



# Huntsman spider phylogeny informs evolution of life history, egg sacs, and morphology

Jacob A. Gorneau<sup>a,b,\*</sup>, Cristina A. Rheims<sup>c</sup>, Corrie S. Moreau<sup>a,d</sup>, Linda S. Rayor<sup>a</sup>

<sup>a</sup> Department of Entomology, Cornell University, Ithaca, NY 14853, USA

<sup>b</sup> Institute for Biodiversity Science and Sustainability, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA

<sup>c</sup> Laboratório de Coleções Zoológicas, Instituto Butantan, Av. Vital Brasil, 1500, 05503-900 São Paulo, SP, Brazil

<sup>d</sup> Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

## ARTICLE INFO

### Keywords:

Sparassidae  
Molecular phylogenetics  
Social evolution  
Stochastic character mapping  
D-test  
Deleninae

## ABSTRACT

Huntsman spiders (Araneae: Sparassidae) are among the most speciose spider families, with a near-worldwide distribution, diverse habitats, equally diverse life histories, and five prolonged subsocial species. Previous molecular phylogenies have focused on individual subfamilies or clades. Here, we provide a phylogenetic inference with broadened sampling from 37 genera and eight of the eleven sparassid subfamilies. We increased taxon sampling by including species not previously sequenced and most available data on GenBank of two mitochondrial (COI, 16S rRNA) and two nuclear (H3, 28S rRNA) genes for a total of 262 ingroup taxa and nine outgroup taxa. Divergence dates were estimated using outgroup fossil taxa suggesting the sparassids evolved ~ 100 mya (stem age), while the clade containing all subfamilies except Sparianthinae evolved ~ 90 mya (stem age). Using a stochastic map approach with 40 species, this is the first sparassid phylogeny to incorporate extensive biology and life history data. Correlations of life history traits with solitary, subsocial, and prolonged subsocial behavior are examined using the D-test. Sparassid sociality is associated with life history traits that allow developing spiders to remain in their natal retreat longer (e.g., larger permanent retreats, plastered egg sacs, and ontogenetically delayed foraging), but is unrelated to body size or lifespan. Detailed morphological scoring of the endemic Australian subfamily Deleninae contextualizes existing molecular data, including in the *Isopeda-Holconia-Isopedella* complex. This study supports the monophyly of many major lineages, including for the first time, the Sparianthinae, but indicates multiple clades (Sparassinae and Eusparassinae) are paraphyletic and need further revision.

## 1. Introduction

Huntsman spiders (Araneae: Sparassidae) comprise 1,319 species described from 91 genera (World Spider Catalog, 2022 23.0), making them the eleventh-most diverse family of spiders (see Fig. 1 for a snapshot of sparassid biodiversity, with a focus on individuals in this study). Although already quite diverse, it is estimated that at least 1,000 huntsman species remain to be described (Agnarsson et al., 2013a). Huntsmen have a near-worldwide distribution and are found in many climates and habitats, ranging from deserts to rainforests, lowlands to highlands, and in caves (Jäger, 2005a, Jäger, 2005b, Rheims, 2010a, Rheims, 2010b, Hsieh & Linsenmair, 2011, Moradmand et al., 2016).

The monophyly of huntsman spiders is unquestioned morphologically and has been corroborated by multiple morphological (Rheims, 2007a, Ramírez, 2014) and molecular phylogenetic inferences (Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017, Tong et al., 2019). Huntsman spiders were first described by Bertkau (1872), though use of the name Sparassidae was disputed until recently, with Heteropodidae and Eusparassidae also in use through the end of the 20th century (Jäger, 1999a). A conspicuous synapomorphy for the family is the presence of a trilobate membrane dorsally on the apex of the metatarsus, as proposed by Petrunkevitch (1928), but other synapomorphies identified by Ramírez (2014) include the apical indentation of claw tuft setae, membranous projections of the tarsi

\* Corresponding author at: Institute for Biodiversity Science and Sustainability, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA.

E-mail addresses: [jag482@cornell.edu](mailto:jag482@cornell.edu), [jgorneau@calacademy.org](mailto:jgorneau@calacademy.org) (J.A. Gorneau), [carheims@gmail.com](mailto:carheims@gmail.com) (C.A. Rheims), [corrie.moreau@cornell.edu](mailto:corrie.moreau@cornell.edu) (C.S. Moreau), [lsl1@cornell.edu](mailto:lsl1@cornell.edu) (L.S. Rayor).

<https://doi.org/10.1016/j.ympev.2022.107530>

Received 29 July 2021; Received in revised form 17 April 2022; Accepted 6 May 2022

Available online 27 May 2022

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around claw tuft plates, and trichobothrial setae with smooth bases.

