Azevedo et al. (2022) to circumscribe the Australian genera formerly placed in Gallieniellidae (*Meedo*, *Neato*, *Oreo*, *Peeto*, and *Questo*) based on the phylogenetic placement recovered from a Combined data set of UCEs, Sanger sequenced markers and phenotypic data. In our UCE, Combined and Dionychan phylogenies, Trachycosmidae was monophyletic (Figures 4, 19, S6), with the exception of *Tinytrema* which was placed as the sister group of Trachelidae (in part) or Gnaphosidae (in part). *Tinytrema* was similarly placed in Wheeler et al.'s (2017) analysis, but was not sampled in the more rigorous analysis of Azevedo et al., (2022).

Azevedo et al., (2022) provided the following diagnosis for Trachycosmidae: anterior lateral spinnerets with a complete distal article and lacking inflatable area, separated by their diameter or more; the presence of two major ampullate gland spigots in males and females; epigynal field formed by an undivided plate, usually with an atrium at the copulatory openings; lens of the anterior lateral eyes are convex, juxtaposed from surrounding cuticle (compared to flat lens of Trochanteriidae).

Clubionidae

This family includes about 662 species classified in 19 genera. In our UCE, Combined and Dionychan phylogenies, Clubionidae was polyphyletic with *Elaver* as a sister group of Anyphaenidae and this clade as the sister group of the remaining Clubionidae (Figures 4, 19, S6). In the morphological cladogram of Ramírez (2014), *Clubiona* and *Elaver* formed a clade which represent the loss of the cylindrical gland spigots. Anyphaenidae and Clubionidae are closely

related families (Platnick 1974), so the placement of *Elaver* recovered in our study is perhaps not surprising. Another clubionid clade including *Carteronius* was a sister group of *Pronophaea* (Corinnidae, in part) (Figures 20, S6) similar to the finding of Wheeler et al. (2017).

Anyphaenidae

This family includes about 614 species classified in 58 genera (World Spider Catalog, 2022). Anyphaenids have an advanced tracheal spiracle and their large and complex tracheal system extends into the prosoma and legs (Platnick, 1974; Ramírez, 2014). The morphological cladogram of Ramírez (2014) included four genera: *Amaurobioides*, *Gayenna*, *Xiruana* and *Anyphaena* which formed a clade. Our UCE and Combined phylogenies recovered a monophyletic Anyphaenidae (Figures 4, 19). In the Combined phylogeny, *Corinnomma* cf. *severum* (Corinnidae) nested with Anyphaenidae, albeit with poor support (59% UB). However, with the Dionychan data set, *Corinnomma* cf. *severum* nested within Corinnidae rendering Anyphaenidae monophyletic (Figure S6).

Gnaphosidae

This is a large family of ground spiders with 2,583 species classified in 163 genera and distributed globally. Gnaphosids are easily identified by the enlarged, cylindrical, widely separated anterior lateral spinnerets (Murphy, 2007). Many gnaphosids have enlarged piriform gland spigots of anterior lateral spinnerets compared to the major ampullate gland spigots (Platnick, 1990). In the gnaphosid subfamily Molycriinae the anterior lateral spinnerets are extremely elongated and placed further anteriorly near middle of the abdomen, away from the remaining spinnerets (Platnick & Baehr, 2006). This configuration of spinnerets is hypothesized

to be an adaptation for efficient use of piriform silk in prey capture (Woff et al., 2017). Another well-studied gnaphosid, *Micaria sociabilis* Kulczyński, 1897 mimics the arboreal *Liometopum microcephalum* (Panzer, 1798) ants using kairomones (a chemical substance produced by *Liometopum* and detected by *Micaria*) (Pekár, 2020). The same species also shows reverse cannibalism where male spiders cannibalized on the older female spiders and showed preference for young females for mating (Sentenská & Pekár 2013). Another gnaphosid, *Drassodes cupreus* (Blackwall, 1834), is known to track polarized light as a compass using its posterior median eyes to navigate to its retreat after the foraging trips (Dacke et al., 1999, 2001).

In our UCE phylogeny, one terminal of *Lampona* (Lamponidae) nested within a clade of four terminals that included three Gnaphosidae taxa (Figure 4). In the Combined and Dionychan phylogenies Gnaphosidae are polyphyletic, although with poor support (<95% UB) (Figures 19, 20, S6). Recent phylogenetic studies using molecular data focussed on systematics of Gnaphosidae also obtained this family as polyphyletic (Azevedo et al., 2018; Rodrigues & Rheims, 2020). Our study recovered relationships similar to the study of Wheeler et al. (2017) since 14 out of 16 taxa representing this family contained six markers, two taxa included UCEs and one with both data.

Lamponidae

This family includes about 192 species classified in 23 genera (World Spider Catalog, 2022) characterized by unisegmented anterior lateral spinnerets (Platnick, 2000). The first cladistic based classification of Lamponidae was proposed by Platnick (2000) using several generic representations and recovered *Lampona*, *Centrothele* and *Asadipus* nested within the family. Ramírez (2014) revised some characters and *Centrothele* and *Lampona* to be monophyletic. The

molecular phylogeny of Wheeler et al. (2017) recovered a polyphyletic Lamponidae similar to the most recent study of Azevedo et al. (2022). However, in our UCE phylogeny rendered a polyphyletic Lamponidae with a clade including *Lampona* (type genus) as a sister group of *Anzacia* (Gnaphosidae) and *Centrothele nardi* (Lamponidae) as a sister group of other Gnaphosidae (Figure 4). Similarly, in our Combined, the *Centrothele* (two terminals) clade was a sister group to Trachycosmidae II whereas *Lampona* (type genus) were a sister group of *Anzacia* (Figure 19, but see Figure S6). Azevedo et al. (2022) recently pointed out that *Anzacia* (SRR6997629) may be a lamponid, but requires examination of the vouchers. The systematics of Lamponidae needs revision and it is possible that a rapid radiation of Lamponidae and Gnaphosidae is generating noise in the resolved phylogenetic signal (Azevedo et al., 2022).

Trochanteriidae

This is a small family with about 50 species classified in six genera (World Spider Catalog, 2022). These spiders have a flattened body and laterigrade legs with greatly elongated posterior trochanters. In our Combined and Dionychan phylogenies, this family was polyphyletic with one clade including *Hemicloea* (three terminals) sister group to *Intruda* (Gnaphosidae) and the other clade including *Doliomalus* and *Vectius* (one terminal each) (Figure 19).

Trachelidae

This family includes about 263 species classified in 20 genera (World Spider Catalog, 2022). Ramírez (2014) provided a diagnosis for this family as follows: claw tufts made of heavily

folded setae, a claw tuft clasper and reduce leg spination on posterior legs and, dorsally on all femora and lacking median apophysis similar to Phrurolithidae, but distinguished by the absence of ventral distal hook on the male palpal femur. In our UCE phylogeny, Trachelidae was monophyletic (Figure 4), however in the Combined phylogeny, it was polyphyletic with two terminals of *Orthobula* sister to *Tinytrema* (Trachycosmidae) (Figure 19). Interestingly, our Dionychan phylogeny recovered a monophyletic Trachelidae (Figure S6). In the UCE phylogeny of Azevedo et al. (2022), Trachelidae was a sister group of Phrurolithidae, however addition of legacy marker data and phenotypic data refuted this placement.

Gallieniellidae

This is a relatively small family with 41 species classified in five genera that are distributed in Argentina (*Galianoella*), South Africa (*Austrachelas*, *Drassodella*), Madagascar (*Gallieniella*, *Legendrena*) and the Comoros (*Gallieniella*). Platnick (1984) diagnosed the family based on sclerotized anterior spinnerets, obliquely depressed endites, and flattened oval posterior median eyes.

Gallieniellids were represented by only one terminal in our UCE phylogeny which was a sister group to Trachelidae (Figure 4). In the Combined and Dionychan phylogenies, the increment of five taxa rendered the family polyphyletic (Figure 19, S6). Gallieniellids are restricted to the Southern hemisphere with five genera (including *Meedo* and *Neato*) found in Australia, four in Africa and Madagascar (including *Gallieniella* and *Legendrena*) and one in Argentina (*Galianoella*, not included in this study). There has been no study aimed at the phylogenetics of Gallieniellidae, to our best knowledge.

Liocranidae

This family includes about 310 species classified in 35 genera (World Spider Catalog, 2022).

Lehtinen (1967) stated that the presence of a secondary conductor in the male palpus is the key characteristic of Liocranidae. The cladistic analysis of Ramírez (2014) recovered a polyphyletic Liocranidae. In all of our phylogenetic analyses, this family was polyphyletic, also similar to Wheeler et al., (2017). The type genus representative *Liocranum* was a sister group of Cithaeronidae (Figures 4, 19, S6). Although the preferred hypothesis of Azevedo et al. (2022) recovered a monophyletic Liocranidae, although they state that another analysis suggests that non-monophyly of this family is equally likely. We recovered a monophyletic *Teutamus* group (*sensu* Ramírez, 2014) which was represented by *Teutamus* and *Sesieutes* in our Combined and Dionycha data sets (Figures 19, S6).

Phrurolithidae

This family includes about 318 species classified in 22 genera (World Spider Catalog, 2022).

Ramírez (2014) diagnosed this family as follows: claw tufts made of heavily folded setae, a claw tuft clasper and reduce leg spination on posterior legs and, dorsally on all femora and lacking median apophysis similar to Trachelidae, but distinguished by modifications on the ventral median apophysis and usually a ventral apical hook, a globose receptacle on the epigynum, in addition to the primary and secondary spermathecae. Our UCE phylogeny recovered a monophyletic Phrurolithidae as a sister group of *Xenoplectus* (Liocranidae) (Figure 4), however, addition of *Otacilia* in the Combined phylogeny recovered a polyphyletic placement (Figure 19). Interestingly, the Dionychan data set recovered a monophyletic Phrurolithidae (Figure S6). The

taxon sample of Azevedo et al. (2022) was similar to our UCE data set and they recovered a monophyletic Phrurolithidae as a sister group of Trachelidae, however the placement was not robust to addition of legacy marker or phenotypic data set.

Xenoctenidae

This is a relatively small family with 33 species classified in four genera distributed mostly in South America and Australia (World Spider Catalog, 2022). The cladistic analysis of Silva-Dávila (2003) recovered a monophyletic group consisting of *Odo* and *Xenoctenus*. Ramírez (2014) obtained an addition of *Paravulsor* in this clade which he called the *Xenoctenus* group. This group was established formally as a family by Ramírez & Silva-Dávila (2017) in the Wheeler et al. (2017) study. Xenoctenids are diagnosed as being similar to viridasiids and some miturgids owing to two recurved eye rows with grate-shaped tapetum, two claws and well-developed scopulae and claw tufts in some spiders. It is distinguishable by the distal divide in the tegulum in the region where the embolus emerges (Wheeler et al., 2017). In all our analyses, Xenoctenidae was monophyletic (Figures 4, 20, S6), however the placement of Miturgidae as its sister group as in Azevedo et al. (2022) was never recovered.

Philodromidae

Commonly called as running crab spiders, this family includes about 520 species classified in 29 genera (World Spider Catalog, 2022). These spiders lack tapeta on the anterior lateral and the posterior eyes (Azevedo et al., 2022). The first cladistic analysis of Philodromidae by Muster (2009) recovered the family monophyletic. Ramírez (2014) inferred that the claw tuft of tenent

setae in the male and female pedipalps as an unambiguous synapomorphy of Philodromidae. In all our data sets, Philodromidae was a sister group of Salticidae (Figures 4, 20, S6) similar to Wheeler et al. (2017) and Azevedo et al. (2022). This is one of the most robustly supported groupings by molecular data among the Dionychan spider families.

Salticidae

Salticids (jumping spiders) are the largest family of spiders comprising 6,354 species (about 12% of all described spiders) classified in 658 genera distributed globally (World Spider Catalog, 2022). They are easily recognizable by their large anterior median eyes, which likely contribute to their documented ability to learn and problem solving (Jackson 2002). A great diversity of biological features have been documented for jumping spiders, including courtship, foraging behaviors, extreme sexual dimorphism, and aggressive mimicry (reviewed in Richman & Jackson, 1992). Salticidae includes some highly specialized species, such as ant mimics (Ceccarelli & Crozier, 2007), specialists of other spiders, like *Portia* (Jackson & Wilcox, 1998), and even specialization on mosquitoes that have recently had a blood meal (Jackson & Cross, 2015). An example of their charismatic courtship behaviors, the peacock spider, genus *Maratus*, have males that have brightly colored abdomens that enlarge during courtship, and they combine vibrational cues with the visual cues from the abdomen during courtship (Girard et al., 2011).

In our UCE and Combined phylogeny with 31 and 54 taxa respectively, (Figures 4, 20), this family was monophyletic which has been supported by all previous molecular analyses (e.g., Maddison & Hedin, 2003; Maddison et al., 2014; Maddison, 2015; Maddison et al., 2017). Maddison et al. (2017) provided the most updated phylogenetic hypothesis of salticid relationships using anchored hybrid enrichment data. They recovered the

Asemoneinae+Lyssomaninae clade as the sister group to remaining salticids, similar to our study (Figure 20). The internal relationships within Salticinae varied in comparison with Maddison et al. (2017), but it could be attributed to the difference with the taxon sampling in both studies. The baviines were monophyletic in our study similar to Maddison et al. (2020). Until a much necessary effort on the global phylogeny of Salticidae is taken, our study provides the most comprehensive reconstruction of their evolutionary history.

Corinnidae

This family includes about 820 species classified in 73 genera (World Spider Catalog, 2022). In all of our data sets, this family was polyphyletic. In the Dionycha phylogeny, *Pronophaea* (two terminals) as a sister group of *Carteronius* (Clubionidae, in part) and *Olbus* as a sister group to this clade. The remaining Corinnidae taxa (26 terminals) were monophyletic (Figure S6).

Azevedo et al. (2022) recovered *Pronophaea* group within Corinnidae, however, our taxon sample differed from their study and therefore this result could not be tested. Instead, our UCE phylogeny obtained a strongly supported *Pronophaea* group (two terminals) as a sister group of the Viridasiidae + Selenopidae + Cheiracanthiidae clade (Figure 4).

Selenopidae

These cursorial spiders include nine genera and 262 species distributed globally, however with a large diversity in the southern hemisphere (World Spider Catalog, 2022). Selenopids are dorsoventrally flat and extremely agile predators (Crews et al., 2008) and have their posterior

median eyes placed within the row of anterior eyes (Ramírez, 2014). In Wheeler et al. (2017), Selenopidae was a sister group of Viridasiidae. In our UCE and Combined phylogenies, Selenopidae was polyphyletic with the Australian endemic genus placed as a sister group of Miturgidae and the other group (which included the type genus *Selenops*) as a sister group of Viridasiidae (Figures 4, 20). The four gene phylogeny of Crews & Gillespie (2010) included *Karaops* (listed as “New Genus Australia”) which nested within Selenopidae, however with poor support.

Miturgidae

This family includes about 140 species classified into 29 genera (World Spider Catalog, 2022). Miturgidae was monophyletic in our UCE phylogeny placed as a sister group of *Karaops* (Selenopidae), however the addition of *Parapostenus* in the Combined and Dionycha data sets rendered the family polyphyletic (Figures S6). *Parapostenus* was placed as a sister branch to Viridasiidae (in part). Wheeler et al. (2017) mention a possibility that *Parapostenus* may be either a miturgid or a viridasiid. Ramírez (2014) and Azevedo et al. (2022) recovered Miturgidae as a sister group of Xenoctenidae, however, none of our analyses recovered this placement.

Cheiracanthiidae

This family includes 363 species classified in 14 genera with a cosmopolitan distribution (World Spider Catalog, 2022). They are diagnosed by the conical and contiguous anterior lateral and

posterior median spinnerets, an elongated article on posterior lateral spinnerets distally, eyes occupying the caput and curved setae on the opisthosoma (Ramírez, 2014). The cladogram of Ramírez (2014) inferred that Eutichuridae (former name of Cheiracanthiidae, as discussed in the same paper) was a sister group of a clade including Miturgidae, Sparassidae, Philodromidae, Salticidae and Thomisidae. With the six-marker data set, Eutichuridae was a sister group of Viridasiidae and Selenopidae, similar to Azevedo et al. (2022), and our UCE and Combined phylogenies, except that with the Combined data, the sister group of Cheiracanthiidae included *Parapostenus* sp. (Miturgidae) (Figures 4, 20).

Viridasiidae

Viridasiidae is a small family including seven species classified into two genera (*Viridasius* and *Vulsor*) primarily distributed in Madagascar and nearby islands, with one species in Brazil. The natural history of these spiders is poorly known, however, Bauer et al., (2018) and Bauer (2021) reported that in captivity, these spiders constructed silken retreats and a pendulous egg sac covered with debris. In our Combined analysis, *Mahafalytenus* (Ctenidae) nested within Viridasiidae (Figure 22), similar to the result of Wheeler et al., (2017). The recent Azevedo et al., (2022) also recovered this placement and formally transferred *Mahafalytenus* to Viridasiidae.

CONCLUSIONS

(1) The classification of spiders and the hypotheses about their phylogenetic relationships have significantly changed in the last decades. Several morphological features that have been traditionally used to circumscribe higher taxa have evolved or been lost multiple times independently. For example, higher taxa are no longer grouped strictly by presence or absence of

cribellum and several families such as Oecobiidae and Udubidae have both cribellate and ecribellate members. It is clear that this character which once weighed over spider classification has been lost multiple times along the evolution of this group. Although haplogyne spiders are not a clade, a general trend from the haplogyne to the entelegyne condition is suggested by the recent literature, even in the face of multiple convergences both ways. Although the question on whether the orb weavers are a monophyletic group or not seems to have converged onto a stable answer (Orbiculariae is not a clade)—the hypothesis of a single origin of the orbweb remains debated. Thus, in spiders the story tends to be one of groups being defined by a single character, that is later undone and the defining character turns out to be homoplasious. Large scale analyses of genomic data have contributed to a better understanding of both spider phylogeny and the evolution of their morphological features and spinning products. Phylogenetic hypotheses at the interfamilial level have changed in most families, while the intergeneric relationships remain poorly and insufficiently understood.

