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Combining genomic, phenotypic and Sanger sequencing data to elucidate the phylogeny of the two-clawed spiders (Dionycha)

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ABSTRACT

The importance of morphology in the phylogenomic era has recently gained attention, but relatively few studies have combined both types of information when inferring phylogenetic relationships. Sanger sequencing legacy data can also be important for understanding evolutionary relationships. The possibility of combining genomic, morphological and Sanger data in one analysis seems compelling, permitting a more complete sampling and yielding a comprehensive view of the evolution of a group. Here we used these three data types to elucidate the systematics and evolution of the Dionycha, a highly diverse group of spiders relatively underrepresented in phylogenetic studies. The datasets were analyzed separately and combined under different inference methods, including a novel approach for analyzing morphological matrices with commonly used evolutionary models. We tested alternative hypotheses of relationships and performed simulations to investigate the accuracy of our findings. We provide a comprehensive and thorough phylogenetic hypothesis for Dionycha that can serve as a robust framework to test hypotheses about the evolution of key characters. We also show that morphological data might have a phylogenetic impact, even when massively outweighed by molecular data. Our approach to analyze morphological data may serve as an alternative to the proposed practice of arbitrarily partitioning, weighting, and choosing between parsimony and stochastic models. As a result of our findings, we propose Trachycosmidae new rank for a group of Australian genera formerly included in Trochanteriidae and Gallieniellidae, and consider Ammoxenidae as a junior synonym of Gnaphosidae. We restore the family rank for Prodidomidae, but transfer the subfamily Molycriinae to Gnaphosidae. *Drassinella* is transferred to Liocranidae, *Donuea* to Corinnidae, and *Mahafalytenus* to Viridasiidae.

1. Introduction

In the field of phylogenetic systematics, morphology was the earliest and primary source of data used to trace evolutionary history (Hennig, 1965). Later, phylogenies based on Sanger sequencing data of single (or a few) markers brought results sometimes contradicting standard views of morphological evolution (e.g. Aguinaldo, 1997). Advances in nucleotide sequencing technologies allowed the acquisition of impressive amounts of genetic data at relatively low cost, leading to the frequent use of assembled transcriptomes for phylogenetic inference (Lemmon and Lemmon, 2013; McCormack et al., 2013). Most recently, sequence capture methods have emerged as a cost effective alternative for acquiring data at the genomic level for phylogenetic studies (Faircloth et al., 2012; Zhang et al., 2019), and have certainly brought additional insights into the fields of systematics and evolution (Faircloth et al., 2015; Gueuning et al., 2020; Hedin et al., 2019). However, the role of small Sanger sequencing datasets and morphological matrices in phylogenetic studies began to be debated as thousands of genomic loci generated by next generation sequencing techniques became readily available (Ruane et al., 2015; Scotland et al., 2003; Wiens, 2004).

The importance of morphology in the phylogenomic era has gained renewed attention (Flores et al., 2020; Giribet, 2015, 2010; Lee and Palci, 2015; Lopardo et al., 2011), but only a few studies have used both genomic scale data and morphology in a combined analysis (Mongiardino Koch and Thompson, 2020; Neumann et al., 2020; Scarpetta, 2020). Sanger sequencing legacy data can also be important for

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understanding evolutionary and ecological processes (e.g. Fernández et al., 2018; Macías-Hernández et al., 2020). Studies have demonstrated that it is possible to retrieve the traditional Sanger sequencing markers (such as Cytochrome *C* Oxidase I, 28S and others) from genomic libraries obtained with high throughput sequencing methods (Derkarabetian et al., 2019; Do Amaral et al., 2015; Hedin et al., 2018; Zarza et al., 2016). Additionally, ultraconserved elements (UCEs) can be obtained from transcriptome libraries, enabling the combination of data produced with the two different methodologies (Bossert et al., 2019; Hedin et al., 2018; Kulkarni et al., 2021). The possibility of combining these four data types in one analysis seems very compelling, since it could allow the best use of already available data, permitting a more complete sampling and yielding a more comprehensive (and possibly more accurate) view of the evolution of a group of organisms. The use of morphological data may also be important for guiding taxonomic decisions and proposing diagnoses for taxa.

Araneae is a group with an important accumulation of legacy morphological and Sanger sequencing data (Crews et al., 2010; Griswold et al., 2005; Maddison et al., 2014; Ramírez, 2014; Silva Davila, 2003; Wheeler et al., 2017). Phylogenomic and transcriptomic methods have

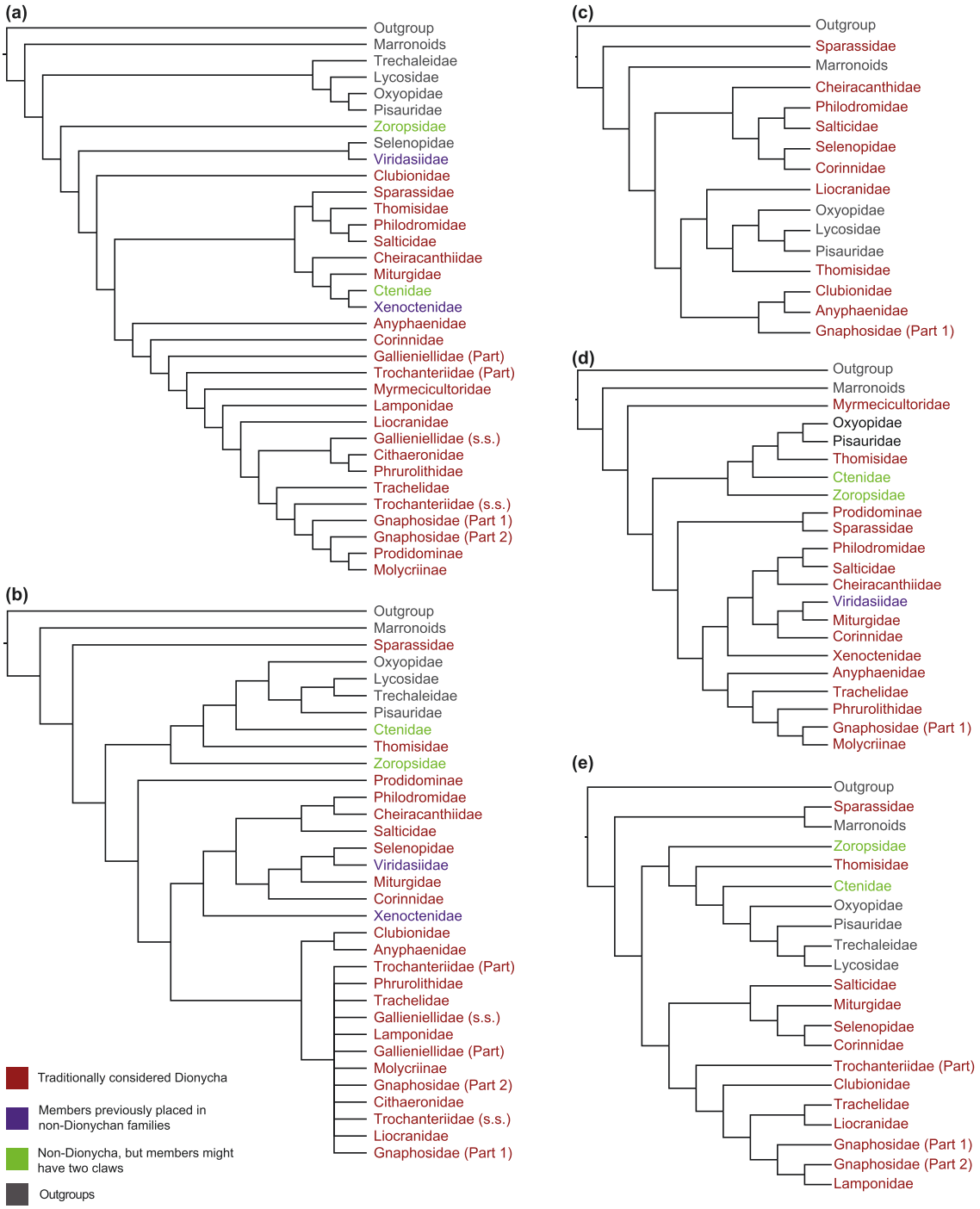

Fig. 1. Previous phylogenetic hypotheses for dionychan relationships. (a) morphological data (Ramírez, 2014); (b) Sanger molecular data (Wheeler et al., 2017); (c) Sanger molecular (Moradmand et al., 2014); (d) total evidence (Ramírez et al., 2019); (e) genomic (UCE) data (Kulkarni et al., 2020).

also recently been applied in higher-level systematics and evolutionary studies of spiders (Fernández et al., 2018; Garrison et al., 2016; Kallal et al., 2020; Kulkarni et al., 2020; Opatova et al., 2020; Ramírez et al., 2021; Xu et al., 2020). Although some spider clades have been studied using a combined analysis of morphology and traditional molecular markers (Blackledge et al., 2009; Bond et al., 2012; Polotow et al., 2015), none have yet been explored using the combination of genomic, transcriptomic, traditional loci and morphological data. This may be worthwhile, particularly in clades in which the phylogenetic relationships are still obscure, as in Dionychan spiders.

