TESIS DE DOCTORADO

Phylogenomic analysis of South American Nemesiidae and Pycnothelidae, and the delimitation of the Crassitarsae clade (Araneae, Mygalomorphae)

Mag. Laura Montes de Oca

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Director: Dr. Jason Bond Davis University Davis, California, EUA. Co-director: Dr. Fernando Pérez-Miles Facultad de Ciencias, Universidad de la República Montevideo, Uruguay

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Dra. Diana Silva







Dr. Martín Ramirez

Dr. Cristian Grismado



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Duniesky Ríos-Tamayo, Nelson Ferretti, Leonela Schwerdt, Sofia Coperi, Gabriel Pompozzi (Argentina), Carlos Perafán (Colombia, Perú), Fernando Pérez-Miles (Colombia, Uruguay), Josh Richards (Perú), Milenko Aguilera (Chile), Rafael Prezzi-Indicati (Brasil).

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Acanthogonatus está en Colombia (Araneae, Mygalomorphae). Laura Montes de Oca, Carlos Perafán, Fernando Pérez-Miles. V Congreso Latinoamericano de Aracnología, Brasil. 2017

Phylogenomics of Neotropical Nemesiidae (Araneae, Mygalomorphae). Laura Montes de Oca, Fernando Pérez-Miles, Vera Opatova, Jason Bond. XXI International congress of arachnology, New Zealand. 2019

Contribución

Opatova V, Hamilton CA, Hedin M, Montes de Oca L, Král J, Bond JE. 2020. Phylogenetic systematics and evolution of the spider infraorder Mygalomorphae using genomic scale data. Systematic Biology, 69(4): 671-707.

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Dr. Miguel Simó. Facultad de Ciencias, Universidad de la República. Dra. Leticia Bidegaray. Instituto de Investigaciones Biológicas Clemente Estable. Dr. José Sotelo. Instituto de Investigaciones Biológicas Clemente Estable.

Tribunal de tesis

Dr. Marcelo Loureiro. Facultad de Ciencias, Universidad de la República. Dra. Ivanna Tomasco. Facultad de Ciencias, Universidad de la República. Dra. Cecilia Da Silva. Centro Universitario de Tacuarembó, Universidad de la República. "...both the relationships within the family and the monophyly of the family itself are far from well established. The two problems are, obviously, interrelated, but the solution to neither of them is likely to be found in the near future."

P. Goloboff, 1995

Phylogenomic analysis of South American Nemesiidae and Pycnothelidae and the delimitation of the Crassitarsae clade (Araneae, Mygalomorphae)

ABSTRACT

Nemesiidae was once one of the largest families of mygalomorph spiders. However, over the past few decades both morphological and molecular studies focusing on mygalomorph phylogeny have continually recovered the group as paraphyletic. Hence the systematics of the family Nemesiidae has more recently been viewed as chaotic and contentious. Presumably due to the lack of informative morphological characters and consensus on diagnostic characters, nemesiid species have been transferred to other genera and families. First described by Simon in 1889, the tribe Nemesiae was placed in the subfamily Ctenizinae Thorell, 1887 which at the time included the genera Genysa, Nemesia, Arbanitis, Hermacha, Spiroctenus, Misgolas and Hermeas. Nearly a century later, Raven elevated the tribe to the family level based and considered Pycnothelidae as junior synonym; however, Nemesiidae were supported by a number of apparently weak diagnostic features. Since 1993, explicit cladistic and phylogenetic assessments have recovered the family as paraphyletic. Indeed, in a recent study by Opatova and collaborators, six nemesiid genera were transferred to the newly reestablished family Pycnothelidae, including Acanthogonatus, Bayana, Pionothele, Pycnothele, Stanwellia and Stenoterommata. Despite these changes, a substantial number of nemesiid genera remain therein, but incertae sedis due to taxon sampling. Accordingly, we evaluate the phylogenetic relationships of a number of South American nemesiid species and genera with the principle aim of resolving their family level placement. Our work represents the most exhaustive phylogenomic sampling for South American nemesiids by including nine of the 13 genera described for the continent. Phylogenetic relationships were reconstructed using 457 loci obtained using the spider Anchored Hybrid Enrichment probe set. Based on these results Neotropical genera of Nemesiidae, Pycnothelidae, Microstigmatidae and Cyrtaucheniidae are not considered monophyletic. Quite

surprisingly, this study also indicates that one lineage may require elevation to the family level considering new affinities among Mygalomorphae families (Fufiidae NEW FAMILY), with basis on the genus *Fufius*, here transferred to new family. In Pycnothelidae, we recognize/delimit five subfamilies (Pionothelinae NEW SUBFAMILY, Pycnothelinae, Diplothelopsinae, Prorachinae NEW SUBFAMILY, Stenoterommatinae NEW SUBFAMILY). We transfer the following Nemesiidae genera to Pycnothelidae: Chaco, Chilelopsis, Diplothelopsis, Flamencopsis, Hermachura, Lycinus, Neostothis, Prorachias, Rachias. Even more we transferred the Microstigmatidae genus Xenonemesia to Pycnothelidae. South American Pycnothelidae genera resulted paraphyletic, except Rachias and Prorachias are recovered monophyletic, and Acanthogonatus and Stenoterommata resulted polyphyletic. We propose the following generic synonymies and species transfers: Neostothis and Bayana as junior synonyms of Pycnothele (NEW SYNONYMY), and Pycnothele gigas and Pycnothele labordai (NEW COMBINATIONS); Hermachura as junior synonym of Stenoterommata (NEW SYNONYMY), and Stenoterommata luederwaldti (NEW COMBINATION); Flamencopsis as junior synonymy of Chilelopsis (NEW SYNONYMY), and Chilelopsis minima (NEW COMBINATION); *Diplothelopsis* as junior synonymy of *Lycinus* (NEW SYNONYMY), and Lycinus ornata and Lycinus bonariensis (NEW COMBINATIONS). Finally, these results provide a robust phylogenetic framework that includes enhanced taxonomic sampling, for further resolving the biogeography and temporal setting for the family Pycnothelidae.

Keywords: Anchored hybrid enrichment, taxonomy, new family (Fufiidae), new subfamilies (Pionothelinae, Prorachinae, Stenoterommatinae)

INTRODUCTION

The infraorder Mygalomorphae is an ancient lineage of spiders that includes tarantulas, trapdoor and funnel web spiders, etc. With a fossil record extending back to Middle Triassic (Selden and Gall 1992), they diverged approximately 300 Ma (Ayoub and Hayashi 2009, Garrison et al. 2016). Mygalomorphs currently comprise 30 families (Opatova et al. 2020), 358 genera and 3098 species (World Spider Catalog 2020; WSC). The systematics of this group has generally been perceived as a challenge due to their remarkably homogeneous morphology (Hedin and Bond 2006). The first exhaustive cladistic analysis of the infraorder was conducted by Raven (1985), at that time analysis were made by hand and only included morphological data; Eskov & Zonstein (1990) followed with a more restricted analysis that disputed some of the hypotheses proposed by Raven (e.g., Atypoidina). In 1993, Goloboff implemented a cladistic computational-based analysis also using morphological data that showed many of the families may be suspect as para or polyphyletic. Subsequently, the first molecular studies using Sanger-sequencing approaches made some advances but lacked the taxon sampling necessary to resolve a number of newly identified and longstanding issues in mygalomorph classification (Hedin and Bond 2006, Ayoub et al. 2007, Bond et al. 2012). Owing to the new genomic technologies now readily available in non-model organismal groups like spiders, a recent work made by Opatova et al. (2020) using more than 400 loci, documents a new and well-supported classification for mygalomorph families. Nevertheless, there are a number of longstanding issues they failed to fully resolve.

Indeed, Nemesiidae Simon, 1889a is currently defined as a family of mygalomorph spiders comprising 22 genera with 184 nominal species (WSC). Since its establishment nearly 35 years ago Nemesiidae has had a tumultuous history; species included in this family have been synonymized, transferred to other genera or families, and placed in any number of disparate families such as Dipluridae Simon, 1889a, Ctenizidae Thorell, 1887, Pycnothelidae Chamberlin, 1917 and Migidae Simon, 1889a. The type genus *Nemesia* was first proposed by Audouin, 1826, and placed in the family Aviculariidae. Simon proposed the tribe Nemesiae Simon, 1889a originally described in Ctenizinae which included the



Figure 1. Phylogenetic reconstructions including the family Nemesiidae. **A.** Raven 1985, morphological characters; **B.** Goloboff 1995, morphological characters; **C.** Bond et al. 2012, morphological and molecular data (Sanger); **D.** Opatova et al. 2020, molecular characters (Anchored Hybrid Enrichment). Bar indicates Nemesiidae species, Circle indicates South American Nemesiidae/Pycnothelidae species.