(2) Using a combination of newly generated and publicly available genome scale data and Sanger sequence based six marker data sets, we produced the most comprehensive phylogenomic inference of the spider tree of life in terms of taxa (128 spider families ~97% sampling, 1,362 terminals). The analyses recovered some highly supported placements that reject the monophyly of certain families, for example, the placement of Gnaphosidae. However, previous studies indicated similar placements based on morphology or molecular data. The subsetting of the Combined data set to Marronoid and Dionychan data sets rendered some polyphyletic families such as Trachelidae to be monophyletic, which reveals an interesting phenomenon that needs further exploration. We are aware of and emphasize the limitations of

our data set and therefore resorted to only review these phylogenetic placements and do not make any formal taxonomic changes.

(3) Our results covered several taxonomic hierarchical levels, cemented various hypotheses on important family level relationships and allowed us to identify the stable phylogenetic relationships across the spider tree of life. We identified the unstable areas of the cladogram and discussed the conflicting hypotheses resulting from various classes of data such as morphology, Sanger sequencing-based markers and genomic scale data such as transcriptomes and UCEs. We recognize that future studies are warranted to focus on certain groups of the spider tree of life (for example, RTA Clade, marronoid clade, Hahniidae and Araneoidea). Our review can help to design studies targeting taxonomic groups in need of systematic revisions.

(4) Some clades supported by morphological characters are corroborated by molecular data (such as in the case of symphytognathoids) whereas some novel groupings have made arachnologists review their classifications over again (such as polyphyly of theridioids). Many new spider phylogenetic studies are published every year, thus recalibrating and refining the synapomorphies of those groups. These continued efforts are helping us to better understand how evolutionary processes have shaped the diversification of spiders. Spider systematics and phylogenetics have never been this close to visualizing a highly comprehensive picture of their evolutionary history at the family level.

(5) Sequencing technologies continue to be increasingly more cost effective and museum specimens are now widely used for both morphology and molecular sequencing. The tools to study morphology have greatly advanced too, such as micro-Computed-Tomography (microCT) scanning. We are now able to see internal anatomical structures in a three-dimensional view (e.g., Michalik et al., 2013; Wood & Parkinson, 2018), when previously morphologists were

restricted to histological sectioning or dissection, typically resulting in a two-dimensional photograph or illustration. MicroCT is of great advantage to observing fossils (e.g., Penney et al., 2007), which is a morphology-based endeavour, and allows for hidden structures to be revealed. This technique also allows for creating 3D digital objects and was recently used to study the evolution of carapace and cheliceral shapes across spiders, with a focus on Araneoidea (Kallal & Wood, 2022).

Taxon sampling has grown comprehensively for molecular data-based phylogeny and fossils, informed by their morphology and ages, provide calibration points for these phylogenies. Beyond the utility of dating phylogenies at nodes, fossils are also used as taxa to be placed in a phylogeny, called “tip-dating”. Wood et al. (2013) used tip-dating to show that Palpimanoidea diversification was shaped by the break-up of Pangaea in the Mesozoic. Recently, using morphology observed under microCT, Magalhaes et al. (2022) discovered that the holotype of *Loxosceles aculicaput* Wunderlich, 2004 (Sicariidae) is actually a misidentified Drymusidae which was the first fossil from the latter family and placed in a phylogeny. Fossils morphology is also useful in reconstructing trait evolution. Morphology also provides observable ontogenetic information in the light of gene regulatory networks, which is detectable to a certain extent in molecular data by the timing and location of gene expression.

Although it is apparent that molecular data are dominating phylogenetic studies, it is likely that this skewed pattern will soon reach a tipping point. The advent of the World Spider Trait database (Pekár et al., 2021) has an enormous potential and will facilitate the study of the evolution of a variety of characters across the spider tree of life. Without morphological, behavioral and natural history data, phylogenetic trees have limited value because their explanatory power is based on their ability to interpret phenotypic and other biological

observations. Both morphology and molecules are gradually converging to unravel a more precise understanding of evolutionary history. It is perhaps the most exciting time so far for advancing the knowledge about the evolution of spiders.

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FIGURE LEGENDS

Figure 1. A schematic figure showing characteristics of spiders. A. Habitus of *Pecanapis* sp. GH2900 (Anapidae). B. Silk secreting spinnerets in *Tylorida striata* (Tetragnathidae). C. A micro-CT graph of the venom glands in *Latrodectus geometricus* (Theridiidae). D. Male pedipalp of *Orsinome* sp. (Tetragnathidae). Scale. A, D-200 μ m, B- 50 μ m.

Figure 2. A. schematic representation of data sampling and curation using target-enrichment and six legacy Sanger-sequenced markers. B. A summary graph of progress in the sampling of Araneoidea, retrolateral tibial apophysis Clade (RTA Clade) and spider families in phylogenetic

studies in comparison to the total number of described families. S- Sanger-sequencing based markers data, T- Transcriptomic data, U- Ultraconserved elements.

Figure 3. A maximum-likelihood phylogeny reconstructed using the 25% occupancy data set of the ultraconserved elements (UCEs) with higher-level groups highlighted. Branch colors correspond to the circles in the top right of the photographs. A. *Liphistius* sp. (Liphistiidae), B. *Theraphosa* sp. (Theraphosidae), C. Pholcidae sp., D. *Eriauchenius workmani* (Archaeidae), E. A typical web of Linyphiidae, F. Orb web of *Ocrepeira darlingtoni* (Araneidae), G. A typical aerial sheet web of *Forstera* (Cyatholipidae), H. The modular vertical web of *Synotaxus* sp. (Synotaxidae), I. *Exechocentrus lancearius* (Araneidae), J. *Deinopis* sp. (Deinopidae) with its cribellate orb web, K. Nicodamidae sp., L. Sparassidae, M. The cribellate web of *Paramatachia* sp. (Desidae), N. *Centroctenus alinahui* (Ctenidae), O. Lycosidae sp., P. *Poecilochroa* sp. (Gnaphosidae). Photo credits. C, L, O, P- Atul Vartak; N- Nicolas Hazzi; remaining photos- Gustavo Hormiga.

Figure 4. A maximum-likelihood phylogeny of the family-level relationships of spiders reconstructed using the 25% occupancy data set of the ultraconserved elements (UCEs).

Figure 5. Phylogenetic relationships of Mesothelae and Mygalomorphae lineages derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 6. Phylogenetic relationships of Synspermiata, Hypochilidae, Filistatidae, Austrochilioidea, Leptonetidae and Archoleptonetidae lineages derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Ultrafast bootstrap values are indicated at nodes, except when they were >95%.

Figure 7. Phylogenetic relationships of Palpimanoidea families derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 8. Phylogenetic interrelationships of the family Theridiidae derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 9. Phylogenetic relationships of the symphytognathoid families derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 10. Phylogenetic relationships of a part of Araneoidea families. A. Pimoidae and Linyphiidae (“linyphioids”), B. Cyatholipidae, C. Araneoidea, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Sub-families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 11. Phylogenetic relationships of a sample of Araneoidea families. A. Tetragnathidae, Arkyidae and Mimetidae (“tetragnathoids”), B. Malkaridae, C. Araneoidea, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Sub-families that are paraphyletic or polyphyletic are

appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 12. Phylogenetic relationships of a sample of Araneoidea families A. Synotaxidae and Araneidae, B. Nesticidae and Physoglenidae, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 13. Phylogenetic relationships of the Nicodamoidea (Nicodamidae and Megadictynidae), Eresidae and the UDOH grade families, Uloboridae, Deinopidae, Oecobiidae and Hersiliidae derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 14. Phylogenetic relationships of a sample of the Tibial apophysis Clade (TA Clade) excluding the Marronoid, Oval Calamistrum, Dionycha Clades and Homalonychidae, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and

the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Sub-families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 15. Phylogenetic relationships of a sample of the Marronoid families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

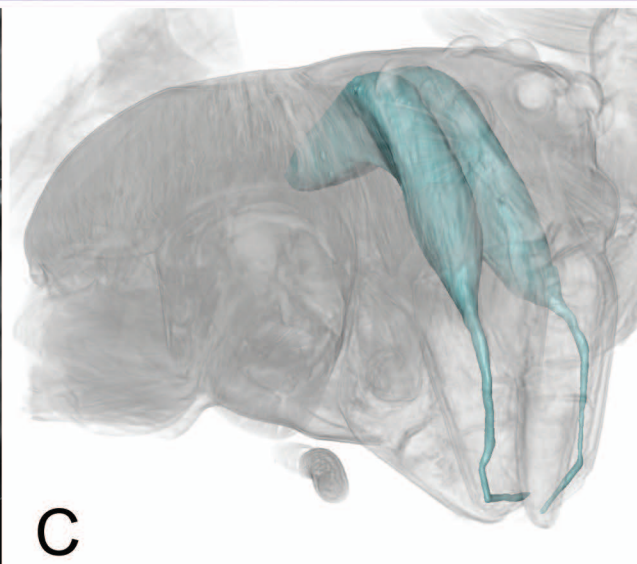
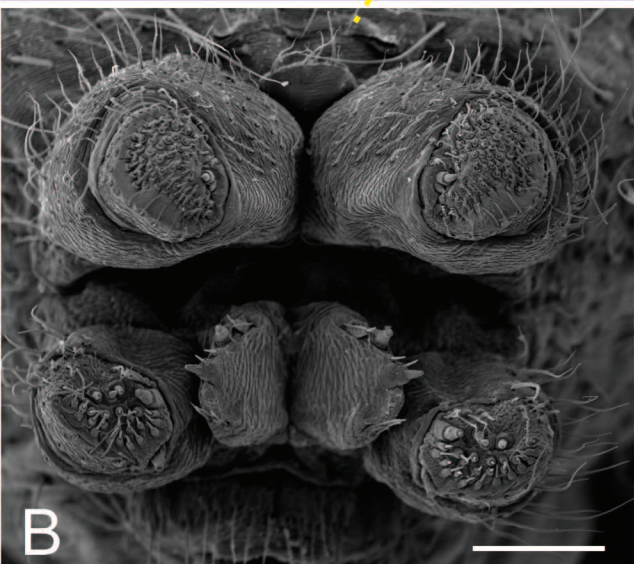
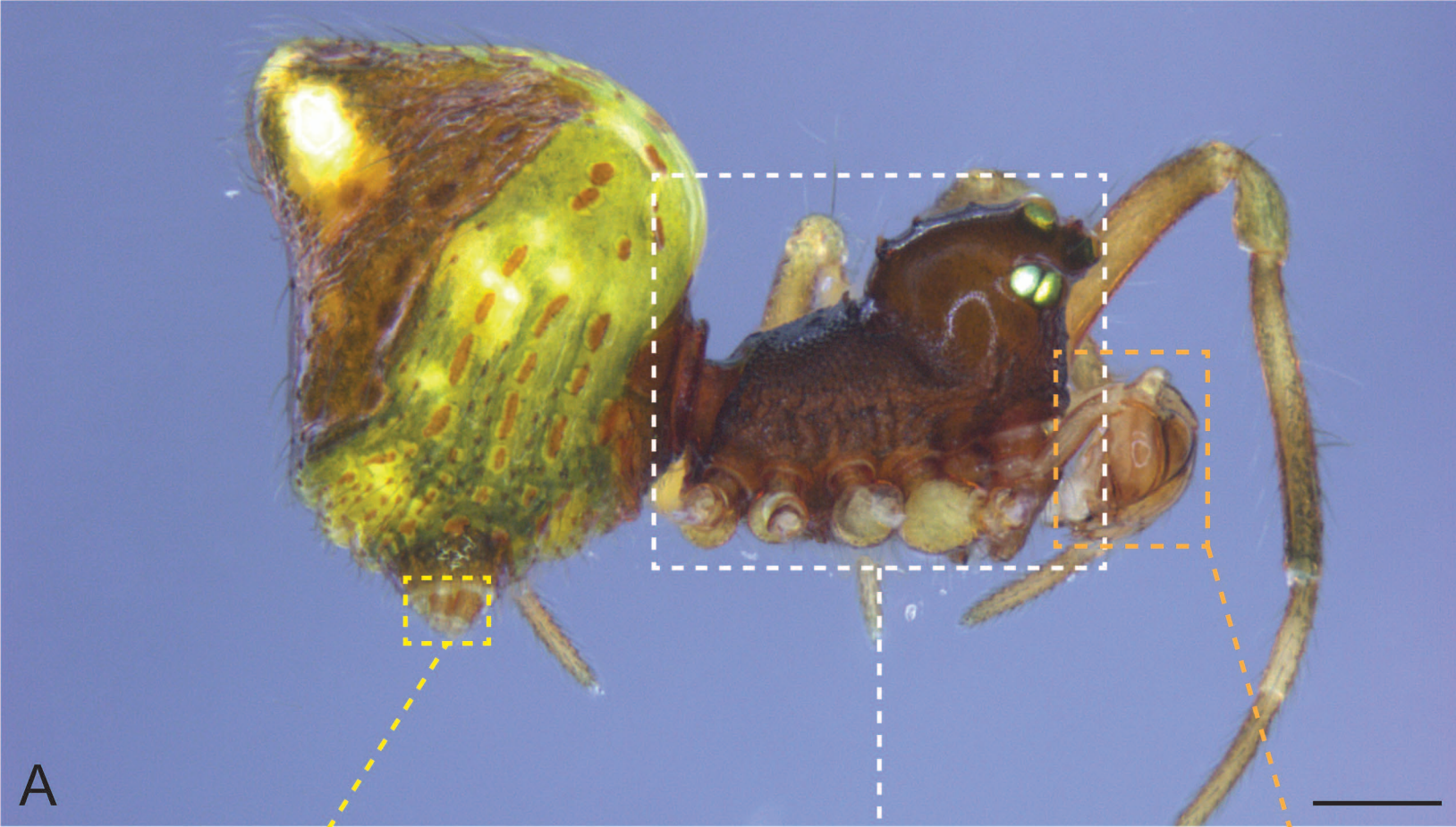
Figure 16. Phylogenetic relationships of a sample of the Marronoid families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

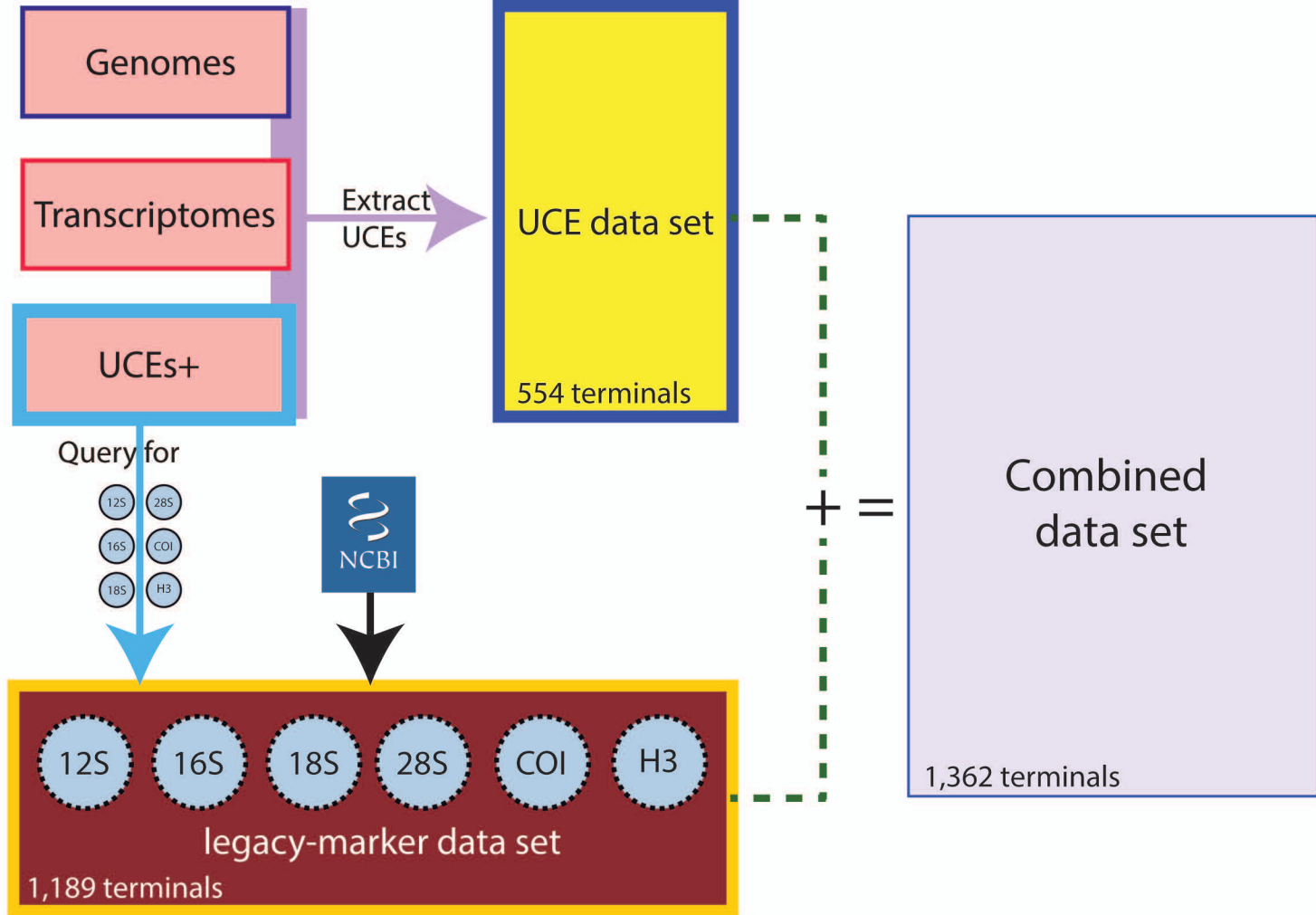
Figure 17. Phylogenetic relationships of Homalonychidae family and a sample of the Oval Calamistrum Clade families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 18. Phylogenetic relationships of a sample of the Oval Calamistrum Clade families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

