



Molecular phylogeny of the orb-weaving spider genus *Leucauge* and the intergeneric relationships of Leucauginae (Araneae, Tetragnathidae)

Jesús A. Ballesteros ^{A,B,C} and Gustavo Hormiga ^A

^ADepartment of Biological Sciences, The George Washington University, Washington, DC 20052, USA.

Email: hormiga@gwu.edu

^BDepartment of Integrative Biology, University of Wisconsin—Madison, Madison, WI 53706, USA.

^CCorresponding author. Email: jeballes@kean.edu

Abstract. The tetragnathid genus *Leucauge* includes some of the most common orb-weaving spiders in the tropics. Although some species in this genus have attained relevance as model systems for several aspects of spider biology, our understanding of the generic diversity and evolutionary relationships among the species is poor. In this study we present the first attempt to determine the phylogenetic structure within *Leucauge* and the relationship of this genus with other genera of Leucauginae. This is based on DNA sequences from the five loci commonly used and Histone H4, used for the first time in spider phylogenetics. We also assess the informativeness of the standard markers and test for base composition biases in the dataset. Our results suggest that *Leucauge* is not monophyletic since species of the genera *Opas*, *Opadometa*, *Mecynometa* and *Alcimosphenus* are included within the current circumscription of the genus. Based on a phylogenetic re-circumscription of the genus to fulfil the requirement for monophyly of taxa, *Leucauge* White, 1841 is deemed to be a senior synonym of the genera *Opas* Pickard-Cambridge, 1896 **revalidated synonymy**, *Mecynometa* Simon, 1894 **revalidated synonymy**, *Opadometa* Archer, 1951 **new synonymy** and *Alcimosphenus* Simon, 1895 **new synonymy**. We identify groups of taxa critical for resolving relationships within Leucauginae and describe the limitations of the standard loci for accomplishing these resolutions.

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Introduction

The orb-weaving spider genus *Leucauge* White, 1841 is one of the most diverse genera in the family Tetragnathidae. In terms of number of described species, *Leucauge* ranks second in the family with 171 (including 8 subspecies) and is surpassed only by the genus *Tetragnatha* Latreille, 1804 that has 347 species (World Spider Catalog, ver. 19.5, Natural History Museum Bern, see <http://wsc.nmbe.ch>). Species of *Leucauge* are known to occur on all continents except Antarctica and Europe and greater diversity occurs in tropical and subtropical regions of the globe.

Leucauge species are usually recognised by the bright, metallic-silvery abdominal pattern (Fig. 1), the presence of feathered trichobothria on the fourth leg femora and the relatively simple male and female genitalia (Álvarez-Padilla and Hormiga 2011). Some species are relatively common and form an abundant component of the spider community. A few *Leucauge* species can be considered model organisms in studies of spider biology including web-building behaviour (Eberhard 1988a, 1988b, 1990; Hénaut *et al.* 2006; Salomon

et al. 2010; Briceño and Eberhard 2011; Barrantes *et al.* 2020); reproductive biology, sexual selection and courtship behaviour (Eberhard and Huber 1998; Preston-Mafham and Cahill 2000, Aisenberg 2009; Aisenberg and Barrantes 2011; Aisenberg *et al.* 2015; Segura-Hernández *et al.* 2020); and various ecological interactions (Buckles 1999; Eberhard 2001; Moya-Laraño *et al.* 2007; Gonzaga *et al.* 2015). Despite this relevance of species of *Leucauge* as arachnid study organisms, the basic taxonomic and phylogenetic research of *Leucauge* has been largely neglected.

The main goals of this study are to establish a phylogenetic scaffolding for *Leucauge* and infer the intergeneric relationships of Leucauginae. A phylogenetic hypothesis for leucaugines would provide a foundation for a better understanding of a great deal of the comparative data at hand and guide future studies on the evolutionary biology of these spiders. We have gathered nucleotide sequences of both mitochondrial and nuclear gene data and combined these with sequences available in public repositories to accomplish these goals.



Fig. 1. Diversity of *Leucauge*. A, *Leucauge argyrobapta* (White, 1841). B, *L. celebesiana* Merian, 1911. C, *L. argentina* (Hasselt, 1882). D, *L. sarawakensis* (Dzulhelmi & Suriyanti, 2015) **new combination** (formerly in *Opadometa*). E, *Leucauge* sp. (Costa Rica, formerly in *Opas*). F, *L. licina* (Simon, 1895) **new combination** (formerly in *Alcimosphenus*). G, *Leucauge* sp. (Costa Rica). H, *L. decorata* (Blackwall, 1864). I, *Mesida gemmea* (Hasselt, 1882). Photos: A, G, G. Hormiga; B-D, H, I, J. Koh; E, G. Kunz; F, T. Shahan.

The taxonomic history and current status of the genus Leucauge

The name *Leucauge* was introduced in 1841 by Adam White at the rank of subgenus for *Linyphia (Leucauge) argyroabpta* and is the only spider name that can be attributed to Charles Darwin (Dimitrov and Hormiga 2010). The specimen described by White (1841) was lost and therefore the identity of *L. argyroabpta* remained dubious for over a century until Dimitrov and Hormiga (2010) designated a neotype for *L. argyroabpta* and proposed synonymy with *Leucauge venusta* (Walckenaer), with priority favouring the later name. Based on a recent species delimitation study using nucleotide sequence data (Ballesteros and Hormiga 2018) we now know that *L. venusta* and *L. argyroabpta* are indeed different species.

Most of the 171 described *Leucauge* species are known from only one sex, 97 from female and 9 from males only, whereas 4 species were described based on juveniles and in the case of *L. pulcherrima ocherrufa* (Franganillo, 1930), the sex of the specimen described is unknown.

Most of the named species of *Leucauge* were described before 1925 and many have not been mentioned in the taxonomic literature since the original description beyond listing in taxonomic catalogues. These older descriptions rarely meet the descriptive standards of the more recent taxonomic literature. In many cases there are no illustrations or these are inadequate for identification, and descriptions often lack differential diagnoses and explicit comparisons to other species. Therefore, even though many species of *Leucauge* have been 'named', this effort does not mean we know a great deal about these species and in many cases, discerning the identity is only possible by examining the type specimens.

Some regional faunas are better known than others. For example, Zhu *et al.* (2003) provide a treatise on Chinese araneofauna with illustrations that allow identification of many of the Asian species of *Leucauge*. In the case of the Americas, Herbert W. Levi's works (Levi 1980, 2008), particularly the unpublished drawings of some *Leucauge* species of the Americas (<http://web.archive.org/web/20150213013325/http://www.oeb.harvard.edu/faculty/levi/leucauge.html>) represent the only resource for species level identification. Nevertheless, these works illustrate only a small fraction of the total number of described species and many regions of the world lack such faunistic treatments for *Leucauge* in which cases often only scattered, isolated species descriptions are available. In addition, many species have yet to be named and described.

Previous phylogenetic work

The monophyly of Tetragnathidae has been consistently corroborated in several phylogenetic studies using both morphological and genetic sequence data (Coddington 1990; Hormiga *et al.* 1995; Álvarez-Padilla 2007; Kuntner *et al.* 2008; Blackledge *et al.* 2009; Dimitrov *et al.* 2012, 2017; Garrison *et al.* 2016; Fernández *et al.* 2018; Álvarez-Padilla *et al.* 2020). Although many of the interfamilial relationships of araneoid spiders remain contentious (Hormiga and

Griswold 2014), there is recent consensus on the monophyly of a clade composed of Tetragnathidae, Mimetidae and Arkyidae (Benavides *et al.* 2017; Dimitrov *et al.* 2017; Wheeler *et al.* 2017; Fernández *et al.* 2018; Kallal and Hormiga 2018; Kallal *et al.* 2020, 2021; Kulkarni *et al.* 2020).

There has also been important progress towards resolving the cladistic relationships of tetragnathid genera and such efforts have led to the classification of tetragnathid species diversity into four subfamilies: Tetragnathinae, Metainae, Nanometainae and Leucauginae, (Álvarez-Padilla 2007; Álvarez-Padilla *et al.* 2009, 2020; Dimitrov *et al.* 2010; Álvarez-Padilla and Hormiga 2011; Dimitrov and Hormiga 2011, 2009). Nevertheless, only a fraction of the tetragnathid genera (25 of 47) have been included in phylogenetic analyses and in many cases, these were represented by a single species and consequently the monophyly of such genera remains untested within a cladistic framework. Additionally, the affinities of several genera remain uncertain and in some cases the phylogenetic position varies among studies; for example, the positions of the genera *Allende* Álvarez-Padilla and *Chrysometa* Simon vary among different analyses (Álvarez-Padilla *et al.* 2009, 2020; Dimitrov and Hormiga 2011).

The subfamily Leucauginae was proposed by di Carporiaco (1955) to include a few species in the genera *Leucauge* and *Plesiometeta* F. O. Pickard-Cambridge, 1903 and the later genus was synonymised with *Leucauge* by Levi (1980). Under the current phylogenetic circumscription, based on morphological and molecular data (Álvarez-Padilla *et al.* 2009; see also Álvarez-Padilla and Hormiga 2011), Leucauginae includes the genera *Leucauge*, *Harlanethis* Álvarez-Padilla, Kallal and Hormiga, 2020; *Metabus* O.P.-Cambridge, 1889; *Mecynometeta* Simon, 1894; *Mesida* Kulczynski, 1911; *Orsinome* Thorell, 1890; *Opadometa* Archer, 1951 and *Tylorida* Simon 1894. In addition, several mostly monotypic genera that have not been included in previous analyses, have been proposed as members of Leucauginae based on the presence of putative leucaugine synapomorphies (Álvarez-Padilla *et al.* 2009): *Alcimospheenus* Simon, 1895 (one species); *Atelidea* Simon, 1895 (one species); *Okileucauge* Tanikawa, 2001 (nine species); *Opas* O. Pickard-Cambridge, 1896 (six species.); and *Pickardinella* Archer, 1951 (one species). The monophyly of the subfamily, as currently defined, seems robust across analyses although the position *Metleucauge* Levi, 1980 and *Azilia* Keyserling, 1881 have been unstable. In some analyses these genera are considered sister to the rest of Leucauginae (Álvarez-Padilla *et al.* 2009). Only two North American species of *Leucauge* have been repeatedly included in previous phylogenetic surveys, *L. venusta* and *L. argyra*. Even with this limited taxon sampling, the results of Dimitrov *et al.* (2012) challenge the monophyly of *Leucauge* because the genus is paraphyletic with respect to *Mecynometeta*.

In this study, we explore the phylogenetic structure of *Leucauge* using a broad sample of species, including representatives from the Americas, Africa, Asia and Australia. This study is the first to explicitly test the monophyly of *Leucauge* and the placement among

leucaugines using genetic sequence data from a broad taxonomic sample of the genera in the subfamily. An exhaustive taxonomic sampling of *Leucauge* is not feasible because of the sheer number of described (and undescribed) species, difficulties in species identification and the wide distribution of the genus. This study is the first attempt to illuminate species level relationships within *Leucauge* based on representatives of 46 species of the genus and representatives of 10 leucaugine genera including, for the first time, samples of *Alcimospheenus*, *Opas* and *Okileucauge*. We also discuss the adequacy of the standard set of genetic markers commonly used in spider phylogenetics, in terms of information content and base composition homogeneity, and evaluate the pattern and tempo of Leucauginae evolution.

Materials and methods

Taxon sampling and specimen acquisition

Taxon sampling focused on *Leucauge* species and closely related genera. We also included representatives of all other tetragnathid subfamilies as outgroups using the edge of Arkyidae + Mimetidae as the root of the tree because the position of Leucauginae within Tetragnathidae has not been robustly resolved. Most of the specimens used in this study were obtained from our own field work and collecting efforts of colleagues around the world. Although an exhaustive sampling of *Leucauge* was not feasible, we selected specimens from across the distribution range of the focal genus. We included specimens from Africa (5 species), Australasia (10 species) and the Americas, including the Caribbean region (28 species). Several species were represented by multiple terminals in our analyses. The identification of *Leucauge* specimens is difficult and identified specimens were given priority over juveniles or unknown species for inclusion in the final dataset. Additional representatives of Leucauginae genera were included in the analyses whenever specimens suitable for sequencing were available with the aim of maximising generic representation. We included the type species of the leucaugine genera in our analyses whenever possible.

All sequenced fragments were derived from the same individual DNA extract and voucher specimens will be deposited at the Museum of Comparative Zoology in Harvard (Table S1 of the Supplementary material). The final dataset was complemented with sequences available in public databases. Genbank accession numbers of all terminals are listed in Table S2 of the Supplementary material.

DNA amplification and sequencing

Total DNA was extracted from two or three legs of each specimen using DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. The selection of genes included in this study aimed to overlap the genetic markers available for other tetragnathids in public databases. The final list of markers included two mitochondrial genes, the ribosomal r16S (16S) and the cytochrome oxidase *c* subunit I (*COI*); and four nuclear genes r18S (18S), r28S (28S), Histone H3 (H3) and Histone H4 (H4). The *H4* gene was used for the first time in spider phylogenetics and was based on custom primer pairs. This locus was identified in the Araneae set of orthologous genes

described in Ballesteros and Hormiga (2016). The primer design used the web interface of primer3 (ver. 0.4.0, see <https://bioinfo.ut.ee/primer3-0.4.0/>; Untergasser *et al.* 2012) and the target sequence was based on the consensus sequence of the *H4* transcripts of *Leucauge venusta*, *Neoscona arabesca* and *Frontinella pyramitela*.

Polymerase chain reactions (PCR) were carried out in 25 µL of total volume using 0.12 µL of PROMEGA Go Taq G2 Flexi DNA polymerase, 5 µL of buffer, 2 µL of MgCl₂ (25 mM), 0.5 µL of dNTP mix (10 mM), 1 µL of each primer (10 mM) and 1–2 µL of template DNA. Primer pairs and annealing temperatures are summarised in Table S3 of the Supplementary material. The basic thermocycle consisted of an initial denaturation step at 94°C for 3–5 min followed by 35 amplification cycles of 94°C for 30 s, annealing for 30 s, extension at 72°C for 30–45 s and a final extension cycle at 72°C for 5 min. PCR products were visualised in 1% agarose gel electrophoresis stained with Biotium GelRed. The unpurified PCR products were sent to MACROGEN (Rockville, MD, USA) facilities for purification and sequencing. Reads were trimmed according to base call quality scores. Contigs were assembled from forward and reverse reads and visually inspected using Geneious (ver. 6.1.8, see <https://www.geneious.com/>; Kears *et al.* 2012) or the Staden package (ver. 2.0.0b11, see <http://staden.sourceforge.net/>; Bonfield *et al.* 1995; Staden 1996).

