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Phylogenomics and biogeography of leptonetid spiders (Araneae : Leptonetidae)

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Abstract. Leptonetidae are rarely encountered spiders, usually associated with caves and mesic habitats, and are disjunctly distributed across the Holarctic. Data from ultraconserved elements (UCEs) were used in concatenated and coalescent-based analyses to estimate the phylogenetic history of the family. Our taxon sample included close outgroups, and 90% of described leptonetid genera, with denser sampling in North America and Mediterranean Europe. Two data matrices were assembled and analysed; the first 'relaxed' matrix includes the maximum number of loci and the second 'strict' matrix is limited to the same set of core orthologs but with flanking introns mostly removed. A molecular dating analysis incorporating fossil and geological calibration points was used to estimate divergence times, and dispersal–extinction–cladogenesis analysis (DEC) was used to infer ancestral distributions. Analysis of both data matrices using maximum likelihood and coalescent-based methods supports the monophyly of Archoleptonetinae and Leptonetinae. However, relationships among Archoleptonetinae, Leptonetinae, and Austrochiloidea are poorly supported and remain unresolved. Archoleptonetinae is elevated to family rank Archoleptonetidae (new rank) and Leptonetidae (new status) is restricted to include only members of the subfamily Leptonetinae; a taxonomic review with morphological diagnoses is provided for both families. Four well supported lineages within Leptonetidae (new status) are recovered: (1) the *Calileptoneta* group, (2) the *Leptoneta* group, (3) the *Paraleptoneta* group, and (4) the *Protoleptoneta* group. Most genera within Leptonetidae are monophyletic, although *Barusia*, *Cataleptoneta*, and *Leptoneta* include misplaced species and require taxonomic revision. The origin of Archoleptonetidae (new rank), Leptonetidae, and the four main lineages within Leptonetidae date to the Cretaceous. DEC analysis infers the *Leptoneta* and *Paraleptoneta* groups to have ancestral distributions restricted to Mediterranean Europe, whereas the *Calileptoneta* and *Protoleptoneta* groups include genera with ancestral distributions spanning eastern and western North America, Mediterranean Europe, and east Asia. Based on a combination of biology, estimated divergence times, and inferred ancestral distributions we hypothesise that Leptonetidae was once widespread across the Holarctic and their present distributions are largely the result of vicariance. Given the wide disjunctions between taxa, we broadly interpret the family as a Holarctic relict fauna and hypothesise that they were once part of the Boreotropical forest ecosystem.

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Introduction

Biology of Leptonetidae

Leptonetids are a lineage of small, rarely encountered spiders that live in moist habitats such as leaf litter, under rocks, and especially in caves. The family includes 21 genera and 355 species placed into two subfamilies, Archoleptonetinae and Leptonetinae (World Spider Catalog, ver. 19.5, see http://wsc.nmbe.ch, accessed 19 August 2020). The archoleptonetines (Fig. 1*A*) include eight species in two genera and are known from the western USA, southern Mexico, Guatemala, and Panama. Leptonetines (Fig. 1*B*) are more diverse (21 genera, 355 species) and have a Holarctic distribution with centres of

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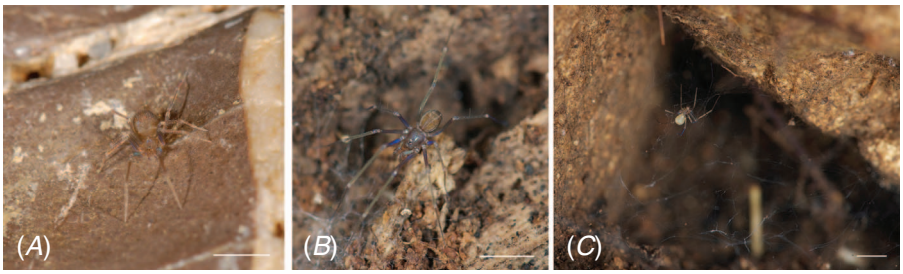

Fig. 1. Images of live spiders in native habitats. (*A*) *Archoleptoneta schusteri*, Marin County, CA, USA; (*B*) *Calileptoneta helferi*, Mendocino County, CA, USA; (*C*) *Tayshaneta myopica* in sheet web, Travis County, TX, USA. Scale: 3 mm.

diversity in North America, Mediterranean Europe, and east Asia. Although the archoleptonetines have few features that make them readily diagnosable by non-specialists, all leptonetines share a unique eye arrangement where the posterior median eyes are displaced from the main eye group (Ledford and Griswold 2010, fig. 24, 27, 28).

Among spiders, leptonetids are best known for their association with caves. Over 50% of described species are known only from caves and many species show a range of troglomorphic morphologies including eye reduction, depigmentation, and appendage elongation (Mammola and Isaia 2017). Most species are small (2–5 mm) and reside in delicate sheet webs from which they hang (Fig. 1*C*). Leptonetids are microhabitat specialists, preferring environments where moisture, temperature and humidity remain stable. Ideal habitat includes breakdown debris in caves and layered rock piles in heavily shaded areas. Observations of reproductive biology have been reported (Cokendolpher 2004; Ledford 2004; Ledford and Griswold 2010) but most aspects of their life history are unknown.

Given their habitat preferences, most species have distributions that are highly localised. Sympatry is rare and known only in a few surface-dwelling populations (Ledford 2004). Even in localities where leptonetids are known to occur, they are rarely encountered and in some regions are recognised as threatened species (US Fish and Wildlife Service 2020). Although gaps in distributional range may be partly explained by inadequate sampling, we propose that the combination of specific habitat preferences and the repeated pattern of narrow endemism for most species worldwide supports a hypothesis of dispersal-limitation for the family. The biological characteristics of limited dispersal ability and high microhabitat preference often lead to biogeographic histories that are dominated by vicariance, with sometimes rare dispersal events, as seen in many other arachnid lineages (e.g. Harrison *et al*. 2016; Hedin and McCormack 2017; Baker *et al*. 2020).

Taxonomic history

Most research on leptonetids has focused on improving understanding of α-level diversity. The western European fauna is arguably the best known due to its long history of study (Simon 1872) and the detailed descriptive efforts of Brignoli (1967*a*, 1967*b*, 1968, 1971, 1974*a*, 1974*c*, 1978, 1979*a*, 1979*b*, 1979*c*), Fage (1913, 1931, 1943), Kratochvíl (1935, 1938, 1978), Machado and Ribera (1986), Ribera (1978, 1988), and Ribera and Lopez (1982). Although most of these works are regionally focused, Fage (1913) treated the European fauna comprehensively and Brignoli (1970, 1979*d*) provided a global interpretation of leptonetid relationships and biogeography. The North American fauna has a history of monographic study starting with Gertsch (1971, 1974) who described the majority of species and Platnick (1986) who delineated three North American genera. Brignoli (1974*b*, 1977, 1979*e*) also worked on the North American fauna, providing a global perspective that sharply contrasted with Gertsch (1971, 1974). Gertsch (1971, 1974) argued that all of the North American leptonetids should be placed in the European genus *Leptoneta* but, unlike Brignoli, had limited understanding of other European leptonetid genera. By contrast, based on his experience working with European leptonetids, Brignoli hypothesised that the North American fauna likely included multiple genera (Brignoli 1972, 1977). Two studies (Ledford and Griswold 2010; Ledford *et al*. 2011) assessed the phylogeny of North American leptonetids using nucleotide sequence data and better defined the genera. During the past 10 years, the most striking advances in leptonetid taxonomy have been in Asia where over 100 new species have been described from China and South Korea (Chen *et al*. 2010; Lin and Li 2010; Wang and Li 2010, 2011; Seo 2015*a*, 2015*b*, 2016*a*, 2016*b*; Guo *et al*. 2016; He *et al*. 2019; Xu *et al*. 2019). Although most of these studies do not include phylogenetic or biogeographic analyses (but see Wang *et al*. 2017), they have greatly improved our understanding of the Asian fauna.

Leptonetidae have a long history of controversy surrounding their relationships to other spiders. Fage (1913) was the first to recognise that leptonetids share convergent morphology with other subterranean-adapted spiders, and Brignoli (1979*d*) suggested that the hypothesised relationships of leptonetids to other spiders were largely based on these convergent features. One example is that leptonetines, Telemidae, and some Ochyroceratidae have independently evolved a peculiar arrangement of the posterior spinnerets where there is a single row of aciniform gland spigots used to build sheet webs (Ledford and Griswold 2010, fig. 65–72). For this reason, many workers have placed leptonetids among the Synspermiata (formerly Haplogynae), usually as sister to Telemidae, despite the fact

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that much of their morphology conflicts with this position. The discovery of a cribellum in *Archoleptoneta* Gertsch, 1974 further compounded these conflicts and, given the traditional placement of leptonetids within Synspermiata, had implications for the interpretation of spinning organs as a whole (Ledford and Griswold 2010). Brignoli (1979*d*) was the first to propose that leptonetids might be more closely related to entelegynes based on the absence of a cheliceral lamina and the presence of expandable male genitalia. Ledford and Griswold (2010) reviewed leptonetid morphology and were the first to suggest the possibility of leptonetid paraphyly, proposing that at least the archoleptonetines are not part of Synspermiata, but instead are more closely related to entelegynes.