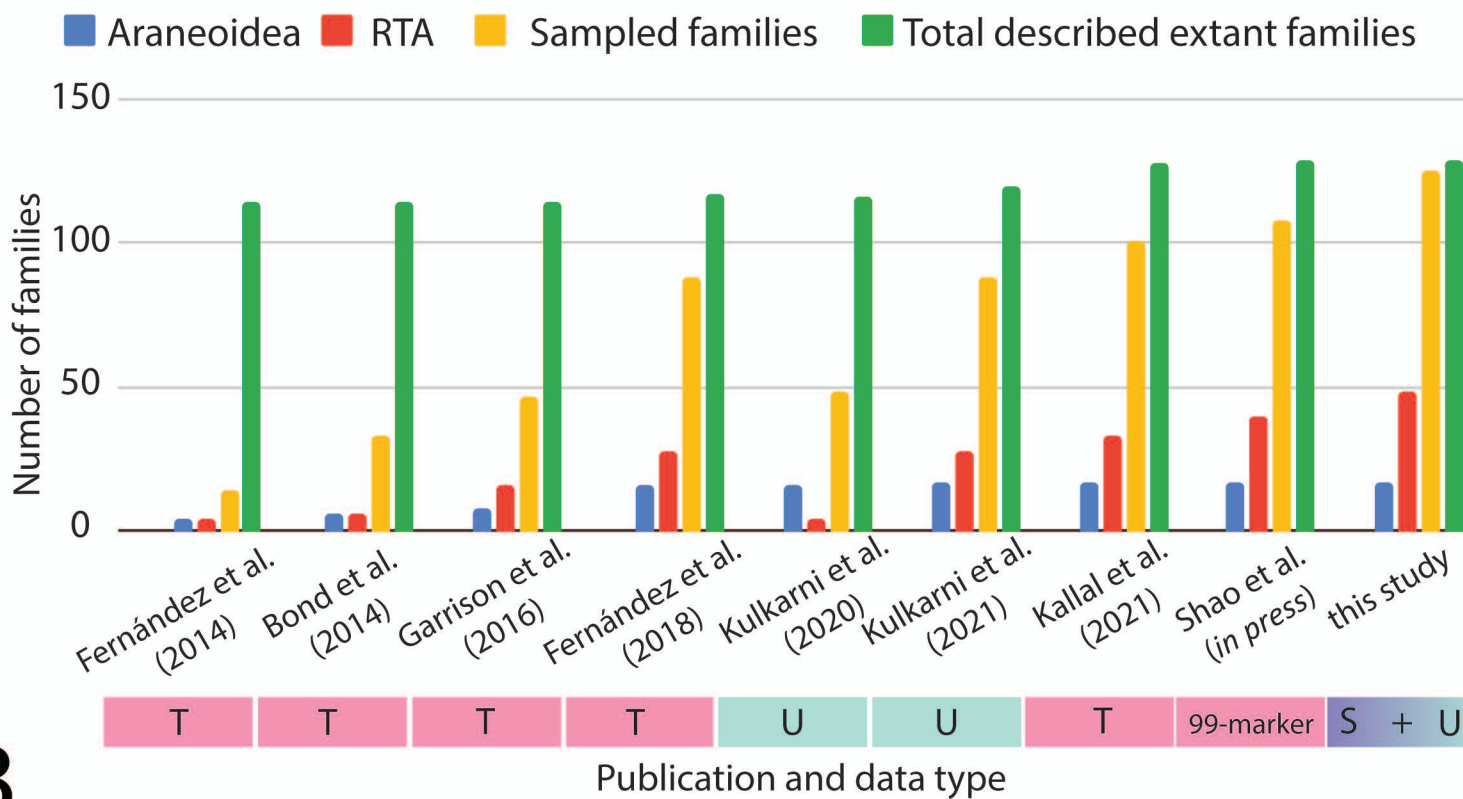
Figure 19. Phylogenetic relationships of a sample of the Dionycha Clade families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Annotated boxes indicate family or subfamily. Coloured squares at tips indicate the following data classes that they represent: Blue- Sanger data only, Red- UCE data only, Green- Sanger+UCE data. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

Figure 20. Phylogenetic relationships of a sample of the Dionycha Clade families, derived using a combination of the 25% occupancy data set of the ultraconserved elements (UCEs) and the Sanger-sequence data set. Coloured boxes indicate family or sub-family. Families that are paraphyletic or polyphyletic are appended with Roman numerals. Ultrafast bootstrap values are indicated at nodes except when they were >95%.

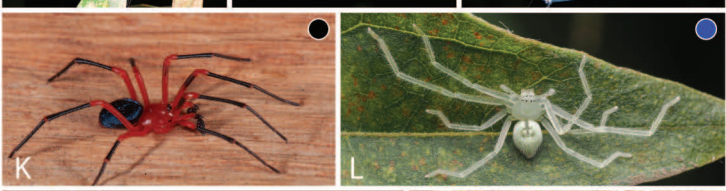
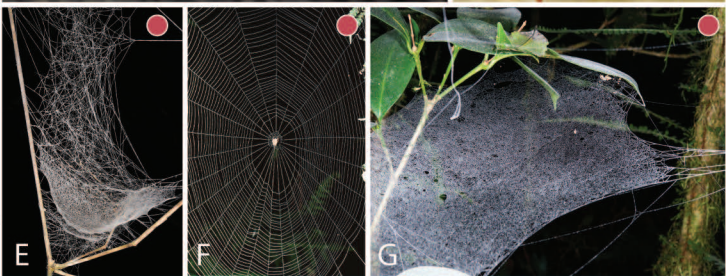
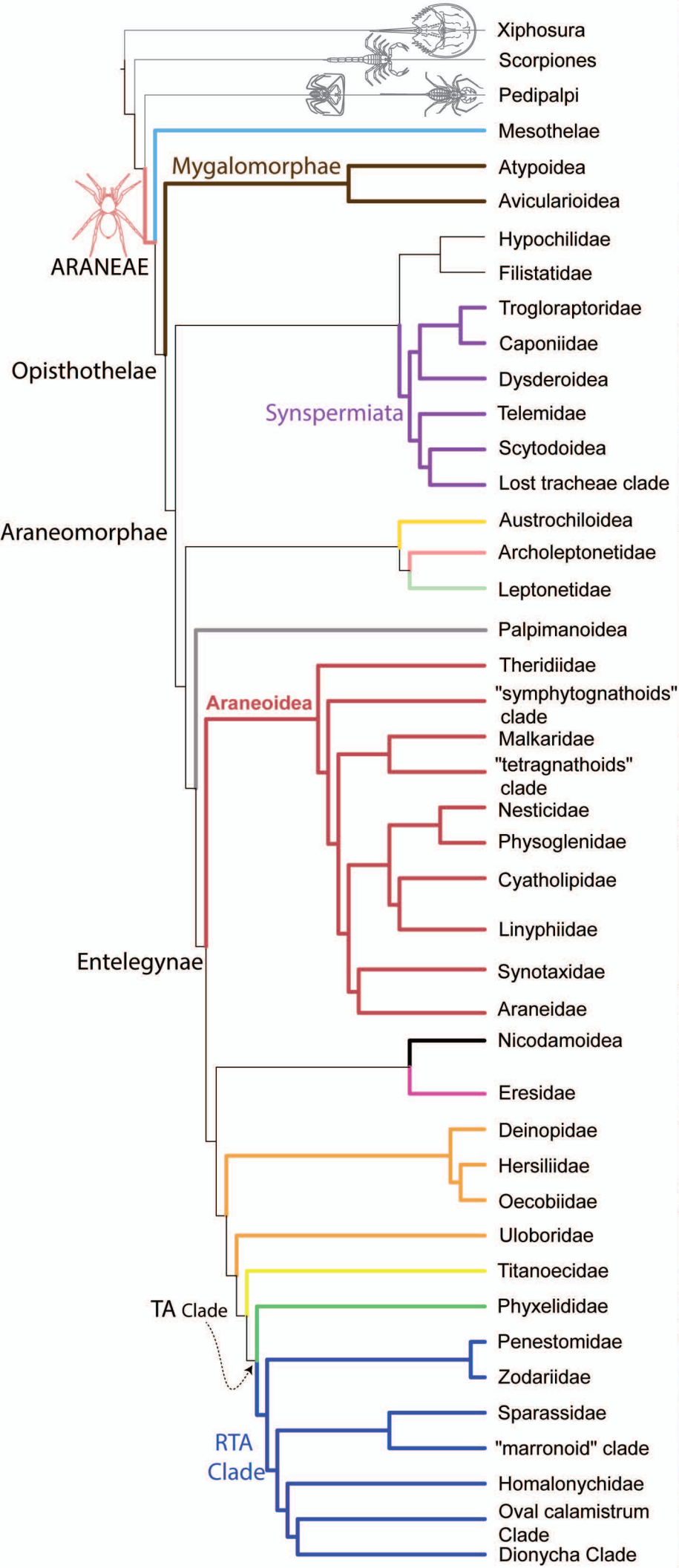


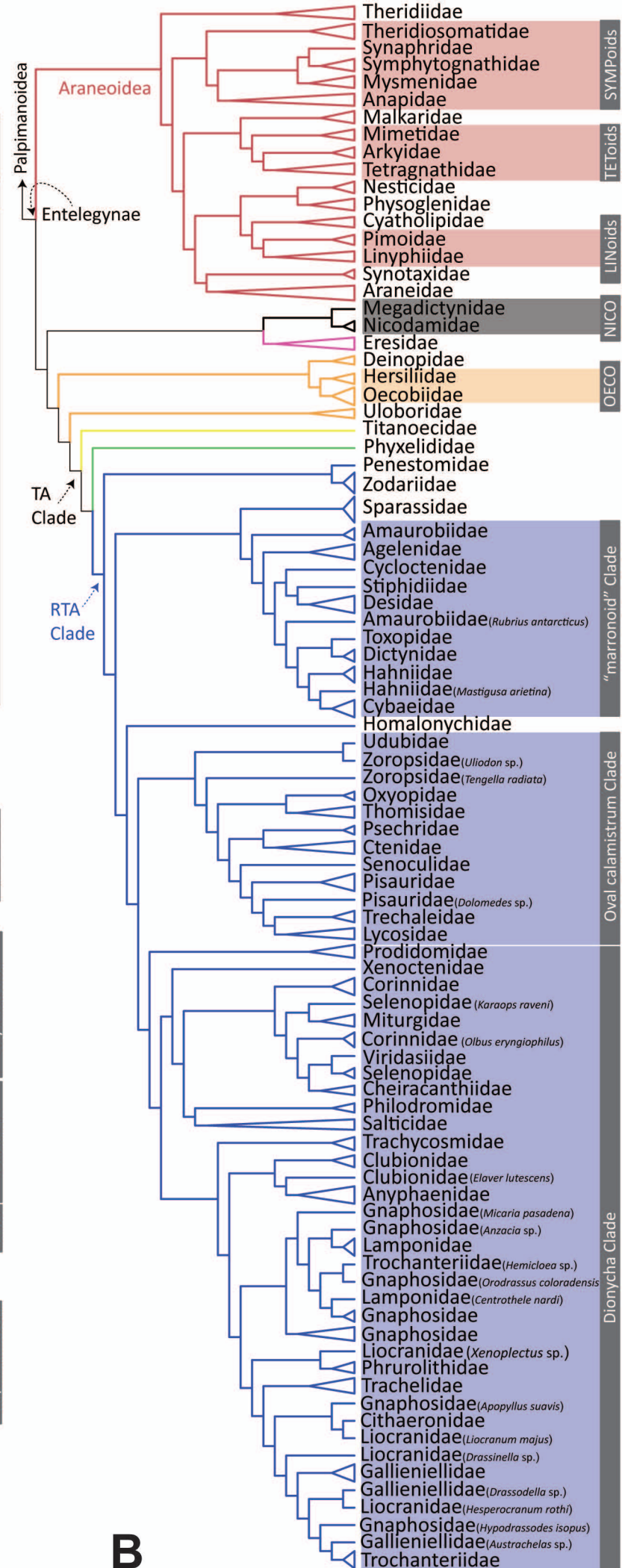
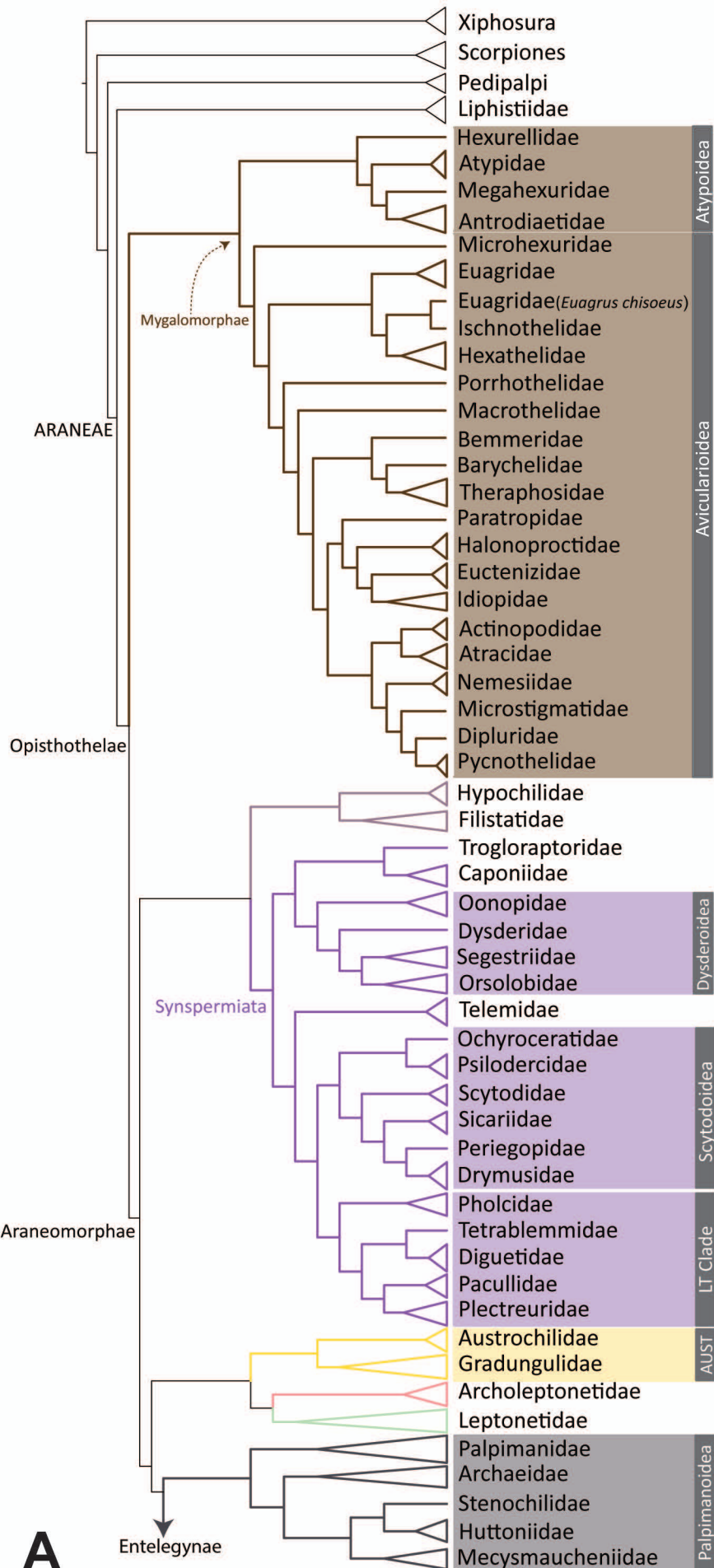


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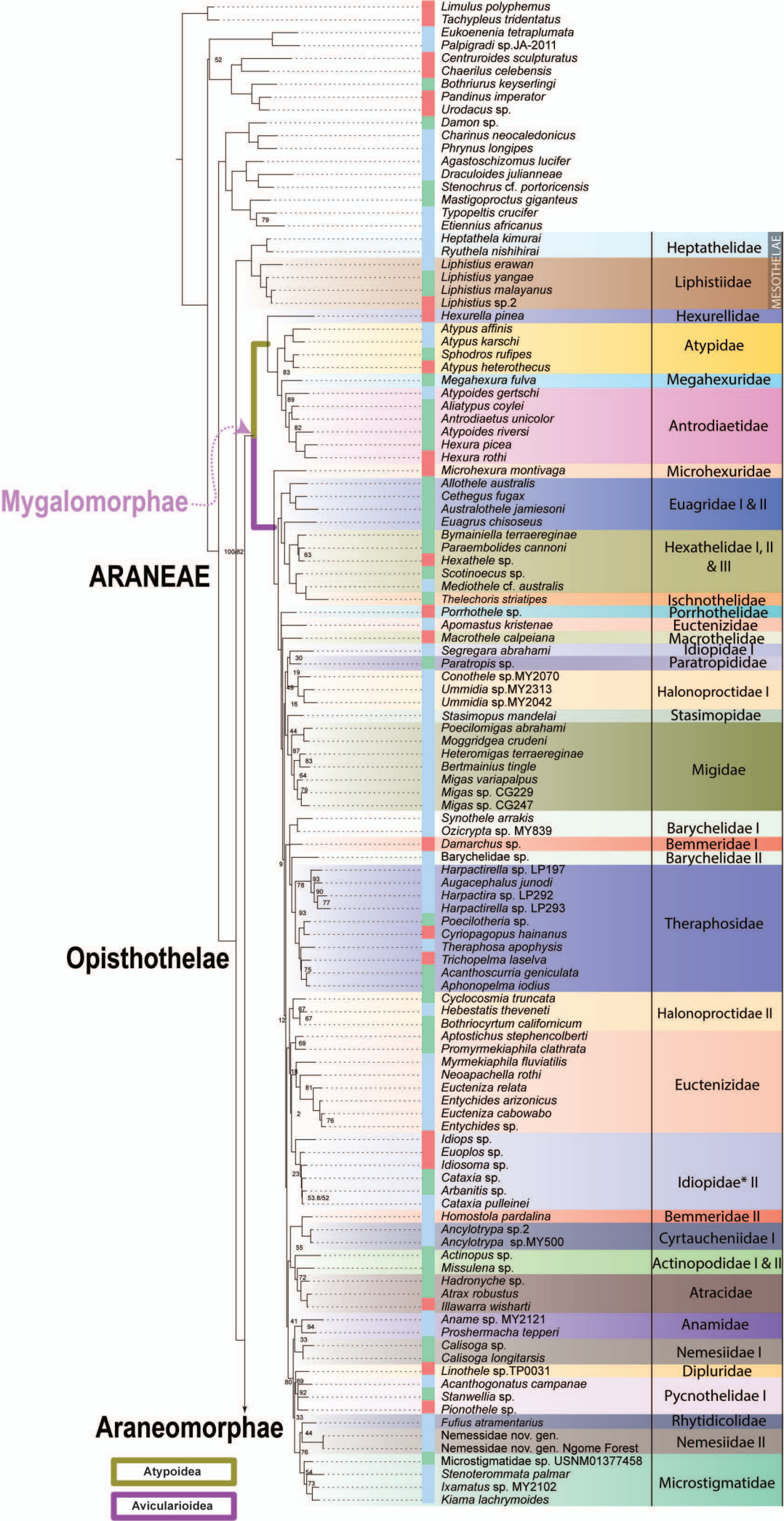