Dionycha is a clade of two-clawed spiders, currently with 19 families and comprises about 30% of all described spider species (WSC, 2021). They can be found in a variety of habitats worldwide, from deserts to tropical forests. Dionychan families are underrepresented in phylogenomic/transcriptomic studies to date, and the few studies addressing higher-level relationships using traditional molecular markers show conflicts with morphological datasets, recovering several families as paraphyletic (Moradmand et al., 2014; Ramírez, 2014; Ramírez et al., 2019; Wheeler et al., 2017). For example, huntsman spiders (Sparassidae), crab spiders (Thomisidae) and a recently described myrmecophagic family (Myrmecicultoridae) were thought to be dionychans based on morphology, but molecular and total evidence suggests that they belong elsewhere in the spider tree of life (Fig. 1). Gnaphosoids, or the Oblique Medium Tapetum Clade, have long been referred to as a monophyletic assemblage inside Dionycha, but molecular phylogenies show this group as polyphyletic. However, Dionycha phylogenies based on traditional molecular markers present low support for internal clades and very short branch lengths (Wheeler et al., 2017). Such conflicts and uncertainties in Dionychan relationships hamper a better understanding of the evolution of this intriguing group of spiders.

In this study we aimed to elucidate the phylogenetic relationships of families in Dionycha. We generated a UCE dataset and complemented our sampling with published transcriptome and Sanger sequencing data. We built upon an existing morphological matrix and scored those characters for additional taxa with molecular data. Datasets were analyzed separately and combined under different inference methods, including a novel approach for analyzing morphological matrices with commonly used stochastic evolutionary models. We also performed topology tests of alternative hypotheses and explored possible inference errors using simulations under the censored coalescent model. Lastly, we explored the influence of morphology in combined evidence phylogenetic inference.

2. Material and methods

2.1. Taxon sampling

Outgroup sampling was designed to include a broad representation of the Retrolateral Tibial Apophysis clade (which includes Dionycha), including most families in the "marronoid" and Oval Calamistrum clade (OCC; Wheeler et al., 2017). A zodariid spider was used to root the tree based on most recent phylogenetic results (Kallal et al., 2020; Wheeler et al., 2017). Representatives of all families currently and formerly placed in Dionycha were sampled for at least two datasets (phenotypic, genomic, or legacy markers). We made sure to sample different lineages of families that are suspected of paraphyly based on previous studies. The list of sampled species with corresponding museum vouchers and GenBank and SRA accession numbers is available in the Supplementary Online Material (Appendix S1). Raw reads generated for this study are deposited under the BioProject PRJNA766666.

2.2. Phenotypic data

Phenotypic (morphological and behavioral) data was based on the matrix published by Ramírez (2014). New characters and adjustments to character states and coding were made based on new observations and recently published data (Azevedo et al., 2018; Rodrigues and Rheims, 2020). Taxa were scored from specimen observations, whenever possible. Published images, matrices and descriptions were used to complement the scoring and reduce missing data. The final matrix consisted of 400 characters scored for 130 species in 129 genera. The character argumentation is available in the Supplementary Online Material (Appendix S2) and the matrix is deposited on FigShare (https://doi.org/10.6084/m9.figshare.14977185).

2.3. Genomic data

Library preparation for UCEs followed Starrett et al. (2017) using the Arachnid probe set (Faircloth, 2017). Sequencing was conducted using an Illumina HiSeq 2000 platform with 150 bp paired end reads at Brigham Young University, UT. Sequences were processed using the programs mentioned below, as implemented in the PHYLUCE v. 1.6.8 (Faircloth, 2016) pipeline. Sequence reads were trimmed using TRIMMOMATIC v. 0.39 (Bolger et al., 2014), with parameters –min_len 40 and –phred 33, and assembled using VELVET v. 1.2.10 (Zerbino and Birney, 2008) with kmer = 31. Probe matching was done using 75 minimum identity and 75 minimum coverage values. To complement our original sampling, published UCE and transcriptome sequences were downloaded from the Sequence Read Archives using fastq-dump v. 2.8.2 (SRA Toolkit Development Team: http://ncbi.github.io/sra-tools/) with the flags –gzip –clip –split-files –qual-filter to remove adapters and trim low-quality base calls. The sequences were assembled, processed and matched to UCE probes, as above. Data were aligned using MAFFT v. 7.455 (Katoh and Standley, 2013), with parameters –max_divergence 0.2, min_length 100, -–no_trim False, –notstrict True, –proportion 0.65, –threshold 0.65 and –window:20, as implemented in PHYLUCE. Alignments were trimmed with GBLOCKS v. 0.31b with parameters –b1 0.60 \–b2 0.75 \–b3 10 \–b4 8.

Incorrect orthology assignments can sometimes be a problem with UCEs (Yuan et al., 2019). To filter out possible paralogs in an automated and less biased manner, we took two complementary approaches. We first used TREESHRINK v. 1.3.1 (Mai and Mirarab, 2018) to objectively remove taxa found on long gene tree branches. We then performed a Blastx search against the *Parasteatoda tepidariorum* genome and excluded UCE alignments with sequences matching different proteins. Details of this process are in the Supplementary Material Appendix S3.

After paralogy and length filtering, a 55% completeness matrix (28776 bp, 197 loci) was created and used for further analyses, except for ASTRAL v. 5.6.3 (Zhang et al., 2018) inferences (see section 2.5). Molloy and Warnow (2018) showed that removing loci because of missing taxa could reduce accuracy in ASTRAL. Therefore, we used all 543 UCE loci, (corresponding to a minimum threshold of 9% completeness) for coalescent-based analyses in ASTRAL. UCE matrices are deposited on FigShare (https://doi.org/10.6084/m9.figshare.14977185).

2.4. Legacy loci data

We used the approach presented in Hedin et al. (2018) to obtain the sequences of six common legacy markers ("the usual suspects") used in spider studies (12S, 16S, 18S, 28S, COI and H3) from our genomic data. Details of this procedure are explained in the Supplementary Material Appendix S3. To complement our original sampling, especially for taxa lacking genomic data, we downloaded sequences from GenBank. Each of the legacy loci matrices were aligned using MAFFT and terminals with long branches were removed from alignments using TREESHRINK (see above). The alignments (totaling 6330 bp) are deposited on FigShare (https://doi.org/10.6084/m9.figshare.14977185).

2.5. Phylogenetic analyses

Phenotypic Matrix. Phylogenetic inference based on the phenotypic

data was conducted under a maximum likelihood approach in IQTREE v1.6.2 (Nguyen et al., 2015). We first considered each character as a partition, similar to a "parsimony" or the non-common mechanism model (Tuffley and Steel, 1997). Then, we ran MODELFINDER (Kalyaanamoorthy et al., 2017) in IQTREE with the –m MFPMERGE option to find the best partitioning scheme and best model for each partition with independent rates (-spp flag). Best model searches included variations of the Mk model (Lewis, 2001), i.e., rate heterogeneity across sites (I + G), different state frequencies and ascertainment bias. With this approach we objectively compared and chose between a parsimony-like model, a variety of Mk partitioned models and a single unpartitioned model. We expected to overcome some problems associated with the application of DNA sequence models to morphological characters, such as the greater heterogeneity between characters (rate, frequency and number of states are very different across the matrix) and the fact that the states do not represent the same thing across the matrix. We also expected to overcome problems related to parsimony, like high level of homoplasy (characters with high rates of evolution), without the need to subjectively choose weights or concavity function parameters, as in implied weighting analyses (Goloboff et al., 2008).

