The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling


aDivision of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th St., New York, NY 10024, USA; bSmithsonian Institution, National Museum of Natural History, 10th and Constitution, NW Washington, DC 20560-0105, USA; cNatural History Museum, University of Oslo, Oslo, Norway; dDepartment of Biological Sciences, The George Washington University, 2029 G St., NW Washington, DC 20052, USA; eUnidad Ejecutora Lillo, FIML-CONICET, Miguel Lillo 251, 4000, SM. de Tucumán, Argentina; fDepartment of Entomology, California Academy of Sciences, 35 Music Concourse Drive, Golden State Park, San Francisco, CA 94118, USA; gMuseo Argentino de Ciencias Naturales ‘Bernardino Rivadavia’—CONICET, Av. Angel Gallardo 470, C1403DJR, Buenos Aires, Argentina; hThe Field Museum of Natural History, 1400 S Lake Shore Drive, Chicago, IL 60605, USA; iLaboratório Especial de Coleções Zoológicas, Instituto Butantan, Av. Angel Gallardo 470, C1405DJR, Buenos Aires, Argentina; jDepartment of Biology, Auburn University Museum of Natural History, Auburn University, Rouse Life Sciences Building, Auburn, AL 36849, USA; kDepartment of Biology, University of California, Davis, CA 95616, USA; lDepartment of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada; mDepartment of Terrestrial Zoology, Netherlands Centre for Biodiversity Naturalis, Postbus 9517 2300 RA, Leiden, The Netherlands; nDepartment of Entomology, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Av. Arenales 1256, Apartado Postal 140434, Lima 14, Peru; oBiodiversity Section, Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, Copenhagen, Denmark; pDepartment of Zoology, University of West Hungary, H9700, Szombathely, Hungary; qCanterbury Museum, Rolleston Avenue, Christchurch, 8013, New Zealand

Accepted 24 October 2016

Abstract

We present a phylogenetic analysis of spiders using a dataset of 932 spider species, representing 115 families (only the family Synaphridae is unrepresented). 700 known genera, and additional representatives of 26 unidentified or undescribed genera. Eleven genera of the orders Amblypygi, Palpigradi, Schizomida and Uropygi are included as outgroups. The dataset includes six markers from the mitochondrial (12S, 16S, COI) and nuclear (histone H3, 18S, 28S) genomes, and was analysed by multiple methods, including constrained analyses using a highly supported backbone tree from transcriptomic data. We recover most of the higher-level structure of the spider tree with good support, including Mesothelae, Opisthothelae, Mygalomorphae and Araneomorphae. Several of our analyses recover Hypochilidae and Filistatidae as sister groups, as suggested by previous transcriptomic analyses. The Synspermiata are robustly supported, and the families Troglocloroporidae and Caponiidae are found as sister to the Dysderoidea. Our results support the Lost Tracheae clade, including Pholcidae, Tetrablemmidae, Digiuetidae,
Plectreuridae and the family Pacullidae (restored status) separate from Tetrablemmidae. The Scytodoidea include Ochyrocercatidae along with Sicularidae, Scytodidae, Drymusidae and Periegiopidae; our results are inconclusive about the separation of these last two families. We did not recover monophyletic Austrochiloidea and Leptonetidae, but our data suggest that both groups are more closely related to the Cylindrical Gland Spigot clade rather than to Synspermiata. Palpimanoidea is not recovered by our analyses, but also not strongly contradicted. We find support for Entelegnaya and Oecobioidae (Oecobiidae plus Hersiliidae), and ambiguous placement of cribellate orb-weavers, compatible with their non-monophyly. Nicodamoidae (Nicodamidae plus Megadictynidae) and Araneoidea composition and relationships are consistent with recent analyses. We did not obtain resolution for the titanoecoids (Titanoceroidea and Phylxielidae), but the Retrolateral Tibial Apophysis clade is well supported. Penestomidae, and probably Holominychidae, are part of Zodarioidea, although the latter family was set apart by recent transcriptomic analyses. Our data support a large group that we call the marronoid clade (including the families Amaurobiidae, Desidae, Dictynidae, Hahniidae, Stiphidiidae, Agelenidae and Toxopidae). The circumscription of most marronoid families is redefined here. Amaurobiidae include the Amaurobiinae and provisionally Macrobuninae. We transfer Malenellinae (Malenella, from Anyphaenidae), Chumnidae (Chumna (new syn.) and Tasmarubriinae (Tasmaborbias, Tasmabrochus and Tseatta, from Ambphonectidae) to Macrobuninae. Cybaeidae are redefined to include Calyommaria, Cynphoeca, Ethobuella and Willisius (transferred from Hahniidae), and Blabomma and Yorima (transferred from Dictynidae). Cycloctenidae are redefined to include Oreopukia (transferred from Agelenidae) and Pakeha and Paravoca (transferred from Amaurobiidae). Desidae are redefined to include five subfamilies: Amphiplectinae, with Amphiplecta, Mamoea, Manilo, Paramamoea and Rangitata (transferred from Amphinectidae); Ischaleinae, with Bakala and Manjila (transferred from Amaurobiidae) and Ischalea (transferred from Stiphidiidae); Metalittellinae, with Austrothria, Buyina, Calacadalia, Connavarria, Jalkarabrura, Keera, Mogua, Metalitella, Nanaoalla and Que-musia; Porterini (new rank), with Baiami, Cambridge, Corosoides and Nanocambridgea (transferred from Stiphidiidae); and Desinae, with Desis, and provisionally Poaka (transferred from Amaurobiidae) and Baralhina (transferred from Stiphidiidae). Argyroneta is transferred from Cybaeidae to Dictynidae. Cicurina is transferred from Dictynidae to Hahniidae. The genera Neoramia (from Agelenidae), and Aoranga, Marplesia and Neolana from Amphinectidae) are transferred to Stiphidiidae. The family Toxopidae (restored status) includes two subfamilies: Myrinoi, with Gasparia, Gohia, Huhua, Neomyro, Myro, Ommaoxa, and Otagoa (transferred from Desidae); and Toxopinae, with Midgee and Jamara, formerly Midgeeinae, new syn. (transferred from Amaurobiidae) and Hapona, Laestrygones, Lamin, Toxops and Toxopsoides (transferred from Desidae). We obtain a monophyletic Oval Calamistrum clade and Dionycha; Sparassidae, however, are not dionychans, but probably the sister group of those two clades. The composition of the Oval Calamistrum clade is confirmed (including Zoropidae, Uloboridae, Ctenidae, Oxyopidae, Senculidae, Pisauroidae, Trechaeidae, Lycosidae, Psechridae and Thomisidae), confirming previous findings on the uncertain relationships of the “ctenids” Ancylometes and Cupiennius, although a core group of Ctenidae are well supported. Our data were ambiguous as to the monophyly of Oxyopidae. In Dionycha, we found a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids. The rest of the dionychans form a first split of core Prodidomidae, excluding the Australian Molycriinae, which fall distantly from core prodidomids, among gnaphosoids.


Introduction

Spiders (Araneae) are a distinctive and megadiverse group of predators, abundant in virtually any terrestrial ecosystem. With over 46 000 described species in ca. 4000 genera (World Spider Catalog—WSC, 2016), all Araneae retain the two synapomorphies of the order: the spinnerets, appendages at the posterior end of the body, used to spin silk through minute hair-like outlets (spigots); and male copulatory organs that have to be charged with sperm prior to copulation. Spinning organs and genital systems represent two of the main sources of characters in spider systematics (e.g. Agnarsson, 2004; Griswold et al., 2005; Álvarez-Padilla and Hormiga, 2011; Ramírez, 2014; Lopardo and Hormiga, 2015, among numerous others).

The silk-producing organs are thought to have played a determinant role in the diversification of spiders, following the diversification of their flying and crawling insect prey, as well as the evolution and increasing complexity of terrestrial habitats (Penney et al., 2003; Dimitrov et al., 2012; Garrison et al., 2016). The diversity of silk structures made by spiders, e.g. lined burrows, trapdoors, aerial webs, cocoons, silken cells and draglines, have an anatomical correlate in their glands and spigots (up to seven types in a given species; e.g. Coddington, 1989; Kovoor and Peters, 1988), combined in a myriad of ways across spider diversity. The resulting wealth of information

*Corresponding author:
E-mail address: wheeler@amnh.org
from spinnerets and web-building behaviour has been at the centre of dispute in spider systematics since the arrival of quantitative phylogenetic methods, underlying the iconic debate on the monophyly of orb-weavers (see Coddington, 1986; Dimitrov et al., 2016; and references therein). While phenotypic data analyses have relied on many characters from spigots and web-building behaviour to obtain orb-weavers as monophyletic (e.g. Coddington, 1990), molecular analyses have had difficulties obtaining supported resolution for the deep, relevant branches of the phylogeny (Blackledge et al., 2009; Dimitrov et al., 2012). The controversy has only lurched to resolution recently through the production of enlarged sequence data sets (Bond et al., 2014; Fernández et al., 2014; Garrison et al., 2016) and extensive taxon sampling for target genes (Dimitrov et al., 2016). Both strategies suggest the non-monophyly of orb-weavers, multiple losses of orb-webs and continuing uncertainty about the placement of important clades despite large quantities of data.

Genital characters are used in spider systematics at every taxonomic level, from species to higher taxa. Some of the largest clades of spiders have been characterized precisely by these characters: the Haplogynae, by a simplified male copulatory bulb with fused sclerites (Platnick et al., 1991); the Entelegynae, by a more complex uni-directional flow of sperm in the female genital system; and within entelegynes, the Retrolateral Tibial Apophysis (RTA) clade, by a process on the male palpal tibia (Griswold et al., 2005). While the more distal groups within these clades are stable across multiple analyses, the basal splits have seen radical rearrangement recently. These changes include the discovery of new taxa and the collection of new data from previously known species. The discovery of Trogloraptoridae, a new spider family allied to haplogynes (Griswold et al., 2012), for example, provided a possible morphological intermediate between dysderoids and other haplogynes. Additionally, a re-examination of Archoleptoneta, thought to be a derived haplogyne, revealed a cibellum, a spinning organ that was thought to be long lost in the group (Ledford and Griswold, 2010). Finally, recent analyses of phylogenomic data have revealed that the most basal splits of entelegynes and former haplogynes require profound remodelling (Bond et al., 2014; Garrison et al., 2016; Hormiga et al., 2016). This reconfiguration has had significant impact on evolutionary hypotheses in spiders, such as the origin and transformations of webs mentioned above, the timing and mode of diversification (Garrison et al., 2016), and the paths of simplification of circulatory and respiratory systems (Huckstorf et al., 2015).

A growing number of multi-family target gene phylogenetic analyses are incrementally covering parts of the spider tree of life. These studies have focused on Mesothelae (Xu et al., 2015b), Mygalomorphae (Bond et al., 2012; and references therein), Palpimanoidea (Wood et al., 2012), many on orb-weavers (Blackledge et al., 2009; Dimitrov et al., 2016; and references therein), symphytognathoids (Rix et al., 2008; Lopardo et al., 2011; Lopardo and Hormiga, 2015), entelegynes (J. Miller et al., 2010; Spagna et al., 2010), the Oval Calamistrum (OC) clade (Polotow et al., 2015), pscheidris (Agnarsson et al., 2012; Bayer and Schönhöfer, 2013), eresids (Miller et al., 2012), plocids (Dimitrov et al., 2013) and sparassids (Moradmand et al., 2014). These analyses show significant agreement as well as important contradictions. A summary analysis compiling sequences from GenBank (Agnarsson et al., 2013) has shown that new, unexpected results emerge when diverse data are combined with broad taxon sampling. Several of these heretofore unexpected results have been corroborated by recent phylogenomic (Bond et al., 2014; Fernández et al., 2014; Garrison et al., 2016) and high taxon sampling, target gene analyses (Dimitrov et al., 2016).

Spider systematics is advancing along multiple fronts: technological advances are making possible the production of unprecedented quantities of sequence data, repositories of morphology based on digital images are documenting the anatomy of large groups, and advances in computational techniques have allowed the analysis of larger, more complex data sets. In this study, we aim to establish the higher-level features of spider history via unprecedentedly broad sampling of spider diversity for multiple target genes.

Materials and methods

Taxonomic sampling

This dataset represents a nearly comprehensive sampling of the arachnid order Araneae at the family rank. The dataset comprised 932 spider species, representing 115 families (only the family Synaphridae is unrepresented), 700 known genera and additional representatives of 26 unidentified or undescribed genera. Eleven outgroup genera of the orders Amblypygi, Palpigradi, Schizomida and Uropygi were included, thus sampling the three orders of the clade Pedipalpi, which is the well-accepted sister group of spiders (Shultz, 1990, 2007; Regier et al., 2010; Sharma et al., 2015). All trees were rooted on the more distantly related order Palpigradi. Several of the sequences generated for this study have already been incorporated into published works on more restricted taxonomic groups, including zodarioids and other entelegynes (J. Miller et al., 2010), araneoids (Lopardo et al., 2011; Lopardo and Hormiga, 2015), palpimanooids (Wood et al., 2012) and Anyphaenidae (Labarque et al., 2015). We also augmented our taxon
sampling by the incorporation of important representatives from other studies prior to 2014 (Supporting Information, Table S1).

**Character sampling**

**Molecular data.** Genes with differing degrees of variability were chosen, and included both mitochondrial and nuclear markers. DNA sequence data from the mitochondrial genome were generated from three regions and included 12S ribosomal RNA (12S), 16S ribosomal RNA (16S) and cytochrome c oxidase subunit 1 (COI) genes. Three nuclear genes were targeted for phylogenetic reconstruction and included the protein-coding histone H3 (H3), as well as the small and large subunits of ribosomal RNA genes (18S and 28S, respectively).

The number of sequence data per terminal varied from 752 bp (Calamoneta sp. MR661) to 6210 bp (Antrodiaetus unicolor), with an average of 4186 bp per taxon (Table S1). The newly generated sequences were deposited in GenBank under accession numbers KY015264–KY018569 (Table S1).

**Laboratory protocols**

**DNA extraction and PCR amplification.** Total genomic DNA was extracted from leg tissue. In cases where the specimen was small, the whole specimen was used for DNA extraction. Sources for tissue samples and locality information are provided in Table S1. DNA extraction was achieved with a DNeasy animal tissue extraction kit (Qiagen). DNA amplification was carried out in a 25-μL volume reaction, using Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare). As a rule, 2 μL of genomic DNA, along with 1 μL of both 10 μM forward and reverse primers were included in each reaction. The primers (and their sources) used in the amplification and sequencing of gene regions in this study are given in Table 1. Molecular-grade distilled water made up the remaining volume. PCRs were executed on an Eppendorf Mastercycler ep gradient thermocycler and subsequently visualized with 1.5% agarose gel electrophoresis.

The amplification profile and annealing temperatures varied for the individual genes and across taxa for the same gene. A number of different PCR profiles were used in this study; initially, all amplifications proceeded with a standard protocol. This PCR profile consisted of an initial denaturing step at 94 °C for 2 min, 30 amplification cycles [94 °C for 30 s, 50 °C or optimal annealing temperature (T_m °C) for 45 s, 72 °C for 45 s], followed by a final extension step at 72 °C for 5 min. The PCR profiles were adjusted for the different primer pair and taxon combinations, until such time that amplification and subsequent sequencing were successful. The annealing temperatures were increased or decreased by 1–2 °C to maximize

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>12S</td>
<td>12S-ai</td>
<td>AAACCTAGGATTAGATAACGATTAT</td>
<td>Köcher et al. (1989)</td>
</tr>
<tr>
<td>12S</td>
<td>12S-bi</td>
<td>AAGAGCGAGGCAGGATGTTG</td>
<td>Köcher et al. (1989)</td>
</tr>
<tr>
<td>16S</td>
<td>16S-A</td>
<td>CGCCTGTTTATCAACAAACAT</td>
<td>Palumbi et al. (1991)</td>
</tr>
<tr>
<td>16S</td>
<td>16S-B</td>
<td>CTCCCTGTTGACTCAAATCA</td>
<td>Palumbi et al. (1991)</td>
</tr>
<tr>
<td>COI</td>
<td>LCO1490</td>
<td>GGTCAACAAATCATAAGATTTGG</td>
<td>Folmer et al. (1994)</td>
</tr>
<tr>
<td>HCOout</td>
<td>GAAATATATGTGAGGTC</td>
<td>Wheeler laboratory fide Schulmeister et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>ExtA</td>
<td>GAAGTTTATATTTATATTCC</td>
<td>Wheeler laboratory fide Schulmeister et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>ExtB</td>
<td>CCTATGGAARACCATRTGAAAT</td>
<td>Wheeler laboratory fide Schulmeister et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>aF</td>
<td>ATGGCTCTGACCCAGAAAACGC</td>
<td>Colgan et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>aR</td>
<td>ATATCCTCGCATRTRGTGAC</td>
<td>Colgan et al. (1998)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-1F</td>
<td>TACCTGTTGATCTCCTGCACTAG</td>
<td>Giribet et al. (1996)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-5R</td>
<td>CTTGCCAAATGCTTTTGCC</td>
<td>Giribet et al. (1996)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-3F</td>
<td>GTCCGATCAGGAGGAAGGA</td>
<td>Giribet et al. (1996)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-bi</td>
<td>GAGTCTCGTTGGTATACGGGA</td>
<td>Whiting et al. (1997)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-a2</td>
<td>ATGGTCTGCAAGCTGAAA</td>
<td>Whiting et al. (1997)</td>
</tr>
<tr>
<td>18S</td>
<td>18S-9R</td>
<td>GATCCCTCCCGAGGTACCTAC</td>
<td>Giribet et al. (1996)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-Rd1a</td>
<td>CCCCGTGAAATTCAGCATAT</td>
<td>Crandall et al. (2000), modification of Van der Auwera et al. (1994): primer 4</td>
</tr>
<tr>
<td></td>
<td>28S-Rd4a</td>
<td>CCTTGGTTCCTGTTTCAAGAC</td>
<td>Crandall et al. (2000), modification of Van der Auwera et al. (1994): primer 10</td>
</tr>
<tr>
<td>28S</td>
<td>28S-Rd3.2a</td>
<td>AGTACCGGAAAACGCTTTACGGGT</td>
<td>Wheeler laboratory fide Whiting (2002)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-B</td>
<td>TCGGAAAGGGCAGCTTACCA</td>
<td>Whiting et al. (1997)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-A</td>
<td>GACCCGTCCTTTGAAGCAGC</td>
<td>Whiting et al. (1997), modification of Nunn et al. (1996)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-Bout</td>
<td>CCCCAGCGCCAGTCTCTTTACC</td>
<td>Wheeler laboratory fide Hovmöller et al. (2002)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-Rd4.8a</td>
<td>ACCTATCTCAACCTTAAATC</td>
<td>Wheeler laboratory fide Whiting (2002)</td>
</tr>
<tr>
<td>28S</td>
<td>28S-Rd7b1</td>
<td>GCCTTCCCTTACCTACAT</td>
<td>Wheeler laboratory fide Whiting (2002)</td>
</tr>
</tbody>
</table>
specificity. For primer pairs that amplified longer fragments, increasing the denaturation and extension time by 30–60 s often yielded better results. Moreover, increasing the number of amplification cycles to 35–40 often helped achieve the necessary results.