Based on morphology and phylogenetic analysis, the placement of huntsman spiders in the Retrolateral Tibial Apophysis (RTA) clade is strongly supported (Moradmand et al., 2014, Ramírez, 2014, Wheeler et al., 2017, Fernández et al., 2018). Moradmand et al. (2014) inferred the Sparassidae as sister to the rest of the RTA clade but did not propose placement of the family beyond this. Wheeler et al. (2017) recovered sparassids as sister to the Oval Calamistrum clade + Dionycha, but this support was weak, and the position of Sparassidae within the RTA clade remained unresolved. Garrison et al. (2016) did not include sparassids, despite including many other major members of the RTA clade. The largest phylogenomic analysis of spiders to date placed the clade containing Sparassidae and the marronoid clade (*sensu* Wheeler et al., 2017) sister to the rest of the RTA clade (Fernández et al., 2018). Azevedo et al. (2022) also recovered sparassids as sister to the marronoid clade.

Nearly a third of sparassid genera are not assigned to a formally described subfamily (Table 1). Currently eleven subfamilies have been described: Chrosiidermatinae Simon, Clastinae Simon, Deleninae Hogg, Eusparassinae Järvi, Heteropodinae Thorell, Palystinae Simon, Polybetinae Järvi, Sparassinae Bertkau, Sparianthinae Simon, Staianinae Simon, and Tibellomatinae Simon. Four previous studies have published phylogenetic hypotheses to evaluate internal relationships within the Sparassidae (Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017, Tong et al., 2019).

Furthermore, some genera are either known or suggested to be paraphyletic (Agnarsson & Rayor, 2013, Moradmand et al., 2014). Based on morphology, Hirst (1990) split the delenine genus *Isopeda* (L.) Koch 1875, into the genera *Isopeda*, *Holconia* Thorell 1877, and

*Isopedella* (Iso.) Hirst 1990. These relationships are not supported in phylogenetic analyses, with all three genera rendered paraphyletic, despite forming a clade when examined as a whole (Agnarsson & Rayor, 2013). The genus *Olios* Walckenaer 1837, formerly the largest genus of sparassids, was until recently known to be paraphyletic but was reduced by Jäger (2020a) to 87 species distributed in Africa, Southern Europe, and Asia. Rheims and collaborators (Rheims, 2007a; Rheims & Jäger, 2008, 2022; Rheims, 2010a, 2010c, 2015, 2019a) have moved many of the New World “*Olios*” species into other previously known or new genera, such as *Vindullus* Simon 1880a, *Macrinus* Simon 1887, *Caayguara* Rheims 2010a, *Curicaberis* Rheims 2015, among others.

While the majority of species are solitary hunters beyond a short period of transient maternal care of egg sacs and newly emerged young, a few sparassids have evolved sociality. To date, five species (*Delena cancerides* Walckenaer 1837, *Delena lapidicola* (Hirst, 1991a), *Delena melanochelis* (Strand 1913), *Delena spenceri* (Hogg, 1903), and an unidentified *Damastes* sp. Simon 1880a) have been observed to live in long lasting mother-offspring groups (Agnarsson & Rayor, 2013, Yip & Rayor, 2014, Rayor unpublished data). These social species are characterized as ‘prolonged subsocial’ and consist of colonies of an interactive family group with a single adult female and her immature or newly mature offspring, who remain together in a retreat to have a ‘prolonged’ association lasting approximately five months to a year depending on the species (Yip & Rayor, 2014). Until the discovery of prolonged sociality in a *Damastes* sp. from Madagascar, all other social sparassids were in the endemic Australian Deleninae, necessitating further phylogenetic investigation to understand the comparative social evolution of the group.