Ethiopian, Palearctic and Australasian genera Genysa Simon, 1889b, Nemesia, Arbanitis L. Koch, 1874, Hermacha Simon, 1889c, Spiroctenus Simon, 1889c, Misgolas Karsch, 1878 and Hermeas Karsch, 1878. Currently, Hermeas and Misgolas were synonymized in to Arbanitis and then transferred along with Genysa to Idiopidae Simon, 1889a by Raven (1985). The first taxonomic revision carried out indirectly in Pycnothelidae (before Nemesiidae was recognized), was undertaken by Schiapelli & Gerschman (1967) which included six species drawn from various South American genera. They also proposed two subfamilies: Pycnothelinae Chamberlin, 1917 which included Lycinus Thorell, 1894, Pycnothele Chamberlin, 1917, Pycnothelopsis Schiapelli & Gerschman, 1942 (currently a junior synonym of *Pycnothele*) and Diplothelopsinae Schiapelli & Gerschman, 1967 including *Diplothelopsis* Tullgren, 1905 (transferred at the time from Barychelidae Simon, 1889a). Raven's (1985) cladistic evaluation of mygalomorphs spider united Pycnothelidae and Nemesiae, elevating Nemesiidae to family status, delimiting and considering it as senior synonym of Pycnothelidae. He hypothesized the monophyly of Nemesiidae based on three synapomorphies: presence of two rows of teeth on the superior tarsal claw (STC), STC wide, and female palpal claw with teeth on the promargin, of which are largely homoplasic features when considered within the context of the entire infraorder. Nevertheless, South American Nemesiidae's were considered paraphyletic (Fig. 1A); Raven recognized six subfamilies: Anaminae Simon, 1889a, Bemmerinae Simon, 1903, Diplothelopsinae, Ixamatinae Raven, 1985, Nemesiinae Simon, 1889a, and Pycnothelinae. Alternatively, a second and subsequent infraordinal level cladistic analysis performed by Goloboff (1993), which included only a few nemesiid genera (Acanthogonatus Karsch, 1880, Stenoterommata Holmberg, 1881 (Neotropical region), Ixamatus Simon, 1887b (Australasian region) and Nemesia (Palearctic region)), did not recover the family as monophyletic, but instead paraphyletic with respect to Microstigmatidae Roewer, 1942. The phylogeny shows three different lineages, one of them shared with Microstigmatidae. In 1995, Goloboff revised and reconstructed the relationships of South American nemesiid species (excepting from Brazil) using morphological data including 12 of the 13 genera described at the time (Acanthogonatus, Lycinus, Diplothelopsis, Chilelopsis Goloboff, 1995,



Figure 2. A-F. Types of burrows. A. Open burrow (*Bayana labordai*); B. Silk tube (*Acanthogonatus tacuariensis*); C. Trapdoor (*Lycinus* sp.); D. Flap-door (*Chaco costai*). E. Trapdoor (*Prorachias* sp.),
F. Open burrow/silk tube (*Fufius lucasae*). Photos: A: Pérez-Miles et al. 2014; B: L. Montes de Oca;
C: L.S. Espinoza; D: Montes de Oca et al. 2013; E, F: R.P. Indicatti. Scale bars = 10 mm.

Flamencopsis Goloboff, 1995, Chaco Tullgren, 1905, Prorachias Mello-Leitão, 1924, Pselligmus Simon, 1892a, Pycnothele, Stenoterommata, Hermachura Mello-Leitão, 1923 and Rachias Simon, 1892b). In this study, he recovered the family as paraphyletic with respect to Theraphosoidina + Microstigmatidae and suggested that the three characters proposed by Raven (1985) as synapomorphies of Nemesiidae are actually just one, described in a different way. Also, South American Nemesiidae were recovered as paraphyletic constituting three different lineages (Fig. 1B). Subsequent infraordinal level works, incorporating a few molecular markers (Hedin and Bond 2006, Bond et al. 2012) re-confirmed the paraphyly of Nemesiidae but chose not to make any taxonomic changes. In both analyses they included the South American genera Acanthogonatus and Stenoterommata which were recovered as paraphyletic with respect to other nemesiids, sharing a clade with the Australian genus Stanwellia Rainbow and Pulleine, 1918 (Fig. 1C). Indeed, a recent exhaustive work made by Opatova et al. (2020) using more than 400 loci scattered members of the family Nemesiidae among a number of other families including Pycnothelidae, and delimited the spider family Nemesiidae to five genera (Mexentypesa Raven, 1987, Calisoga Chamberlin, 1937, Amblyocarenum Simon, 1892b, Iberesia Decae & Cardozo, 2006 and Nemesia), transferring four South American nemesiids genera included in their study (Acanthogonatus, Bayana Pérez-Miles, Costa & Montes de Oca, 2014, Stenoterommata, Pycnothele) to the newly elevated family Pycnothelidae (Fig. 1D). Pycnothelidae now comprises six genera with *Pionothele* Purcell, 1902 (from Africa) as the sister group to the remaining genera; notably the three South American Pycnothelidae species were recovered as paraphyletic clade which included the genus Stanwellia from Australia.

As Nemesiidae currently stands there are 13 South American genera (*Chaco*, *Chilelopsis*, *Damarchus* Siliwal, Molur and Raven, 2015, *Diplothelopsis*, *Flamencopsis*, *Hermachura*, *Longistylus* Indicatti & Lucas, 2005, *Lycinus*, *Neostothis* Vellard, 1925, *Prorachias*, *Psalistopoides* Mello-Leitão, 1934, *Pselligmus*, *Rachias*) within 108 species (WSC) listed as *incertae sedis*. Nemesiids *sensu lato* are tiny to median sized spiders (3.8 - 38.7 mm); with a transverse foveal groove; the eyes are typically grouped on a tubercle; 2-4 short spinnerets; anterior tarsus without spines; scopula on tarsus III and IV light or absent; lacks claw tufts; and superior tarsal claws are bipectinated with numerous teeth. They are generally thought to be nocturnally active and are fossorial. Their burrows are constructed with a J or Y-shaped with one or two entrance that is usually covered by silk (Goloboff 1995). These silk tubes are often found under stones or logs (some *Acanthogonatus*); in open or closed burrows under the soil (*Stenoterommata, Rachias, Pycnothele*) or sometimes a typical trapdoor or flap-door burrower builders (*Chaco, Prorachias*) (Fig. 2) (Goloboff 1995, Lucas et al. 2005, Montes de Oca & Pérez-Miles 2013, Indicatti, 2013).

The aim of this study was to definitively place the South American Nemesiidae genera by reconstructing their phylogenetic relationships using a target enriched genomic approach via Anchored Hybrid Enrichment. Our newly derived phylogenetic framework which includes many field-sampled taxa allows us to revise the family's classification; affinities with other groups are evaluated providing a substantive contribution to resolving some long-neglected branches on the Spider Tree of Life.

METHODS

Specimen sampling and DNA extraction. We included nine of 13 Nemesiidae genera described for South America (WSC) (Chaco, Chilelopsis, Diplothelopsis, Flamencopsis, Hermachura, Lycinus, Neostothis, Prorachias, Rachias) and six Pycnothelidae genera (Acanthogonatus, Bayana, Pionothele, Pycnothele, Stanwellia, Stenoterommata). We use representatives of 17 and 14 species respectively. For the outgroup, we incorporated Nemesiidae sensu lato samples, as well as other samples representative of the Crassitarsae clade (sensu Bond et al. (2012)) such as Anamidae Simon, 1889a, Barychelidae, Bemmeridae Simon, 1903, Cyrtaucheniidae Simon, 1889a, Dipluridae, Entypesidae Bond, Opatova & Hedin, 2020, Microstigmatidae and Theraphosidae Thorell, 1869 providing a robust evolutionary framework totaling a sample of 101 taxa (Table S1). Some samples were borrowed from Museums Collections (Facultad de Ciencias, Montevideo, Uruguay: FCE-MY; Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina: MACN; Coleção Aracnológica de Diamantina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rio Claro, Brazil: CAD) as well as field collections by the first author and RPI (South American samples except Ecuador in Fig. 3). All samples were stored in 100% ethanol and deposited in scientific collections (MACN, CAD, FCE-MY) (see Table S1). In this study, we incorporate 87% new samples of Nemesiidae sensu lato, whereas 40% of the total data set was obtained from previous works (Hamilton et al. 2016, Godwin et al. 2018, Opatova et al. 2020). DNA was extracted using DNeasy blood and tissue kit (Qiagen) following manufacture's protocol. RNase A was added to each sample after the lysis with proteinase K and before the addition of the Buffer AL in order to digest all the RNA. Library preparation, enrichment and sequencing were carried out at the Center of Anchored Phylogenomics, Florida State University following the methodology described in Lemmon et al. (2012) and Hamilton et al. (2016). The 585 loci Spider Probe kit v1 (Hamilton et al. 2016) was used for the targeted capture through Anchored Hybrid Enrichment (AHE).