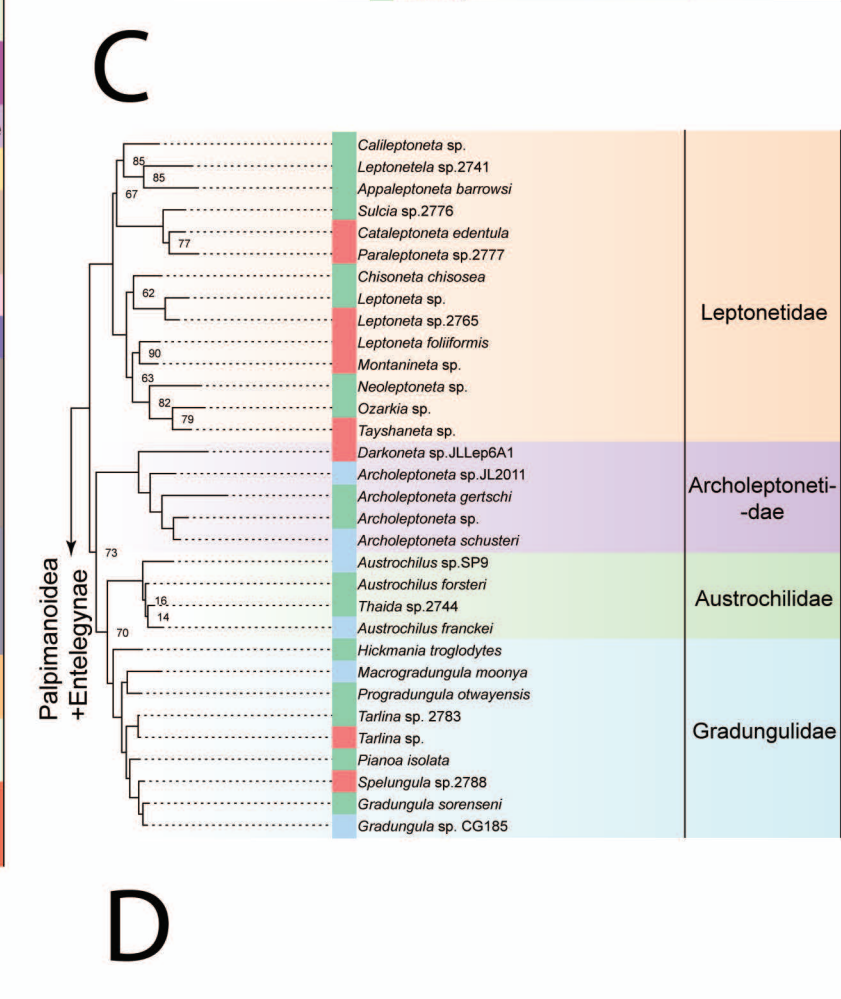
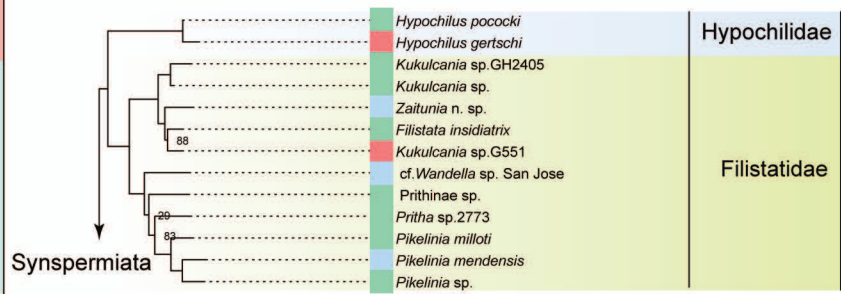
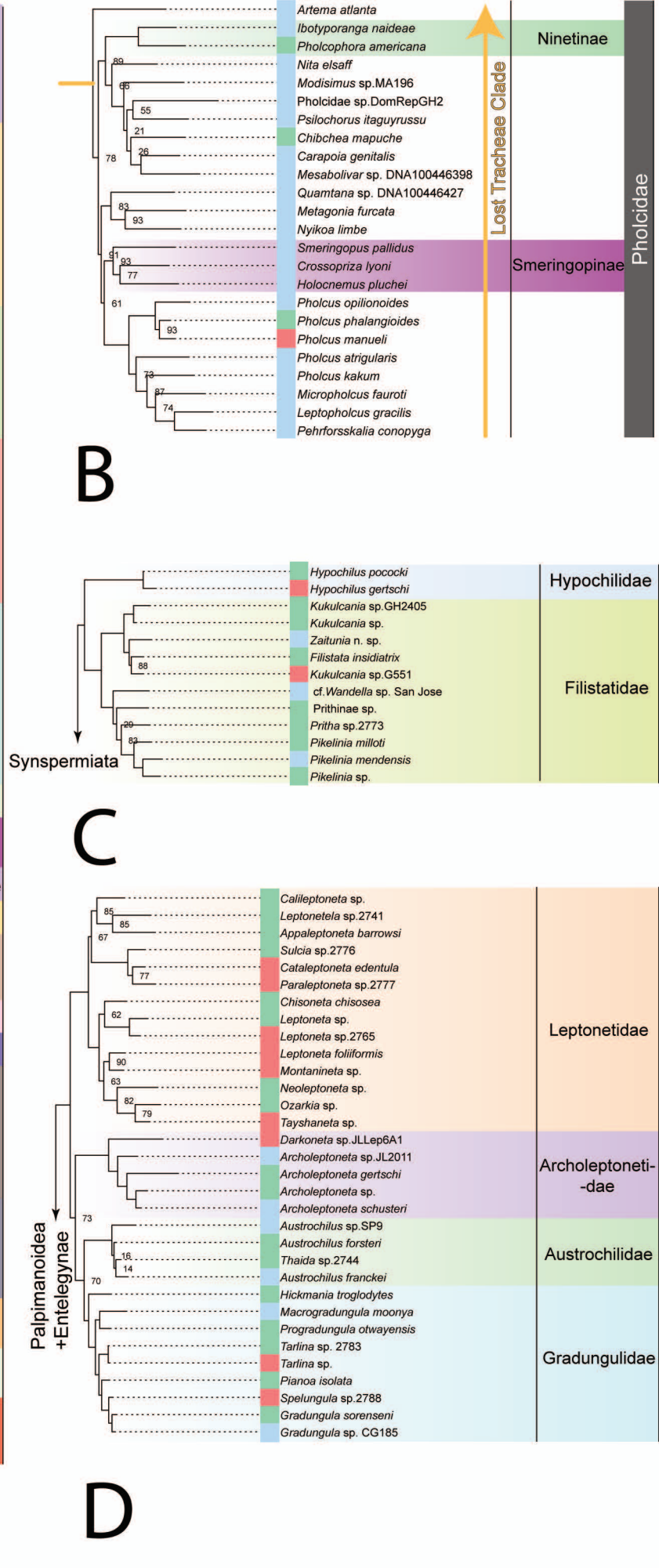
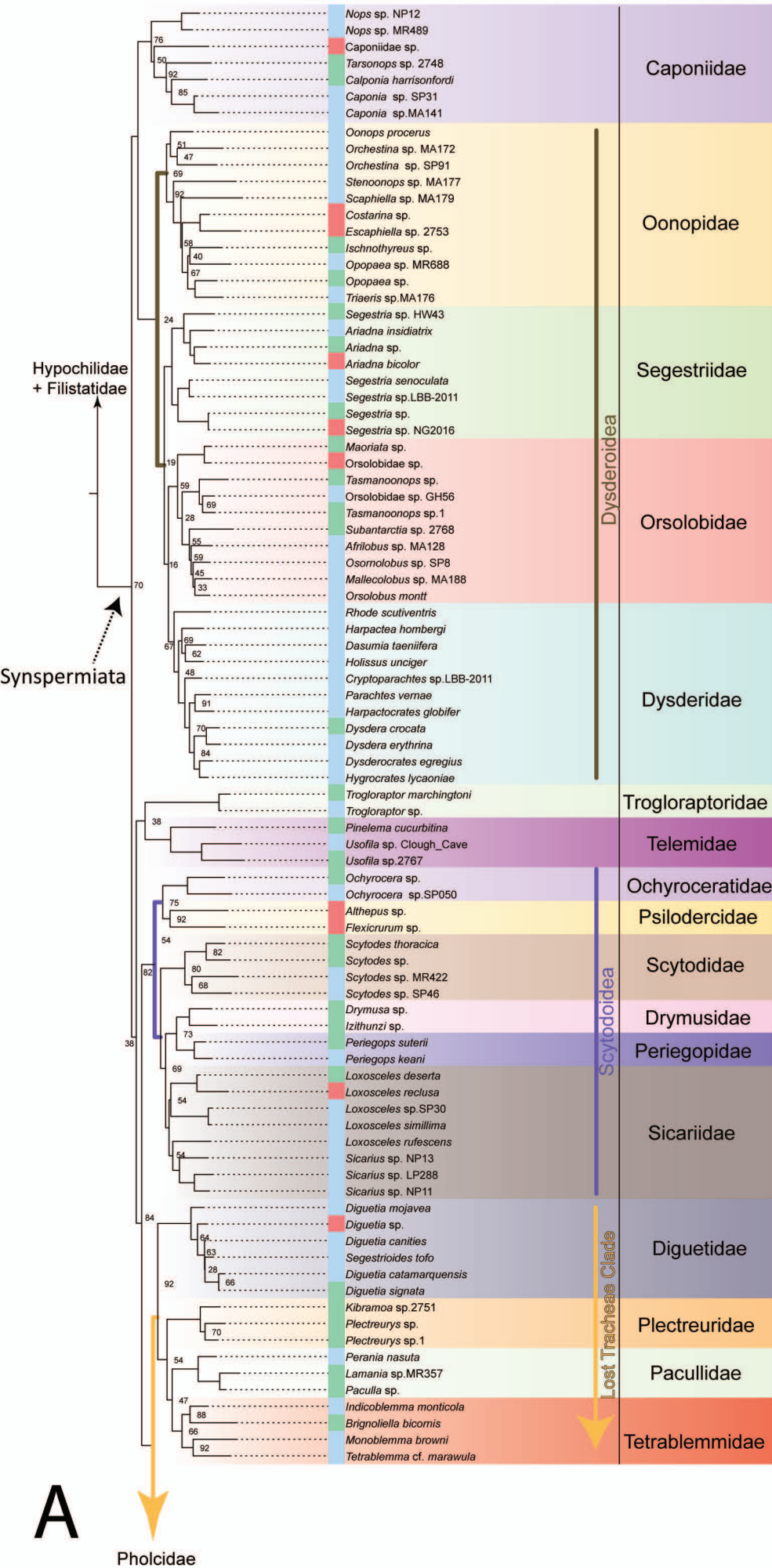


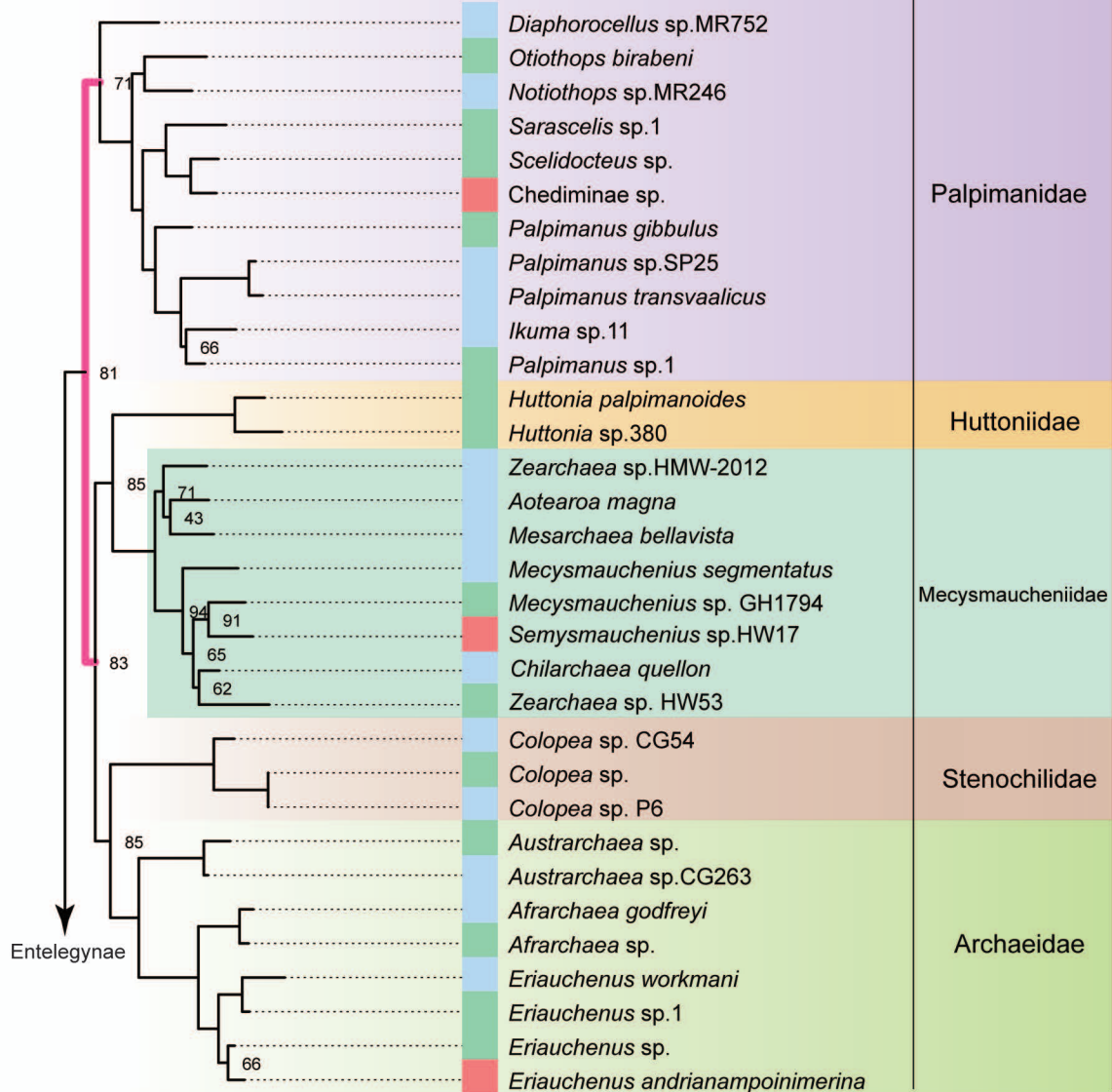
Abbreviations

AUST- Austrochiloidea	RTA Clade- Retrolateral tibial apophysis Clade
LT Clade- Lost Tracheae Clade	SYMPoids- symphytognathoids
LINoids- linyphioids	TA Clade- Tibial apophysis Clade
NICO- Nicodamoidea	TEToids- tetragnathoids
OECO- Oecobioidae	