Agnarsson *et al*. (2013) used a supertree approach and was the first to suggest a relationship between *Archoleptoneta* and austrochiloids, but thought that the result was caused by long-branch attraction. Using transcriptomes, Garrison *et al*. (2016) recovered leptonetids outside of Synspermiata, placing *Calileptoneta* Platnick, 1986 as sister to Entelegynae. Several studies built upon the foundation of Garrison *et al*. (2016), including Shao and Li (2018) who recovered leptonetines as sister to entelegynes but did not include austrochiloids as part of their study. Fernández *et al*. (2018) added transcriptomes for both leptonetid subfamilies (*Archoleptoneta* and *Calileptoneta*) and in their preferred topology recovered a monophyletic Leptonetidae sister to austrochiloids (Fig. 2). Based on a combination of multigene nucleotide data and morphology, Wheeler *et al*. (2017) recovered a polyphyletic Leptonetidae, but did support a relationship between *Archoleptoneta* and austrochiloids. As part of a study on basal araneomorphs, Ramírez *et al*. (2021) included better representation across Leptonetidae and used ultraconserved elements (UCEs) to hypothesise a sister-group relationship between Leptonetinae and austrochiloids, rendering Leptonetidae paraphyletic.

Systematics and biogeography of Leptonetidae

In this paper, we present a phylogenomic analysis of Leptonetidae including representatives from across the geographic range of the family. Both genera of archoleptonetines and 90% of described leptonetine genera are sampled. Where possible, multiple exemplars for each genus are used, including several from type localities. We include a broad sample of austrochiloids and use Telemidae as the outgroup. Because this study is an extension of a parallel study on basal araneomorphs (Ramírez *et al*. 2021), we do not include a broad sampling of entelegynes in order to assess the relationships of leptonetids to other spiders. Instead, we focus on testing the monophyly of leptonetid subfamilies and explore relationships among leptonetid genera. We use our results to review the current taxonomy of Leptonetidae and identify areas in need of taxonomic revision. As the first study to produce a global phylogeny of Leptonetidae, we also gain insight into the historical biogeography of the group. Using a combination of fossils and dates for continental events, we present a chronogram and assess the biogeography of the family using dispersal–vicariance analysis. Our results provide a robust phylogenetic framework for the family that can be used as a scaffold for forthcoming taxonomic works.

Methods

Taxon sampling

Representatives for 19 of the 21 described genera in Leptonetidae were sampled from localities worldwide (Fig. 3). Our sample does not include the genera *Masirana* Kishida, 1942 and *Rhyssoleptoneta* Tong & Li, 2007. We gathered original UCE data for 28 specimens, combined with data for 17 specimens taken from previous studies (Wood *et al*. 2018; Ramírez *et al*. 2021) (Table S1 of the Supplementary material). Depending on availability, multiple species within a genus were used. We included a broad sample of Austrochiloidea and used *Usofila pacifica* Banks, 1894 (Telemidae) as the outgroup.

DNA extraction

Most specimens were preserved for DNA studies (preserved in high percentage ethyl alcohol at –80°C), and genomic DNA was extracted from leg tissue using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). For a handful of

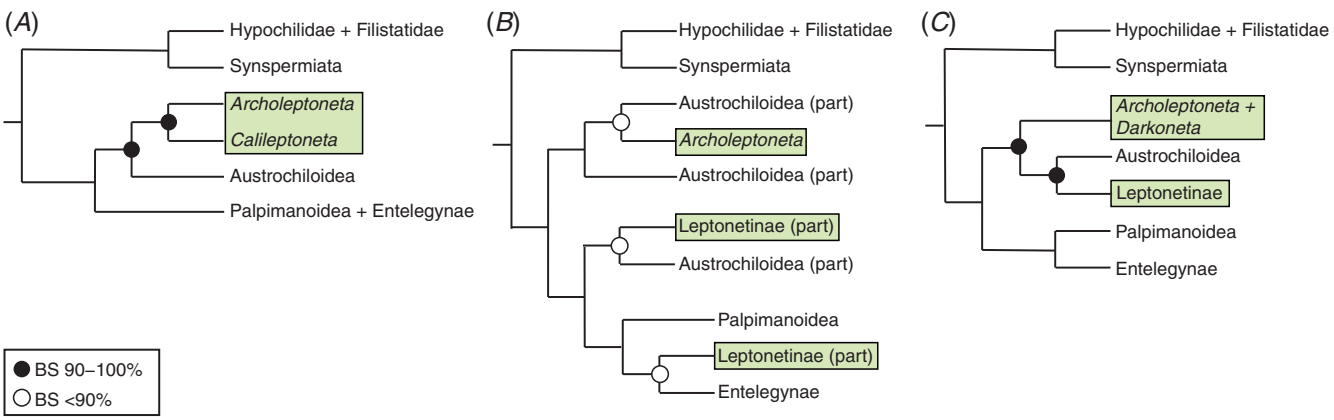

Fig. 2. Alternate hypotheses of leptonetid relationships from recent studies: (A) Fernández *et al*. (2018), fig. 1A (transcriptomes); (B) Wheeler *et al*. (2017), fig. 3 (morphology + single genes); and (C) Ramírez *et al*. (2021), fig. 2 (ultraconserved elements). Figures represent summary trees. Support values for relevant nodes are indicated.

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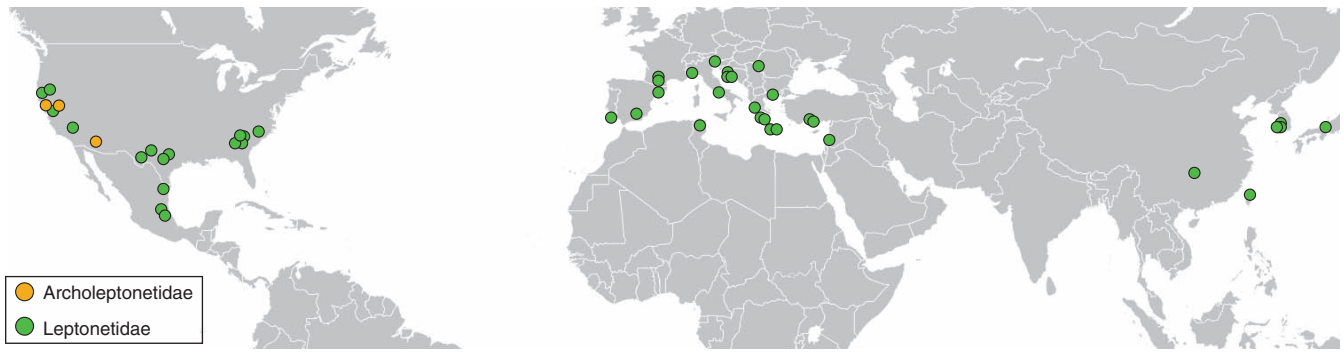

Fig. 3. Distribution map of archoleptonetid and leptonetid samples included in this study.

tissues preserved in 70–80% ethyl alcohol we used standard phenol chloroform extractions with 24-h incubation for lysis. Extraction type for each specimen is indicated in Table S1. Extractions were quantified using a Qubit Fluorometer (Life Technologies, Inc.) and quality was assessed by agarose gel electrophoresis. Between 11 and 500 ng of total DNA was used for UCE library preparation.

UCE data collection

UCE data were collected in multiple library preparation and sequencing experiments. Up to 500 ng of genomic DNA was used in sonication, using a Covaris M220 Focused-ultrasonicator. Library preparation followed methods previously used for arachnids, as in Starrett *et al*. (2017), Derkarabetian *et al*. (2018, 2019), and Hedin *et al*. (2018*a*, 2018*b*). Target enrichment was performed using the MYbaits Arachnida 1.1K kit (ver. 1, Arbor Biosciences; Faircloth 2017) following the Target Enrichment of Illumina Libraries protocol (ver. 1.5, see http://ultraconserved.org/#protocols). Libraries were sequenced with an Illumina HiSeq 2500 with 125 bp paired-end reads (Brigham Young University DNA Sequencing Center).

Matrix filtering and assembly

Raw demultiplexed reads were processed with the PHYLUCE pipeline (ver. 1.6, see https://phyluce.readthedocs.io/en/latest/; Faircloth 2016). Quality control and adaptor removal were conducted with the Illumiprocessor wrapper (B. C. Faircloth, see https://github.com/faircloth-lab/illumiprocessor). Assemblies were created with Velvet (ver. 1.2.10, see https://www.ebi.ac.uk/~zerbino/velvet/; Zerbino and Birney 2008) and Trinity (ver. 2.11.0, see https://github.com/trinityrnaseq/trinityrnaseq/wiki; Grabherr *et al*. 2011), both at default settings. Assemblies were combined for probe matching, retrieving assembly-specific UCEs and overall increasing the number of UCEs per sample relative to using only a single assembly method. Contigs were matched to probes using minimum coverage and minimum identity at liberal values of 65. UCE loci were aligned with MAFFT (ver. 7.471, see https://mafft.cbrc.jp/alignment/software/ Katoh and Standley 2013) at default settings and trimmed with Gblocks (ver. 0.91b, see http://molevol.cmima.csic.es/castresana/Gblocks/Gblocks_documentation.html; Castresana 2000; Talavera and Castresana 2007), with settings –b1 0.5 –b2 0.5 –b3 6 –b4 6 in the Phyluce pipeline.