Sequences of 12S, ranging in length from 287 to 356 bp, were successfully obtained using the standard primer combination, 12S-ai and 12S-bi. This region, located in the 3' end of the gene, corresponds to approximately half of the entire 12S rRNA gene found in this group. Primers developed by Palumbi et al. (1991) were used to amplify approximately half of the complete 16S rRNA gene. This fragment, ranging from 421 to 552 bp, is located in the 3' region of the gene. Sequences for a large fragment of COI were determined using the primers pairs LCO1490 and HCOOutout, and ExtA and ExtB. These overlapping primers amplified a region approximately 1100 bp in size (the complete gene being ~1500 bp in length).

In addition to these mitochondrial genes, a single nuclear protein-coding gene (H3) was also targeted. The standard primer combination of Colgan et al. (1998) successfully amplified a 328-bp fragment of this gene. The complete 18S (approximately 1.7 kb) was amplified in three overlapping fragments, using the primer pairs 18S-1F and 18S-5R, 18S-3F and 18S-bi, and 18S-a2.0 and 18S-9R. Multiple primers were employed in the amplification of a large region of 28S (approximately 2.2 kb). These overlapping primers include the pairs 28S-Rd1a and 28S-Rd4b, 28S-Rd3.2A and 28S-B, 28S-A and 28S-Bout, and 28S-4.8A and 28S-Rd7b1.

PCR clean up and sequencing. PCR products were cleaned by use of a Beckman Coulter Laboratory Automation Workstation using Agencourt magnetic bead technology (AMPure). Double-stranded cycle-sequencing of purified products was achieved using dye-labelled terminators (BigDye Terminator v.3.1 Cycle Sequencing Reaction Kit, Applied Biosystems) in a thermocycler. The cycle-sequencing reactions consisted of 0.5 μL BigDye, 2.0 μL BigDye Extender Buffer, 2.0 μL 3.2 μM primer and 3.5 μL purified PCR product (for a total of 8 μL). In all cases, the same primer used in the amplification process was used for sequencing. The cycle-sequencing programme consisted of 25 amplification cycles (96 °C for 15 s, 50 °C for 15 s and 60 °C for 4 min). Tagged products were subsequently cleaned on the workstation using CleanSEQ (Agencourt). Sequencing of the purified PCR products was conducted by the dideoxy termination method (Sanger et al., 1977) using an automated ABI Prism 3730xl DNA sequencer (Applied Biosystems).

Sequence editing and error checking. Sequence chromatograms were assembled, visualized and edited using Sequencher 4.1 (Gene Codes Corp.). Once assembled, contigs were then queried against the online NCBI BLAST database. The resulting BLAST hits were checked to highlight possible contaminants (from external sources). The sequences were subsequently aligned using the CLUSTALW (Thompson et al., 1994) package (under the default settings), as spawned through BioEdit (Hall, 2007). The alignment was also visualized in BioEdit. While a multiple sequence alignment (MSA) is not necessary for the analysis of sequences with dynamic homology, it is desirable for two reasons. The first relates to quality control—an MSA can highlight problems within a sequence (e.g. extraneous nucleotides disrupting the reading frame in protein coding sequences), as well as highlight aberrant sequences (e.g. sequences that may have been inadvertently reverse complemented during contig assembly are clearly evident in an MSA). The second concerns the partitioning of the sequence data into smaller fragments for analysis under direct optimization and is discussed below.

Phylogenetic analyses

Datasets and analyses. A number of analytical methods of phylogenetic inference were explored in this study and are discussed in turn below. These methods were conducted using the dynamic homology approach (POY analyses) or with the use of static alignments (all other analyses).

Direct optimization (DO). The MSAs generated by CLUSTALW were used to partition the data. The procedure for partitioning the MSA is explained in detail elsewhere (see Wheeler et al., 2014; chapter 4). The partitioning of sequences of unequal length (due to incomplete sequencing) reduces the amount of ambiguity (IUPAC X or N coding) in these regions. Hence, these regions of the MSA are subsequently treated as missing data in the analysis, and therefore do not influence subsequent phylogenetic analysis. Furthermore, partitioning allows more rapid and efficient optimization of the sequences (see Giribet, 2002). These MSAs in no way constrained the subsequent analysis of the data under DO, given that gaps in the MSA are removed prior to analysis with DO. This procedure is commonly used in the analysis of molecular data under dynamic homology (e.g. Arango and Wheeler, 2007; Lindgren and Daly, 2007; Liu et al., 2009; Padial et al., 2014).

Analysis of these datasets was conducted on two 1024-GB RAM, AMD Opteron 6380 Series 2.5-GHz, parallel 64-core computers at the AMNH using dynamic homology (Wheeler, 2001), with the DO method (Wheeler, 1996), as implemented in a parallel version of POY 5 (Wheeler et al., 2015) version 5.1.1 (source code Wheeler et al., 2014).
Multiple datasets were analysed in this study—each molecular partition, as well as a combined analysis of all the molecular data. The separate analysis of these data partitions facilitated the assessment of the pattern of relationships from each. An exploration of different parameter costs [gap opening, insertion/deletion (indel) and transversion/transition (tv/ts)] was undertaken as a sensitivity analysis (Wheeler, 1995). Three indel cost ratios (1, 2, 4) and three tv/ts cost ratios (1, 2, 4) were employed. Character congruence was measured using the incongruence length difference index (ILD) (Farris, 1973). The parameter set that minimized the incongruence among the partitions was considered optimal.

The analyses were performed in two stages. The initial analyses consisted of random addition sequence Wagner builds, followed by TBR branch swapping. Tree fusing (Goloboff, 1999) followed. Each run held a maximum of eight trees per replicate. A final round of TBR branch swapping was performed, prior to reporting the resulting trees [command line: build(8) swap() fuse(iterations:240) select(best:8) swap() select()]. The first round of analyses was performed for each of the nine parameter combinations under investigation. All subsequent analyses involved combining the trees from the previous analyses and using them as input trees in the next round. The process was repeated until such time that the results of all the parameter combinations were stable (27 rounds). This strategy (Simulated Annealing Tree Fusing, SATF) has proven to be quite effective in identifying stable and heuristically optimal results (D’Haese, 2003; Wheeler et al., 2004; Boyer et al., 2005; Sørensen et al., 2006; Giribet, 2007; Schuh et al., 2009) in other analyses.

Parsimony analysis ‘static’ (EW, IW). MSAs were carried out with MUSCLE (Edgar, 2004) under default options, and analysed with the parallel version of TNT (Goloboff et al., 2008) running on 32 processors in the cluster of the Fundación Miguel Lillo—CONICET. For the equal weights analysis (EW) each processor produced ten replicates of RAS + TBR, followed by sectorial searches and tree-drifting, and the trees from all processors were combined by tree-fusing. The extended implied weighting analysis (IW; Goloboff, 2014) used the same search, computing average weighting against homoplasy in blocks of 50 sites for the ribosomal markers, and the average of 1st, 2nd and 3rd positions for protein coding genes; in both cases the reference constant of concavity for the weighting function was set to 500. For each group of characters, this reference concavity was decreased based on the proportion of missing entries, so that the weight for the block decreases more rapidly with homoplasy as there are more missing entries, assuming missing entries have 0.75 of the homoplasy in observed entries, and using a concavity ratio within 5 [command line: xpiwe (*0.75 < 5) (see Goloboff, 2014: 264). Bootstrap proportions were calculated with 100 pseudoreplicates, each of five replicates of RAS + TBR, followed by sectorial searches and tree drifting. The resulting trees from the five replicates were subsequently subjected to tree-fusing.

Maximum-likelihood (ML). MSAs were carried out with MAFFFT v7.243 (Katoh and Standley, 2013). Alignments of the protein-coding H3 and COI genes were trivial due to the lack of gaps and were produced using the L-INS-i method [command line: mafft—thread 3—threadit 0—reorder—maxiterate 1000—retree 1—genafpair]. Due to the highly variable nature of ribosomal genes, the E-INS-i method, which incorporates affine gap costs, was used to generate alignments of 12S, 16S, 28S and 18S [commands line: mafft—thread 3—threadit 0—reorder—maxiterate 1000—retree 1—genafpair]. A concatenated dataset of these alignments was generated. An additional dataset was constructed where ambiguously aligned regions in the ribosomal genes were excluded. To detect and exclude ambiguously aligned regions, alignments of the ribosomal genes were processed with the program trimAl v1.3 (Capella-Gutiérrez et al., 2009) using the heuristic automated1 method, except for the hypertervarible region of 28S. This region of 28S was processed using the gappyout method, as the automated1 failed to provide a plausible solution (i.e. 99% of the characters were removed).

ML analyses of the concatenated dataset were conducted with the program RAxML (Stamatakis, 2014) on the Abel Cluster at the University of Oslo or using the CIPRES Science Gateway (M. Miller et al., 2010). The concatenated gene matrix was partitioned by gene. Bootstrap and optimal trees were computed in the same run using the—fa option using 1000 bootstrap-ping replicates. The GTRCAT model was used for the fast bootstrap replicates and GTRGamma for optimal topology searches. The raxmlHPC-HYBRID binaries were employed and the job was run using 12 mpi processors, each with four threads [command line: raxmlHPC-HYBRID -T 4 -n result -s infile.txt -q part.txt -p 12345 -x 12345 -N 1000 -c 25 -f a -m GTRCAT -o Palpigrad sp. JA-2011].

The recent phylogenomic study of Garrison et al. (2016) was used to construct a backbone constraint tree for additional rounds of RAxML analyses. This study, which included 50 spider families, and involved extensive molecular sampling (3398 gene regions and 696 652 amino acid sites), was based on transcriptomic data. Therein, many of the relationships in the resulting trees were well supported, albeit for a restricted sample of families. The topology of the preferred tree of Garrison et al. (2016) was modified, such that terminals not included in our study were pruned from the tree and nodes for the terminals that were not highly supported (e.g. with bootstrap supports below 100).
Bayesian inference (BI). Bayesian analyses were carried out in ExaBayes 1.4.1 (Aberer et al., 2014) using the same dataset that was subjected to RAxML analyses. All ExaBayes analyses were run using the Abel cluster at the University of Oslo. Because the current version of ExaBayes does not allow the use of topological constraints, only unconstrained analyses, using the same partitioning scheme and model settings as in the RAxML analyses, were run. Two independent analyses, each with four independent chains and using Metropolis-Coupling (numCoupledChains was set to 2) to speed up convergence, were run. Each run was started using a parsimony starting tree. All model parameters were unlinked among partitions except for branch length. Results were checked for convergence and effective sampling sizes of all parameters using the tools distributed with the ExaBayes package. Support values are expressed as posterior probabilities.

Taxonomic congruence and selection of working tree. Results from the different phylogenetic analyses are summarized in the form of a preferred working tree (Figs 1–8). Selection of this tree, among all the trees produced by the different optimality criteria, was achieved by computing the correspondence of each candidate tree with previously and herein established families and infraorders of spiders (taxonomic congruence). The tree with the greatest correspondence with previous taxonomic hypotheses was reasoned to be conservative by definition, and probably more robust, by having a better fit to previous knowledge and independent data, including morphology. We also calculated how many of the groups most stable to all optimality criteria were recovered by each individual criterion. Furthermore, the quantity of shared groups with the transcriptomic study of Garrison et al. (2016) was also measured (transcriptomic congruence). For this comparison, only those groups with high support values and consistency across the different analyses (Garrison et al., 2016: fig. 2; see next paragraph for groups and mapping of terminals) were considered. The correspondence with taxonomy was checked using the [command line: symcoeff]. To control for possible biases in the taxonomic congruence measures, the taxonomic congruence was calculated twice, for the families as here relimited, or excluding the relimited families (conservative taxonomic tree). Graphics of trees were obtained with FigTree v. 1.4.2 (Rambaut, 2014), TNT and POY.

Constrained analyses using a highly supported backbone tree from transcriptomic (TR) data. Results from the Garrison et al. (2016) analysis of transcriptomic data were used as a skeleton tree to produce additional, constrained tree searches. Fifty-eight of the 74 terminals of this phylogenomic study were mapped to our representative taxa (Table S2). Of these mapped individuals, 25 were the same species (thus mapping was unproblematic), 28 were congeneric terminals and the remaining five mapped individuals were representatives of the same subfamily or family (three and two, respectively). The latter five were added to recover the monophyly of additional, important groups. This strategy is justified on the basis that highly supported groups (bootstrap 1) and groups that were robust to all types of analyses in the transcriptomic analysis were likely to remain well supported upon the addition of our six target genes. Having removed all unmatched terminals from the TR tree, nodes that were unstable across analyses or with bootstrap values < 1 were collapsed. The resulting tree (Fig. S1) was then used as a skeleton to constrain the analysis, only affecting the 58 mapped terminals; all the remaining terminals in our dataset were left unconstrained, free to connect anywhere as dictated by our data. To assess whether the six molecular markers included in this study might overturn the highly supported groups of the transcriptomic dataset, an analysis of the smaller dataset of Bond et al. (2014), consisting of 110 808 characters and 43 terminals, combined with our sequence data, was performed. This dataset was too large for full analysis (not shown; 122 001 characters by 1005 terminals, 89% missing entries), but, as expected, superficial parsimony searches of this dataset did not challenge the monophyly of any of the groups that were highly supported in the original transcriptomic analysis.

Results

Phylogenetic analyses

The primary phylogenetic analyses of the concatenated markers are presented as Figs S6–S10. The sensitivity analysis for the DO analysis (Table S3) found minimum character incongruence at an indel cost ratio of 1 and tv/ts cost ratio of 2, and thus we used that tree for subsequent congruence comparisons with other methods.
Congruence with taxonomy (TX) and transcriptomic tree (TR)

A taxonomic classification of our spider terminals is presented in Fig. S2. This reference tree (TX) contains 105 multi-sampled families (103 of spiders, two of outgroups), and four higher groups (the order Araneae, the suborder Opisthothelae, and the infraorders Mygalomorphae and Araneomorphae). This taxonomy is based on the current classification of the WSC (2016), with additional taxonomic changes as introduced by Dimitrov et al. (2016) and herein (see Taxonomy section below). A second calculation was made with the groups that are not relimited, or whose rank is not
changed here (see Table S4). This conservative reference tree (TX-C) has only 84 spider families (Fig. S3, Table S4), and produced the same result as the full taxonomic reference, but with slightly more marked differences. This is expected, because the taxonomic changes introduced here are robust to analytical strategies. In the unconstrained analyses, the ML tree (Fig. S6) recovered the most taxonomic groups (74%), followed by BI (68%; Fig. S7), DO (60%; Fig. S8), IW (52%; Fig. S10) and EW (50%; Fig. S9) (Table 2). The second reference is the transcriptomic tree from Garrison et al. (2016); after pruning taxa that did not map to our terminals and collapsing groups not highly supported (bootstrap below 100%, or variable across analytical regimes), this tree has 45 groups (Fig. S1). Of these, 36 of the 45 groups are recovered by the BI tree, 34 by the ML and IW trees, 33 by the DO tree, and 31 by the EW tree. A topological measure (a symmetric distortion coefficient modified after Farris, 1973; implemented in TNT, Goloboff et al., 2008) produced the same preferences for the BI and ML trees when compared with the reference trees. The majority rule consensus tree of the five optimality criteria (EW, IW, DO, BI, ML) has 728 groups shared by at least three criteria, and hence more stable (Fig. S12). We calculated how many of those stable groups were recovered by each criterion: ML and BI recovered the most (90.2 and 89.8%, respectively), followed by IW, DO and EW (85.6, 85.4 and 83.1%, respectively) (Table 2). The ML criterion was subsequently used for the constrained analysis; the BI criterion also performed well in our taxonomic tests, but the current version of ExaBayes does not allow constrained tree searches.

