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Rounding up the usual suspects: a standard target-gene approach for resolving the interfamilial phylogenetic relationships of ecribellate orb-weaving spiders with a new family-rank classification (Araneae, Araneoidea)

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Abstract

We test the limits of the spider superfamily Araneoidea and reconstruct its interfamilial relationships using standard molecular markers. The taxon sample (363 terminals) comprises for the first time representatives of all araneoid families, including the first molecular data of the family Synaphridae. We use the resulting phylogenetic framework to study web evolution in araneoids. Araneoidea is monophyletic and sister to Nicodamoidea **rank. n.** Orbiculariae are not monophyletic and also include the RTA clade, Oecobiidae and Hersiliidae. Deinopoidea is paraphyletic with respect to a lineage that includes the RTA clade, Hersiliidae and Oecobiidae. The cribellate orb-weaving family Uloboridae is monophyletic and is sister group to a lineage that includes the RTA Clade, Hersiliidae and Oecobiidae. The monophyly of most Araneoidea families is well supported, with a few exceptions. Anapidae includes holarchaeids but the family remains diphyletic even if *Holarchaea* is considered an anapid. The orb-web is ancient, having evolved by the early Jurassic; a single origin of the orb with multiple "losses" is implied by our analyses. By the late Jurassic, the orb-web had already been transformed into different architectures, but the ancestors of the RTA clade probably built orb-webs. We also find further support for a single origin of the cribellum and multiple independent losses. The following taxonomic and nomenclatural changes are proposed: the cribellate and ecribellate nicodamids are grouped in the superfamily Nicodamoidea **rank n.** (Megadictynidae **rank res.** and Nicodamidae **stat. n.**). Araneoidea includes 17 families with the following changes: Araneidae is recircumscribed to include nephilines**,** Nephilinae **rank res.,** Arkyidae **rank n.**, Physoglenidae **rank n.**, Synotaxidae is limited to the genus *Synotaxus*, Pararchaeidae is a junior synonym of Malkaridae (**syn. n.**), Holarchaeidae of Anapidae (**syn. n.**) and Sinopimoidae of Linyphiidae (**syn. n.**).

Introduction

The orb-weaving spiders ("Orbiculariae") include at least one of the most diverse branches of the spider tree of life—Araneoidea. More than 12 500 species (approximately 28% of the more than 45 000 described spider species) have been classified as members of one of the former 21 extant "orbicularian" families. Although the defining trait of orbicularians, as their name suggests, is the orb-web itself, web architecture in this putative lineage is extraordinarily variable (Fig. 1), ranging from the well-known bidimensional highly geometric snare with a framed set

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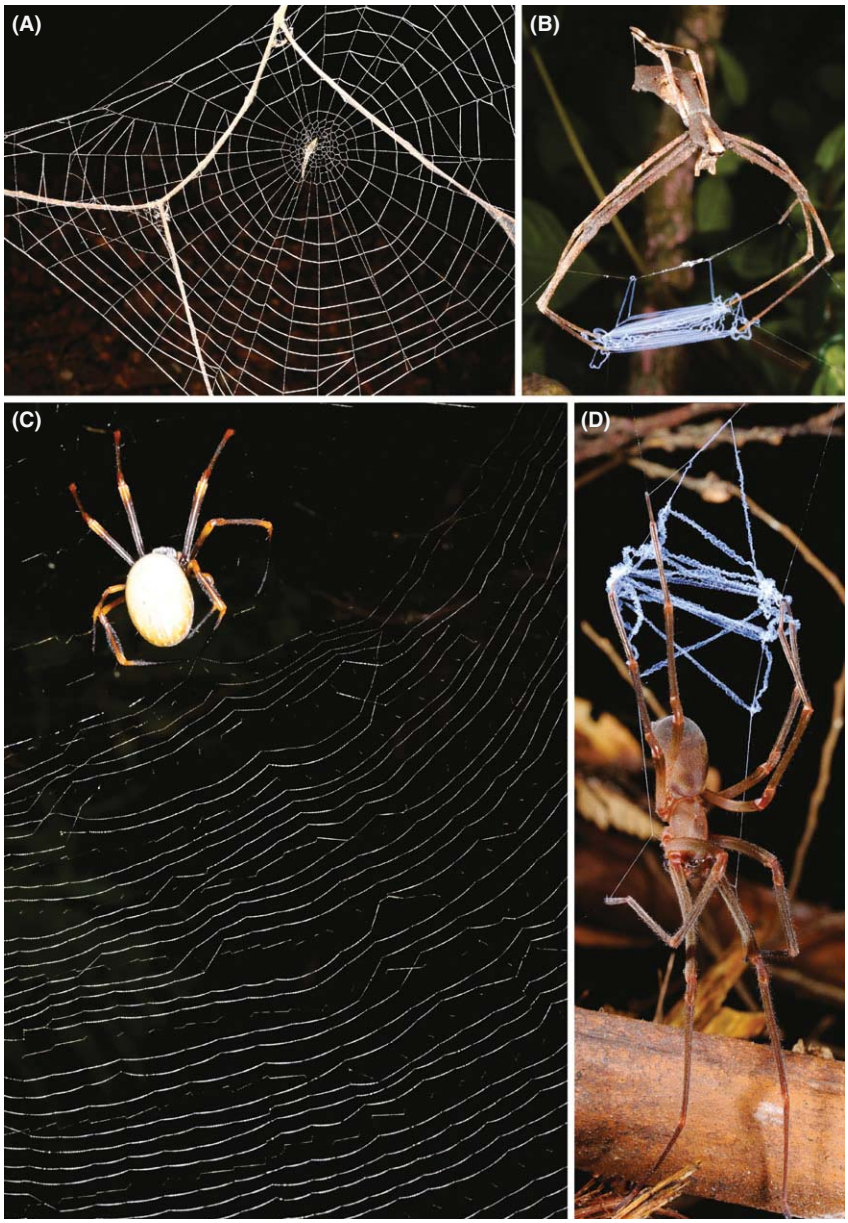

Fig. 1. (A) The cribellate web of *Sybota* sp. (Uloboridae), from Chile (DSC_2250). (B) The cribellate ogre-face spider *Deinopis* sp. (Deinopidae), from Australia (DSC_0983). (C) The ecribellate *Nephila plumipes* building its orb-web, Australia; the highly reflective silk lines in this image are the viscid capture spiral turns covered with a sticky glycoprotein, a synapomorphy of Araneoidea. The less reflective silk lines in between sticky turns are part of the temporary nonsticky spiral, which in *Nephila* and its relatives are left in the finished web (DSC_6451). (D) *Progradungula otwayensis* (Gradungulidae), from Australia, with its ladder cribellate web; an example of an early-branching araneomorph that illustrates the antiquity of cribellate silk (DSC_1424). Photos: G. Hormiga.

of radii and a sticky spiral (e.g. in Tetragnathidae; Fig. 6F) to highly irregular tridimensional webs (e.g. in Linyphiidae; Fig. 6D, G, H). Almost everything in between these architectural extremes seems to exist and most of this web diversity is still undiscovered or undocumented (e.g. Scharff and Hormiga, 2012). In some cases foraging webs have been abandoned altogether, such as in the pirate spiders (Mimetidae; Fig. 4C).

Two groups of orb-weavers—deinopoids and araneoids—build similar webs that differ significantly in the structure and composition of the silk of their capture spiral. Traditionally regarded as a lineage, these two groups are now hypothesized not to form a clade (Dimitrov et al., 2013; Bond et al., 2014; Fernández et al., 2014). Deinopoids (Deinopidae, Uloboridae) use cribellate silk for their sticky spiral (Fig. 1A, B), while the allegedly homologous counterpart in araneoids is

made of a type of viscid silk that is unique to araneoids (e.g. Fig. 1C). Cribellate silk is ancient (e.g. Fig. 1D)—it evolved in the early araneomorph lineages—and thus sharing such type of silk among deinopoid taxa is expected to be symplesiomorphic. This type of silk is spun by a spinning plate (the cribellum) in combination with a combing structure on the fourth leg metatarsus consisting of a row of modified macrosetae (the calamistrum). Cribellate silk is "dry" and is formed of thousands of fine looped fibrils woven on a core of two axial fibres (e.g. Opell, 1998, fig. 1). Its adhesive properties are attained by van der Waals and hygroscopic forces (Hawthorn and Opell, 2003). In contrast, araneoids produce a novel type of sticky silk in which the axial fibres are coated with a viscid glycoprotein. This type of composite sticky thread is produced faster, presumably more economically, and attains a much higher stickiness than the dry deinopoid cribellate silk. A large body of empirical work has studied and compared the biological and physicochemical properties of these types of silks (see review in Blackledge, 2012).

There is a marked disparity in species richness between cribellate and ecribellate orb-weavers. The majority of orb-weaving spiders are members of the superfamily Araneoidea (the ecribellate orb-weavers, 17 families, more than 12 000 species described). In comparison, Deinopoidea, the cribellate orb-weavers, include only 331 described species in two families. Nicodamidae, a small Austral group (29 species named) with both cribellate and ecribellate members, appears to be phylogenetically related to the ecribellate orb-weavers (Blackledge et al., 2009; Dimitrov et al., 2012). This asymmetry in species diversity between deinopoids and araneoids has been attributed to the shift in type of capture thread from dry, fuzzy cribellate silk (Fig. 1B) to viscid, sticky silk (Fig. 1C), combined with changes in the silk spectral reflective properties and a transition from horizontal to vertical orb-webs (references summarized in Hormiga and Griswold, 2014). However, recent studies (Dimitrov et al., 2013; Bond et al., 2014; Fernández et al., 2014) and the results presented here show that the contrast Deinopoidea–Araneoidea is no longer valid and it is likely that evolution of webs and diversification into new ecological niches are responsible for the differences in diversity of these spider clades (e.g. Dimitrov et al., 2012).

The question of whether cribellate and ecribellate orb-webs can be traced to a single origin or have evolved independently began to be debated in the 19th Century (summarized in Coddington, 1986), and has been discussed extensively in the literature. It was not until the late 1980s that a consensus began to emerge on the answer to this problem. During the last three decades, the combination of comparative behavioural data (such as the seminal work of Eberhard, 1982) and cladistic approaches to analyse the available evidence has favoured a monophyletic origin of orb-webs and the monophyly of Orbiculariae (e.g. Levi and Coddington, 1983; Coddington, 1986, 1990), with the preponderance of evidence supporting this view coming from the webs and the concomitant stereotypical behaviours used to build them. Most research in the last two decades has supported a single origin of the orb-web. Because the monophyly of orb-weavers has been supported primarily by behavioural and spinning organ characters, it has been challenging to test the possibility that orb-webs were not convergent in the cribellate and ecribellate orb-weavers without referring to the building behaviours and silk products. Genetic data have played an increasingly important role in resolving spider phylogenetic relationships, mostly in the form of nucleotide sequences from a few genes (the nuclear and mitochondrial rRNA genes 18S, 28S, 12S and 16S and a handful of protein-encoding genes from which the most commonly used are the nuclear histone H3 and the mitochondrial COI), often humorously described as "the usual suspects". However, the success of these markers as an independent test to resolve orbicularian relationships has been limited, particularly at the interfamilial level (e.g. Blackledge et al., 2009; Dimitrov et al., 2012).

Only one phylogenetic analysis of molecular data with a sufficiently dense taxon sample to properly address interfamilial relationships has recovered Orbiculariae as a clade, albeit without support (Dimitrov et al., 2012). Furthermore, these nucleotide data failed to resolve or provide support for the relationships among most orbicularian families: the majority of deep internodes are short. Although most phylogenetic analyses of DNA sequence data have found that orbicularians are not monophyletic, this particular result has often been dismissed as "artefactual" (e.g. due to taxon sampling effects) or "misleading"—such has been the convincing power of the orbicularian monophyly hypothesis. For example, in an analysis of the spider sequences available in GenBank, Agnarsson et al. (2013) explicitly stated that the placement of *Uloborus* as sister group to the RTA clade "can be presumed to be false".

Moreover, molecular data analyses often fail to find support for the monophyly of Deinopoidea—the cribellate orb-weavers (Uloboridae + Deinopidae) (e.g. Dimitrov et al., 2012, 2013; Bond et al., 2014; Fernández et al., 2014). In contrast, the monophyly of Araneoidea (the ecribellate orb-weavers) is well supported by both morphological and molecular data, but relationships among families remained unresolved for the most part (Hormiga and Griswold, 2014; and references therein) until publication of two recent transcriptome-based phylogenetic analyses (Bond et al., 2014; Fernández et al., 2014).

As the present study shows, the long-held hypothesis of Orbiculariae monophyly continues to be overturned by molecular data, using both standard PCR-amplified genetic markers (Dimitrov et al., 2013) and, more persuasively, transcriptomic data (Bond et al., 2014; Fernández et al., 2014). These recent studies place the cribellate orb-weavers (Deinopoidea; which do not form a clade) with other groups, rather than with the ecribellate orb-weavers (Araneoidea), as the monophyly hypothesis demands.

Spurious groupings in orbicularian analyses could result from a number of well-known causes. Missing data have long been discussed with respect to their potential for affecting phylogenetic results (e.g. Kearney, 2002; Wiens, 2003; Wiens and Morrill, 2011). For the cladistic problem discussed herein, missing data occurred because of variable success in obtaining sequences for all markers and because of a certain lack of overlap across published analyses. Sparse taxon sampling can also be a concern (e.g. Pollock et al., 2002; Hillis et al., 2003), particularly at higher levels, because it may produce results that are difficult to interpret in the absence of relevant higher taxa (e.g. insufficient representation of symphytognathoids in Blackledge et al., 2009) or that are refuted with a denser taxon sample (e.g. in Lopardo and Hormiga, 2008; the addition of the family Synaphridae to the data of Griswold et al., 1998 changed the sister group of Cyatholipidae from Synotaxidae to Synaphridae). Another potential pitfall stems from unrecognized paralogy (or lack of concerted evolution) of nuclear ribosomal genes widely used in spider phylogenetic studies. Nuclear rRNAs of some orbicularian spiders have attracted attention because of their high variability not only in total length, but also at the nucleotide composition level (e.g. Spagna and Gillespie, 2006). Recently, a study specifically designed to test for paralogues of the 28S rRNA gene in jumping spiders found multiple copies of this gene in a single specimen (Vink et al., 2011).

Furthermore, reconstructing the evolutionary chronicle of orb-weavers is a particularly onerous task because araneoid family-level phylogeny is likely the result of an ancient radiation compressed in a relatively narrow timespan (Dimitrov et al., 2012), as has also been shown when reconstructing rapid radiations of other major arthropod lineages, such as in the lepidopteran phylogeny problem (e.g. Bazinet et al., 2013).

Published data (e.g. Dimitrov et al., 2012; and references therein) suggest a Late Triassic origin of orb-weavers and a late Jurassic–Early Cretaceous origin for most araneoid families (but see Bond et al., 2014, for a proposed early Jurassic origin for the orb-web).