We expected some paralogy at the low minimum coverage values used, but considered this as a tradeoff given the ancient divergences considered. In addition to internal checks for paralogy in Phyluce, we checked for paralogy by conducting RAxML analyses on all individual Phyluce alignments. We excluded individual loci that failed to recover the well supported clade Austrochiloidea. We did not exclude duplicate UCE loci, as found in Hedin *et al*. (2019) – these are distinct UCE alignments that match the same protein, but are expected to be well separated by long introns. Two data matrices were assembled for phylogenomic analyses: (1) 65% (36 of 55 terminals) occupancy matrix, exon + intron, no 'paralogs' matrix, and (2) the same matrix as above using very strict Gblocks settings (–b1 0.5 –b2 0.85 –b3 4 –b4 8) to further trim alignments. We visually checked to confirm that these trimmed alignments comprised mostly exon data. These two matrices were developed to test the sensitivity of results to different alignment parameters (e.g. see Portik and Wiens 2020), and are referred to as 'relaxed' and 'strict' throughout the paper.

Phylogenomic analyses

Partitioned concatenation and coalescent-based analyses were conducted. Maximum likelihood analysis of the concatenated datasets was performed using RAxML (ver. 8, see https://cme.h-its.org/exelixis/web/software/raxml/; Stamatakis 2014) with the data partitioned by locus, the GTR+Γ substitution model, and support estimated by 500 bootstrap pseudoreplicates. Maximum likelihood analysis of the concatenated datasets was also performed using IQ-TREE (ver. 1.7-beta9, see http://www.iqtree.org/; Nguyen *et al*. 2015; Minh *et al*. 2020*a*) in order to calculate gene (gCF) and site (sCF) concordance factors for the relaxed and strict data matrices (Minh *et al*. 2020*b*). These measures were used as alternative measures of support (Ane *et al*. 2006), especially for nodes where topology conflicted between analysis type. An SVDQuartets analysis (ver. 1.0, see https://www.asc.ohio-state.edu/kubatko.2/software/SVDquartets/; Chifman and Kubatko 2014, 2015) was conducted on both matrices using PAUP* (ver. 4.0a, see https://paup.phylosolutions.com/), implementing a multispecies coalescent tree model with exhaustive quartets sampling and 100 bootstrap replicates.

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Molecular dating analysis

A molecular clock analysis was conducted in a Bayesian framework with the MCMCtree module in the PAML package (ver. 4.9i, see http://abacus.gene.ucl.ac.uk/software/paml.html; Yang 2007). For the clock analysis we used the relaxed matrix RAxML topology, and treated the concatenated data as unpartitioned. The outgroup was removed from the tree and dataset, and a maximum boundary of 240 Ma was applied to the root node, based on the upper boundary of the 95% highest posterior density credible interval of the Leptonetidae + Austrochiloidea node from Fernández *et al*. (2018). The analysis was run with a model that assumes independent rates among branches (Yang and Rannala 2006; Rannala and Yang 2007). Estimation of the parameters (shape and scale) of the gamma distribution for the substitution rate prior (μ) was done with Baseml based on four fossil and biogeographic-based calibration points treated as fixed (shape = 1, scale = 5). We used shape = 1 and scale = 4.5 for the gamma distributed prior for σ2, or the variability in substitution rate among branches. The HKY sequence model was used and the analysis was run with birth rate, death rate, and species sampling priors of 2, 2 and 0.1 respectively. Gamma priors for κ (the transition/transversion ratio) and α (shape parameter for among site rate variation) were left as default (Yang 2007). Calibrations (see below) were treated as soft boundaries (i.e. 0.025 probability date falls beyond boundary; Yang and Rannala 2006; Inoue *et al*. 2010). The first 40 000 iterations were discarded as burnin, followed by 40 000 iterations sampled with 100 iterations (4 million generations). The analysis was run twice to ensure MCMC convergence, with negligible differences in posterior date estimates and 95% highest posterior density credible intervals occurring between the two runs.

Our clock analysis was calibrated based on fossils in Burmese Amber (Wunderlich 2008, 2012) and two continental events in Mediterranean Europe (Lymberakis and Poulakakis 2010; Garcia-Castellanos and Villaseñor 2011). Leptonetinae was assigned a minimum age of 98.19 Ma based on the fossil *Palaeoleptoneta calcar* Wunderlich, 2008 from Burmese Amber. Burmese Amber has been radiometrically dated to 98.79 ± 0.6 Ma (Selden and Ren 2017). Although this fossil cannot be confidently placed among extant genera (Magalhaes *et al*. 2020), it appears to share the ocular arrangement of leptonetines and we interpret it as part of the Leptonetinae (Wunderlich 2012). The Messinian Salinity Crisis, MSC (5.96–5.33 Ma) refers to the large-scale desiccation of the Mediterranean basin leading to reconnection of Mediterranean islands and part of north Africa to mainland Europe (Garcia-Castellanos and Villaseñor 2011). We set the nodes uniting *Sulcia violacea* (Greece) + *S. cretica* (Crete) and *Paraleptoneta spinimana* (Italy) + *S. bellesi* (Tunisia) to a minimum age of 5.33 Ma because these areas were most recently connected during the MSC. Greece and Turkey were formerly connected in a contiguous landmass called Ägäis. The breakup of Ägäis resulted in the formation of Aegean Islands 12–9 Ma (Dermitzakis and Papanikolaou 1981; Papadopoulou *et al*. 2010; Lymberakis and Poulakakis 2010). The node uniting *Cataleptoneta sengleti* (Greece) + *C. aesculpii* (Turkey) was set to a minimum age of 12 Ma based on their present distributions. We acknowledge that using biogeographic events as calibrations for divergence dating to assess biogeographic history is potentially circular in its logic. However, given the scarcity of fossils for Leptonetidae, we feel that using these recent and localised events, and the benefit they provide for estimating the age of leptonetid lineages across the Holarctic, outweighs the negative aspects.

Biogeographic reconstructions

Biogeographic analysis was performed with the Reconstruct Ancestral State in Phylogenies package (ver. 4.2, see http://mnh.scu.edu.cn/soft/blog/RASP/; Yu *et al*. 2015) using the dispersal–extinction–cladogenesis model (DEC) (Ree and Smith 2008) implemented in the C++ version of Lagrange (S. A. Smith, see http://mnh.scu.edu.cn/soft/blog/RASP/). We use the APE package (Paradis *et al*. 2004) in R (ver. 4.0, R Foundation for Statistical Computing, Vienna, Austria, see https://www.R-project.org/) to convert the relaxed RAxML tree topology into a relative-rate scaled ultrametric tree ('chronopl' using an assigned lambda value of 0.1). Analysis settings allowed for two unit areas in ancestral distributions and equal probabilities of dispersal events between all areas. Terminal taxa were assigned to six distribution ranges: (A) eastern North America, (B) western North America, (C) South America, (D) Mediterranean Europe, (E) Australia or New Zealand, and (F) East Asia. Four of the assigned regions (eastern North America, western North America, East Asia, Mediterranean Europe) correspond to infraregions identified in meta-analyses of Holarctic biogeography (Sanmartín *et al*. 2001; Donoghue and Smith 2004).

Results

Data

Voucher data, input DNA values, assembled contig numbers, and UCE locus numbers are provided in Table S1. In total, 408 loci were included in the final matrices. The relaxed data matrix included 97 094 basepairs (mean locus length of 238) and 39 114 parsimony informative sites; the strict data matrix included 49 656 basepairs (mean locus length of 122) and 17 638 parsimony informative sites. Raw reads from our 30 original samples have been submitted to the SRA (PRJNA694694); aligned matrices and .TRE files are available at Dryad (doi:10.25338/B8SS5F).

Phylogenomic analyses

Maximum likelihood analysis using RAxML and IQ-TREE for both data matrices resulted in identical topologies with the exception of the RAxML strict matrix (Fig. 4 and S1–S3 of the Supplementary material). All ML analyses recovered a sister-group relationship between Leptonetinae and Austrochiloidea that is highly supported with a bootstrap value of 100%. However, genealogical and site concordance factor analysis for this node resulted in relatively low values of gCF 33% and sCF 40.5% for the relaxed data matrix (Fig. S2) and gCF 21.5% and sCF 40.3% for the strict data matrix (Fig. S3). We

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Fig. 4. Concatenated RAxML results for the relaxed data matrix. Unless otherwise indicated, bootstrap support for all nodes is 100%. Taxa indicated in bold represent the type species for that genus.

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present these values as contrasting measures of support, noting that for both data matrices a majority of gene trees (over 60%) support alternative resolutions of this node despite the 100% bootstrap support.