Genomic Matrices. The genomic data were analyzed under a concatenated maximum likelihood approach and two coalescent approaches. For the concatenated analysis, the 55% completeness matrix was partitioned by UCE locus. MODELFINDER in IQTREE with –m MFPMERGE and -mset mrbayes options were used to find the best partitioning scheme and best model for each partition. The tree search was done in IQTREE using partition proportional branch lengths (-spp flag). The same matrix was used for the coalescent method SVDquartets (Chifman and Kubatko, 2014) in PAUP* (Swofford, 2002) using the default settings.

For the coalescent method in ASTRAL (Zhang et al., 2018), all UCEs that passed through our filters were used. However, the UCEs were curated to make sure only independent loci were used for gene tree inference (Hedin et al., 2018; Van Dam et al., 2021). Details can be found in Appendix S3.

Legacy Loci Matrices. All six legacy loci matrices were analyzed in a concatenated maximum likelihood framework in IQTREE, as for genomic matrices. Besides concatenation, we also estimated separate gene trees for the mitochondrial (12S, 16S and COI), ribosomal (18S and 28S) and nuclear (H3) genes.

Combined Legacy and Genomic Matrices. Legacy data and the 55% completeness genomic matrix were combined and analyzed in a concatenated maximum likelihood approach in IQTREE. The best models and partitions found in the previous individual analyses were used for this inference.

Total Evidence Analysis. The 55% completeness genomic matrix, the legacy Sanger matrices and the phenotypic data were combined for a total evidence maximum likelihood analysis using IQTREE. Best models and partitions found in previous individual analyses were used for this inference. To explore the effects of morphology, we compared the topology and ultrafast bootstrap values of the total evidence analysis with the results yielded by the molecular (legacy and genomic matrices) data analysis.

Topology tests. Based on previous phylogenetic and taxonomic hypotheses, we ran topology tests with constrained topology searches in IQTREE and compared the constraint tree results to trees found in total evidence unconstrained analyses. The main hypotheses tested involved the position of Sparassidae, Myrmecicultoridae and Thomisidae as dionychans; the position of Prodidominae as a gnaphosid offshoot or as sister to the remaining Dionycha; Ammoxenidae as a gnaphosid offshoot or as sister to Cithaeronidae; the monophyly of Gnaphosidae; Gallieniellids as monophyletic, diphyletic or polyphyletic; Cheiracanthiidae as monophyletic (Supplementary Material Fig. S1). Tree topology tests were conducted with the approximately unbiased (AU) test (Shimodaira, 2002) in IQTREE. Based on the results and in order to have a tree that is more concordant with the current taxonomic knowledge, we considered the tree that constrained the monophyly of Cheiracanthiidae and non-Australian gallieniellids as our working hypotheses tree (see section 3.1). We also completed an ASTRAL analysis on the working hypothesis constrained tree searches, including the legacy gene trees, to estimate branch lengths in coalescent units and to calculate posterior local probability support, site concordance and gene concordance factors.

For simple exploration of morphological diagnoses and putative synapomorphies for clades of interest, all phenotypic characters were optimized using parsimony on the working hypothesis tree with WinClada (Nixon, 2002)

2.6. Accuracy of phylogenetic inference

To test whether taxa may have actually evolved under an alternative topology, but high levels of gene tree incongruence lead to inference errors resulting in trees similar to our working hypothesis, we used simulations under the censored (or "multispecies") coalescent model (Rannala and Yang, 2003). We simulated 200 gene trees using alternative hypotheses (with clades that do not appear in the working hypothesis) as a containing species tree. After that, sequences were generated for each gene tree using the JC model, and a rate of 0.005. Rate and model choice were chosen because they generate sequences with nucleotide diversity values similar to that found in the empirical sequences. Alignments were concatenated and IQTREE was used to estimate the species tree. Fifty replications were done for each alternative hypothesis. The frequency of specific clades (which are found in our working hypothesis) was counted in the simulated species trees. If the inference of the empirical data is not accurate, we would expect to find the working hypothesis relationships with relevant frequency in the simulated data (here considered if frequency > 0.05). We focused our tests on the position of Sparassidae, Myrmecicultoridae, Thomisidae, Prodidominae, as well as the monophyly of Gnaphosidae (excluding Prodidominae) and Trochanteriidae (Supplementary Material Fig. S2). Simulations were done using DENDROPY (Sukumaran and Holder, 2010). Branch lengths in coalescent units for each alternative species tree were estimated with ASTRAL using the constrained search with empirical matrices, and ultrametricized using the R function force. ultrametric (method="extend") in PHYTOOLS (Revell, 2012). Trees were pruned to simplify simulations (Supplementary Material Fig. S2). All trees and matrices resulting from the simulations, as well as scripts used, are deposited on FigShare (https://doi.org/10.6084/m9.figshare.14977185).

3. Results

3.1. Phylogenetic analyses

All matrices, as well as the selected partition scheme and trees (with branch lengths and support values) are available as nexus files deposited on FigShare (https://doi.org/10.6084/m9.figshare.14977185). The inference with only the phenotypic matrix shows great discordance to previous analyses with the same data type (Ramírez, 2014), and to previous (Wheeler et al., 2017) and present analyses with molecular and combined data (Supplementary Material Figs S3–S16). The genomic, genomic and legacy data combined, and the total evidence results agree with the previous analyses regarding the first two basal splits within Dionycha: Prodidominae as sister to the remaining dionychans, which is divided into Dionycha A and Dionycha B clades (Figs. 2 and 3). Our analyses also recover Sparassidae, Myrmecicultoridae and Thomisidae as non-dionychans, as suggested by previous molecular studies. Relationships of some families inside Dionycha A and Dionycha B are quite unstable regarding the data and analytical approach (Figs S3–S16).

The addition of phenotypic data to the combined molecular matrix (genomic and legacy) alters the resolution inside Prodidomidae, Dionycha A and Dionycha B (Fig. 4, Fig. 5, S7, S12). The morphological data