The diversity of orbicularian species and lifestyles, including web architecture, remains poorly understood, in part because of the lack of a robust phylogenetic framework. Standing questions include whether orb-webs were transformed into sheets, cobwebs and other forms (see Figs 6 and 7 for examples) multiple times or if there was a single "loss" of the typical orb architecture defining a large clade of araneoids (for example, as suggested in Griswold et al., 1998). Of course, at shallow phylogenetic levels many such orb transformations are known; for example, within Anapidae there are transitions from orb- to sheet-webs. Understanding web evolution and diversification requires an empirically robust hypothesis about the underlying phylogenetic patterns.

In this study, we have expanded the taxonomic sample used in our previous work (Dimitrov et al., 2012), both within araneoids and their potential outgroup taxa. The main goal of this study is to test the limits of Araneoidea using standard polymerase chain reaction (PCR)-amplified molecular markers and including all current and former members of the superfamily, and to reconstruct the interfamilial relationships of araneoids. In addition, our analyses aim to provide a phylogenic framework with which to study web evolution and diversification in araneoids and to set up a roadmap for future studies of araneoid relationships using phylogenomic data.

Materials and methods

Taxon sampling

The current study builds on the recent analyses of Dimitrov et al. (2012), expanding greatly the taxon sampling of araneoid lineages with specific emphasis on families and putative groups within families that were poorly represented or absent in former molecular phylogenies. We have emphasized the addition of data for families that were under-represented in our previous study, as well as those whose phylogenetic placement is critical to understand web evolution (e.g. in Synotaxidae: synotaxine webs ("regular"; Fig. 6C) vs. pahorine, physoglenine webs ("irregular" sheets; Fig. 7A–F)). We also provide the first molecular data for the araneoid family Synaphridae. In addition, an extended number of Palpimanoidea and other outgroup taxa have been included in order to test the limits of Araneoidea and the controversial placement of some araneoid linages (e.g. Holarchaeidae) in Palpimanoidea. The present matrix thus brings together, for the first time, representatives of all orbicularian families. We have sequenced *de novo* 98 species and added 265 species to the analyses using data from other studies and those available in GenBank (Arnedo et al., 2007, 2009; Rix et al., 2008; Álvarez-Padilla et al., 2009; Blackledge et al., 2009; Miller et al., 2010; Dimitrov and Hormiga, 2011; Lopardo et al., 2011;

Dimitrov et al., 2012; Wood et al., 2012). The complete list of taxa, 363 terminals in total, and the GenBank accession numbers are listed in Table S1. Taxon names and nomenclatural changes are discussed in the "Systematics of Araneoidea and Nicodamoidea" section.

Molecular methods

For each specimen, up to three legs were used for total DNA extraction using the DNeasy tissue kit (Qiagen, Valencia, CA, USA); the remainder of the spider was kept as a voucher. Purified genomic DNA was used as a template in order to target the following six genes or gene fragments: two nuclear ribosomal genes, 18S rRNA (18S hereafter, ~1800 bp) and 28S rRNA (28S hereafter, fragment of ~2700 bp); two mitochondrial ribosomal genes, 12S rRNA (12S hereafter, ~400 bp) and 16S rRNA (16S hereafter, ~550 bp), the nuclear protein-encoding gene histone H3 (H3 hereafter, 327 bp) and the mitochondrial protein-encoding gene cytochrome *c* oxidase subunit I, (COI hereafter, 771 bp). We did not generate additional wingless sequences as part of the current study. All wingless sequences used in the analyses come from previous studies and were already available in GenBank. The PCRs were carried out using IllustraTM puReTaq Ready-To-Go PCR beads (GE Healthcare, UK, www.gelifesciences.com/), as described in the Supporting Information.

PCR-amplified products were sent to the High Throughput Sequencing (htSEQ) Genomics Center facility at the University of Washington (Seattle, WA, USA), for enzymatic cleanup and double-stranded sequencing. The resulting chromatograms were read and edited and overlapping sequence fragments assembled, visually inspected and edited using Sequencher v.4.7 (Gene Codes Corporation, Ann Harbor, MI, USA) and Geneious v.6.0.5 (Biomatters; available at http://www.geneious.com/). In order to detect contamination, individual fragments were submitted to BLAST (Basic Local Alignment Search Tool), as implemented on the NCBI website (http://blast.ncbi.nlm.nih.gov). A consensus was compiled from all sequenced DNA fragments for each gene and taxon and deposited in GenBank (Table S1). The biological sequence alignment editor Bioedit v.7.1.11 (Hall, 1999; available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used to edit the complete sequences.

Phylogenetic analyses

All molecular phylogenetic analyses were run on the Abel Cluster at the University of Oslo, the CIPRES science gateway (Miller et al., 2011) and at a Linux server at the Natural History Museum, Oslo. Parsimony analyses were run on a fast desktop computer at the Natural History Museum of Denmark, University of Copenhagen.

Alignments. Multiple sequence alignments were carried out with MAFFT v.7.058b (Katoh and Standley, 2013) run on the Ubuntu server at the Natural History Museum, University of Oslo. Alignments of protein-encoding genes were trivial due to the lack of gaps (except few insertions/deletions in *wingless*) and were produced using the L-INS-i method. Ribosomal genes, however, contain variable regions. In addition, the distribution of insertions and deletions is nonrandom in stem regions due to structural constraints such as compensatory mutations, and, consequently, taking rRNA secondary structure into consideration is also important (Rix et al., 2008; Murienne et al., 2010). To that end, we have used the Q-INS-i method, which implements the four-way consistency objective function (Katoh and Toh, 2008). Because the Q-INS-i method is computationally very demanding, long fragments such as 18S and 28S were aligned in shorter blocks (based on amplicon limits), which were assembled after alignment.

In a few cases, sequences were found to be a contamination or potential paralogues and were excluded from the final analyses (see supporting information). However, to exemplify the effect of indiscriminately including all data, we ran a round of maximum-likelihood (ML) analyses keeping these sequences. These results are not discussed further here but are shown in Fig. S1. Additional data sets were created using different approaches to improve data completeness or decrease potential ambiguities. To increase data completeness, we excluded taxa that were not sequenced for most of the genes in a stepwise fashion, retaining taxa with data for at least three genes and taxa with data for at least four genes. In order to reduce ambiguously aligned regions in the data set, we processed the ribosomal genes with the program trimal v.1.3 (Capella-Gutiérrez et al., 2009) using the heuristic *automated1* method and the *gappyout* method for the 28S1 fragment for which *automated1* failed to provide plausible solution. The list of all matrices and the treatments that were applied to generate them are summarized in Table S2.

Maximum-likelihood. The ML analyses were carried out with the program RAxML (Stamatakis, 2014) on CIPRES or on Abel. The concatenated gene matrix was partitioned by gene and the protein-encoding genes were further partitioned into 1st + 2nd position and 3rd position partitions. Bootstrap and optimal trees were computed in the same run using the *–fa* option using 1000 bootstrapping replicates. Trees were

rooted using the mygalomorph spider *Euagrus chisoseus* (Dipluridae).

Nonparametric methods and mixture models. Because each position in a gene can be under different selective pressures, a site-specific approach to the estimation of substitution rates and other model parameters may be most appropriate. To investigate the effects of this approximation, we used the nonparametric models of site-specific rates of equilibrium frequency profiles as implemented in PhyloBayes v.3.3e (Lartillot et al., 2009). We used the CAT-GTR model, which is the most appropriate for DNA (*-cat -gtr -dgam* 4). Two independent runs were launched and checked for convergence, and the results are summarized in the topology presented in Fig. S2.

Parsimony methods. The parsimony analyses of the concatenated molecular matrix were carried out with the computer program TNT v.1.1 (Goloboff et al., 2008). Given the size of the matrix (363 taxa and 7 genes), a driven search combining new technology algorithms using equal weights (i.e. tree drifting, mixed sectorial searches and tree fusing) was performed (50 initial addition sequences, initial level: 10, cycles of drifting: 10) until it stabilized onto a strict consensus five times (with default factor of 75). This is one of the most efficient search strategies when dealing with large, difficult data sets (Goloboff, 1999). Most other search settings were left as default values. Commands used were included in, and run from, a script file, which was generated by modifying an automatically generated TNT batch file. The detailed sequence of commands is given in the Supporting Information.

Nodal support was estimated via 1000 replicates of parsimony jackknifing (Farris et al., 1996; Farris, 1997) under new technology (using default values).

Divergence time estimation. In order to estimate divergence times, we used a relaxed uncorrelated lognormal approximation (Drummond et al., 2006) as implemented in the program BEAST v.2.1.1 (Bouckaert et al., 2014). Analyses in BEAST were run with exponential distribution for the probability density of the *tmrca* prior and birth–death model for the tree prior. Calibration points and relevant prior parameters are listed in Table S3. Parameters were chosen in such a way that 95% of the priors' distributions fell between the minimum (the offset) and the maximum values reported for the dating uncertainty of the corresponding fossil. Because it is unknown how far the fossil is from the most recent common ancestor of the node that it is constraining (e.g. what is its position along the stem), we used a noninformative hyper prior with gamma distribution to incorporate the uncertainty of the calibration-density (Heath, 2012). All constraints were applied as stem calibrations. In the results presented here we have not included as a constraint the fossil spider *Mongolarachne jurassica* (Selden et al., 2011, 2013; formerly classified as a *Nephila* species), from the Middle Jurassic deposits of China (Inner Mongolia, Daohugou, China), because of recent concerns about its taxonomic placement (e.g. Kuntner et al., 2013). However, the fossil described by Selden et al. (2011) does seem to have morphological characters compatible with those of other nephilids. A male specimen described two years later was assigned to the same species (Selden et al., 2013) and because the male did not fit the Nephilidae diagnosis, the female (described as *N. jurassica*) and the male were placed in a new family—Mongolarachnidae. Selden et al. (2013) did not present convincing evidence that these two specimens are conspecific (e.g. the male resembles *Ectatosticta*, a hypochilid genus endemic to China), so in our view the question of where *M. jurassica* belongs is still in need of further research. For example, recent description of *Geratonephila burmanica* from Early Cretaceous Burmese amber (97–110 Myr old; Poinar and Buckley, 2012; see also Penney, 2014) challenges the hypothesis of Kuntner et al. (2013) that the clade of *Nephila* and its close relatives is only 40–60 Myr old.

As a starting tree in all BEAST runs, we used the best tree from the ML analysis of the full data set that was processed with the program treePL (Smith and O'Meara, 2012) and the same sets of calibration constraints as for the corresponding BEAST analyses. Nodes where fossil calibrations were applied were also constrained as monophyletic (note that these were already selected in order to reflect well-supported monophyletic groups as found by the ML analyses: see arrows on Fig. 3); however, the starting tree topology was not strictly constrained in order to account for topological uncertainties. Conversion of the ML tree to ultrametric with treePL was necessary in order to provide BEAST with a starting tree that satisfies all priors and topological constraints. Clock and substitution models were unlinked between gene partitions except for the mitochondrial genes (16S and COI). Analyses were run for at least 200 million generations with second runs for at least 70 million generations to test for convergence of the results. Chain mixing, effective sample sizes of estimates and other relevant statistics were evaluated in Tracer v.1.5 (Rambaut and Drummond, 2007). Trees were summarized with the program TreeAnnotator, which is distributed as part of the BEAST package. Two different sets of dating analyses were run with calibrations applied in such a way that the nephilids are treated as a clade with araneids (Araneidae) and as an independent clade (see discussion in the "Systematics of Araneoidea and Nicodamoidea" section). In addition to the partitioned

analyses, we also ran an analysis treating the whole data set as a single partition. This was done in order to compare both approaches and because it has been shown that in some cases partitioning may cause statistical problems in dating analyses (e.g. Dos Reis et al., 2014).

Comparative analyses

We used the web architecture data matrix from Dimitrov et al. (2012) as a base for the current analyses. Additional taxa were added to this data set and despite the number of species with unknown web architecture, representatives from all orb-weaving families were scored in the data set (the web character matrix is available as supporting information). Comparative analyses were carried out using the ultrametric trees from the dating analyses and the R packages ape (Paradis, 2012) and phytools (Revell, 2012). Likelihood models for discrete characters may be based on three general assumptions about the rates of character transformation: (1) equal rates of transition between states (ER); (2) a symmetric model where forward and reverse rates of transition between two states are equal but other rates may vary (SYM), and (3) the most parameterized case of all rates being different (ARD). We fitted these three models to our data and selected the one that resulted in the highest likelihood. To do this, we used the function *ace* in ape with *type = "discrete"*. The best-performing model was then used to reconstruct web evolution using a stochastic character mapping approach (SIMMAP) as implemented in phytools (with the make.simmap function). A thousand stochastic maps were generated using 1000 values for the Q matrix obtained from the posterior distribution using the *Q = "mcmc"* command and *nsim = 1000* as a prior and results were summarized on the corresponding BEAST summary tree. The stochastic character mapping is a Bayesian approximation to ancestral state reconstruction (Bollback, 2006). We preferred SIMMAP to other likelihood approaches to ancestral state reconstruction of discrete traits because it allows changes to occur along branches and for assessing the uncertainty in character history.

In addition to web architecture, we also scored the presence or absence of a cribellum for all taxa in our matrix. The cribellum is a part of a complex spinning apparatus present in all cribellate spiders regardless of their web architecture. For example, some cribellates build orb-webs whereas others may build sheet or irregular webs. The presence of the calamistrum (a fourth metatarsus comb made out of modified macrosetae) as well as a diversity of silk "combing" behaviours, are correlated with the cribellum in the production of the cribellate silk that we observe in their webs. In earlier classification systems, the presence or absence of a cribellum had been used as an important diagnostic character separating araneomorph spiders into two large groups—cribellates and ecribellates. This early view has been replaced by the current paradigm of cribellum evolution, which treats this character system (and the associated cribellate web) as a symplesiomorphic araneomorph feature that has undergone multiple losses during the evolutionary history of this lineage (e.g. Lehtinen, 1967; Griswold et al., 1999, 2005; Spagna and Gillespie, 2008; Miller et al., 2010). The most recent study of cribellum evolution (Miller et al., 2010) used a large sample of araneomorph lineages and parsimony and Bayesian methods to infer the history of this character. Because of the complexity of the cribellate spinning apparatus Miller et al. (2010) argued that it is likely to expect that rates of transition between character states are asymmetrical for these particular characters. Although this is a plausible expectation, in their analyses they had to manually alter rates of character transformation in order to find a minimum threshold at which the cribellum is reconstructed as symplesiomorphic in araneomorphs, that is, with a single origin and the implied multiple losses. They also suggested that additional data might improve the results reconstructing the cribellum as homologous and allowing for actual estimation of the rates of cribellum gain and loss. We agree with the arguments for rates asymmetry presented in Miller et al. (2010) and here we test if the combined use of a different approach to ancestral state reconstruction with a larger data set is capable of further elucidating this problem. The methods used to study the evolution of the cribellum are the same as those described above for web architecture.

Results

The ML analyses of the full data set (Figs 2, S3) recover Araneoidea as a clade with Nicodamoidea as its sister group, both with a bootstrap support > 75% (bootstrap support values are given in Table S4 and also shown on Figs 2, S3). The monophyly of cribellate and ecribellate nicodamids receives high support and this clade is what we now rank as the superfamily Nicodamoidea.