SVDQuartets results are presented for both data matrices in Fig. S4–S7 of the Supplementary material. As suggested by the low gCF and sCF values, the SVD trees and associated 50% majority rule consensus trees show two conflicting resolutions for relationships within Leptonetidae. For both data matrices, SVD optimal trees recover leptonetid monophyly; archoleptonetines are sister to leptonetines and each subfamily is monophyletic. By contrast, the SVD 50% majority rule consensus trees show leptonetid paraphyly identical to our ML results, although support values are low. Despite the ambiguous resolution between the leptonetid subfamilies and Austrochiloidea, all three major lineages (Archoleptonetinae, Leptonetinae, Austrochiloidea) are strongly supported as monophyletic in all analyses. We therefore elevate the subfamily Archoleptonetinae to Archoleptonetidae (new rank) and restrict Leptonetidae to include only members of the subfamily Leptonetinae. This taxonomic structure is used throughout the remainder of the paper.

Relationships among the three species representing Archoleptonetidae are consistent and well supported across all analyses. We recover four main lineages within Leptonetidae which we refer to as the *Paraleptoneta*, *Leptoneta*, *Protoleptoneta*, and *Calileptoneta* groups. Although relationships among these groups vary by analysis, the *Calileptoneta* group is consistently sister to the *Paraleptoneta* + *Leptoneta* + *Protoleptoneta* groups. RAxML analyses of the relaxed data matrix results in a sister group relationship between the *Paraleptoneta* and *Leptoneta* groups (Fig. 4). However, RAxML analysis of our strict data matrix results in a sister group relationship between the *Protoleptoneta* and *Leptoneta* groups (Fig. S1). This node has low bootstrap support in both cases, with 91% support for the relaxed data matrix and 58% for the strict data matrix. Further, gCF and sCF values show that only 6–11% of the gene trees support a relationship between the *Paraleptoneta* and *Leptoneta* groups which we interpret as additional evidence of topological uncertainty (Fig. S2–S3). SVDQuartets analysis for both data matrices result in a sister group relationship between the *Protoleptoneta* and *Leptoneta* groups (Fig. S4–S7); however, 50% majority rule consensus trees show lower support for this node.

The European genera *Leptoneta* Simon, 1872, *Leptonetela* Kratochvíl, 1978, *Paraleptoneta* Fage, 1913, *Protoleptoneta* Deltshev, 1972, and *Sulcia* Kratochvíl, 1938 are all supported as monophyletic. However, the type species *Cataleptoneta edentula* Denis, 1955 does not group with *C. aesculapii* (Brignoli, 1968) or *C. sengleti* (Brignoli, 1974). The type species *Barusia maheni* (Kratochvíl & Miller, 1939) is sister to *B. insulana* (Kratochvíl & Miller, 1939) but *B. laconica* (Brignoli, 1974) is sister to *Cataleptoneta semipinnata* Wang & Li, 2010 and does not group with the types of *Barusia* or *Cataleptoneta*. *Teloleptoneta* Ribera, 1988 is weakly supported as sister to *Protoleptoneta*. One undetermined specimen from Croatia (AR4354) is sister to *Appaleptoneta* Platnick, 1986 + *Leptonetela* Kratochvíl, 1978. *Leptonetela thracia* Gasparo, 2005 is supported as sister to *L. jiulong* Lin & Li, 2010. All North American genera are monophyletic, including *Appaleptoneta* Platnick, 1986, *Calileptoneta* Platnick, 1986, *Chisoneta* Ledford & Griswold, 2011, *Neoleptoneta* Brignoli, 1972, *Ozarkia* Ledford & Griswold, 2011, and *Tayshaneta* Ledford & Griswold, 2011. *Montanineta* Ledford & Griswold, 2011 is weakly supported as sister to *Protoleptoneta* + *Teloleptoneta*. The Asian genera *Falcileptoneta* Komatsu, 1970 and *Longileptoneta* Seo, 2015 are both monophyletic. A single female specimen from Taiwan (CASENT9056030) is sister to *Longileptoneta* but is a juvenile and unable to be confidently assigned to this genus.

Molecular dating

Results from our dating analysis are presented in Fig. 5. We summarise posterior mean divergence times for major splits below and provide a list of key divergence times in Table S2 of the Supplementary material. The age of the root node is estimated as early Jurassic (189 Ma). Both Archoleptonetidae (112 Ma) and Leptonetidae (126 Ma) are of Cretaceous origin and the most recent common ancestor of Austrochiloidea + Leptonetidae dates to the mid-Jurassic (163 Ma). Most of the major divergences within Leptonetidae occur in the middle to late Cretaceous. The *Paraleptoneta*, *Leptoneta*, *Protoleptoneta*, and *Calileptoneta* groups arose 87–110 Ma and the majority of more recent divergence occurred in the mid-Paleogene.

Biogeography

Results for our biogeographic analysis are presented in Fig. 6. Multiple dispersal and vicariance events are inferred and, with few exceptions, their probabilities are 1.0 (Table S3 of the Supplementary material). No extinction events are reconstructed on the phylogeny. Archoleptonetidae is reconstructed to have an ancestral distribution in western North America. Leptonetidae is predicted to have originated in a region spanning western North America and Mediterranean Europe. The ancestor of the *Paraleptoneta*, *Leptoneta*, and *Protoleptoneta* groups most likely originated in Mediterranean Europe with a low probability of dispersal (0.52) to eastern North America. The *Paraleptoneta* and *Leptoneta* groups both have reconstructed origins in Mediterranean Europe. The *Protoleptoneta* group most likely had an ancestral distribution spanning eastern North America and Mediterranean Europe with a low probability of dispersal (0.54) to east Asia. The *Calileptoneta* group is reconstructed to have originated in a region spanning western North America and Mediterranean Europe.

Discussion

Systematics

Recent studies of spider phylogeny have recovered conflicting results for the relationships of Archoleptonetidae and Leptonetidae, ranging from hypotheses of monophyly (Fernández *et al*. 2018), to paraphyly (Ramírez *et al*. 2021), and polyphyly (Wheeler *et al*. 2017). Given the conflict observed between these studies (Fig. 2), and the results

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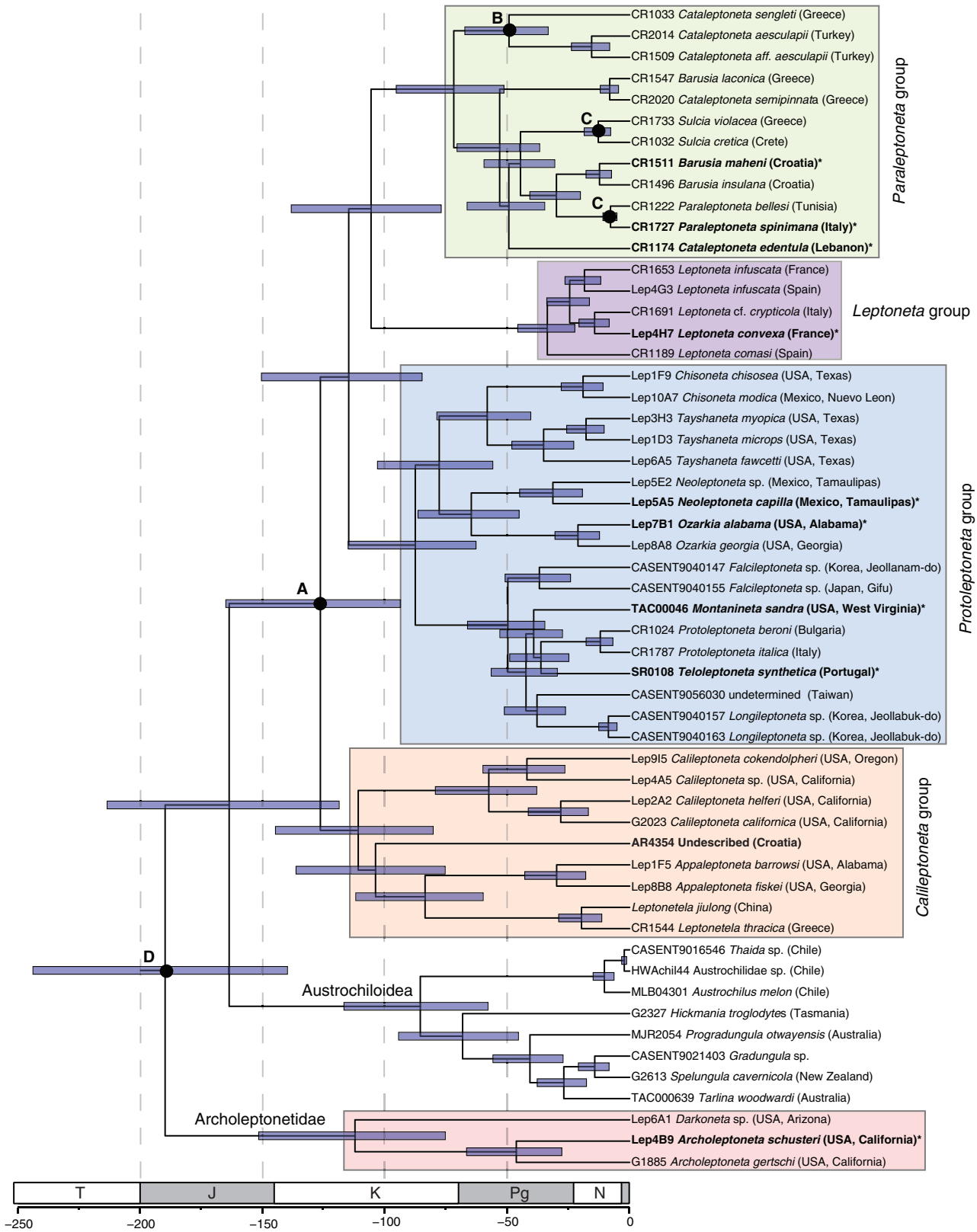

Fig. 5. MCMC tree chronogram generated from the relaxed data matrix. Nodes A–D represent calibration points used in our analysis: A, *Palaeoleptoneta calcar* from Burmese Amber (98.19 Ma); B, Breakup of Ägäis (12 Ma); C, Messinian Salinity Crisis (5.33 Ma); D, Fernández *et al*. (2018).