**Constrained analysis**

The constrained ML tree (C-ML; Fig. S4), obtained after constraining the backbone topology from the transcriptomic analysis, is topologically very similar to the unconstrained ML analysis, sharing 84% of the recovered groups (788 out of 941 groups; see Table 2). Support values are also similar, with slightly lower overall group support (average bootstrap 0.71 in ML vs. 0.69 in C-ML). It shows, however, a few important differences in the resolution of some higher-level
Fig. 3. Non-entelegyne Araneomorphae (same conventions as in Fig. 2).
Fig. 4. Nicodamoidea and Araneoidea (same conventions as in Fig. 2).
Fig. 5. The marronoid clade and other groups of Entelegynae: Eresidae, Deinopidae, Uloboridae, Oecobioidae, Titanocoidea and Zodarioida, with alternative resolution for Myroinae from Bayesian analysis (BI) (same conventions as in Fig. 2).
Fig. 6. Sparassidae, Homalonychidae and the Oval Calamistrum clade (same conventions as in Fig. 2).
Fig. 7. Prodidomidae and Dionycha part A (same conventions as in Fig. 2).
Fig. 8. Dionycha part B, with alternative resolutions from Bayesian analysis (BI) (same conventions as in Fig. 2).
The results presented are based on molecular data analyzed using a variety of optimality criteria (Figs 1–8). Morphological characters, where discussed, are used as ad hoc and were not included in the phylogenetic analyses. Bootstrap support values (Figs 2–8) were calculated for the maximum likelihood analyses (Fig. S11). Additional results from analyses under different analytical criteria are given in Table S4. Relationships

### Table 2

<table>
<thead>
<tr>
<th>Tree</th>
<th>Groups in tree</th>
<th>TR groups recovered</th>
<th>TX groups recovered</th>
<th>TXC groups recovered</th>
<th>Sym. Coeff. vs. TR</th>
<th>Sym. Coeff. vs. TX</th>
<th>Sym. Coeff. vs. TXC</th>
<th>C-ML groups recovered</th>
<th>Stable groups recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TR) Transcriptic skeleton</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>0.913</td>
<td>1</td>
<td>1</td>
<td>623</td>
<td>85.6%</td>
</tr>
<tr>
<td>(TX) Taxonomy reference</td>
<td>109</td>
<td>109</td>
<td>94</td>
<td>100%</td>
<td>0.905</td>
<td>1</td>
<td>1</td>
<td>605</td>
<td>83.1%</td>
</tr>
<tr>
<td>(TXC) Taxonomy reference,</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>100%</td>
<td>0.905</td>
<td>1</td>
<td>1</td>
<td>605</td>
<td>83.1%</td>
</tr>
<tr>
<td>conservative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>654</td>
<td>89.8%</td>
</tr>
<tr>
<td>(IW) Parsimony, implied weights</td>
<td>941</td>
<td>34</td>
<td>57</td>
<td>52%</td>
<td>0.996</td>
<td>0.722</td>
<td>0.722</td>
<td>542</td>
<td>58%</td>
</tr>
<tr>
<td>(EW) Parsimony, equal weights</td>
<td>907</td>
<td>31</td>
<td>54</td>
<td>50%</td>
<td>0.995</td>
<td>0.736</td>
<td>0.736</td>
<td>528</td>
<td>58%</td>
</tr>
<tr>
<td>(DO) Direct optimization</td>
<td>940</td>
<td>36</td>
<td>65</td>
<td>60%</td>
<td>0.995</td>
<td>0.736</td>
<td>0.736</td>
<td>546</td>
<td>60%</td>
</tr>
<tr>
<td>(ML) Maximum likelihood</td>
<td>941</td>
<td>34</td>
<td>81</td>
<td>74%</td>
<td>0.997</td>
<td>0.766</td>
<td>0.766</td>
<td>710</td>
<td>76%</td>
</tr>
<tr>
<td>(C-ML-T) Trimmed alignments, maximum likelihood constrained by skeleton TR tree</td>
<td>941</td>
<td>44</td>
<td>70</td>
<td>64%</td>
<td>61</td>
<td>65%</td>
<td>0.998</td>
<td>0.761</td>
<td>0.761</td>
</tr>
<tr>
<td>(C-ML) Maximum likelihood constrained by skeleton TR tree</td>
<td>941</td>
<td>44</td>
<td>76</td>
<td>70%</td>
<td>66</td>
<td>70%</td>
<td>0.999</td>
<td>0.779</td>
<td>0.779</td>
</tr>
</tbody>
</table>

The removal of these four terminals from the dataset and running a new ML analysis (C-ML-P) produced an increment in bootstrap support of several branches in the vicinity of these species, bringing their support above 0.69 (Fig. S11). Hence, we present this analysis of 939 terminals as our working hypothesis (C-ML-P tree, Figs 1–8).
analyses are reported in the supplementary materials and discussed briefly below.

A number of taxonomic changes are suggested based on these analyses, and are in general conservative. In a few cases, we refer to the results of clade-specific total evidence (DNA sequences and morphology) analyses recently performed for Mygalomorphae (Bond et al., 2012; with morphological characters for Euctenizidae), Palpimanoidea (Wood et al., 2012, 2013) and Lycosoidea (Polo et al., 2015). Although our results here differ in some ways from those studies, we rely on the total evidence results to guide taxonomic suggestions for Mygalomorphae, Palpimanoidea and Lycosoidea.

Outgroups

We recover a strongly supported Tetrapulmonata, consisting of a monophyletic Pedipalpi, sister to Ara

aneae, following Shultz (1990) and others (Fig. 2). Amblypygi is monophyletic, with Charinidae placing basally in the order, following Weygoldt (1996). Pedi

palpi comprise the monophyletic Schizomida plus Uro

pygi, which follows Weygoldt and Paulus (1979), and Amblypigi. Protopsychomidae place basally within Schizomida, following Cokendolpher and Reddell (1992).

Mesothelae versus Opisthothelae

The Mesothelae (family Liphistiidae, Liphistiomor-

phae) retain external abdominal segmentation; at least vestiges of all four pairs of spinnerets and body segments 12–18 are present, extending behind the spinnerets such that these appear to be beneath the middle of the abdomen, hence “meso-thele”. Four book lungs and orthognath fangs are also retained in this group. The Mesothelae sampling herein includes three species of Liphistiis, and one each of Ryuthela and Heptathela. We find that Liphistiis, Liphisti-

iniae, Heptathelinae, Liphistiidae, Mesothelae and Opisthothelae are all monophyletic with strong sup-

port (Fig. 2), in agreement with the recent analyses of Xu et al. (2015a, b).

Opisthothelae

This group represents spiders in which the abdomi-

nal segments 12–18 are suppressed such that the spin-

nerets appear to be beneath the apex of the abdomen, hence “opistho-thele”. We find a monophyletic Opisthothelae with strong support (Fig. 2).

Mygalomorphae

These taxa retain the primitive four book lungs and orthognath fangs but have reduced spinning organs. None has a homologue of the anterior median spinnerets (AMS), and only a few retain anterior lateral spinneret (ALS) homologues; most have only four pos-
terior spinnerets. A monophyletic Mygalomorphae is recovered and strongly supported. Like previous molecular studies that have included a broad sampling of mygalomorph taxa (Hedin and Bond, 2006; Bond et al., 2012, 2014; Garrison et al., 2016), we recover a basal split between the Atypoidea and the Aviculari-

oidae (Fig. 2). The Atypoidea (Antrodiaetidae, Atypi-
dae and Mecicobothriidae) are a clade of behaviourally disparate mygalomorphs that retain ves-
tiges of segmentation as dorsal abdominal tergites. Holarctic Atypidae are monophyletic, whereas Holarc-
tic Antrodiaetidae are rendered paraphyletic, by the inclusion of Mecicobothriidae. The latter show a bipa-

lar (North and southern South America), disjunct dis-

tribution. North America is a centre of diversity and endemism for Atypoidea.

We recover a monophyletic Avicularioidea, compris-
ing all non-atypoidine mygalomorphs, which have lost all vestige of external abdominal segmentation. Reso-

lution with Avicularioidea has similarities and differ-

ces with previous molecular studies of Mygalomorphae (Hedin and Bond, 2006; Bond et al., 2012, 2014; Garrison et al., 2016). When we compare our results to the analyses recently performed by Bond et al. (2012) (combining sequence data with morpho-

logical characters for Euctenizidae), we notice several differences. Of the groupings found by Bond et al. (2012), we recover Mygalomorphae, Atypoidea and Avicularioidea, although arrangements within differ, Theraphosoidina including Barychelidae and Thera-

phosidae, and the families Atypidae, Euctenizidae, Migidae and Idiopidae (the latter with weak support). We obtained a monophyletic Actinopodidae in several of our analyses (but not C-ML or BI); our results find a close association of actinopodids with some Hexathelidae, reproducing previous results by Bond et al. (2012) and Opatova and Arnedo (2014). Nemesi-

dae are a large group paraphyletic with respect to Microstigmatidae and at least Fusius of Cyrtauchen-

idae. Our results differ from those of Bond et al. (2012) in that our data do not recover any of the fol-

lowing taxa as monophyletic: Domothelina, Crassi-
tarsae, Ctenizoidina, Euctenizoidina, Antrodiaetidae, Dipluridae or Ctenizidae. Since these differences are all weakly supported, we do not draw further conclu-
sions on this basis.

Ctenizidae, represented by eight taxa, are diphylectic. The American Ummidia and Eurasian Conothele form a strongly supported group (Ctenizidae part A), apart from a weakly supported group of South African (Stasimopus) and North American genera (Ctenizidae part B). The three diplurid exemplars in this study are not monophyletic, although the genera Australothele...
analyses also recover the group Hypochilidae and Filistatidae, with four exemplars, break into two parts—*Homostola* plus *Ancylotrypa* are strongly related and are supported as related to the Theraphosoidina, a result consistent with Bond et al. (2012), while *Fufius* fell within Nemesiidae. We suspect that the non-monophyly of Ctenizidae and Dipluridae may be due to inadequacies in our data, and we defer to the total evidence results of Bond et al. (2012), and refrain from making any taxonomic changes for these families. Only the unconstrained ML analysis retrieves a monophyletic Ctenizidae, although with weak support.

**Araneomorphae**

As with some mesotheles, the AMS are present in some araneomorphs, but with many functional spigots. The araneomorph AMS are unrecognizable as spinnerets, as they are modified into a cribleum. The cribleum is a synapomorphy for Araneomorphae, although in a majority of species it is reduced to a non-functional colulus, or lost altogether. Another synapomorphy for Araneomorphae are piri-form silk glands, which produce a sticky glue that bonds threads to each other or to the substrate, making possible a much more varied and precise use of silk than in mesotheles and mygalomorphs. The basal branches of Araneomorphae are in a state of flux. The most recent analysis from transcriptomes (Garrison et al., 2016) finds strong support for some basal clades, which we reflect in our backbone constraint. As expected, it is in the deeper nodes of Araneomorphae where the transcriptomic data have a stronger stabilizing effect, even with a limited taxon sampling.

**Hypochilidae and Filistatidae**

This clade is one of the most exciting findings of the recent transcriptomic analyses of Bond et al. (2014) and Garrison et al. (2016). It not only refutes important higher level groups (Paleo- and Neocribellatae, Araneoclada, Haplogynae; see *Taxonomy* below), but it begs for a substantial re-interpretation of the evolution of important characters, such as respiratory and circulatory systems (see Huckstorf et al., 2015). This clade is well supported after the addition of the TR backbone tree, which contains *Hypochilus* and *Filistata* (Fig. 3). We recover a monophyletic Filistatidae for our eight exemplars. The unconstrained BI and IW analyses also recover the group Hypochilidae + Filistatidae (although in IW a representative of Filistatidae, Prithinae sp. Costa Rica MR11, goes to an odd place, in Dysderidae, seemingly due to a misalignment problem in the parsimony analyses).

**Synspermiata**

This group was recently named by Michalik and Ramírez (2014) for all the ecribellate “haplogynes”, after the unique fusion of several spermatids into one synsperm (Alberti and Weinmann, 1985). Synspermiata includes the superfamilies Dysderoidea, Scytodoidea and the Lost Tracheae Clade. Synspermiata is recovered with low support, but is obtained in all analyses except for two unstable terminals (the telemid *Usofila* and the filistatid Prithinae sp. Costa Rica MR11 mentioned above) (Fig. 3).

**Dysderoidea**

The four dysderoid families (Dysderidae, Segestriidae, Oonopidae and Orsolobidae) are recovered as monophyletic in all analyses (Fig. 3), except for a few unstable representatives that may go, or come from, elsewhere (the oonopid *Stenoonops* and the telemid *Usofila*). The relationships among the families are homogeneous across analyses, including a new sister group relationship between Orsolobidae and Dysderidae, although weakly supported. This latter result agrees well with the partly fused testes in dysderids, as an intermediate step towards total fusion that is distinctive of oonopids (Burger and Michalik, 2010; Michalik and Ramírez, 2014). Within Oonopidae, the C-ML tree is consistent with the progressive hardening of the body from lower to higher gamasomorphines proposed by Grismado et al. (2014: 7) (see also de Busschere et al., 2014: 186), although not solving the orchestinines and soft-bodied clades, which are undersampled in our study. In Orsolobidae, we obtain a monophyletic group of South American genera, represented by *Orsolobus*, *Osornolobus* and cf. *Mallecolobus* sp. Chile MA188.

**Trogloraptoridae and Caponiidae**

Trogloraptoridae are the only entirely new spider family described during this century. This taxon, known only from *Trogloraptor marchingtoni*, was discovered in caves and old growth forest in the Klamath-Siskiyou region of Oregon and California. Their morphology suggests a kinship with the Dysderoidea and Caponiidae (Griswold et al., 2012). Most analyses obtain Trogloraptoridae and Caponiidae as sister groups (Fig. 3); C-ML and BI bring the unstable telemid *Usofila* in between, but the removal of *Usofila* increases the support of the group to moderate. This latter clade is the sister group of Dysderoidea after the TR backbone is enforced, and in the BI unconstrained tree; in general, this is in good agreement with the morphology, except that it implies homoplasy in the posterior respiratory system of caponiids, remarkably similar to that of dysderoids (Ramírez, 2000).
Scytodoidea

The scytodoids are a group of Synspermiata with six eyes grouped in three pairs. They include, among others, the brown recluse spiders (Loxosceles, Sicariidae) and spitting spiders (Seytoidae). Scytodoids are recovered in the BI tree, and their monophyly is only weakly supported by the transcriptomic constraint, although only Loxosceles and Seytodes are included in the skeleton tree. According to this result, and also supported by all the analyses, the tropical Ocyeroceratidae are a member of Scytodoidea (Fig. 3). This relationship had previously been suggested by Lehimen (1986), and we have confirmed the presence of bipectinate proclaws and a distal dorsal hood covering the claw bases in Ocyerocera and Theotima (A. Pérez-González and M. Ramírez, unpublished data); both are synapomorphies of Seytoidoidea (see Labarque and Ramírez, 2012). Although Drymusidae are monophyletic in several of our analyses (C-ML-T, ML, BI, DO), our results are overall inconclusive about the separation or inclusion of Periegopidae in Drymusidae.

Lost Tracheae Clade

This clade is a group of families of Synspermiata that have lost their posterior respiratory system (Ramírez, 2000). This clade is strongly supported after the stabilization of the skeleton TR tree (which includes Pholcus and Digueta as the only representatives of the clade) (Fig. 3), and also in the BI unconstrained tree. The resolution is novel, and implies the separation of the armoured spiders Tetrablemmidae from Pacullidae, currently grouped in Tetrablemmidae, but previously considered separate families (see Taxonomy below). Pholcidae are strongly supported, but only the subfamilies Ninetinae and Smeringopinae are recovered in our analysis; we refer to the densely sampled analysis of Dimitrov et al. (2013) for intra-familial relationships.

Austrochiloidea and Leptonetidae

These two groups of spiders seem to be an endless source of surprises. Austrochilooids comprise two austral families, Gradungulidae and Austrochilidae, which include some of the largest araneomorphs (Forster et al., 1987). Since Platnick (1977), these families have been placed as an early branching lineage of the higher spiders, as suggested by the retention of the primitive arrangement of four book lungs. Within the last decade, field observations and careful scanning electron microscopy examinations have revealed derived characters in common with higher spiders, thereby challenging their grouping near the araneomorph base. At least some austrochilids have cylindrical gland spigots and card their cribellate silk in a supposedly derived manner, using one leg IV braced against the other, mobile leg IV (Lopardo et al., 2004; Griswold et al., 2005). Leptonetids, tiny fragile spiders of caves and other dark places, were traditionally placed within the simple-genitalia haplogyne spiders. Again, detailed scanning electron microscopy studies have changed things: by discovering, first, a class of spigots that are probably cylindrical gland spigots (Platnick et al., 1991) and, second, a cribellum in Archeoleptoneta (Ledford and Griswold, 2010). Morphology has suggested a leptonetid affinity to entelegynes (Ledford and Griswold, 2010) and phylogenomics added further support (Garrison et al., 2016). Previous molecular studies have suggested an Archeoleptoneta-austrochiloid affinity (Agnarsson et al., 2013). Herein, we continue to expand novel interpretations of austrochiloid and leptonetid placement (Fig. 3), underscoring the pivotal importance of these obscure animals.

Gradungulidae

This bizarre family, known only from Australia and New Zealand, is characterized by grotesquely asymmetric tarsal claws and includes cribellate and ecribel-late, four-lunged spiders. Gradungulidae are well represented in our study, including the huge Australian cribellate Macrogradungula moonya and the ecribellates Gradungula sorenseni from New Zealand and Tarlina woodwardi from Australia. Gradungulids unite with high support, and appear related to the cribellate, four-lunged sheet-web builder from Tasmania, Hickmania troglodytes (Austrochilidae). Gradungulids and Hickmania appear also related to the cribellate leptonetid Archeoleptoneta in a clade of moderate support, although the precise relationships among them are uncertain (Fig. 3).

Leptonetidae

The family Leptonetidae, exclusively Holarctisch, had long been placed among the haplogyne spiders. The discovery of a cribellum in Archeoleptoneta, a relict leptonetid from California (Ledford and Griswold, 2010), changed this scenario. Leptonetids had already been recognized for having a category of spigots with sexual dimorphism like cylindrical gland spigots, and Brignoli (1979) had already remarked on the similarities to Entelegynes. Ledford and Griswold (2010) suggested that Archeoleptoneta might be a proto-entelegyne, and that the whole family might be misplaced in Synspermiata. Wunderlich (2015: 287) placed leptonetids as part of his LAE clade (Leptonetoids, Archaeoids and Entelegynes), based on cylindrical gland spigots, loss of the posterior lungs and absence of eye triads. Molecular data supported placement of Leptonetidae
outside of the Haplologynae (Agnarsson et al., 2013), as did phylogenomic data (Garrison et al., 2016), probably as sister to Entelegynae, although the latter study lacked some other strong candidates for leptonetid relatives, e.g. austrochiloids and palpimanoids (see below). Our study corroborates the placement of leptonetids outside the Synspermiata and nearer the Entelegynae; in addition, our data challenge leptonetid monophyly (Fig. 3). In our study, the cribletate Archholeptoneta, with a simple eye pattern, are separate from the cribletates, with posterior median eyes displaced behind the lateral eyes. The cribletate leptonetids of our sample group are, however, diphylectic in the optimal trees, and thus we prefer to defer taxonomic changes in Leptonetidae (see comments below).