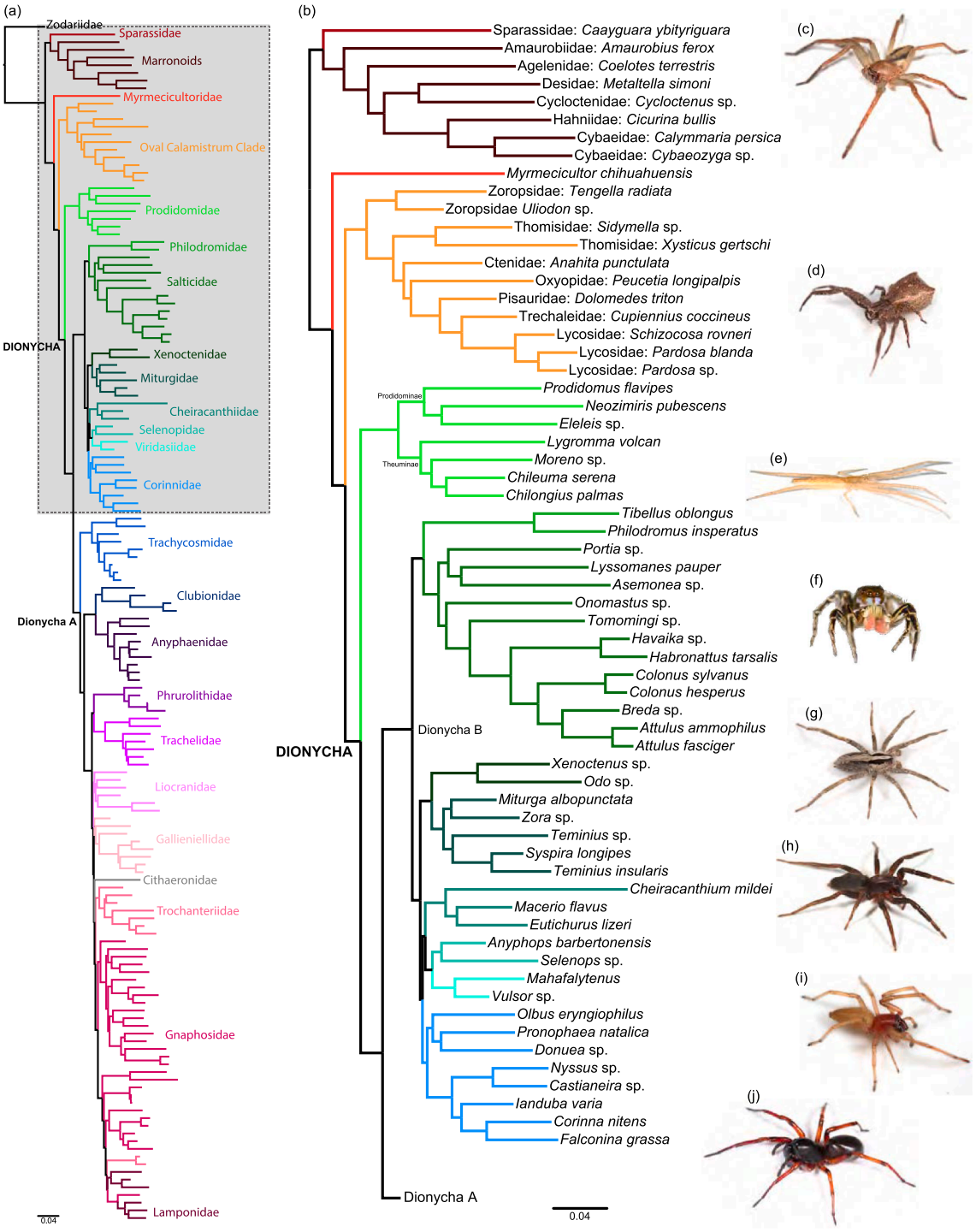

Fig. 2. Working hypothesis for Dionycha phylogeny inferred with genomic, phenotypic and Sanger data. (a) Overview of the entire tree depicting general relationship of families represented by different colors. (b) Detailed relationship of outgroups, Prodidomidae and Dionycha B. (c) Sparassidae: *Polybetes*. (d) Thomisidae: *Sidymella*. (e) Philodromidae: *Tibellus*. (f) Salticidae: *Habronattus*. (g) Xenoctenidae: *Odo*. (h) Miturgidae: *Teminius*. (i) Cheiracanthiidae: *Macerio*. (j) Corinnidae: *Corinna*.

contributed to a general increase in the branch supports in Dionycha B, especially regarding the clade that comprises corinnids and allies (Fig. 4, S7, S12). In the Dionycha A clade the topological changes were more complex, with many branches increasing support, while in others the support decreased, leading to the same median but different distribution of the support values (Fig. 5, S7, S12). The most drastic changes involve members of the families Corinnidae, Selenopidae, Cithaeronidae, Trachycosmidae **n. rank** (see section 6) and Lamponidae (Fig. 4, Fig. 5, S7, S12).

Topology tests show that the hypotheses constraining the non-Australian gallieniellids as monophyletic and Cheiracanthiidae as monophyletic cannot be rejected when compared to the total evidence unconstrained hypothesis (Fig. S1; Table S1). The hypotheses that constrain Sparassidae to be inside Dionycha also cannot be rejected. However, in that case, the resultant tree recovers a sister group relationship with these two taxa, but the branch length is estimated to be

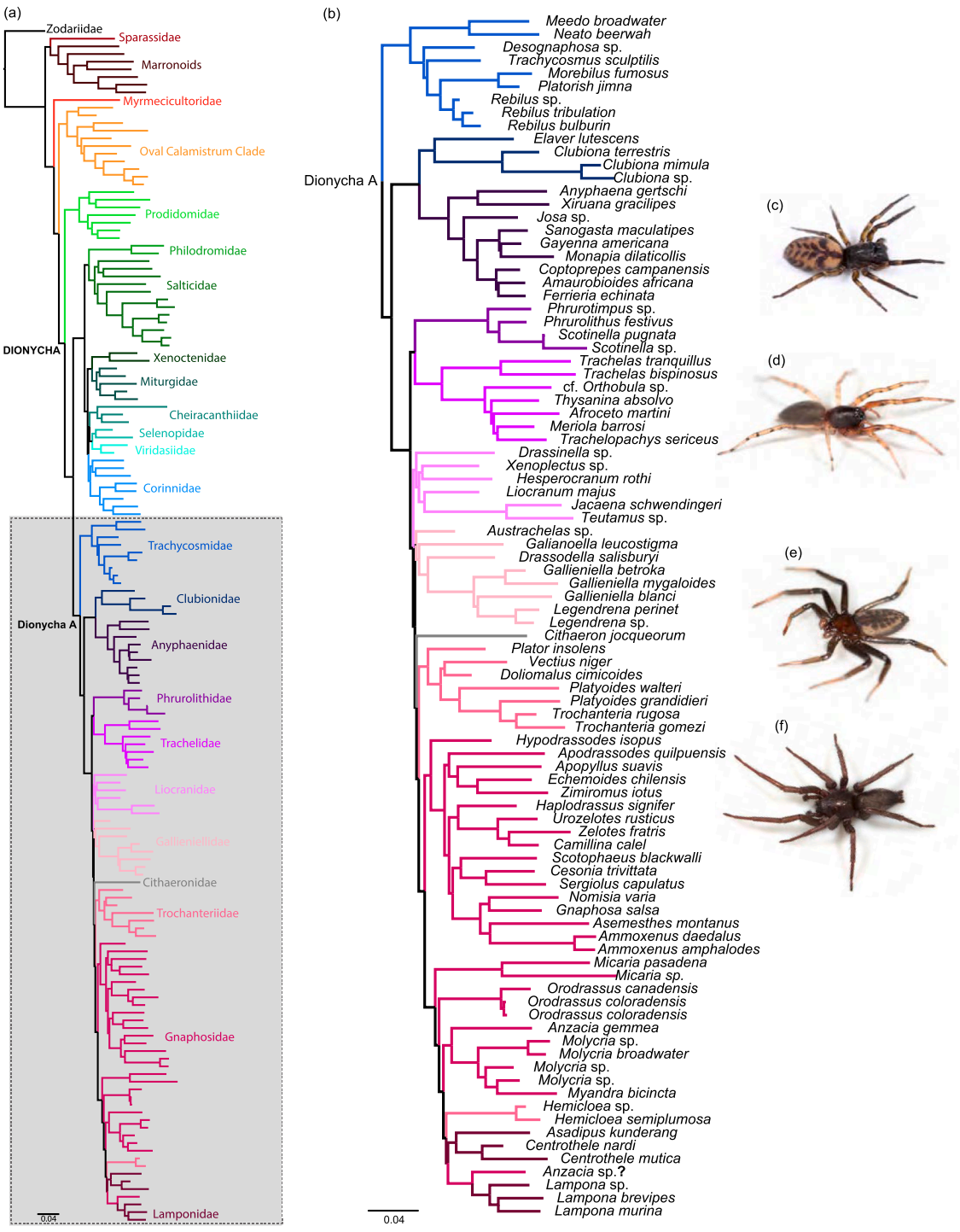

Fig. 3. Working hypothesis for Dionycha phylogeny inferred with genomic, phenotypic and Sanger data. (a) Overview of the entire tree depicting general relationship of families represented by different colors. (b) Detailed relationship of Dionycha A clade. (c) Anyphaenidae: *Amaurobioides*. (d) Trachelidae: *Meriola*. (e) Trochanteriidae: *Vectius*. (f) Gnaphosidae: *Apodrassodes*.

zero (Fig. S1b). The same happens with the constraint tree that forces Myrmecicultoridae to be a dionychan (Fig. S1c). Therefore, we chose the hypothesis that constrains non-Australian gallieniellids, and Cheiracanthiidae as each monophyletic, as a working hypothesis for Dionycha phylogeny (Figs. 2 and 3; Fig S1a). This hypothesis is more congruent with current taxonomy.

Most basal nodes for interfamilial relationships inside Dionycha A have relatively low support (Figs S12, S13), and the branch length estimation in coalescent units and concordance factors suggest possible high degrees of gene tree incongruence (Figs S14–S16). However, the support and resolution of nodes within each family are generally better than previous findings.

Parsimony reconstruction shows that most branches are supported by homoplasious characters, but several are useful for diagnosing certain clades (Supplementary Material Fig. S17; see also Taxonomy below).