**Fig. 1.** Survey of some of the adult female huntsman spiders in this study. A: *Pandercetes* cf. *malleator*. B: *Pandercetes* cf. *nigrogularis* on plastered egg sac embedded into curl of leaf. C: *Barylestis occidentalis* with plastered egg sac and first instar young. The young have emerged from a split between the front and back sheets of the egg sac. D: *Heteropoda jugulans* with plastered egg sac. E: *Heteropoda lunula* immature female. F: *Heteropoda venatoria* with lenticular egg sac carried under abdomen. G: *Heteropoda boiei*. H: *Damastes* sp. gravid. I: *Delena cancerides* with plastered egg sac, non-feeding first instar young. J: *Delena gloriosa* with plastered egg sac. K: *Neosparassus calligaster* with round egg sac, silken cage, first instar. White at base of silken cage is a bottle cap pulled into the silk. L: *Pediana horni*. M: *Isopeda villosa*. N: *Isopeda leishmanni* on lenticular egg sac with a newly opened emergence hole. All Deleninae females chew a hole in the egg sac for the young to emerge from. O: *Isopedella pessleri* with first instar young on lenticular egg sac. P: *Isopeda canberrana* within an hour after young emerge from the chewed hole in sac. The young emerge as nymphs and upon emergence molt to first instar with longer legs. Silk lines attach the egg sac to the bark. Q: *Isopedella leai* in silk cage with access hole on bottom. R: *Isopedella conspersa* outside a silk cage with a lenticular egg sac. S: *Holconia nigrigularis* with newly laid lenticular egg sac with silk attachments. T: *Holconia insignis* on lenticular egg sac with silk attachments. Photos of *Beregama aurea* (L) and *Iso. conspersa* (S) by Alan Henderson, all other photos by Linda Rayor.

Table 1

Information on taxonomic placement of 91 current genera in sparassid subfamilies. Genera with molecular data represented in this study are bolded.