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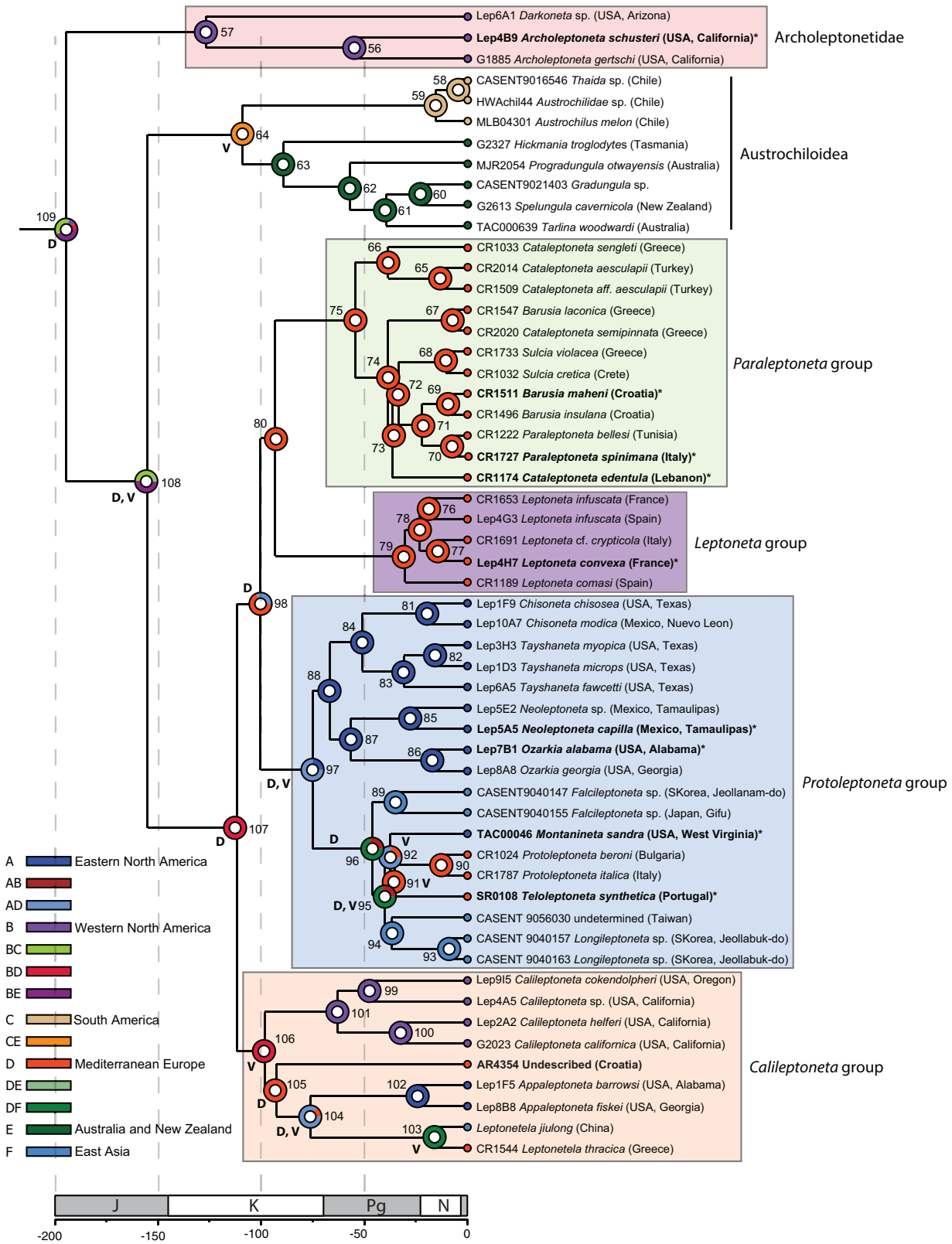

Fig. 6. Inferred ancestral range reconstruction based on the DEC model, based on the relaxed RAxML topology. D, dispersal; V, vicariance. Numbers at nodes are used for reference only and do not indicate measures of statistical support (see Table S3).

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of our analyses, it is not unreasonable to question the elevation of Archoleptonetidae. Further, what are the possible causes for the discordance between these results and how does our study address their limitations?

Among the primary strengths of our study is taxon sampling. We include specimens collected over the past 16 years, many species of which are monotypic, rare, or live in difficult to access habitats such as caves. Admittedly, our sampling is limited in Asia, but efforts to access additional material from this region have been challenging. By contrast, most recent studies have relatively sparse sampling of archoleptonetids and leptonetids. Fernández *et al*. (2018) includes two exemplars (*Archoleptoneta* and *Calileptoneta*) and provides insight into their relationships to other spiders, but lacks sufficient depth to test the monophyly of Archoleptonetidae and Leptonetidae. Wheeler *et al*. (2017) has broader representation with *Archoleptoneta*, *Calileptoneta*, *Leptoneta*, and *Neoleptoneta* but does not include the ecribellate *Darkoneta* in order to test the monophyly of Archoleptonetidae. Ramírez *et al*. (2021) includes denser sampling within Archoleptonetidae and Leptonetidae but is mostly focused on the evolution of tracheael systems in basal araneomorphs. Our study expands on Ramírez *et al*. (2021) by adding representatives of most leptonetid genera, thereby providing a robust test of the monophyly of Archoleptonetidae and Leptonetidae while gaining insight into relationships among the genera.

In addition to differences in sampling, each of these studies used a different type of data. Wheeler *et al*. (2017) used nucleotide data from six genes routinely used in spider systematics. Their results are perplexing as Leptonetidae is polyphyletic with *Leptoneta* sister to Austrochilidae and *Calileptoneta* + *Neoleptoneta* sister to Entelegynae (Fig. 3). Analytical instability was recognised as a problem by the authors, who also argued that a polyphyletic Leptonetidae was unlikely to be based on morphological synapomorphies. Given the problems of the Wheeler *et al*. (2017) analysis, the question becomes focused on whether or not Archoleptonetidae and Leptonetidae are sister groups.

Fernández *et al*. (2018) expanded on the results of Garrison *et al*. (2016), which used transcriptomes to infer relationships across the spider tree of life. Given that both transcriptomes and the UCEs in our study are exonic (following results of Hedin *et al*. 2019), the core data should be comparable and we expected similar results. As part of their study, Fernández *et al*. (2018) developed several data matrices, most of which recovered a monophyletic Leptonetidae (Archoleptonetinae + Leptonetinae). However, their ML analysis of a truncated, strict orthology matrix resulted in a paraphyletic Leptonetidae with *Calileptoneta* sister to austrochiloids. Our study also shows conflicting results for the resolution of this node with ML analyses supporting leptonetid paraphyly (Austrochiloidea + Leptonetidae) and some SVDQuartets results showing leptonetid monophyly (Archoleptonetidae + Leptonetidae), although weakly supported. Similar to Fernández *et al*. (2018), our strict matrix was partly intended as a sensitivity test by restricting our data to a core set of orthologs and removing flanking introns where alignment may be uncertain. Even with nearly 50% of the data removed, the strict matrix still does not consistently resolve the node as some SVDQuartets results recover leptonetid monophyly (Fig. S4, S6). Interestingly, despite the fact that our ML analyses show 100% bootstrap support for a sister group relationship between Austrochiloidea and Leptonetidae (Fig. 4, S1–S3), the relatively low gCF and sCF values suggest that most of the gene trees recover alternate resolutions for the node. In summary, our results and those of Fernández *et al*. (2018) show ambiguity in the resolution of this node as reflected in both analytical sensitivity and gCF and sCF values.