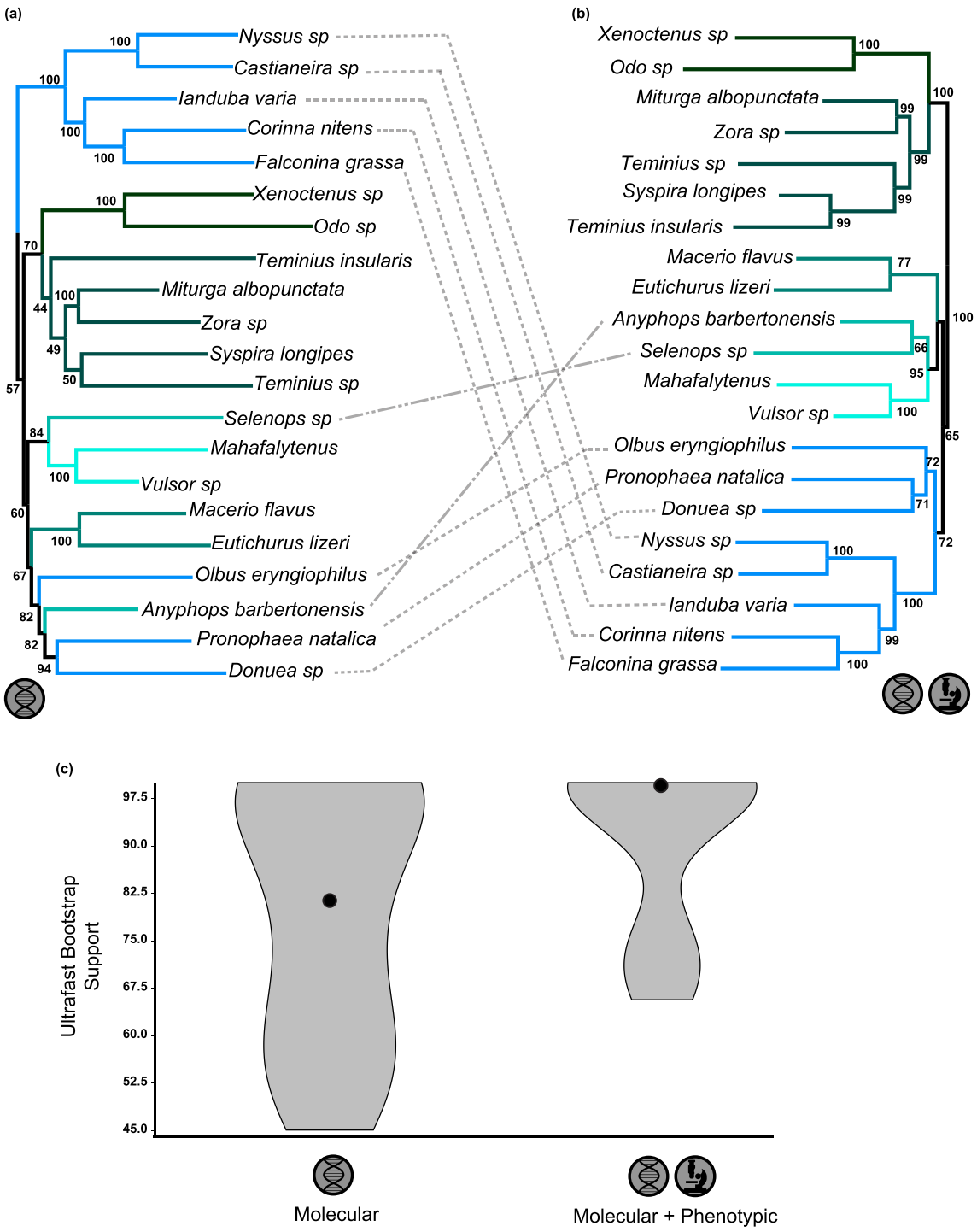

Fig. 4. Dionycha B (excluding Salticidae and Philodromidae) phylogenetic relationships inferred with the (a) molecular data (UCE, transcriptome and the legacy Sanger sequences) and with (b) molecular and phenotypic data combined (total evidence). Changes in topology discussed in the text are shown with dashed lines. Branches are colored by family. Numbers on nodes are ultrafast bootstrap support values. (c) Violin plot showing the kernel density distribution of the branch support values for the molecular and total evidence trees. Dots mark the median value of the distribution: 82 for the molecular and 99 for total evidence tree.

3.2. Accuracy of phylogenetic inferences

Simulations under the censored coalescent model show that if Sparassidae were sister to the remaining Dionycha and if the divergence between OCC, Sparassidae and Dionycha had happened in a very short interval of time (and/or the ancestral effective population size was very large), it is unlikely that the tree inference method used here would erroneously yield sparassids as sister to marronoids, since this relationship was never recovered with simulated data (Fig. S2a, S2h). The same is true for the data simulated under the scenario of Thomisidae included in Dionycha: the simulation which considers rapid divergence (and/or large ancestral populations) between OCC, thomisids and Dionycha inferred Thomisidae as a core OCC (Fig. S2c, S2h). Data simulated under the hypothesis that Prodidominae are closely related to other gnaphosoids inside Dionycha A (but with rapid radiation or large ancestral populations) also never recovered a tree where prodidomines are not within Dionycha A (Fig. S2d, S2h). A zero probability of recovering the Australian Trochanteriids as sister to remaining Dionycha A is