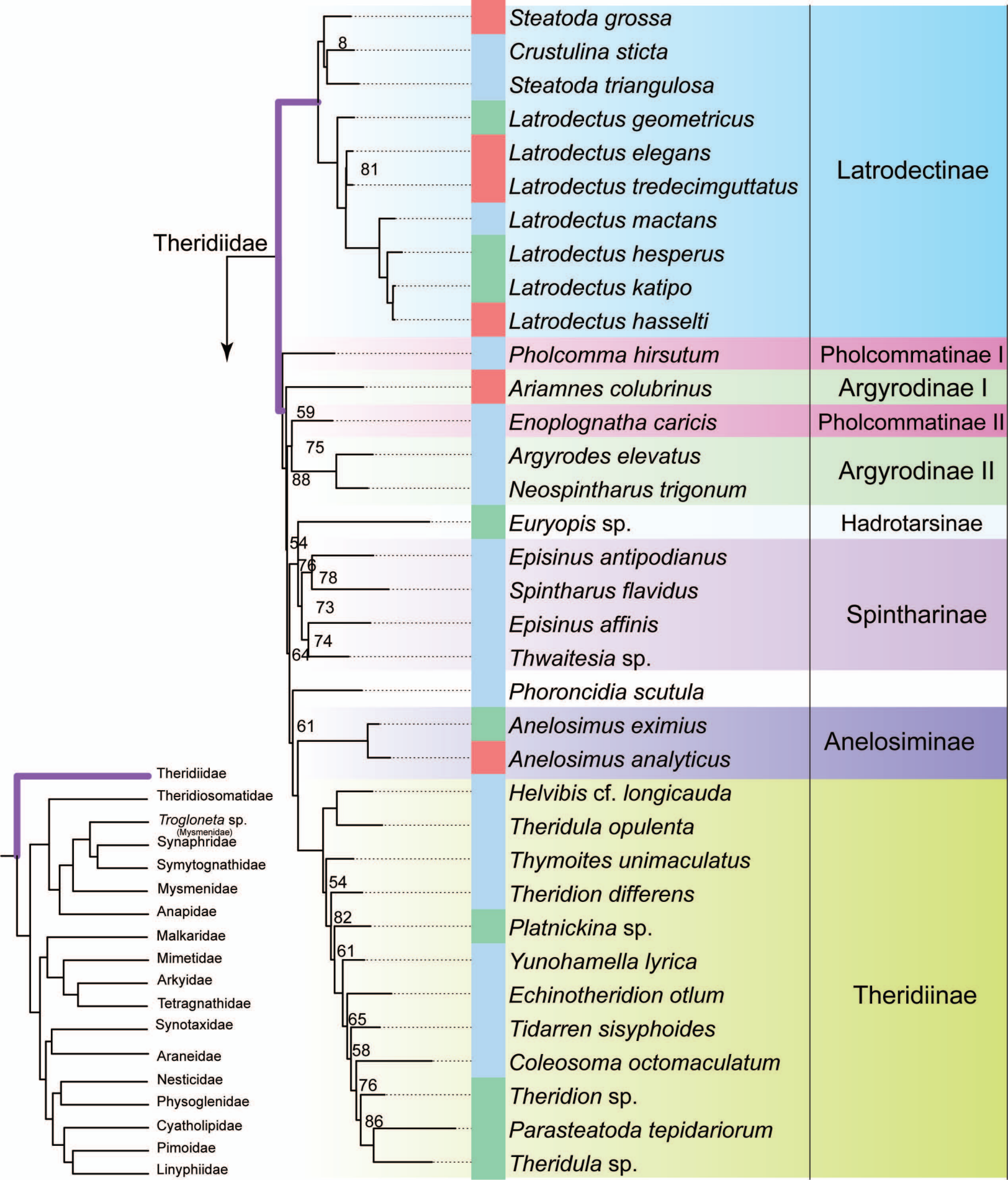
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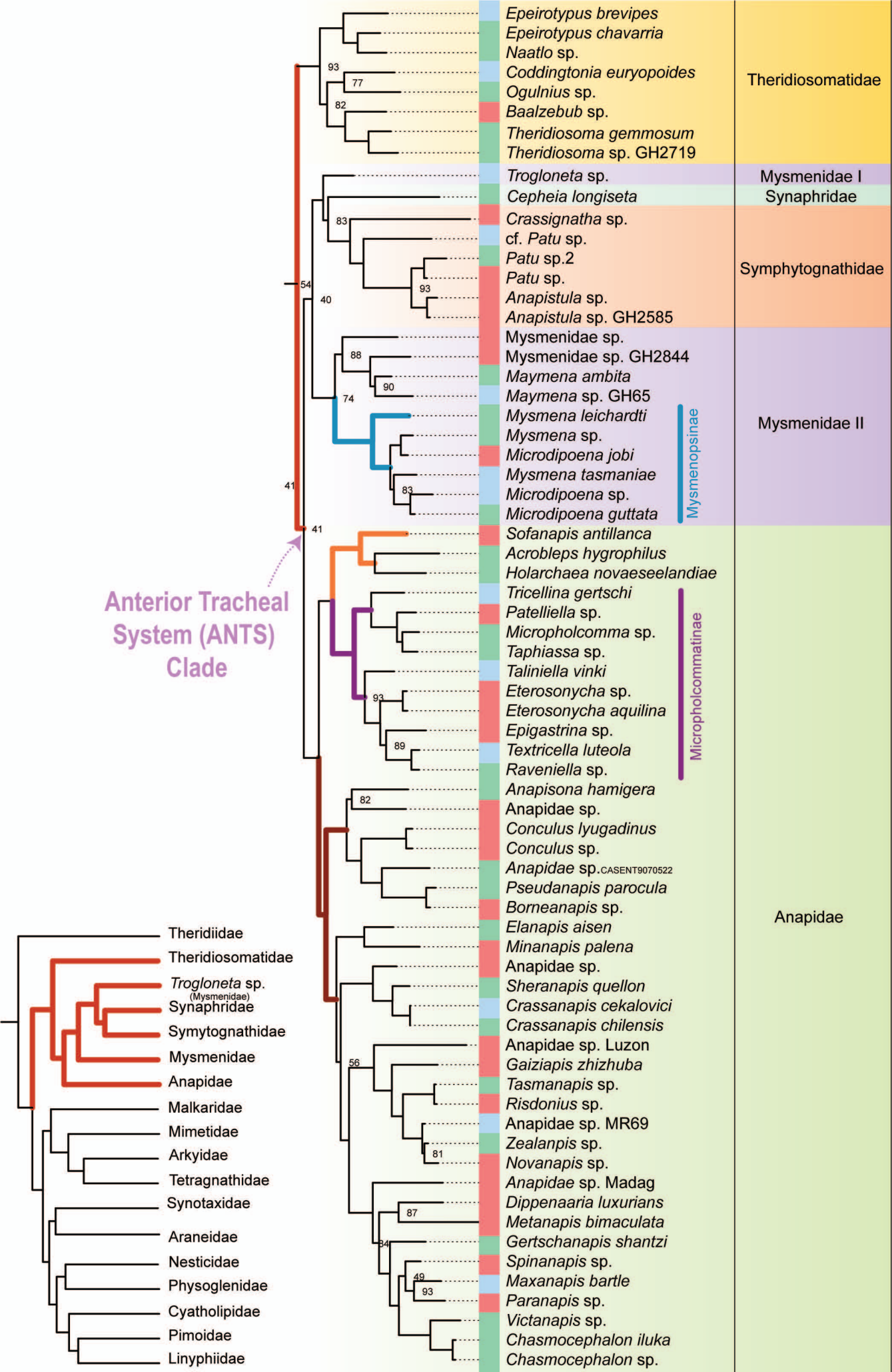


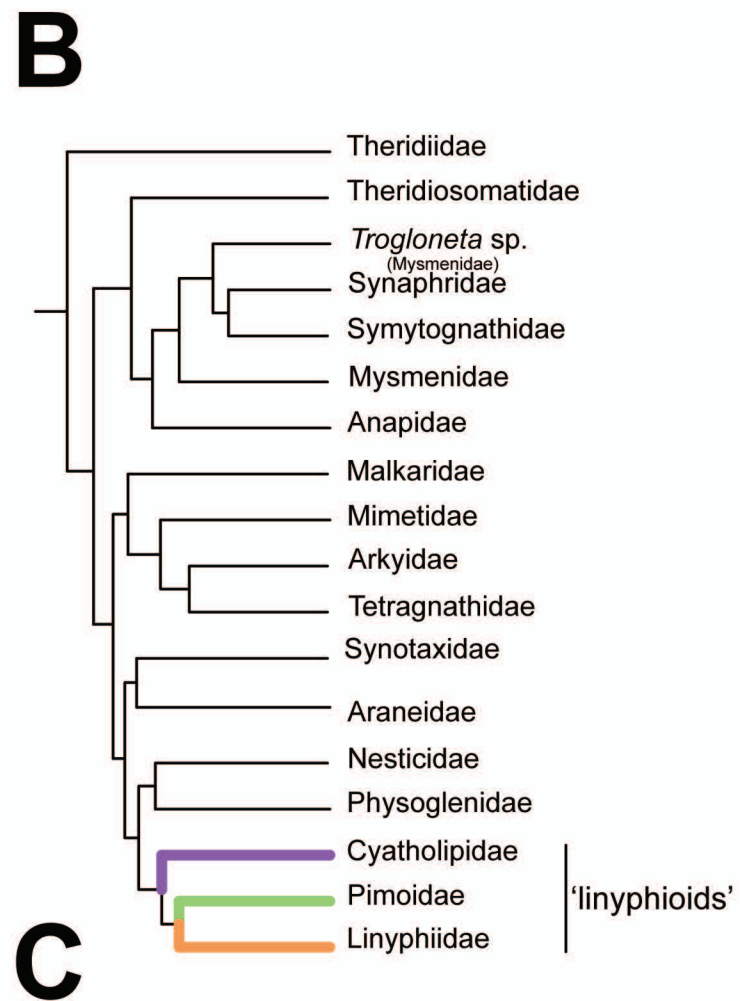
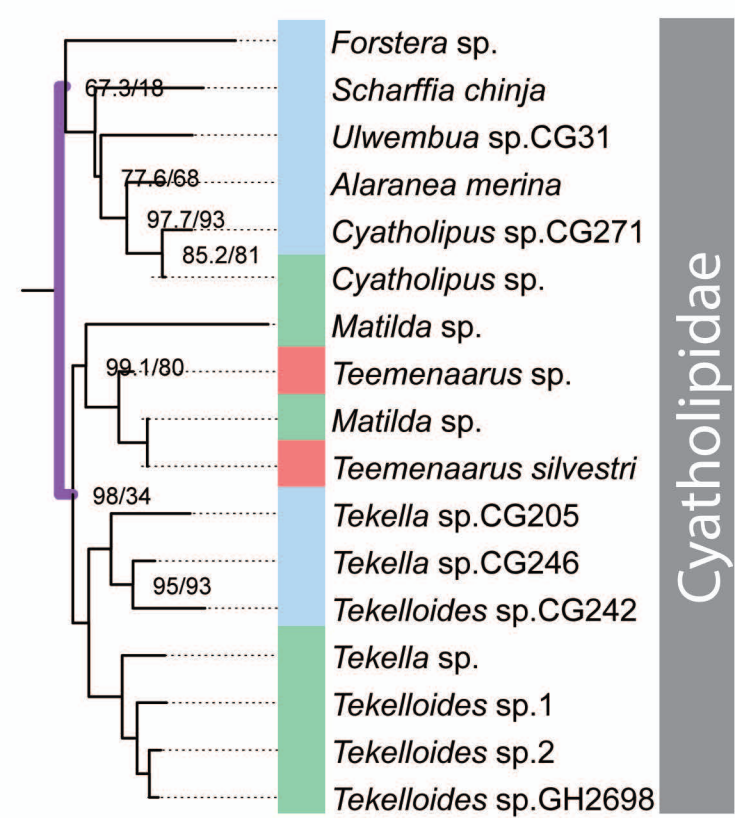
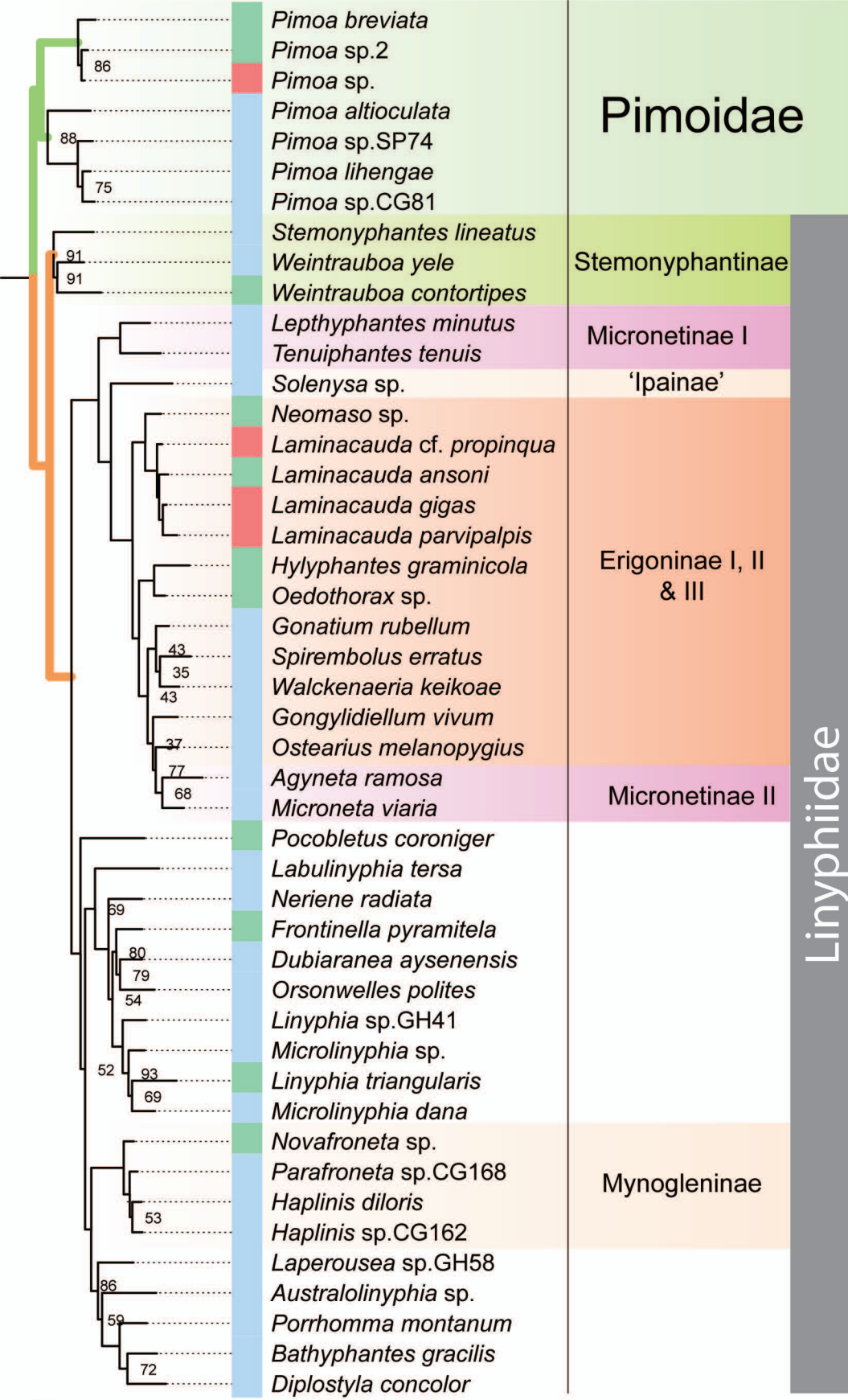


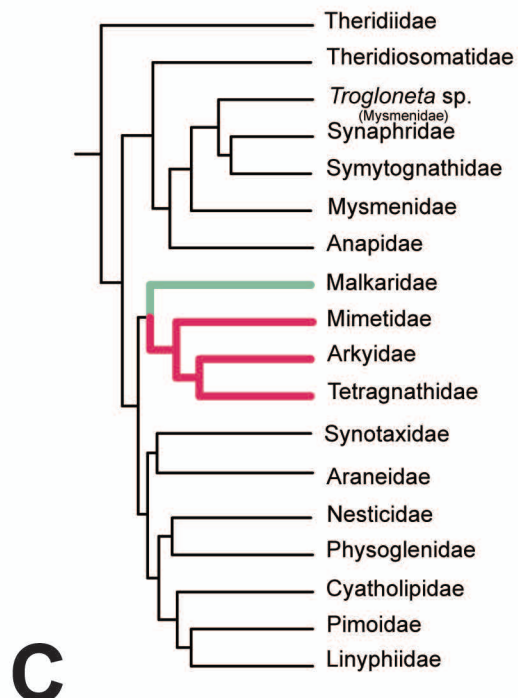
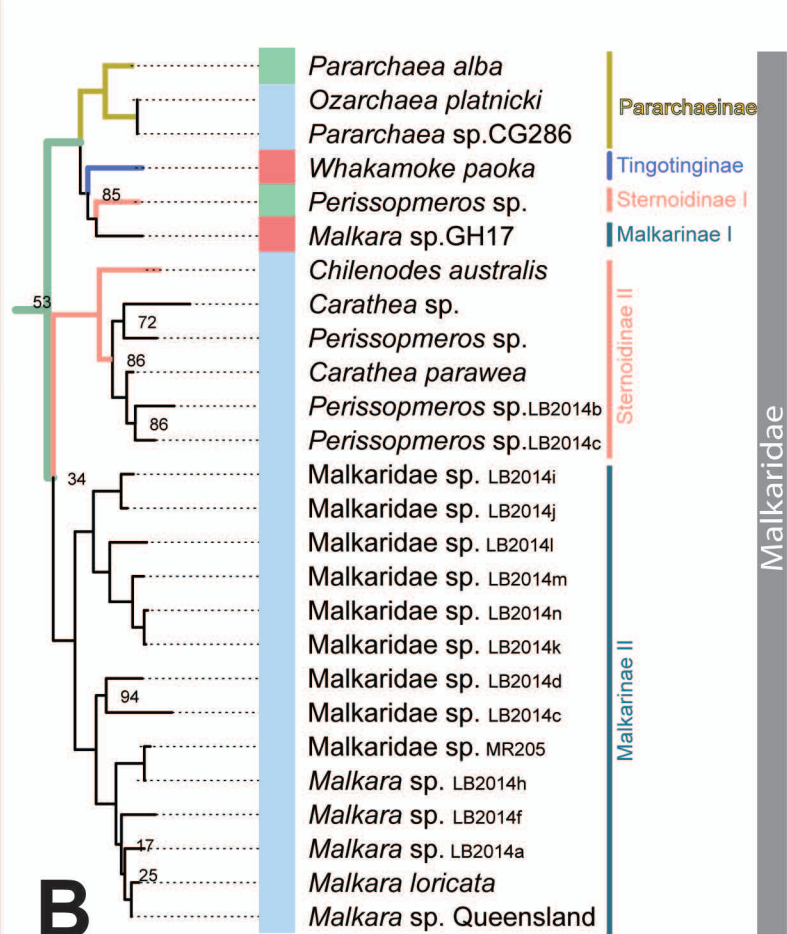
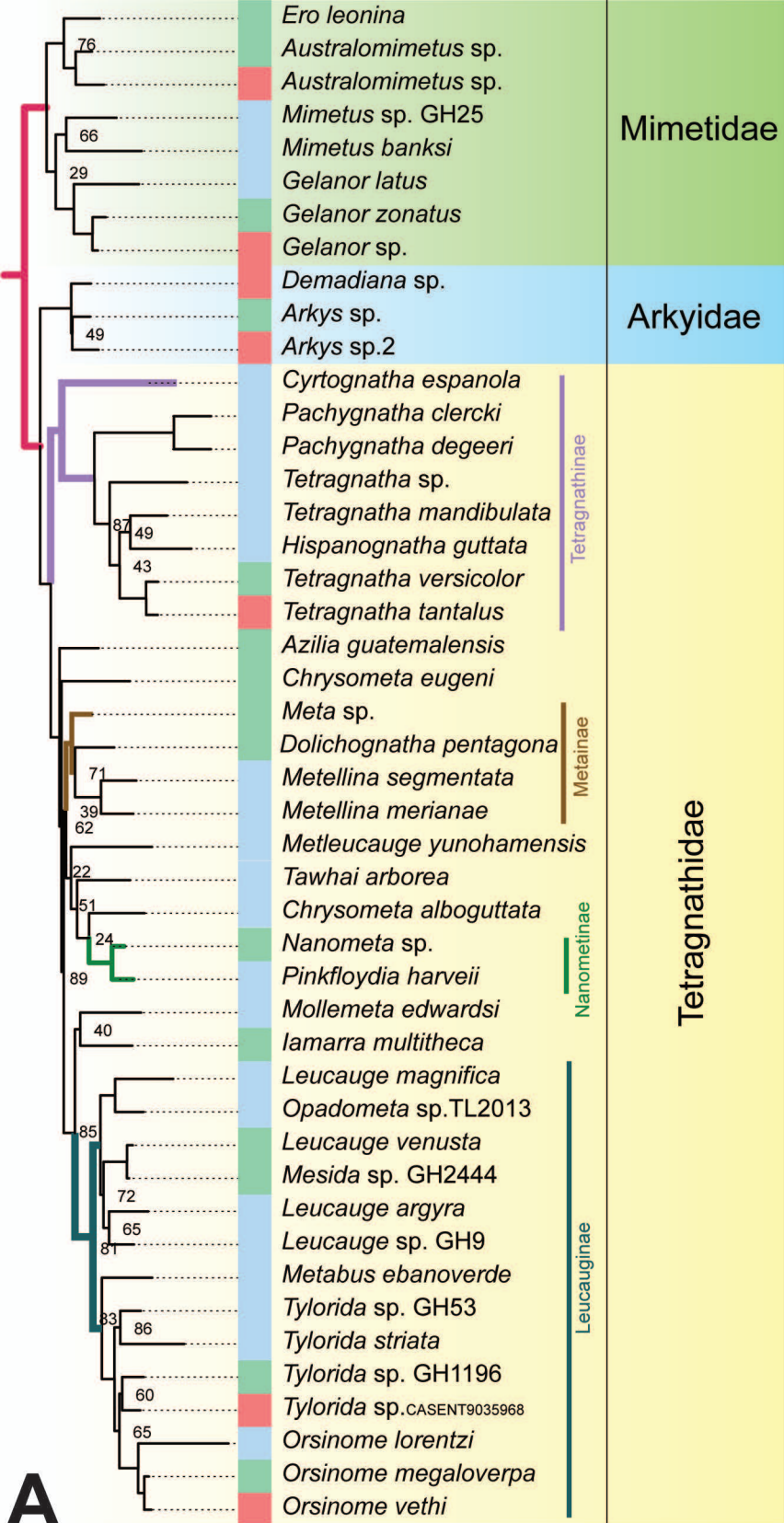