Although we are encouraged by the results in our study, we recognise that conflict in the relationships of Austrochiloidea, Archoleptonetidae and Leptonetidae persists. As a consequence, we argue that the node is best viewed as a trichotomy in need of further study. One key outcome of all recent studies is that Archoleptonetidae and Leptonetidae are not part of Synspermiata, a result predicted by morphology (Brignoli 1979*d*; Ledford and Griswold 2010) and corroborated by all phylogenomic analyses. Regardless of the eventual placement of Archoleptonetidae and Leptonetidae, the elevation of Archoleptonetidae is supported by their morphology (Ledford and Griswold 2010), facilitates their diagnosis, and serves a grouping function. We therefore view the elevation of Archoleptonetidae as both warranted and timely, especially as our understanding of spider phylogeny improves with increased adoption of phylogenomic methods. As newly defined, Archoleptonetidae is a small family consisting of two genera; however, many areas within their distributional range are undersampled and we expect that more species likely await discovery.

In each of our analyses, we recover four main lineages within Leptonetidae which we refer to as the *Paraleptoneta*, *Leptoneta*, *Protoleptoneta*, and *Calileptoneta* groups. The *Paraleptoneta* and *Leptoneta* groups include species limited to Mediterranean Europe, whereas both the *Protoleptoneta* and *Calileptoneta* groups include species distributed on multiple continents. The North American fauna is well represented in our study, and all genera have high statistical support corroborating the hypotheses of Ledford *et al*. (2011, 2012). *Chisoneta*, *Neoleptoneta*, *Ozarkia*, and *Tayshaneta* comprise a single lineage that is distributed from the south-western USA to Mexico and is sister to the remainder of the *Protoleptoneta* group. Representation of the Asian fauna is weaker, but *Falcileptoneta* and *Longileptoneta* are monophyletic and well supported as part of the *Protoleptoneta* group. Although our sample includes only a single *Leptonetela* species from China, *L. jiulong* Lin & Li, 2010 is sister to *L. thracia* Gasparo, 2005, supporting the synonymies of the genera *Guineta*, *Qianleptoneta*, and *Sinoneta* (Lin & Li, 2010). With the exception of *Cataleptoneta*, the European genera are supported as monophyletic. However, as discussed below, several species are likely misplaced. Although our study does not include Asian representatives of *Leptoneta*, several *Leptoneta* species are part of our analyses, including the type *L. convexa* Simon, 1872. Based on the features of their genitalic morphology (J. Ledford, pers. obs.) and the distribution of *Leptoneta* in western Mediterranean Europe, we predict that all of the currently described Asian *Leptoneta* are misplaced.

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As the first study to assess leptonetid phylogeny using a sample of most genera, it is not surprising to discover taxonomic problems and learn that some genera need revision. In particular, the generic limits among *Barusia*, *Cataleptoneta*, and *Paraleptoneta* are unclear. The type species *Cataleptoneta edentula* (Denis 1955) does not group with *C. semipinnata* (Wang and Li 2010) and *C. sengleti* (Brignoli 1974*a*). *Barusia* has similar problems as *B. laconica* (Brignoli 1974*a*) does not group with the type species *B. maheni*. Taxonomic issues within *Barusia* and *Cataleptoneta* date to Kratochvíl (1978), who based his diagnoses on spination differences on the male palpal tibia and not details of palpal bulb morphology as had been done by previous workers. *Barusia*, *Cataleptoneta*, *Paraleptoneta*, and *Sulcia* are all characterised by complex spination patterns on the male palpal tibia (Le Peru 2011), making the morphological distinction between them unclear. Although recent studies on *Cataleptoneta* (Wang and Li 2010; Deltshev and Li 2013; Gavish-Regev *et al*. 2016; Demircan 2020) have contributed to our understanding of its diversity, they have not provided a broad perspective on the diagnosis, distribution, or relationships of the genus as a whole. Work in progress (C. Ribera, unpubl. data) further shows that the genetic diversity within *Paraleptoneta* is higher than expected despite a history of synonymy (Brignoli 1979*c*). Based on our results, we hypothesise that at least two additional genera remain undescribed; the first includes *B. laconica* and *C. semipinnata* and the second includes *C. sengleti* and *C. aesculapii*. However, we believe that taxonomic changes to *Barusia* and *Cataleptoneta* need the context of a more comprehensive revision and we defer any changes until the lineage can be more carefully studied.

Within Leptonetidae, there are three described monotypic genera: *Montanineta* Ledford & Griswold, 2010 from the eastern USA, *Teloleptoneta* Ribera, 1988 from Portugal, and *Rhyssoleptoneta* Tong & Li, 2007 from China. Until this study, none of these taxa have been included in a phylogenetic analysis and the justification used in their descriptions was based solely on the degree of difference in their genitalic morphology. Although our study does not include *Rhyssoleptoneta*, both *Montanineta* and *Teloleptoneta* group with *Protoleptoneta* (Deltshev 1972) although support within this clade is low. Unpublished single-gene analyses also show an ambiguous relationship between *Protoleptoneta* and *Teloleptoneta* (C. Ribera, unpubl. data). Close inspection of the genitalic morphology of *Montanineta*, *Protoleptoneta*, and *Teloleptoneta* show some similarities (J. Ledford, pers. obs.) and one solution is that all of these genera could be combined into a single genus. Although this would simplify the nomenclature of the group, it would not change our interpretation of the relationships in the lineage as a whole. Namely, given the wide geographic disjunction between these genera we view them as relictual; the closest relative of *Montanineta*, for example, lives on a different continent. One specimen from Croatia (AR4354) likely represents yet another monotypic genus given the geographic disjunction with its closest relatives (*Appaleptoneta* and *Leptonetela*). Lastly, although *Rhyssoleptoneta* was not included in our analysis examination of its genitalic morphology shows similarity in palpal structures to *Appaleptoneta* (J. Ledford, pers. obs.) and we predict that the genus may be part of the *Calileptoneta* group.

Biogeography

Given that our sampling of Archoleptonetidae is limited to representatives from California and Arizona, it is not surprising that the ancestral distribution for the group is reconstructed as western North America (B). As more species are described, we expect that the biogeography of Archoleptonetidae will become better understood.

Results of our molecular dating analysis estimate the origin of Leptonetidae at 126 Ma and its ancestral distribution is inferred to encompass an area spanning western North America and Mediterranean Europe (BD, node 107, Fig. 6). Although the *Paraleptoneta* and *Leptoneta* groups are reconstructed to have ancestral distributions restricted to Mediterranean Europe (D, node 80, Fig. 6), the *Protoleptoneta* and *Calileptoneta* groups are relatively older and have ancestral distributions that include combinations of areas including eastern and western North America, Mediterranean Europe, and east Asia (nodes 97 and 106, Fig. 6).

During the mid-Jurassic, Pangaea split into the northern and southern supercontinents Laurasia and Gondwana. The origin of Leptonetidae (mid-Cretaceous) coincides with a time when Laurasia was largely connected, although North America and Asia remained separated. Most of the major divergences within Leptonetidae occurred 100–70 Ma. Within this time, Laurasia split into two palaeocontinents: Euramerica (Europe + eastern North America) and Asiamerica (western North America + Asia). North America was divided by the Mid-Continental Seaway and western North America was connected to east Asia through Beringia. Europe was divided from Asia by the Turgai Strait and eastern North America remained connected to Europe through multiple, periodic land bridges across the Atlantic (reviewed in Sanmartín *et al*. 2001).

During the early Tertiary a continuous region of vegetation, the Boreotropical forest, was distributed across Laurasia. The boreotropics hypothesis (Wolfe 1975; Tiffney 1985) postulates that the biogeographical disjunctions often seen in plants between eastern North America–Europe and western North America–Asia resulted from the fragmentation of Laurasia and the recession of the Boreotropical forest. Based on the age estimates, ancestral distributions, and the biology of leptonetids, we hypothesise that they were once widespread across Laurasia. One possibility is that they were associated with the Boreotropical forest ecosystem and their phylogeny is largely the product of vicariance as Laurasia fragmented. This scenario not only aligns well with our results, but also explains the repeated pattern of narrow endemism for most leptonetid species worldwide. As Laurasia drifted apart, the climate became drier and leptonetids were restricted to areas where mesic conditions similar to those found in the Boreotropical forest persisted. This may also explain the close association of leptonetids with caves, which, due to their cool and moist environments, may have functioned as refugia. As such, we argue that leptonetids may be regarded as a largely relictual

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fauna, similar to Holarctic relict floras (Tiffney 1985; Lavin and Luckow 1993; Milne and Abbott 2002).

As a relictual fauna, we predict that extinction has played a significant role in shaping the present distribution of leptonetids. Although our DEC analyses recover no extinction, we view this result as improbable given the age and reconstructed ancestral distributions for most groups in our analyses (Fig. 5, 6). The underestimation of local extinction is also a known issue with DEC analysis (Ree and Smith 2008). Although dispersal is inferred in our DEC results, the probabilities associated with these events are low (Table S3). We also find the pattern of sympatry within Leptonetidae of particular interest; in most cases leptonetid genera do not have overlapping distributions, even among distantly related lineages. In areas where the distributions of genera are close, such as eastern Europe, our phylogeny reveals taxonomic problems. There are few cases of sympatry within genera although sampling error may be a contributing factor given the relative rarity of leptonetids in collections. We interpret these patterns as evidence for niche conservatism in the group through deep geologic time, a pattern predicted by their biology and supported by phylogeny.