Figure 3. Neotropical taxon sampling used for the phylogenetic reconstruction.

Phylogenomic analyses. Loci were aligned using MAFFT v7.023b (Katoh 2013) implementing L-INS-I method (--localpair --maxiterate 1000). Aliscore and Alicut (Misof and Misof 2009, Kück et al. 2010) were used to score and filter ambiguous or random similar sites within the multiple sequence alignment. Alignments were examined in Geneious Pro v5.6 (Kearse et al. 2012) for consistency and, for removing short sequences or potential paralogy. Loci were then concatenated using FASconCAT (Kück and Meusemann 2010). For the DNA matrix, partition scheme and substitution models were defined using PartitionFinder 2 (Lanfear et al. 2016) under the Akaike Information Criterion (AIC) with rcluster algorithm (Lanfear et al. 2014). Maximum likelihood (ML) analyses were conducted using RAxML v8 (Stamatakis 2014) selecting the best tree using the parameters -m GTRGAMMA, -N 1000, and the partitions scheme for each locus. Bootstrap support values were inferred from 50 replicates computed via autoMRE (Pattengale et al. 2010). Tree was rooted with Bothriocyrtum sp (Halonoproctidae Pocock, 1901). Genealogical and sites concordance factors (gCF and sCF, respectively) were also calculated using IQ-TREE (Minh et al. 2018); gCF and sCF analyses are alternative measures of topological support and calculate the proportion of loci or sites from which a particular node in the preferred tree is inferred (Ane et al. 2006). Bayesian (BI) analyses were inferred using Exabayes version 1.4.1 (Aberer et al. 2014) with two independent runs of 20 million generations, four coupled chains each, starting from a parsimony tree resampling every 1000 generations with 0.33 burn in proportion discarded. Species tree estimation was inferred from 454 gene trees under ASTRAL (Mirarab and Warnow 2015). Node support was estimated using ASTRAL's local posterior probabilities. Single gene tree was first inferred from AHE nucleotide alignments implementing GTR+G model in RAxML, selecting the best ML tree from 1000 independent iterations for each locus individually. Divergence times. Node divergences were estimated using penalized likelihood method (Sanderson, 2002) implemented in treePL (Stephen et al. 2012) with the tree topology obtained in RAxML. The setting for the analysis was determined with the following smoothing values = 0.01, 0.1, 1, 110, and 100. Since there are not relevant fossil calibration points for the Crassitarsae clade lineages we used the Opatova et al. 2020 estimated the

divergence times for the Mygalomorphae families and their dates inferred from 95% confidence intervals from 100 bootstraps. Calibration was as follow (Fig. 6): (1) Most recent common ancestor (MRCA) of Idiopidae and Bemmeridae: 123 Ma (corresponding to the minimum bound of the Idiopidae clade's node) as minimum bound and 163 Ma (maximum bound from the MRCA between Idiops and Spiroctenus) as maximum bound; (2) Theraphosoidina clade, MRCA between Bemmeridae and Barychelidae: 125 – 160 Ma (minimum bound corresponding to MRCA between Spiroctenus and Artrophothele and maximum bound to the Crassitarsae node; (3) MRCA of Artrophothele and Ozicrypta: 82 – 94 Ma (corresponding to the MRCA between the same pair of samples); (4) Nemesoidina clade, MRCA of Mexentypesa and Calisoga: 94 – 106 Ma (corresponding to the MRCA between the same pair of samples); and (5) MRCA of *Kiama* and *Kwonkan*: 81 – 92 Ma (corresponding to the same pair of samples). All phylogenetic analyses were run on the Hopper Community Cluster at Auburn University and the Farm Community Cluster at UC Davis. All supplementary material is uploaded in Figshare Repository. *Proposed taxonomic changes and images*. All diagnostic characters were provided by Rafael Prezzi-Indicatti and used posteriori to discriminate the clades obtained through the phylogenetic analyses. Stereomicroscope and scanning electron microscopy (SEM) images were prepared and taken as in Indicatti et al. (2015).

RESULTS

Concatenated analyses. The total data set comprised 456 loci (of 85374 nucleotides) for 101 terminals (upload to Figshare Repository) with 13.2% proportion of missing data. We recovered a similar tree topology from the ML (-In 940239.669658) and BI analysis. In general, nodes in all trees have high relative support. At the family level all clades are fully supported (bootstrap = 100, pp = 1) (Fig. 4, Supplementary Figs. S1-2). Maximum likelihood analysis performed with IQ-tree recovered the same topology (Fig. S3). The Crassitarsae clade was recovered with two main clades including the Theraphosoidina and "Nemesoidina", the two clades were strongly supported (bootstrap = 100, pp = 1). Theraphosoidina clade plus Bemmeridae was recovered as sister group of Barychelidae plus Theraphosidae forming the Theraphosoidina clade. "Nemesoidina" clade comprises the family Nemesiidae as sister group of a clade which includes the families Pycnothelidae, Microstigmatidae, Entypesidae, Anamidae, Cyrtaucheniidae, Dipluridae plus two lineages (see above). The family Nemesiidae is here represented by a clade including *Mexentypesa* as sister group of a clade comprising Calisoga (Nemesia, Iberesia); all nodes are strongly supported (bootstrap = 100, pp = 1) (Fig. 4). In Pycnothelidae five major lineages are recovered (each one with pp = 1, bootstrap = 100) (Fig. 5). The monogeneric Pionothelinae (NEW SUBFAMILY) represented by *Pionothele* from Africa is recovered as sister group of Pycnothelinae (including Pycnothele, Bayana, Neostothis, Xenonemesia); Prorachinae (NEW SUBFAMILY, including only the genus Prorachias); and Stenoterommatinae (NEW SUBFAMILY, including Stenoterommata, Hermachura, Rachias and two new genera (one from Peru and one from Brazil). The genera Pycnothele, Diplothelopsis, Lycinus, and Chilelopsis resulted paraphyletic, whereas *Prorachias* and *Rachias* are recovered monophyletic, and Acanthogonatus and Stenoterommata polyphyletic. Anamidae is the sister group to Entypesidae + Microstigmatidae, all of which are recovered with high support (bootstrap = 100, pp = 1). Cyrtaucheniidae was recovered as sister group of a clade with two lineages comprising Dipluridae, Fufiidae (NEW FAMILY). Species tree analysis. Multi-species coalescent analysis produced a species tree from 454 input gene trees obtained using RAxML. The resulting quartet-based



Figure 4. Phylogenetic tree summarizing concatenated and species tree reconstructions using the topology from the maximum likelihood analysis. Support values are denoted in the boxes on the nodes following the order left to right: RAXML bootstrap support, BI: Bayesian posterior probabilities, A: ASTRAL support values, IQ-TREE gCF support values. Each support level is denoted by different colors described in the references at the left bottom. One filled box indicates the same support for all analyses and white box topology not recovered in the species tree analysis.

super tree estimated in ASTRAL (Supplementary Fig. S4) comprises 990,021,192 induced quartet trees from the input gene trees, representing the 74.6% of all quartets presents in the species tree. ASTRAL produced a slightly different topology than the concatenated analysis (Supplementary Fig. S3). The major differences are the inclusion of Idiopidae in the Crassitarsae clade (pp = 0.65) and Cyrtaucheniidae clade is recovered as a sister group of (((Microstigmatidae, Entypesidae), Anamidae), (Dipluridae, Fufiidae)) with a low support (pp = 0.55). Theraphosoidina and Nemesoidina clades are recovered highly supported (pp = 1) as well as all family clades.



Figure 5. Topology representing Pycnothelidae subfamilies. Each subfamily support level is denoted by different colors described in the reference in Figure 4. One filled box indicates the same support for all analyses.



Figure 6. Divergence time estimates derived from treePL on a topology obtained in RAxML. Calibration points are marked in red circles. The x axis represents the time in million years as well as the numbers given in the nodes. Geologic time abbreviations: (J) Jurassic, (K) Kretaceus, (P) Paleogene, (N) Neogene. In red and within a circle are placed the calibration (minimum and maximum bound respectively).

Divergence time. The dated topology suggests the origin of Pycnothelidae ~ 84 Ma during the Cretaceous era, but South American Pycnothelids started to diverge ~ 60 Ma in the Paleogene (Cenozoic era) as well as the other South American taxa sampled in this study (Cyrtaucheniids ~ 63 Ma, Theraphosinae ~ 55 Ma, Diplurids ~ 50 Ma, Barychelids ~ 23 Ma). An exception is the new family Fufiidae that shows it origin earlier during the late Cretaceous ~ 70 Ma but diversification, according to our sample, is during the Neogene and Quaternary periods from the Cenozoic era. Likewise, the Theraphosidae clade in our dated topology split ~ 75 Ma during the Cretaceous between the Ischnocolinae (including the South American genus *Catumiri* Guadanucci, 2004) and the other lineages.