All sequences were subject to quality control inspection against the non-redundant nucleotide public sequence database (nr-NCBI) using BLAST+ (ver. 2.5, see <https://blast.ncbi.nlm.nih.gov/Blast.cgi/>; Camacho *et al.* 2009).

Multiple sequence alignment of the ribosomal genes was performed using the PASTA routine (see <https://github.com/smirarab/pasta>; Mirarab *et al.* 2015), with MAFFT (ver. 7.463, see <https://mafft.cbrc.jp/alignment/software/>; Katoh and Standley 2013) and RAXML (ver. 8.2.9, see <https://github.com/stamatak/standard-RAXML>; Stamatakis 2014) as aligner and tree estimator respectively. Alignment of protein coding loci was done with MAFFT verifying the open reading frame.

Model selection and base composition homogeneity test

The selection of data partitions and models of molecular evolution was investigated by comparing alternative partition schemes and models using the Bayesian Information Criterion (BIC) as implemented in PartitionFinder 2 (ver. 2.1.1, see <https://www.robertlanfear.com/partitionfinder/>; Lanfear *et al.* 2012, 2016) and using 'phym1' for maximum likelihood estimates (ver. 3.0, see <http://www.atgc-montpellier.fr/phym1/binaries.php/>; Guindon *et al.* 2010). Base composition homogeneity assumption was evaluated with the χ^2 test and the compositional homogeneity test using simulations proposed by Foster (2004). Both tests were performed in the program p4 (ver. 0.89, see <http://p4.nhm.ac.uk/>; Foster 2004). The simulation test is hereafter referred as the p4 test because the test using simulation was specifically implemented in this program. The p4 test is a modified χ^2 where the null distribution for the statistics derived from values is simulated on a given tree topology. The simulations and tests were performed on the full concatenated data using the PartitionFinder partition scheme and on the partitioning by gene and codon position of the

whole dataset (12 partitions) for the p4 test. The latter partitioning was applied for the homogeneity tests because the base composition heterogeneity was known to vary across codon positions (Foster 2004).

The individual data partitions for which the composition homogeneity assumption was rejected were recoded as purines and pyrimidines (RY coded) and subsequently concatenated for phylogenetic analyses to account for potentially biased results due to non-stationarity. Owing to differences in the results of the base composition tests, RY coded matrices were identified as RY- χ^2 and RY-p4.

Phylogenetic informativeness

The explanatory power of the data was evaluated using phylogenetic informativeness profiles (PIP; Townsend 2007) as implemented in the webserver version of PhyDesign (see <http://phydesign.townsend.yale.edu/>; López-Giráldez and Townsend 2011; Townsend *et al.* 2012). Site rates were estimated using HyPhy (ver. 2.5, see <https://www.hyphy.org/>; Kosakovsky Pond *et al.* 2005) using the maximum clade credibility tree from the Bayesian inference (BI) analysis (working hypothesis). Both net and per-site informativeness profiles were estimated for the full dataset, partitioned by gene and codon position.

Phylogenetic analyses and dated phylogeny

Maximum likelihood (ML) analyses were performed in RAxML (ver. 8.2.9, see <https://github.com/stamatak/standard-RAxML/>; Stamatakis 2014) using a 500 rapid bootstrap routine using with GTRGAMMAI substitution model.

For Bayesian inference (BI) analyses, the root node was constrained at the split of Tetragnathidae with Arkyidae + Mimetidae. Bayesian analyses were carried out in Beast2 (ver. 2.3.3, see <https://www.beast2.org/>; Bouckaert *et al.* 2014) using four independent Markov Chain Monte-Carlo (MCMC) runs, each 100×10^6 generations long, sampling every 10 000 states. Nucleotide substitution model and partitioning followed PartitionFinder’s best scheme; rates of evolution of each gene used the relaxed uncorrelated clock model (see details of time calibration below; Drummond *et al.* 2006) and tree modelled with birth-death model (Kendall 1948). Sampled states and trees obtained from each run were combined using Logcombiner (ver. 2.4.2, see <https://www.beast2.org/>; Drummond and Rambaut 2007) discarding 20% of each run as burn-in. Convergence of individual and combined runs was assessed in Tracer (ver. 1.6, see <https://www.beast2.org/>; Drummond and Rambaut 2007) and maximum clade credibility produced in TreeAnnotator (ver. 2.4.2, see <https://www.beast2.org/>; Drummond and Rambaut 2007).

Four matrices were analysed phylogenetically (Table 1). Trees for the full and condensed matrices were estimated both in the Bayesian and maximum-likelihood frameworks. The RY recoded matrices were analysed with only ML as optimality criterion. All analyses used models and partitions selected by PartitionFinder.

Topological congruence across analyses was based on the comparison of bipartitions (splits) in the resulting trees using the maximum credibility tree from the BI analyses of the full matrix. For the reason that measures of branch support between ML and Bayesian trees are fundamentally different (Simmons *et al.* 2004) and do not allow direct numerical comparisons, differences in the support value of shared bipartition were evaluated qualitatively. The support value associated with the shared splits was considered high for bootstrap values ≥ 70 in ML trees and $PP \geq 90$ for Bayesian trees. Instances where the support of the shared split improved or worsened when compared to the full BI tree (our working hypothesis; see ‘Results’ section) are reported.

Time calibration

There are no described fossils of *Leucauge* (Dunlop *et al.* 2017). Previous analyses have used the Cretaceous *Macryphantes cowdeni* Selden, 1990 as a calibration point for the Tetragnathidae node but recent studies have questioned the position of this fossil within Tetragnathidae, suggesting the fossil may rather be a member of Deinopidae (Selden *et al.* 2015). Another Cretaceous fossil originally attributed to Tetragnathidae, *Huergina diazromerali* Selden & Penney, 2003 was not included because of the lack of tetragnathid synapomorphies (Selden and Penney 2003) and the uncertainty produced by the circumscription of Tetragnathidae used in the original description that included the nephilines currently classified at the rank of subfamily in Araneidae (Dimitrov *et al.* 2017, Kallal *et al.* 2018, 2020). Magalhaes *et al.* (2020) recommend not using *Macryphantes* and *Huergina* as calibration points because these lack any clear synapomorphies of Tetragnathidae.

In the absence of reliable calibration points, the only option to provide a temporal framework is relying on previous estimated clock rates of the four certain genes. In our Bayesian analyses, we implemented independent uncorrelated log normal relaxed clocks (ucln) for each gene; fixing the mean ucln for *COI* at 0.0115 substitutions per million years based on estimates of empirical rates used in three previous studies: Brower (1994) for Lepidoptera, and Bidegaray-Batista and Arnedo (2011) and Peres *et al.* (2015) for spiders. The use of this clock was only complementary in this study, as no temporal hypotheses were being tested and the implementation had no effects on the topology because the

Table 1. List of datasets and analyses performed
BI, Bayesian Inference; ML, Maximum Likelihood; RY, purine and pyrimidine recoding

Matrix	Number of sites	Analyses		Description
full	4695	BI	ML	All genes (6)
RY- χ^2	4695		ML	All genes, two partitions RY recoded based on χ^2 test.
RY-p4	4695		ML	All genes, 5 partitions RY recoded based on p4 test.
condensed	2289	BI	ML	Four genes (<i>COI</i> , <i>18S</i> , <i>28S</i> , <i>H3</i>).

clock rates of all other genes were estimated as free parameters.

Results

Model selection and base composition homogeneity test

The best fitting partition scheme selected by PartitionFinder divided the concatenated alignment into three data partitions and models as follow: (a) for the partition composed of *16S*, *COI* first and second codons, second codon of *H3* and *H4* (all codons); Generalised Time Reversible (GTR) substitution model (Tavaré 1986) with Γ distributed across-site rate variation (G), estimating the proportion of invariant sites (I) and with estimated base frequencies (X), and this may be summarised as GTR+I+G+X; (b) The Tamura–Nei model (TN93) (Tamura and Nei 1993) +G+X for the third codon position of *COI*; and (c) GTR+I+G+X for a partition composed of *18S*, *28S*, the first and third codons of *H3*. Results of the composition homogeneity tests are summarised in Table 2. Consistent with the justification provided by Foster (2004), the p4 test rejected the null hypothesis on partitions where χ^2 failed to reject the null hypothesis. The χ^2 tests rejected homogeneity of two partitions, the third codon positions of *COI* and *H3*. The p4 test identified heterogeneity in the ribosomal genes (*16S*, *18S* and *28S*) and the third codon positions of the three protein coding genes (*COI*, *H3*, *H4*). These partitions were RY-recoded and the results were compared with those from the original dataset.

Achieving full or nearly full matrix completeness is often a challenge in phylogenetics. The selection of markers and primers plays an important role if a significant overlap with publicly available data is desired. To address potential problems due to the high proportion of missing data in the complete matrix, we produced a subset in which the loci with the highest amount of missing data (*H4* and *16S*) were removed. These two loci are substantially underrepresented in the terminals for different reasons. The *16S* locus showed poor amplification for our samples and *H4* is not represented in any of the terminals retrieved from public databases. The results of both ‘full’ and ‘condensed’ matrices are shown in Fig. S1–S4 of the Supplementary material and compared in Fig. 2. These matrices have the same number of terminals (164) but differ in the number of sites and the proportion of undetermined characters (including gaps, and missing and

ambiguous characters). The full matrix includes 4695 sites and 56.25% undetermined characters, and the condensed matrix includes 2289 positions of which 34.41% are undetermined sites.

Phylogenetic informativeness profiles

Net and per-site PIP are shown in Fig. 3. These graphs, read from the tips to the base of the tree, depict the explanatory power of the data and show an increase until reaching the maximum informativeness value, after which the decrease in informativeness indicates the potential of noise or saturation to overcome the phylogenetic signal. The phylogenetic informativeness score itself represents a normalised likelihood score for the probability of the marker to show unreversed substitutions at a given tree height. Whereas net PIP allows the evaluation of the relative informativeness of each gene in total, per-site PIPs are useful for comparing relative power partitioning without the confounding influence of gene length.

Informativeness varied in magnitude and depth across different partitions. The net PIP graphs show the partitions of *28S*, *COI* (3rd codon position) and *16S* with the highest overall phylogenetic informativeness; all the marker profiles show peaks between 10 and 21 Ma. In the per-site profiles, the 3rd codon of *COI* stands as the most informative with peak *c.* 15 Ma, followed by the 3rd codon positions of *H4*, *H3* and *16S*.

The newly introduced sequences of *H4* showed similar informativeness profiles to those of *H3*, both with low net information content. The per site PIP indicates a favourable cost benefit in terms of sequence length and information content.

Phylogeny

We selected the maximum clade credibility tree (MCCT) obtained from the analysis of the full dataset in BEAST as our working phylogenetic hypothesis (reference tree). Discussions regarding Leucauginae systematics are based on the topology of this tree. The overall topology of this tree and congruence with the other analyses are summarised in Fig. 2 and 4. The individual resulting trees with full leaf names and branch support are provided in Fig. S1–S5 of the Supplementary material. As indicated by the low support values and sensitivity to analytical procedures, many groupings shown in this tree must be considered effectively unresolved.

Despite the base composition heterogeneity results, the trees obtained from the RY coded matrices showed no improvement in branch support when compared with the reference tree. As an example, the reference topology shared 96 splits with the RY- χ^2 tree; improving the support on 9 splits, and impairing this on 8 and 79 splits showed no change. The p4 based RY analyses showed the lowest number of bipartitions shared with the reference tree at 88 splits, of which the support improved on 6, 14 were impaired and 66 remained the same.

In the same manner, the condensed matrix did not produce better supported trees, suggesting that missing data were not the main factor of uncertainty for this dataset.

Table 2. Summary results of composition homogeneity tests based on χ^2 and the composition corrected by simulations tests (p4)
Significant values ($P < 0.05$) are highlighted in bold

		<i>COI</i>		<i>16S</i>	<i>18S</i>	<i>28S</i>
	1st	2nd	3rd	–	–	–
χ^2	1.0000	1.000	0.000	0.781	1.0000	0.702
p4	0.937	1.000	0.000	0.000	0.000	0.000
		<i>H3</i>			<i>H4</i>	
	1st	2nd	3rd	1st	2nd	3rd
χ^2	1.00	1.000	0.000	1.00	1.00	1.00
p4	0.991	1.000	0.000	0.127	0.677	0.007