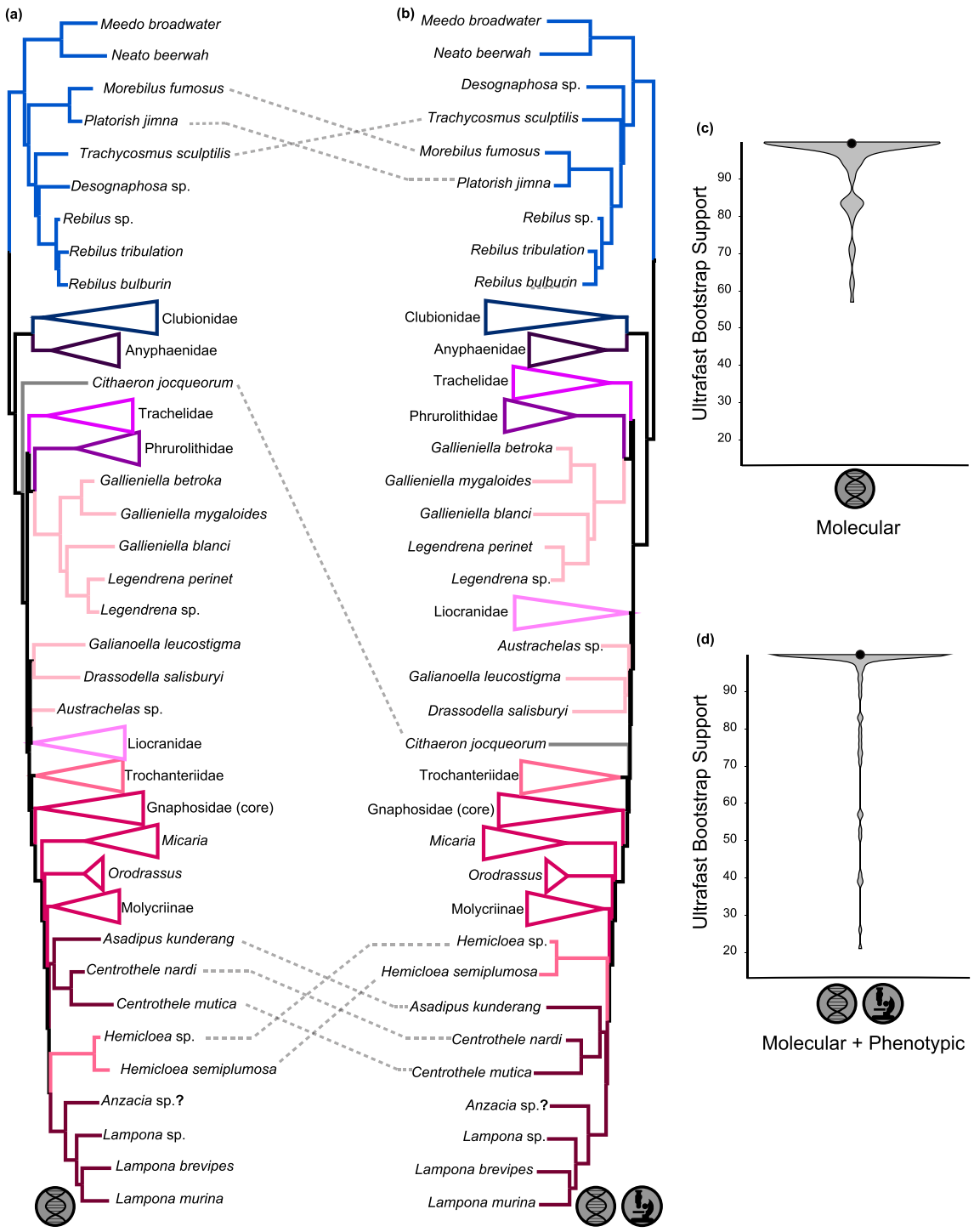

Fig. 5. Dionycha A phylogenetic relationships inferred with the (a) molecular data (UCE, transcriptome and the legacy Sanger sequences) and with (b) molecular and phenotypic data combined (total evidence). Changes in topology discussed in the text are shown with dashed lines. Branch colored by family. For ultrafast bootstrap values see Supplemental Figs. S7 and S19. Violin plot showing the kernel density distribution of the branch support values for the molecular and total evidence trees are shown in (c) and (d) respectively. Dots mark the median value of the distribution: 100 for the molecular and 100 for total evidence tree.

found when data is simulated considering all Gallieniellidae as a monophyletic, following a rapid radiation (Figs S2e, S2h).

The analyses of simulated data under the hypothesis that Myrmecicultoridae is sister to the remaining dionychans recovered a topology similar to the tree inferred with empirical data in 8% of cases (i.e. Myrmecicultoridae sister to OCC + Dionycha; Fig. S2b, S2h). When a restricted Gnaphosidae (i.e., excluding the prodidomines) was simulated to be monophyletic and sister to lamponids, forming a rapidly diverged clade, Gnaphosidae was recovered as paraphyletic in 40% of inferences (Fig. S2b, S2h). In conclusion, from all scenarios tested, only the position of Myrmecicultoridae and the paraphyly of a restricted Gnaphosidae with respect to Lamponidae are likely to be due to inference method errors.

4. Discussion

4.1. Dionycha phylogeny and evolution

4.1.1. Outgroups and the monophyly of Dionycha

The term Dionycha has traditionally been used to designate a group of spiders with the derived conditions of the absence of a median tarsal claw and the presence of a claw tuft of tenent setae (Petrunkevitch, 1928). These characters were acknowledged as weak, since they have certainly arisen convergently several times in spiders that walk on smooth surfaces (Coddington, 2005; Coddington and Levi, 1991; Lehtinen, 1967; Wolff et al., 2013). We may be able to better understand the evolution of these traits with a more robust phylogenetic hypothesis for traditional dionychan families and outgroups. Although the monophyly of Dionycha has long been questioned, it was only recently formally tested using morphological (Ramírez, 2014) and Sanger data (Moradmand et al., 2014; Wheeler et al., 2017). Here we present a comprehensive and thorough analysis of Dionycha relationships using genomic, Sanger loci and phenotypic data.

One of the key families to understanding dionychan evolution is Sparassidae. This family has been phylogenetically placed in Dionycha (Ramírez, 2014), as sister to the RTA clade (Moradmand et al., 2014), as sister to Dionycha + OCC (Wheeler et al., 2017) and as sister to marronoids (Fernández et al., 2018; Kallal et al., 2020; Kulkarni et al., 2020). Here we corroborated the latter hypothesis with strong support. The sparassid branch is not atypically long, the taxon does not represent an outlier in GC content, there is high support for the sister relationship with marronoids, and is not highly contradicted by individual gene trees. Moreover, alternative hypotheses are not supported by topology tests, and simulations showed that the family placement is unlikely to be phylogenetic inference error. Although its position may change with additional outgroup and sparassid taxa, it is very unlikely that Sparassidae is closely related to dionychan families.

Thomisidae is another family previously and questionably placed in Dionycha in prior research. Morphological phylogenetic analyses slightly supported the family as a dionychan (Ramírez, 2014). The grate-shaped tapetum in the eyes is a morphological character that links Thomisidae with the OCC (Polotow et al., 2015), although this evidence is ambiguous, since *Borboropactus*, sister to the rest of the thomisids, has canoe-shaped tapeta (Benjamin, 2011; Ramírez, 2014). Molecular and total evidence analyses also suggest that thomisids are closely related to wolf spiders and relatives (Wheeler et al., 2017), and our analyses strongly support the close relationship of thomisids and lycosoids inside the OCC.

The Myrmecicultoridae are enigmatic myrmecophagic, two-clawed spiders with puzzling phylogenetic relationships that hampered the formal description of the family for almost 20 years (Ramírez et al., 2019). Morphological analyses place the family inside Dionycha, but molecular and total evidence analyses place it as sister to Dionycha + OCC (Ramírez et al., 2019). Here we corroborate the latter. However, there is a chance that the position found here might be inference method error. If the Myrmecicultoridae is a result of rapid radiation in early branches of Dionycha, it is likely that the amount of incomplete lineage sorting would cause the inference to erroneously yield the same results found in our empirical data. Unfortunately, we were unable to obtain genomic data for this taxon, and it is possible that the results found here might be biased towards the information contained only in the legacy loci data. Ribosomal genes suggest a possible sister taxon relationship to prodidomines, while mitochondrial place the family inside OCC. Genomic data would certainly help to place Myrmecicultoridae in the spider tree of life, but currently it seems that the best hypothesis is that this family is sister to Dionycha + OCC.

4.1.2. The root of Dionycha

The two earliest divergences in our Dionycha tree gave rise to the three main clades in the group, Prodidominae (*sensu* Rodrigues and Rheims, 2020), Dionycha Part A and Dionycha Part B. The latter two clades comprise the greatest component of dionychan diversity, with ghost spiders (Anyphaenidae), ground spiders (Gnaphosidae) and relatives being members of Dionycha A, and jumping spiders (Salticidae), ant-mimic spiders (Corinnidae) and relatives belonging to Dionycha B. Prodidominae is currently taxonomically placed in Gnaphosidae and has always been traditionally associated to that family based on morphological characters (Azevedo et al., 2018; Platnick, 2002, 1990; Platnick and Baehr, 2006; Ramírez, 2014; Rodrigues and Rheims, 2020). However, previous molecular data (Wheeler et al., 2017) and our genomic and total evidence analyses indicate that prodidomines are distantly related to gnaphosids. It is unlikely that the results found here could be caused by topology estimation errors. Therefore, the subfamily should be removed from Gnaphosidae and have its familial status resurrected (see section 6); we will be treating it as such below. We recover as monophyletic the two subfamilies Prodidominae and Theuminae. The position of Prodidomidae as sister to remaining dionychans poses several intriguing questions about the similarities between remarkable morphological features present in Prodidomidae and the gnaphosids, such as the OMT, the clasping mechanism and the piriform gland spigots with elongated bases (Ramírez, 2014).

4.1.3. Dionycha A

The Dionycha A clade is recovered here, corroborating previous molecular studies. The group is also supported by one unambiguous morphological synapomorphy: the cylindrical gland spigots (Cy) on the posterior median spinnerets are clustered posteriorly and isolated from the other spigots (char. 290). This character state is only found convergently in *Lygromma* (Prodidomidae) in our dataset. However, the Cy spigots are completely lost in the most recent common ancestor of Clubionidae and Anyphaenidae, families that form a clade inside Dionycha A. The complete loss of Cy is also found in Salticidae and scattered in other Dionycha B families.

The polyphyletic nature of the Trochanteriidae and Gallieniellidae as currently delimited is reproduced here, corroborating previous hypotheses based on molecular and morphological data (Ramírez, 2014; Wheeler et al., 2017). In our results, the Australian trochanteriids and gallieniellids are grouped in a clade sister to all remaining Dionychan A, supported by six non-ambiguous, homoplasious synapomorphies (see Section 5.1). Three of them include: the anterior lateral spinnerets separated by at least its diameter (char. 245); the retention of two major ampullate gland spigots in adult males (char. 270); and the epigynal field formed by an undivided plate (char. 373). Therefore, this clade deserves a familial status, Trachycosmidae **n. rank** (see Section 5).