The clade that includes both the cribellate and ecribellate orb-weavers also includes the RTA clade, Oecobiidae and Hersiliidae and is the sister group to a monophyletic Eresidae, albeit with low support. The superfamily Deinopoidea is paraphyletic with respect to a lineage that includes the RTA clade, Hersiliidae and Oecobiidae. Consequently, the Orbiculariae are not monophyletic. The cribellate orb-weaving family Uloboridae is monophyletic and well supported, and is sister group, albeit with low support, to a lineage that

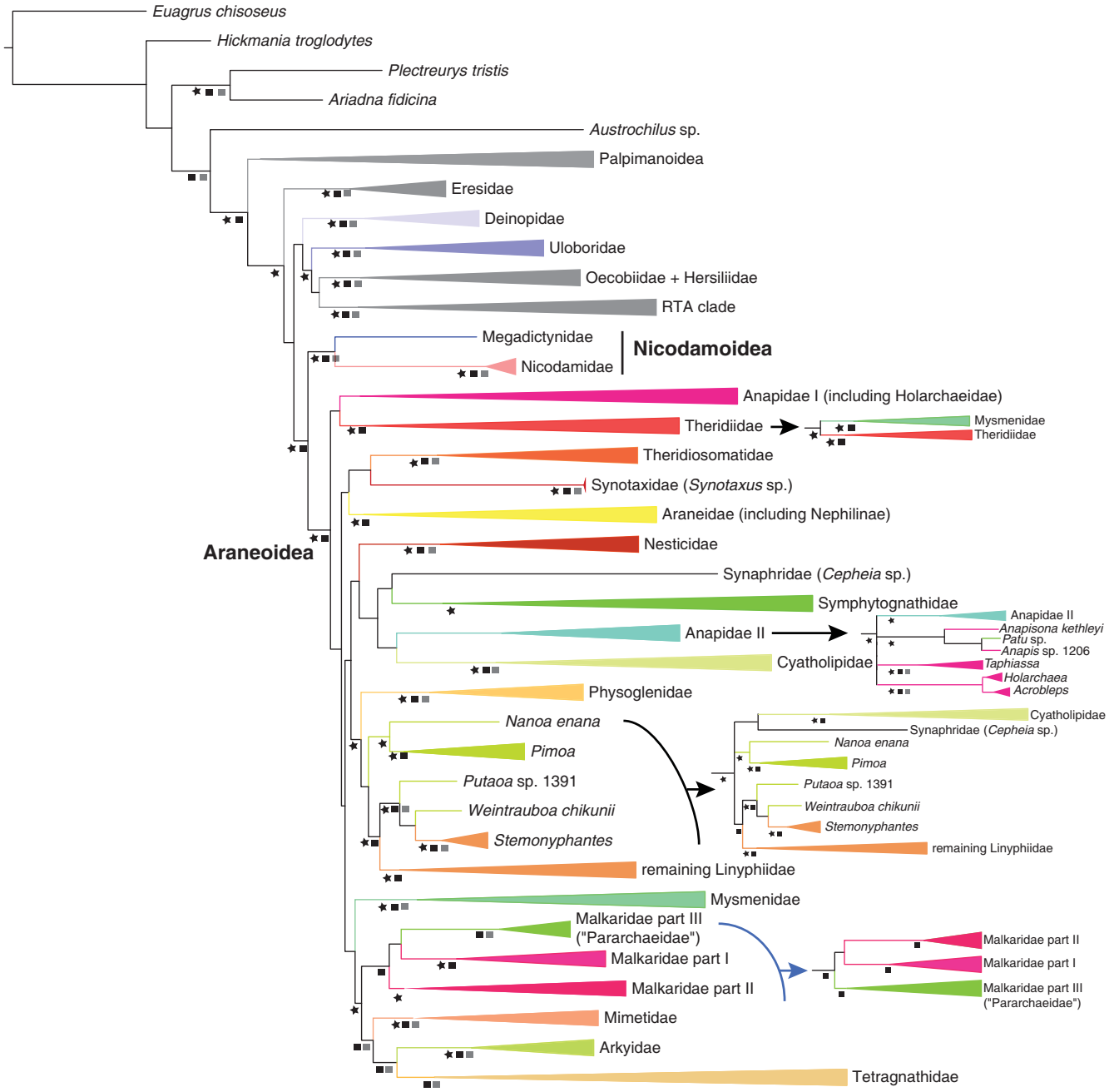

Fig. 2. Summary of topologies and clade supports from the different phylogenetic analyses described in the materials and methods section. Family crown groups are collapsed into coloured triangles. Most triangles are equally sized; their sizes are not proportional to the number of representatives included in the analyses (a total of 363 terminals were included in the analyses). The base topology is the maximum-likelihood (ML) result from the analyses of the complete data set. Black squares denote ML bootstrap values >70, grey squares indicate maximum parsimony (MP) bootstrap value > 70 and black stars show posterior probabilities from the PhyloBayes analyses which are ≥ 95%. Alternative topologies are shown on the right: black arrows correspond to PhyloBayes results and blue arrows show alternative ML resolutions. Because the MP tree showed more differences, these are not summarized here but the full MP topology is available in Fig. S7.

includes the RTA clade, Hersiliidae and Oecobiidae. The monophyly of the RTA clade is well supported, however. Although lacking nodal support, in the optimal tree Deinopidae is sister group to a lineage that includes Uloboridae, (Hersiliidae + Oecobiidae) and the RTA clade; Deinopidae is well supported.

The results show high support for the monophyly of most Araneoidea families, with a few exceptions. In general, bootstrap support values improve when partition completeness is optimized (see Table S4 and Figs S4, S5). Anapidae includes *Anapis*, the micropholcommatines and the holarchaeids; the family is never

recovered as monophyletic even if *Holarchaea* is considered an anapid, because a second "anapid" clade, comprising *Gertschanapis*, *Maxanapis* and *Chasmocephalon*, resolves elsewhere. The family Synotaxidae appears as diphyletic because the synotaxines are not closely related to the pahorine + physoglenine clade. However, the monophyly of the latter two subfamilies as a clade is well supported.

Linyphiidae plus Pimoidae form a clade, but neither family is supported as monophyletic due to the clustering of the Asian pimoid genera *Weintrauboa* and *Putaoa* with the early branching linyphiid genus *Stemonyphantes* (this clade is strongly supported). Support values for most nodes at the base of linyphioids (Linyphiidae plus Pimoidae) are low, as well as that of the node that indicates that the sister group of 'linyphioids' is the Physogleninae plus Pahorinae synotaxid clade (which we group now under the family name Physoglenidae).

Nodal support for interfamilial relationships is generally low across Araneoidea, except in a few instances: the clade of Mimetidae plus Arkyidae + Tetragnathidae and the clade of Malkaridae plus Pararchaeidae. The arkyines (which we rank at the family level in our revised classification), represented here by nine terminals, are monophyletic and well supported but do not fall within Araneidae (where they are currently classified); instead the arkyine clade is sister group to Tetragnathidae and this lineage is sister to Mimetidae. Nephilidae plus Araneidae form a well-supported clade, and although both groups appear reciprocally monophyletic in some analyses, nodal support for Araneidae is low whereas it is high for the clade of *Nephila* and its closest relatives. The symphytognathoid families constitute a polyphyletic group, although all the nodes involving these interfamilial relationships receive low support values. *Cepheia longiseta*, the single representative of Synaphridae in our analyses, is sister group to the Symphytognathidae lineage.

The ML analyses of the data sets where ambiguously aligned blocks of data were excluded (*matrix_trimal*) and those based on data sets where taxa with low gene representation were excluded (*matrix_3g* and *matrix_4g*) recovered results that were highly congruent with those from the full data set. Different resolutions involved only groupings that received lower support and did not involve any of the clades discussed above. Results from these analyses are summarized in Fig. 2 and full topologies are presented in Figs S4–S6. Given this high congruence of the results from different data treatments, we used only the full data set (as it contains the highest amount of data and retains all taxa) for the Bayesian and parsimony analyses.

Results from PhyloBayes (Fig. S2) are highly congruent with those from ML except for a handful of instances that are highlighted on Fig. 2. From those, the most significant are the recovery of a monophyletic Anapidae that includes Holarchaeidae and the move of Cyatholipidae to a clade together with Pimoidae, Linyphiidae and Synaphridae. Parsimony analyses in TNT found 211 shortest trees and after collapsing and filtering out zero length branches, a single tree was retained (shown in Fig. S7). TNT results are mostly congruent with ML and Bayesian results, but the support for some groups is lower, showing once more that the amount of information available to resolve these families is limited, particularly at the interfamilial and deeper levels. Only some of the interfamilial groupings, such as the clade [Mimetidae + (Arkyidae + Tetragnathidae)], were recovered with high support.

Molecular dating results

The annotated highest clade credibility tree from the BEAST analyses with dating scheme applying the oldest fossil described as araneid to Araneidae s.l. is presented in Fig. 3. Additional trees from the different BEAST runs are available as supporting information (Figs S8 and S9). The results showed convergence for most of the parameters but in some cases effective sampling sizes (ESS) of relevant estimates were not optimal (higher than 150 but less than 200). Independent runs of dating analyses showed a tendency to converge but, because of the size of the current data set and the time required to run a large number of generations, only one instance of each analysis was allowed to sample more than 200 million states from the posterior distribution. Close examinations of the results and lack of improvement when extending the sampling suggest that many of these problems are likely due to topological uncertainties in combination with missing data. The best example for this is the case of *Pimoa* and the clade *Pimoa* + *Nanoa* in which the estimate for the age of its stem varies significantly between the two most common topologies presented in the posterior sample: either as sister group to the other pimoids + linyphiids or as closely related to physoglenids. As expected, different dating strategies and use of partitioned versus unpartitioned analyses resulted in slightly different age estimates.

Despite these differences in the inferred median ages, 95% intervals of probability densities from all analyses are congruent and show overlap. It is worthwhile specifically mentioning the case of nephilids, because they have been the subject of a detailed study recently (Kuntner et al., 2013). In our analyses we did not implement a constraint for this group due to the unclear status of some of the available fossils. The age of *Nephila* in all of our analyses was found to be younger than that suggested by *Mongolarachne jurassica*, and the estimated age of the genus and the whole subfamily was closer to the estimates of Kuntner et al. (2013). The median ages from our unpartitioned

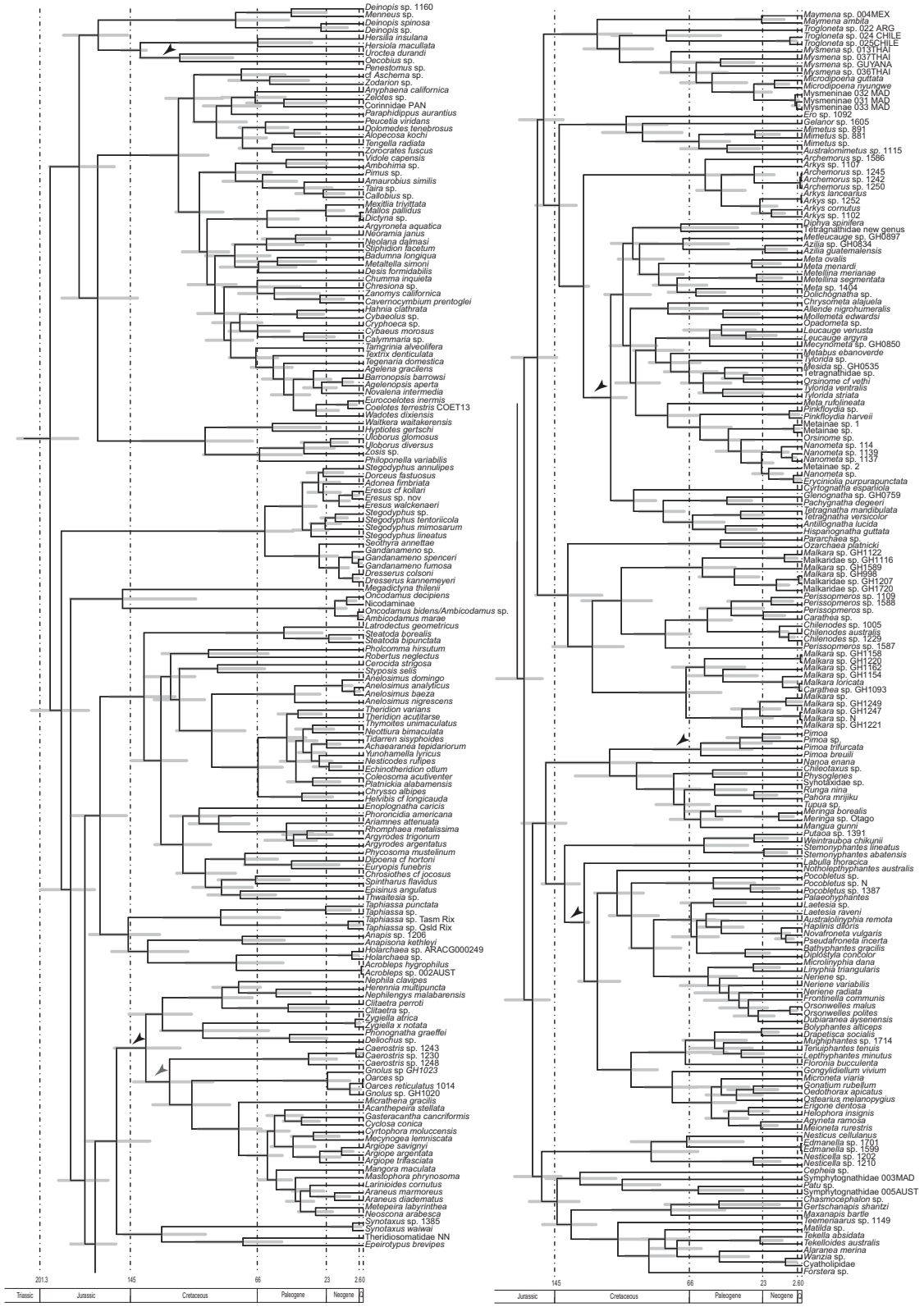

Fig. 3. Results from molecular dating in BEAST using the Araneidae constraint to the redefined Araneidae (including Nephilinae). Grey bars at nodes represent the 95% credibility interval for node age estimates. Some outgroup clades that are not discussed in the text are not shown due to space constraints. Black arrows show the branches to which dating constraints were applied (grey arrow shows the branch of the alternative application of the Araneidae constraint; see also Fig. S8).

analyses are particularly close to the findings of Kuntner et al. (2013). Clearly, all "nephilid" fossils deserve further study. Additional results based on the tree from the alternative dating scheme for Araneidae are presented in Fig. S10.

Web architecture and cribellum evolution

The Araneidae calibration was applied both including the nephilids and excluding them, because these two alternatives result in some slight topological differences and minor discrepancies of the branch length estimates of the ultrametric trees. For this reason, we ran comparative analyses on both dated trees. Fitting the three general models for rates of character transformation applicable to discrete characters (ER, SYM and ARD) on the web architecture data set always resulted in ER giving the highest log-likelihood. Because, conceptually, ER is also the simplest model, we selected these results and ran SIMMAP using the ER model. SIMMAP results from both topologies were highly congruent and here we present only the result from running the analyses with the tree that was dated with an araneid circumscription that includes the nephilids (Fig. 4).