DISCUSSION

Pycnothelidae and Nemesiidae. Our results more closely resemble the classification proposed by Schiapelli & Gerschman (1967) where they classified the family Pycnothelidae as containing five South American species from the genera *Lycinus, Pycnothele*, and *Diplothelopsis*. Beforehand Raven (1985) united them within the tribe Nemesiae, elevating that group to family level (Nemesiidae). Our results alternatively infer the non-monophyly of South American Nemesiidae. Indeed, all South American Nemesiidae *sensu lato* included in our analysis are recovered within the Pycnothelidae family as a robustly supported clade. Furthermore, South American Pycnothelidae resulted paraphyletic as in Opatova et al. (2020) including the genus *Stanwellia* (found in Australia and New Zealand).

Based on the Nemesiidae subfamilies proposed by Raven (1985) and following the reclassification scheme proposed by Opatova el at. (2020), the available pycnothelid subfamilies are Pycnothelinae and Diplothelopsinae; our results identify three others subfamily rank lineages: Pionothelinae NEW SUBFAMILY, Stenoterommatinae NEW SUBFAMILY and Prorachinae NEW SUBFAMILY (see below). The subfamily Pycnothelinae is delimited to include the genera *Xenonemesia* and *Pycnothele*.

It is noteworthy that the genus *Xenonemesia* currently belongs to the family Microstigmatidae and is transferred herein to Pycnothelidae. *Xenonemesia was* originally described as a nemesiid genus by Goloboff (1988) based on *X. platensis* Goloboff, 1988 specimens from Argentina and Uruguay. Goloboff (1993) suggested that *Xenonemesia* be transferred to Microstigmatidae or alternatively that microstigmatids be considered as a subfamily of Nemesiidae. Goloboff (1995) transferred *Xenonemesia* to Microstigmatidae, a family diagnosed as having round book lungs openings, extremely short posterior lateral spinnerets, glabrous tegument and anterior tarsi with absent (or very light) scopula. According to Goloboff (1993) the book lung openings of *Xenonemesia*, was coded as for *Microstigmata*, *Pseudonemesia*, and *Micromygale* despite some apparent differences. The book-lung openings are not as small, and the posterior margin is not as sclerotized as *Microstigmata*, *Pseudonemesia*. All of them, however, seem to share a common type of modification (which progresses further in *Microstigmata*)

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and *Pseudonemesia*), and are therefore coded as having the derived state as being small and rounded (state "1" in his character scoring scheme as opposed to "Book-lung openings normal = 0"), whereas *Xenonemesia* should be coded as having normal openings (see Indicatti et al. 2007 fig. 27, Indicatti et al. 2008 fig. 12). On the other hand, *Xenonemesia* has several characteristics that distinguish it from all other microstigmatid genera (except *Spelocteniza* Gertsch, 1982, not examined) (see proposed changes below). Based in our results and features mentioned we transfer *Xenonemesia* from Microstigmatidae to Pycnothelidae (Pycnothelinae). Furthermore, we are confident that the inclusion of more cyrtaucheniid or nemesiid (*sensu lato*) genera in a new analysis facilitate elevating the *Xenonemesia* lineage as a new subfamily, as first suggested by Goloboff (1993).

Indeed, more taxa are needed for future studies in order to accurately delimit many of these genera as well as create effective identification keys that employ diagnostic characters principally for *Stenoterommata* and *Acanthogonatus*, genera that remain ostensibly polyphyletic. The systematics of these two genera has been chaotic – *Stenoterommata* has been transferred from Ctenizidae to Nemesiidae by Raven (1985) and recently to Pycnothelidae by Opatova et al. (2020), and some species have been transferred to *Acanthogonatus* or synonymized (Goloboff 1995). *Acanthogonatus* was transferred from Barychelidae to Nemesiidae (Raven 1985) and recently to Pycnothelidae by Opatova et al. (2020), and some species have been transferred to the genus *Fufius* (Raven 1985) and recently to Pycnothelidae by Opatova et al. (2020), and some species have been transferred to the genus *Fufius* (Raven 1985) and to *Stenoterommata* (Goloboff 1995). Thus, the taxonomy of these genera clearly remains in need of a comprehensive revision.

Finally, in light of our results we propose the following generic level synonymies and transferred species: (1) *Neostothis* and *Bayana* a junior synonyms of *Pycnothele*, and its type species, *Neostothis gigas* Vellard, 1925 and *Bayana labordai* Pérez-Miles et al., 2014, respectively, transferred to Pycnothele; (2) *Hermachura* a junior synonym of *Stenoterommata*, and its type species, *Hermachura luederwaldti* Mello-Leitão, 1923 transferred to *Stenoterommata* (as mentioned and corroborated by Goloboff 1995, Indicatti 2013); (3) *Flamencopsis* as a junior synonym of *Chilelopsis*, and its type species, *Flamencopsis minima*

Goloboff, 1995 transferred to *Chilelopsis*; (4) *Diplothelopsis* as junior synonym of *Lycinus* and its type species, *Diplothelopsis ornata* Tullgren, 1905 and *Diplothelopsis bonariensis* Mello-Leitão, 1938 transferred to *Lycinus*; and (5) *Xenonemesia* (Microstigmatidae) transferred to Pycnothelidae. See proposed taxonomic changes below.

Crassitarsae reclassification. Since the first cladistic analysis using morphological characters by Raven (1985) followed by a number of molecular studies (Hedin and Bond 2006, Ayoub et al. 2007, Bond et al. 2012, Garrison et al. 2016) and the more recent exhaustive genomic based analysis (Opatova et al. 2020), the families composing the Crassitarsae clade have been both increased and the relationships among them have been shuffled. Within the context of this study, we recovered the Crassitarsae lineage with high support (bootstrap = 100, pp = 1). The Theraphosoidina clade is recovered as in Opatova et al. (2020) comprising the family Bemmeridae as sister to Barychelidae and Theraphosidae. However, for the clade Nemesoidina we document three noteworthy departures. First, the clade including the genus *Fufius*, although within the Nemesoidina clade, is strongly supported as an independent lineage from other cyrtauchenids (Fig. 2F). The genus has been also controversy, first placed in Ctenizinae (Aviculariidae) (Simon 1888, 1891) and then transferred to Diplurinae (Aviculariidae) (Simon 1892a, b). In 1985, Raven considered as a Cyrtaucheniidae (a family suggested to be paraphyletic according to Goloboff (1993, 1995)). Indeed, the phylogenetic analysis by Bond et al. (2012) recovered *Fufius* as being more closely related to Nemesiidae than other cyrtaucheniids. Consequently, we propose the new family rank taxon Fufiidae (NEW FAMILY). Secondly, the sister group of the Fufius clade is a second divergent lineage comprising an unknown species from Peru. The samples included here are females and juveniles and differ from all other known mygalomorph taxa described to date (LMO pers. observation). Rather than describe this lineage as a new family we believe the conservative approach is to attribute these to the family Fufiidae until more material (particularly male specimens) have been collected. Third, relationships among the Nemesoidina clade are appreciably changed: Dipluridae is not the sister group of Cyrtaucheniidae as recovered in Opatova et al. (2020) instead Cyrtaucheniidae is

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the sister group of the clade comprising Dipluridae + Fufiidae (NEW FAMILY). Although Dipluridae and Cyrtaucheniidae were not exhaustively sampled for this study, the Nemesoidina clade is highly supported, allowing us to formulate this new phylogenetic hypothesis.

Divergence time. According to the dated topology the family Pycnothelidae diverged ~ 84,3 Ma during the Cretaceous where we observe the split between the lineage comprising *Pionothele* from the rest of the Pycnothelids. *Pionothele* is an African genus and is recovered as the sister group to the South American pycnothelids. According to our results the divergence time of these lineages coincides with the separation of the African and South American continents as a consequence of the Gondwanan break up during the Cretaceous (see also Opatova et al. 2020). In that sense the divergence of South American taxa of Pycnothelidae, as well as Dipluridae, Theraphosinae, Cyrtaucheniidae clades appears to have occurred during the Paleogene after the Cretaceous-Paleogene boundary event (~ 66 Ma; Vandenberghe at al. 2012). Principally, they started to diversify after the Paleocene-Eocene Thermal Maximum (~ 56 Ma, Vandenberghe at al. 2012) During these epochs there is evidence for diversification of terrestrial and marine lineages (Keller et al. 2018, Molina 2015). Moreover, in South America, since ~ 66 Ma the Andes Mountain range started to form, creating vicariant events which may have facilitated allopatric speciation/divergence among some of these groups. Stanwellia (an Australian and New Zealand genus) is recovered within the subfamily Diplothelopsinae that includes a number of South American taxa. The relationship between those clades may be explained by the divergence time of this lineages (~ 31 Ma) which coincides with the Australia/Antarctica separation, with Antarctica forming the last connection between Australia and South America (see also Opatova et al. 2020). Also, from the dated topology we infer that the split between Cyrtaucheniids and Fufiids clades occurred during the Cretaceous, ~ 88 Ma. Most significantly, according to our sample, Fufiidae diversification started ~ 70 Ma during the Cretaceous but the "actual species" are dated since ~ 11 Ma during the Neogene. On the other hand, Cyrtaucheniidae family diversification started during the Eocene (~ 50 Ma).