The true Gallieniellidae and Trochanteriidae are grouped together with Trachelidae, Phrurolithidae, Liocranidae, Cithaeronidae, Lamponidae, Ammoxenidae and Gnaphosidae in a clade in which the main synapomorphy is the oblique median tapetum (with convergences, discussed above). Although this clade is very stable and well supported, the relationships within it and the limits of some families are still somehow contentious. For instance, a phylogenetic hypothesis that shows Liocranidae and Gallieniellidae as monophyletic families is just as likely as trees that present these taxa as paraphyletic. The *Teutamus* group is recovered as closely related to *Liocranum*, but the absence of genomic data and the long branch suggest caution is needed when considering the relationship of liocranids. Also, our test suggests that the paraphyletic Gnaphosidae could be a result of inference error caused by a rapid radiation of Lamponidae and Gnaphosidae. These four families require deeper phylogenetic investigation. Our results indicate that Ammoxenidae is most likely a derived Gnaphosidae and both families should be synonymized (see Section 5.5).

4.1.4. Dionycha B

The remaining dionychans are grouped in the Dionycha B, a clade also previously proposed based on molecular data. Although this group is supported by two unambiguous synapomorphies, both of them are

homoplasious and present several state changes inside the clade. Dionycha B is divided into two relatively stable and well supported clades. One of them includes Salticidae and Philodromidae, the main synapomorphies including the loss of tapeta on the indirect eyes (chars 22 and 25) and the loss of cylindrical gland spigots (char. 285). The loss of the tapeta may be associated with the evolution of diurnal activity. In salticids, such diurnal activity together with the development of accurate vision may have facilitated the exploitation of resources and contributed to its enormous diversification (more than 6300 species described; Maddison, 2015; WSC, 2021).

The other clade includes the Xenoctenidae, Miturgidae, Viridasiidae, Selenopidae and Corinnidae, morphologically supported predominantly by the presence of a retrocoxal hymen on leg I, a very homoplastic character (with about 8 additional independent origins). Although relationships between these families are unstable across data type and analyses, our total evidence results are more resolved than previous hypotheses and provide important clues about their phylogeny and family limits. For instance, the maximum likelihood and ASTRAL analyses of UCE data and the total evidence analysis suggests that the *Pronophaea* group may indeed be a Corinnidae, contrary to previous findings (Wheeler et al., 2017). The presence of a precoxal triangle in females (char. 96) and a smooth tarsal cuticle (char. 101) are morphological synapomorphies that support the relationship of the *Pronophaea* group with the remaining corinnids.

4.2. The role of morphology

It has recently been demonstrated that the inclusion of morphological matrices together with genomic data can significantly impact certain phylogenetic hypotheses (Neumann et al., 2020). However, to produce a change in topology, sometimes morphological characters need to be weighted (Giribet, 2010; Neumann et al., 2020). The choice of weighting is hardly justifiable, and even in an implied weighting analysis it is still necessary to arbitrarily choose the concavity value (Goloboff et al., 2008). Weighting is usually necessary when there are various levels of homoplasy in the data. Since the level of homoplasy is related to the rate of character change, the use of Markov (Mk) models that incorporate rate variation offer a similar impact as character weighting, without the need to arbitrarily choose parameters. This can be achieved by including a rate variation parameter in the model (Yang, 1994) and/or using partitioning schemes (Rosa et al., 2019). Nevertheless, there is still a problem of defining the morphological partitions and testing the best partition scheme (Rosa et al., 2019). There are also concerns regarding the use of Mk models with morphological data, for instance, that character states (represented as 0 or 1) are not equivalent across the entire matrix and therefore, a unique transition matrix for such different characters would not be meaningful (contrary to molecular data in which nucleotide states are equivalent across all taxa). We attempted to resolve these problems by taking advantage of the ModelFinder algorithm in IQTREE. We used ModelFinder to search for partition schemes that group characters with similar profiles of the parameters of the transition matrix in an objective and automated way, without the need of *a priori* specification of partitions.

With our approach we obtained small, but considerable changes in topology in both Dionycha A and B clades (Fig. 4, Fig. 5, S7, S12). The addition of the morphological matrix to the combined molecular data changed the resolution and increased the support of clades within Trachycosmidae **n. rank** and among lamponid genera (Fig. 5, S7, S12). On the other hand, the phylogenetic placement of *Cithaeron* seems to be less supported and less resolved (shorter branches) in the total evidence analyses than in the molecular only data (Fig. 5, S7, S12). Unsurprisingly, the relationships of the aforementioned taxa in the total evidence analysis are more in agreement with previous taxonomical and phylogenetic hypotheses based on morphology (Platnick, 2002, 2000).

The monophyly of Corinnidae, and especially Selenopidae, seem to have been beneficially influenced by the phenotypic dataset. Selenopidae is a family of dorsoventrally flattened spiders with unique eye disposition and which the monophyly have been supported before (Crews and Gillespie, 2010; Ramírez, 2014; Wheeler et al., 2017). Our tree resulting from all molecular data combined shows Selenopidae as paraphyletic, but the molecular and morphological data combined (as well as in the morphology alone data) yielded this family as monophyletic (Fig. 4, S11). A clade of corinnids known as the *Pronophaea* group has been recovered as distantly related to other corinnids in previous analyses (Ramírez, 2014; Wheeler et al., 2017) and in our combined molecular data (Fig. S7). In our morphological analysis, the core corinnids and *Pronophaea* group appear as two separated clades in a polytomy with a third clade (Fig S11). However, the total evidence analyses recovered this group as sister to other corinnids (Fig. 4). Curiously, the resolution of the Dionycha B clade in the total evidence tree was more similar to the resolution in the ML tree obtained with UCE/transcriptome data alone, than to the molecular tree that also includes the traditional legacy Sanger sequencing data together with genomic (compare the topology inside Dionycha B in the Figs S3, S7, S12). The total evidence tree is also very different from the morphological tree alone (Fig. S11). This pattern could suggest that information added by legacy molecular data is noisy and that, somehow, this noise is overcome by the addition of the phenotypic data, even if the phenotypic matrix alone presents considerable noise itself.

It is worth noting that the changes in topology were in regions of the tree with high gene tree discordance. Areas of great discordance in phylogenies are usually associated with higher rates of morphological changes and innovations (Parins-Fukuchi et al., 2020). This could be an explanation for the observed influence of the morphology in the Dionycha phylogeny. However, it is hard to determine whether such topology changes represent a gain in accuracy or noise in the phylogenetic signal introduced by the high rates of morphological change. Since our approach accounted for differences in rates across the matrix by extensively and objectively selecting the best morphological partition scheme and testing models that include within-partition rate variation, it is possible that the signal is overcoming the noise in our data, leading to increased accuracy. It is also possible that rapid radiations may lead to different signatures in phenotypic and molecular characters. While the combination of high neutral mutations and great amount of incomplete lineage sorting may result in confusing signal in the nucleotide sequences, selection in several phenotypic characters may help to define the *bauplan* of the higher-level clades (families in our case), leaving a strong phylogenetic signal (even if some phenotypic characters alone might be homoplastic). Dionychan spiders may be a good model system, and our analytical approach may serve as a good option for understanding the dynamics of rapid radiations. Nevertheless, a deep investigation on the influence of morphology in phylogenetic inferences in rapid radiations using simulated and empirical data is still needed to develop better models and analytical tools for morphological characters.

5. Taxonomy

5.1. Trachycosmidae Platnick, new rank

Type genus: *Trachycosmus* Simon 1893

Morebilinae (Platnick, 2002) (type genus *Morebilus* (Platnick, 2002). **New synonymy.**

Diagnosis. The family Trachycosmidae can be diagnosed by the anterior lateral spinnerets separated by the length of their diameter or more; the presence of two major ampullate gland spigots in males and females; anterior lateral spinnerets with complete distal article and without inflatable area; epigynal field formed by an undivided plate, usually with an atrium where the copulatory openings are located; lens of the anterior lateral eyes are convex, raised from surrounding cuticle (compared to Trochanteriidae, in which lens is flat).