The comparison between ER, SYM and ARD models for the cribellate data resulted in the ARD reconstruction having a slightly better likelihood (although not statistically significant under the likelihood ratio test—χ^2 *P*-value of 0.7148122). Because Miller et al. (2010) have discussed at length the arguments for adopting an approach where the rate of cribellum state transformations are asymmetrical, we follow this approach in our SIMMAP analyses and do not try to further optimize and achieve higher significance for the ARD results (see Miller et al., 2010 for such results and discussion). Ancestral state reconstruction of the cribellum (and hence the ecribellate web) under an ARD model corroborates the homology of this structure and the cribellate web without *ad hoc* manipulation of the *rates* or other model parameters. The results from the SIMMAP analyses using the araneid calibration (including nephilines) are summarized in Fig. 5. Additional results based on dated tree using the alternative dating scheme for Araneidae are presented in Fig. S11. It is worth mentioning here that, as in previous analyses using ER (see discussion in Miller et al., 2010), our results under ER and SYM models (which are equivalent for a two state character) also contradicted the single origin of the cribellum and the cribellate web.

Discussion

In general, the phylogenetic signal provided by the analysed sequences finds support for the monophyly of most araneoid families, as well as for relationships within families. Most interfamilial nodes, however, involve short internal branches with low nodal support. Although some of the relationships with low support values were deemed suspicious in previous Sanger-based sequence analyses (such as the placement of the RTA clade among orbicularians), some are now being corroborated by larger transcriptomic analyses (Bond et al., 2014; Fernández et al., 2014). This phenomenon, corroboration of "unsupported" nodes through phylogenomics, should council against hastily discarding topologies simply because of poor support values.

Increased taxon sampling (relative to the taxa used in Dimitrov et al. (2012), the direct predecessor of this study) has improved the support values for the monophyly of a few araneoid families (e.g. Tetragnathidae), resolved some controversial placements (e.g. increased sample of cyatholipids from two to eight representatives has moved out this lineage from an earlier placement within a Linyphiidae + Pimoidae clade) and supported the circumscription of a few new families (e.g. Arkyidae, Physoglenidae), but for the most part has not resolved araneoid interfamilial relationships. The dating analyses done so far (e.g., Ayoub et al., 2007; Dimitrov et al., 2012; Bond et al., 2014; this paper) agree in suggesting that the cladogenetic events and the diversification of araneoid families are both ancient and compressed in a relatively narrow time interval (Fig. 2). Because most araneoid families were already present during the Cretaceous (Fig. 3), we can hypothesize that web architectures similar to those that characterize their extant species were already diverse at the time of the spectacular diversification of holometabolous insects (primarily Hymenoptera, Diptera and Lepidoptera) (e.g. Misof et al., 2014), which coincide with the angiosperm radiation. Although in the present study we are not explicitly testing hypotheses of insect–spider codiversification (e.g. Penney, 2003), we should point out that the findings reported here are concordant with our previous hypothesis (Dimitrov et al., 2012) suggesting that the diversification of araneoid webs, which includes numerous shifts in web architecture, and of web-building behaviours likely have been driven by environmental factors (such as increasing complexity of habitats), availability of prey and intraguild competition. The subject of orb-weavers' diversification requires special attention, and we will address it in a separate paper.

Our data refute the long-held paradigm of orbicularian monophyly (e.g. Coddington, 1986; Dimitrov et al., 2012) by including the RTA clade in the same lineage that groups the cribellate (Deinopoidea) and ecribellate (Araneoidea) orb-weavers. This latter result, based on DNA sequence data, is by no means new

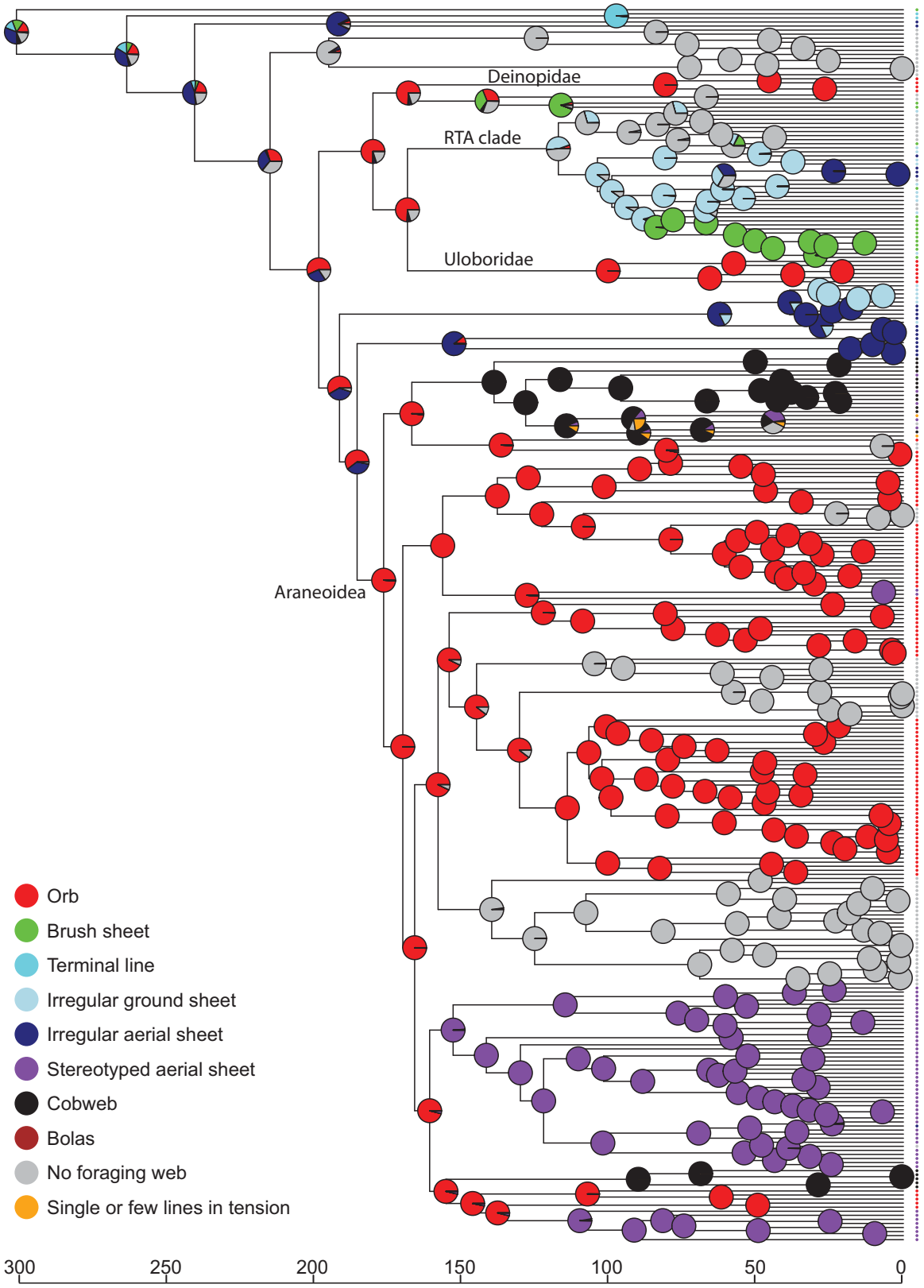

Fig. 4. Web architecture evolutionary history: summary of 1000 SIMMAP characters maps using the dated tree based on the redefined Araneidae (including Nephilinae) dating. Colours represent different web types; sectors of pies at nodes are proportional to the probabilities of each state at that node; scale is in Myr.

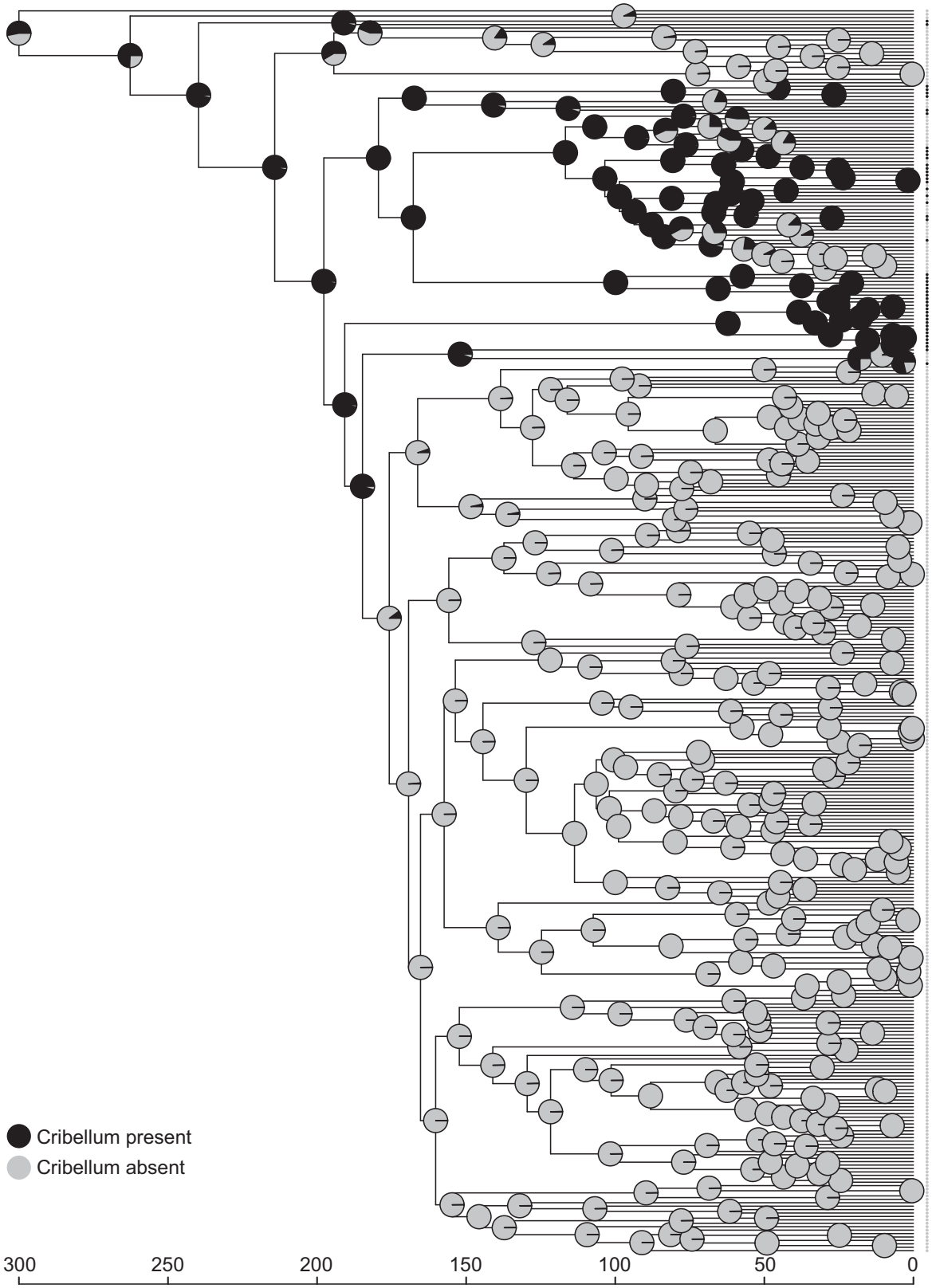

Fig. 5. Cribellum evolutionary history: summary of 1000 SIMMAP characters maps using the dated tree based on redefined Araneidae (including Nephilinae) dating. Presence or absence of cribellum is represented by different colours; sectors of pies at nodes are proportional to the probabilities of each state at that node; scale is in Myr.

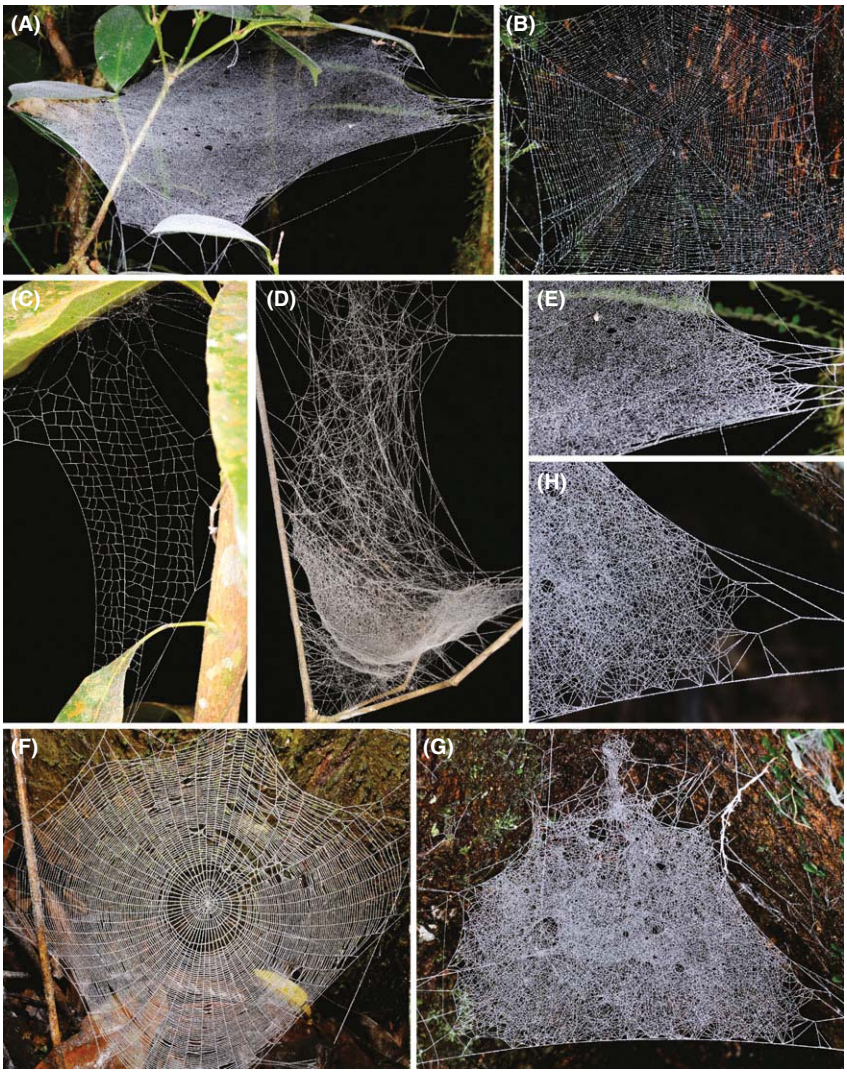

Fig. 6. (A) The horizontal sheet-web of an undescribed Cyatholipidae from Australia (DSC_3145). (B) The micro-orb of *Tasmanapis strahan* (Anapidae), from Tasmania (DSC_0497). (C) The "chicken-wire" modular web of *Synotaxus* sp. (Synotaxidae) from Brazil (DSC_9305). (D) The bowl-shaped sheet-web of an undescribed linyphiid from Taiwan (DSC_0971). (E) Detail of (A); the spider, extremely small relative to the size of the web, is the light "dot" in the upper left corner (DSC_3146). (F) The closely woven, horizontal orb-web of an undescribed Tetragnathidae from Australia (DSC_8075). (G) The horizontal sheet-web of an undescribed Linyphiidae from Australia (DSC_2794). (H) Detail of (G) (DSC_2801). Photos: G. Hormiga.