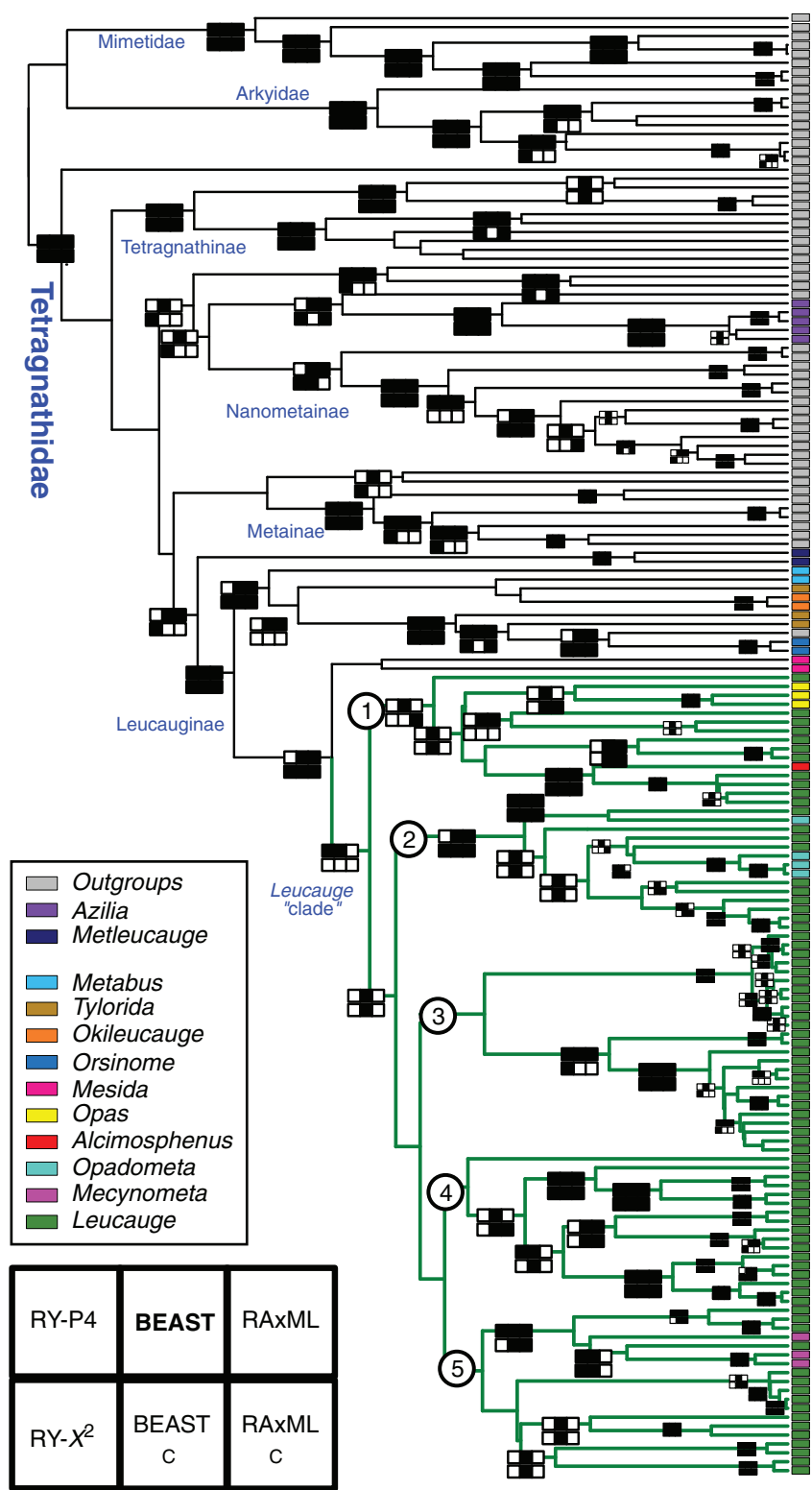


Fig. 2. Maximum clade credibility tree (BI) from the full dataset. Bipartitions shared by this tree and alternative analyses are indicated with black squares on a grid with the key shown in the lower left corner; analyses of the condensed dataset are represented by 'c'. Leaves associated with the Leucauginae subfamily are highlighted and colour-coded according to genus. Details of numbered clades are discussed in the text. The fully labelled tree is given in Fig. S1.

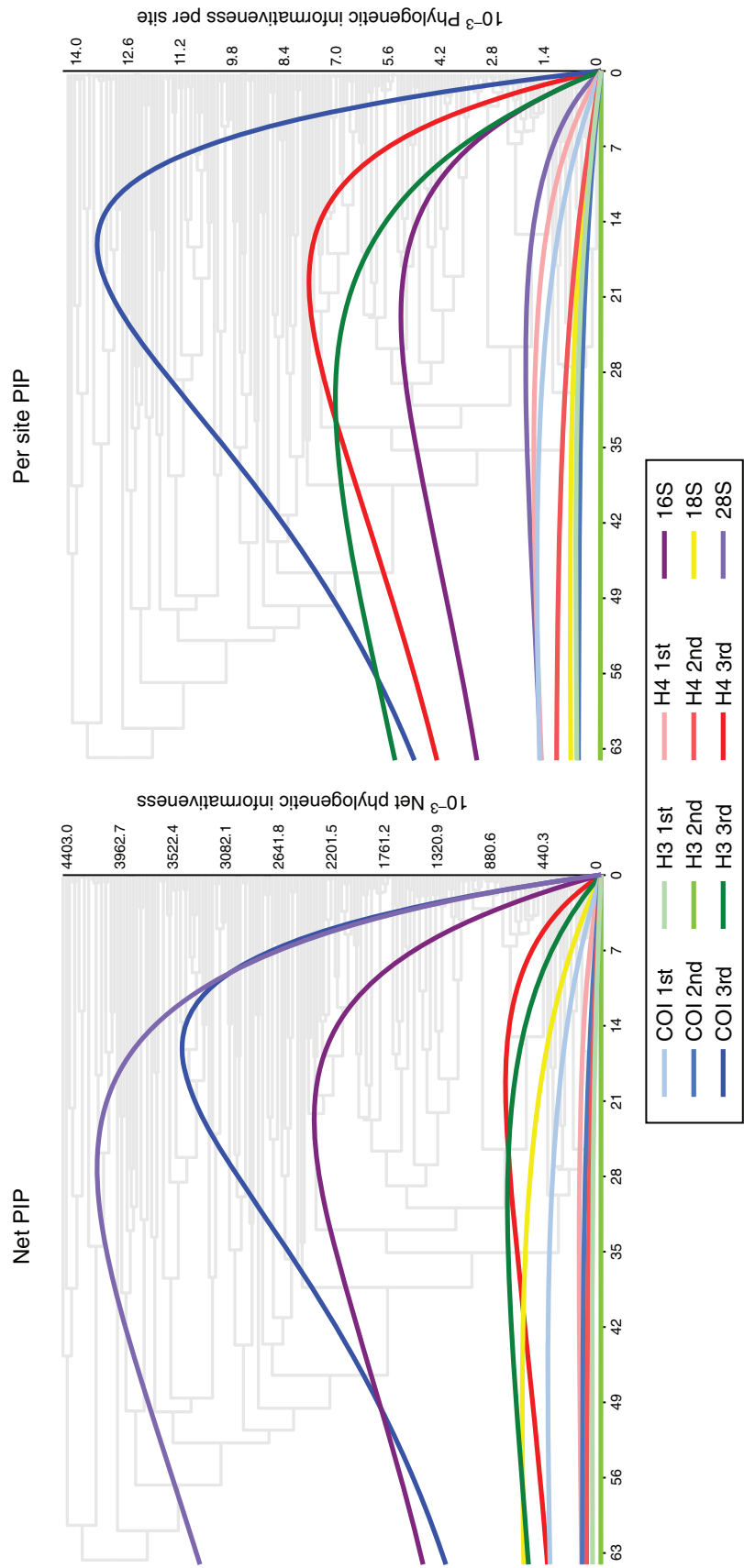


Fig. 3. Phylogenetic informativeness profiles (PIP) from the full dataset divided by gene and codon. Left graph shows net profiles and per-site profiles are shown on the right. The tree BI tree is shown in the background with x-axis indicating node age in millions of years, and y-axis shows normalised likelihood score. See text for details.

The families Arkyidae, Mimetidae and Tetragnathidae formed a monophyletic group across all analyses (Fig. 2), although the taxon sampling was not designed to specifically test the monophyly of each of these well-established families. In the case of the Bayesian analyses, the node of Tetragnathidae was constrained. The internal relationships of the main tetragnathid lineages were unstable across analyses. The reference tree (Fig. 2) showed *Mollemeta edwardsi* (Simon, 1904) as sister to a clade containing the rest of the tetragnathids but the position of *Mollemeta* varied across analyses and was found to be sister to *Diphya spinifera* Tullgren, 1902 in all other trees (Fig. S1–S6) albeit with low support value. All analyses were congruent in supporting the monophyly of the subfamilies Tetragnathinae, Metainae and Nanometinae. Some genera could not be robustly placed in any of the subfamilies. *Taraira rufolineta* (Urquhart, 1889) from New Zealand and formerly classified in the genus *Meta*, did not form part of the group with other *Meta* species in Metainae in any of the analyses.

Metleucauge was sister to Leucauginae but the propinquity of this genus to Leucauginae was inconsistent across analyses. The Leucauginae clade included the genera *Metabus*, *Tylorida*, *Orsinome*, *Mesida*, *Okileucauge*, *Opas*, *Alcimosphenus*, *Mecynometa* and *Leucauge* and this was found in all analyses with relatively high support values (Fig. 2). A clade including *Metabus*, *Tylorida*, *Okileucauge* and *Orsinome* was consistently observed across analyses. Note that *Metabus* and *Tylorida* were not monophyletic in any of our analyses but further sampling would be required to resolve the phylogenetic status of these genera.

Mesida, represented in the analyses by two species, was monophyletic and sister to a clade that grouped all *Leucauge* species together, although the position and monophyly of *Mesida* varied in the other analyses, with some analyses placing this genus within *Leucauge*.

All *Leucauge* species were consistently grouped in a clade that also included species currently placed in the genera *Alcimosphenus*, *Opas*, *Opadometa* and *Mecynometa* that were nested within the clade at various positions. This grouping, that we have named the ‘*Leucauge*’ clade (Fig. 2), was supported across all analyses (except the p4 RY-coded tree). These results suggest that *Leucauge*, as currently circumscribed, is not monophyletic. To facilitate discussions, the ‘*Leucauge* clade’ was partitioned into five lineages labelled 1 to 5 in Fig. 2. However, these clades had low branch support values and the relationships within the *Leucauge* clade were not stable across analytical treatments.

Clade number 1 (Fig. 2) included *Opas*, *Alcimosphenus* and some of the South-east Asian species of *Leucauge*, namely *L. argentina* (Hasselt, 1882), *L. granulata* (Walckenaer, 1841), *L. nanshan* Song & Zhang, 2003 and *L. crucinota* (Bösenberg & Strand, 1906), three unidentified species from Africa and the neotropical *L. moerens* (O. Pickard-Cambridge, 1896). Most branches in this clade find little congruence in other analyses, except for the clade grouping *Alcimosphenus licinus* with *L. moerens* that occurs in all trees across analyses. The species of *Opas* are monophyletic only in the reference tree and the two condensed matrix analyses.

A clade grouping South-east Asian species of *Opadometa*, *Leucauge celebesiana* (Walckenaer, 1841), *L. decorata* (Blackwall, 1864), *L. taiwanica* Yoshida, 2009, and two unidentified African species of *Leucauge* occurred consistently in all analyses with relatively high support; this clade is hereafter referred as the *Opadometa* clade (Clade number 2 in Fig. 2). Some of the *Leucauge* species in this clade (*L. taiwanica*, *L. celebesiana* and *L. tessellata*) share a dense group of setae on the distal tibia of the fourth legs (‘gaiters’) with *Opadometa*. The sequences of one of the *Opadometa* that were retrieved from GenBank, published in Álvarez-Padilla *et al.* (2009), were consistently grouped apart from the other *Opadometa* terminals (sister to *Leucauge taiwanica*) suggesting either that *Opadometa* is not monophyletic or this may have been a case of misidentification of the voucher specimen.

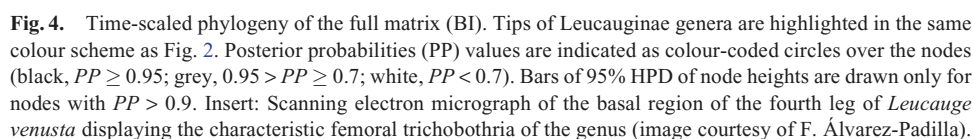
Clade number 3 contained the type species of the genus, *L. argyrobapta*, and the neotropical species *L. bituberculata*, *L. argyra* and *L. venusta*. The resolution within this clade (Fig. 2, 4) did not occur in any other analyses but *L. argyrobapta*, *L. bituberculata* and *L. venusta* were always found to be closely related; either in the same clade or in a paraphyletic arrangement, including other *Leucauge* species or excluding *L. argyra* from this grouping. Despite the apparent almost identical morphology of *L. argyrobapta* and *L. venusta* (Levi 1980; Dimitrov and Hormiga 2010; Ballesteros and Hormiga 2018), all analyses found *L. bituberculata*, a species endemic to the Galapagos archipelago, to be the closest relative of *L. argyrobapta*.

Clade number 4 exclusively comprised neotropical species; including *Leucauge mariana* and *L. branicki* (Taczanowski, 1874), *L. aurostriata* (O. Pickard-Cambridge, 1897), *L. funebris* (Mello-Leitão, 1930), *L. atrostricta* Badcock, 1932, *L. cf. turbida* (Keyserling, 1893) and *L. roseosignatha* Mello-Leitão, 1943. The internal relationships within this clade also received strong support. The lack of topological congruence with other analyses was caused by the unstable position of *L. roseosignatha* because the ML tree of the full dataset (Fig. S3), included *Opas* in this clade.

Clade number 5 (Fig. 2) exclusively comprised neotropical species of *Leucauge*, most of which are known only from South America and some specimens have been identified as undescribed species closely related to *Mecynometa globosa* O. Pickard-Cambridge, 1889. This clade conformation occurred only in the BEAST tree but the clade grouping *Mecynometa cf. globosa* with *L. simplex* F. O. Pickard-Cambridge, 1903 and *L. gemminipunctata* Chamberlin & Ivie, 1936 occurred in all but one analysis (the RY recoded dataset based on χ^2 , Fig. S5).

Dated phylogeny

The time-calibrated reference tree is shown in Fig. 4 against the geological scale, branch support (PP) and bars indicating the 95% highest posterior density interval (HPD) of the node height. Despite using identical priors and the same mean rate for *COI*, the full and condensed dataset produced different mean age nodes, with the condensed dataset showing older



node ages than the full dataset and this was potentially due to topological uncertainties within and between analyses and the confounding effects of missing data (Fig. S1–S2). The full tree showed the mean age of Leucauginae to be 46.7 Ma (Eocene, 95% HPD interval 37–56 Ma), whereas for the same node the condensed dataset mean age is 57.4 Ma (Paleocene, 95% HPD interval from 42–66 Ma). In relation to the origin of the *Leucauge* clade (including *Mesida*), age is inferred from the Eocene period in both dated matrices, 38 Ma (HPD = 29–48) and 45.38 (HPD = 32–55) in the full and condensed datasets respectively.

Taxonomy

Order Araneae Clerck, 1757

Family Tetragnathidae Menge, 1866

Genus *Leucauge* White, 1841

Leucauge White, 1841: 473. *Type species*: *Linyphia* (*Leucauge*) *argyrobapta* (White, 1841).