The CY Spigot clade, and the austrochiloids

Cylindrical gland spigots (CY), also known as “tubuliform”, characterize a large clade of spiders comprising all Araneomorphae except Hypochilidae, Filistatidae and Synspermiata. These spigots appear only in mature females and presumably take part in making the eggsac, but the exact role is still unknown. The aforementioned Leptonetidae join the austral family Austrochilidae in having a category of spigots with the same sexual dimorphism as CY. This last family was long placed in Austrochiloidea near the base of the Araneomorphae, because several (the austrochilid Hickmania, together with Gradungulidae) retain four book lungs. Our results place the two-lunged Austrochiloidea Austrochilus and Thaida as a monophyletic group with high support, and ally these with the cribletate type genus of Leptonetidae, Leptoneta, but with low support (Fig. 3). Austrochilids could be giant austral counterparts of leptonetids; at least the peculiar patella/tibia autospasy characteristic of leptonetids and austrochiline supports this hypothesis. Leptonetidae are not without problems though, in that the cribletate leptonetines Neoleptoneta and Calileptoneta fall far from Leptoneta, forming a group near the Entelegynae, and the cribletate Archholeptoneta joins with Gradungulidae (see above). We suspect that this result, especially the diphylectic Leptonetidae, is artefactual, as there are numerous morphological synapomorphies uniting the Leptonetinae, and the results are unstable across analyses. Our data suggest, although with moderate to low support, that the austrochiloids and leptonetids are more closely related to palpimanoids and entelegnynes than to Synspermiata (Fig. 3).

Palpimanoidea

Assassin spiders are restricted to five living families, Archaeidae, Huttoniidae, Mecysmaucheniidae, Palpimanidae and Stenochilidae. Palpimanoids were once more widespread, with fossils dating back to the Jurassic in Northern Hemisphere deposits (Selden et al., 2008; Wood et al., 2013). The fossil family Lagonomogopidae was widespread in the Mesozoic world, and 12 fossil genera of Northern Hemisphere Archaeidae contrast to the only four known today from Africa, Australia and Madagascar.

Wood et al. (2012, 2013), in total-evidence analyses including fossil taxa, established the monophyly of a Palpimanoidea comprising the above five families, which we accept as the most robust solution. Molecular data alone repeatedly fail to recover Palpimanoidea. Each of the five palpimanoid families is represented here by multiple exemplars and all of them are recovered with strong support (Fig. 3). Huttoniidae and Palpimanidae unite with moderate support, but the rest of the families, especially Archaeidae, are unstable across analyses, clustering to other long-branched regions of the tree; this situation improves after the removal of Theridiosoma gemmosum and in the BI tree. This instability might be the effect of long-branch attraction or indicative of undetected paralogy in some of the nuclear ribosomal genes (see comments in Dimitrov et al., 2016). We prefer to retain the classification of Wood et al. (2012, 2013) and accept a monophyletic Palpimanoidea; among other morphological characters, they have peg teeth on the promargin of the chelicerae and a cheliceral gland mound.

All palpimanoids appear to be prey specialists often feeding on other spiders, hence the broadly applied term “assassin spiders”. Araneophagic palpimanids enter the retreats of other spiders, seize them with scopulate anterior legs and prey on them, at least pelican spiders (Archaediae) attack Araneoidea in the host’s webs, and captive stenochilids and huttoniids seem to prefer spider prey (see Wood et al., 2012). Huttoniidae, Palpimanidae and Stenochilidae have extensive, lateral scopulae on their forelegs, which enable them to seize and manipulate prey, and even the web-invading archaediae retain a vestige of these scopulae. Mecysmaucheniiidene have a remarkable trap-jaw mechanism (Wood et al., 2012, 2016). They hold the chelicerae widely open and locked before a strike; when prey contacts one of the long, forwardly directed setae (possibly triggers), the chelicerae snap closed at remarkable speed, enabling them to capture such speedy prey as springtails (Collembola). Prey preferences of extinct Palpimanoidea are unknown, but archaediae from amber have the same prey-specialized morphologies as living forms. Within Palpimanidae, our data support the monophyly of the subfamilies Oioithopinae (represented by Notioithops and Oioithops), Chediminae (by Scelidocteus and Saracelis) and Palpimaninae (with Palpimanus and Ikuma); our Ikuma representative is nested in an African group of Palpimanus species, suggesting that the generic limits should be re-examined.
Enteleynae

This large clade of spiders is characterized by a “flow-thru” female genital system with separate ducts for copulation and fertilization. Enteleynae monophyly is supported, but with weak support (Figs 4–8). The enteleynae condition and the sclerotized genital plate near the copulatory openings (the epigynum) have long been recognized as a synapomorphy for this clade, with three reversals to a haplogyne organization in tetragathines, in the uloborids Waitkera and Tangaroa and in the anapid Comaroma. A close examination of the genital system of anapids revealed fertilization ducts in all except Comaroma (Lopardo and Hormiga, 2015).

Nicodamoidea and Araneoidea

Araneoidea are a rich, successful clade characterized by a triplet of one flagelliform gland and two aggregate gland spigots on the posterior lateral spinnerets (PLS; variations occur): the flagelliform glands each produce an axial line and the aggregate coat these with viscid glue. The superfAMILY Araneoidea is a clade and its sister group is the superfAMILY Nicodamoidea, but, unlike the results in Dimitrov et al. (2016), here the Araneoidea and sister group association of Nicodamoidea and Araneoidea have only low support (Fig. 4). Each included nicodamoid family, represented by two terminals of the same cribellate species (Mega dictynidae, New Zealand) and three cribellate Nicodamidae (Australia and New Guinea), and the superfAMILY Nicodamoidea, are all strongly supported.

The Araneidae have received much attention in phylogenetic studies and have a checkered history (e.g. Hormiga et al., 1995; Scharff and Coddington, 1997; Kuntner, 2006; Kuntner et al., 2008, 2013; Alvarez-Padilla et al., 2009; Dimitrov and Hormiga, 2009, 2011; Gregorić et al., 2015). Our coverage of these taxa is good, with 13 nephilines and 24 non-nephiline Araneidae including the contentious oarces (Gnolus and Oarces), Caerostris, Zygella and Phonognatha, plus Arkys, formerly an araneid but placed in its own family, sister to the tetragnathids by Dimitrov et al. (2016). We obtained good support for Araneidae (excluding Arkys), with a strongly supported Nephilinae sister to the rest of the family, as in Dimitrov et al. (2016; but that study included many more terminals). Caerostris sexcuspidata, one of the species that we pruned from the analysis, fluctuated across several clades and erased their support (compare Figs 2 and S4).

A poorly supported but robust arrangement emerging from our study and mirroring Dimitrov et al. (2016) is the relationship among Tetragnathidae, Arkyidae and Mimetidae. Our Tetragnathidae comprise 27 exemplars, whose monophyly is strongly supported. These are in turn sister group to the Australian Arkyidae (Arkys), with good support. Finally, our six exemplars of the worldwide pirate spiders (Mimetidae) comprise a well-supported family.

The peculiar, poorly known Austral spiders formerly placed in Holarchaeidae and Pararchaeidae long posed classification mysteries, and were alternatively classified with orb builders (Araneoidea) and assassin spiders (Palpimanoidea). Holarchaea is strongly allied to the anapid Acrobeleps and our two pararchaeid exemplars fit within the Malkaridae, compatible with the synonymies proposed by Dimitrov et al. (2016), but with weak support and unstable across analyses. We include Australian and Chilean malkarid exemplars, but none of the undescribed taxa from New Zealand used in that study.

Of the families formerly placed in the symphytognathoids (Griswold et al., 1998) or symphytognathids (Rix and Harvey, 2010), we only recover a weakly supported group of Anapidae and Symphytognathidae, but the families themselves are in general well supported. Anapidae, comprising 16 exemplars including Holarchaea and a variety of micropholcomatines, are well supported, as are Mysmenidae (ten exemplars). Symphytognathidae (six exemplars) have moderate support. We obtained low support for Theridiosomatidae, even after pruning Theridiosoma gemmosum due to its unstable placement near Titanocidae and Archaeidae (e.g. in ML), in a group seemingly characterized only by long branches, suggesting either an analytical artefact or a sequencing problem.

Synotaxus, the sole remaining genus in Synotaxidae, allies strongly to the nesticid Gaucelmus. Diphly of the old Synotaxidae is well justified on molecular, morphological and behavioural grounds: the Neotropical Synotaxus builds a unique, “chicken-wire” web with modules of glue-sticky silk (Eberhard, 1995), whereas the southern South American, Australian and New Zealand physoglenids make ordinary sheet or dome webs (Dimitrov et al., 2016; and references therein). We obtain a strongly supported core Nesticidae of four genera (Nesticus, Nesticodes, Eidmannella and Carpathonesticus) but Gaucelmus fluctuates among analyses, always together with Synotaxus. Separation of the cob-web building Nesticidae (five exemplars) and Theridiidae (27 exemplars) is found here, as it has been in previous molecular studies (e.g. Dimitrov et al., 2012, 2016; Agnarsson et al., 2013; Bond et al., 2014; Garrison et al., 2016). The sticky-silk apparatus of these two families is strikingly similar (Coddington, 1989), with enlarged aggregate gland spigots on the PLS (also present in Arkyidae and Synotaxus) and a ventral comb on the fourth tarsus for throwing silk blobs (hence, “comb foot spiders”), but nesticid and theridiid genitalia are very different (Agnarsson, 2004). Males of the former have
a typical araneoid paracymbium (a process on the cymbium of the palp), whereas theridiid males have a distal locking mechanism within the alveolus. Theridiidae are well supported but only allied with low support to the remaining Araneoidea. The propinquity between Theridiidae and some Mysmenidae and Anapidae, as found in Dimitrov et al. (2016), is excluded by the backbone constraint, but a liaison with Mysmenidae is obtained in some of the unconstrained analyses (ML, BI).

We recover strongly supported groups for the 11 Physoglenidae exemplars, ten Cyatholipidae and a clade of 39 exemplars comprising Linyphiidae plus Pimoidae (the pimoid Weintrauboa clusters with the linyphiid Stenonyphantes, otherwise these two families are reciprocally monophyletic; see Dimitrov et al., 2016 for a more detailed discussion).

**Eresidae**

Velvet spiders comprise an almost exclusively Old World taxon (one species, possibly introduced, is reported from Brazil) that has many species and genera in the arid parts of Africa. Subsociality has evolved at least three times within *Steatodyphus*, once in south Asia (S. sarasinorum) and twice in Africa (S. dunicola and S. mimosarum) (Johannesen et al., 2007). The phylogeny of velvet spiders has recently been studied in detail (Miller et al., 2012). Our nine eresid exemplars form a robust monophyletic group arising near the base of the Entelegynae, but more detailed placement is impossible, as support for relations to other entelegynes is essentially non-existent (Fig. 5). The concept of Eresoida (Eresidae plus Oecobiidae) was established based on morphological data (Platnick et al., 1991; Griswold et al., 1999, 2005) but molecular data have challenged this (J. Miller et al., 2010) and the Eresoida hypothesis now seems definitively refuted. The transcriptomic studies are indecisive on the relationships of Eresoida (only two species of the three putative eresid families have been included), and hence we did not constrain their placement.

**Oecobiioidea**

These two families, Hersiliidae and Oecobiidae, have modified, elongated PLS that spin sheets or curtains of silk to tie down their prey, which is applied while the spider whirls rapidly in circles around the prey. Tiny, ecribellate *Oecobius* include cosmopolitan and pantropical species that are common in houses; ecribellate hersiliids have a worldwide distribution. We include four exemplars of each family; Hersiliidae are strongly supported and the ecribellate *Oecobius* and ecribellate *Uroctea* form a moderately supported Oecobiidae (Fig. 5). Grouping of Hersiliidae and Oecobiidae as superfamily Oecobiioidea is also well supported in the ML and C-ML trees, while the remaining methods did not recover *Oecobius* and *Uroctea* together.

**Orb-web weaving spiders**

Perhaps the most dramatic change in spider phylogeny in the last decade is the realization that the complex suite of stereotypical behaviours used to construct an orb-web do not define the corresponding clade “Orbiculariae”, formerly comprising all cribellate and ecribellate orb-building spiders (Bond et al., 2014; Fernández et al., 2014; Hormiga and Griswold, 2014; Dimitrov et al., 2016; Garrison et al., 2016). Instead, these studies suggest that the orb-webs are most likely an ancient development of a more inclusive group, probably of all entelegynes, and that the orb-web was subsequently modified or lost in several lineages.

**Cribellate orb-weavers**

The monophyly of Deinopoidea (Uloboridae and Deinopidae) is disputed by molecular data, as revealed in the molecules-only partitions of Blackledge et al. (2009), as clearly shown by Dimitrov et al. (2012, 2016) and Agnarsson et al. (2013), and now solidly refuted by transcriptomic analyses (Bond et al., 2014; Fernández et al., 2014; Garrison et al., 2016) and herein (Fig. 5).

Not surprisingly, the monophyly of Deinopidae, represented by five exemplars (two *Menneus* and three *Deinopis*), is strongly supported by sequence data. The Uloboridae representatives, seven exemplars, group together with low support. Both families vary their placement across analyses; other than the well-established non-monophyly of Orbiculariae and Deinopoidea, a clear understanding of the placement of cribellate orb-weavers remains a future goal. Although “Orbiculariae” now appears to be an artificial taxonomic concept, recent analyses of character evolution still show some support for a single origin of the orb web, but with multiple losses (e.g. Dimitrov et al., 2016; Garrison et al., 2016), either by modifying the web into architectures no longer recognizable as orbs (e.g. in Cyatholipidae) or by dispensing altogether with foraging webs (e.g. the pirate spiders, Mimetidae).

**Ecribellate orb-weavers**

The phylogeny of Araneoidea has received extensive recent attention (e.g. Blackledge et al., 2009; Schütt, 2009; Lopardo et al., 2011; Dimitrov et al., 2012, 2016; Agnarsson et al., 2013; Hormiga and Griswold, 2014). The study of Dimitrov et al. (2016) includes representatives of all valid araneoid families, including Synaphridae, which are unrepresented in the present
study. Common results from this study and that of Dimitrov et al. (2016) are the sister group relationship of Nicodamoidea and Araneoidea, of Nephilinae and the rest of Araneidae, close association among Mimetidae, Arkyidae and Tetagnathidae, the inclusion of former Pararchaeidae within Malkaridae, of Holarchaeidae and Micropholcommatidae within Anapidae, isolation of Synotaxus from other former Synotaxidae and the remarkable diphyly of the comb-footed “theridioids” (Theridiidae and Nesticidae). Several of those findings are strongly supported in our study as well. Another similarity is the generally low support for interfamilial relations. Disappointingly, the evolution and diversification of ecribellate orb-builders and their kin as yet cannot be understood. Compared to the results of Dimitrov et al. (2016), we do find similarities and differences in family placement, which are detailed in the previous section, Nicodamoidea and Araneoidea.

**Spiders with male palpal tibial apophyses**

A process or set of processes on the male palpal tibia occurs widely within the Entelegynae. Occurrence of as many as four lateral processes, e.g. in Amaurobiidae, suggests that there may be up to four homologies (Griswold et al., 2005). The retrolateral tibial apophysis (RTA) characterizes a large clade of spiders (see below). The dorsal tibial apophysis (DTA) of Nicodamoidea and some Linyphiidae, Titanoecidae and Phyxelididae is not homologous to the RTA; some phyxelidids (Vytfutia) and phrurolithids (Phonotimpus) and most Amaurobiidae have both RTA and DTA.

**Titanoecidae**

Titanoecids lack tarsal trichobothria, a primitive condition, and have a complex DTA but lack an RTA on the male palp. In our dataset, Titanoecidae are well represented by six exemplars and form a robust, strongly supported monophyletic family (Fig. 5).

**Phyxelididae**

These spiders occur in Africa, Madagascar and Asia, and have been suggested as the sister group of Titanoecidae (Griswold et al., 1999, 2005). We include ten representatives including African Vidolini and also Phyxelidini from both Africa and Madagascar, but unfortunately the enigmatic Asian Vytfutiini are absent from our analysis. Phyxelididae are strongly supported and robustly allied to the RTA clade, although with low support (Fig. 5). Phyxelididae and Titanoecidae fall apart, provisionally refuting the clade Titanoecoidea (Griswold et al., 1999). A broader representation of phyxelidids, including Vytfutiini, plus enhanced molecular, as well as morphological data, will be necessary to better test the monophyly and the limits of Titanoecoidea.

**The RTA clade**

These taxa have an RTA on the male palp and also one to many trichobothria on the tarsi and metatarsi, representing advances in mating stabilization and vibration sensitivity, respectively. Spiders with dorsal but not retrolateral male palpal tibial apophyses, i.e. Nicodamoidea, Phyxelididae and Titanoecidae, are historically excluded from the RTA clade (Griswold et al., 1999, 2005). Herein, we recover the RTA clade, albeit with low support (Fig. 5).