(e.g. Hayashi, 1996; Hausdorf, 1999), but has been dismissed repeatedly in favour of the orbicularian monophyly hypothesis (e.g. Blackledge et al., 2009; Agnarsson et al., 2013). Our results, based on the largest sample of orbicularians analysed to date, corroborate recent findings about the origin of Orbiculariae, which used transcriptomic data for a more modest taxon sample (Bond et al., 2014; Fernández et al., 2014). Furthermore, the results presented herein suggest that nicodamids are the closest relatives to a clade that includes all ecribellate orb-weavers, as suggested in the combined analysis of Blackledge et al. (2009) and Dimitrov et al. (2012) (see also systematic discussion below).

Web architecture and web type evolution

Despite the diversity of web architectures represented by the taxon sample analysed herein (e.g. see Figs 1A–C, 6–10), the lack of robust nodal support at the interfamilial level does not allow us to address web architecture evolution within Araneoidea satisfactorily. Additional difficulties stem from the lack of a good fossil record and uncertainties in the dating and the systematic circumscription of some of the oldest known orb-weaver fossils. There are, however, several general trends that emerge from the results presented here. The orb-web is ancient, having evolved at least by the early Jurassic. By the late Jurassic, the orb-web

Fig. 7. Webs of Physoglenidae. (A) *Physoglenes* sp., from Chile (GH001230_R03_14). (B) *Mangua* sp., from New Zealand (DSC_7925). (C) *Chileotaxus* sp., from Chile (DSC_2028). (D) Undescribed physoglenid from Australia (DSC_1392). (E) *Pahora parakaunui*, from New Zealand (CASENT9062577_CRW_0363). (F) *Runga* sp., from New Zealand (DSC_7972). Photos: G. Hormiga, except (E) (C. Griswold).

had already been transformed into significantly different architectures such as those found in linyphioids (sheet-webs) and theridiids (cob- and sheet-webs). The ancestors of the RTA clade—a lineage that includes many ground and cursorial spiders, such as wolf (Lycosidae) and jumping spiders (Salticidae)—may have built orb-webs. Throughout their diversification, orb-weavers have often abandoned foraging webs to adopt a cursorial lifestyle (e.g. Fig. 8A, B, C, F). Independent and well-supported cases of araneoids that have abandoned ancestral foraging snares in favour of active hunting for prey include the oarcine araneids (e.g. *Oarces* sp., Fig. 8B), the leaf-litter inhabiting family Malkaridae (Figs 8F, 9A–C), Mimetidae (a largely araneophagic lineage; Fig. 8C), the arkyids (which we now classify in the family Arkyidae; Fig. 8A) and the holarchaeids (which we now classify in the family Anapidae; Fig. 9E, F). There are some striking convergent morphological features associated with some of these independent instances of evolution of cursorial foraging behaviour, such as the leg spination pattern of mimetids (Fig. 8C), New Zealand malkarids (Fig. 10H) and of some of the oarcine araneids (Fig. 8B), in which the anterior leg or legs share an arrangement of macrosetae, alternating distinctively long and short spiniform setae.

Orbs are old (Late Triassic to early Jurassic; Fig. 4) and likely have a single origin (e.g. Bond et al., 2014; Fernández et al., 2014), but the RTA clade taxa have either abandoned building orb-webs or have shifted to different web architectural types, such as the sheet-webs of agelenids or the irregular ground-webs of amaurobiids. It seems now that, from a systematic point of view, the orb-web itself is not a good character (or character complex) with which to define clades. Thus, a logical consequence of these results (see also Bond et al., 2014; Fernández et al., 2014) is to abandon the concepts of Orbiculariae (Araneoidea plus Deinopoidea) and Deinopoidea (Deinopidae plus Uloboridae), because neither of them correspond to monophyletic groups; orbicularian could still be used in the vernacular sense, but not to refer to a taxon or a natural group.

Similarly to web architecture, web type (cribellate or ecribellate) has also had a very dynamic evolutionary history. However, it has been dominated by a general

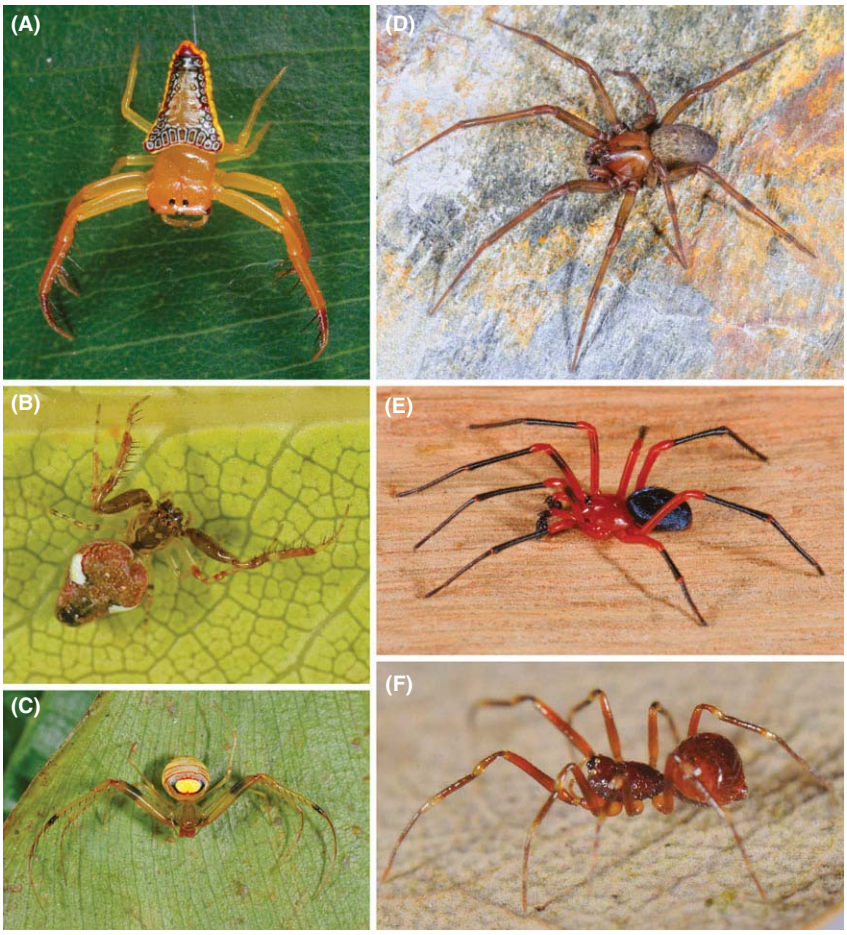

Fig. 8. (A) *Arkys* sp. (Arkyidae), a web-less araneoid from Australia (DSC_0191). (B) *Oarces* sp. (Araneidae), a web-less araneoid from Chile (DSC_2399). (C) The pirate spider *Gelanor latus* (Mimetidae), from Brazil (DSC_9119). (D) The cribellate *Megadictyna thilenii* (Megadictynidae), from New Zealand (DSC_2599). (E) An Australian member of the ecribellate family Nicodamidae (DSC_2729). (F) An undescribed, cursorial species of *Malkara* (Malkaridae, MALK_GH_017) from Australia (DSC_8196). Photos: G. Hormiga.

trend of loss of the cribellum and shift to either ecribellate webs or cursorial (non web-building) lifestyles. As in previous analyses, when a model of character transformations with equal rates is considered, the data are best explained by multiple independent origins of the cribellum and the cribellate web. This is, however, highly unlikely as already argued (e.g. Miller et al., 2010). Nevertheless, the use of models that allow for asymmetric rates of character transformations provides strong support for the single origin of the cribellum, in agreement with the current view on cribellate web evolution.

Systematics of Araneoidea and Nicodamoidea

In this section we discuss the taxonomic and systematic implications for Araneoidea based on the phylogenetic results of this study (as well as data presented elsewhere). Membership and composition of higher-level groups are discussed for extant taxa only. We have chosen the results of the ML analyses of the full data matrix to guide our taxonomic decisions (Figs 2 and S3), but the taxonomic decisions take into account the results from other methods, degrees of support and morphological characters that aid the diagnoses of groups discussed here.

Based on the phylogenetic results of this study the superfamily Araneoidea includes the following 17 families: Anapidae, Araneidae, Arkyidae, Cyatholipidae, Linyphiidae, Malkaridae, Mimetidae, Mysmenidae, Nesticidae, Physoglenidae, Pimoidae, Symphytognathidae, Synaphridae, Synotaxidae, Tetragnathidae, Theridiidae and Theridiosomatidae. Micropholcommatines constitute a lineage within Anapidae. The latter would be rendered paraphyletic if the former were treated at the family rank, as demonstrated by Lopardo et al. (2011) (see also Lopardo and Hormiga, 2015 and discussion below).

We highlight the following higher-level taxonomic changes that are discussed in more detail below:

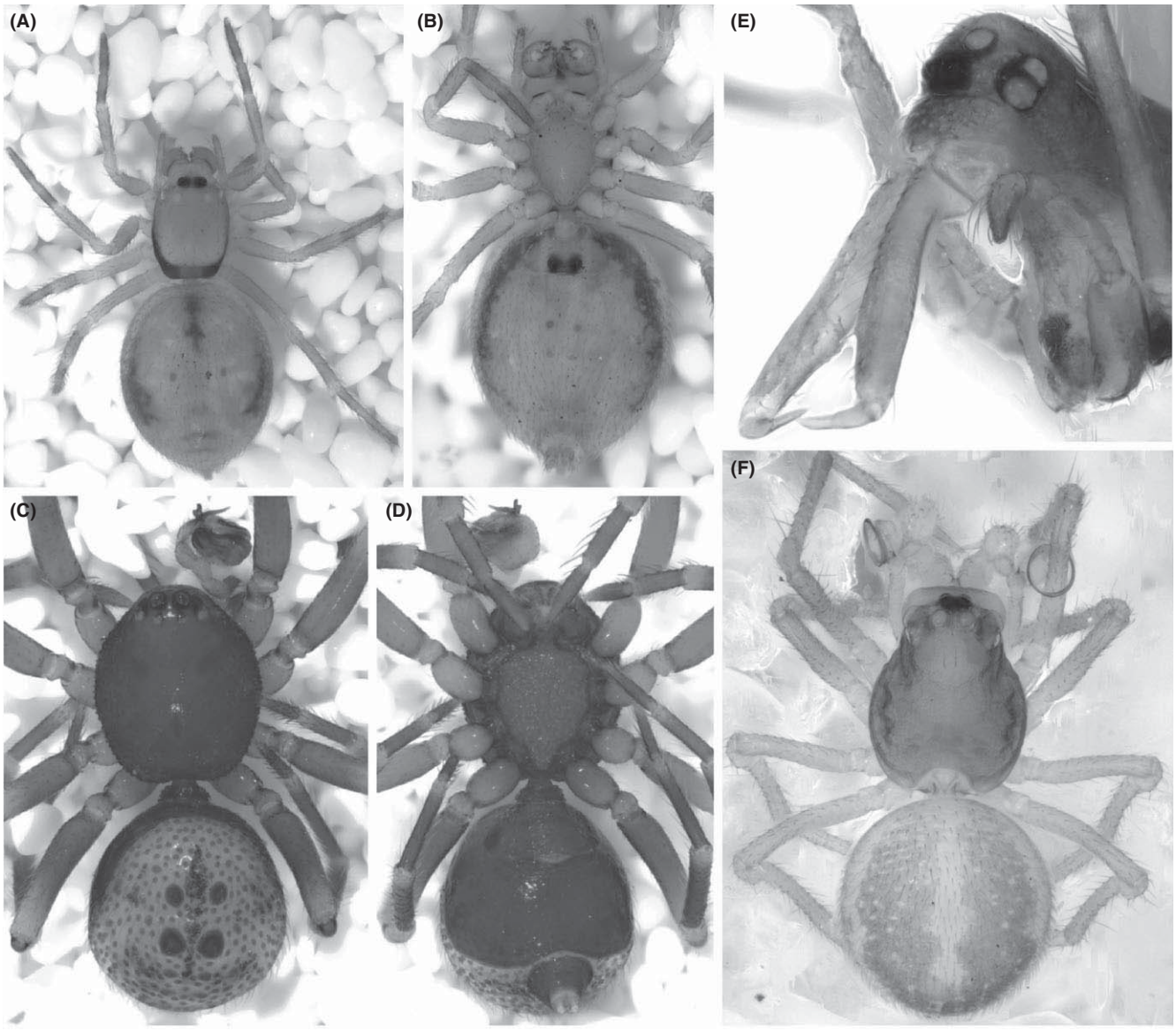

Fig. 9. (A, B) A female of the Tasmanian malkarid *Ozarchaea ornata* (Malkaridae, formerly Pararchaeidae), dorsal (A), ventral (B). (C, D) The male of an undescribed species of *Malkara* (Malkaridae, MALK_GH_013) from Australia, dorsal (C), ventral (D). (E) Lateral view of the anterior region of the prosoma of a female of *Holarchaea* (Anapidae), from New Zealand, showing its highly modified chelicerae. (F) A male of *Holarchaea* (Anapidae), from New Zealand, dorsal. Photos: G. Hormiga (E, F, Griswold lab-ATOL project).

The cribellate and ecribellate nicodamids are now ranked at the family level (Megadictynidae **rank res.** and Nicodamidae **stat. n.**, respectively) and grouped under the superfamily Nicodamoidea **rank n.** Synotaxidae are now circumscribed to include only the genus *Synotaxus*. The formerly synotaxid subfamilies Physogleninae and Pahorinae are now grouped under the family Physoglenidae **rank n.** Arkyinae, formerly in Araneidae, is now classified as the family Arkyidae **rank n**. Nephilinae **rank res.** is now classified as a subfamily under the re-circumscribed family Araneidae. The results also corroborate the placement of Oarcinae in Araneidae, rather than in Mimetidae, as formally proposed by Dimitrov et al. (2012). The morphology of *Sinopimoa bicolor*, the only member of the family Sinopimoidae (Li and Wunderlich, 2008), as described so far, is congruent with that of Linyphiidae (Hormiga, 2008) and thus we consider Sinopimoidae a junior synonym of the family Linyphiidae (**syn. n.**). Holarchaeidae is a junior synonym of the family Anapidae (**syn. n.**) and Pararchaeidae a junior synonym of the family Malkaridae (**syn. n.**).