Subfamily	Genera Included	Status	Distribution	Notes
Chrosiodermatinae	<i>Chrosioderma</i> Simon 1897a	Monotypic	Africa (Madagascar)	
Clastinae	<i>Clastes</i> Walckenaer 1837	Monotypic	Oceania	
Deleninae	<b><i>Delena</i></b> Walckenaer 1837, <b><i>Beregama</i></b> Hirst 1990, <b><i>Holconia</i></b> Thorell 1877, <b><i>Isopeda</i></b> L. Koch 1875, <b><i>Isopedella</i></b> Hirst 1990, <b><i>Pediana</i></b> Simon 1880a, <b><i>Neosparassus</i></b> Hogg 1903, <b><i>Typostola</i></b> Simon 1897a, <b><i>Zachria</i></b> L. Koch 1875, some former <i>Olios</i> rendered genus <i>incertae sedis</i>	Monophyletic (Agnarsson & Rayor 2013, Wheeler et al., 2017, Moradmand et al., 2014)	Oceania	See Jäger (2020a) for specifics on subset of former <i>Olios</i> now designated in Deleninae <i>incertae sedis</i>
Eusparassinae	<b><i>Arandisa</i></b> Lawrence 1938, <b><i>Carparachne</i></b> Lawrence 1962, <b><i>Eusparassus</i></b> Simon 1903a, <b><i>Leucorchestris</i></b> Lawrence 1962, <b><i>Microrchestris</i></b> Lawrence 1962, <b><i>Orchestrella</i></b> Lawrence 1965, <b><i>Palystella</i></b> Lawrence 1928, <b><i>Pseudomicrommata</i></b> Järvi 1912	Paraphyletic: subset not related to <i>Eusparassus</i> forms monophyletic African clade (Moradmand et al., 2014); <i>Eusparassus</i> monophyletic	Africa, Asia, Europe, South America	
Heteropodinae (sensu stricto)	<b><i>Barylestis</i></b> Simon 1909, <b><i>Bhutaniella</i></b> Jäger 2000, <b><i>Heteropoda</i></b> Latreille 1804, <b><i>Martensikara</i></b> Jäger 2021, <b><i>Martensopoda</i></b> Jäger 2006, <b><i>Pandercetes</i></b> L. Koch 1875, <b><i>Platnickopoda</i></b> Jäger 2020c, <b><i>Pseudopoda</i></b> Jäger 2000, <b><i>Sinopoda</i></b> Jäger 1999b, <b><i>Spariolenus</i></b> Simon 1880a, <b><i>Thunberga</i></b> Jäger 2020b, <b><i>Yünthi</i></b> Davies 1994	Monophyletic (Agnarsson & Rayor 2013, Moradmand et al., 2014)	Africa, Asia, Oceania; <i>Barylestis variatus</i> introduced widely in Europe; <i>Heteropoda venatoria</i> introduced nearly worldwide	
Heteropodinae (sensu lato)	As above, and including: <i>Anaptomecus</i> Simon 1903b, <i>Guadana</i> Rheims 2010d, <i>Sparianthina</i> Banks 1929, some former <i>Olios</i> rendered genus <i>incertae sedis</i>	Polyphyletic, with <i>Meri</i> cf. <i>sanctivincenti</i> included the sole representative in Moradmand et al. (2014), but see text	North America, South America	<i>Caayguara</i> moved to <i>incertae sedis</i> by Pinto & Rheims (2016); see Jäger (2020a) for specifics on subset of former <i>Olios</i> now designated <i>incertae sedis</i>
Palystinae	<b><i>Anchonastus</i></b> Simon 1898b, <b><i>Palystes</i></b> L. Koch 1875, <b><i>Panaretella</i></b> Lawrence 1937, <b><i>Parapalystes</i></b> Croeser 1996, <b><i>Sarotesius</i></b> Pocock 1898, at least one former <i>Olios</i> rendered genus <i>incertae sedis</i>	Monophyletic (Agnarsson & Rayor 2013, Moradmand et al., 2014)	Africa, Asia	See Jäger (2020a) for specifics on subset of former <i>Olios</i> now designated <i>incertae sedis</i>
Polybetinae	<b><i>Polybetes</i></b> Simon 1897a	Monotypic	South America	
Sparassinae	<b><i>Adcatomus</i></b> Karsch 1880, <b><i>Cebrennus</i></b> Simon 1880b, <b><i>Cerbalus</i></b> Simon 1897a, <b><i>Curicaberis</i></b> Rheims 2015, <b><i>Olios</i></b> Walckenaer 1837, <b><i>Macrinus</i></b> Simon 1887, <b><i>Micrommata</i></b> Latreille 1804, <b><i>Quemedice</i></b> Mello-Leitão 1942, <b><i>Sivalicus</i></b> Dyal 1957, <b><i>Vindullus</i></b> Simon 1880a, some former <i>Olios</i> rendered genus <i>incertae sedis</i>	Polyphyletic (Moradmand et al., 2014)	Asia, Africa, Europe, North America, South America	Rheims (2015) placed <i>Curicaberis</i> in Sparassinae; see Rheims (2007b) and Jäger (2020a) for specifics on subset of former <i>Olios</i> now designated <i>incertae sedis</i>
Sparianthinae	<b><i>Decaphora</i></b> Franganillo 1931, <b><i>Diminutella</i></b> Rheims & Alayón 2018, <b><i>Extraordinarius</i></b> Rheims 2019b, <b><i>Neostasina</i></b> Rheims and Alayón 2016, <b><i>Pleorotus</i></b> Simon 1898c, <b><i>Rhacocnemis</i></b> Simon 1898b, <b><i>Sparianthis</i></b> Simon 1880a, <b><i>Stasina</i></b> Simon 1877, <b><i>Stipax</i></b> Simon 1898c, <b><i>Thecticopsis</i></b> Karsch 1884, <b><i>Thomasettia</i></b> Hirst 1911, <b><i>Uaiuara</i></b> Rheims 2013	Sister to the rest of Sparassidae (Moradmand et al., 2014, Wheeler et al., 2017); Sparianthinae represented only by <i>Neostasina</i> in Tong et al. (2019), so monophyly not evaluated	Asia, Africa, North America, Oceania, South America	<i>Diminutella</i> included by Rheims & Alayón (2018), <i>Extraordinarius</i> included by Rheims (2019), <i>Neostasina</i> included by Rheims & Alayón (2016), <i>Uaiuara</i> placed here by Rheims (2013)
Staianinae	<b><i>Staianus</i></b> Simon 1889	Monotypic	Africa (Madagascar)	
Tibellomatinae	<b><i>Tibellomma</i></b> Simon 1903a	Monotypic	South America (Venezuela)	Described only from immature, see text
Generic group 1 sensu Moradmand et al., 2014	<b><i>Damastes</i></b> Simon 1880a, <b><i>Megaloremnius</i></b> Simon 1903a	Designated by Moradmand et al., 2014	Africa (primarily Madagascar)	
Generic group 2 sensu Moradmand et al., 2014	<b><i>Gnathopalystes</i></b> Rainbow 1899, <b><i>Prychia</i></b> L. Koch 1875, <b><i>Tychicus</i></b> Simon 1880a	Designated by Moradmand et al., 2014	Asia, Oceania	
Generic group 3 sensu Moradmand et al., 2014	<b><i>Remnius</i></b> Simon 1897b, <b><i>Rhitymna</i></b> Simon 1897b	Designated by Moradmand et al., 2014	Africa, Asia	
“African Clade” sensu Moradmand et al., 2014	<b><i>Arandisa</i></b> , <b><i>Carparachne</i></b> , <b><i>Leucorchestris</i></b> , <b><i>May</i></b> Jäger & Krehenwinkel 2015, <b><i>Microrchestris</i></b> , <b><i>Palystella</i></b> , <b><i>Pseudomicrommata</i></b>	Designated by Moradmand et al., 2014	Africa	May included by Jäger & Krehenwinkel (2015)  <i>Arandisa</i> , <i>Carparachne</i> , <i>Leucorchestris</i> , <i>Microrchestris</i> , <i>Palystella</i> , <i>Pseudomicrommata</i> all still formally in Eusparassinae
<i>incertae sedis</i>	<b><i>Berlandia</i></b> Lessert 1921, <b><i>Caayguara</i></b> Rheims 2010a, <b><i>Deelemanikara</i></b>			See note at Heteropodinae s.l. regarding inclusion of <i>Caayguara</i> ;