**Zodarioidea**

A gestalt of morphological data long associated the Zodariidae and Homalonychidae, to which the peculiar African Penestomidae were added by J. Miller et al. (2010) based on molecular evidence. One morphological synapomorphy for these three families is that the ALS have the major ampullate gland spigots placed deep within the piriform spinning field (J. Miller et al., 2010; Ramírez et al., 2014), thus providing independent corroboration of the molecular results. We find a robust and well-supported Zodariidae comprising 27 exemplars (Fig. 5). Zodariids are easily distinguished by a combination of characters (see Jocqué, 1991)—an unambiguous synapomorphy for the family was recently discovered by Jocqué and Henrard (2015)—the distal dorsal rim of the leg tibia has a ball-shaped projection, fitting in a cavity of the metatarsus. Within zodariids, we corroborate the kinship of Cryptothelidae with former cyrulinids, and thus Cryptothelinae, as indicated by morphological data (Ramírez et al., 2014). Our only representative of Lachesaninae (Lutica) is deeply nested among zodariines, although most internal nodes have weak support. Penestomidae (Penestomus) are moderately supported as the sister group of Zodariidae. Homalonychidae are placed with zodarioids in our DO, BI and ML analyses, with weak support, but are driven elsewhere in the tree by the backbone constraint of Garrison et al. (2016), close to the OC Clade and Dionycha, but with weak support. Considering the congruence in morphology and the molecular data found here, we suspect that the breakup of Zodarioidea forced by the transcriptomic analysis backbone tree may be artefactual.

**The marronoid families**

Our informal name marronoid (marrón, brown in Spanish) comes from grouping together several spider families lacking striking characters and formerly classified together in such “tailor’s drawer” families as...
Ageelenidae, Desidae, Dictynidae and Amaurobiidae. Most are brown, grey or mottled but also have complex, distinctive genitalia with an elaborate RTA and several bulbal sclerites, all retain three claws, and most families have both cribellate and ecribellate species. The globally representative taxon sample in Griswold et al. (1999, 2005) suggested a dichotomy roughly between the spiders placed in Amaurobioidea and Dictyinoidea, but not equivalent to the tracheae-based groups Amaurobioidea and Dictyinoidea as defined by Forster and Wilton (1973). Instead, their results hinted at a basic dichotomy between Holartic (Amaurobiidae, Dictynidae) and Austral (Desidae, Stiphidiidae) spiders, the latter informally referred to as the “Fused Paracribellar clade” (FPC) after a peculiarity of their spinning organs. A series of molecular phylogenetic studies beginning with Spagna and Gillespie (2008) and continued through J. Miller et al. (2010), Spagna et al. (2010), Agnarsson et al. (2013) down to the current study have divided and reassembled these taxonomic concepts, hence requiring the reclassification of Forster and Wilton’s concepts of Amaurobioidea and Dictyinoidea. Our study comprises by far the densest and broadest taxon sampling for target genes. This convinces us to formalize the taxonomic reorganization presaged by the works cited above and corroborated and extended here. The families Ageelenidae, Amaurobiidae, Amphinectidae, Cybaeidae, Desidae, Dictynidae, Hahniidae, Neolaniidae and Stiphidiidae all require radical reclassification due to our analysis, and below we provide the transfers and in some cases new synonymies necessary for a phylogenetically robust classification. The “marronoid” group is recovered by all our analyses albeit with weak support, only differing in the inclusion of Sparassidae as a member in the unconstrained analyses (sister to Amaurobiidae, with weak support), or exclusion after the constraint skeleton from Garrison et al. (2016), which did not have sparassids in their sample (Fig. 5).

**Amaurobiidae**

This family is strongly supported only if drastically redelimited (Fig. 5). Of the 15 taxa in our dataset formerly classified as amaurobids, only four species in three genera, *Amaurobius* (two species), *Callobius* and *Pimus*, remain to comprise the core of Amaurobiidae, i.e. subfamily Amaurobiinae. Others are distributed to Ageelenidae (*Paracoeleotes*), Cycloctenidae (*Pakeha* and *Paravoca*) and Toxopidae (*Midgee*). This classical family has been picked apart so that of the nine subfamilies recognized by Lehtinen (1967), only the Holarctic Amaurobiinae, the principally austral Macroobuninae and the mysterious Neotropical Altellopsinae remain. The subfamily Macroobuninae was proposed by Lehtinen (1967) within Amaurobiidae, but clear synapomorphies were discovered in recent years, in the male palps: a peculiar gland discharging in the retrolateral tibial apophysis (Compagnucci and Ramírez, 2000), and a stridulatory area on the cymbium (Griswold et al., 2005). The molecular study of J. Miller et al. (2010) indicated that the African and North American macrobunines *Chresiona* and *Zanomys* allied with the monogeneric South African Chummidae, falling far from the core Amaurobiidae. We link here these taxa with the core South American macrobunines, confirming that they are not amaurobids, although their nomenclatorial status is still unsolved (under study by Almeida-Silva, pers. comm., Almeida-Silva, 2013; unpublished thesis). Macroobunines generally occur in temperate and subantarctic habitats in Africa, Australia and South America, where they predominate in collections of undetermined “amaurobids”. This group received good support in our analysis. Our constellation of Macroobuninae includes genera listed in Amaurobiidae (*Anisacate, Callevopsis, Chresiona, Livius, Naevius, Rubrius* and *Zanomys*), Chummidae (*Chuma*), Amphinectidae (*Tasmarubrius*) and most surprisingly, Anyphaenidae (*Malenella*). *Malenella nana* exhibits one of the many striking convergences into a dionychan-like morphology. This Chilean species has the extensive tracheal system and expanded claw tuft setae typical of Anyphaenidae, where it was originally described (Ramírez, 1995). The morphological analysis of Ramírez (2014) obtained *Malenella* in the vicinity of anyphaenids, but still uncovered several convergences in tracheal systems and claw tuft setae strikingly similar to those of anyphaenids (e.g. in some Eutichuridae and in *Hortipes*). The present analysis strongly supports the placement of *Malenella* among macrobunines, independently supported by each molecular marker; we sequenced several specimens with identical results, so the possibility of contamination is discounted.

**Ageelenidae**

This is a large Holarctic family, only native to Africa, south of the Equator. This family received high support in our analysis. As noted above, austral “ageelenids”, e.g. *Neoarachna*, were misclassified and belong elsewhere. Invasive *Tegenaria* occurs in South America, New Zealand and Australia; otherwise ageelenids comprise numerous genera and myriad species in North America and temperate Eurasia, extending to South Africa. There is a single cribellate ageelenid genus, *Tamgrinia* (J. Miller et al., 2010), a little known taxon from central Asia. Most are ecribellate funnel or sheet web builders with lengthened PLS and a characteristic divided colulus; in tropical Africa there are even subsocial species. Of our ageelenid representatives, two genera are moved to other families: *Orepukia*, from New Zealand, allies with Cycloctenidae and *Neoarachna* is placed in Stiphidiidae. Among the Ageelenidae, Coelotinae and a core
Ageleninae are each robust and strongly supported, but we obtain Textrix and Tegenaria outside these subfamilies, grouped with moderate support; the ecribellate Tamigrinia is sister to the rest of the family (Fig. 5). All these results were first suggested by J. Miller et al. (2010). Bolzern et al. (2013) obtained a different composition for ageelenines, including Textrix and Tegenaria.

The “Dictynidae”, a polyphyletic group

The current catalogue of spiders lists 52 genera and 578 species in the family Dictynidae, and includes cribellate and ecribellate spiders (WSC, 2016). Whereas morphological data are adequate to group the cribellates together (Griswold et al., 2005), the family falls apart in molecular analyses that include cribellate and ecribellate members (Spagna and Gillespie, 2008; J. Miller et al., 2010; Spagna et al., 2010). Dictynidae are clearly in need of a major overhaul, which was begun by Spagna et al. (2010) and Zamani et al. (2016). In our analysis, former dictynids are scattered among at least three clades, including the newly circumscribed families Cybaeidae, Hahniidae and Dictynidae (Fig. 5).

Cybaeidae

In our analysis, Cybaeidae receive strong support (Fig. 5), but differ from tradition by including Calymmaria (formerly Hahniidae) but excluding Argyroneta (moved to Dictynidae, see below). The genus Cybaeus was subject to a revision (Bennett, 1991; unpublished thesis), revealing several new genera and many new species. Calymmaria has been revised (Heiss and Draney, 2004). Like Calymmaria, little-known genera with patella/tibia autospasy probably belong here as well. The ecribellate Cryptoecea, Blabomma, Willisus and Yorima have been associated with Cybaeus and Calymmaria by Roth and Brame (1972) and Spagna et al. (2010) and we follow the latter study in classifying these ecribellate genera in our redefined Cybaeidae.

Hahniidae

This family contains the clearly monophyletic Hahniiinae with a strongly modified arrangement of spinnerets in a single transverse row, here represented by Hahnia and Neoantistea (Fig. 5). These group with the austral Cybaeolinae (Cybaeohus), forming a strongly supported core Hahniidae. In our analysis, the monophyly of the Holarctic Cicurina group with our core Hahniidae is only moderately supported.

Dictynidae

This group represents a dumping ground for a diverse array of cribellate and ecribellate three-clawed Entelegynae. Of the several genera representing Dictynidae in our analysis, Cicurina departs to Hahniidae, leaving Dictyna, Mallos, Mexitlia and Saltonia in Dictynidae (Fig. 5). We add to these the ecribellate Argyroneta (formerly Cybaeidae). Corroborating Zamani et al. (2016), the intertidal genus Paratheuma also falls here. All these together form a strongly supported Dictynidae. The cribellate residue of Dictynidae contains Dictyna, of course, and similar genera such as Mexitlia, and Mallos, but the cribellate Lathys is not clearly placed in any of the above families (Spagna et al., 2010), but is provisionally kept in Dictynidae.

Toxopidae

We resurrect the family Toxopidae to accommodate an array of small spiders, typically with strongly curved eye rows, from Australia and New Zealand. In addition to the Tasmanian Toxops, a small, flattened spider with large eyes, typically found running on forest vegetation, Toxopidae include the genera Hapona and Laestrygones from New Zealand, which are similar in morphology and lifestyle. Also circumscribed in this family is the tropical Australian genus Midgee, formerly Amaurobiidae; these together as the subfamily Toxopinae are strongly supported. A second group of toxopids comprises Laminia, Onnmatauxesis, Otagoa and Myro. The first three are restricted to Australia and New Zealand but Myro is scattered across sub-Antarctic islands south of New Zealand and in the southern Indian Ocean (e.g. Kerguelen, Marion and the Crozets). Toxopidae might be an austral analogue of the Dictynidae. Like dictynids, toxopines, e.g. Toxops, Hapona and Midgee, are small hunters on foliage or in soil, whereas myroines, e.g. Otagoa, Myro and Onnmatauxesis, are largely intertidal. Myroines are monophyletic in several of our analyses, only varying in the placement of cf. Gasparia sp. New Zealand CG105 (occasionally with Desidae, Amphinectinae).

Relationships among the above six families generally receive extremely weak support and are unstable across analyses, as is typical in target gene analyses. A relationship of at least Hahniidae plus Cybaeidae receives support from phylogenomics; Garrison et al. (2016) associate Cicurina and Calymmaria, and were placed by them in Dictynidae and Hahniidae, respectively. In the context of our revised classification, this is equivalent to uniting Hahniidae and Cybaeidae.

“Austral Cribellates” or the “Fused Paracribellar clade”

Many species of this group are characterized by unusual arrangements of the paracribellar spigots within the posterior median spinneret (PMS) spinning field, typically with two to many shafts arising from common bases—the function of these multi-shaft spigot
arrangements, as opposed to the more widely encountered single shaft spigot, is unknown. The “Fused Paracribelllar Clade” was named by Griswold et al. (1999) and corroborated by Spagna and Gillespie (2008) (“Austral Cribellates”); this is now known to be one of the dominant spider clades in the southern hemisphere, but is represented by only a few invasives in North America. Of the families previously associated with the FPC, Agelenidae are here excluded: cribellate agelenids, e.g. Neoramia, are not related to true Agelenidae and phylogenies relying on Neoramia for agelenid placement (e.g. Griswold et al., 2005; Blackledge et al., 2009) are erroneous. We find a weakly supported group fairly coincident with the FPC clade (Desidae, Stiphidiidae, newly joined by Cycloctenidae), although the composition of these families will differ radically from former classifications (see Taxonomy) (Fig. 5).

**Cycloctenidae**

In our dataset, the genera *Cycloctenus* and *Toxopsisella*, traditionally placed in Cycloctenidae, group with high support (Fig. 5). The family may be expanded to a group with lower support to include *Paravoca* (formerly Amaurobiidae) and *Orepuikia* (formerly Agelenidae) plus *Pakeha* (formerly Amaurobiidae), which we circumscribe as Cycloctenidae *sensu lato*. The New Zealand genera *Pakeha* and *Orepuikia* are closely related, and all the cycloctenids in our analysis are restricted to Australia and New Zealand.

**Stiphidiidae**

Twelve taxa in our dataset formerly assigned to Stiphidiidae are distributed among a few families, rejecting the monophyly of traditional Stiphidiidae. The newly circumscribed Stiphidiidae recover a well-supported core Stiphidiidae, comprising the type genus *Stiphidion*, together with *Therlyna* and *Procambrigea*. The former Neolanidae *Marpleia* and *Neolana* (incorrectly synonymized with Amphinectidae; Jocqué and Dippenaar-Schoeman, 2006) group with the former agelenid *Neoramia*; these also refer to the core Stiphidiidae (*Neolanidae = Stiphidiidae, new synonymy*). *Aorangia*, a New Zealand genus formerly attributed to Amphinectidae, is related to core Stiphidiidae with moderate support (Fig. 5).

**Desidae**

We recognize a broadly circumscribed Desidae with weak support (Fig. 5). Classification of the “Austral Cribellates” or “FPC” remains one of the largest unsolved family-level problems in spiders, in spite of 30 years of effort, and we leave most uncertainty within our broad Desidae.

*Desis* is quite distinct from other genera placed in this family, and other genera unstably associated near *Desis*, i.e. *Poaka* and *Barahna*, do so with weak support. Forster and Wilton (1973) assigned many pre-existing and new genera to families first recognized from the northern hemisphere, e.g. Agelenidae or Amaurobiidae, but the presence of any true Agelenidae or Amaurobiidae native to the Australia–New Zealand region is doubtful. Davies took the most careful approach to classifying her new Australian genera, placing them with quantitative analyses (Davies, 1990, 1997, 1998), but the shortage of outgroups in most of her analyses renders the conclusions uncertain. We are also unable to test another of her discoveries, e.g. the Kababinia, for which no material was available. Within our loosely conceived Desidae, we identify at least five subgroups with stronger support. One could recognize as families the taxon clusters around *Desis*, *Amphinecta*, *Porteria*, *Metalabella* and *Paramatachia*, but this risks stranding many genera, particularly those not treated in our phylogeny, without family assignment. Instead, we retain the broad Desidae and treat subgroups as subfamilies.

**Subfamily Amphinectinae**

We have six exemplars representing four genera, including *Amphinecta*, which cluster within Desidae as Amphinectinae with weak support. All of our exemplars, *Amphinecta*, *Mamoea*, *Paramamoea* and *Rangitata*, are from New Zealand. We obtained the species cf. *Gasparia* sp. New Zealand CG105 within Amphinectinae in the preferred tree, but it falls among myroines (where *Gasparia* is currently listed) in the EW, IW, BI and DO analyses.

**Subfamily Metaltellinae**

The South American and Australian metaltellines, formerly attributed to Amphinectidae, but distant from amphinectines here, are easily recognized by a unique male palpal conformation. *Calacadia* and *Metalabella* group with high support. The enigmatic former stiphidiid *Ischalea* from New Zealand groups near these, representing Davies’ (1990) *Ischaleinae*.

**Subfamily Matachiinae**

*Matachia* and *Paramatachia* (our exemplar) are elongate spiders with large chelicerae sometimes likened to cribellate *Desis*. Their genitalia and spinning organs differ greatly from *Desis*, however. *Badumna* exhibit genitalia strikingly similar to *Paramatachia*, and the other genera that fall here are also similar. Matachiinae as a whole and all subgroups are strongly supported.
Subfamily Porteriinae

Five genera include some striking genital, somatic and behavioural similarities, regardless of their disparate distribution among families. *Porteria* (South America) runs atop its sheet web and the abdomen is boldly marked with guanine; *Corasoides* (Australia) could be a giant version of *Porteria*, with which they group with good support. *Cambridgea* and *Nanocambridgea* are closely allied, long-legged spiders from New Zealand that hang beneath their sheet webs. All four genera mentioned above have a unique configuration of the piriform gland spigot field on the anterior lateral spigots. *Baiami* (Australia) also hangs beneath sheet webs, and shares genital similarities with the above. The Porteriinae have strong support; *Porteria* remains in the Desidae; the other four genera are transferred here from Staphidiidae.

Evidence for combining the subfamilies and main groups of Desidae is weak and unstable across analyses. Scores of genera attributed to Desidae and Amphinectidae are not included in our dataset, so it would be premature to suggest finer familial subdivisions. In the Taxonomy section below, we reclassify several genera, but others we leave where they are because knowledge of their characters and phylogenetic placement remains poor.

Sparassidae

Huntsman spiders are difficult to place even though they have many synapomorphies (e.g. the fleshy, trilobate metatarsal dorsal tip) and are indisputably monophyletic (Moradmand et al., 2014); except for the sparianthines (first split in the family), all the rest have a unique tapetum for acute night vision, as a shiny, regularly perforated plate (Nørgaard et al., 2008). Phylogeny within the family has been investigated using DNA sequences (Agnarsson and Rayor, 2013; Moradmand et al., 2014) and morphology (Rheims, 2007; unpublished thesis); although our sample of 12 representatives is not very extensive, we recover the Heteropodinae and Deleninae with strong support (Fig. 6). Morphology places Sparassidae among the Dionycha (Ramirez, 2014) but molecular data are ambiguous and to date there is no clear association with other families, Dionycha or not; Moradmand et al. (2014) found sparassids near the base of the RTA clade, far from other Dionycha, a result mirrored by Agnarsson et al. (2013). Sparassidae, comprising 12 exemplars in our analysis, are recovered with high support. Unconstrained versions of our analyses occasionally grouped Sparassidae with Amaurobiinae (Amaurobiidae), but with weak support. In our constrained analysis, Sparassidae are possibly related to the combined OC clade and Dionycha, although this has very weak support as well. Sparassid placement remains a goal for future studies.