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DELIMITATION OF PYCNOTHELIDAE CHAMBERLIN, 1917 Pycnothelidae Chamberlin, 1917 (new circumscription)

Genus type: *Pycnothele* Chamberlin, 1917 (type species *Pycnothele perdita* Chamberlin, 1917).

Diagnosis and remarks. Pycnothelidae was reestablished to the family level by Opatova et al. (2020). Based on our results and the inclusion of 13 Neotropical genera, we can reorganize the family classification and propose diagnostic characters. In that sense, Pycnothelidae can be recognized by the following unique combination of characters: (1) presence of small to large, yellow pallid, soft, developed intercheliceral tumescence covered with few to many setae (Fig. 7A, D-H) (not evident/absent in *Pionothele* (Raven 1985, Zonstein 2016)); (2) cymbium lacking dorsal spines; (3) patella III with 1-1-1 prolateral spines or more in same three positions (except *Pionothele straminea* 1-1); (4) male tarsi flexible (one or more legs) (except *Pionothele, Xenonemesia* and new genus from Brazil); (5) tarsal organ located on apical central region (Fig. 9A); (6) absence of claw tufts. Additionally, all Pycnothelidae genera have the palpal bulb with low to high keels or low ridges (R.P. Indicatti pers. observation), all individuals, except *Pionothele*, were examined under SEM or light microscope. These ridges are homologous to keels (Goloboff 1995).

List of included genera for Pycnothelidae (* indicates taxa included in our analysis): *Acanthogonatus Karsch, 1880 *Chaco Tullgren, 1905 *Chilelopsis Goloboff, 1995 *Lycinus Thorell, 1894 *Pionothele Purcell, 1902 *Prorachias Mello-Leitão, 1924 *Pycnothele Chamberlin, 1917 *Rachias Simon, 1892b *Stanwellia Rainbow and Paulleine, 1918 *Stenoterommata Holmberg, 1881

*Xenonemesia Goloboff, 1988

Transferred species (on the basis of its phylogenetic position and similar morphological features)

Chilelopsis minima (Goloboff, 1995) NEW COMBINATION Lycinus bonariensis (Mello-Leitão, 1938) NEW COMBINATION Lycinus ornatus (Tullgren, 1905) NEW COMBINATION Pycnothele gigas (Vellard, 1925) NEW COMBINATION Pycnothele labordai (Pérez-Miles, Costa, Montes de Oca, 2014) NEW COMBINATION

Stenoterommata luederwaldti (Mello-Leitão, 1923) NEW COMBINATION

Delimitation of Pycnothelidae subfamilies

Pycnothelinae (new circumscription)

Genera included: Pycnothele Chamberlin, 1917; *Xenonemesia* Goloboff, 1988 *Distribution*: Argentina, Brazil, Uruguay

Diagnosis and remarks. The genus *Xenonemesia* does not share most of the diagnostic morphological characters for *Pycnothele*, *Neostothis*, *Bayana*, making it difficult to find features that unite them as a subfamily. Even so we highlight some characteristics that combined distinguish Pycnothelinae from other subfamilies: (1) chelicerae with weak rastellum composed of thickened setae (Fig. 10 A, B, G); (2) chelicerae projected at the apex (Fig. 10 A, B, G); (3) maxillary serrula found only in males; (4) tibial spur on male leg I absent; (5) metatarsal preening combs absent; (6) female scopulae on legs I, II symmetric; (7) inferior tarsal claws (ITC) I-IV absent (Fig. 8D, for leg IV); (8) posterior lateral spinnerets (PLS) with domed apical article.

Diplothelopsinae (new circumscription)

Genera included: Chaco Tullgren, 1905; Acanthogonatus Karsch, 1880; Chilelopsis Goloboff, 1995; Stanwellia Rainbow & Pulleine, 1918; Lycinus Thorell, 1894

Distribution: Argentina, Australia, Brazil, Chile, Uruguay

Diagnosis and remarks. Diplothelopsinae can be recognized by the combination of the following characters: (1) male tarsi flexible; (2) female scopulae on legs I, II symmetric; (3) ITC on tarsi IV or absent. Even though not recognizing the subfamily Diplothelopsinae in his phylogeny, Goloboff (1995) proposed the Diplothelopsini tribe, comprising Chilelopsis, Flamencopsis, Diplothelopsis and Lycinus. It can be recognized by having the anterior median eyes much larger than the minute posterior median eyes; posterior eyes row slightly procurved (Goloboff 1995 figs 118A, 119A, 123A); and by the short, wide caput of females (Goloboff 1995). However, these characters cannot be applied to Chaco (Goloboff 1995) and recently, to Brazilian species of Lycinus with posterior eyes row slight recurved (Indicatti and Lucas 2010 figs. 5, 12). Moreover, the inclusion of Acanthogonatus and Stanwellia in Diplothelopsinae make it difficult to find features that diagnosis the subfamily. Due to the absence of characters, the differentiation among specimens of Diplothelopsinae and other subfamilies will depend from the exclusion of the characters combination proposed in other subfamilies, as well as, the inclusion of possible new taxa.

Pionothelinae NEW SUBFAMILY

Genera included: Pionothele Purcell, 1902

Distribution: Africa

Diagnosis and remarks. Pionothele was first described by Purcell (1902) as a ctenizid. The new subfamily Pionothelinae proposed here can be diagnosed by the genus and species descriptions following Purcell (1902), Tucker (1917), Raven (1985, figs. 73-78) and Zonstein (2016, figs. 1-7): (1) rastellum weak, composed by slightly thickened setae; (2) clypeus narrow; (3) thoracic fovea short, more or less straight in males and females; (4) sternum broad posteriorly, narrowing anteriorly (as in *Prorachinae*); (5) posterior sternal sigilla oval, very small, away from margin *ca*. three times length (as in *Prorachinae*); (6) male tibia I with one sessile retroventral megaspine; (7) metatarsal preening combs absent; (8) male tarsi I, II swollen in the middle or distal region; (9) tarsi I-IV not flexible; (10) ITC very small. Actually, *Pionothele* does not share most of the morphological characters existing in Pycnothelidae, e.g., presence of intercheliceral tumescence and palpal bulb probably with low ridges on embolus (homologous to keels, as in others

Pycnothelidae genera currently considered without keels, all examined in SEM (R.P. Indicatti pers. observation). As a matter of fact, the inclusion of more genera in future studies would probably indicate elevating *Pionothele* as a new family.

Prorachinae NEW SUBFAMILY

Genus included: Prorachias Mello-Leitão, 1924

Distribution: Brazil

Diagnosis and remarks. Prorachias was first described by Mello-Leitão (1924) as a ctenizid. The genus was redescribed by Lucas et al. (2005, figs 1-10) based on the type species, *Prorachias bristowei* Mello-Leitão, 1924 and additional characteristics from Raven (1985 figs. 161-164) and Goloboff (1995). The subfamily can be diagnosed by the combination of the following characters: (1) chelicerae with very strong rastellum, composed of 5-9 stout coniform spines being 2-3 on raised mound (Figs. 7G, 10H); (2) projected chelicerae on apical region (Fig. 10D, H); (3) clypeus wide; (4) sternum broad posteriorly, narrowing anteriorly, triangular-shaped (Fig.10D) (as in *Pionothele straminea*); (5) posterior sternal sigilla small, oval, away from margin *ca*. three times their length (Fig.10D); (6) tibial spur or megaspine on male leg I absent; (7) female scopulae on legs I, II more developed on prolateral side, asymmetric; (8) female tibiae I, II densely scopulate;(9) ITC on all legs; (10) PLS with domed apical article.