(continued on next page)

Table 1 (continued)

Subfamily	Genera Included	Status	Distribution	Notes
	Jäger 2021, <i>Defectrix</i> Petrunkevitch 1925, <i>Dermochrosia</i> Mello-Leitão 1940, <i>Exopalystes</i> Hogg 1914, <i>Geminia</i> Thorell 1897, <i>Irileka</i> Hirst 1998, <i>Keilira</i> Hirst 1989, <i>Nolavia</i> Kammerer 2006, <i>Nungara</i> Pinto & Rheims 2016, <i>Meri</i> Rheims & Jäger 2022, <i>Origes</i> Simon 1897b, <i>Paenula</i> Simon 1897b, <i>Prusias</i> O. Pickard-Cambridge 1892, <i>Sadala</i> Simon 1880, <i>Sagellula</i> Strand 1942, <i>Stasinoides</i> Berland 1922, <i>Strandiellum</i> Kolosváry 1934, some former <i>Olios</i> rendered genus <i>incertae sedis</i>		Africa, South America, North America, Asia, Oceania	<i>Nungara</i> included by Pinto & Rheims (2016); see Jäger (2020a) for specifics on subset of former <i>Olios</i> now designated <i>incertae sedis</i>

Based on long-term study by LSR, most huntsman species reach adulthood in their tenth instar, with individual *Delena lapidicola* starting to disperse in the fifth or sixth instar or later; *D. melanochelis*, *D. spenceri*, and *Damastes* sp. in the sixth instar or later; and *D. cancerides* occasionally in the eighth instar, but most as penultimate ninth instars or young adults. Prolonged subsocial species are characterized by a single breeding adult female and the simultaneous presence of multiple clutches of her offspring of all ages in the same retreat, sharing prey, and extensive interaction, tolerance, and (in *D. cancerides*) intolerance to older immigrants (Rowell & Avilés, 1995, Auletta & Rayor, 2011, Yip et al., 2012, Yip & Rayor 2013, Yip & Rayor, 2014, Jones & Rayor, 2017). Subadult or recently mature females disperse independently to establish their own retreats, and similarly aged males disperse to find receptive females. In all species studied to date, a single adult female establishes a colony and other mature females are not tolerated. Additionally, all five prolonged subsocial species have delayed development where the spiderlings emerge from the egg sac at the first instar with large yolk-filled abdomens, but do not feed until they molt to the second instar (Cardillo & Rayor, in preparation, using terminology which refers to the first instar to emerge from the egg sac as the first instar or one instar earlier than referred to in earlier Rayor publications). All other solitary and subsocial species start foraging during the first instar.

Like other sparassids, but unlike most subsocial and social spiders in other families, prolonged subsocial species do not live on prey capture webs, do not exhibit a skewed sex ratio, and do not show evidence of the inbreeding typical of cooperative ‘social’ spiders (Avilés & Guevara, 2017). Instead, they are found in bark or rock retreats. Most other social spiders use webs as a substrate for chemical and vibrational cues, so the presence of social behavior in a group lacking such a hallmark of spider communication is especially noteworthy (Avilés, 1997, Avilés & Guevara, 2017).

Two more delenine species exhibit subsocial behavior in the laboratory: *Isopedella leai* (Hogg, 1903) and *I. pessleri* (Thorell, 1870). During the four to five weeks (late third instar) that these two species remain with their mother and siblings, they are notable for the relatively high frequency of prey sharing, and tolerant associations compared to the solitary huntsman species (Rayor & Giulian unpublished data, Yip & Rayor, 2014). Solitary species typically disperse during the second instar.

With varied life history, behavioral, physiological, and developmental traits, sparassids represent a model clade (*sensu* Sanger & Rajakumar, 2019). This diversity makes phylogenetic study especially informative for understanding the evolution of social behavior. We infer a robust molecular phylogeny of the Sparassidae by broadening taxon sampling in the group. We then employ this phylogeny to evaluate hypotheses about life history traits and social evolution based on extensive field and laboratory observations. Group living in huntsman spiders is characterized by mothers who remain associated with one or more cohorts of their offspring for long periods. Thus, we hypothesized that prolonged subsociality could be phylogenetically associated with certain life history traits, such as more permanent or well-protected retreats,

less movement while guarding eggs, spatial or developmental characteristics that may facilitate interactions among group-members, or even a longer lifespan. In contrast, we anticipated that solitary species should have less permanent or protective retreats, could be more vagile while guarding egg sacs or have shorter lifespans. As such, we tested life history traits, including retreat features, egg sac structure and attachment, development, and lifespan, across the Sparassidae to determine whether certain character states are associated with group-living in a phylogenetic context.