Oval Calamistrum clade and Dionycha

Molecular, morphological and total evidence analyses agree on associating the venerable Dionycha, or two-clawed spiders, with a more recently discovered clade (Griswold, 1993) characterized by a calamistrum with several rows of setae, typically forming a patch, hence the Oval Calamistrum clade. These spiders have numerous tarsal and metatarsal trichobothria, sharpening their vibration detection, most have extensive scopulae at least on the forelegs and usually claw tufts for feet adherence; and several clades have remarkable specializations of the eyes as befits fast moving, visually aware predators. Each clade has good support, i.e. the OC Clade and the Dionycha, and their sister group status is strongly supported (Fig. 6); these higher groups are also obtained in the transcriptomic analysis of Garrison et al. (2016), but we obtain higher support values with our target genes without applying a constrained skeleton tree (see Figs S6 and S7). This may be related to the position of Homalonychidae, which we believe is an artefact of the TR analysis (see above).

The OC clade

Phylogenetic studies (e.g. Griswold, 1993) have associated two remarkable morphologies: a cibellum comprising several lines or a patch of modified setae and an autospasy joint through the bases of the tibia of males (“male tibial crack”). A series of studies based on morphology grouped the Lycosoidae, including Lycosidae, Pisauridae, Oxyopidae and Ctenidae, with the less known Trechaleidae, Senoculidae and even the cribellate Psechridae, Tengellidae, Zorocratesidae and Zoropsidae (see Raven and Stumkat, 2005; Piacentini et al., 2013). A recent total evidence phylogeny (Polotow et al., 2015) differs in some details from our tree: the findings of Polotow et al. (2015) are used as the basis for our classification here.

The Udubidae are well supported (Fig. 6); our exemplars comprise *Raecius* from Africa and two species of *Uduba* and three of *Zorodictyna*, all from Madagascar. For the Zoropsidae, we found good support for several of the subfamilies as found in Polotow et al. (2015), but the family as a whole fluctuates across analyses (Fig. 6). The cribellate Griswoldiinae, *Griswoldia* and *Phanotea*, from South Africa ally with strong support. *Tengella*, *Zorocrates* and *Liocranoides* unite as Tengellinae with strong support; *Kilyana* and *Uliodon* unite as Uliodontinae, again with high support, to which are allied *Austrotengella*, forming a moderately supported Australia and New Zealand clade. The
analyses are unstable for the relationships among these three subfamilies, the South American Cinifella and Zoropsis, and in some of them Udubidae are nested within Zoropsidae, although with negligible support. This extends to all the branches near the base of the OC clade.

Ctenidae are a large, mainly tropical family that has been persistently hard to circumscribe. Griswold (1993) made the first quantitative attempt using a few exemplars, and the first extensive phylogeny was produced by Silva-Dávila (2003). Polotow et al. (2015) provided the first analysis including sequence data and morphology. We find a core Ctenidae of Anahita, Ctenus and Phoneutria grouped with high support (Fig. 6). Ctenidae continues to be a problematic taxon. Polotow et al. (2015) solved some problems by revealing that Vulsor, Viridiasus and some other genera from Madagascar are not ctenids at all but belong among the Dionycha as family Viridasiidae, a result corroborated here (see below). Other genera traditionally placed in Ctenidae also fall outside this family. The model genus Cupiennius here allies with Trechaleidae with good support, and these together ally with the Lycosidae, again with strong support. This is similar to the result from Polotow et al. (2015), with Cupiennius allied to Lycosidae and a paraphyletic Pisauridae. Ancylopelotes bogotensis sits alone as sister to the higher Lycosoidea, but with low support; Ancylopelotes placement is unsolved here, except as Lycosoidea, and its affinities remain another goal for future studies.

The wolf spiders (Lycosidae), fishing and nursery web spiders (Pisauridae) and lynx spiders (Oxyopidae) are well known Lycoidea. Most are cursorial hunters relying on their excellent eyesight to find prey, but at least some members of each family construct sheet webs, which may have evolved in parallel (e.g. Murphy et al., 2006). Lycosidae, represented by 17 exemplars, are recovered with good support and in turn are related with the clade Trechaleoides (Trechaleidae) plus Cupiennius (“Ctenidae”) (Fig. 6). Sister group to this clade, with high support, we have Pisauridae, with low support, which may be divided into three subfamilies, each with high to good support: Halinae, comprising Hala and Tolma; Dolomedinae, comprising Bradystichus and Dolomedes; and Pisaurinae, represented here by Dendrolycosa, Eurychora, Paracladycnis, Pisaura and Nilus.

The East Asian Psechriderae are monophyletic only in some of our analyses, although with weak support, and usually ally with Thomisidae (Fig. 6). A grate-shaped tapetum and oval calamistrum place them in the OC Clade (Homann, 1971; Griswold, 1993; Griswold et al., 1999, 2005; Aagnarsson et al., 2012), but psechrids possess a bizarre combination of characters: true claw tufts in addition to a third tarsal claw at all life stages. All psechrids hang from cribellate sheet webs (Robinson and Lubin, 1979; Griswold et al., 2005: fig. 208A, B, D, E). Psechrider monophyly and placement deserve further study. Male genitalia and adult web types differ greatly. Psechrider placement was also dubious for Polotow et al. (2015), with their exemplar Psechrus placed within a paraphyletic Ctenidae, far from the Thomisidae. The more detailed analysis of Bayer and Schönhofer (2013) also obtained low support, if any, for the monophyly of psechrids.

Crab spiders (Thomisidae) are well represented in our analysis with 44 terminals (Fig. 6). All thomisids, except Borboropactus, group with good support; our two Borboropactus join these with weaker support, and then only in the BI, ML and C-ML analyses, resembling the results of Benjamin et al. (2008). We prefer to keep the more traditional Thomisidae sensu lato with weak support, but our results are also compatible with the split of a robust Thomisidae sensu stricto and a separate Borboropactidae as proposed by Wunderlich (2004). We also obtain a strongly supported and robust Thomisus group, including all genera with few or no cheliceral teeth, also including the bird-dropping genus Phrynanchae and the ant-specialists Aphantochilinae as obtained by Benjamin (2011). Our resolution inside Aphantochilinae is slightly different from the one obtained by Teixeira et al. (2013) using morphological data, but the conflicting clades are not strongly supported in either analysis. Of the thomisids retaining several cheliceral teeth (“stephanopines”), we obtain a lengthy multinode grade separated by branches with weak support. Homann (1971) suggested thomisid affinity to Lycoidea based on their eye structure (a grate-shaped tapetum as in lycosoids), but Griswold (1993) discounted Homann’s opinion. Sequence data settled the placement of crab spiders among lycosoids (Bayer and Schönhofer, 2013; Polotow et al., 2015), while the morphological data were slightly in favour of dionychans (Ramirez, 2014). Here, our data place Thomisidae firmly among the Lycosoidea.

In addition to the above stated problems with ctenid circumscription, the families Senoculidae and Oxyopidae are also problematic (Fig. 6). Polotow et al. (2015) found a monophyletic Oxyopidae, not surprising given the distinctive eye pattern and spinose legs of the lynx spiders. Senoculidae (the sole genus Senoculus) emerged from a long branch within Zoropsidae in Polotow et al. (2015), a dubious result and one different from our current analysis. Here, Senoculus is allied to some Oxyopidae, a result that harkens back at least to Griswold (1993). Several of our analyses find Oxyopidae diphyletic. Each clade (Tapinillus plus Petuetia and Oxyopes plus Hamataliwa) is recovered with high support, but these and Senoculus combine in different ways and with other lycosoid branches, always with weak support. It is possible that the long branch of
Senoculus is responsible for instability in the higher lycosoids.

Dionycha

We obtained a relatively robust Dionycha with moderate support, without the use of the backbone constraint (Figs 7 and 8). Some of the larger clades within dionychans are quite unexpected, and we are cautious in their interpretation. Three main clades compose dionychans in our tree: (A) Prodidomidae sensu stricto (see below) (Fig. 7); (B) a well-supported clade of Gnaphosoidea sensu lato (herein referred to as Dionycha part A), for lack of a better name, roughly equivalent to the morphology-based groupings of gnaphosoids delineated by Platnick (2002) or the Claw-Tuft Clasper and the Oblique Median Tapetum (Fig. 7). We obtain the family as strongly supported mirroring the results of Labarque et al. (2015), with a well-supported Anyphaenidae and Amaurobioidinae, and Josa as a basal split in Amaurobioidinae (Fig. 7).

Prodidomidae

The first and surprising split of dionychans is the Prodidomidae sensu stricto, containing three genera of Prodidomidae (Prodidomus, Austrodomus and Neozimiris) and three of Theuminae (cf. Tricongius sp. Argentina NP18-MR16, Lygromma and Chilongius), all with high support. The bizarre Australian Molycriinae, here represented by three species of Molycria and Myandra, fall distantly from other prodidomids, among other gnaphosoid-like taxa separated from Prodidomidae sensu stricto by several branches with high support; molycriines have extremely long ALS placed anteriorly, far removed from the other spinnerets (Platnick, 1990; Platnick and Baehr, 2006) (Fig. 7). We defer taxonomic action until other prodidomids are more adequately studied, especially Zimiris, which has an intermediate morphology between prodidomines and molycriines (Platnick and Penney, 2004), and is also bearer of a family-level group name.

Dionycha part A

This large clade includes the gnaphosoids, Trachelidae, Phrurolithidae, members of the poorly defined “Liocranidae” and, most surprisingly, Anyphaenidae plus Clubionidae (Fig. 7). The linking of these last two families in a group confirms Platnick’s (1990) suspicions due to their common absence of cylindrical gland spigots, but their inclusion among “gnaphosoids” is unexpected. The North American liocranid Apostenus serves as a link between these two families and the rest, in a well-supported clade.

Clubionidae

We obtain a strongly supported core Clubionidae except for the American Elaver, which group with Anyphaeninae with low support (Fig. 7); we believe that this is an artefactual result, as the DO analysis obtains a monophyletic Clubionidae, and Elaver have many morphological characters in common with clubionines (Ramirez, 2014), but a basal placement in the family seems plausible, as it is the only genus of clubionids that retains a median apophysis on the male palp (Saturnino and Bonaldo, 2015).

Anyphaenidae

We obtain the family as strongly supported mirroring the results of Labarque et al. (2015), with a well-supported Anyphaeninae and Amaurobioidinae, and Josa as a basal split in Amaurobioidinae (Fig. 7). Our larger sample of outgroups allowed the detection of a remarkable morphological convergence in the Chilean Male Bellina nana, the sole representative of Malenellinae, hereby transferred to Macrobuninae (Amaurobiidae).

Trachelidae

We obtain trachelids together but with weak support, probably due to two terminals floating nearby through the different analyses; one of them is Cithaeron, the only representative of Cithaeronidae, and the other is an unidentified Liocranidae from the Dominican Republic (Fig. 7). We obtain the minute soil dweller Orthobula deep inside Trachelidae, a possibility considered by Ramirez (2014) as suboptimal but still plausible; a re-examination of the foot morphology of Orthobula calceata revealed that they have the claw lever file projections interlocking with the claw tuft bases, uniquely found in some trachelids (Ramirez, 2014: character 171 and fig. 76D). With this evidence, we thus transfer the genus Orthobula from Phrurolithidae to Trachelidae.

Phrurolithidae

We obtained strong support for the family after the removal of Orthobula to Trachelidae, although our sampling is limited to two genera from North America and South East Asia (Scotinella and Otacilia, respectively). We confirm also that the Teutamus group, here represented by Teutamus and Sesieutes and joined with high support, are not phrurolithids, although its family association remains to be solved with a better sampling (Fig. 7).

Liocranidae

We were unable to get suitable samples of the South European Liocranum. Of our five representatives, one
is very unstable (Liocranidae sp. Dominican Republic MR160), two are species of the North American *Apostenus*, linked with good support to Anyphaenidae plus Clubionidae, and two are species of the Malagasy *Donnea*, which remain mysterious and only loosely associated to a few rogue terminals in Dionycha part B. Liocranids are poorly defined and are a target for future research (Fig. 7).

**Gallieniellidae, Trochanteriidae and Lamponidae**

We obtain a core group of the Malagasy *Gallieniella* and *Legendrena* with strong support (Fig. 7), but the Australian gallieniellids *Neato* and *Meedo* are grouped, also with high support, with five Australian trochanteriids (*Trachycosmus, Desogaphosa, Rebilus, Platorith and Morebilius*). These trochanteriids are in turn isolated from other Australian (*Tinytrema*), American (*Dolionalus*) and South African (*Platyoides*) genera placed in Trochanteriidae. Most trochanteriids are extremely flat bark-dwellers, but this is a syndrome that has occurred many times in spider phylogeny producing remarkable convergences, including the gnaphosid *Hemicloea* (see below). Ramírez (2014) already detected at least two unrelated groups of “trochanteriids” using morphological data. Our results are in partial agreement with this, in having the Australian trochanteriids in a basal split, and the African and South American genera more closely associated to Gnaphosidae, but otherwise gnaphosoids are quite mixed up. This is another target of future research, especially after the name-bearer genus *Trochanteria* is better studied. Our three lamponid representatives (*Lampona, Centrothele* and *Asadipus*) fall nearby but not together.

**Ammoxenidae**

We did not retrieve the South African ammoxenids *Rastellus* and *Ammoxenus* in a monophyletic group [Ramírez (2014) did not find morphological support for ammoxenid monophyly either], but obtain them nested within gnaphosids, together with *Eilica* and *Asemesthes* although with weak support (Fig. 7). This is biologically interesting; *Asemesthes* are sand-dwelling spiders, as are *Rastellus* and *Ammoxenus*, and *Eilica* are specialized feeders on ants, while *Rastellus* and *Ammoxenus* specialize on termites.

**Gnaphosidae**

We included 14 genera placed in Gnaphosidae, with a wide geographical and morphological breadth (Fig. 7). Our results are daunting, as they are all involved in weakly supported, unstable groups, mixed with other gnaphosoid families. The three *Hemicloea* species, all flat bark dwellers from Australia, are joined with moderate support with *Intruda*, a typical gnaphosid from Australasia, thus confirming the placement suggested by their gland spigot morphology (Platnick, 1990). It seems that Gnaphosidae will have to be profoundly reorganized, but this will have to wait for a better taxon and character sampling. A comprehensive morphological phylogenetic assessment of gnaphosids and their kin is underway (G. Azevedo, pers. comm.).

**Dionycha part B**

This clade includes the Salticidae, Philodromidae, Eutichuridae, Miturgidae, Corinnidae, Selenopidae, Viridasiidae and the new family Xenocenidae. The group is strongly supported and robust, even if not constrained by the transcriptomic backbone (only represented by the salticid *Habronattus* in the study of Garrison et al., 2016). The internal relationships among families have, however, weak support and are unstable (Fig. 8).

**Salticidae**

Jumping spiders are well supported and robust (Fig. 8). Our results agree in general with those of Maddison and collaborators for the family (see Maddison et al., 2014, 2016; Maddison, 2015), using a larger taxon sampling and more markers. The three subfamilies Lyssomaninae, Onomastinae and Asemoninae are joined in an unstable group with low support. The Spartaenae and a clade of Hisponinae plus Salticinae are strongly supported, although we did not recover the Hisponinae, probably because of shallow taxon sampling (*Hisco joins the Salticinae*, leaving *Massagris* behind). The sister group to salticids is, unfortunately, not solved by our analyses and will remain a subject of future studies.

**Philodromidae**

The family is strongly supported and robust (Fig. 8). We recover the tribe Thanatini with good support, represented by *Tibellus* and *Thanatus*, sister to a group of all other philodromids with moderate support, but this is unstable and does not agree well with the morphological analysis of Muster (2009). The two Hawaiian endemics *Pedinoptista* and *Pagiopalpus* are together with good support, thus suggesting a single colonization event to the islands.

**Eutichuridae**

A core Eutichuridae is retrieved with strong support and robustness; the entire family including *Eutichurus,*
however, is only retrieved in the BI analysis and the MUSCLE alignments (EW, IW), with very low support; in DO and ML Eutichurus floats around a few rogue terminals of unclear affiliation (see the Pronophaea group). Four species of Cheiracanthium group with strong support (Fig. 8).

**Xenocentidae**

We reproduce previous morphological results of Silva-Dávila (2003) and Ramírez (2014) by obtaining a strongly supported clade of three lycosoid-looking genera with a distal division in the tegulum of the male copulatory bulb (Xenocenus, Odo, Paravulsor). These were previously assigned to Zoridae and Miturgidae for lack of a better affiliation (see the Xenocenus group in Ramírez, 2014). This analysis has a wide taxonomic coverage allowing us to formalize the new family here (Fig. 8).

**Viridasiidae**

A core Viridasiidae is obtained with strong support, and resting among the Dionycha, confirming the results of Polotow et al. (2015) for this recently established Malagasy family (Fig. 8). We included an undescribed genus from Madagascar (Viridasiidae sp. Madagascar CG28) that is probably allied to the family as well.

**Selenopidae**

The flatties, or Selenopidae, are well supported, with two to three successive basal splits from Africa and Madagascar (Anyphops, Hovops, Garcorops); the remaining terminals in our analysis are three American Selenops species (Fig. 8). This is in agreement with the much more detailed findings of Crews and Gillespie (2010).