Paraleptoneta *and* Leptoneta *groups*

The origin of the *Paraleptoneta* and *Leptoneta* groups dates to 105 Ma and coincides with the separation of Europe and Asia by the Turgai Strait. The *Paraleptoneta* group includes *Barusia*, *Cataleptoneta*, *Paraleptoneta*, and *Sulcia*, which are primarily distributed in the eastern Mediterranean but also have records in the Levant and Tunisia (Ribera and Lopez 1982; Gavish-Regev *et al*. 2016). At the time of the origin of this group, the eastern Mediterranean formed a continuous plate from Turkey to the Balkans, including the Aegean and Croatian Islands. The present geographical distribution of the *Paraleptoneta* group includes all of southern Europe (except for the Iberian Peninsula), where Pleistocene glaciations had minimal impact (Clark *et al*. 2009; Batchelor *et al*. 2019). It is likely that the group extends further north, although currently there are no known species at higher latitudes. Our analysis suggests an eastern origin (Turkey or Greece) with subsequent colonisation to the Balkan Mountains, from Albania to Croatia and Italy, extending southward to Tunisia during the Messinian Salinity Crisis (Garcia-Castellanos and Villaseñor 2011).

The *Leptoneta* group includes 30 species distributed throughout the western Mediterranean and 38 species in Asia (World Spider Catalog, see http://wsc.nmbe.ch). Based on the species included in our analysis, and the distributions of all other *Leptoneta* from Europe, we interpret the genus to be of Iberian origin; all *Leptoneta* species in Europe occur in regions that were formerly part of or connected to the peninsula. Results from our molecular dating analysis estimate the divergence of *Leptoneta* at 33 Ma. During this time the Iberian Peninsula was located between Eurasia and North America but separated from the northern mainland of Africa. This timing also precedes the drying of the Turgai Strait and subsequent uplift of the Himalayas. A key consequence of this timing is that *Leptoneta* is restricted to the western Mediterranean. In conjunction with our observations of its morphology, we view this as evidence that all currently described Asian *Leptoneta* are misplaced and belong to other genera.

Protoleptoneta *group*

The *Protoleptoneta* group is estimated to have diverged 87.5 Ma and includes genera from eastern North America, Mediterranean Europe, and east Asia. The ancestral distribution of the lineage has a mixed probability of origin, but most likely includes an area spanning eastern North America and Mediterranean Europe (AD, node 97, Fig. 6). Two main lineages occur within the group; the first includes four genera (*Chisoneta*, *Neoleptoneta*, *Ozarkia*, and *Tayshaneta*) distributed in the southern USA and Mexico. This group is estimated to have arisen 77 Ma in eastern North America after the closure of the Mid-Continental Seaway and the subsequent uplift of the western mountain ranges as part of the Laramide orogeny. The westernmost species in this lineage, *Ozarkia apachaea* (Gertsch, 1974) is known from the Chiricahua Mountains and we predict that the western mountains presented a barrier to further dispersal of the group in western North America.

The second *Protoleptoneta* group lineage includes five widely disjunct genera: *Falcileptoneta* (east Asia), *Longileptoneta* (east Asia), *Montanineta* (eastern North America), *Protoleptoneta* (Mediterranean Europe), and *Teloleptoneta* (Mediterranean Europe). Given the geographic complexity, the DEC analysis infers a mixed probability of origin for this group (node 96, Fig. 6). Two possibilities are presented: (1) Mediterranean Europe + east Asia (DF, 70.9%), and (2) eastern North America + east Asia (AF, 29%). The first scenario requires dispersal to eastern North America, perhaps by the Thulean or De Greer land bridges, both of which are recognised as important routes between Mediterranean Europe and eastern North America (Sanmartín *et al*. 2001; Brikiatis 2014). The second scenario requires dispersal to Mediterranean Europe which seems less likely given the persistence of the Turgai Strait until the late Tertiary (30 Ma). One possible explanation for the ambiguity is insufficient sampling in Asia. *Falcileptoneta* and *Longileptoneta*, for example, are broadly distributed in Japan, Korea, and Taiwan and together include over 50 species. Our data includes only four representatives of these genera and we predict that increased sampling will greatly inform the inferred ancestral distribution of the group. Based on the number of described species, the centre of diversity for the *Protoleptoneta* group appears to be east Asia. The genera *Montanineta* and *Teloleptoneta* are monotypic and *Protoleptoneta* includes only four species, mostly known from isolated caves. Given the age of the *Protoleptoneta* group and its inferred ancestral distribution, we hypothesise that *Montanineta*, *Teloleptoneta*, and *Protoleptoneta* are relictual and perhaps the last extant members of formerly widespread lineages.

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Calileptoneta *group*

The origin of the *Calileptoneta* group is estimated at 110 Ma and is reconstructed as having an ancestral distribution spanning western North America and Mediterranean Europe (BD, node 106, Fig. 6). This timing is close to the origin of the mid-Continental Seaway, which separated North America into two subcontinents (Laramidia in the west and Appalachia in the east). Given the age of the group, a plausible scenario is that the group was formerly widespread and then divided by the formation of the mid-Continental Seaway.

Within the *Calileptoneta* group, one lineage corresponds to *Calileptoneta*, which is known only from western North America (B, node 101, Fig. 6). Although we see no relationships between *Calileptoneta* and taxa from east Asia, the western North America–east Asia disjunction is well established and we predict that some unsampled taxa in Asia will have close affinity with *Calileptoneta*. The monotypic genus *Rhyssoleptoneta*, for example, shares palpal homologies with *Calileptoneta* (J. Ledford, pers. obs.) and may prove important to understanding relationships and biogeography within this group.

The second lineage includes taxa from Mediterranean Europe, eastern North America, and east Asia. The group is reconstructed to have originated in Mediterranean Europe (D, node 105, Fig. 6) with an estimated age of 103 Ma. Given the reasonably ancient age of the group, we predict that it was formerly widespread and some lineages (AR 4354 and *Appaleptoneta*) are relictual. Within this group, the genus *Leptonetela* is the most diverse with over 100 species described from Asia (China and Vietnam) and 12 from the eastern Mediterranean (Turkey, Caucasus, and Greece). Although we only have two *Leptonetela* species in our dataset, both Mediterranean Europe and east Asia are represented. The estimated age for the split of *L. jiulong* (Guizhou, China) and *L. thracia* (Greece) is 19 Ma (the origin of the genus *Leptonetela* is older, *c*. 80 Ma) after the closure of the Turgai Strait (Tangelder 1988; Sanmartín *et al*. 2001). We interpret the occurrence of *Leptonetela* in Europe as a recent dispersal from South Asia, that occurred before the rise of the Tibetan plateau or the formation, further north, of the Gobi Desert, which prevented connection between Central Asia and the Eastern Mediterranean. Increased sampling will likely improve our understanding of the biogeography of *Leptonetela*, especially in the region from Turkey to India, which remains undersampled and may hold more species.

Conclusions

Archoleptonetids and leptonetids are ancient lineages of spiders whose relationships have been enigmatic for over 100 years. Brignoli (1979*d*) was the first to recognise that they were likely not part of Synspermiata (then Haplogynae) although he did not have a clear conception of where they fit on the spider tree of life. He also recognised that the placement of leptonetids with telemids and ochyroceratids was a result of the poor state of knowledge about these families at the time. Since Brignoli, most workers have placed Leptonetidae (including Archoleptonetidae) as sister to Telemidae (Platnick *et al*. 1991; Ramírez 2000) although it was widely recognised that aspects of their morphology (absence of cheliceral lamina, cylindrical gland spigots, expandable male genitalia, respiratory structures) argued against this arrangement. Ledford and Griswold (2010) reviewed the morphology of both groups and found additional problems, proposing that they were more closely related to entelegynes.

Among the challenges with both families is that they are rarely encountered spiders, even by specialists, and their small size and delicate features make them difficult to study. For example, in the absence of scanning electron microscopy, the intricate features of the male palpal bulb are rarely observed and most species worldwide remain inadequately described and diagnosed (but see Ledford 2004; Ledford and Griswold 2010; Ledford *et al*. 2011, 2012). The increasingly widespread adoption of phylogenomics has provided insight into relationships among all spiders, although, as demonstrated in this study, this is not a panacea. The deep splits inherent on the spider tree of life are difficult to resolve, especially among early-diverging araneomorph groups such as archoleptonetids and leptonetids. As shown in a recent study on spider fossils (Magalhaes *et al*. 2020), many of the early-diverging araneomorphs known from fossils are difficult to place and extinction of these lineages is likely a confounding factor in understanding present-day spider phylogeny. However, despite the inability of our study and recent phylogenomic studies to resolve relationships among austrochiloids, archoleptonetids, and leptonetids it is clear that they are not part of Synspermiata, a hypothesis supported by both their morphology and molecular data. Resolving relationships among these groups will require special attention, likely incorporating data from fossils and careful analysis of their morphology.