## 2. Materials and methods

Life history data for 40 species were based upon laboratory and field observations by L. Rayor and were collected between 2002 and 2021. These data include observations on mother-offspring dynamics and sociality, egg sac structure, how the egg sac was attached to the retreat or carried, retreat type, modifications to the retreat, adult female body mass and cephalothorax width, age at sexual maturity, and lifespan for the following subfamilies: Sparianthinae (represented by *Thelcticopsis*), Heteropodinae, Palystinae, Sparassinae (represented by a true *Olios* and one “*Olios*”), and Deleninae. Legs, or whole bodies, in the case of spiderlings, were pulled from representative specimens for molecular analysis and stored in 95% ethanol in a  $-20^{\circ}\text{C}$  freezer until extraction. Additionally, voucher specimens representing species in the molecular and life history dataset were deposited at the National Museum of Natural History (NMNH, USA).

A total of 82 DNA samples were extracted in this study. PCR was performed for nuclear genes H3 and 28S rRNA and mitochondrial genes 16S rRNA and COI using primers described in Palumbi et al. (1991), Folmer et al. (1994), Colgan et al. (1998), Hedin & Maddison (2001), and Vink et al. (2005). Protocols were adapted from Agnarsson et al. (2007, 2013b) and Agnarsson (2012). After purification with ExoSAP-IT (Thermo Fisher, Waltham, MA, USA), samples were sent to the Cornell Genomics Facility (Ithaca, NY, USA) for bidirectional Sanger sequencing. Sequences were assembled using Geneious Prime 2020.0.5 (<https://www.geneious.com/>). Sequences generated in this study were combined with sequences from 201 additional samples from GenBank to increase taxon representation within the Sparassidae and to provide outgroups. Outgroups included the following: *Deinopsis spinosa* Marx 1889 (Deinopidae), *Uloborus diversus* Marx 1898 (in Banks 1898; Uloboridae), *Oecobius Blackwall* 1862 (Oecobiidae), *Uroctea durandi* ((Latreille, 1809); Oecobiidae), *Peucetia viridans* ((Hentz, 1832); Oxyopidae), *Dolomedes tenebrosus* Hentz 1844 (Pisauridae), *Salticus scenicus* (Clerck, 1757; Salticidae), *Selenops muehlmannorum* Jäger & Praxaysombath, 2011 (Selenopidae), and *Tibellus chamberlini* Gertsch 1933 (Philodromidae). Sequences were aligned using MAFFT and were manually examined using Mesquite version 3.6 (Katoh & Standley, 2013, Maddison & Maddison, 2018). Aligned sequences were concatenated and phylogenies were inferred in IQ-TREE using ModelFinder and ultrafast bootstraps (UFBoot) as support values (Nguyen et al., 2015, Kalyaanamoorthy et al., 2017, Hoang et al., 2018). With the IQ-TREE

phylogeny as a starting tree, a maximum likelihood phylogeny was also inferred using the program RAxML (Stamatakis, 2014). Due to a lack of convergence, a starting tree was used for Bayesian inference in MrBayes (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003). Divergence dating was implemented in BEAST, calibrated with fossil outgroups, while a fossil sparassid exists, it is not useful for calibration purposes (Drummond et al., 2012, Suchard et al., 2018, Bouckaert et al., 2019, Magalhães et al., 2020). Models of molecular evolution were selected using jModelTest2 (Darriba et al., 2012). Fossil outgroups included *Zamilia aculeopectens* Wunderlich 2015 (Oecobiidae) for the node representing Oecobiidae, *Oxyopes succini* Petrunkevitch 1958 (Oxyopidae) for Oxyopidae + Pisauridae, *Almolinus ligula* Wunderlich, 2004 (Salticidae) for the node containing the outgroups Salticidae + Philodromidae, and ‘*Selenops*’ sp. indet. Wunderlich, 1988 (Selenopidae) for the node with outgroups Selenopidae sister to Salticidae + Philodromidae. BEAST runs were examined using the program Tracer (Rambaut et al., 2018). Trees were initially examined using FigTree (Rambaut, 2018). MAFFT, MrBayes, jModelTest2, and BEAST were all run using the CIPRES Science Gateway (Miller et al., 2010).