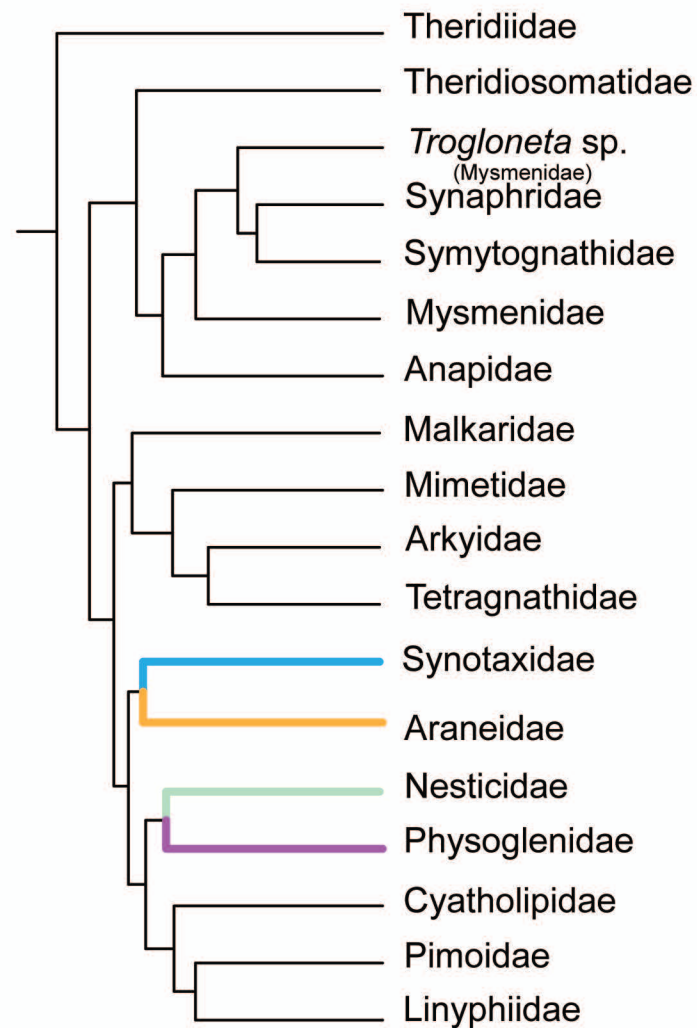
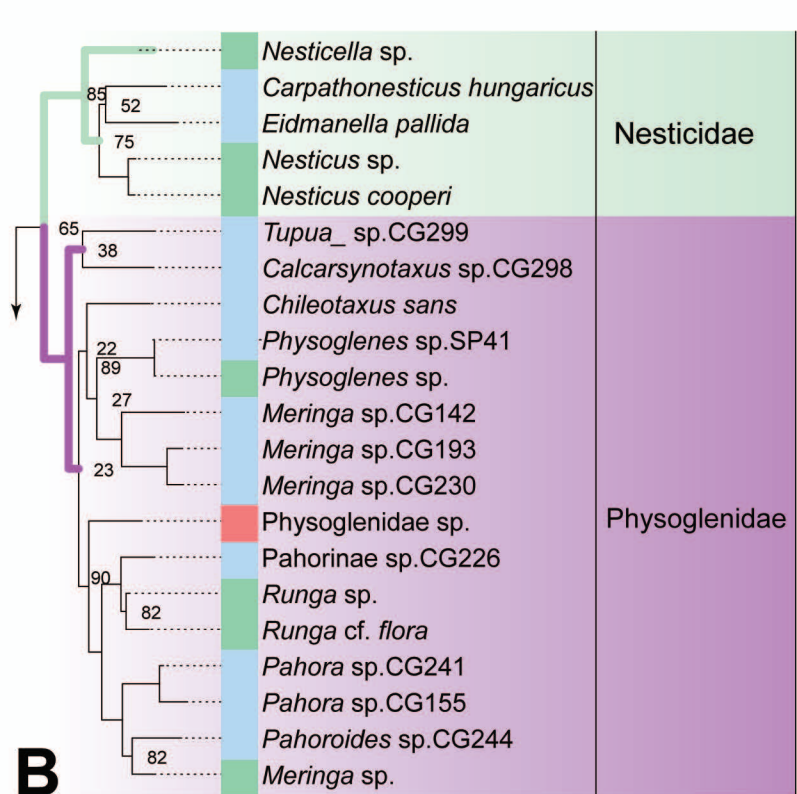
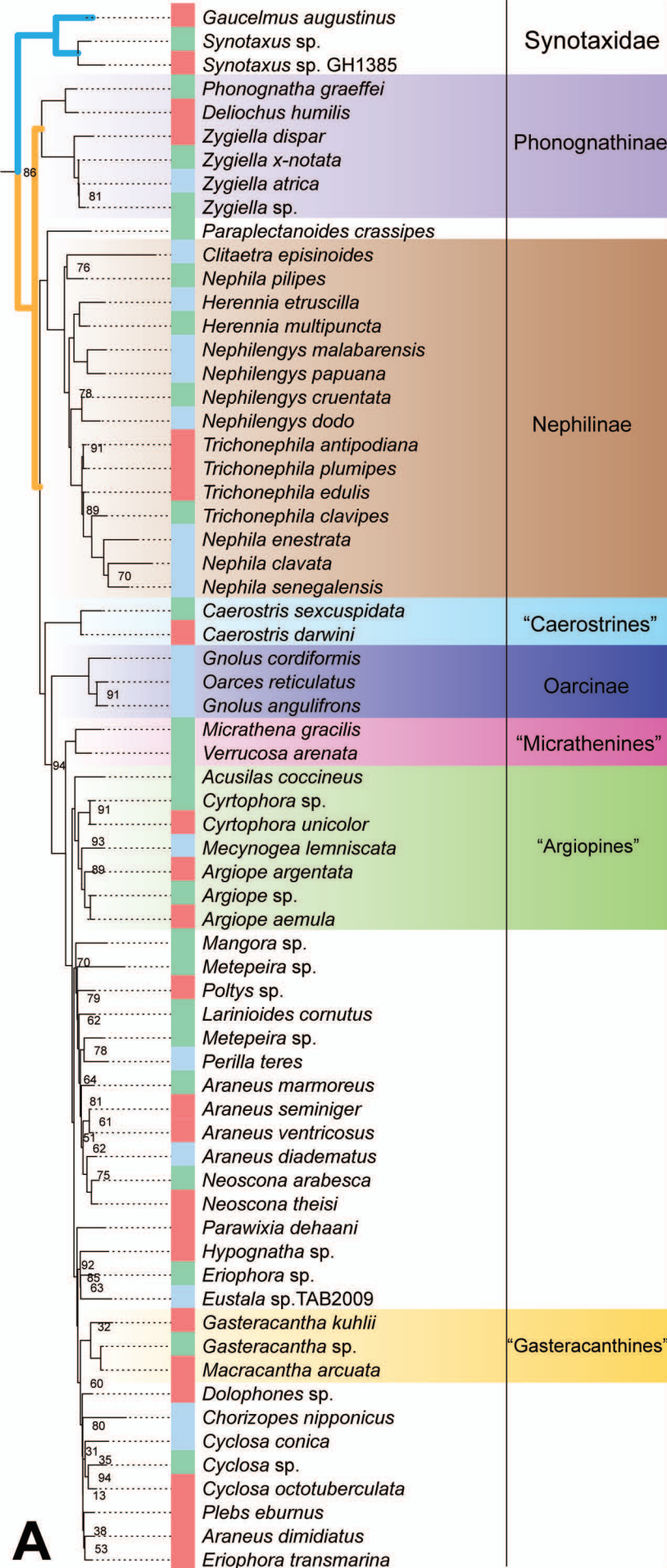
Palpimanoidea