**Miturgidae**

The family is retrieved with weak support, probably because of several rogue terminals of unclear association (see below). Two genera of former zorids (Zora, Argoctenus) plus a zorid-like undescribed genus (Miturgidae sp. Queensland MR629) are grouped as sister to three Australian miturgines (Miturga, Mituliodon, Nuliodon). The American miturgines Teminius and Syspira are on a basal split. According to our results, the enigmatic African genus Parapostenus could be a miturgid or a viridasiid (ML and BI analyses, respectively) (Fig. 8).

**Corinnidae**

We included a good breadth of Corinnidae (21 representatives of 20 genera), and recovered the family as a group, confirming its separation from the Trachelidae and Phrurolithidae as proposed by Ramírez (2014). Corinnidae are obtained with weak support; the subfamily Castianeirinae, well known from the many ant-mimicking genera, is moderately supported, and the recently described genus Allomedmassa is suggested as its sister group. Although the details differ, we confirm the findings of Haddad (2013) that the non-ant-mimicking castianeirines (Copa sp. MR46 and Echinax, in our dataset) have derived from an ant-mimicking ancestor through loss of myrmecomorphy (Fig. 8).

**The “Pronophaea” group**

A few corinnid-looking genera retaining a median apophysis on the male copulatory bulb were loosely grouped in the Pronophaea group by Ramírez (2014), although it is not clear that they are all closely related to each other. In that study, it was rejected that they were basal corinnids, as proposed before by Bonaldo (1997) and Ramírez et al. (2001). We include here two species of Pronophaea, two of Donuea, and one each of Olbus and Carteronius. The congeners grouped with high support, but besides a weak but consistent grouping of Donuea with Pronophaea (all analyses except EW) and sometimes also Olbus (BI), our study only indicates that they belong to the Dionycha part B (Fig. 8); solving their affiliation remains a future goal.

**Taxonomy**

**Family Agelenidae C. L. Koch, 1837**

Type genus Agelena Walckenaer, 1805 (type species Araneus labyrinthicus Clerck 1757).

**Diagnosis.** RTA clade spiders with a divided colulus, i.e. with two separate patches of hairs, and, in most species, PLS with the apical segment pointed and greater than or equal to the length of the basal; the cribellate genus Tamgrinia may be recognized by details of the genitalia, as presented by Wang (2000).

**Composition.** The WSC (2016) lists 73 genera and 1195 species in this family. As discussed below, it is unlikely that 13 genera and nearly 100 species endemic to Australia and New Zealand truly belong here, but even discounting these, the more than 1000 species distributed across Africa and the Holarctic region make this one of the largest families of spiders.

**Remarks.** Lehtinen (1967) noted that the presence of a divided colulus, i.e. with two separate patches of hairs, allows recognition of the family. This bifid
colulus serves as an agelenid synapomorphy (Lehtinen, 1967; Spagna and Gillespie, 2008; J. Miller et al., 2010) for the taxa represented in this study and include Ageleninae, Textricinae, Tegenariinae and Coelotinae. As pointed out by J. Miller et al. (2010) and in this study, at least the New Zealand Neoramina, a cribellate “agelenid” exemplar (Griswold et al., 2005; Blackledge et al., 2009), is not an agelenid; other austral agelenid genera may also be misplaced. At least 13 genera, most described by Forster and Wilton (1973), are recorded as endemic to New Zealand (WSC, 2016). These authors note that “the ecribellates ... do not possess a divided colulus. The colulus is then a more or less triangular structure, and the hairs are not separated into two bunches” (Forster and Wilton, 1973: 11). Absence of the agelenid synapomorphy, plus placement elsewhere by our study for some of the New Zealand genera, e.g. Neoramina to Stiphidiidae, Orepkia to Cycloctenidae, suggests that none of the New Zealand genera may belong in true Agelenidae. We defer transfer of these genera to other families until they can be studied in more detail, and preferably with molecular data.

Family Amaurobiidae Thorell, 1870 (new circumscription)

Type genus Amaurobius C. L. Koch, 1837 (type species Aranea fenestrallis Ström 1768).

Diagnosis. This diverse and probably non-monophyletic taxon is difficult to diagnose. All are entelegyne Araneomorphae in the RTA clade, and most have male palp tibial processes in addition to male palpal tibial processes in addition to the RTA (Griswold et al., 2005). Amaurobiinae have a “pseudocalamistrum”, a row of prominent setae adjacent and parallel to the calamistrum (Lehtinen, 1967: 321), only three remain: Altellopsinae, Amaurobiinae and Macrobuninae. To these have subsequently been added Arctobiinae, Midgeeinae and Ovtchinnikoviinae. The enigmatic Altellopsinae Lehtinen, 1967 is too poorly known to diagnose or even confirm as an amaurobiid, whereas affinities of the Arctobiinae and Ovtchinnikoviinae remain to be tested: we provisionally leave them here for lack of a better alternative. The Macrobuninae are discussed in detail below. Our results suggest that some of Forster and Wilton’s (1973) austral amaurobiid genera are misplaced: we move Pakêha and Paravaoco to Cycloctenidae and Poaka to the Desidae. Ischalea (from Stiphidiidae) is transferred to Desidae Ischaleinae: our phylogeny places Ischalea within Desidae near Metaltellinae. Davies (1990: 102) suggested that Ischalea, Bakala and Manjala form the Ischaleinae. Although the latter genera are listed in Amaurobiidae (WSC, 2016) we transfer all to Desidae Ischaleinae. Midge and Jamara [placed in Amaurobiidae, Midgeeinae by Davies (1995: 93)] are here transferred to Toxopidae Toxopinae. Pending resolution of the taxonomic situation for Macrobuninae, there may be no true amaurobiids native to the southern hemisphere. See below for a discussion of Macrobuninae, including the new synonyms Tasmarubiinae (Amphinectidae), Malenellidae (Anyphaenidae) and Chummidae.

Subfamily Macrobuninae Lehtinen, 1967

Type genus Macrobunus Tullgren, 1901 (type species Myro backhauseni Simon, 1896).


Diagnosis. Entelegyne RTA clade spiders with a single row of tarsal trichobothria distinguished by the presence of denticles on the cheliceral retromargin (Almeida-Silva et al., 2015: fig. 2E), an oblique cheliceral groove (e.g. Griswold et al., 2005: 231; fig. 130G, I) and reduced anterior median eyes (Almeida-Silva et al., 2015: figs 1A, 2A–B).
Composition. Macrobuininae comprise taxa from Australia, western North America, South Africa and South America. Currently included are Anisacate, Auximella, Callevopsis, Cavernocymbium, Chresiona, Chumna, Emmenomma, Hicanodon, Livius, Macrobusus, Malenella, Naevius, Neoporertia, Obatala, Parazanomys, Pseudauximus, Retiro, Rubrius, Tasmarrubrius, Tasnabrochus, Teeatta, Urepus, Yupanquia and Zanomys.

Remarks. Lehtinen (1967: 333) recognized the distinctness of a group of genera related to Macrobunus. Compagnucci and Ramírez (2000) discovered a gland in the male palpal tibia of Anisacate, Emmenomma and Naevius, and Griswold et al. (2005) noted a stridulatory area on the retrobasal area of the cymbium of Anisacate, Emmenomma, Macrobusus and Rubrius, comprising characters that may be useful in outlining the phylogeny of the group. J. Miller et al. (2010) treated the macrobunine Chresiona in a molecular phylogeny and found Chresiona to fall far from the core amaurobiids, represented by Amaurobius, Callobius and Pimus, and also found the South African Chumna and North American Cavernocymbium and Zanomys closely allied to Chresiona. Although noting the distinctness from the core Amaurobiidae, no taxonomic changes were made. Malenella, formerly Anyphaenidae, and Tasmarrubrius, formerly Amphinectidae, are newly transferred here. Macrobuinine placement and taxonomy are currently under study by Almeida-Silva (pers. comm.), who has provided diagnostic characters and putative synapomorphies (Almeida-Silva, 2013; unpublished thesis; Almeida-Silva et al., 2014, 2015). Macrobuininae is provisionally kept in Amaurobiidae.

Family Cybaeidae Banks, 1892 (new circumscription)

Type genus Cybaeus L. Koch, 1868 (type species Amaurobius tetricus C. L. Koch, 1839).


Diagnosis. Araneomorphs with three claws and branched tracheae; most have an apophysis on the male palpal patella in addition to the RTA.

Composition. This family comprises several genera from the Holarctic, and especially genera endemic to North America. The family Cybaeidae has been revised in North America (Bennett, 1991; unpublished thesis; Bennett et al., 2016).

Remarks. Our exemplars include Cybaeus and Calymmria, the latter transferred here from Hahniidae. Closely related to Cybaeus are Cybaeina, Cybaeota and Cybaeozyga (Lehtinen, 1967; Bennett, 1991; unpublished thesis). Calymmria has a peculiar form of autospasy, with a break through the leg patella: this was recognized by Roth and Brame (1972), who considered this a synapomorphy for a group of several poorly known genera. Following their suggestion, we include other genera with patellar autospasy: Cryphoea, Ethobuella and Willisius (from Hahniidae) and Blabomma and Yorima (from Dictynidae) are here transferred to Cybaeidae. Based on our results and those of J. Miller et al. (2010) and Spagna et al. (2010), we transfer Argyroneta from Cybaeidae to Dictynidae.

Family Cycloctenidae Simon, 1898 (new circumscription)

Type genus Cycloctenus L. Koch, 1878 (type species Cycloctenus flaviceps L. Koch, 1878).

Diagnosis. Araneomorphs with three claws, simple posterior tracheae, a complex RTA and a hyaline conductor on the male palp. At least Cycloctenus and Toxopsiella have a strongly recurved anterior eye row, and Cycloctenus a latergrade habitus, resembling Ctenidae.

Composition. All the classic and newly assigned genera occur in New Zealand and Australia.

Remarks. Our exemplars comprise the classic (Lehtinen, 1967; Forster and Wilton, 1973) cycloctenids Cycloctenus and Toxopsiella. To these we add Orepuia (from Agelenidae) and Pakeha and Paravoca (from Amaurobiidae). There may be other taxa currently placed in Agelenidae and Amaurobiidae that more appropriately conform to Cycloctenidae.

Family Desidae Pocock, 1895 (new circumscription)

Type genus Desis Walckenaer, 1837 (type species Aranea maxillosa Fabricius 1793).


Diagnosis. Araneomorphs with three claws, tarsal trichobothria, cribellate or ecribellate. Our phylogenetic concept brings together a diverse set of spiders, including some with simple and some with complex, branched posterior tracheae. The male palpal tibia of most included taxa has a complex RTA, with several separate processes (Matachiinae) or separate distal and proximal processes (Metaltellinae and many Amphinectidae). The included subfamilies have strong diagnostic characters, e.g. the peculiar palpal bulbs of...
metaltellinae and matachiines and narrowed piriform gland spigot field on the ALS of Porteriinae.

**Composition.** In addition to Desis, which occur worldwide on tropical seacoasts, our Desidae occur in Australia and New Zealand and nearby parts of Asia and in southern South America. We recognize Desis and the subfamilies Metaltellinae, Matachiinae, Amphinectinae, Ischaleinae and Porteriinae (new rank).

**Remarks.** Our dataset includes numerous exemplars that we group together in our enlarged Desidae. In addition, we follow the suggestions of authors to include presumed close relatives of our exemplars. Desis, which branches basally in our enlarged Desidae, is the most divergent genus; we could establish it as a monotypic family and retain (Amphinectidae) or establish family rank for the several related subfamilies, e.g. “Metaltellidae” and “Porteriidae”, but the low support values for the basal branches and numerous additional taxa not treated give us pause, and we feel the most cautious and conservative approach is to recognize a large, polythetic Desidae. On our tree, Desis is close to Poaka, which is transferred from Amaurobiidae to Desidae. Our exemplars include the former amphinectids Amphinecta, Manoeea, Manihoo, Paramanhoee and Rangitata; along with Manihoo, as suggested by Forster and Wilton (1973), these are placed in Desidae Amphinectinae. Our phylogeny also places Barahna in Desidae Amphinectinae, transferred from Stiphidiidae. Our exemplars of Amphinectidae Metaltellinae (Calacadia and Metalletella) form a group transferred to Desidae Metaltellinae; to these we add Austmusia, Buyina, Cunnawarra, Jalkaraburra, Keera, Magua, Penaoela and Quemusia, following the classification of Davies (1998). Ischalea falls near the metaltellines; we transfer this genus from Stiphidiidae, along with Bakala and Manjala from Amaurobiidae, to comprise Desidae Ischaleiinae (Davies, 1990; 102). Our exemplars Goyenia, Badunna, Matachiinae sp. Queensland CG275 and Paramanatachia form a group; these we add Matachia, Notomatachia and Nuisiana, following Forster (1970) and Griswold et al. (1999, 2005), to comprise the Desidae Matachiinae. Finally, to the Chilean desid genus Porteria, we add Baitami, Cambridgea, Corosoides and Nanocambridgea from Stiphidiidae to comprise Desidae Porteriinae, a group first recognized by Lehtinen (1967, as Porteriini) and that is currently under study (Morrill, 2014; unpublished thesis).

**Family Dictynidae O. Pickard-Cambridge, 1871**

**Diagnosis.** Araneomorphae with three claws and branched median tracheae; cribellate or ecribellate. All have a characteristic male palpus: the conductor is fleshy, and embraces the embolus, and the conductor apex extends proximally along the retrolateral side of the cymbium.

**Composition.** A worldwide taxon of cribellate web builders; ecribellates appear to be associated with water or former wet places, i.e. Argyroneta, Saltonia and Paratheauma.

**Remarks.** The family Dictynidae has long been a receptacle for a miscellany of cribellate and ecribellate RTA clade spiders. Cribellate Dictynidae are easy to recognize and seem to be a natural group: all have the characteristic male palpus, and many males have chelicerae that are concave medially. With our transfer of many ecribellates to other families, e.g. of Blabomma and Yorima to Cybaeidae, Dictynidae become more consistent and the remaining ecribellate genera also have the characteristic male palpus. Spagna and Gillespie (2008), J. Miller et al. (2010) and Spagna et al. (2010) have explored the molecular phylogenetics of the RTA clade and our results corroborate many of theirs. We find support for inclusion of Saltonia and Paratheauma, and based on our results and those of J. Miller et al. (2010) and Spagna et al. (2010), Argyroneta is transferred from Cybaeidae to Dictynidae. The cribellates Mexitlia, Dictyna and Mallos fall in the Dictynidae; Lathyia also groups here but with little support.

**Family Hahniiidae Bertkau, 1878**

Hahniiidae Bertkau, 1878. Type genus Hahnia C. L. Koch, 1841 (type species Hahnia pusilla C. L. Koch, 1841).

**Diagnosis.** Araneomorphae with three claws, an RTA and in many species a process on the patella as well; typical Hahniiinae have the spinnerets in one transverse row.

**Composition.** Hahniiinae are worldwide; Cybaeolinae (Cybaeolus) are endemic to southern South America; Cicurina is Holarctic.

**Remarks.** Our dataset includes the hahniiid exemplars Hahnia and Neoantistea (Hahniiinae), Cybaeolus (Cybaeolinae) and Calymmaria (Cyphoeicinae). We find that the Hahniiinae and Cybaeolinae are valid members of Hahniiidae, but that Calymmaria, along with Cyphoea, Ethobuella and Willius, must be transferred to Cybaeidae (see
above and J. Miller et al., 2010). *Cicurina*, also in our dataset, is transferred from Dictynidae to Hahniidae.

**Family Pacullidae Simon, 1893 (restored status)**

Type genus *Paculla* Simon, 1887 (type species *Phaedima granulosa* Thorell, 1881).

Pacullinae, Lehtinen, 1981.

**Diagnosis.** Ecribellate, three-clawed Synspermiata with six eyes, similar to Tetrablemmidae by the abdomen with dorsal and ventral scuta and laterally with thin, sclerotized strips or lines of platelets, distinguished from those by the much larger size (greater than 5 mm length), heavily rugose cuticle and by lacking a pair of large membranous receptacles in the female genitalia (Shear, 1978; Lehtinen, 1981).

**Composition.** Four genera, all from Southeast Asia: *Paculla*, *Perania*, *Lamania* and *Sabahya* (Lehtinen, 1981; WSC, 2016).

**Remarks.** Our matrix includes the paculline exemplars *Paculla* sp., *Lamania* sp. and *Perania nasuta*, which form a family group distinct from the tetrablemmines and sister to the Diguettidae within the “lost tracheae clade” (Diguettidae, Pacullidae, Plectreuridae and Tetrablemmidae).

**Family Stiphidiidae Dalmas, 1917 (new circumscription)**

Type genus *Stiphidion* Simon, 1902 (type species *Stiphidion facetum* Simon, 1902).


**Diagnosis.** Three-clawed RTA clade araneomorphs with a simple posterior respiratory system of four tubes that may be cribellate or ecribellate; cribellates have PMS paracribellars with multiple shafts arising from single, enlarged bases. A comprehensive diagnosis is not yet possible, but potential diagnoses are made in the original descriptions of the subfamilies Borralinae, Kababinae and Neolaninae and by the characters of included genera.

**Composition.** Stiphidiidae incertae sedis comprise *Aorangia*, *Neoramia*, *Procambridgea* and *Stiphidion*; Neolaninae comprise *Marplesia* and *Neolana*; Borralinae comprise *Borrala*, *Couranga*, *Elleguna*, *Jamberoo*, *Karriella*, *Pillara* and *Therlinya*; Kababinae comprise *Carbinea*, *Kababina*, *Malarina* and *Wabua*.

Remarks. Lehtinen (1967: 331) placed the group as a subfamily of Amaurobiidae; Forster and Wilton (1973: 128) raised them to family. Our phylogeny suggests several changes in stiphidiid composition: *Neoramina* (from Ageilenidae) and *Aorangia*, *Marplesia* and *Neolana* (from Amphinectidae) transferred to Stiphidiidae; *Batami*, *Cambridgea*, *Corasoides* and *Nanocambridgea* transferred to Desidae Porteriinae, where they are closely related to *Porteria* (Morrill, 2014; unpublished thesis); *Ischalea* is transferred to Desidae Ischaleinae. Our matrix includes the incertae sedis genera *Aorangia*, *Neoramia*, *Procambridgea*, *Stiphidion*, *Marplesia* and *Neolana* from the Neolaninae; and *Therlinya* from the Borralinae. We use the discussion of Gray and Smith (2008) to place the other borraline genera, and also to associate Kababinae with Borralinae.