Stenoterommatinae NEW SUBFAMILY

Genera included: Stenoterommata Holmberg, 1881*; Rachias* Simon, 1892b *Distribution*: Argentina, Brazil, Peru, Uruguay

Diagnosis. This subfamily can be diagnosed by the combination of the following characters: (1) chelicerae with weak (Fig. 10 E,I) to strong rastellum, composed of long thickened setae to short or long coniform spines not on raised mound (Fig.10 E,I); (2) clypeus narrow; (3) posterior sternal sigilla small, oval, away from margin *ca.* one (Fig. 10E) or two times their length; (4) female scopulae on legs I, II symmetric; (5) metatarsal preening combs present; (6) narrow to wide band of pumpkiniforms spigots on inner edge of the spinning field of the PLS articles (except on a new genus from Brazil); (7) enlarged pumpkiniform spigots present (except on a new genus from Brazil).

DELIMITATION OF NEMESIIDAE SIMON, 1889

Nemesiidae Simon, 1889 (new circumscription)

Genus type: Nemesia Audouin, 1826 (type species *Nemesia cellicola* Audouin, 1826)

Remarks: Since Opatova et al. (2020) delimited the family Nemesiidae many taxa remain as *incertae sedis*. In light of our results, we were able to transfer some of the South American taxa to Pycnothelidae. Much work still remains to clarify the correct position for many of these taxa.

List of included genera (* indicates taxa included in our analysis):

*Nemesia Audouin, 1826

Amblyocarenum Simon, 1892b

*Calisoga Chamberlin, 1927

*Iberesia Decae and Cardoso, 2006

*Mexentypesa Raven, 1987

Incertae sedis

Atmetochilus Simon, 1887a Brachythele Ausserer, 1871 Damarchilus Siliwal, Molur and Raven, 2015 Gravelyia Mirza and Mondal, 2018 Longistylus Indicatti and Lucas, 2005 Psalistopoides Mello-Leitao, 1934 Pselligmus Simon, 1892a Raveniola Zonstein, 1987 Sinopesa Raven and Schwendinger, 1995

FUFIIDAE NEW FAMILY

Genus type: *Fufius* Simon, 1888 (type species by monotypy *Fufius atramentarius* Simon, 1888)

Distribution: Argentina, Bolivia, Brazil, Colombia, Ecuador, Guatemala, Peru, Trinidad, Venezuela

Diagnosis: The monotypic family Fufiidae is here proposed on the basis of phylogenetic position (Fig. 2F) and morphological characters. Based on

examination of several specimens of both sexes and descriptions of Raven (1985 figs. 225-228), Guadanucci and Indicatti (2004 figs. 1-10), Ortega et al. (2013 figs. 1-45) for *Fufius* and additional features to an new taxa from a second lineage in the family, Fufiidae may be diagnosed on the basis of the following unique combination of characters: (1) rastellum absent (Fig. 10F); (2) intercheliceral tumescence absent (Fig. 7I); (3) low eye tubercle; (4) anterior eyes row recurved; (5) male with incrassate tibia I with short retroventral apical spur and megaspine; (6) metatarsus I basally arcuate, prolaterally sigmoid; (7) PLS long with digitiform apical article; (8) separated spinnerets; (9) bulb without keels with thin, long embolus.

Included genus (* indicates taxa included in our analysis): *Fufius Simon, 1888

DELIMITATION OF CYRTAUCHENIIDAE SIMON, 1889

Cyrtaucheniidae Simon, 1889 (new circumscription)

Genus type: Cyrtauchenius Thorell, 1869 (type species *Cyrtauchenius terricola* Thorell, 1846).

Remarks: Our study allows us to confirm the placement of *Bolostromus* Ausserer, 1875 in the family Cyrtaucheniidae and transfer *Fufius* to a newly named family Fufiidae. Nonetheless, more work still remains done to clarify the position of the *incertae sedis* genera.

List of included genera (* indicates taxa included in our analysis):

*Cyrtauchenius Thorell, 1869 Ancylotrypa Simon, 1889c *Bolostromus Ausserer, 1875 Incertae sedis

> Acontius Karsch, 1879 Anemesia Pocock, 1875 Bolostromoides Schiapelli and Gerschman, 1945 Rhytidicolus Simon, 1889a

DELIMITATION OF MICROSTIGMATIDAE ROEWER, 1942 Microstigmatidae Roewer, 1942 (new circumscription)

Genus type: Microstigmata Strand, 1932 (type species *Microstigmata geophila* (Hewitt, 1916)).

Remarks: Based on our phylogenetic and morphological analysis Xenonemesia is transferred here to Pycnothelidae. The taxon included in our analysis corresponds to a new species from Brazil which was diagnosed as Xenonemesia by the combination of following features: carapace color pattern (three longitudinal yellowish light brown bands); wide sternum; keelless palpal bulb; slightly raised tarsal organ; absence of male tibia I spur; absence of thickened setae on cymbium; absence of inferior tarsal claw (Goloboff 1988, Indicatti et al. 2007); presence of black markings on legs and abdomen (wide and narrow intercalated marks on a central longitudinal band) (Indicatti et al. 2007 figs. 10, 13, 18, 23, 2008 figs. 30, 31). On the other hand, Xenonemesia has several characteristics that distinguish it from all Microstigmatidae genera (except Spelocteniza Gertsch, 1982, not examined): (1) body color pattern; (2) patella III with 1-1-1 prolateral spines or more in same positions (instead of 1-1 or absent); (3) only thin setae on cymbium (lacking spines); (4) inferior tarsal claw absent; (5) well developed, pallid and soft intercheliceral tumescence covered with few setae (Fig. 7A) (instead of almost inconspicuous, not soft, asetose in *Microstigmata* Strand, 1932 (Fig. 7B) or similar as in Ixamatus (Raven, 1981 Fig. 7C)); (6) wide and flattened book lungs openings (Indicatti et al. 2007 fig. 27, Indicatti et al. 2008 fig. 12), intermediate width among Microstigmata and Ixamatus, Xamiatus Raven, 1981, Kiama Main and Mascord, 1969 that are wider; (7) posterior margin of the book-lung openings is not as sclerotized as Microstigmata, Pseudonemesia Caporiacco, 1955 (Goloboff 1995, Indicatti and Villarreal 2016 figs. 6C, 9A), and other Microstigmatinae or Micromygalinae genera; (8) absence of clavate setae on legs, abdomen and spinnerets (Indicatti et al. 2007 figs. 18, 20, 23); (9) weak serrula with 5-35 isolated cuticular thorns in males (Fig. 8 A-B) (absent in Xamiatus and Kiama (Raven 1981, 1985)); (10) slightly raised tarsal organ (Fig. 9A), located on apical central position (Fig. 9A); (11) adhesive setae on ventral tarsi of all legs (Fig. 8 C, D); (12) integument with rounded ridges (Fig. 9A) (more close related to

Pycnothele (Fig. 9B) and *Neostothis* (Fig. 9C) (differing in the density) than in all Microstigmatidae genera, not presenting digitiform cuticular pustules (Fig. 9D) or flattened scaly cuticle (Fig. 9D)). Microstigmatidae probably remains as non-monophyletic, mainly in the Neotropical genera.

List of included genera (* indicates taxa included in our analysis):

*Microstigmata Strand, 1932 Angka Raven and Schwendinger, 1995 Envia Ott and Höfer, 2003 Ixamatus Simon, 1887b *Kiama Main and Mascord, 1969 Micromygale Platnick and Forster, 1982 Ministigmata Raven and Platnick, 1981 Pseudonemesia Caporiacco, 1955 Spelocteniza Gertsch, 1982 Tonton Passanha, Cizauskas and Brescovit, 2019 Xiamatus Raven, 1981



Figure 7. A-I. Intercheliceral tumescence: A. *Xenonemesia* sp., B. *Microstigmata longipes*, C. *Ixamatus barina*, D. *Pycnothele perdita*, E. *Neostothis gigas*, F. *Stanwellia grisea*, G. *Prorachias* sp., H. *Stenoterommata* sp. (RPI18 in our work), I. *Fufius* sp. Photos: R.P. Indicatti. Scale bars = 0.5 mm.



Figure 8. A, B. Serrula, male, C, D. Adhesive setae, male: A, C *Xenonemesia* sp., B, D. *Xenonemesia otti*. C. Tarsus I, retrolateral view, D. Tarsus IV, retrolateral view. Photos: B. Mauricio and R.P.Indicatti. Scale bars = 50 µm.



Figure 9. A-F. Integument and tarsal organ (arrows), dorsal view: A. *Xenonemesia* sp, B. *Pycnothele perdita.* C. *Neostothis gigas.* D. *Microstigmata longipes.* E. *Pseudonemesia tabiskey.* F. *Fufius* sp. Photos: A, D: B. Mauricio and R.P. Indicatti; B: Passanha et al. 2014; C: D.F. Candiani and R.P. Indicatti; E: Indicatti and Villarreal 2016; F: J.P.L. Guadanucci and R.P. Indicatti.