Plesiometeta F. O. Pickard-Cambridge, 1903: 438. *Type species*: *Tetragnatha argyra* Walckenaer, 1841. Synonymised with *Leucauge* by Banks (1909) and Levi (1980), contra Archer (1951).

Opas O. Pickard-Cambridge, 1896: 185. *Type species*: *Opas lugens* O. Pickard-Cambridge, 1896. Synonymised with *Leucauge* by F. O. Pickard-Cambridge (1903, p. 438). Removed from the synonymy of *Leucauge* White, 1841 by Levi (2008, p. 167), after Archer (1951), contra Levi (1980, p. 23). **REVALIDATED SYNONYMY.**

Mecynometeta Simon, 1894: 737. *Type species*: *Meta globosa* O. Pickard-Cambridge, 1889. Synonymised with *Leucauge* by Levi (1986, p. 105) and later implicitly revalidated by Levi (1991, p. 178), when placing *Epeira caudacuta* Taczanowski, 1873 in *Mecynometeta*. *Mecynometeta* was also treated as a valid genus by Levi (2008). **REVALIDATED SYNONYMY.**

Opadometa Archer, 1951: 8. *Type species*: *Epeira grata* Guérin, 1838. **NEW SYNONYMY.**

Alcimosphenus Simon 1895: 931. *Type species*: *Alcimosphenus licinus* Simon 1895. **NEW SYNONYMY.**

Diagnosis

An accurate diagnosis of *Leucauge* will only be possible once all Leucauginae genera have been revised. Only *Metabus* has been revised (Álvarez-Padilla 2007). *Leucauge* can be distinguished from other tetragnathids by the presence of two parallel rows of dorsal or ectal ‘feathery’ trichobothria on the proximal part of the fourth femur (Fig. 4). *Leucauge* species are similar to those of *Mesida* but the males of the latter are easily distinguished by the presence of a tooth-like projection on the prolateral surface of the paturon (Álvarez-Padilla and Hormiga 2011). *Leucauge* is distinguished from *Tylorida* by the fourth femoral trichobothria, which in the latter genus do not form two clear rows and have smooth, unbranched shafts. *Orsinome* species also have two rows of fourth femoral trichobothria with unbranched, smooth shafts, but this genus can be distinguished by the prominent mesal mastidion in the male chelicerae. *Leucauge* can be distinguished from *Metabus* by the absence of femoral trichobothria in the latter genus (Álvarez-Padilla 2007).

Based on the phylogenetic position of the terminals representing the genera *Opas*, *Opadometa*, *Mecynometeta* and

Alcimosphenus, that are nested within the clade that includes all the *Leucauge* representatives, we propose synonymy with *Leucauge* to render the latter genus monophyletic. In the case of *Mecynometeta*, *Opas* and *Opadometa* the synonymy status is reinstated from previous proposals (e.g. Levi 1980), either from explicit synonymy or by the transfer of the type species to *Leucauge*. In relation to the monotypic genus *Alcimosphenus*, a new synonymy with *Leucauge* is proposed (Levi 1980, p. 23, had already suggested the possibility that *Alcimosphenus* could not be separated from *Leucauge*).

Despite the low support values of the placement in the optimal cladogram, the representatives of the herein proposed synonyms are consistently associated in all trees with typical *Leucauge* species. Future studies aiming to further resolve the phylogeny of Leucauginae should include a broader sample of *Leucauge* species and those in the genera considered the closest relatives in this study (*Metabus*, *Okileucauge*, *Tylorida*, *Orsinome* and *Mesida*).

In the case of *Mesida*, we refrain from proposing nomenclatural changes because the two species analysed form a clade independent of *Leucauge* in at least one analysis, the working hypothesis (that is, with *Leucauge*, as circumscribed here, and *Mesida* being reciprocally monophyletic; Fig. 2). A more detailed justification of the taxonomic changes proposed here is given in the ‘Discussion’ section, under the heading ‘Phylogenetic structure’.

Discussion

Base composition biases

The two base compositional tests showed heterogeneous base composition in at least two partitions. The p4 test (Foster 2004), in which the null distribution of expected base composition is produced by simulations, rejected homogeneity on partitions where the simple χ^2 favoured the null hypothesis (type II error). Base homogeneity tests are usually performed to find explanations for topological anomalies. Given the absence of an empirically robust hypothesis of Leucauginae relationships, to *a priori* identify spurious groupings is not possible. The negative effects of heterogeneous base composition have been demonstrated in empirical and simulated data (e.g. Lockhart *et al.* 1994; Van Den Bussche *et al.* 1998; Tarrío *et al.* 2001; Foster 2004), yet models that include compositional heterogeneity are rare in phylogenetic software or the implementation is too computationally demanding for use in empirical datasets. The recoding of the biased partition to purines and pyrimidines, RY coding, has proven to be a reasonably good solution to ameliorate the effects of base composition biases (Phillips *et al.* 2004; Ishikawa *et al.* 2012). Nevertheless, the use of this coding strategy is not exempt from caveats. This recoding can reduce resolution by reducing the information content in the data and the implementation in phylogenetic inference software must be considered as different programs vary in treatment of ambiguity codes. For example, in the case of BEAST, the default is to treat ‘RY’ ambiguity codes the same as missing data. This behaviour can be manually overridden at the expense of

increasing parameter complexity and consequently the associated computational time required to analyse the data.

Ultimately, there is no standard procedure that universally corrects for the effects of compositional heterogeneity in phylogenetic analysis. Recoding of heterogeneous partitions has been reported to ameliorate the topological effects but results vary from case to case (Ishikawa *et al.* 2012). Although base composition heterogeneity has been previously reported in other spider datasets (e.g. Hedin 2001; Wood *et al.* 2007), the lack of well implemented heterogeneous models has led to few attempts to correct for the potential biases. In other groups of organisms or in genomic datasets, avoiding base composition biases is facilitated by selecting loci that comply with the base composition assumptions implemented (Regier *et al.* 2008; Romiguier *et al.* 2013). The potential ubiquity of compositional heterogeneity in spider datasets should prioritise efforts for increasing the array of loci available for spider phylogenetics.

Phylogenetic structure

Although the taxon sampling used in this study is not intended to test araneoid interfamilial relationships, all our analyses found the families Arkyidae, Mimetidae and Tetragnathidae to be monophyletic (Fig. 2). Phylogenetic relationships of the araneoid families have been difficult to resolve (Hormiga and Griswold 2014; Dimitrov *et al.* 2017; Fernández *et al.* 2018; Kulkarni *et al.* 2020; Kallal *et al.* 2021) but most analyses agree on Mimetidae being the sister group of the clade Arkyidae and Tetragnathidae (Dimitrov *et al.* 2012, 2017; Benavides *et al.* 2017; Wheeler *et al.* 2017; Kallal and Hormiga 2018), a hypothesis more recently supported by transcriptomic data (Fernández *et al.* 2018; Kallal *et al.* 2021) and ultra-conserved elements (UCEs) sequences (Kulkarni *et al.* 2020).

In terms of the composition of the subfamilies of Tetragnathidae, our results are congruent with previous studies on the monophyly and circumscription of Tetragnathinae, Nanometinae, Metainae and Leucauginae (Álvarez-Padilla and Hormiga 2011 and references therein; Kallal and Hormiga 2018; Hormiga 2017; Álvarez-Padilla *et al.* 2020). Similarly to previous molecular studies, there is poor resolution and low branch support values on how these subfamilies are related to each other, and on the placement of some taxa such as *Diphya* Nicolet, 1849, *Allende* Álvarez-Padilla, 2007 and *Mollemeta* Álvarez-Padilla, 2007 (Fig. S1–S4). The position of *Azilia* has also been unstable in previous studies. Morphological matrices show *Azilia* either as sister to Leucauginae or sister to the rest of Tetragnathidae (Álvarez-Padilla 2007; Álvarez-Padilla *et al.* 2009; Álvarez-Padilla and Hormiga 2011). In all our analyses, *Azilia* grouped with *Allende* as sister to the Nanometinae in agreement with Dimitrov and Hormiga (2009) but differed from the recent analysis of Álvarez-Padilla *et al.* (2020) in which *Azilia* + *Diphya* are the sister clade of Leucauginae. Two sister clades occur consistently within Leucauginae. One clade groups *Metabus*, *Tylorida*, *Orsinome* and *Okileucauge* and this grouping is congruent with the results of Dimitrov *et al.* (2017), including a non-monophyletic *Tylorida*.

In our working hypothesis (Fig. 2), the genus *Mesida* (represented by *M. argentiopunctata* (Rainbow, 1916) and *M. yini* Zhu Song & Zhang, 2003) is the sister group of the newly circumscribed *Leucauge* clade. All other analyses, however, suggest a non-monophyletic *Mesida* with these two species nested in different places within the *Leucauge* clade, albeit with low branch support. *Mesida* groups 14 species that occur in Australia and South-east Asia. Members of this genus are remarkably similar to *Leucauge* but the males of *Mesida* are easily distinguished from those of *Leucauge* by the presence of a tooth-like projection on the prolateral surface of the paturon. The low support values and the modest taxonomic representation of *Mesida* (we could not include the type species *M. humilis* Kulczyński, 1911 in our sample) prevent nomenclatural actions. Future studies should be tailored to properly address the monophyly and position of *Mesida* because our data suggest that at least some *Mesida* species may be nested within *Leucauge*.

None of the optimal trees resulting from our analyses suggest that *Leucauge* is a monophyletic group as currently circumscribed. Despite topological conflict in the internal structure, all analyses show that the group circumscribed to include all the *Leucauge* species in the analyses (the *Leucauge* clade), also includes species currently placed in the genera *Mecynomeya*, *Opadometa*, *Opas*, *Alcimosphenus* and potentially *Mesida*. The close affinity of *Alcimosphenus*, *Opas* and *Mecynometa* with *Leucauge* species had been previously noted. Levi (2008) regarded these three genera as valid and distinguished these from *Leucauge* based on sexual size dimorphism. In *Alcimosphenus*, *Opas* and *Mecynometa*, adult males are much smaller than females, whereas in *Leucauge* the degree of size dimorphism is only moderate. *Mecynometa* Simon, 1894 includes three tropical species and is distinguished from *Leucauge* by the aforementioned sexual size dimorphism and having much more slender and longer legs. Two species are known only from female specimens: *M. argyrosticta* Simon, 1907, from West Africa and the Congo, and *M. gibbosa* Schmidt & Krause, 1993 from the Comoro Islands. The type species of the genus, *M. globosa* (O. Pickard-Cambridge, 1889), is widely distributed in the Neotropics, ranging from Guatemala to Brazil. Three *Mecynometa* terminals are included in our analyses identified as *Mecynometa* sp. based on similar habitus (distinctive long legs, spherical abdomen with characteristic guanine patches pattern) to the type species *M. globosa* but differing in the epigynal morphology.

Although the support values are moderate across analyses, *Alcimosphenus* consistently occurs as sister to *Leucauge moerens* (O. Pickard-Cambridge, 1896); this later species was originally described in the genus *Opas*. The females of both *Opas* and *Alcimosphenus* have an elongated posterior abdomen overhanging the spinnerets, although some species of *Leucauge* also show an elongated abdomen, most species in the latter genus have cylindrical abdomens. Additionally, females of *Alcimosphenus* have distinctive bright red-black colouration of the abdomen (Levi 2005), whereas most *Leucauge* species have silvery and metallic coloured abdomens. Despite the low branch support values, the results consistently demand that *Opas* and *Alcimosphenus*

are synonymised with *Leucauge* to circumscribe the latter genus as a monophyletic group.

Both sexes of the type species of *Opadometa* (*O. grata* Guérin, 1838) show several differences from those of the type species of *Leucauge* (*L. argyrobapta*) in somatic morphology and genitalia, suggesting placement in two different genera, at least based on overall morphological features. The genus currently includes five species from Asia and Papua New Guinea. Previous analyses have placed *Opadometa* (represented by an undescribed species highly similar to the type species, *O. grata*) as the sister group of *Leucauge* (represented by *L. argyra* and *L. venusta*) (Álvarez-Padilla *et al.* 2009; Álvarez-Padilla and Hormiga 2011; Dimitrov *et al.* 2012). Our phylogenetic results suggest that *Opadometa* is a clade within *Leucauge* and sister group to the common pantropical species *L. decorata* (Blackwall, 1864). *Opadometa* needs to be synonymised with *Leucauge* because taxa should be circumscribed to be monophyletic (Hennig 1950, 1966). Our results also suggest that future studies aiming to test the monophyletic status of *Opadometa* with any rigor, must include the *Leucauge* species in the *Opadometa* clade (Clade 2, Fig. 2) in addition to *L. argyrobapta* (the type species), *L. venusta* and *L. argyra*.

Although *Leucauge argyrobapta* (the type species) and *L. venusta* are morphologically highly similar and have been synonymised (Dimitrov and Hormiga 2010; Ballesteros and Hormiga 2018), our results suggest that these two species (each represented by 11 terminals in the analyses) are not the closest relatives of each other (but both species form monophyletic groups). In our working hypothesis (Fig. 2, 4 and S1) the relationships of these species can be summarised as follows: (*L. venusta* (*L. argyra* (*L. bituberculata*, *L. argyrobapta*))). Considering this topology and given that *L. venusta* and *L. argyrobapta* have been treated as a single species based on the morphology until recently, the finding that these two species are not sister taxa is surprising. *Leucauge bituberculata* Baert, 1987 is endemic to the Galapagos Archipelago and is morphologically highly similar to *L. venusta* and *L. argyrobapta*. The optimal cladogram implies that the morphological similarities between *L. argyrobapta* and *L. venusta* are most parsimoniously interpreted as symplesiomorphic, and are also shared with *L. bituberculata* to some extent, and therefore the unusual features of the genitalia of *L. argyra* (such as the conical, protruding epigynal region and the large conductor and dorsal cymbial process; see Levi 1980, fig. 60–71) are autapomorphic and have evolved from an ancestor whose morphology resembled that of *L. venusta* and *L. argyrobapta* (see Levi 1980, fig. 44–59; and Dimitrov and Hormiga 2010, fig. 2–9).