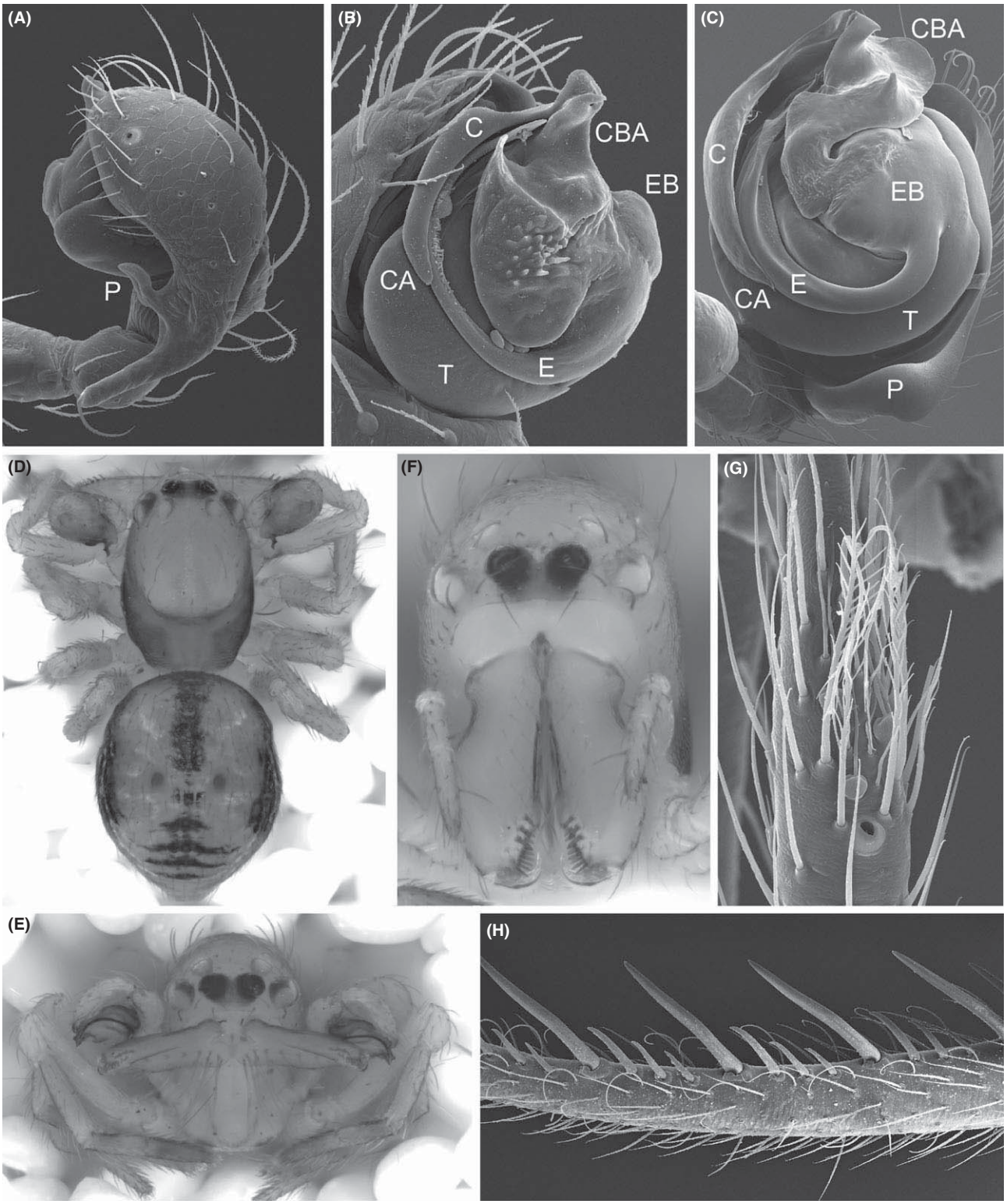

Fig. 10. (A, B) SEM of the male pedipalp (right, reversed) of *Pararchaea* sp. (Malkaridae) from Australia, ectal (A), ventral (B). (C) SEM of the male pedipalp (left) of an undescribed Malkaridae (MALK_GH_009) from New Zealand, ventral. (D, E) Male of *Pararchaea* sp. (Malkaridae) from Australia, dorsal (D), anterior, with open chelicerae (E). (F) Female of *Pararchaea* sp. (Malkaridae) from Australia, anterior. The cheliceral peg teeth can be seen next to the fangs. (G) SEM of the male tarsal organ of *Holarchaea* (Anapidae), from New Zealand. (H) SEM of the femur I spination pattern of an undescribed Malkaridae (MALK_GH_009) from New Zealand. C, Conductor; CA, Conductor Apex; CBA, Conductor Basal Apophysis; E, Embolus; EB, Embolus Base; T, Tegulum; P, Paracymbium. Photos: G. Hormiga (A, B, G, Griswold lab-ATOL project).

Taxonomy

Araneae Clerck, 1757

Superfamily Nicodamoidea Simon, 1897 rank n.

Diagnosis (after Harvey (1995) and Griswold et al. (2005)): male palpal tibia with large dorsal apophysis; tarsi without trichobothria. Cribellate nicodamoids differ from Phyxelididae in lacking a clasping spine on male metatarsus I and lacking thorn-like setae on the anterior of the palpal femora. They differ from Titanoecidae in having a simple dorsal tibial apophysis on the male palp and having paracribellar spigots on the PMS.

Putative synapomorphies: dorsal tibial apophysis in the male palp (Harvey, 1995; Griswold et al., 2005; Ramírez, 2014), the complex conformation of this process (Ramírez, 2014, p. 241), branched median tracheae (Griswold et al., 2005) and a single cheliceral tooth (Harvey, 1995) have been suggested as providing morphological evidence of Nicodamoidea monophyly.

Composition: Two families: Nicodamidae Simon, 1897 **stat. n.** and Megadictynidae Lehtinen, 1967 **rank res**.

Family Nicodamidae Simon, 1897 stat. n.

Nicodamidae Simon, 1897: 15.—Forster, 1970: 177; Davies, 1985: 92.

Nicodaminae Simon.—Simon, 1898: 221-3; Bonnet, 1958: 3101.

Type species: *Theridion peregrinum* Walckenaer, 1841: 297; = *Nicodamus peregrinus* (Walckenaer, 1841).

Diagnosis (based in part on Harvey (1995)): Ecribellate, entelegyne spiders with a large dorsal apophysis on the male palpal tibia (Griswold et al., 2005, fig. 172A–D) and a row of three to four stiff, dark setae in an otherwise large bare area on the dorsal surface of the ALS (Griswold et al., 2005, fig. 41A, C) (Fig. 8E).

Putative synapomorphies: Harvey (1995) suggests the following synapomorphies for this taxon: loss of the cribellum; a row of three to four stiff, dark setae in an otherwise large bare area on the dorsal surface of the ALS; bright red carapace, legs and sternum; fertilization duct openings facing mesally.

Composition: Seven genera with 27 species, found in Australia and New Guinea. Included are: *Ambicodamus* Harvey, 1995; *Dimidamus* Harvey, 1995; *Durodamus* Harvey, 1995; *Litodamus* Harvey, 1995; *Nicodamus* Simon, 1887; *Novodamus* Harvey, 1995; and *Oncodamus* Harvey, 1995.

Family Megadictynidae Lehtinen, 1967 rank res.

Megadictynidae Lehtinen, 1967: 247, 296. Synonymized with Nicodamidae by Forster, 1970: 177.

Type species: *Megadictyna thilenii* Dahl, 1906: 62.

Diagnosis: (based in part on (Harvey, 1995)): Cribellate, entelegyne spiders with a large dorsal apophysis on the male palpal tibia (Griswold et al., 2005, fig. 171A–C), entire cribellum (Griswold et al., 2005, fig. 41A, B), a posterior mAP spigot on the PLS (Griswold et al., 2005, fig. 39C) and enlarged spinning field of the PLS (Forster, 1970, fig. 523; Griswold et al., 2005, figs 39A, D, 40A, D) (Fig. 8D).

Putative synapomorphies: Harvey (1995) suggests the following synapomorphies for this taxon: the enlarged spinning field of the posterior lateral spinneret and the location of the copulatory duct openings onto the dorsal surface of the epigynum.

Composition: Two genera with two species, found in New Zealand: *Forstertyna* Harvey, 1995 and *Megadictyna* Dahl, 1906.

Comments. The superfamily Nicodamoidea, sister group to the Araneoidea, is readily diagnosed, but the same can be said for each included family. We propose that two families be recognized here, resurrecting the status of both Megadictynidae and Nicodamidae. The association of the cribellate *Megadictyna* with the ecribellate Nicodamidae was first proposed by Ray Forster, based on a suggestion by C. L. Wilton (Forster, 1970, p. 177). This taxonomic grouping was corroborated by Harvey (1995), Griswold et al. (2005), Blackledge et al. (2009), Dimitrov et al. (2012, 2013), Ramírez (2014) and by this study. Nevertheless, the conventional Nicodamidae *sensu* Forster (1970) are heterogeneous. Synapomorphic and diagnostic characters of Megadictynidae and Nicodamidae, respectively, serve grouping functions and justify the recognition of two families.

The ecribellate nicodamids had long been associated with Araneoidea, perhaps because of their somatic similarity to theridiids (e.g. Fig. 8E), and indeed, ecribellate nicodamids were attributed originally to the comb-footed spiders. The first described was *Theridion peregrinum* Walckenaer (1841) from 'Brazil'; shortly thereafter L. Koch (1865) named three others from Australia including *Theridium semijlavum* from Wollongong, New South Wales. Although Simon (1898) suggested that *Nicodamus* was not a theridiid, and placed this genus in the subfamily Nicodaminae in Agelenidae (Simon, 1897), *Nicodamus* continued to be catalogued under Theridiidae (Roewer, 1942; Bonnet, 1958). Herbert and Lorna Levi, world experts on Theridiidae, rejected theridiid placement for *Nicodamus* and, after discussing the issue with Forster (Forster, 1970, p. 177), moved *Nicodamus* to Zodariidae (Levi and Levi, 1962), thereby ending their association with theridiids, and more broadly, Araneoidea.

The cribellate *Megadictyna* was described in Dictynidae by Dahl (1906), which placement was followed by Marples (1959). Lehtinen (1967) thought *Megadictyna* so distinct from dictynids, and from other spiders, that he created the family Megadictynidae.

Harvey (1995) revised Nicodamidae and followed Forster (1970) by including cribellate and ecribellate members, providing a diagnosis and suggesting as

synapomorphies the male palpal tibia with large dorsal apophysis, metatarsus IV without a trichobothrium and the chelicera with a single distal tooth on the promargin. Harvey (1995) placed the nicodamids in the "RTA clade" (i.e. spiders with any process on the male palpal tibia) and further could only suggest placement in the "Amaurobioidea", RTA clade spiders with simple, entire or weakly branched tracheal systems.

Suggested orb-weaver affinities for Nicodamidae began to appear a few years later: in one of the equally most parsimonious trees for Entelegynae suggested by Griswold et al. (1999, p. 60), Nicodamidae and Orbiculariae appeared as sister groups, although this result was based in part on character codings (e.g. serrate accessory setae on the tarsi) that were later discovered to be more widespread, orbicularian affinities of Nicodamidae appeared again in the cladistic analyses of Griswold et al. (2005, figs 218B, C). Morphological evidence for this arrangement remains weak: like Araneoidea, *Megadictyna* have the minor ampullate gland spigot (mAP) on the posterior median spinnerets (PMS) posterior (Griswold et al., 2005, fig. 140C), but in ecribellate nicodamids the PMS mAP is median (not anterior nor posterior) and therefore not informative. Placement of nicodamids outside the RTA-clade saves some evolutionary steps: the cribellum of *Megadictyna* is entire, like uloborids and deinopids, and different to most RTA-clade spiders, and the palpal tibial apophysis is dorsal, not retrolateral. Nevertheless, the morphological evidence for placing nicodamids near or far from orb-weavers is not robust. It is molecular evidence, albeit from the same genes but with a diverse array of taxon samples, that strongly associates Nicodamoidea with Araneoidea (Blackledge et al., 2009; Miller et al., 2010; Spagna et al., 2010; Dimitrov et al., 2012, 2013; Agnarsson et al., 2013), although Nicodamoidea was contradicted by Agnarsson et al. (2012). That result is corroborated by our analysis, with relatively good (73) bootstrap support, and we consider this the best supported working hypothesis. This implies a notable course of web evolution from the primitive, homologous orb of deinopoids and araneoids to a substrate-limited sheet of cribellate nicodamids, unrecognizable architecturally as an orb. The evolution of the whole RTA clade from an orbicularian ancestor is thus conceivable, an idea that has been recently corroborated by phylogenomic data (Bond et al., 2014; Fernández et al., 2014).

Superfamily Araneoidea Clerck, 1757

Family Anapidae Simon, 1895

Type species: *Amazula hetschkii* Keyserling, 1886

Micropholcommatidae Hickman, 1944 (implied but not formalized in Brignoli (1970) and Schütt (2003); synonymy formally proposed in Lopardo et al. (2011); see also Lopardo and Hormiga (2015)).

Type species: *Micropholcomma caeligenum* Crosby and Bishop, 1927

Holarchaeidae Forster and Platnick, 1984 **syn. n.**

Type species: *Archaea novaeseelandiae* Forster, 1949

Diagnosis: Minute Araneoidea with the labium fused to the sternum, a huge posterior PLS cylindrical gland spigot, pore-bearing prosomal depressions on the lateral margin of the carapace (except most micropholcommatines, which do not have pores), and abdomen with conspicuous sigilla and provided with scattered, sclerotized spots.

Putative synapomorphies: Anapid synapomorphies comprise at least the labium fused to the sternum, the carapace with pore-bearing prosomal depressions (lost in most micropholcommatines), and fatiscent leg cuticle. Additional morphological synapomorphies are discussed and illustrated in Lopardo et al. (2011) and Lopardo and Hormiga (2015).

Composition: Fifty-eight genera and 238 species, worldwide. Of these, 19 genera and 66 species are placed in Micropholcommatinae, and found in South Africa, South America, Australia and New Zealand, and one genus with two species in *Holarchaea*, occurring in Australia and New Zealand. Many more species remain to be discovered, especially in the tropics.

Comments. The family-level taxa treated here as synonyms have had a convoluted and troubled history. Rix and Harvey (2010a, p. 13) pointed out that "Anapidae are … at the center of all problems 'symphytognathidan' in nature." Micropholcommatidae were long associated with Araneoidea but in 1984, along with Mimetidae and the newly created family Holarchaeidae, they were placed far away in the Palpimanoidea (Forster and Platnick, 1984). The study of Griswold et al. (1998) did not address the Palpimanoidea/Araneoidea problem explicitly and treated Araneoidea circumscription as firmly established (the symphytognathoid families were included but not the Mimetidae). Schütt (2000, 2003) placed Micropholcommatidae and Mimetidae back among the araneoids, and suggested that Micropholcommatidae should be synonymized under Anapidae. In spite of her clear argumentation, her results were not widely accepted. More recently several studies, some of which included molecular data (Lopardo and Hormiga, 2008, 2015; Rix et al., 2008; Rix and Harvey, 2010a; Lopardo et al., 2011) have firmly placed micropholcommatines within Araneoidea, and Wood et al. (2012, 2013) definitively distinguished Palpimanoidea and Araneoidea.

The status of Micropholcommatidae remained unsettled, with Lopardo and Hormiga (2008) agreeing with Schütt (2000) in synonymizing them with Anapidae, Rix and Harvey (2010a,b) rejecting this synonymy, Lopardo et al. (2011) reasserting the synonymy on the basis of a new suit of synapomorphies, and Lopardo and Hormiga (2015) corroborating

this. The placement of Micropholcommatidae as a subgroup of Anapidae can now be considered to be strongly corroborated.