**Family Tetrablemmidae O. Pickard-Cambridge, 1873 (new circumscription)**

Type genus *Tetrablemma* O. Pickard-Cambridge, 1873 (type species *Tetrablemma medioculatum* O. Pickard-Cambridge, 1873).

**Diagnosis.** Ecribellate, three-clawed Synspermiata with six to no eyes, similar to Pacullidae by the abdomen with dorsal and ventral scuta and laterally with thin, sclerotized strips or lines of platelets, distinguished from those by the much smaller size (less than 3 mm length), smooth cuticle and by having a pair of large membranous receptacles in the female genitalia (Shear, 1978; Lehtinen, 1981).


Remarks. Our matrix includes the tetrablemmine exemplars *Indicoblemma monticola*, *Tetrablemma thamin* and *Shearella browni*, which form a family group distinct from the pacullines and sister to the remaining families of the “lost tracheae clade” (Plectreuridae, Diguettidae and Pacullidae).

**Family Toxopidae Hickman, 1940 (restored status)**

Type genus *Toxops* Hickman, 1940 (type species *Toxops montanus* Hickman, 1940).

Toxopidae Hickman, 1940 (included in Desidae by Forster and Wilton, 1973).
Diagnosis. Three-clawed RTA clade cribellate (Jamara) or ecribellate Araneomorphae with highly branched posterior median tracheae. Toxopines are small spiders, many laterigrade, that have a T-shaped branched posterior median tracheae. Toxopines are transferred to Toxopidae Myroinae. Our exemplars Desidae) group together in our phylogeny, far from the other analyses. Forster (1970) associated the Desidae in the ML trees, but with myroines in most placed Gohia Myro... McCallum. Mooreines may have strongly recurved eye rows (Myro and Ommatauxesis); all have vulvae with slender, convoluted copulatory ducts.

Composition. Two subfamilies, Toxopinae (Hapona, Jamara, Laestrygones, Lamina, Midgee, Toxops and Toxopsoides) and Myroinae (Gasparia, Gohia, Hulua, Neomyro, Myro, Ommatauxesis and Otagoa), occur in Australia and New Zealand. Myro also occurs on subantarctic islands around the Southern Ocean.

Remarks. Myro, Ommatauxesis and Otagoa (all from Desidae) group together in our phylogeny, far from the taxa that we place in Desidae. Gasparia groups among the Desidae in the ML trees, but with myroines in most other analyses. Forster (1970) associated Gasparia with Myro, and we follow his suggestion. Forster (1970) also placed Gohia, Hulua and Neomyro near Myro (in Myroinae, sic.). Gasparia, Gohia, Hulua, Neomyro, Myro, Ommatauxesis and Otagoa (all from Desidae) are transferred to Toxopidae Myroinae. Our exemplars include Toxops, Hapona, Laestrygones and Lamina, which fall together in a group with the Australian amaurobiid Midgee. We transfer Midgeeinae (Midgee plus Jamara) from Amaurobiidae and Hapona, Laestrygones, Lamina, Toxops and Toxopsoides, all from Desidae, to Toxopidae Toxopinae.

Family Trachelidae Simon, 1897 (new circumscription)

Diagnosis. Trachelids are similar to prurolithids in having claw tufts arising from a non-articulated area of the distal tarsi, made of heavily folded setae, tarsal claws with a claw tuft clasper and reduced leg spination especially on posterior legs and dorsally on all femora, and lacking a median apophysis on the male copulatory bulb. Most trachelids (except Orthobula) can be distinguished by lacking a ventral distal hook on the male palp femur. Most trachelids have also uniquely shaped bases of the claw tuft setae, in the form of rectangular blocks, and frequently the claw lever file projections interlock with the claw tuft bases. With a few exceptions such as Orthobula and Spinotrachelas, most of the trachelid species lack macrosetae altogether, and the males have leg cusuples.


Remarks. Orthobula is transferred from Prurolithidae to Trachelidae.

Family Prurolithidae Banks, 1892 (new circumscription)

Diagnosis. Prurolithids are similar to trachelids in having claw tufts arising from a non-articulated area of the distal tarsi, made of heavily folded setae, tarsal claws with a claw tuft clasper and reduced leg spination especially on posterior legs and dorsally on all femora, and lacking a median apophysis on the male copulatory bulb. They can be distinguished by having ventral modifications on the male palp femur, especially a ventral median apophysis and usually a ventral apical hook. Prurolithids (except Drasinella) have a globose, often flexible receptacle on the female internal genitalia, in addition to the primary and secondary spermathecae (this receptacle is also present in the trachelid Orthobula). Prurolithids differ from most trachelids by having several pairs of ventral macrosetae on the anterior tibiae, and by lacking characters unique to trachelids (projections of the claw lever file interlocking with claw tuft bases, expanded bases of the claw tuft setae).

Composition. Abdosetae, Dorymetaecus, Drasinella, Liophurillus, Otacilia, Phonotimpus, Prurolinillus, Prurolithus, Phruronellus, Phrurotimpus, Piabuna, Plymon and Scotinella.

Remarks. Orthobula is transferred from Prurolithidae to Trachelidae.

Family Xenocenidae Ramírez and Silva-Dávila, new family

Type genus Xenoctenus Mello-Leitão, 1938 (type species Xenoctenus unguiculatus Mello-Leitão, 1938) by present designation. Xenoctenus group, Ramírez, 2014.

Diagnosis. Xenocenids are similar to viridasids and some miturgids by having the eyes in two recurved rows and with grate-shaped tapetum in the indirect eyes, two claws and well-developed scopulae and
sometimes claw tufts, but can be distinguished by having a distal division in the tegulum of the male copulatory bulb, in the region where the embolus arises.


Remarks. The group was already distinguished as deserving family-level status by Silva-Dávila (2003), and later confirmed by Ramírez (2014) as the ‘*Xenoctenus* group’; both authors identified the tegular distal division at the embolar base as a synapomorphy (see Ramírez, 2014: character 343). This analysis confirmed with high support that Xenocenididae are members of Dionycha. The closer relatives to xenocenids are, however, uncertain in this and previous analyses, but their monophyly is well supported by all these analyses.

Summary

The present paper is the culmination of years of intensive research on spider systematics and, although mainly based on the findings of the spider AToL project, also summarizes relevant findings from the last decade of systematic studies of that group. Our results reach far into the spider evolutionary tree and many branches are reorganized or newly discovered. We hope that these new insights on spider relationships will stimulate a plethora of follow-up studies on spider evolution and systematics, especially for the areas of the spider tree where we still have too little information to gain insight on taxonomic relationships. We also show the relevance of target gene approaches in the phylogenomics era and how both can be used to build on each other’s findings. In our study, the unprecedented taxon sampling would have been very challenging in a phylogenomic framework whilst recent phylogenomic studies have been invaluable assets in improving our results.

Acknowledgements

This work was primarily supported by a grant from the United States National Science Foundation (NSF) (EAR-0228699—‘Assembling the Tree of Life: Phylogeny of Spiders’) awarded to W. C. Wheeler (P.I.) and J. Coddington (JC), G. Hormiga (GH), L. Prendini (LP) and P. Sierwald (PS) (co-P.I.s). JC acknowledges support from the NMNH Neotropical Lowlands Program, Small Grants Program and Biodiversity of the Guyanas Program, as well as the NSF grants DEB-9712353 (GH, P.I.; JC, co-P.I.) and DBI-0956426 (N. Davies, P.I.; G. Roderick, C. Meyer, JC and T. Orrell, co-P.I.s). Various aspects of this research in the GH Lab at GWU were supported by NSF awards DEB-0328644, DEB-1144492 and DEB-1457300 (to GH and Gonzalo Giribet), by NSF award DEB-0613928 (to N. I. Platnick (P.I.), R. Gillespie, CEG, GH, and PS (co-P.I.s)) and by three NSF REU supplementary grants. GH’s stay at the Scharff’s lab in the Zoological Museum (University of Copenhagen) was supported by a Villum Kann Rasmussen Fund, VELUX Visiting Professorship. Further support to GH for this project was provided by several awards from GWU. LL acknowledges the Cosmos Club Foundation Program of Grants-in-Aid to Young Scholars and a Weintraub Fellowship from The George Washington University. The participation in this project of F. Álvarez-Padilla, L. Benavides, S. Benjamin, D. Dimitrov and E. Hasan was made possible in part by support of The George Washington University and the aforementioned NSF awards to GH. F. Álvarez-Padilla acknowledges support from a scholarship from the Consejo Nacional de Ciencia y Tecnología de Mexico (CONACYT).

Aspects of this research conducted in the LP lab at the AMNH, including field collections that contributed material to the project, were supported by NSF awards DEB-0413453 (to LP and W. D. Sissom) and DEB-0640219 (to LP and P. E. Cushing), by a grant from the Richard Lounsbery Foundation (to R. DeSalle, LP and M. E. Siddall), and a Constantine Niarchos Expedition Grant (to LP). PS’s fieldwork in South Africa and Myanmar was supported by Field Museum’s Marshal Field Fund.

C. E. Griswold (CEG) and the CAS Arachnology Lab (L. Almeida-Silva, F. Álvarez-Padilla, J. Ledford, D. Silva-Davila, D. Polotow, F. Labarque, D. Ubick and H. Wood) acknowledge financial support from the CAS Exline-Frizzell Fund for Arachnological Research and CAS Lindsay Expedition Fund and by The Schlinger Foundation; D. Polotow is grateful for CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico (PDE programme), D. Polotow and L. Almeida-Silva both acknowledge Bill and Maria Peck Fellowships (CAS); and J. Ledford, D. Silva-Davila, F. Álvarez-Padilla, F. Labarque and L. Almeida-Silva acknowledge Schlinger Chair of Arachnology Postdoctoral Fellowships from CAS. T. Szüts’ participation was funded by a Marie Curie Intra European Fellowships (Life Sciences Panel, No. 025850) and he acknowledges support from the OTKA/NKFI 106241. Aspects of this research were supported by NSF grants: DEB-0072713 (to CEG and B. Fisher), DEB-9296271 (to CEG), DEB-0613775 (to NIP, CEG, GH, MJR) and BSI-0103795 (to Jablonski and Fritsch, P.I.s). Participation by undergraduate students (Robin Carlson, Joel Ledford, Hillary Gutman, Nibia Soto-Rolón, Christopher Vo, Vanessa Knutson, Jasper
Aspects of this research in the Scharff lab (NS) at the Zoological Museum, University of Copenhagen, were supported by a grant from the Danish National Science Research Council (SNF21-02-0502). NS acknowledges travel grants for fieldwork in Thailand from DANIDA (Danish development cooperation), as well as from the Carlsberg Foundation (grant no. 0537/60) for fieldwork in Australia. Participation by undergraduate student Jesper Birkedal Schmidt in the NS lab was made possible by the above-mentioned grants.

Fieldwork was made possible thanks to the support of Andrés A. Ojanguren-Affilastro, Luis Compagnucci, Gonzalo Rubio and Ana Quaglino (Argentina); Barbara Baehr, Lisa Boutin, Mark S. Harvey, Robert Raven and Michael Rix (Australia); Valerio Vignoli (Benin); Joseph Koh and Natalia Chousou-Polydouri (Brunei Darussalem); Elizabeth Arias, Juan Enrique Barriga Tuñón, Camilo I. Mattoni, Peter Michalik and Jose A. Ochoa (Chile); Brian D. Farrell, Kelvin Guerrero, Jeremy Huff and Erich S. Volschenk (Dominican Republic); Jeremy Huff (French Guiana); Jeremy Huff and Valerio Vignoli (Guinea Bissau and Senegal); Alexander Gromov, Alex and Elena Kreuzberg (Kazakhstan and Uzbekistan); Oscar Francke, Andrew Ghuesenkamp, Edmundo González-Santillán, Randy Mercurio, Hector Montaño, Ricardo Paredes, Javier Ponce-Saavedra, Charles Savvas and Peter Sprouse (Mexico); Da Aye Aye Cho, Dong Lin, U Kin Mawng Zaw, U Tin Mya Soe and Joe Slowinski (Myanmar); Tharina and Chris Bird, Quinton and Nicole Martins and Elizabeth Scott (Namibia); Ray Forster, Lyn Forster and Steven King (New Zealand); Pedro Cardoso (Portugal); Lauren Esposito (Puerto Rico); Ansie Dippeear-Schoeman, Ian Engelbrecht, Charles Haddad, Norman Larsen, Robin Lyle, Randy Mercurio, Audrey Ndaba, Elizabeth Scott and Esther van der Westhuizen (South Africa); and Weerachai Nanakorn, Suyanee Vessabutr and Chaweewan Hutacharern (Thailand).

Specimens for this project were provided by Andrés A. Ojanguren-Affilastro, Ingi Agnarsson, Elizabeth Arias, Barbara Baehr, Domir De Bakker, Jorge Barneche, Jesper Birkedal Schmidt, Marius Burger, Tomás Cekalovic, Fred Coyle, Sara Crews, Michael Driessen, Ian Engelbrecht, Mike Fitzgerald, Charles Haddad, Mark S. Harvey, Bernhard Huber, Jeremy Huff, Joseph Joh, Mattazj Kuntner, James Lazell, Steven Lew, Camilo Mattoni, José Ochoa, Vladimir Ovt-sharenko, Pierre Paquin, Robert Raven, Carles Ribera, Michael Rix, Sergio Rodriguez Gil, Cristina Scioscia, Carlos Viquez and Peter Weygoldt.

DNA sequence data were generated at the AMNH by Rebecca B. Dikow, Torsten Dikow, Jeni Kuszak, Diana Pietri and Dana Price. We thank Bryce McQuillan for providing a photograph of Megadictyna thilienii.

**Author contributions**

Led the project: WCW. Directed the study: WCW, JAC, GH, LP, PS. Directed a lab/group: WCW, JAC, CEG, GH, LP, MJR, PS, JEB, MH, WPM, NS. Participated in taxon selection, collected, identified specimens, processed samples: JAC, CEG, GH, LP, MJR, PS, LAS, FAP, MA, LBS, SB, JEB, CJG, EH, MH, MAI, FML, JL, LL, WPM, JM, LNP, NIP, DP, DSD, NS, TS, DU, CV, HMW, JZ. Managed tissue sample and voucher flow among project participants and AMNH: LP. Directed molecular lab: WCW. Edited sequences and generated data files: LMC. Provided additional sequences: DD, CEG, GH, LP, MJR, PS, JEB, MH, FML, JL, LL, WPM, JM, DP, NS, HMW, JZ. Curated and maintained tissue samples and voucher data: LMC, LP.

Examined preliminary analyses, detected contaminations: LMC, DD, CEG, GH, MJR. Performed phylogenetic analyses: WCW (direct optimization), DD (maximum-likelihood and Bayesian), PAG (parsimony). Performed congruence analyses: MJR.

Drafted manuscript sections: LMC (Methods); MJR (Results); CEG, MJR (Relationships, Taxonomy). Made illustrations: MJR. Made tables: LMC, MJR.

Submitted sequences to GenBank: LMC. All authors approved the final text.

**References**


testing morphological and ethological homologies. Cladistics 24, 147–217.


Rambaut, A., 2014. FigTree, tree figure drawing tool. v. 1.4.2. Institute of Evolutionary Biology, University of Edinburgh.


Supporting Information

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

Fig. S1. Transcriptomic skeleton tree (TR). Skeleton tree obtained from the transcriptomic analysis of Garrison et al. (2016), with terminals mapped to our dataset. All clades have bootstrap values of 100% and are robust to analytical methods.

Fig. S2. Taxonomic reference tree (TX). Includes multi-sampled families of spiders and outgroups.

Fig. S3. Taxonomic reference tree, conservative (TX-C). Same as TX, but excluding the families that are relimited here.

Fig. S4. Maximum-likelihood tree constrained by TR skeleton tree (C-ML). With bootstrap values.

Fig. S5. Maximum-likelihood tree constrained by TR skeleton tree based on the dataset excluding areas of ambiguously aligned position in non-coding genes (C-ML-T). With bootstrap values.

Fig. S6. Unconstrained maximum-likelihood tree (ML). With bootstrap values.

Fig. S7. Unconstrained Bayesian inference tree (BI). With posterior probabilities.

Fig. S8. Direct optimization tree (DO). Tree obtained for InDel cost ratio of 1 and tv/ts cost ratio of 2.

Fig. S9. Parsimony equal weights tree (EW). With bootstrap values.

Fig. S10. Parsimony extended weighting tree (IW). With bootstrap values.

Fig. S11. Tree with robust groups. Majority rule consensus tree from all analyses in Figs S4–S10.

Fig. S12. Tree with groups stable to optimality criteria. Majority rule consensus tree from the five optimality criteria (EW, IW, DO, BI, ML), complete alignments without constraints.

Table S1. Voucher and DNA locus information. Specimen and locus information is provided below. The identification code (Voucher Codes) of the tissue or DNA sample from which newly generated sequences were obtained is given. Institution codes (Museum Voucher Code) are also provided. Locality data are also supplied, with localities for conspecific tissues separated by a semicolon. All newly generated sequences, as well as those attributable to the NSF grant EAR-0228699, are highlighted in bold type; all others refer to sequences that were previously deposited in GenBank by other authors.

Table S2. Mapping of our terminals to those of Garrison et al.’s (2016) analysis of transcriptomic data.

Table S3. Combined and partial tree costs and incongruence length difference (ILD) for different schemas of InDel and tv/ts cost ratios.

Table S4. Monophyly of selected taxonomic groups and multi-sampled families as found in the different analyses, and list of taxa used for the taxonomic congruence analyses.