Stochastic character mapping was selected as the method of choice for trait mapping to allow for examination of character evolution among nodes and the generation of posterior probabilities from stochastic character map runs at each node. This was completed in the R using the packages corHMM, phylotools, and phytools (Revell, 2012, Beaulieu et al., 2013, Zhang et al., 2017, R Core Team, 2022). Using stochastic character maps also enabled a pairwise examination of correlation between life history traits and sociality through the D-test, developed by Huelsenbeck et al. (2003) for identifying correlations of morphological characters and implemented in phytools.

Morphological data for the subfamily Deleninae as well as the social species of *Damastes*, currently undescribed, were scored by C.A. Rheims (see the corresponding Data in Brief paper by Gorneau et al. (in review) for more information as well as images of the *Damastes* species studied). These characters were mapped on the tips of the Deleninae + *Damastes* phylogeny using the packages phylotools, phytools, and RColorBrewer (Revell, 2012; Neuwirth, 2014; Zhang et al., 2017).

For more detailed information on all materials and methods with respect to data collection and analysis including specific model parameters for phylogenetic inferences, please refer to the corresponding Data in Brief article (Gorneau et al., in review).

### 3. Results

#### 3.1. Sequencing results and matrix completeness

Of 82 samples, 70 had at least two genes that sequenced successfully and were included in these analyses. The concatenated matrix with GenBank data included was a total of 2173 basepairs in length (GenBank data sourced from: Agnarsson & Blackledge, 2009, Blackledge et al., 2009, Crews & Gillespie, 2010, Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017, Tong et al., 2019). Within this matrix were 1687 distinct patterns, 1142 parsimony-informative sites, 303 singleton sites, and 728 constant sites. For H3, 12 of 271 samples lacked sequence data. For 28S rRNA, 27 of 271 samples lacked sequence data. For COI, 38 of 271 samples lacked sequence data. For 16S rRNA, 21 of 271 samples lacked sequence data. Overall, the entire matrix contained about 14% missing data.

#### 3.2. IQ-TREE maximum likelihood phylogeny of Sparassidae

The IQ-TREE maximum likelihood inference included a total of nine outgroup taxa and 262 ingroup samples, representing 37 of the 89 described genera. A topology in support of a monophyletic Sparassidae was recovered (Fig. 2, UFBoot = 100). Support for the monophyly of subfamilies in the Sparassidae was varied, consistent with previous work (Agnarsson & Rayor, 2013, Moradmand et al., 2014). The sister group to

all other huntsman spiders, the Sparianthinae, were recovered as monophyletic (UFBoot = 96). The Heteropodinae *sensu stricto* were recovered as monophyletic (UFBoot = 98), and while the Heteropodinae *sensu lato* (represented with the inclusion of *Sparianthina* Banks, 1929) were also recovered as monophyletic, the support was comparably low (UFBoot = 78). Moradmand et al. (2014) considered *Meri* cf. *sancti-vincenti* Simon 1898a as part of Heteropodinae *sensu lato*, though this representative is not considered part of the group in this study, as its inclusion renders the group paraphyletic. A clade of exclusively Neotropical sparassids formerly placed in *Olios* was recovered as monophyletic (UFBoot = 84) and weakly recovered as sister to the Neotropical Polybetinae, represented solely by *Polybetes pythagoricus* (Holmberg, 1875), and thus unable to be evaluated on itself. The Palystinae were recovered as monophyletic (UFBoot = 100). The monotypic Staianinae, represented by a single *Staianus* Simon 1889, was recovered as sister to two undetermined “*Olios*” from the pet trade (UFBoot = 96). The genus *Damastes* was recovered as monophyletic (UFBoot = 100), but weakly supported nodes make it difficult to make any confident inferences about which group it is most closely related to. *Micrommata virescens* (Clerck, 1757), the type of the subfamily Sparassinae, was recovered with poor support sister to Eusparassinae + Deleninae, (UFBoot = 67). The remaining members of the subfamily examined (*Olios*, *Cerbalus* Simon 1897a) formed a monophyletic group with “*Olios*” *giganteus* Keyserling 1884 and related taxa from the Neotropics (UFBoot = 97), rendering the Sparassinae non-monophyletic. The Eusparassinae were recovered as paraphyletic, with the proposed members of the ‘African clade’ *sensu* Moradmand et al. (2014) recovered as their own group and *Eusparassus* Simon 1903a recovered as monophyletic (UFBoot = 100), and weakly recovered as sister to the Deleninae (UFBoot = 57). The endemic Australian Deleninae were recovered as monophyletic (UFBoot = 67). An uncollapsed view of the tree is available in Fig. S1. The results of a RAxML inference, as well as a Bayesian inference via MrBayes, both using the IQ-TREE phylogeny as a starting tree, were largely similar and are available in the corresponding data paper (Gorneau et al., in review).