When the morphology of the *Leucauge* clade is examined within the cladistic context provided by the molecular phylogenetic tree, an overall pattern emerges. Most *Leucauge* species share common somatic and genitalic morphologies, similar to those of the type species (*L. argyrobapta*). Several taxa within the *Leucauge* clade have diverged from this plesiomorphic morphological ‘syndrome’. In most such cases, under the pre-cladistic

taxonomic approach of maximising overall similarity of species within a genus, and invoking gaps in character space between genera, several genera have been erected. For example, *Alcimosphenus licinus* Simon, 1895 (the sole species of this West Indies genus; see Levi 2005, 2008) is simply a species of *Leucauge* that has been taxonomically highlighted on account of the unique, beautiful, bright red-orange colouration and extreme sexual size dimorphism (female body size ranges between 6–10 mm long whereas males are less than 2 mm long). Similarly, *Mecynometa globosa* departs morphologically from the *Leucauge* ‘syndrome’ in being much smaller (females ~2.7 mm and males ~2 mm long) (Pickard-Cambridge 1903; Chickering 1963) and having long, thin legs (Levi 2008). Resolving the taxonomy of *Leucauge* is a Herculean task with 171 described species. This requires a monographic approach, the study of extensive global museum collections including numerous types, world-wide collection of specimens for molecular work and a phylogenetic analysis with as many species terminals as possible. This study represents a small first step in this long systematic journey. Biological nomenclature is by nature a conservative science because the aim is to provide the maximum universality and continuity of scientific names (International Commission on Zoological Nomenclature 1999). Our study should perhaps refrain from making taxonomic or nomenclatural changes in *Leucauge*, on account of the relatively small number of species studied (46 species, 26% of the currently valid species), small number of characters (provided by only six loci) when recent spider phylogenetic studies have identified thousands of potential loci (e.g. see Fernández *et al.* 2014; Garrison *et al.* 2016; Ballesteros and Hormiga 2016; Fernández *et al.* 2018; Kulkarni *et al.* 2020, 2021) and low branch support values in various parts of the optimal tree. On the other hand, a taxonomic problem of this magnitude can only be realistically tackled by taking incremental steps. We have demonstrated that *Leucauge*, as currently circumscribed, is not monophyletic. The most inclusive and stable clade to include all the *Leucauge* species in our study, the *Leucauge* clade, provides a phylogenetically based circumscription of the genus (none existed before our work). Circumscribing *Leucauge* to be monophyletic requires treating *Opas*, *Mecynometa*, *Opadometa* and *Alcimosphenus* as junior synonyms of *Leucauge*. After these synonymisations, *Leucauge* increases by 14 additional species (*Alcimosphenus* is monotypic and the other three genera lack modern taxonomic revisions). Our revised classification is grounded on phylogenetic reasoning and provides a testable framework for future work in *Leucauge*.

Limitations of available loci

Progress in molecular phylogenetics in spiders has been hampered by a reduced repertoire of genetic markers (e.g. Hedin and Bond 2006; Maddison *et al.* 2008; Lopardo *et al.* 2011; Agnarsson *et al.* 2013; Hormiga and Griswold 2014; Hamilton *et al.* 2016; Rix *et al.* 2017; Wheeler *et al.* 2017). In addition to the reduced number of available markers, most studies (this one included) use long-established

‘universal primer pairs’ for the amplification and sequencing of the regions of interest. The wide use of these primers is based on the assumption of high sequence conservation of the primer binding region across a wide range of taxa and broad evolutionary scales. Nevertheless, nothing makes primer-binding regions mutation-proof and some gene fragments are well known to be ‘easier’ or ‘harder’ to amplify with universal primers in particular groups (Sharma and Kobayashi 2014). Despite these difficulties, few efforts have been made to develop and document alternative spider PCR primers for known markers for Sanger sequencing, and to develop primers for new loci (Ayoub *et al.* 2007; Vink *et al.* 2008). Tuning the amplification conditions to increase amplification success has been favoured instead but these strategies may fail at the sequencing stage or increase the risk of contamination.

Several authors have discussed the importance of the selection of appropriate genetic markers for estimating phylogenies (Townsend *et al.* 2012; Salichos and Rokas 2013). The PIPs show that only a few of the loci, and specifically some data partitions, have relatively strong phylogenetic signal. These profiles also show that most of the explanatory power is concentrated at relatively shallow temporal scales (less than 30 Ma) and potentially explain the lack of topological support at certain nodes. As discussed in Townsend (2007) and López-Giráldez and Townsend (2011) these profiles are notably not definitive indicators, nor do these invalidate the utility of a given marker beyond the peak of informativeness. Ultimately these profiles can be used as additional criteria to guide decisions and prioritise use of loci most likely to inform nodes of interest. A highly relevant example of this approach is provided by Rix *et al.* (2017) who developed new orthologous nuclear markers for the phylogenetic inference of mygalomorph spiders. These markers were subsequently used to infer the phylogeny of Australasian Idiopidae with a 12-gene parallel tagged amplicon next-generation sequencing approach. A limitation in spider phylogenetics is that the selection of loci used in most phylogenetic surveys is pragmatically dominated by what can be sequenced, based on costs or amplification success, rather than favouring the genetic markers most likely to resolve the nodes of interest. Lower cost and target capture strategies will likely improve the resolution required to advance the taxonomy of spiders and understudied groups (e.g. Kulkarni *et al.* 2020, 2021).

Conclusion

Leucauge is currently not monophyletic as the genera *Alcimosphenus*, *Opas*, *Opadometa* and *Mecynometa* are included in the circumscription. We synonymised these four genera with *Leucauge* to render this latter genus monophyletic based on our phylogenetic results. All the trees suffered from low nodal support and many clades should be considered unresolved. There is evidence of composition heterogeneity in at least three of the loci but assessing the effects of this heterogeneity in the phylogenetic inference is difficult. Additionally, the phylogenetic informativeness profiling suggests heterogeneous information content across partitions. These profiles indicate a potential lack of power of the traditional markers to solve relationships at the hierarchy

of subfamilies and genera, with some markers suffering from rapid saturation and others evolving too slowly to resolve nodes at the depth of interest (including histone *H4*). These limitations suggest that for certain nodes, a broader sampling of loci or taxon coverage is required to achieve stable phylogenetic resolution.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Supplementary material

Molecular phylogeny of the orb-weaving spider genus *Leucauge* and the intergeneric relationships of Leucauginae (Araneae, Tetragnathidae)

Jesús A. Ballesteros^{A,B,C} and Gustavo Hormiga^A

^ADepartment of Biological Sciences, The George Washington University, Washington, DC 20052, USA.

Email: hormiga@gwu.edu

^BDepartment of Integrative Biology, University of Wisconsin—Madison, Madison, WI 53706, USA.

^CCorresponding author. Email: ballesterosc@wisc.edu

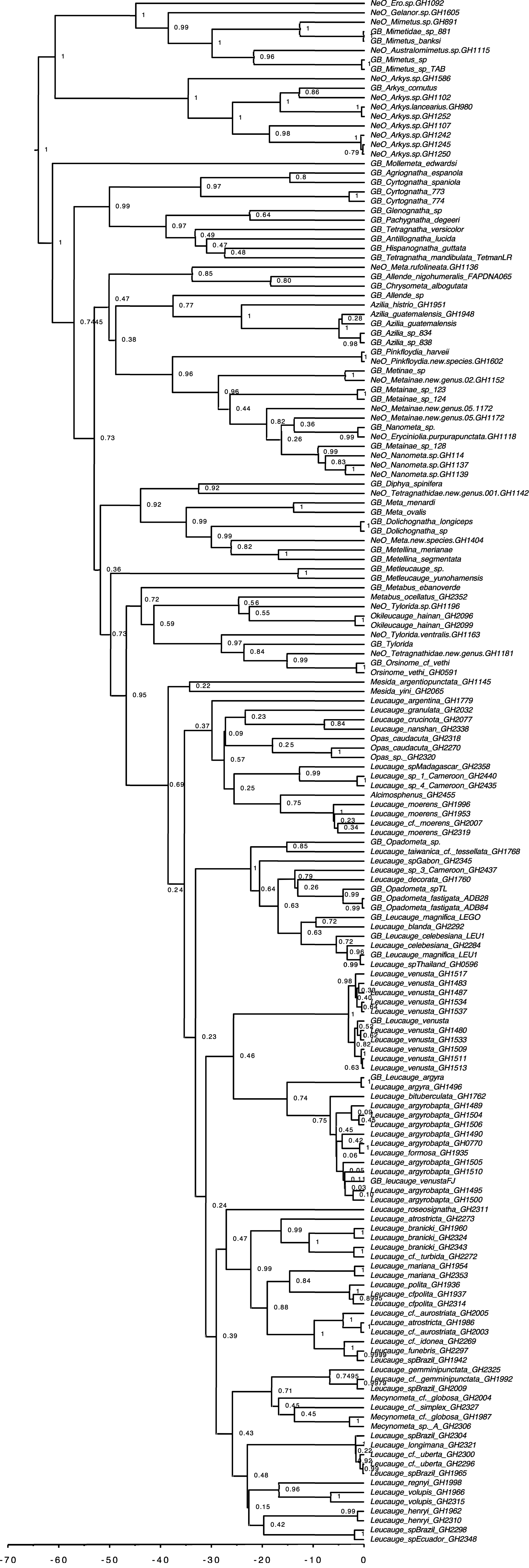


Fig. S1. Maximum clade credibility tree (BI) from the full dataset.

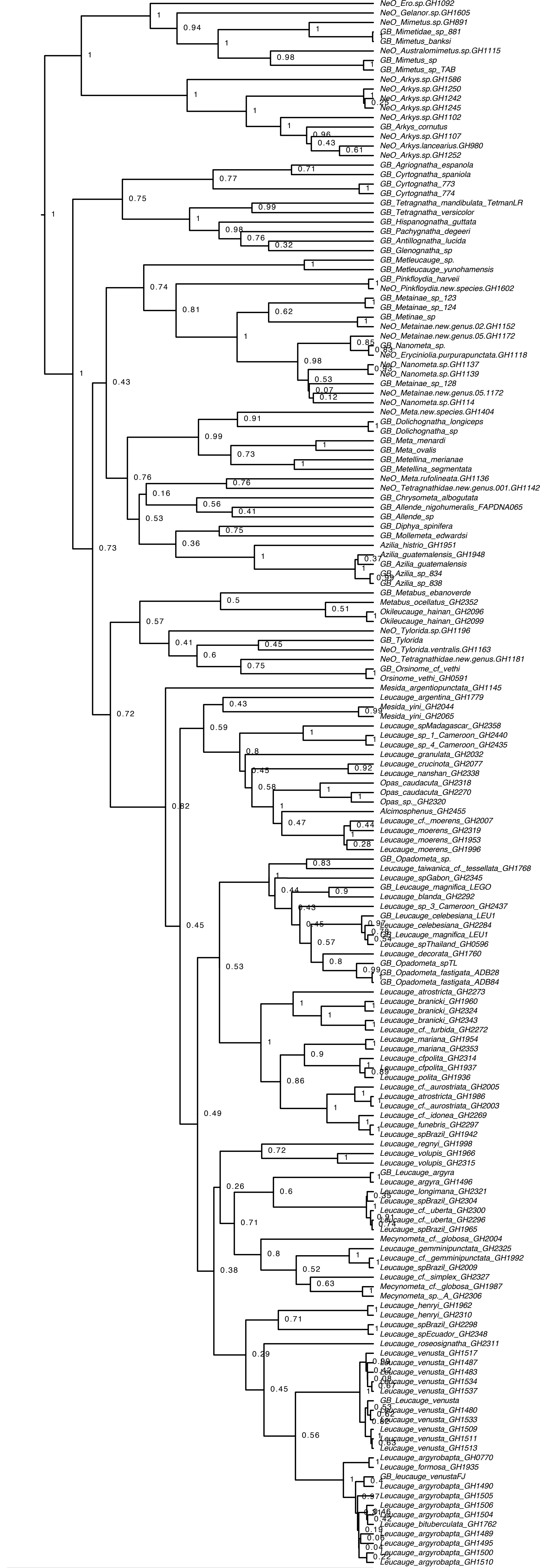
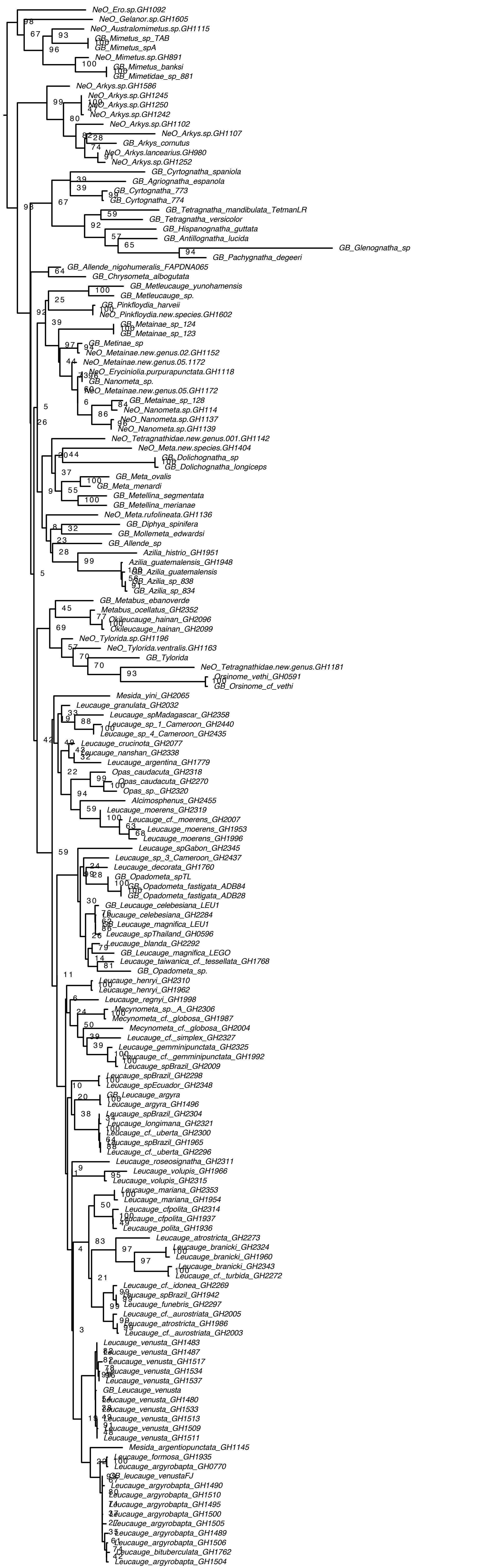


Fig. S2. Maximum clade credibility tree (BI) from the condensed dataset.