Figure 10. A-F. Cephalothorax and rastellum, ventral view. A-D, F. female. E. Male: A. *Pycnothele auronitens*. B. *Xenonemesia* sp. C. *Chaco obscura*. D. *Prorachias* sp. E. *Stenoterommata iguazu*. F. *Fufius lucasae*. G-I. Rastellum. G. *Pycnothele modesta*. H. *Prorachias* sp. I. *Rachias timbo*. **Abbreviations:** ra, rastellum; ma, maxilla; cb, maxillary cuspules; lb, labium; ste, sternum; sig, posterior sigillum, ram, rastellum on raised mound. Photos: R.P. Indicatti. Scale bars: A-F = 1 mm, G-I = 0.5 mm.

CONCLUSIONS

The lack of informative morphological characters and general homogeneity in mygalomorph spiders has long complicated the precise classification of this group. The use of genomic scale data appears to have contributed to a more comprehensive and well supported framework of phylogenetic relationships within the infraorder Mygalomorphae. We present for the first time the most complete sampling of the family Nemesiidae *sensu lato* since Goloboff's treatment of the group in 1995, over a quarter of a century ago. Based on extensive fieldwork throughout South America (previously undersampled in molecular studies) and the inclusion of a more broader sampling scheme, we are able to delimit the families Nemesiidae and Pycnothelidae along with the composition of the Crassitarsae clade. Further studies, including more taxa, are necessary to obtain a more accurate hypothesis about the evolution of some unresolved lineages (e.g., *Acanthogonatus, Stenoterommata*).

PERSPECTIVES

- Make the description of new taxa.
- Add more taxa to delimit *Stenoterommata* and *Acanthogonatus* genera.
- Make an integrative approach adding morphological characters to the phylogenomic analyses as well as conduct historical biogeography analyses.
- Increase the sampling in Peru and elucidate the new taxa status.

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REPOSITORY UPLOAD SUPPLEMENTARY MATERIAL

Table S1: Specimen locality data. Specimen sequenced for this study: ^^ new samples collected (by the first author LMO) in the field and deposited in Facultad de Ciencias, Montevideo, Uruguay: FCE-MY; new saples obtained from musseum collections + FCE-MY, ++ Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina: MACN, +++ Coleção Aracnológica de Diamantina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rio Claro, Brazil: CAD (collected by the co-author RPI). Sequence data proceeding from: * Hamilton et al. (2016b), **data proceeding from Godwin et al. (2018) *** data proceeding from Opatova et al. (2020). Coordinates are given in G.g.

Specimen ID	Genus	Species	Family	Country	Locality	Latitude	Longitude
MY3741**	Hebestatis	theveneti	Halonoproctida e	USA	CA, San Benito	36,43238	-121,22751
FCEMY1496 ^^	Idiops	sp.	Idiopidae	Peru	San Martin, Tarapoto	-6,4593333	-76,350005
AUMS19292***	Spiroctenus	flavopunctatus	Bemmeridae	South Africa	Hogsback	-32,588806	26,935194
AUMS19298***	Homostola	sp.	Bemmeridae	South Africa	Vernon Crookes	-30,274889	30,609222
AUMS22158	Spiroctenus	sp.	Bemmeridae	South Africa	Ndnumo	-26,306944	32,742169
MY2146***	Ozicrypta	sp.	Barychelidae	Australia	Queensland, Amama Park	-26,36168	152,64028
MY2135***	Synothele	sp.	Barychelidae	Australia	Western Australia, N of Menzies	-29,44338	121,26
AUMS19285***	Artrophothele	socotrana	Barychelidae	Yemen	Socrota	12,520972	53,889594
FCEMY1510 ^^	Strophaeus	sp.	Barychelidae	Peru	San Martin, San Antonio de Cumbaza	-6,4028833	-76,4062
FCEMY1377 ^^	Strophaeus	sp.	Barychelidae	Colombia	Nariño, Palmas Bajo	1,32083	-77,27972
FCEMY1383 ^^	Strophaeus	sp.	Barychelidae	Colombia	Quindio, La Virginia	4,48028	-75,62639
FCEMY1381 ^^	Strophaeus	sp.	Barychelidae	Colombia	Cali, Buenaventura	3,52519	-76,61992
FCEMY1389 ^^	Strophaeus	sp.	Barychelidae	Colombia	Quindio, Filandia	4,67519	-75,60278
FCEMY032***	Catumiri	parvum	Theraphosidae	Uruguay	Lavalleja, Aguas Blancas	-34,533333	-55,4
MY507***	Brachionopus	sp.	Theraphosidae	South Africa	Gauteng Province, Roodeplat Nature Preserve	-25,65095	28,34425
AUMS19414***	Cyriopagopus	sp.	Theraphosidae	Vietnam	Ba Vi	21,061056	105,36334
AUMS19580	Ischnocolus	sp.	Theraphosidae	Morocco	Ouzoud	31,959	6,768
AUMS19576***	Ischnocolus	sp.	Theraphosidae	Morocco	Casablanca	33,56509	-7,36581
FCEMY1397 ^^	Theraphosdiae	sp.	Theraphosidae	Colombia	Merchan, Sutamarchan	5,75028	-73,66722
AUMS11401/MY3820 *	Theraphosidae	sp.	Theraphosidae	Panama	Chiriqui Province, Blanco Arriba	8,36625	-82,935633
FCEMY1556 ^^	Theraphosidae	sp.	Theraphosidae	Peru	Pasco, Rincon de Chacos	-10,628917	-75,325967
FCEMY1555 ^^	Theraphosidae	sp.	Theraphosidae	Peru	Pasco, Por Ruta 108	-10,676683	-75,315333
AUMS10968*	Stichoplastoris	sp.	Theraphosidae	Costa Rica	La Selva	10,429	-84,0095
APH3101*	Aphonopelma	mojave	Theraphosidae	USA	CA, E of Mojave, Cache Creek	35,1261	-118,18484
AUMS16044/CNAN- Ar3658***	Mexentypesa	sp.	Nemesiidae	Mexico	Tepoztlan, San Juan Tlacotenco	19,0099	-99,0924
MY3485**	Calisoga	sp.	Nemesiidae	USA	CA, Santa Clara	37,15198	-121,58653
AUMS15439***	Nemesia	sp.	Nemesiidae	Italy	Savona, Erli	44,1219	8,1158
AUMS19577***	Nemesia	sp.	Nemesiidae	Morocco	Casablanca	33,56509	-7,36581
AUMS19583***	Iberesia	sp.	Nemesiidae	Morocco	Rhafsai	34,63257	-4,93249
AUMS15472***	Iberesia	sp.	Nemesiidae	Portugal	Quarteira	37,0795	-8,0898
MY2061***	Kwonkan	sp.	Anamidae	Australia	Western Australia, N of Menzies	-29,44338	121,26
MY2131***	Aname	sp.	Anamidae	Australia	Western Australia, N of Leonora	-28,10172	120,90417
MY2064***	Teyl	sp.	Anamidae	Australia	Western Australia, Korrelocking	-31,201722	117,47728
MY2117***	Pseudoteyl	sp.	Anamidae	Australia	Western Australia, West Cape Howe NP	-35,094972	117,62669
MY2096*	Proshermacha	tepperi	Anamidae	Australia	Western Australia, Wungong Damn	-32,19487	116,05917
MY2051***	Proshermacha	sp.	Anamidae	Australia	Western Australia, Widgiemooltha	-31,462889	121,56419
AUMS19305***	Entypesa	sp.	Entypesidae	South Africa	Drakensberg	-28,690947	28,941185