Composition. The family, as here relimited, includes the Trachycosminae, Morebilinae and the Australian gallieniellids revised by

(Platnick, 2002). It is composed of the following genera: *Boolanthana*, *Desognanops, Desognaphosa, Fissarena, Hemicloeina, Longrita, Meedo, Morebilus, Neato, Olin, Oreo, Peeto, Platorish, Pyrnus, Questo, Rebilus, Tinytrema, Trachycosmus, Trachyspina,* and *Trachytrema*. Although many of these genera are not present in our study, their morphology and the original study of (Platnick, 2002) suggest a close relationship with the genera included here. We stress that a deeper investigation of the limits of this family is necessary. A group of Australian genera formerly placed in Gallieniellidae (*Meedo*, *Neato, Oreo, Peeto*, and *Questo*) may also deserve family status, since they seem morphologically distinct from other trachycosmids.

Nomenclatorial note. The two subfamilies Trachycosmidae and Morebilinae were proposed by Platnick (2002) in the same work. We, as first reviewers, are here setting priority of Trachycosminae over Morebilinae (ICZN, Articles 24.2.1, 24.2.2).

5.2. Prodidomidae rank res.

Prodidomides Simon 1884: CCCII (type genus *Prodidomus* Hentz 1847).

Prodidominae (*sensu* Rodrigues and Rheims (2020)) is resurrected to family rank. Refer to Rodrigues and Rheims (2020) for diagnosis and composition. Molycriinae is maintained as a subfamily in Gnaphosidae.

5.3. Gallieniellidae

Gallieniellidae Millot 1947: 159 (type genus *Gallieniella* Millot 1947).

Diagnosis. Gallieniellidae is redefined and can be diagnosed by the legs I and II with virtually no spines; absence of tarsal macroseta; contiguous anterior lateral spigots; presence of two major ampullate gland spigots in females and usually one in males (*Galianoella* have two); absence of interlocking interaction between the claw lever file and claw tuft base.

Transfers. The Australian genera previously placed in Gallieniellidae are transferred to Trachycosmidae n. rank (see above).

Composition: *Austrachelas*, *Drassodella*, *Galianoella*, *Gallieniella*, *Legendrena*.

5.4. Trochanteriidae

Trochanterioidae Karsch, 1879: 536 (type genus *Trochanteria* Karsch 1878).

Platoridae Simon, 1897: 15 (type genus *Plator* Simon, 1880).

Diagnosis. Trochanteriidae can be diagnosed by an extremely flat carapace, with a reflexed border; a flat posterior median eyes lens that is contiguous with carapace cuticle; by a clypeus produced in a median lobe; laterigrade legs; absence of claw tufts; anterior lateral spinnerets with incomplete distal article and with inflatable area; and epigynal field formed by a divided plate.

Composition. *Plator*, *Platyoides*, *Trochanteria*, *Vectius*, and, provisionally, *Hemicloea*. The Australian *Hemicloea* could be closely related to members of Lamponidae, Gnaphosidae or Trochanteriidae, as found here and in previous studies (Azevedo et al., 2021; Azevedo et al., 2018; Platnick, 2002; Wheeler et al., 2017). It is unlikely that *Hemicloea* is closely related to the Australian flattened dionychans now placed in the Trachycosmidae n. rank, since no phylogenetic analyses so far suggested this relationship. The genus is retained here until more information is available to redefine the limits of Gnaphosidae, Lamponidae and Trochanteriidae.

5.5. Gnaphosidae Banks 1892

Gnaphosi Banks 1892: 94 (type genus *Gnaphosa* Latreille 1804).

Ammoxenidae Simon 1893: 331, 452 (type genus *Ammoxenus* Simon 1893). **New synonymy.**

Synonymy. Sanger and genomic markers indicate that Ammoxenidae is deeply nested within Gnaphosidae, closely related to the African gnaphosine *Asemesthes*. *Ammoxenus* shares with Gnaphosinae the presence of promarginal escort seta, a conductor, a transverse subtegulum visible proximally on the bulb in ventral view. The genus also shares with *Asemesthes* characters related to claw tuft seta, a broad sternum and the posterior median eyes smaller than posterior lateral eyes. The male genitalia of *Ammoxenus* also resembles that of *Asemesthes*, and both are suspected termitophagous (Charles Haddad, personal communication). The genus *Rastellus*, also placed in Ammoxenidae, is related to gnaphosines, according to Sanger data (Wheeler et al., 2017). The only remaining ammoxenid genera are the Australian *Austrammo* and *Barrowammo*, which are placed tentatively in Gnaphosidae as well.

The limits between Gnaphosidae, Lamponidae and Trochanteriidae still need further investigation. Our results suggest that lamponids and *Hemicloea* could belong to Gnaphosidae, but the evidence is not strong. We refrain to propose taxonomic acts for those taxa until a more thorough analysis is made with the aim to the delimit these families.

Nomenclatorial note. The family name Gnaphosidae was usually attributed to Pocock (1898), who first proposed the family name to replace Drassidae Sundevall 1833, because of the synonymy of *Drassus* Walckenaer 1804 with *Gnaphosa* Latreille 1804. Although the replacement was probably unnecessary, it became established and widely used (see Bonnett, 1956: 1554). According to the principle of coordination (ICZN, Article 36), the family name is attributed to the first author who established the name in any rank at the family level (e.g., tribe, subfamily, family, superfamily). Banks (1892: 94) used the tribes Gnaphosi and Micari, thus Gnaphosidae and Micariinae should be attributed to Banks (1892). Accordingly, Gnaphosidae has priority over Ammoxenidae.

5.6. Further taxonomic transfers

The genus *Drassinella* is transferred from Phrurolithidae to Liocranidae based on the presence of an indented socket of the scopular setae and the presence of a row of paired long, strong spines on metatarsus and tarsus of the legs I and II, and molecular data.

The genus *Donuea* is transferred from Liocranidae to Corinnidae, based on the absence of an oblique tapetum in the posterior median eyes, the presence of precoxal triangles in females, a smooth tarsal cuticle, and molecular data.

Mahafalytenus Silva-Dávila, 2007 is transferred from Ctenidae to Viridasiidae.

5.7. Misidentifications

The specimen identified as “Liocranidae sp.” in Fernández et al. (2018; MCZ IZ-71291, SRR6997875) is a phrurolithid, probably *Scotinella pugnata*, as it is joined by a zero-length branch with our identified sequenced specimen. We labeled that specimen as “*Scotinella* sp.”. We also suspect that the specimen Anzacia_sp_SRR6997629 might be misidentified in that genus and might be a lamponid, although a close examination of the specimen is needed.

6. Conclusions

Our study demonstrates how the combination of genomic data with Sanger legacy data and a phenotypic matrix can yield a better understanding of the systematics and evolution of a group of organisms. We provide a comprehensive and thorough phylogenetic hypothesis for the Dionycha that can be used as a robust framework to test hypotheses about the evolution of key characters. Given the group’s impressive diversity, there are many morphological and ecological features that would be interesting to study and would benefit from this framework. For instance, a deeper study of claw and claw tuft evolution,

araneophagy, silk use, or myrmecophagy using this phylogeny could aid in a better understanding the diversification patterns in the two-clawed spiders. Prospective studies could also explore the processes behind the patterns found here, for example, identifying whether Dionycha passed through an adaptive radiation, leaving a confusing genetic and morphological signal.

We also show that morphological data might have an impact on some parts of the tree even when greatly outnumbered by molecular data (phenotypic data corresponds to 1.1% of the molecular matrix). The approach we used to analyze the combined data may serve as an alternative to the practice of arbitrarily weighting or choosing between parsimony and Markov models. Tests with empirical data and simulations could help to better understand if the proposed approach is improving the accuracy or amplifying noise in the phylogenetic reconstruction. In our case, given the branches of the tree in which the morphological matrix affected the topology, we believe it may be improving accuracy. Independently of the accuracy and the impact on the topology, a comprehensive morphological matrix as presented here can reveal the patterns of character distribution in the tree, identify possible synapomorphies and diagnoses of clades, as well as provide insights about phenotypic evolution.

CRediT authorship contribution statement

Guilherme H.F. Azevedo: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. **Tierney Bougie:** Formal analysis, Investigation, Writing – review & editing. **Martin Carboni:** Formal analysis. **Marshal Hedin:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Martín J. Ramírez:** Conceptualization, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107327.

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