Fig. S3. Maximum likelihood tree from the full dataset.



0.05

Fig. S4. Maximum likelihood tree from the condensed dataset.

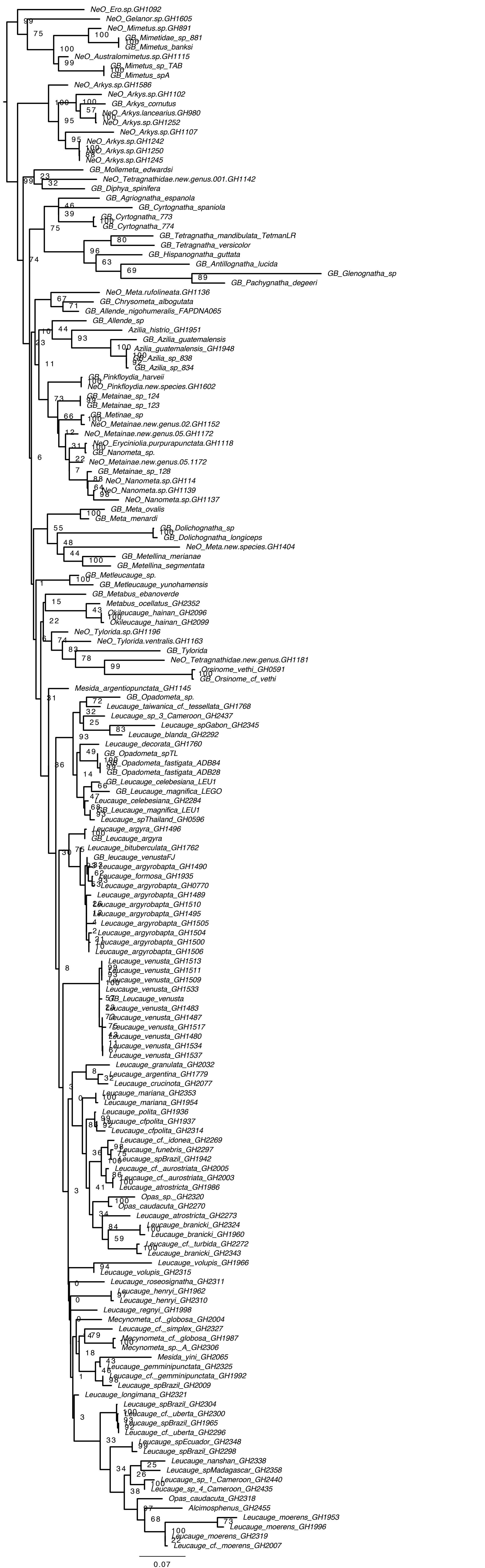


Fig. S5. Maximum likelihood tree from the full dataset using RY coding for composition biased positions based on χ^2 test.

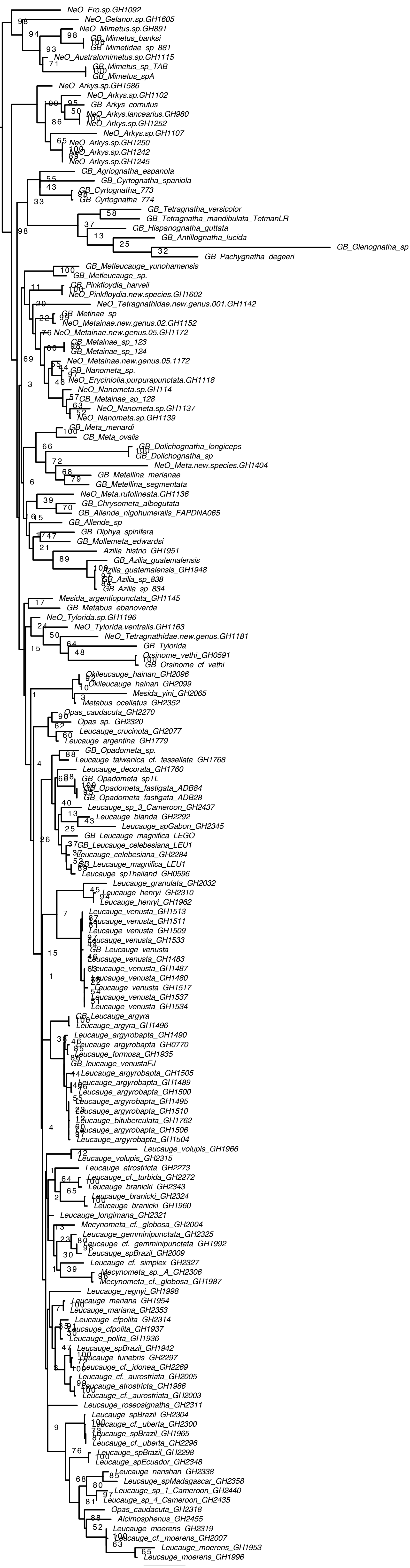


Fig. S6. Maximum likelihood tree from the full dataset with biased positions RY based on p4 test.

Table S1. Specimen collection metadata

Voucher	Species	Date	Country: state	Locality name	Longitude	Latitude
GH2455	<i>Alcimosphenus licinus</i>		Dominican Republic	NA	NA	NA
GH1948	<i>Azilia guatemalensis</i>	09-Aug-13	Costa Rica: Puntarenas	Estacion Biologica Monteverde, Sendero Principal (bridge)	-84.8079	10.3198
GH1951	<i>Azilia histrio</i>	25-May-12	Brazil: Roraima	Trail leading E. of Caicubi community	-62.0866	-1.02925
GH1779	<i>Leucauge argentina</i>	28-Apr-11	Singapore:	Bukit Timah Nat. Res.	1.35472	103.777
GH1496	<i>Leucauge argyra</i>	07-Jul-14	USA: Florida	Archbold Biological Station	-81.3504	27.1818
GH0770	<i>Leucauge argyrobapta</i>	21-Aug-07	Brazil: Rio de Janeiro	Jardin Botanico, Museu Nacional do Rio de Janeiro	-43.2235	-22.9084
GH1489	<i>Leucauge venusta</i>	06-Jul-14	USA: Georgia	Near Jekyll Island	-81.4155	31.1145
GH1490	<i>Leucauge venusta</i>	06-Jul-14	USA: Florida	Near Osceola National Forest	-82.4417	30.2468
GH1495	<i>Leucauge venusta</i>	07-Jul-14	USA: Florida	Archbold Biological Station	-81.3504	27.1818
GH1500	<i>Leucauge venusta</i>	08-Jul-14	USA: Florida	Lake Annie, Archbold Biological Station	-81.3491	27.2106
GH1504	<i>Leucauge venusta</i>	09-Jul-14	USA: Florida	Near Goethe State Park	-82.6331	29.1805
GH1505	<i>Leucauge venusta</i>	09-Jul-14	USA: Florida	Otter Springs Campground	-82.9415	29.6453
GH1506	<i>Leucauge venusta</i>	10-Jul-14	USA: Florida	Near Ecofina River State Park	-83.9065	30.059
GH1510	<i>Leucauge venusta</i>	11-Jul-14	USA: Louisiana	Near Tickfaw State Park	-90.637	30.3838
GH1986	<i>Leucauge atrostricta</i>	14-Apr-12	Brazil: Para	Fazenda Bom Retiro	-49.238	-4.84498
GH2273	<i>Leucauge atrostricta</i>	29-Oct-14	Brazil: Para	FLONA Tapajos, km 83	-54.9418	3.043
GH1762	<i>Leucauge bituberculata</i>	23-Mar-09	Ecuador: Galapagos	El Junco, Isla San Cristobal	-89.4813	-0.895278
GH2292	<i>Leucauge blanda</i>	10-Jul-13	Taiwan:	Dasyueshan National Forest, Recreation Area	121.007	24.2574
GH1960	<i>Leucauge branicki</i>	11-Aug-11	Brazil: Para	Mirinzal	-44.8285	-2.12743
GH2324	<i>Leucauge branicki</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2343	<i>Leucauge branicki</i>	12-Dec-09	Ecuador: Napo	Fundacion Jatun Sacha	-77.6167	-1.06597
GH2284	<i>Leucauge celebesiana</i>	07-Jul-13	Taiwan:	Luhu, near Nanzhuang Township	121.047	24.5407
GH2003	<i>Leucauge cf. aurostriata</i>	04-Nov-14	Brazil: Para	Parque Estadual do Utinga	-48.4293	-1.42308
GH2005	<i>Leucauge cf. aurostriata</i>	16-Jul-14	Mexico: Veracruz-Llave	Estacion de Biologia Los Tuxtlas	-95.0742	18.5854
GH1992	<i>Leucauge cf. gemminipunctata</i>	14-Mar-12	Brazil: Maranhao	Reserva Biologica Gurupi (Norte)	-46.7463	-3.69273
GH2269	<i>Leucauge cf. idonea</i>	17-Jun-14	Brazil: Roraima	Vila Tepequem, Cachoeira Paiva	-61.7164	3.75511
GH2007	<i>Leucauge cf. moerens</i>	15-Jun-14	Brazil: Roraima	Vila Tepequem, Pousada PSJ	-61.7219	3.78206
GH2327	<i>Leucauge cf. simplex</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2272	<i>Leucauge cf. turbida</i>	04-Nov-14	Brazil: Para	Parque Estadual do Utinga	-48.4293	-1.42308
GH2296	<i>Leucauge cf. uberta</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2300	<i>Leucauge cf. uberta</i>	27-May-12	Brazil: Roraima	Bacaba, Comunidad de Caicubi	-62.1031	-0.977952
GH1937	<i>Leucauge sp.</i>	26-Aug-12	Mexico: Distrito Federal	Bosque de Tlalpan	-99.1949	19.2955
GH2314	<i>Leucauge venusta</i>	12-Oct-12	Mexico: Veracruz-Llave	Trail to plot II, Pico de Orizaba Volcano. Atotonilco de Calchualco	-97.2071	19.1419
GH2077	<i>Leucauge crucinota</i>	13-Oct-09	Vietnam: Ninh Binh	Cuc Phuong National Park, Cay Dang Co Thu trail	105.656	20.299
GH1760	<i>Leucauge decorata</i>	07-Jul-13	Taiwan:	Luhu, near Nanzhuang Township	121.047	24.5407
GH1935	<i>Leucauge argyrobapta</i>	23-Jan-14	Brazil: Rio de Janeiro	Pista Claudio Coutinho	-43.1588	-22.9498
GH2297	<i>Leucauge funebris</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2325	<i>Leucauge gemminipunctata</i>	25-May-12	Brazil: Roraima	Trail leading E. of Caicubi community	-62.0866	-1.02925
GH2032	<i>Leucauge granulata</i>	08-Feb-12	Australia: Queensland	Land Rd, Rose Gums Wilderness Retreat ~13 km N.E. of Malanda ridge trail	145.703	17.3141
GH1962	<i>Leucauge henryi</i>	31-Oct-10	Brazil: Para	Parque Estadual do Utinga	-48.4293	-1.42308
GH2310	<i>Leucauge henryi</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2321	<i>Leucauge longimana</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH1954	<i>Leucauge mariana</i>	08-Aug-13	Costa Rica: Heredia	INBIO parque	-84.0928	9.97303
GH2353	<i>Leucauge mariana</i>	01-Aug-2013	Costa Rica: Puntarenas	University of Georgia	-84.7986	10.2819
GH1953	<i>Leucauge moerens</i>	01-Aug-2013	Costa Rica: Heredia	La Selva Biological Station, near Puerto Viejo	-84	10.4306
GH1996	<i>Leucauge moerens</i>	10-Sep-14	Panama: Chiriqui	David	-82.4974	8.43772
GH2319	<i>Leucauge moerens</i>	31-May-12	Brazil: Roraima	Tucano, Arquipelago de Mariui e Baixo Rio Branco, Rio Jufari Comunidad de Caicubi	-62.1058	-1.01484
GH2338	<i>Leucauge nanshan</i>	27-Sep-09	Vietnam: Ha Tinh	Vu Quang National Park, forest near Don Bien Phong (border station) 567)	105.439	18.3313

