

## Phylogenomic reclassification of the world's most venomous spiders (Mygalomorphae, Atracinae), with implications for venom evolution

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### Supplemental Information

**Supplemental Text.** Summary of prior hexathelid phylogeny and classification studies.

Although our overall results contrast with earlier morphology-only studies of mygalomorph relationships, several points of agreement can be found with more recent molecular studies. Raven [12, 27] re-limited the family Hexathelidae, and identified one morphological synapomorphy (possession of numerous labial cuspules). Raven [12] discussed at length the uncomfortable placement of *Atrax* (then the only described atracine), including hypothesized morphological homoplasy with more derived mygalomorph taxa. Goloboff [22] conducted a formal cladistic analysis of morphology, and recovered a paraphyletic Hexathelidae as an early-diverging Avicularioidea lineage, but noted that near parsimonious trees included hexathelids as monophyletic. Using ribosomal DNA sequences, Hedin & Bond [23] found a paraphyletic Hexathelidae, with taxa intermixed with non-diplurine diplurids at the base of Avicularioidea. Sampled atracines were not recovered with the single sampled actinopodid. Using elongation factor-1 gamma sequences, Ayoub *et al.* [17] recovered a *Hadronyche* plus *Missulena* clade, separate from the hexatheline *Bymainiella*. Using multiple genes alone, or molecules plus morphology, Bond *et al.* [13] recovered *Atrax* + *Hadronyche* with *Missulena* + *Actinopus* (F Actinopodidae). Again, hexathelids were not monophyletic, with two hexathelines intermixed with early-diverging non-diplurine diplurids. Opatova and Arnedo [18] used the Bond *et al.* [13] multigenic matrixes, adding data for multiple *Macrothele* specimens. These authors found four separate hexathelid lineages (i.e., hexathelid non-monophyly), a monophyletic *Macrothele*, and an atracine plus actinopodid sister relationship. Using a 6-gene dataset, Wheeler *et al.* [20] recovered a core hexathelid lineage (including the important South American taxa *Mediothele* and *Scotinoecus* as sister taxa), separate from a well-supported atracine plus actinopodid clade. Finally, Hamilton *et al.* [19] used anchored hybrid enrichment (AHE) sequence capture data to show hexathelid non-monophyly, and an atracinae plus actinopodid relationship (see **FIG 1C**).

**Supplemental Table 1.** Excel file with voucher, identification and UCE information.

**Supplemental Figure 1.** ASTRAL phylogenies.