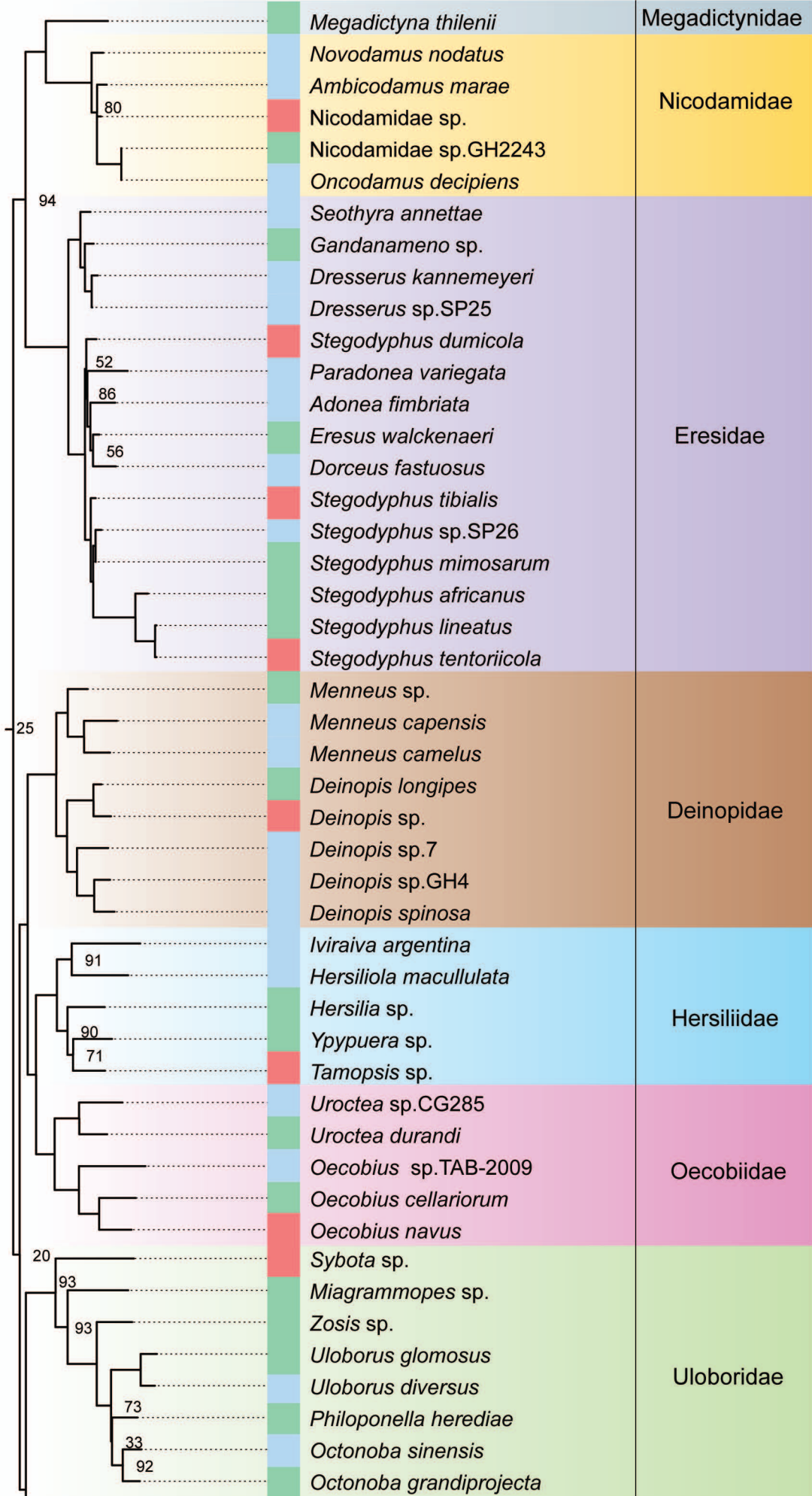




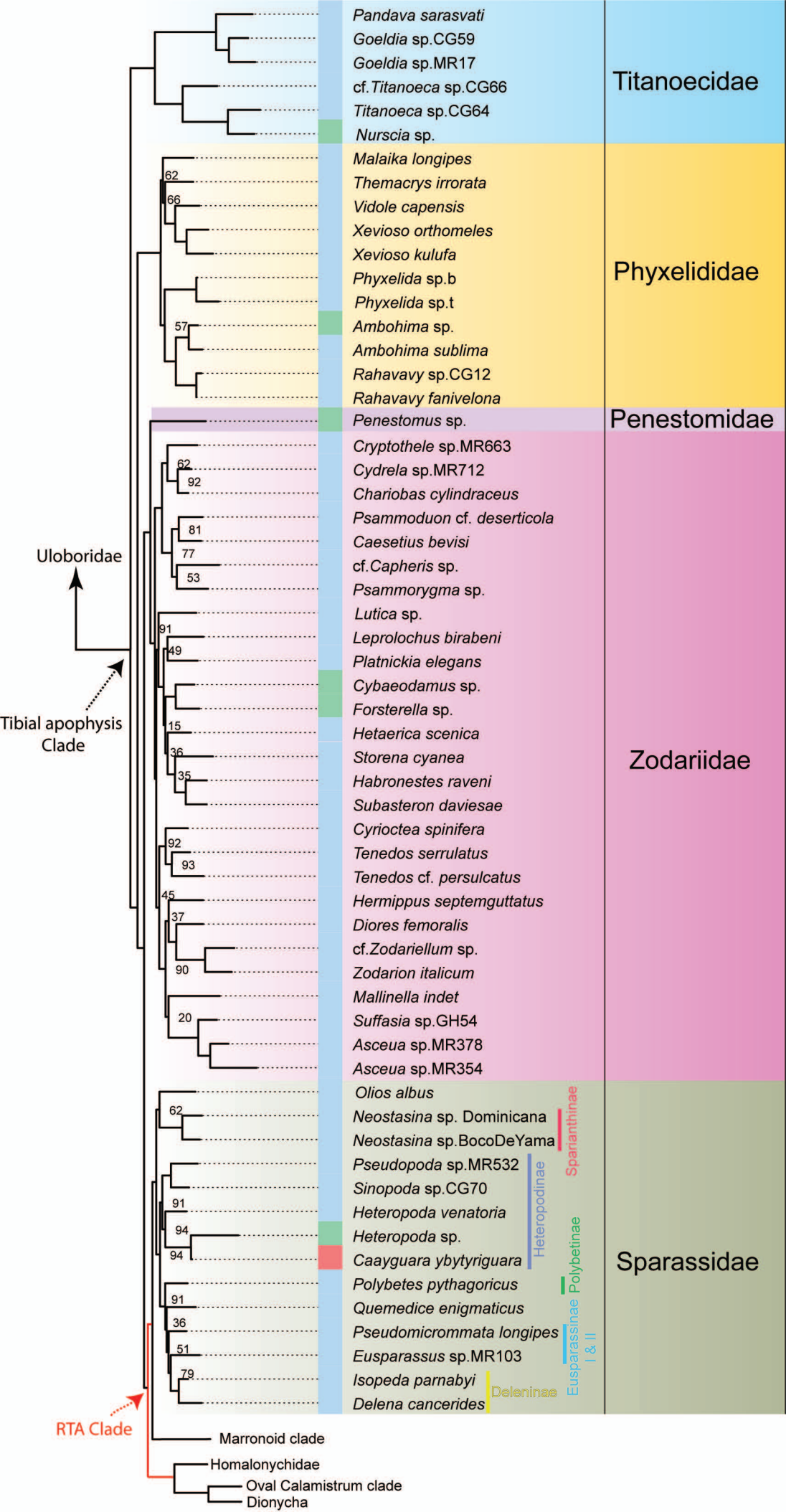


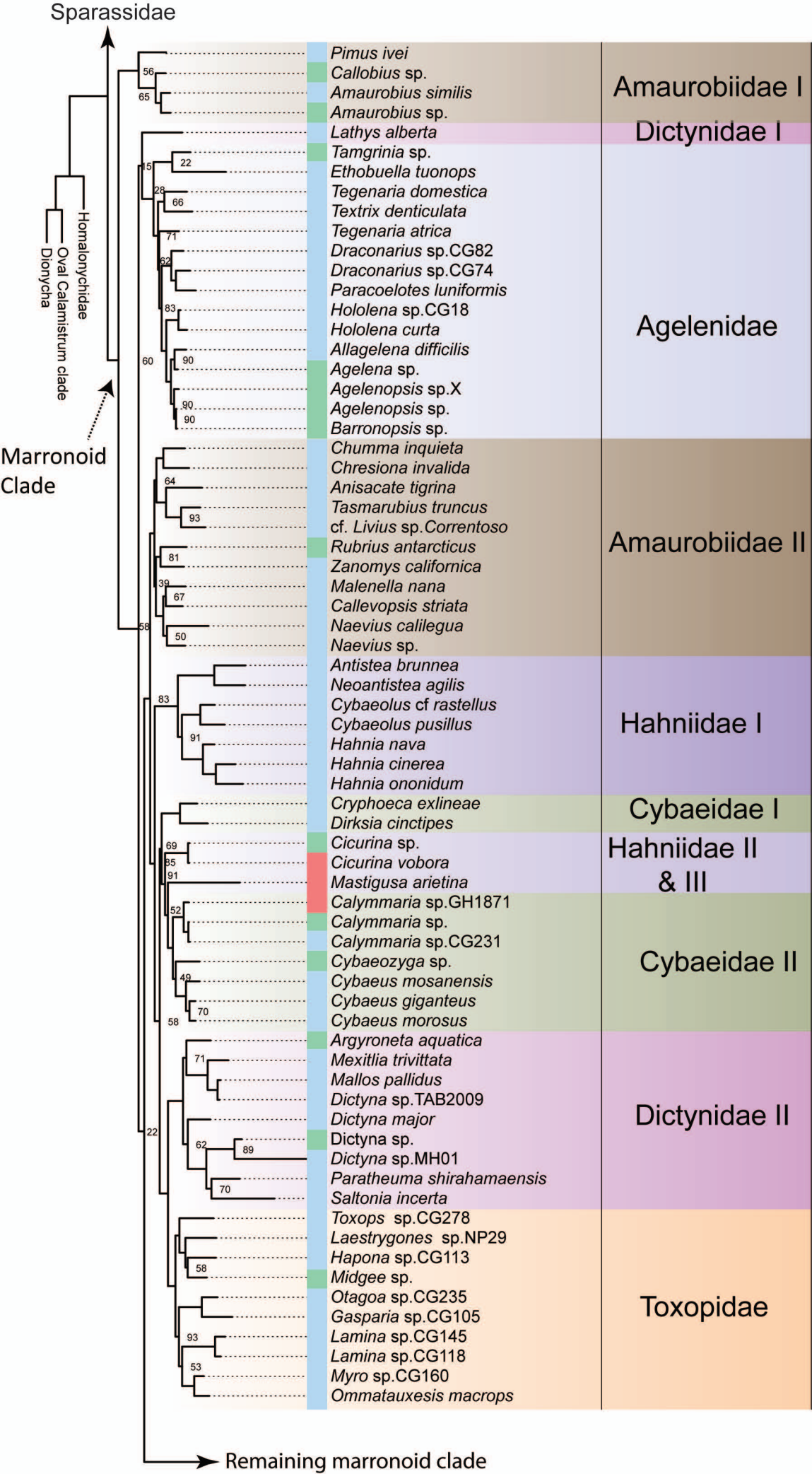




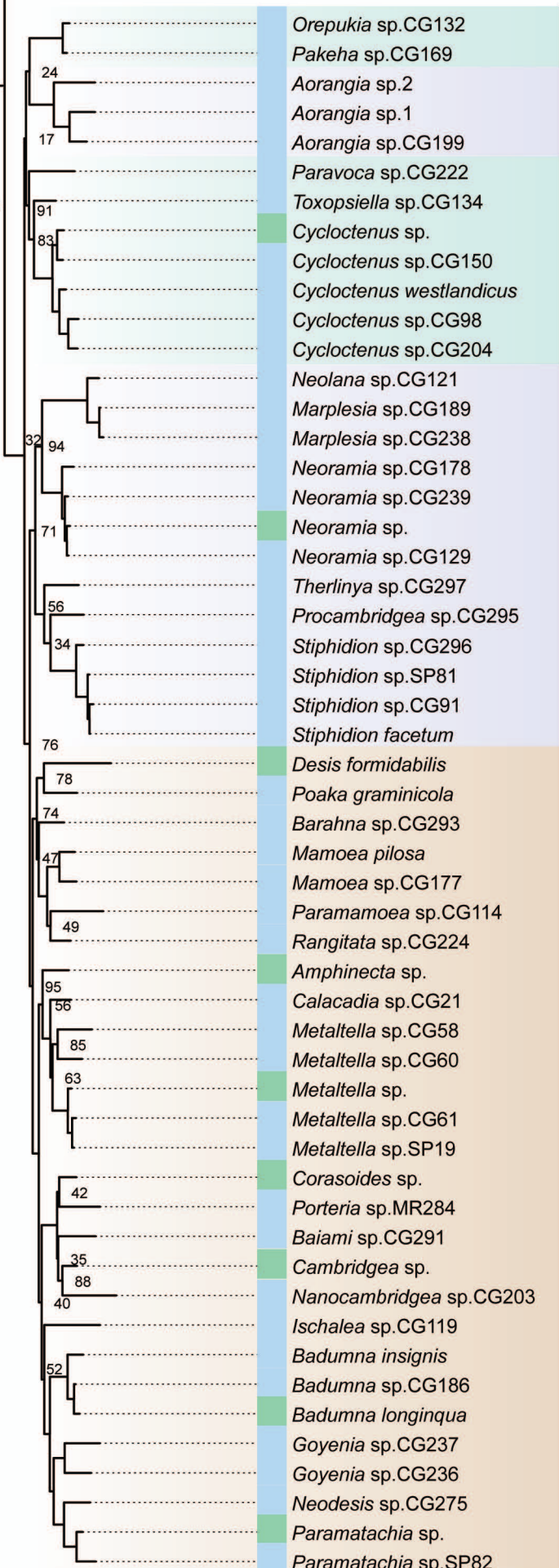
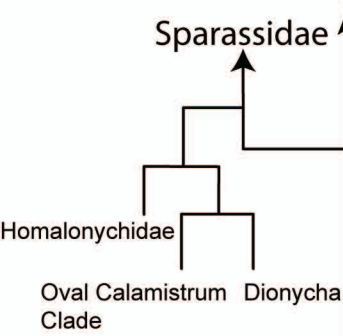


Tibial apophysis Clade





Remaining marronoid clade



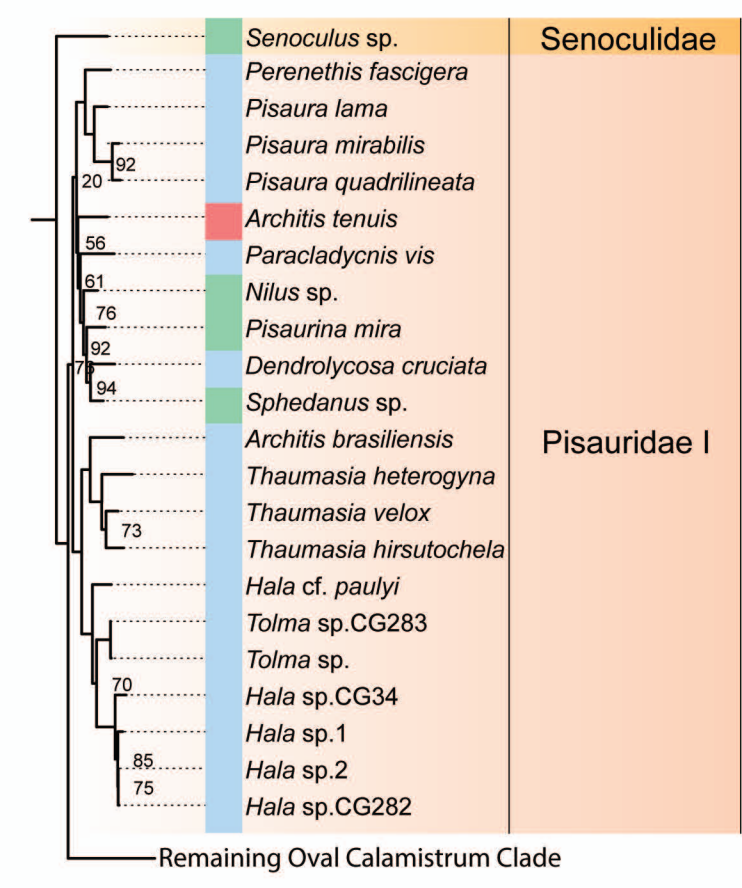
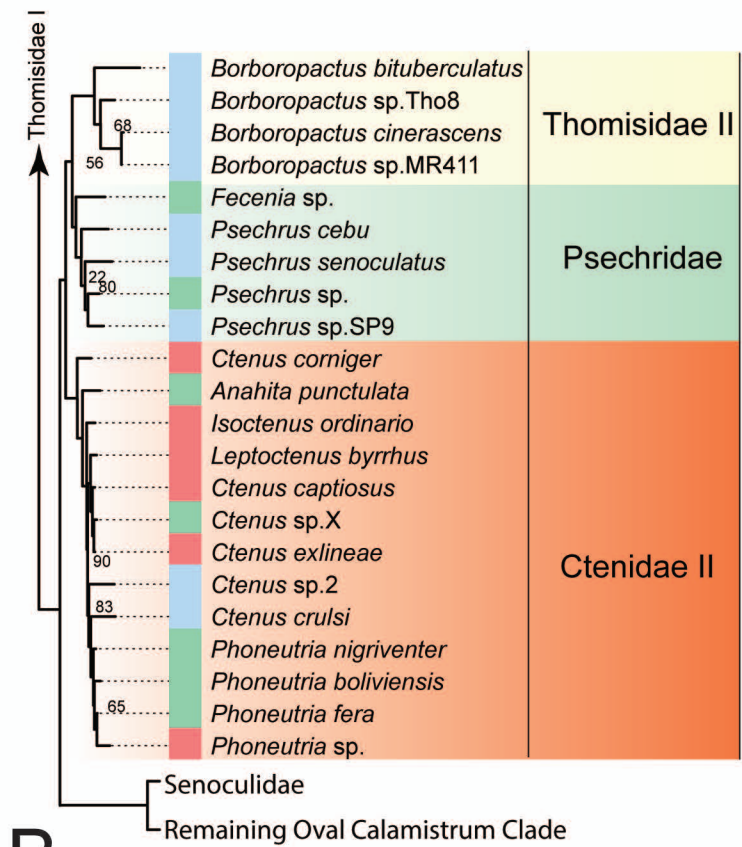
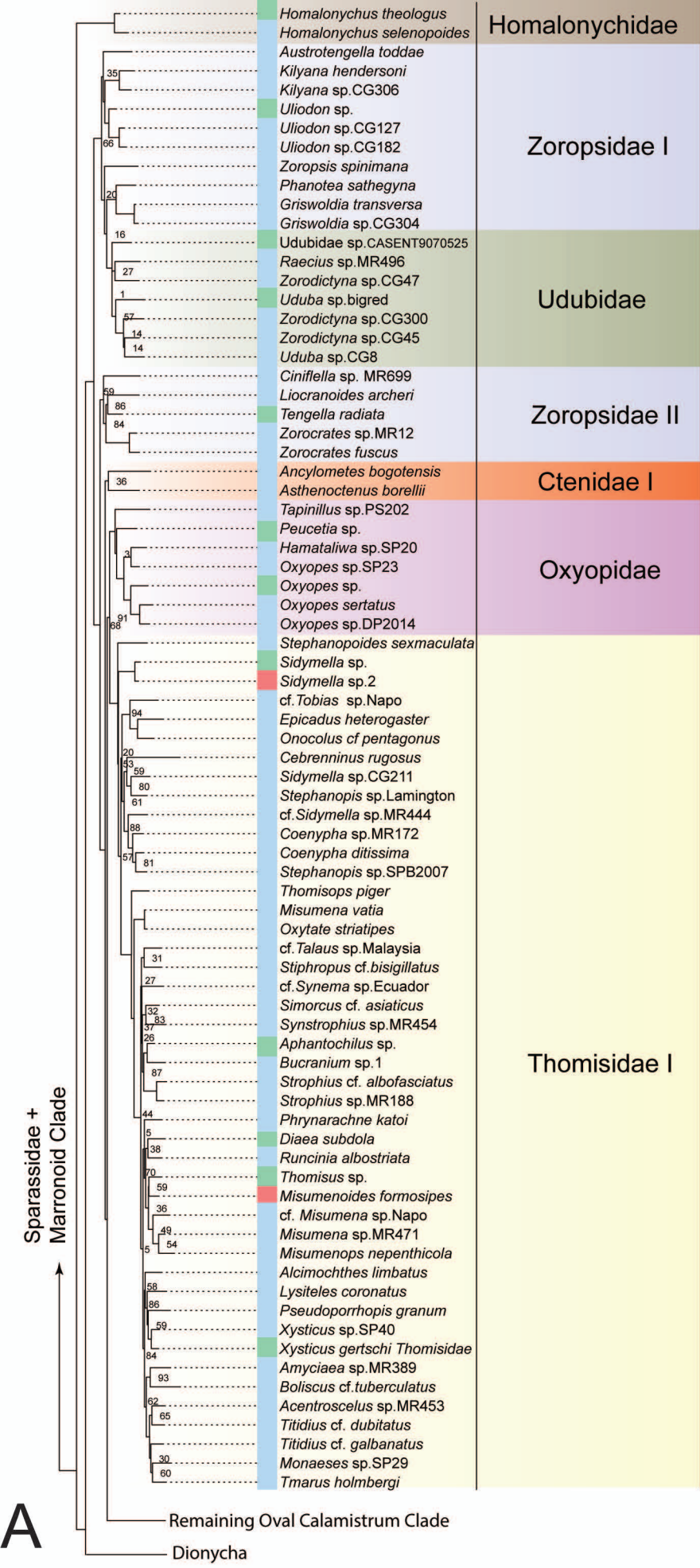
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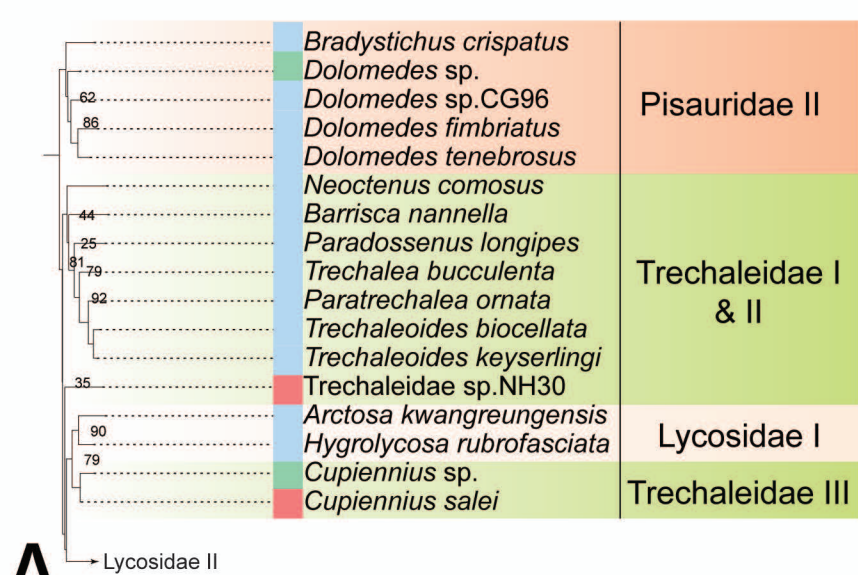
Stiphidiidae I