Voucher	Species	Date	Country: state	Locality name	Longitude	Latitude
GH1936	<i>Leucauge polita</i>	19-Sep-12	Mexico: Mexico	Acueducto Vista del Valle # 38, CP 53296	-99.2659	19.4738
GH1998	<i>Leucauge regnyi</i>	16-Apr-15	Dominican Republic: La Vega	Reserva Cientifica Ebano Verde	-70.5419	19.0324
GH2311	<i>Leucauge roseosignatha</i>	23-Jan-12	Brazil: Bahia	Parque Estadual Sete Passagens, Miguel Calmon	-40.5221	-11.3998
GH1942	<i>Leucauge</i> sp.	29-May-12	Brazil: Roraima	Trail leading E. of Caicubi community	-62.0898	-1.0285
GH1965	<i>Leucauge</i> sp.	19-Feb-11	Brazil: Para	Acampamento Mutum	-56.2229	-2.555
GH2009	<i>Leucauge</i> sp.	29-Oct-14	Brazil: Para	FLONA Tapajos, km 83	-54.9418	3.043
GH2298	<i>Leucauge</i> sp.	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH2304	<i>Leucauge</i> sp.	28-May-12	Brazil: Roraima	Pupunha, Comunidad de Caicubi	-62.0974	-0.988992
GH2348	<i>Leucauge</i> sp.	12-Dec-09	Ecuador: Napo	Fundacion Jatun Sacha	-77.6167	-1.06597
GH2345	<i>Leucauge</i> sp.	28-Jun-09	Gabon: Moyen-Ogooue	Lambarene	10.1992	-0.65825
GH2358	<i>Leucauge</i> sp.	22-Jan-03	Madagascar: Fianarantsoa	Foret d'Antsirakambiaty	46.564	-20.594
GH0596	<i>Leucauge</i> sp.	02-Oct-03	Thailand: Chiang Mai	Amphen Chiangdao, below guest house along road	98.8297	19.3203
GH2440	<i>Leucauge</i> sp. 1 Cameroon	14-Jun-09	Cameroon: South-west Region	Mount Cameroon, Buea, track from prison farm	9.21822	4.17018
GH2437	<i>Leucauge</i> sp. 3 Cameroon	13-Jun-09	Cameroon: South-west Region	Mount Cameroon, Track to Ekona Lelu	9.31117	4.26873
GH2435	<i>Leucauge</i> sp. 4 Cameroon	14-Jun-09	Cameroon: South-west Region	Mount Cameroon, Buea, track from prison farm	9.21822	4.17018
GH1768	<i>Leucauge taiwanica</i> cf. <i>tessellata</i>	29-Jul-13	Taiwan:	Li-Lung Mountain, N. of Dungyuan Township, off County Road 199	120.855	22.2406
GH1480	<i>Leucauge venusta</i>	02-Jul-14	USA: Virginia	Moonshine Dell Trails, Mountain Lake Biological Station	-80.5186	37.3727
GH1483	<i>Leucauge venusta</i>	03-Jul-14	USA: North Carolina	Near Arrowhead Campground, Uwharrie National Forest	-80.0713	35.4396
GH1487	<i>Leucauge venusta</i>	05-Jul-14	USA: Georgia	Near Magnolia Springs, State Park	-81.9531	32.8853
GH1509	<i>Leucauge venusta</i>	11-Jul-14	USA: Alabama	Near Little River State Park	-87.4853	31.2402
GH1511	<i>Leucauge venusta</i>	12-Jul-14	USA: Louisiana	Near Three Rivers Wildlife Management Area	-91.6461	31.0099
GH1513	<i>Leucauge venusta</i>	12-Jul-14	USA: Mississippi	Near St Catherine Creek National Wildlife Refuge	-91.4514	31.4073
GH1517	<i>Leucauge venusta</i>	13-Jul-14	USA: Arkansas	Poison Creek	-93.0053	33.6391
GH1533	<i>Leucauge venusta</i>	18-Jul-14	USA: Ohio	Near Shawnee State Park	-83.1802	38.7268
GH1534	<i>Leucauge venusta</i>	19-Jul-14	USA: Ohio	Hanging Rock Recreation Area	-82.7133	38.5732
GH1537	<i>Leucauge venusta</i>	19-Jul-14	USA: West Virginia	Near Cedar Creek State Park	-80.8707	38.8769
GH1966	<i>Leucauge volupis</i>	25-Feb-11	Brazil: Para	Varzea Piranha	-56.1224	-2.21003
GH2315	<i>Leucauge volupis</i>	02-Nov-12	Brazil: São Paulo	Botucatu, Rio Bonito	-48.6658	-22.6779
GH1987	<i>Mecynometa</i> cf. <i>globosa</i>	31-Oct-10	Brazil: Para	Parque Estadual do Utinga	-48.4293	-1.42308
GH2004	<i>Mecynometa</i> cf. <i>globosa</i>	04-Nov-14	Brazil: Para	Parque Estadual do Utinga	-48.4293	-1.42308
GH2306	<i>Mecynometa</i> sp. <i>A</i>	18-May-12	Brazil: Amazonas	Reserva Florestal Adolfo Ducke	-59.9733	-2.93216
GH1145	<i>Mesida argentiopunctata</i>	09-Feb-12	Australia: Queensland	Crater Lakes National Park, Lake Barrine, Rainforest Walk Trail	145.642	-17.2449
GH2065	<i>Mesida yini</i>	13-Oct-09	Vietnam: Ninh Binh	Cuc Phuong National Park, Cay Dang Co Thu trail	105.656	20.299
GH2352	<i>Metabus ocellatus</i>	11-Aug-13	Costa Rica: Puntarenas	Estacion Biologica Monteverde, sendero Congo	-84.8083	10.3157
GH2096	<i>Okileucauge hainan</i>	27-Sep-09	Vietnam: Ha Tinh	Vu Quang National Park, forest near Don Bien Phong (border station) 567)	105.439	18.3313
GH2099	<i>Okileucauge hainan</i>	27-Sep-09	Vietnam: Ha Tinh	Vu Quang National Park, forest near Don Bien Phong (border station) 567)	105.439	18.3313
GH2270	<i>Opas caudacuta</i>	16-Apr-14	Brazil: Roraima	Vils Tepequem, SESC	-61.722	3.782
GH2318	<i>Opas caudacuta</i>	28-May-12	Brazil: Roraima	Pupunha, Comunidad de Caicubi	-62.0974	-0.988992
GH2320	<i>Opas</i> sp.	27-May-12	Brazil: Roraima	Bacaba, Comunidad de Caicubi	-62.1031	-0.977952
GH0591	<i>Orsinome vethi</i>	15-Oct-03	Thailand: Yala	Bang Lang National Park	1.16414	6.19653

Table S2. GenBank accession numbers

Taxon name	16S	18S1	18S2	28S2	28S3	COI	H3	H4	References
GB_Agriognatha_espanola	NA	EU003344	NA	EU153162	EU003402	EU003283	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Allende_nigohumeralis_FAPDNA065	EU003271	EU003368	EU003369	EU003396.1	NA	NA	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Allende_sp.	NA	GU129574	NA	NA	NA	GU129635	GU129649	NA	Dimitrov and Hormiga (2011)
GB_Antillognatha_lucida	NA	GU129576	GU129577	GU129603	NA	GU129631	GU129647	NA	Dimitrov and Hormiga (2011)
GB_Arkys_cornutus	NA	NA	FJ607482	NA	NA	FJ607556	FJ607595	NA	Blackledge <i>et al.</i> (2009)
GB_Azilia_guatemalensis	EU003262	EU003371	EU003372	EU003399	NA	EU003280	EU003313	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Azilia_sp._834	GU129570	GU129581	NA	GU129606	NA	GU129624	GU129641	NA	Dimitrov and Hormiga (2011)
GB_Azilia_sp._838	NA	GU129582	NA	GU129607	NA	GU129625	GU129642	NA	Dimitrov and Hormiga (2011)
GB_Chrysometa_albogutata	NA	EU003389	NA	EU153160	EU003400	NA	EU003314	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Cyrtognatha_773	NA	NA	NA	GU129609	NA	GU129630	GU129645	NA	Dimitrov and Hormiga (2011)
GB_Cyrtognatha_774	NA	NA	NA	GU129610	NA	GU129629	GU129646	NA	Dimitrov and Hormiga (2011)
GB_Cyrtognatha_spaniola	NA	EU003344	NA	EU153162	EU003402	NA	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Diphya_spinifera	NA	GU129584	GU129585	GU129611	NA	GU129626	GU129643	NA	Dimitrov <i>et al.</i> (2012)
GB_Dolichognatha_longiceps	NA	GU129578	GU129579	GU129604	GU129605	GU129632	GU129648	NA	Dimitrov <i>et al.</i> (2012)
GB_Dolichognatha_sp.	NA	EU003346	NA	EU153165	EU003405	EU003285	EU003317	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Glenognatha_sp.	NA	GU129586	NA	GU129612	NA	GU129627	GU129644	NA	Dimitrov and Hormiga (2011)
GB_Hispanognatha_guttata	NA	GU129587	GU129588	GU129613	NA	GU129633	GU129652	NA	Dimitrov and Hormiga (2011)
GB_Leucauge_argyra	EU003264	EU003364	NA	EU003427	NA	EU003291	EU003339	NA	Dimitrov and Hormiga (2011)
GB_Leucauge_celebesiana_LEU1	JN816497.1	JN816719.1	NA	JN816928.1	NA	JN817131.1	NA	NA	K. H. Jang and U. W. Hwang (unpubl. data)
GB_Leucauge_magnifica_LEGO	JN816496	JN816718	NA	JN816927	NA	JN817130	NA	NA	K. H. Jang and U. W. Hwang (unpubl. data)
GB_Leucauge_magnifica_LEU1	HQ441966	HQ441985	NA	NA	NA	HQ441946	NA	NA	Su <i>et al.</i> (2011)
GB_Leucauge_venusta	EU003263	EU003350	NA	EU153169	EU003409	EU003290	EU003322	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Meta_menardi	EU003268	EU003353	NA	EU153173	EU003413	EU003295	EU003325	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Meta_ovalis	FJ607460	FJ607497	NA	NA	NA	FJ607571	FJ607609	NA	Blackledge <i>et al.</i> (2009)
GB_Metabus_ebanoverde	EU003265	EU003354	NA	EU153174	EU003414	EU003296	EU003326	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Metainae_sp._123	NA	GU129591	NA	GU129616	NA	NA	NA	NA	Dimitrov and Hormiga (2011)
GB_Metainae_sp._124	NA	GU129592	GU129593	GU129617	GU129618	NA	NA	NA	Dimitrov and Hormiga (2011)
GB_Metainae_sp._128	NA	GU129595	GU129596	GU129619	GU129620	NA	NA	NA	Dimitrov and Hormiga (2011)
GB_Metellina_merianae	EU003270	EU003356	NA	EU153176	EU003416	EU003298	EU003328	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Metellina_segmentata	FJ607461	FJ607498	NA	FJ607536	NA	FJ607572	FJ607610	NA	Blackledge <i>et al.</i> (2009)
GB_Metinae_sp.	EU003272	EU003357	NA	EU153177	EU003417	EU003299	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Metleucauge_sp.	NA	GU129599	NA	GU129621	NA	GU129636	NA	NA	Dimitrov and Hormiga (2011)
GB_Metleucauge_yunohamensis	JN816500	JN816722	NA	JN816931	NA	JN817134	NA	NA	K. H. Jang and U. W. Hwang (unpubl. data)
GB_Mimetidae_sp._881	NA	NA	NA	JN010191.1	NA	NA	NA	NA	K. H. Jang and U. W. Hwang (unpubl. data)
GB_Mimetus_banksi	NA	GU129600	NA	GU129622	NA	GU129637	GU129651	NA	Dimitrov and Hormiga (2011)
GB_Mimetus_sp.	FJ607463	FJ607500	NA	FJ607538	NA	FJ607574	FJ607612	NA	Blackledge <i>et al.</i> (2009)
GB_Mimetus_sp._TAB	FJ607463.1	FJ607500.1	NA	FJ607538.1	NA	FJ607574.1	FJ607612	NA	Blackledge <i>et al.</i> (2009)
GB_Mollemeta_edwardsi	EU003269	EU003374	EU003375	EU003419	NA	NA	EU003330	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Nanometa_sp.	NA	EU003391	NA	EU153179	EU003420	NA	EU003331	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Opadometa_fastigata_ADB28	NA	NA	NA	NA	NA	KT383690	NA	NA	DNA barcoding of spiders from Pune, Maharashtra, India (unpubl. data)
GB_Opadometa_fastigata_ADB84	NA	NA	NA	NA	NA	KT383716	NA	NA	DNA barcoding of spiders from Pune, Maharashtra, India (unpubl. data)
GB_Opadometa_sp.	EU003266	EU003361	NA	EU003423	NA	EU003304	EU003336	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Opadometa_spTL	KC849142.1	KC848955	NA	KC849016	NA	KC849101	KC849057	NA	Kuntner <i>et al.</i> (2013)
GB_Orsinome_cf_vethi	EU003267	EU003362	NA	EU153181	EU003424	EU003305	EU003337	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Pachygnatha_degeeri	NA	NA	NA	EU153182	NA	NA	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)