Although problems with the current taxonomy of leptonetids are identified in our study, most described genera are monophyletic. The problems within the *Paraleptoneta* group are especially intriguing as they have a long history of study, including detailed illustrations and descriptions of the male and female genitalia. However, none of these groups has undergone comprehensive revision and no European genera have ever been quantitatively analysed. Further, significant sampling gaps remain, especially in eastern Europe, where a wealth of diversity undoubtedly remains undiscovered as evidenced by the undescribed genus from Croatia included in this study. The most substantial challenge in leptonetid systematics is the Asian fauna. Recent efforts focused on Asian leptonetids have provided insight into their diversity, but only one of these studies (Wang *et al*. 2017) include quantitative analyses of their phylogeny. The result of these descriptive efforts is that most Asian leptonetids remain unplaced, and genera such as *Leptoneta* continue to be used as placeholders thereby preventing a complete picture of their biogeography. Given the age and distribution of the family, we predict that connections especially to the western North American fauna await discovery. Among the goals of this study is to provide a comprehensive phylogenetic scaffold such that new species in Asia can be more confidently placed.

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Taxonomy

Justification

We have decided to raise both subfamilies Archoleptonetinae and Leptonetinae to family status on the grounds that to do so is practical, making each easier to diagnose and that it does no phylogenetic harm, i.e. each is monophyletic. Further, if our preferred tree is correct (Fig. 4), with Leptonetinae sister to the Austrochiloidea to the exclusion of the Archoleptonetinae, Leptonetidae *sensu lato* becomes paraphyletic and relimiting the family becomes essential.

Austrochiloidea, Archoleptonetidae and Leptonetidae

This analysis and other recent analyses (e.g. Fernández *et al*. 2018) suggest that Archoleptonetidae and Leptonetidae are closely related to Austrochiloidea (Austrochilidae, including *Hickmania*, and Gradungulidae). Whereas molecular data unite these taxa, they do not share any obvious morphological synapomorphies. Austrochiloids are readily distinguished from Archoleptonetidae and Leptonetidae. The austrochiloid families share the morphological synapomorphies of a clypeal hood, i.e. a median projection over the middle cheliceral base (Griswold *et al*. 2005, character 30), a distal notch in the orifice of the trichobothrial base (Griswold *et al*. 2005, character 9), and a peculiar genitalic morphology in which the gonopore is visible anterior to the epigastric fold (Griswold *et al*. 2005, character 135; Ramírez 2014 character 363). Austrochiloids are large spiders, and all have austral distributions. Members of *Kaiya*, smallest of the gradungulids, still have a body length of 1.2 cm and a leg span of more than 3 cm, whereas some austrochiloids are veritable giants. *Hickmania troglodytes* (Austrochlidae) may have a body length of 2 cm and a leg span of 17 cm, and *Macrogradungula moonya* (Gradungulidae) may have a body length of 2 cm and a leg span of more than 18 cm! By contrast, temperate Archoleptonetidae and Leptonetidae are small spiders (less than 5 mm body length) with long, slender legs, with a leg spread of less than 2.5 cm. All have only six eyes (or none), three claws with the STC (superior tarsal claws) simple (unlike the asymmetrical claws of Gradungulidae, raptorial claws of Trogloraptoridae or enlarged, bipectinate STC of many Dysderoidea), no cheliceral lamina, unusual iridescence, especially on the legs and carapace (shared with Ochyroceratidae and Psilodercidae, among others), autospasy at the patella–tibia joint (shared with Filistatidae, Austrochilinae (*Thaida* and *Austrochilus*) and linyphioids), patella–tibia gland plates (shared with the Telemidae; similar structures occur in various Entelegynae), no external epigynum (unlike Entelegyne and some Palpimanoidea and Synspermiata), a single posterior spiracle, a divided cribellum (unlike the entire cribellum of Hypochilidae and cribellate Austrochiloidea) or a small colulus (unlike the large coluli of Telemidae, Ochyroceratidae and Psilodercidae and some Synspermiata), clypeus with margin evenly concave (unlike the median hood of Austrochiloidea), and no peg teeth (unlike Palpimanoidea).

Ledford and Griswold (2010, p. 4) diagnosed Leptonetidae *sensu latu* based on a set of homoplastic putative synapomorphies, which assumed placement of Leptonetidae in the Haplogynae, an hypothesis and grouping now discarded. Features cited were unusual iridescence, especially on the legs and carapace, patellar–tibial gland plates, autospasy at the patella–tibia joint, the presence of tartipores on the ALS (shared widely but absent in Synspermiata), male palpi with a fused tegulum and subtegulum but with an expandable basal haematodocha (a peculiar morphology, which requires further study in austrochiloids), and a respiratory system consisting of a pair of short median branches with long laterals that open to a single spiracle anteriad of the ALS (also found in many Entelegynae). This diagnosis overlooks notable differences between Archoleptonetinae and Leptonetinae in eye position, spinning organs and male and female genitalia: recognising Archoleptonetidae and Leptonetidae as families will make each far easier to diagnose.

Family **ARCHOLEPTONETIDAE** Gertsch, 1974 (new rank)

Archoleptonetinae Gertsch, 1974: 198

Diagnosis

Archoleptonetidae may be diagnosed from all other spiders except Leptonetidae by the characters listed above. Archoleptonetids may be diagnosed from leptonetids by having the endites with a pair of conspicuous stout setae, by the simple ocular arrangement with PME and PLE contiguous, and by the form of the tarsal organ, spinning organs and genitalia. All Archoleptonetidae have the tarsal organ with at least one elongate sensillum, multiple MAP (major ampullate gland) spigots on the ALS (anterior lateral spinnerets) and have only a few scattered AC (aciniform gland) spigots on the PMS and PLS (posterior median and lateral spinnerets); at least *Archoleptoneta* have a divided cribellum. Archoleptonetidae have the male palpal tibia and tarsus simple and cylindrical and the palpal bulb with an elongate embolus and three accessory sclerites, two of which straddle the embolus at the base (PRS, MS) and one that is situated prolaterally (RLS). In addition, the simple female genitalia with two receptacula and oval to elongate patellar–tibial glands may distinguish Archoleptonetidae from Leptonetidae.

Type species: *Archoleptoneta schusteri* Gertsch, 1974.

Composition

Two genera: *Archoleptoneta* Gertsch, 1974 (2 species) and *Darkoneta* Ledford & Griswold, 2010 (6 species).

Family **LEPTONETIDAE** Simon, 1890 (new status)

Leptonetidae Simon, 1890: 80

Diagnosis

Leptonetidae may be readily diagnosed from all other spiders by the unique ocular arrangement with PME displaced behind the ALE and PLE, AME lost. Another character seemingly

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unique to the leptonetids is the metatarsus III with an apical preening comb. Leptonetids may be distinguished from archoleptonetids by the endites lacking conspicuous stout setae and by the form of the tarsal organ, spinning organs and genitalia. Leptonetid tarsal organs may have an elongate base but the sensillum(a) are short and inconspicuous. All leptonetids are ecribellate with a small colulus (like the archoleptonetids *Darkoneta*) but the ALS have only a single MAP (major ampullate gland) spigot and at least the PLS have tightly packed rows of AC (aciniform gland) spigots. Leptonetid male palpi typically have the tibia and tarsus modified: the lateral surfaces of the tibia typically have a variety of spines and twisted setae that in many genera are produced into large spine-like apophyses and the palpal tarsus is dorsally constricted and often modified apically and retrolaterally, usually bearing chemosensory and a variety of other specialised setae. The leptonetid female genitalia that present a vulva with a large, central atrium with a pair of lateral twisted spermathecae bearing numerous flagellate pores, which are connected laterally to the atrium by short, twisted tubes, are unique. In addition, leptonetids have patellar–tibial glands but these differ in shape from those of archoleptonetids and from the family Telemidae.

Type species: *Leptoneta convexa* Simon, 1872.

Composition

In total, 19 genera and 347 species: *Appaleptoneta* (Platnick 1986) (7 species), *Barusia* (Kratochvíl 1978) (5 species), *Calileptoneta* (Platnick 1986) (9 species), *Cataleptoneta* (Denis 1955) (8 species), *Chisoneta* (Ledford *et al*. 2011) (4 species), *Falcileptoneta* (Komatsu 1970) (50 species), *Leptoneta* (Simon 1872) (70 species), *Leptonetela* (Kratochvíl 1978) (108 species), *Longileptoneta* (Seo 2015*b*) (5 species), *Masirana* (Kishida, 1942, in Komatsu 1942) (26 species), *Montanineta* (Ledford *et al*. 2011) (1 species), *Neoleptoneta* (Brignoli 1972) (8 species), *Ozarkia* (Ledford *et al*. 2011) (9 species), *Paraleptoneta* (Fage 1913) (2 species), *Protoleptoneta* (Deltshev 1972) (4 species), *Rhyssoleptoneta* (Tong and Li 2007) (1 species), *Sulcia* (Kratochvíl 1938) (10 species), *Tayshaneta* (Ledford *et al*. 2011) (19 species) and *Teloleptoneta* Ribera, 1988 (1 species).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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