AUMS19301	Entypesa	sp.	Entypesidae	South	Drakensberg	-28,690947	28,941185
AUMS19286***	Entypesa	sp.	Entypesidae	South	Vernon Crookes	-30,274889	30,609222
MY2094*	Kiama	lachrymoides	Microstigmatida	Australia	New South Wales	-34,6993	150,8064
AUMS19321***	Microstigmata	sp.	e Microstigmatida	South	Hogsback, Big tree trail	-32,602222	26,943361
AUMS19578***	Cyrtauchenius	sp.	e Cyrtaucheniida	Africa Morocco	Rhafsai	34,63257	-4,93249
AUMS19416***	Cyrtaucheniida	sp.	e Cyrtaucheniida	Ecuador	Napo province	-1,06727	-77,11671
FCEMY1386 ^^	e Bolostromus	sp.	e Cyrtaucheniida	Colombia	Quindio, Salento	4,63806	-75,57306
FCEMY1384 ^^	Bolostromus	sp.	e Cyrtaucheniida	Colombia	Quindio, La Virginia	4,48028	-75,62639
FECMY1494 ^^	Bolostromus	sp.	e Cyrtaucheniida	Peru	San Martin, Tarapoto	-6,45	-76,35
FCEMY1495 ^^	Bolostromus	sp.	e Cyrtaucheniida	Peru	San Martin, Tarapoto	-6,45	-76,35
FCEMY1391 ^^	Bolostromus	sp.	e Cyrtaucheniida	Colombia	Antioquia, Santa Elena	6,29578	-75,50131
FCEMY1380 ^^	Linothele	sp.	e Dipluridae	Colombia	Alto de Daza, Nariño	1,25744	-77,27039
FCEMY1388 ^^	Linothele	sp.	Dipluridae	Colombia	Quindio, La Virginia	4,67278	-75,66056
FCEMY1392 ^^	Linothele	sp.	Dipluridae	Colombia	Antioquia, Santa Elena	6,29578	-75,50131
FCEMY1393 ^^	Linothele	SD.	Dipluridae	Colombia	Antioquia, Santa Elena	6.29578	-75.50131
	Linotholo	op.	Dipluridaa	Boru	Hueneoveliee	10 20222	10,00101
FCENIT 1536	Linothele	sp.	Dipiuridae	Peru	Huancavelica	-12,30333	-12,30333
AUMS19303***	Linothele	sp.	Dipluridae	Panama	NA	NA	NA
FCEMY1523 ^^	Diplura	sp.	Dipluridae	Peru	San Martin, Biodiversidad	-6,48333	-76,35
FCEMY1498 ^^	Diplura	sp.	Dipluridae	Peru	San Martin, Tarapoto	-6,45	-76,35
AUMS19295***	Diplura	cf. petrunkevitchi	Dipluridae	Venezuela	San Cristóbal	7,80373	-72,17756
FCEMY1497 ^^	Diplura	sp.	Dipluridae	Peru	San Martin, Tarapoto	-6,45	-76,35
FCEMY1554 ^^	Fufiidae	sp.	Fufiidae	Peru	Junin, Rumbo a la Merced	-11,216667	-75,48333
FCEMY1549 ^^	Fufiidae	sp.	Fufiidae	Peru	Junin, Rumbo a la Merced	-11,283333	-75,55
FCEMY1548 ^^	Fufiidae	sp.	Fufiidae	Peru	Junin, Rumbo a la Merced	-11,283333	-75,55
ECEMY1552 ^^	Fufiidae	sp	Fufiidae	Peru	Junin Rumbo a la Merced	-11 283333	-75 55
	Fufiidao	ep.	Fufiidao	Poru	Junin, Rumbo a la Morcod	11 283333	75,55
	Fufiidaa	эр. ор	Fufiidae	Doru		11.25	75 52222
	Tullidae	sp.	Tufiidae	Peru	Son Martin Ducalna	-11,55	75,00007
FCEMIT 1493	Fullus	sp.	Fullidae	Peru	San Martin, Pucaipa	-6,5105556	-/5,61100/
FCEMY1524 M	Fufius	sp.	Fufildae	Peru	San Martin, Biodiversidad	-76,35005	-76,35
FCEMY1507 ^^	Fufius	sp.	Fufiidae	Peru	San Martin, Tarapoto	-6,45	-76,35005
FCEMY1509 ^^	Fufius	sp.	Fufiidae	Peru	San Martin, San Antonio de Cumbaza	-6,4028833	-76,4062
AUMS6720*	Pionothele	sp.	Pycnothelidae	Namibia	Gobabeb	-23,5653	15,0398
RPI13 +++	Chaco	sp.	Pycnothelidae	Brasil	Rio de Janeiro	-22,286	-44,604833
RPI5 +++	Stenoterommat a	sp.	Pycnothelidae	Brasil	Rio de Janeiro	-22,4525	-42,990833
RPI22 +++	Stenoterommat a	pavesii	Pycnothelidae	Brasil	Santa Catarina	-27,053611	-49,085833
RPI23 +++	Hermachura	luederwaldti	Pycnothelidae	Brasil	Rio de Janeiro	-22,4205	-44,6265
RPI18 +++	Stenoterommat	sp.	Pycnothelidae	Brasil	Rio de Janeiro	-22,286	-44,604833
MACN36488***	Stenoterommat	iguazu	Pycnothelidae	Argentina	Misiones, Yacuy	-25,583333	-54,216667
FCEMY1275 +	Stenoterommat a	palmar	Pycnothelidae	Uruguay	Salto, Ruta 31, Arroyo Arerungua	-31,323056	-57,06583
RPI21 +++	Stenoterommat a	sp.	Pycnothelidae	Brasil	Santa Catarina	-71,73975	-49,085833
FCEMY1492 ^^	Pycnothelidae	sp.	Pycnothelidae	Peru	Cusco	-13,617472	-71,73975
RPI6 +++	Rachias	sp.	Pycnothelidae	Brasil	Rio de Janeiro	-22,4525	-42,990833
RPI3 +++	Rachias	dispar	Pycnothelidae	Brasil	Rio de Janeiro	-22,4525	-42.990833
RPI15 +++	Rachias	sp	Pycnothelidae	Brasil	Rio de Janeiro	-22 286	-44 604833
BPI10 +++	Prorachias	sn.	Pycnothelidao	Brasil	Rio de Janeiro	-22 368611	_// 7/5
	Prorochioo	op.	Dyenothelidae	Brasil	São Doulo	-22,500011	46 755922
	Proracillas	sp.	Pychothelidae	DIASI		-23,43/5	-40,700000
FCEMY12/3 +	Chaco	costai	Pycnothelidae	Uruguay	Rocha, Benicio	-34,416667	-53,85
MACN34037 ++	Acanthogonatu s	huanquen	Pycnothelidae	Chile	Cuesta Melon	NA	NA
FCEMY1400/FCEMY130 0 ^^	Chilelopsis	serena	Pycnothelidae	Chile	Elqui, Coquimbo	-29,378056	-71,089167
FCEMY1399/FCEMY129 9 ^^	Chilelopsis	calderoni	Pycnothelidae	Chile	Vallenar, Atacama	-28,087778	-70,598889
FCEMY1290 ^^	Flamencopsis	minima	Pycnothelidae	Chile	Diego de Almagro, Atacama, Quebrada Aspera	-26,590556	-70,676389
MY2092*	Stanwellia	hoggi	Pycnothelidae	Australia	New South Wales, N of Stanwell Park	-34,22273	150,99
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FCEMY1280 +	Acanthogonatu s	centralis	Pycnothelidae	Argentina	Buenos Aires, Sierra de la Ventana	-38,072222	-62,051944
FCEMY1274***	Acanthogonatu s	tacuariensis	Pycnothelidae	Uruguay	Rivera, Amarillo	-31,583333	-55,1
MACN34591 ++	Diplothelopsis	bonariensis	Pycnothelidae	Argentina	La Pampa	NA	NA
MACN30949 ++	Lycinus	longipes	Pycnothelidae	Argentina	San Luis	NA	NA
FCEMY1398/FCEMY129 8 ^^	Lycinus	tofo	Pycnothelidae	Chile	Elqui, Coquimbo, Peaje Punta Colorada	-29,378056	-71,089167
FCEMY1401/FCEMY130 1 ^^	Lycinus	sp.	Pycnothelidae	Chile	Elqui, Coquimbo, cerca de Choros	-29,296667	-71,368056
FCEMY1294 ^^	Licinus	gajardoi	Pycnothelidae	Chile	Elqui, Coquimbo, N de La Serena.	-29,756667	-71,326667
RPI2 +++	Xenonemesia	sp.	Pycnothelidae	Brasil	Santa Catarina	-27,780278	-48,5075
RPI25 +++	Pycnothele	auronitens	Pycnothelidae	Brasil	Rio Grande do Sul	-29,158333	-50,07972
RPI24 +++	Neostotis	gigas	Pycnothelidae	Brasil	São Paulo	-23,774444	-46,312778
FCEMY1278***	Bayana	labordai	Pycnothelidae	Uruguay	Rivera, Cerro Miriñaque	-31,5	-55,65
MACN17843 ++	Pycnothele	modesta	Pycnothelidae	Argentina	Jujuy	NA	NA

Supplementary figure 1. Topology obtained from RaxML in the maximum likelihood (ML) analysis. Bootstrap values supports are indicated on the nodes.



Supplementary figure 2. Topology obtained from Exabayes in the Bayesian Inference (BI) analysis. Posterior probability (PP) values supports are indicated on the nodes.



Supplementary figure 3. Topology obtained from IQ-TREE in the Maximum likelihood (ML) analysis. Bootstrap and genealogical and sites concordances factor (bootstrap/gCF/sGF/ respectively) values supports are indicated on the nodes.



Supplementary figure 4. Topology obtained from ASTRAL in the species tree analysis. ASTRAL support values are indicated in the nodes.