Taxon name	16S	18S1	18S2	28S2	28S3	COI	H3	H4	References
GB_Pinkfloydia_harveii	NA	GU129571	GU129572	GU129601	GU129602	GU129628	GU129640	NA	Dimitrov and Hormiga (2011)
GB_Tetragnatha_mandibulata_TetmanLR	NA	NA	NA	AY231069	NA	NA	NA	NA	Arnedo <i>et al.</i> (2004)
GB_Tetragnatha_versicolor	NA	EU003394	NA	EU153185	EU003429	EU003308	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Tylorida	NA	EU003365	NA	EU153186	NA	EU003309	NA	NA	Álvarez-Padilla <i>et al.</i> (2009)
GB_Leucauge_venustaFJ	NA	NA	NA	NA	NA	FJ607568	FJ607606	NA	Blackledge <i>et al.</i> (2009)
NeO_Arkys.lancearius.GH980	KM486279	NA	NA	KM486346	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1102	KM486280	KM486131	NA	KM486347	NA	KM486422	KM486474	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1107	KM486281	NA	NA	KM486348	NA	KM486423	KM486475	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1242	KM486275	KM486127	NA	KM486342	NA	NA	KM486476	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1245	KM486276	KM486128	NA	KM486343	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1250	KM486277	KM486129	NA	KM486344	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1252	KM486282	KM486132	NA	KM486349	NA	KM486424	KM486477	NA	Dimitrov <i>et al.</i> (2017)
NeO_Arkys.sp.GH1586	KM486278	KM486130	NA	KM486345	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Australomimetes.sp.GH1115	NA	KP271653	NA	KP271728	NA	KP271798	KP271855	NA	Dimitrov <i>et al.</i> (2017)
NeO_Ero.sp.GH1092	KP271663	KP271663	NA	KP271738	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Eryciniolia.purpurapunctata.GH1118	KM486295	KM486146	NA	KM486362	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Gelanor.sp.GH1605	NA	KP271678	NA	KP271750	NA	KP271817	KP271881	NA	Dimitrov <i>et al.</i> (2017)
NeO_Meta.new.species.GH1404	NA	KM486172	NA	KM486384	NA	NA	KM486502	NA	Dimitrov <i>et al.</i> (2017)
NeO_Meta.rufolineata.GH1136	KM486316	KM486173	NA	KM486385	NA	KM486456	KM486503	NA	Dimitrov <i>et al.</i> (2017)
NeO_Metainae.new.genus.02.GH1152	KM486314	KM486170	NA	KM486382	NA	NA	KM486501	NA	Dimitrov <i>et al.</i> (2017)
NeO_Metainae.new.genus.05.1172	KM486315	KM486171	NA	KM486383	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Metainae.new.genus.05.GH1172	NA	NA	NA	NA	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Mimetes.sp.GH891	KP271633	KP271702	NA	KP271774	NA	KP271838	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Nanometa.sp.GH1137	KM486317	KM486175	NA	KM486387	NA	KM486458	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Nanometa.sp.GH1139	KM486318	KM486176	NA	KM486388	NA	KM486459	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Nanometa.sp.GH1114	KM486319	KM486177	NA	KM486389	NA	KM486460	KM486505	NA	Dimitrov <i>et al.</i> (2017)
NeO_Pinkfloydia.new.species.GH1602	KM486328	KM486190	NA	KM486401	NA	NA	KM486515	NA	Dimitrov <i>et al.</i> (2017)
NeO_Tetragnathidae.new.genus.001.GH1142	KM486334	KM486202	NA	KM486412	NA	NA	KM486524	NA	Dimitrov <i>et al.</i> (2017)
NeO_Tetragnathidae.new.genus.GH1181	NA	KM486203	NA	KM486413	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Tylorida.sp.GH1196	NA	KM486206	NA	KM486416	NA	NA	NA	NA	Dimitrov <i>et al.</i> (2017)
NeO_Tylorida.ventralis.GH1163	NA	KM486207	NA	KM486417	NA	KM486470	KM486526	NA	Dimitrov <i>et al.</i> (2017)
Alcimospheus_GH2455	NA	MZ604143		MZ604205	NA	MZ562645	MZ562795	MZ562841	
Azilia_guatemalensis_GH1948	NA	NA	NA	NA	NA	NA	NA	NA	
Azilia_histrio_GH1951	NA	MZ604144	NA	MZ604206	NA	MZ562628	NA	MZ562842	
Leucauge_argentina_GH1779	NA	NA	NA	NA	NA	MZ562647	MZ562796	MZ562843	
Leucauge_argyra_GH1496	NA	NA	MZ604145	NA	NA	MG738495	NA	NA	
Leucauge_argyroabapta_GH0770	NA	MZ604146		MZ64207	NA	MG738517	NA	NA	
Leucauge_argyroabapta_GH1489	NA	NA	MZ604147	NA	NA	MG738556	NA	NA	
Leucauge_argyroabapta_GH1490	NA	NA	MZ604148	NA	NA	MG738539	NA	NA	
Leucauge_argyroabapta_GH1495	NA	NA	MZ604149	NA	NA	MG738543	NA	NA	
Leucauge_argyroabapta_GH1500	NA	NA	MZ604150	NA	NA	MG738544	NA	NA	
Leucauge_argyroabapta_GH1504	NA	NA	MZ604151	NA	NA	MG738547	NA	NA	
Leucauge_argyroabapta_GH1505	NA	NA	MZ604152	NA	NA	MG738548	NA	NA	
Leucauge_argyroabapta_GH1506	NA	NA	MZ604153	NA	NA	MG738549	NA	NA	
Leucauge_argyroabapta_GH1510	NA	NA	MZ604154	NA	NA	MG738560	NA	NA	
Leucauge_atrostricta_GH1986	NA	MZ604155	NA	MZ604208	NA	MZ562622	NA	NA	
Leucauge_atrostricta_GH2273	NA	NA	NA	MZ604209	NA	MZ562642	MZ562797	NA	
Leucauge_bituberculata_GH1762	NA	MZ604156		MZ604210	NA	MZ562624	NA	NA	
Leucauge_blanda_GH2292	NA	MZ604204	NA	MZ604211	NA	MZ562667	MZ562798	MZ562844	
Leucauge_branicki_GH1960	NA	NA	NA	MZ604212	NA	MZ562631	NA	NA	
Leucauge_branicki_GH2324	NA	NA	NA	MZ604213	NA	MZ562632	MZ562799	NA	
Leucauge_branicki_GH2343	NA	NA	MZ604157	NA	NA	MZ562640	MZ562800	NA	
Leucauge_celebesiana_GH2284	NA	NA	NA	MZ604214	NA	MZ562669	MZ562801	MZ562845	
Leucauge_cf_aurostriata_GH2003	NA	MZ604158	NA	MZ604215	NA	MZ562652	NA	NA	

Taxon name	<i>16S</i>	<i>18S1</i>	<i>18S2</i>	<i>28S2</i>	<i>28S3</i>	<i>COI</i>	<i>H3</i>	<i>H4</i>	References
Leucauge_cf_aurostriata_GH2005	NA	NA	NA	MZ604216	NA	MZ562651	MZ562802	MZ562846	
Leucauge_cf_gemminipunctata_GH1992	NA	NA	NA	NA	NA	MZ562653	MZ562803	MZ562847	
Leucauge_cf_idonea_GH2269	NA	MZ604159	NA	MZ604217	NA	MZ562650	MZ562804	MZ562848	
Leucauge_cf_moerens_GH2007	NA	NA	NA	NA	NA	MZ562661	NA	NA	
Leucauge_cf_simplex_GH2327	NA	MZ604160	NA	MZ604218	NA	MZ562639	MZ562805	MZ562849	
Leucauge_cf_turbida_GH2272	NA	MZ604161	NA	MZ604219	NA	MZ562641	MZ562806	MZ562850	
Leucauge_cf_uberta_GH2296	NA	MZ604162	NA	MZ604220	NA	MZ562634	MZ562807	MZ562851	
Leucauge_cf_uberta_GH2300	NA	MZ604163	NA	MZ604221	NA	MZ562623	MZ562808	NA	
Leucauge_cf.polita_GH1937	NA	NA	NA	MZ604222	NA	MG738512	MZ562809	MZ562852	
Leucauge_cf.polita_GH2314	NA	NA	MZ604164	MZ604223	NA	MG738514	NA	MZ562853	
Leucauge_crucinata_GH2077	NA	MZ604165	NA	MZ604224	NA	NA	MZ562810	MZ562854	
Leucauge_decorata_GH1760	NA	MZ604166	NA	MZ604225	NA	MZ562625	NA	NA	
Leucauge_formosa_GH1935	NA	MZ604167		MZ604226	NA	MZ562655	MZ562811	MZ562855	
Leucauge_funebris_GH2297	NA	MZ604168		NA	NA	MZ562648	MZ562812	NA	
Leucauge_gemminipunctata_GH2325	NA	NA	NA	MZ604227	NA	MG738505	MZ562813	MZ562856	
Leucauge_granulata_GH2032	NA	MZ604169	NA	MZ604228	NA	NA	MZ562814	MZ562857	
Leucauge_henryi_GH1962	NA	NA	NA	MZ604229	NA	MG738507	NA	NA	
Leucauge_henryi_GH2310	NA	NA	MZ604170	MZ604230	NA	MG738506	MZ562815	MZ562858	
Leucauge_longimana_GH2321	NA	MZ604171	NA	MZ604231	NA	NA	MZ562816	MZ562859	
Leucauge_mariana_GH1954	NA	NA	NA	NA	NA	NA	NA	NA	
Leucauge_mariana_GH2353	NA	NA	NA	MZ604232	NA	MG738508	MZ562817	MZ562860	
Leucauge_moerens_GH1953	NA	NA	NA	MZ604233	NA	MZ562663	MZ562818	MZ562861	
Leucauge_moerens_GH1996	NA	MZ604172	NA	MZ604234	NA	MZ562664	NA	NA	
Leucauge_moerens_GH2319	NA	MZ604173	NA	MZ604235	NA	MZ562662	MZ562819	NA	
Leucauge_nanshan_GH2338	NA	NA	NA	MZ604236	NA	NA	MZ562820	NA	
Leucauge_polita_GH1936	NA	NA	NA	MZ604237	NA	MG738513	MZ562821	MZ562862	
Leucauge_regnyi_GH1998	NA	MZ604174		MZ604238	NA	MG738515	NA	NA	
Leucauge_roseosignatha_GH2311	NA	NA	NA	MZ604239	NA	MZ562646	MZ562822	MZ562863	
Leucauge_sp.Brazil_GH1942	NA	NA	NA	MZ604240	NA	MZ562649	MZ562823	MZ562864	
Leucauge_sp.Brazil_GH1965	NA	MZ604175	NA	MZ604241	NA	MZ562621	NA	NA	
Leucauge_sp.Brazil_GH2009	NA	MZ604176		MZ604242	NA	MZ562654	NA	NA	
Leucauge_sp.Brazil_GH2298	NA	NA	NA	MZ604243	NA	MZ562643	MZ562824	MZ562865	
Leucauge_sp.Brazil_GH2304	NA	MZ604177	NA	MZ604244	NA	MZ562635	MZ562825	NA	
Leucauge_sp.Ecuador_GH2348	NA	NA	MZ604178	MZ604245	NA	MZ562644	MZ562827	NA	
Leucauge_sp.Gabon_GH2345	NA	NA	MZ604179	MZ604246	NA	MZ562665	MZ562826	NA	
Leucauge_sp.Madagascar_GH2358	NA	NA	NA	MZ604247	NA	MZ562667	NA	NA	
Leucauge_sp.Thailand_GH0596	NA	MZ604180		MZ604248	NA	MZ562669	NA	NA	
Leucauge_sp._1_Cameroon_GH2440	NA	MZ604181	NA	MZ604249	NA	MZ562658	MZ562828	MZ562866	
Leucauge_sp._3_Cameroon_GH2437	NA	MZ604182	NA	MZ604250	NA	MZ562626	MZ562829	MZ562879	
Leucauge_sp._4_Cameroon_GH2435	NA	MZ604183	NA	MZ604251	NA	MZ562659	MZ562830	MZ562880	
Leucauge_taiwanica_cf_tessellata_GH1768	NA	MZ604184	NA	MZ604252	NA	MZ562666	MZ562831	MZ562867	
Leucauge_venusta_GH1480	NA	NA	MZ604185	NA	NA	MG738588	NA	NA	
Leucauge_venusta_GH1483	NA	NA	MZ604186	NA	NA	MG738578	NA	NA	
Leucauge_venusta_GH1487	NA	NA	MZ604187	NA	NA	MG738554	NA	NA	
Leucauge_venusta_GH1509	NA	NA	MZ604188	NA	NA	MG738530	NA	NA	
Leucauge_venusta_GH1511	NA	NA	MZ604189	NA	NA	MG738561	NA	NA	
Leucauge_venusta_GH1513	NA	NA	MZ604190	NA	NA	MG738574	NA	NA	
Leucauge_venusta_GH1517	NA	NA	MZ604191	NA	NA	MG738531	NA	NA	
Leucauge_venusta_GH1533	NA	NA	MZ604192	NA	NA	MG738579	NA	NA	
Leucauge_venusta_GH1534	NA	NA	MZ604193	NA	NA	MG738580	NA	NA	
Leucauge_venusta_GH1537	NA	NA	MZ604194	NA	NA	MG738593	NA	NA	
Leucauge_volupis_GH1966	NA	NA	NA	MZ604253	NA	NA	NA	MZ562868	
Leucauge_volupis_GH2315	NA	MZ604195	NA	MZ604254	NA	MZ562660	NA	MZ562869	

Taxon name	<i>16S</i>	<i>18S1</i>	<i>18S2</i>	<i>28S2</i>	<i>28S3</i>	<i>COI</i>	<i>H3</i>	<i>H4</i>	References
Mecynometa_cf_globosa_GH1987	NA	NA	NA	NA	NA	MZ562636	NA	NA	
Mecynometa_cf_globosa_GH2004	NA	NA	NA	NA	NA	MZ562633	NA	NA	
Mecynometa_sp._A_GH2306	NA	MZ604196	NA	MZ604255	NA	MZ562637	MZ562832	MZ562870	
Mesida_argentiopunctata_GH1145	NA	MZ604197	NA	MZ604256	NA	NA	MZ562833	MZ562871	
Mesida_yini_GH2065	NA	NA	NA	MZ604257	NA	MZ562627	MZ562834	MZ562872	
Metabus_ocellatus_GH2352	MZ604142		MZ604198	MZ604258	NA	MZ562629	NA	MZ562873	
Okileucauge_hainan_GH2096	NA	NA	NA	MZ604259	NA	NA	MZ562835	MZ562874	
Okileucauge_hainan_GH2099	NA	NA	MZ604199	MZ604260	NA	MZ562630	MZ562836	NA	
Opas_caudacuta_GH2270	NA	MZ604200	NA	MZ604261	NA	NA	MZ562837	MZ562875	
Opas_caudacuta_GH2318	NA	MZ604201	NA	MZ604262	NA	MZ562656	MZ562838	MZ562876	
Opas_sp._GH2320	NA	MZ604202	NA	MZ604263	NA	NA	MZ562839	MZ562877	
Orsinome_vethi_GH0591	NA	MZ604203	NA	MZ604264	NA	MZ562638	MZ562840	MZ562878	

Table S3. Primer pairs and annealing temperatures

Locus	Temperature	Primer sequence (5'-3')	References
<i>COI</i>	42–48	Fwd: GGT CAA CAA ATC ATA AAG ATA TTG G Rev: CCA GGT AAA ATT AAA ATA TAA ACT TC	Folmer <i>et al.</i> 1994 Carpenter and Wheeler 1999
<i>16S</i>	40–45	Fwd: CGC CTG TTT ATC AAA AAC AT Rev: CTC CGG TTT GAA CTC AGA TCA	Palumbi <i>et al.</i> 1991 Palumbi <i>et al.</i> 1991
<i>18S</i>	48–52	Fwd: TAC CTG GTT GAT CCT GCC AGT AG Rev: CTT GGC AAA TGC TTT CGC,	Giribet <i>et al.</i> 1996 Giribet <i>et al.</i> 1996
	48–52	Fwd: CCA GCA GCC GCG CTA ATTC, Rev: GCA TCA CAG ACC TGT TAT TGC	Giribet <i>et al.</i> 1996 Giribet <i>et al.</i> 1996
<i>28S</i>	50	Fwd: GAC CCG TCT TGA AGC ACG Rev: CCA CAG CGC CAG TTC TGC TTA C	Whiting <i>et al.</i> 1997 Schwendinger and Giribet 2005
	45–50	Fwd: ACC TAT TCT CAA ACT TTA AAT GG Rev: GAC TTC CCT TAC CTA CAT	Schwendinger and Giribet 2005 Schwendinger and Giribet 2005
<i>H3</i>	56	Fwd: ATG GCT CGT ACC AAG CAG ACV GC Rev: ATA TCC TTR GGC ATR ATR GTGAC	Colgan <i>et al.</i> 1998 Colgan <i>et al.</i> 1998
<i>H4</i>	56	Fwd: ATG TCC GGC CGT GGA AAA GG Rev: AAC CAC CCG AAG CCG TAC AGA G	This study This study

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