The family Holarchaeidae (Fig. 9E, F) is another story. Despite a striking superficial resemblance to the palpimanoid "pelican spiders" (Archaeidae), placing Holarchaeidae in the Palpimanoidea presents a number of problems—such as their entelegyne female genitalia, the absence of cheliceral peg teeth and the lack of leg I scopulae. Our molecular analysis groups *Holarchaea* with the anapid *Acrobleps* with strong support in all data treatments and, in turn, these taxa group with other Anapidae, including the type genus *Anapis*, albeit with low support. What the molecular data suggest is strongly corroborated by morphology. Lopardo et al. (2011) and Lopardo and Hormiga (2015) suggest a number of morphological synapomorphies for Anapidae and *Holarchaea* shares most of these. The labium is fused to the sternum; carapace with pore-bearing prosomal depressions, including a large depression near the carapace lateral margin; sternal cuticle is punctate; leg cuticle is fatiscent; the tarsal organ opening is huge, subequal or larger than setal sockets (Fig. 10G); abdomen with conspicuous sigilla and it is also provided with scattered, sclerotized spots; anterior respiratory system comprises modified book-lungs; females have internal copulatory openings; spermatic duct simple, with no loops before entering the embolus; and thick embolus. Like Symphytognathidae, males lack epiandrous fusules and the posterior PLS cylindrical gland spigot is enlarged: whereas Lopardo and Hormiga (2015) regard these as anapid plus symphytognathid synapomorphies, on our tree they may optimize as anapid synapomorphies. Lastly, the absence of a paracymbium from the male palp has also been interpreted as an anapid plus symphytognathid synapomorphy (Lopardo et al., 2011). Nevertheless Anapidae continue to be problematic (Rix and Harvey, 2010a, p. 124) because the family optimizes as diphyletic: true Anapidae include *Anapis*, micropholcommatines and the holarchaeids, but a second "anapid" clade, comprising *Gertschanapis*, *Maxanapis* and *Chasmocephalon*, resolves elsewhere. Only in the parsimony analyses are these two anapid clades recovered as sister groups, albeit with low support (Fig. S7). Understanding anapid phylogenetic relationships is essential to study evolutionary transitions between orb-webs and other architectures. Most Anapidae build micro-orbs (e.g. Fig. 6B; see also Miller et al., 2009) but the family also includes species that build sheet-webs similar to those of Cyatholipidae (Hormiga, unpublished).

Family Synotaxidae Simon, 1894

Synotaxeae Simon, 1894: 494.

Synotaxidae Forster, Platnick and Coddington, 1990.

Type genus: *Synotaxus* Simon, 1895

Diagnosis: Diagnostic characters for Synotaxidae (circumscribed here to include only the genus *Synotaxus*) include the unique "chicken-wire" web comprising modular rectangles of sticky silk (Fig. 6C); the following character combination further distinguishes synotaxids: spiniform setae on the male palpal patella (though at least *S. ecuadorensis* is depicted as having spiniform setae on the tibia instead (Exline and Levi, 1965, figs 25–27; Griswold et al., 1998, fig. 19C); enlarged (but not flattened) aggregate gland spigots on the PLS (Griswold et al., 1998, figs 38A, D); leg femora not basally thickened; a retrolateral groove on the paracymbium and a dorsally-excavated and cup-shaped integral paracymbium (Griswold et al., 1998, fig. 19C; Agnarsson, 2004a, fig. 3).

Putative synapomorphies: The unique "chicken-wire" web comprising modular rectangles of sticky silk (Eberhard, 1977, 1995); other, homoplastic synapomorphies comprise spiniform setae on the male palpal patella (shared with some Physoglenidae, e.g. *Nomaua crinifrons*); enlarged (but not flattened) aggregate gland spigots on the PLS; a retrolateral groove on the paracymbium (shared with Physoglenidae) and a dorsally-excavated and cup-shaped integral paracymbium (shared with Cyatholipidae and Physoglenidae).

Composition: Only the genus *Synotaxus*, with 10 species; endemic to the American tropics.

Comments. Forster et al. (1990) associated *Synotaxus* with *Physoglenes*, *Pahora* and other similar genera in the new family-ranked Synotaxidae. We distinguish Synotaxidae and Physoglenidae as separate families to recognize the separate affinities on our tree and to make each family easier to diagnose. Such differences in genealogical relationships help to explain the great disparity in web architecture between synotaxids (vertical "chicken-wire" modular webs; Fig. 6C) and the physoglenids (horizontal sheet and irregular webs; Fig. 7). In addition, the different geographical distribution of these two groups better fits the current phylogenetic re-circumscription.

Family Physoglenidae Petrunkevitch, 1928 rank n.

Type Genus: *Physoglenes* Simon, 1904

Diagnosis: Physoglenids have lost the basal PLS cylindrical spigot and any cylindrical spigots from the PMS (Griswold et al., 1998, figs 40, 42, 44); like Synotaxidae they have a retrolateral cymbial incision and like Synotaxidae and Cyatholipidae they have a small, basal, dorsally-excavated paracymbium (Griswold et al., 1998, figs 18C–F). Physoglenids differ from Cyatholipidae in having the posterior tracheal spiracle narrower than the width of the spinnerets. Members of subfamilies Physogleninae and Pahorinae have modifications of the male abdomen and carapace and/or abdomen that may function in stridulation.

Putative synapomorphies: The loss of the cylindrical gland spigots from the PMS is a unique synapomorphy; homoplastic synapomorphies include the paracymbium and cymbial form, elongate but basally thickened femora, truncate posterior apex of the sternum, and complex tegular apophysis, which may be homologous either to the conductor (Griswold et al., 1998) or the theridiid tegular apophysis (Agnarsson, 2004b).

Composition: Thirteen genera and 72 species, found in Australia, New Zealand and southern South America (Argentina and Chile); additional genera and species remain to be described.

Comments. *Synotaxus* and genera here newly assigned to the Physoglenidae were associated in the Synotaxidae by Forster et al. (1990). They suggested that potential synapomorphies were the small, basal, dorsally-excavated paracymbium, a retrolateral cymbial incision, dorsal macrosetae on the male palp (though the segment varies, and some lack such setae altogether), and greatly elongated, spineless legs (Forster et al., 1990). Our analyses consistently separate *Synotaxus* from other former members of Synotaxidae, although support values for the intervening nodes are low. Nevertheless, we recognize Physoglenidae and Synotaxidae as separate families. The monophyly of Physoglenidae in our analysis (*Pahora*, *Runga*, *Meringa*, *Tupua*, *Physoglenes*, *Mangua*, *Chileotaxus* and Synotaxidae sp. (GH1194) an undescribed genus from New Zealand) receives maximum clade support. Physoglenids are sister group to the pimoid/linyphiid lineage albeit with a low support value. As discussed above, *Synotaxus* appears elsewhere in our tree, distantly related to physoglenids. Recognizing Physoglenidae and Synotaxidae as separate families is cognizant of these separate phylogenetic affinities, and makes each family easier to diagnose. A diagnostic character for the Physoglenidae is the absence of any cylindrical gland spigots from the PMS. Other potential physoglenid synapomorphies are shared with other families: only a single cylindrical gland spigot remaining on the PLS (shared with Cyatholipidae), retrolateral groove on the paracymbium (shared with Synotaxidae) and dorsally-excavated, cup-shaped, integral paracymbium (shared with Cyatholipidae and Synotaxidae). Dorsal macrosetae or cuticular spurs on the male palp are not universal, and may characterize genera or subgroups of Physoglenidae. Most physoglenid genera have some form of carapace/abdomen stridulating mechanism, although nothing of the sort is found in *Chileotaxus*, which nevertheless agrees with the other Physoglenidae in the PMS and PLS spinneret synapomorphies. In addition to explaining the differences in web architecture between synotaxids (Fig. 6C) and physoglenids (Fig. 7A–F), our phylogenetic hypothesis also helps to explain the similarities in the sheet-webs of some physoglenids and some linyphiids. For example, the sheet-web of the Chilean *Physoglenes puyehue* (Fig. 7A) could easily be taken as a linyphiid web (Fig. 6G).

Subfamily Physogleninae Petrunkevitch, 1928

Type Genus: *Physoglenes* Simon, 1904

Diagnosis: The anterior part of the abdomen of physoglenine males is sclerotized in association with an expanded, heavily sclerotized pedicel (Forster et al., 1990).

Composition: Five genera and 20 species. Included are *Physoglenes* Simon, 1904 from South America; *Meringa* Forster, 1990 and *Zeatupua* Fitzgerald and Sirvid, 2009 from New Zealand; and *Tupua* Platnick, 1990 and *Paratupua* Platnick, 1990 from Australia.

Subfamily Pahorinae Forster, 1990 (in Forster et al., 1990: 36)

Type Genus: *Pahora* Forster, 1990 (in Forster et al., 1990: 40).

Diagnosis: Forster et al. (1990) suggest that pahorines can be diagnosed by an area on the posterior margin of the carapace that engages with a stridulatory file on the antero-dorsal surface of the abdomen of males.

Composition: Four genera and 34 species, all from New Zealand. Included are *Pahora* Forster, 1990, *Pahoroides*, Forster, 1990, *Nomaua* Forster, 1990 (a senior synonym of *Wairua* Forster, 1990: see (Fitzgerald and Sirvid, 2009)) and *Runga* Forster, 1990.

Comments. There are two unplaced physoglenid genera from New Zealand (*Mangua* Forster, 1990 and a new genus, discussed below), one (*Chileotaxus* Platnick, 1990) from South America, and two (*Calcarsynotaxus* Wunderlich, 1995 and *Microsynotaxus* Wunderlich, 2008) from Australia. All of these genera lack the peculiar carapace/abdomen modifications for stridulation that are found in Pahorinae and Physogleninae. *Chileotaxus* and *Mangua* have the palpal and spinneret modifications characteristic of Physoglenidae; *Chileotaxus* is sister group to *Physoglenes* in our analysis, with high support value, and *Mangua* groups with these two genera with lower support. An undescribed New Zealand physoglenid (Synotaxidae sp. [GH1194]) has been found as either a commensal or a kleptoparasite in the webs of cyatholipids (Forster, 1988, pp. 8–9; Forster and Forster, 1999; p. 195; Paquin et al., 2010, p. 61), stiphidiids and hexathelids (CG and GH, pers. obs.). This small (2 mm) spider with a round abdomen and enlarged, divergent male chelicerae, closely resembles cyatholipids in the genus *Tekella* in whose webs they may live. In contrast to cyatholipids, the hexathelids and stiphidiids and the host sheet-webs in which these undescribed physoglenids live are both significantly larger than the commensal/kleptoparasites. In every mention they have been identified as theridiids, but their palpal form,

especially the small, cup-shaped paracymbium, places them in Physoglenidae. In our analysis these group with the Pahorinae genera *Runga* and *Pahora* with a BS = 72. The Australian genera *Calcarsynotaxus* and *Microsynotaxus* are of dubious affinities. *Calcarsynotaxus* has only one PLS cylindrical gland spigot (like Physoglenidae), a small, basal, dorsally-excavated paracymbium and a pair of strong male palpal patella spiniform setae (like Synotaxidae and many Physoglenidae); like Synotaxidae, *Calcarsynotaxus* has a cylindrical gland spigot on the PMS. *Microsynotaxus* lacks a PMS cylindrical and has only one PLS cylindrical, but the male palp is unlike any Synotaxidae or Physoglenidae. Based on our phylogenetic hypothesis, the Pahorinae are monophyletic (and include the undescribed genus from New Zealand) and the Physogleninae, as currently circumscribed, which may include *Chileotaxus* and *Mangua*, are paraphyletic. Additional taxa need to be added to the analysis (especially *Calcarsynotaxus* and *Microsynotaxus*) before taking further taxonomic actions.

Family Malkaridae Davies, 1980 stat. n.

Type genus: *Malkara* Davies, 1980

Type species: *Malkara loricata* Davies, 1980

Family Pararchaeidae Forster and Platnick, 1984 **syn. n.**

Type species: *Pararchaea alba* Forster, 1955

Diagnosis: Small to very small cryptic entelegyne three clawed spiders. Male palp with basal paracymbium, no median apophysis and a conductor that circles the embolus in opposite direction to most araneoids (counterclockwise, left palp ventral view; Fig. 10B, C). Body armored with a ventral abdominal scutum around the pedicel in males (sometimes also in females) and sclerotized ring around spinnerets in both sexes. Abdomen with sclerotized sigilla (Figs 9A, C, 10D). Like mimetids, both sexes lack aggregate and flagelliform gland spigots on posterior lateral spinnerets. Some malkarids, particularly some of the New Zealand species, have leg I and II spination very similar to that of mimetids (alternating long and short spines; Fig. 10H), but malkarids can be distinguished from the latter by the unique orientation of the palpal conductor (Fig. 10C).

Putative synapomorphies: Abdomen with ventral abdominal scutum that surrounds pedicel (at least in males), sclerotized ring around spinnerets (Fig. 9D), abdominal setae arise from sclerotized discs (Fig. 9A, C), abdomen with sigilla (Fig. 9A, C), sternum fused around petiole to carapace, conductor encircling the embolus in a counterclockwise direction and with a conspicuous basal apophysis (Fig. 10C), PLS araneoid triad absent (Rix and Harvey, 2010b, figs 16–17).

Composition: Eleven genera and 46 described species. Included are the genera *Anarchaea*, *Carathea*, *Chilenodes*, *Flavarchaea*, *Forstrarchaea*, *Malkara*, *Nanarchaea*, *Ozarchaea*, *Pararchaea*, *Perissopmeros* and *Westrarchaea*. Numerous new malkarid species remain to be described from New Zealand (at least 12 new species) and Australia (Hormiga and Scharff, unpublished).

Comments. The spider family Pararchaeidae was erected by Forster and Platnick (1984) to accommodate five Australian and two New Zealand *Pararchaea* species described by Forster (1955). Forster and Platnick placed this family within the superfamily Palpimanoidea together with two other new families established in the same paper, Mecysmaucheniidae and Holarchaeidae. Schütt (2000) tested the limits of Palpimanoidea and Araneoidea in a phylogenetic study and concluded that Pararchaeidae, Holarchaeidae and Mimetidae belonged in the superfamily Araneoidea. This placement was confirmed by a molecular study (Rix et al., 2008), and the placement of Holarchaeidae and Pararchaeidae within Araneoidea was further corroborated by Wood et al. (2012) based on both molecular and morphological data. In our study we find strong support for a placement of Pararchaeidae within the current family Malkaridae, thereby rendering this latter family paraphyletic, and we therefore synonymize Pararchaeidae with Malkaridae. Some of our analyses support a sister-group relationship between Pararchaeidae and Malkaridae. If both current families (Malkaridae and Pararchaeidae) turn out to be reciprocally monophyletic they could be ranked as subfamilies while retaining the family diagnosis that we have provided here for the recircumscribed Malkaridae.

Our results support four clades within the re-circumscribed Malkaridae (but see the parsimony results): a lineage with the representatives of *Perissopmeros*, *Carathea* and *Chilenodes* (i.e. subfamily Sternoidinae Harvey, 2002); a lineage with the New Zealand taxa (all of which are currently undescribed and including at least 12 new species); a lineage with *Malkara* (currently monotypic, but there are no less than 30 undescribed species in Australia); and a lineage with the former pararchaeid representatives (see Rix, 2006). It is worth mentioning that in the results from the parsimony analyses, pararchaeids did not cluster with malkarids but with a clade containing mostly cyatholipids; however, this grouping and all intermediate branches between malkarids and that clade did not receive significant support.