Cyclotenidae II

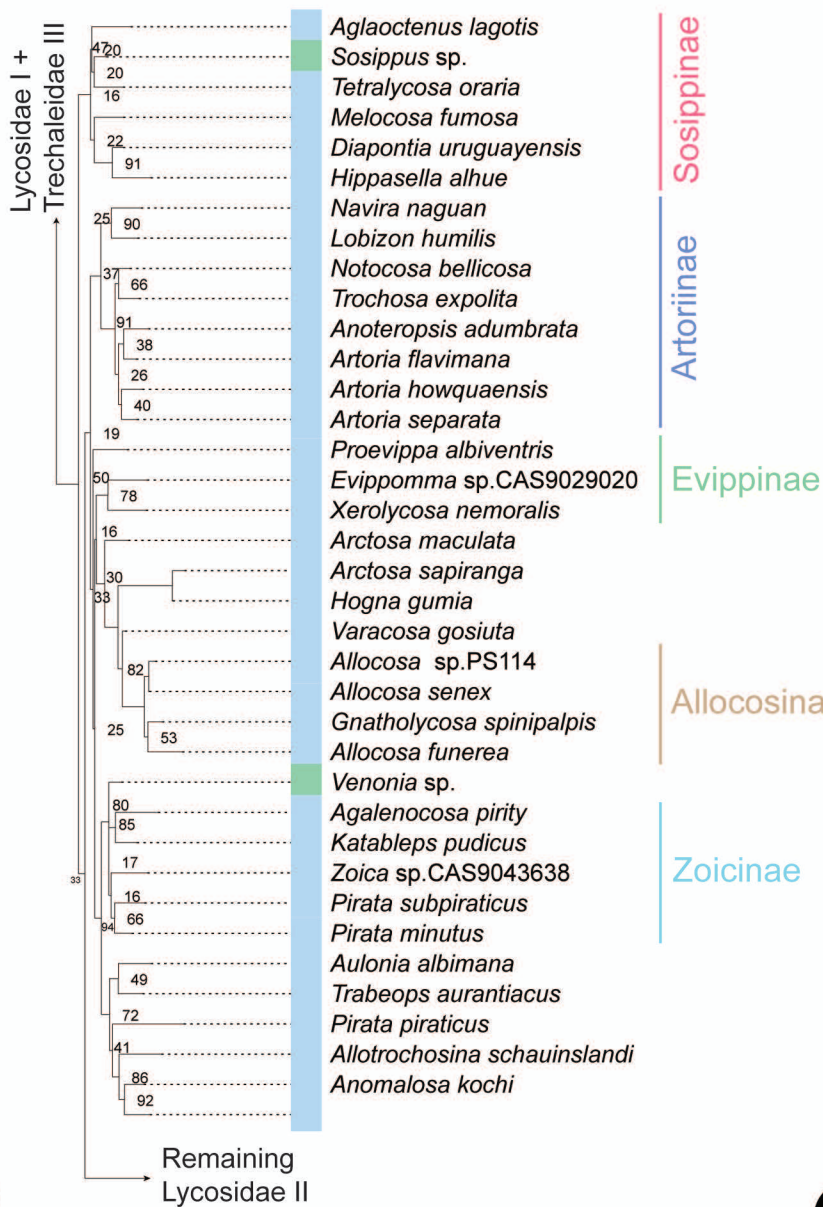
Stiphidiidae II

Desidae I

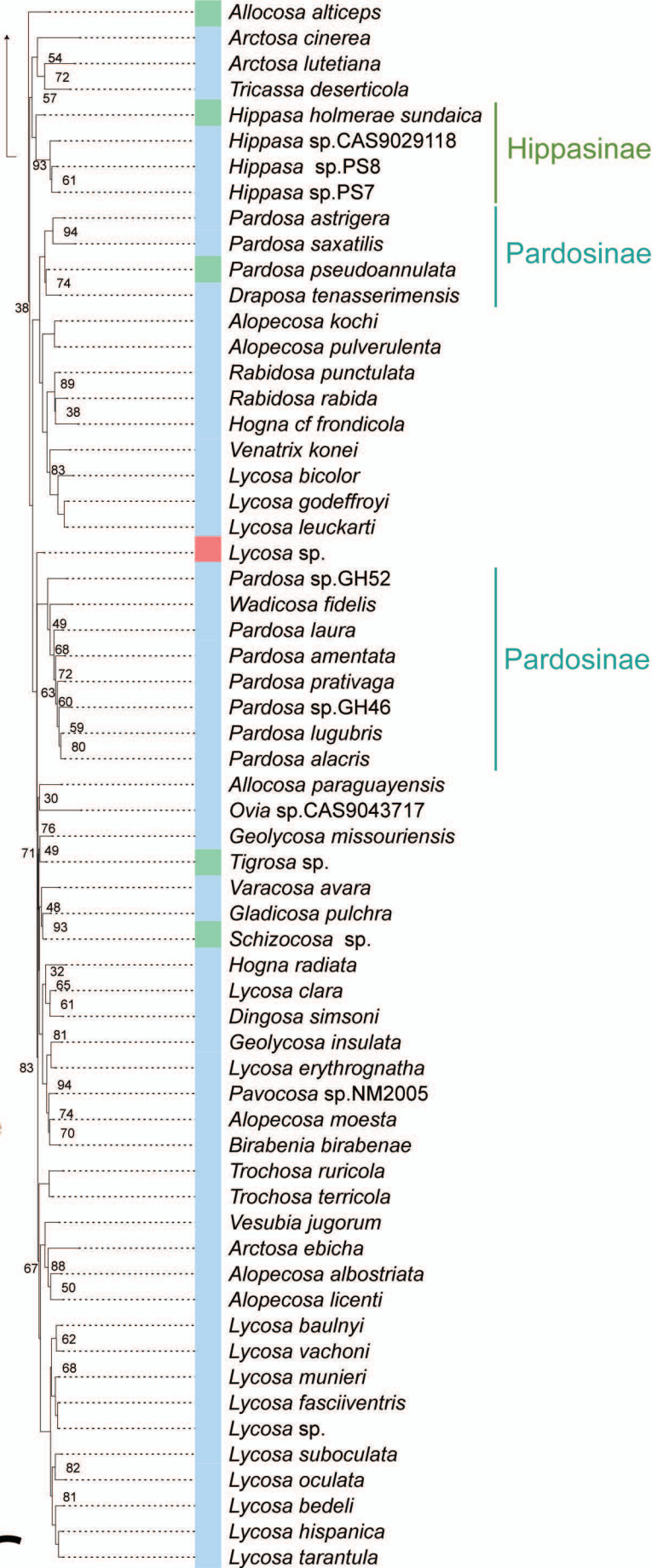




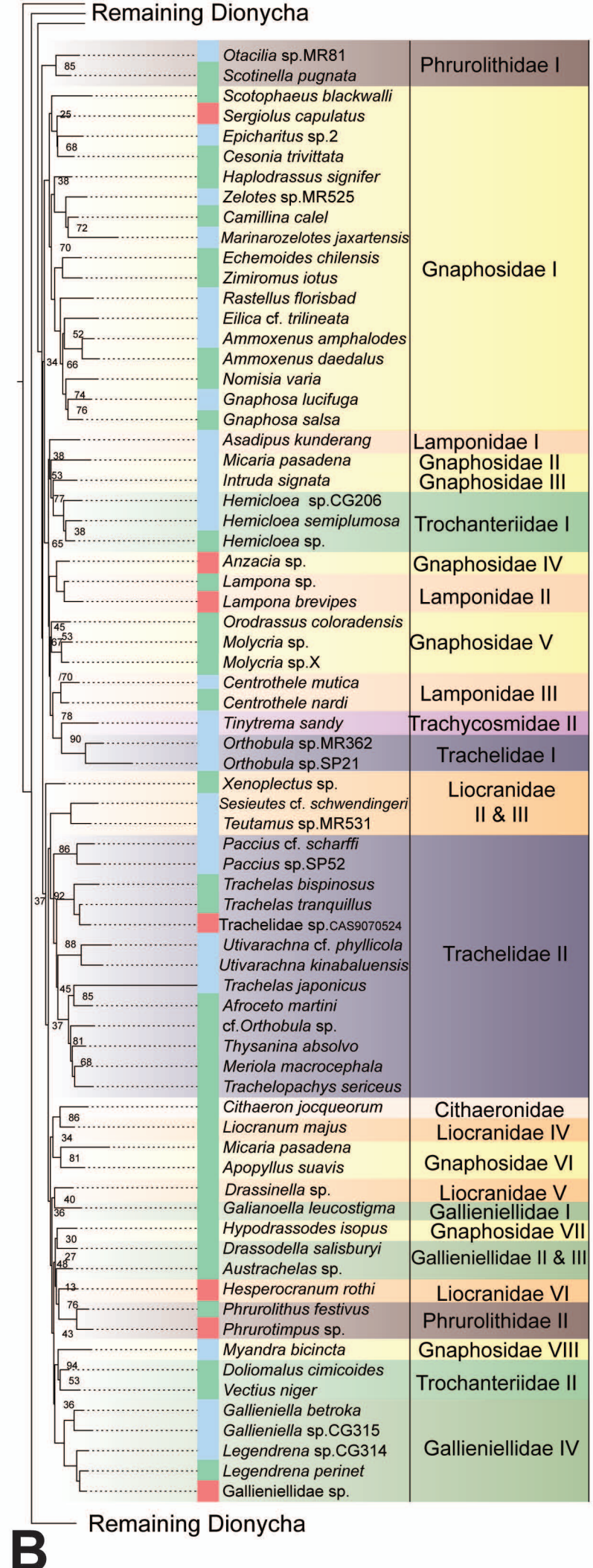
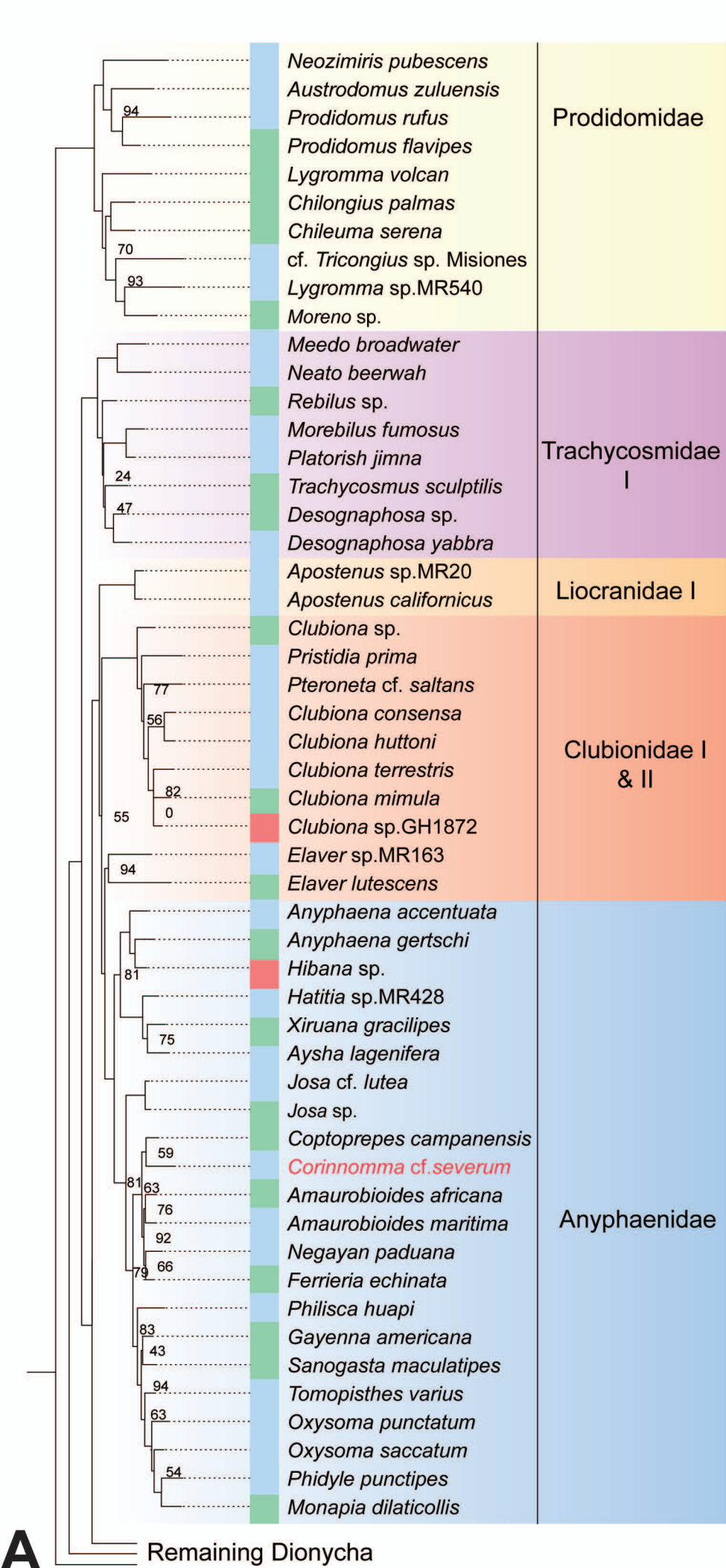
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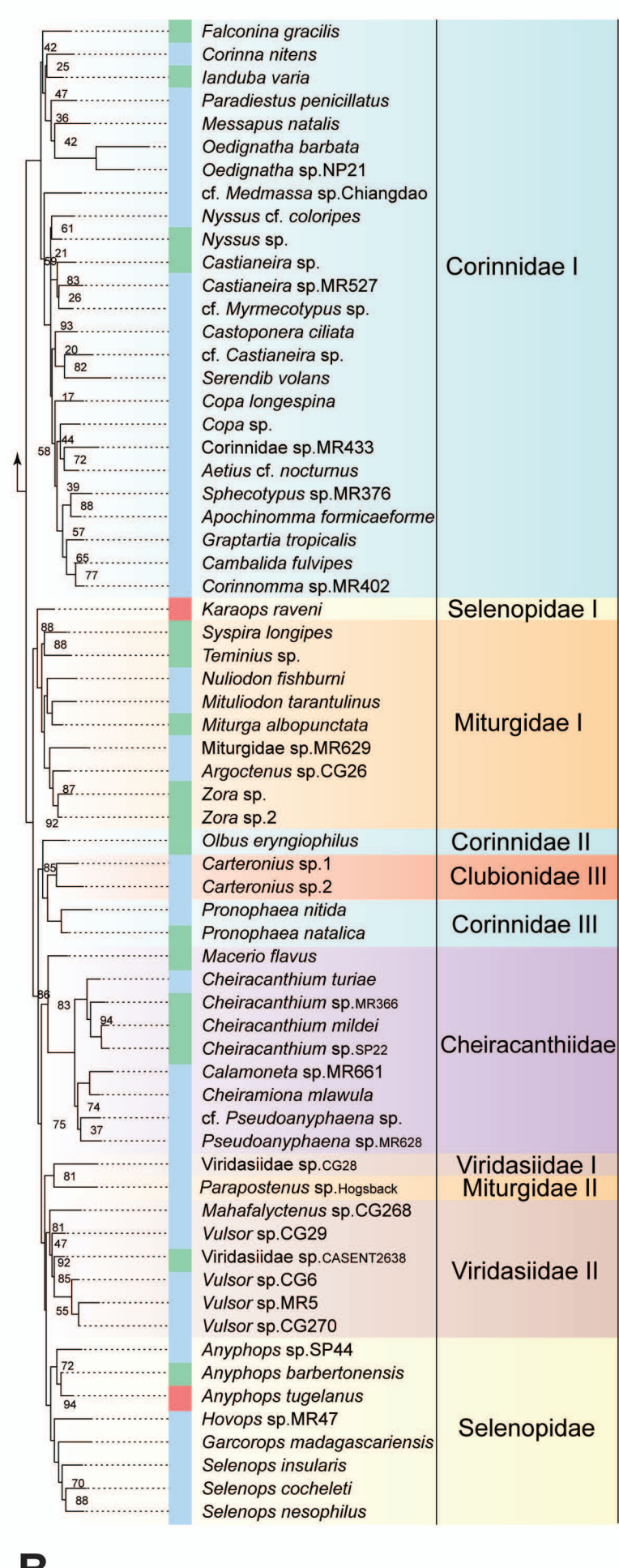
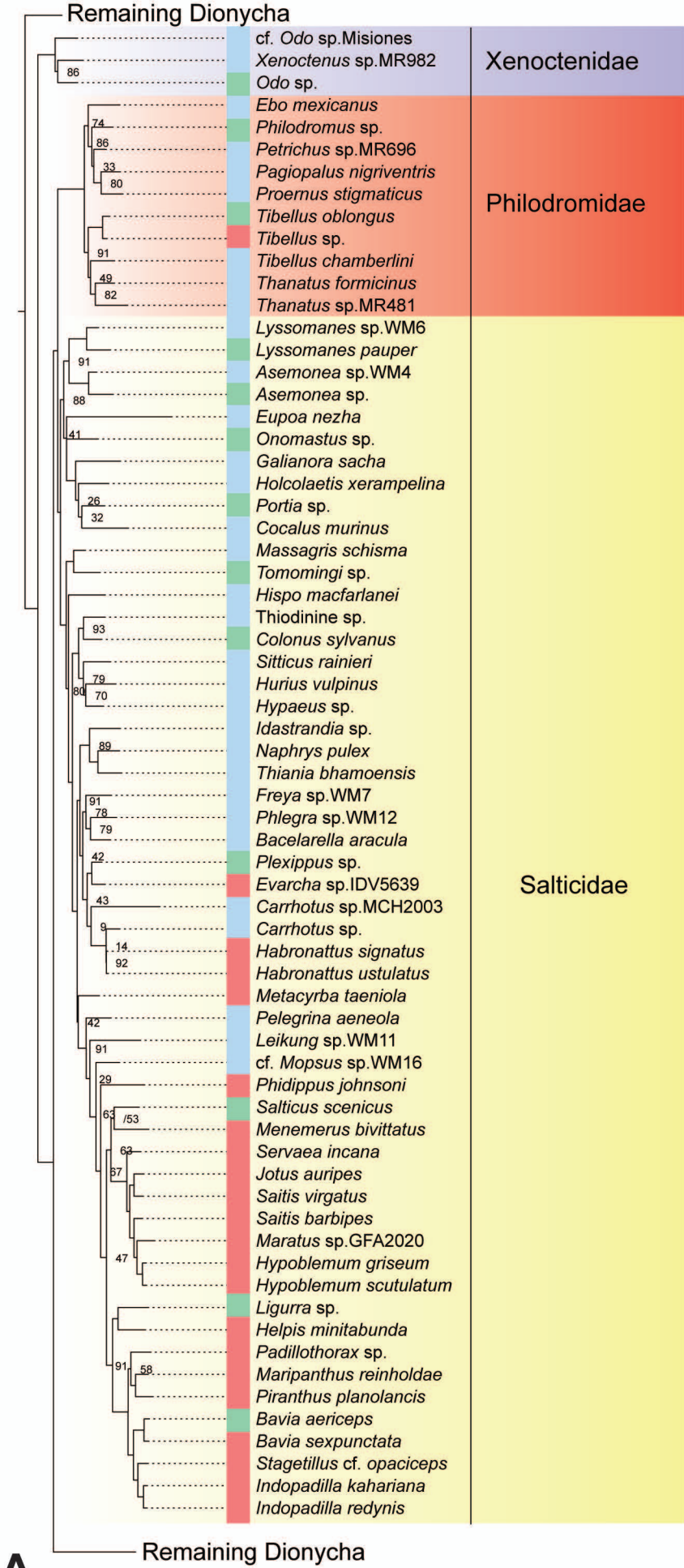


B



C





A

B