The new, expanded Malkaridae consist of species found mainly in Australia and New Zealand. Only two of the 46 known species have been found outside this region. That is *Flavarchaea humboldti* Rix and Harvey, 2010a,b from New Caledonia and *Chilenodes australis* Platnick and Forster, 1987 from Argentina and Chile.

Family Arkyidae rank n.

Arkyinae L. Koch, 1872

Type genus: *Arkys* Walckenaer, 1837

Type species: *Arkys lancearius* Walckenaer, 1837

Diagnosis (mainly from Framenau et al., 2010): the prolateral field of short dense setae on tarsus I of males (Heimer et al., 1982) and the enlarged aggregate gland spigots on the PLS of both sexes are unique to Arkyidae. Arkyidae can be further diagnosed by the following combination of characters: both sexes with a procurved posterior eye row and with posterior median eyes more widely spaced than the anterior median eyes; absence of radix and abdomen distinctively triangular in males (Fig. 8A); and a pattern of abdominal sigilla in two rows in females. Arkyids are distinguished from most araneids and tetragnathids by the absence of foraging webs.

Putative synapomorphies: prolateral field of short dense setae on tarsus I of males (Framenau et al., 2010, fig. 2), enlarged aggregate gland spigots on the PLS (Framenau et al., 2010, figs 4A, C–D, 22D and 23D) in both sexes and absence of a flagelliform gland spigot (Framenau et al., 2010, figs 22D and 23D).

Composition: Two genera (*Arkys* Walckenaer, 1837 and *Demadiana* Strand, 1929) and 37 species (World Spider Catalog, 2016) v.17.0.

Comments. Eight species of *Arkys* have been included in this study, a broad and representative sample of the morphological variation within the genus. The genus *Demadiana* could not be included, because DNA quality tissue was not available, but there are strong morphological synapomorphies that unite *Demadiana* and *Arkys* (see diagnosis) as a monophyletic group (Framenau et al., 2010). In this study, based entirely on molecular data, there is strong support for the monophyly of the genus *Arkys* (BS = 100) and strong support for the sister-group relationship to Tetragnathidae (BS = 100). Together with Mimetidae, Tetragnathidae and Arkyidae constitute a monophyletic group with high support (BS = 98).

The systematic position of *Arkys* has been controversial. Previous authors have placed the genus in such different families as Thomisidae, Araneidae, Tetragnathidae and Mimetidae (for the taxonomic history of this group, see Framenau et al., 2010). Heimer (1984) placed *Arkys* in Mimetidae based on the complicated paracymbium of the male palp, and this placement was supported by Platnick and Shadab (1993). However, Platnick and Shadab (1993) reported the presence of aggregate gland spigots on the posterior lateral spinnerets of *Arkys* and thereby contradicting the mimetid placement: aggregate gland spigots are known only from Araneoidea, and Platnick and Shadab (1993) considered mimetids to be palpimanoids, not araneoids (Forster and Platnick, 1984). Scharff and Coddington (1997) tested the monophyly and phylogenetic placement of *Arkys* within Araneoidea in a morphological matrix and found *Arkys* to be nested within Araneidae, where until now *Arkys* has been classified. The molecular analysis of Blackledge et al. (2009) found strong support for the placement of *Arkys* as sister group to Tetragnathidae, and for a sister-group relationship between a clade consisting of Tetragnathidae + *Arkys* and Mimetidae, as also found by Dimitrov et al. (2012) and the current study. The combined analyses of Dimitrov and Hormiga (2011) also refuted araneid affinities of *Arkys* but could not unambiguously resolve its placement. Some analyses suggested that *Arkys* was sister group to Tetragnathidae (all Bayesian analyses; as in Blackledge et al., 2009), whereas in other analyses *Arkys* appears to be a mimetid (dynamic and static homology parsimony analyses and the morphological partition).

Our analyses, as well as the above-cited molecular analyses, place *Arkys* as the sister group of Tetragnathidae with high support values. In a guide to the orb-weaving spiders of Australia, Davies (1988, p. 282) "tentatively placed within the metines" the genus *Arkys*, based solely in the absence of mimetid/palpimanoid characters. Davies did not offer any explicit character support for a metaine/tetragnathid grouping. This is not surprising, as no characters had ever been suggested to justify a circumscription of Tetragnathidae that would include *Arkys*. We treat Tetragnathidae and Arkyidae as separate families, thereby fulfilling the reciprocal monophyly requirement and making both families easier to diagnose morphologically.

Family Araneidae Clerck, 1757

Type: *Araneus* Clerck, 1757

Type species: *Araneus angulatus* Clerck, 1757

Subfamily Nephilinae Simon, 1894 **rank res.**

Type: *Nephila* Leach, 1815

Type species: *Aranea pilipes* Fabricius, 1793

Diagnosis: Araneidae are small to very large entelegyne three-clawed spiders that build typical vertical orb-webs above ground. Legs spiny, clypeus usually low. Male palp typically complex, with at least one tegular sclerite (usually the conductor), with an enlarged embolus base (radix), fused to the proximal part of the embolus in nephilines (Kuntner et al., 2008). Adult males often smaller than females and with pear-shaped carapace. Females with chilum and denticles on chelicerae. Fourth leg with sustentaculum (Scharff and Coddington, 1997; Griswold et al., 1998; Kuntner et al., 2008).

Putative synapomorphies: The presence of modified setae (sustentaculum) on the tip of the fourth tarsi and the presence of a radix in the embolic division of the male palp are putative synapomorphies of Araneidae. The radix is fused to the proximal part of the embolus in nephilines and in a few other araneids (e.g. *Neogea*). Coddington (1986, pp. 339–340) suggested the presence of nonbirefringent cement at all SS-line and radius junctions (SS-R cement) as another potential synapomorphy of araneids.

Composition: Araneidae, excluding Arkyinae (*Arkys* and *Demadiana*, now family Arkyidae), but including the subfamily Nephilinae (*Clitaetra*, *Nephila*, *Herennia*, *Nephilengys* and *Nephilingis*), holds 174 genera and 3160 species (World Spider Catalog v.17.0, 2016); found worldwide; many additional species and genera remain to be described.

Comments. Throughout history, the family Araneidae has been recognized as a natural group, even though the taxonomic composition has changed over time and defining morphological characters have been difficult to identify. The family is diverse morphologically, ecologically and behaviorally, and this adds to the difficulties of circumscribing the family. The last comprehensive classification is that of Simon (1892). His concept of Araneidae (Argiopidae) was more similar to the modern-day superfamily Araneoidea than to modern-day Araneidae. Subsequent attempts to circumscribe the superfamily have been done mainly through re-delimitation and redefinition, especially within the large families Araneidae and Theridiidae (Coddington and Levi, 1991). Until recently the family Araneidae included present-day Theridiosomatidae and Tetragnathidae, but these families were removed from Araneidae thereby making it more compact and diagnosable. For most of the 20th Century and before, *Nephila* and its relatives were considered as a subfamily (Nephilinae) of Araneidae (Simon, 1864, 1892; Roewer, 1942; Bonnet, 1955; Benoit, 1962; Brignoli, 1983; Heimer and Nentwig, 1983; Wunderlich, 1986, 2004; see Kuntner et al., 2008, for a historical overview), until Levi (1986) suggested that *Nephila* and *Nephilengys* belonged in Tetragnathidae, based on male palpal characters. The association of nephilids with tetragnathids was first shown by the cladistic analysis of Coddington (1990), based on morphological and behavioral data, and further corroborated later on with more morphological characters and additional taxa by Hormiga et al. (1995), Griswold et al. (1998) and Dimitrov and Hormiga (2009). Nevertheless, the sister-group relationship of nephilids and tetragnathids was refuted on the basis of redefined and new morphological, as well as behavioral characters (Kuntner et al., 2008, and simplified versions of this matrix in few other earlier publications). These new studies suggested that nephilines were not closely related to Araneidae or Tetragnathidae, but could not satisfactorily resolve the placement of nephilines among araneoids. The analysis of Kuntner et al. (2008) suggested that nephilines were the sister group of a clade that included all other araneoid taxa sampled, although this was only weakly supported. Kuntner (2006) removed nephilines from Tetragnathidae and raised the group to family rank (Nephilidae). The first molecular study including nephilines is that of Pan et al. (2004), who found in all of their analyses that *Nephila* was sister group to the araneid taxa (a clade of two species) rather than to their two *Tetragnatha* species. These authors suggested that nephilines should be moved back into the Araneidae. Their results were, however, based on a sparse taxon sample and few genes (12S rRNA and 18S rRNA and major ampullate spidroin-1, MaSp1, for a total of nine species) and thus required further testing with more taxa and genes. Studies by Blackledge et al. (2009), using six genes and 44 genera, and by Álvarez-Padilla et al. (2009), using six genes and 213 morphological and behavioral characters coded for 47 genera, confirmed the sister-group relationship between nephilids and araneids with high support values. Further analyses combining morphological and behavioral data (Dimitrov and Hormiga, 2011), molecular data only (Dimitrov et al., 2012) and phylogenomic data (Bond et al., 2014) also corroborated the araneid affinities of nephilids. A more recent analysis of nephilid relationships (Kuntner et al., 2013), based on morphological and molecular data, and analyzing the largest sample of nephilid species to date, places nephilids within Araneidae. Most of their analyses offered high support to a clade that included all the nephilid and araneid taxa (12 representatives) studied. Their results rather consistently imply that the sister group of nephilids are either "araneids *sensu stricto* in fig. 1 and "zygiellids" in fig. 2" (Kuntner et al., 2013, p. 972).

Our study, including 363 taxa and seven genes, strongly supports the monophyly of a group that includes nephilids plus araneids. Not surprisingly, the combined group is difficult to define morphologically, but because all recent phylogenetic analyses (see above), and this study, have found strong support for a monophyletic Araneidae, including nephilids, we decided to return the nephilid lineage to its classical position as a subfamily (Nephilinae) within Araneidae. We are currently only able to list a few putative morphological synapomorphies to define the re-circumscribed Araneidae, and have therefore given preference to the strong molecular support to guide our decision for this taxonomic change. Araneidae, without nephilines, are also difficult to define morphologically and such a group has low support in all analyses. This change in rank better reflects our improved understanding of the phylogenetic position and evolutionary history of nephilines while maintaining the diagnosability of Nephilinae, and avoids the paraphyly of Araneidae implied by several recent published studies (e.g. Kuntner et al., 2013; but see Gregorič et al., 2015) and by the Bayesian results of this study (Fig. S2).

Family Linyphiidae Blackwall, 1859

Type genus: *Linyphia* Latreille, 1804

Type species: *Araneus triangularis* Clerck, 1757

Sinopimoidae Li and Wunderlich, 2008 **syn. n.**

Type species: *Sinopimoa bicolor* Li and Wunderlich, 2008

Comments. Although we could not include in our analysis *Sinopimoa bicolor* Li and Wunderlich, 2008, the sole member of Sinopimoidae, we formalize here the hypothesis of Hormiga (2008) stating that *Sinopimoa* is a member of the family Linyphiidae. As detailed by Hormiga (2008, p. 4), the study of Li and Wunderlich (2008) is missing essential morphological data for a convincing phylogenetic justification of a new family. As those authors point out, two characters support membership of *Sinopimoa* in the "linyphioid" clade (Pimoidae + Linyphiidae): cheliceral stridulatory striae and patella-tibia leg autospasy. The apparent absence of conductor and median apophysis in the male palp (one of these sclerites, or both, are found in Pimoidae) supports the conjecture that *Sinopimoa bicolor* is a linyphiid. *Sinopimoa*, as described by Li and Wunderlich (2008) does not have any pimoid synapomorphies. In addition, *Sinopimoa* shares two Erigoninae synapomorphies (Hormiga, 2000; Miller and Hormiga, 2004): absence of the female palpal claw and a retrolateral tibial apophysis in the male palp, and like many erigonines, is of very small size and has only one dorsal tibial spine in legs III and IV. The most parsimonious interpretation of the available data is that *Sinopimoa* is a linyphiid and consequently we treat Sinopimoidae as a junior synonym of Linyphiidae.

Our results suggest with high support that the pimoid species *Weintrauboa* and *Putaoa* group with the linyphiid genus *Stemonyphantes*. The monophyly of Linyphiidae including the latter clade is also robustly supported in our ML and Bayesian results (but see results from parsimony analyses for alternative topology; Fig. S7). *Pimoa* plus *Nanoa* are the sister group of such a Linyphiidae circumscription. These results could support a transfer of *Weintrauboa* and *Putaoa* to Linyphiidae, as members of the subfamily Stemonyphantinae (which would need a significant revision of its morphological diagnosis), and re-circumscribe Pimoidae to include only *Pimoa* and *Nanoa*. Such a hypothesis is in conflict with the results of morphological analyses (e.g. Hormiga, 2008; Hormiga and Tu, 2008). A recent interpretation of the male palp sclerites of *Stemonyphantes* (Gavish-Regev et al., 2013) suggests that in this linyphiid genus the tegular sclerites could be homologues of the conductor and median apophysis, but supported a sister group relationship of *Weintrauboa* and *Pimoa* (and thus, Pimoidae monophyly) and of Pimoidae plus Linyphiidae. We are currently studying the phylogeny of pimoids with additional morphological and molecular data and a much larger taxon sample, including undescribed taxa (Hormiga and Dimitrov, unpublished). Preliminary analyses of the combined and molecular data robustly support pimoid monophyly including *Weintrauboa* and *Putaoa*, and linyphiid and linyphioid monophyly (Hormiga and Dimitrov, 2010). We will address this problem with a more extensive data set elsewhere.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Maximum likelihood of a version of the dataset that includes 28S and 18S sequences that might be problematic (see supporting information for discussion).

Figure S2. Results from the Bayesian analyses of the full dataset (in PhyloBayes).

Figure S3. Full topology from the maximum likelihood analyses of the full dataset (simplified version is presented in Fig. 2).

Figure S4. Full topology from the maximum likelihood analyses of the matrix_3g dataset.

Figure S5. Full topology from the maximum likelihood analyses of the matrix_4g dataset.

Figure S6. Full topology from the maximum likelihood analyses of the dataset treated with trimAL to remove ambiguously aligned regions.

Figure S7. Single best topology from the maximum parsimony analyses of the full dataset (length = 298012).

Figure S8. Results from the molecular dating analyses in BEAST using constraint of Araneidae excluding Nephilinae.

Figure S9. Results from the molecular dating analyses in BEAST using constraint of the redefined Araneidae (including Nephilinae) and an unpartitioned dataset.

Figure S10. Web architecture evolutionary history—summary of 1000 SIMMAP characters maps using the dated tree based on Araneidae excluding Nephilinae dating.

Figure S11. Cribellum evolutionary history—summary of 1000 SIMMAP characters maps using the dated tree based on Araneidae excluding Nephilinae dating.

Table S1. GenBank accession numbers for all sequences used in this study.

Table S2. List of the different data matrices with the corresponding number of taxa and characters.

Table S3. List of fossil constraints and the relevant settings for the fossils constantans implementation in the molecular dating analyses.

Table S4. Summary of the support under different analytical treatments for the main clades discusses in the present manuscript.

Data S1. traits.nex—annotated nexus with the information for the web and cribellum used in the comparative analyses.