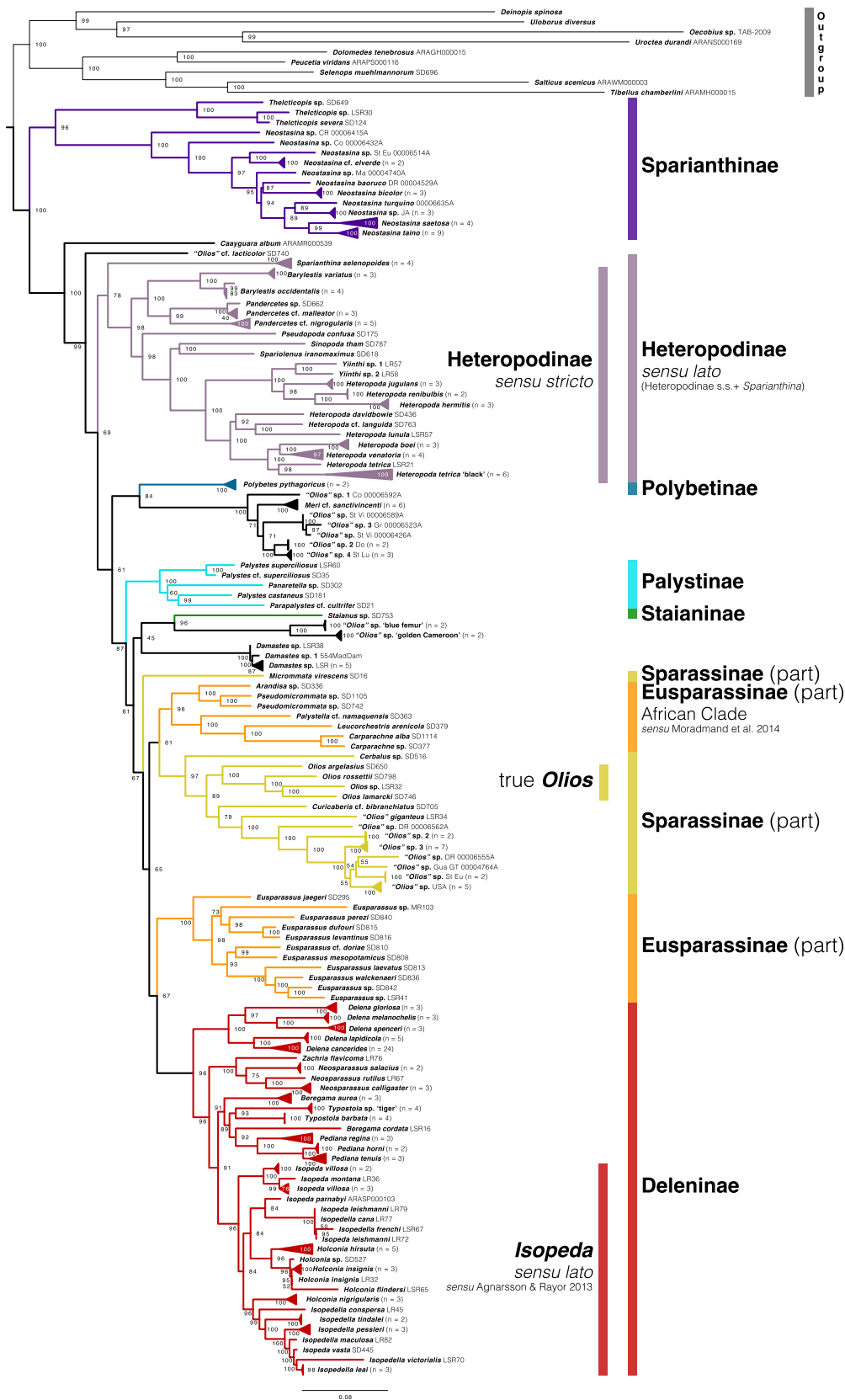
#### 3.3. BEAST fossil-calibrated estimated divergence of Sparassidae

The results of the BEAST fossil-calibrated divergence time for the Sparassidae suggest an estimated stem age of ~ 98 million years and a crown age of ~ 90 million years (Fig. 3). The crown age for the social genus *Delena* Walckenaer 1837 is approximately 24 million years old, representing the oldest generic lineage within the Deleninae. The crown age for the Sparianthinae, sister to all other huntsmen, represented an age of approximately 79 million years old. The 95% confidence intervals overlapped for most inferences.

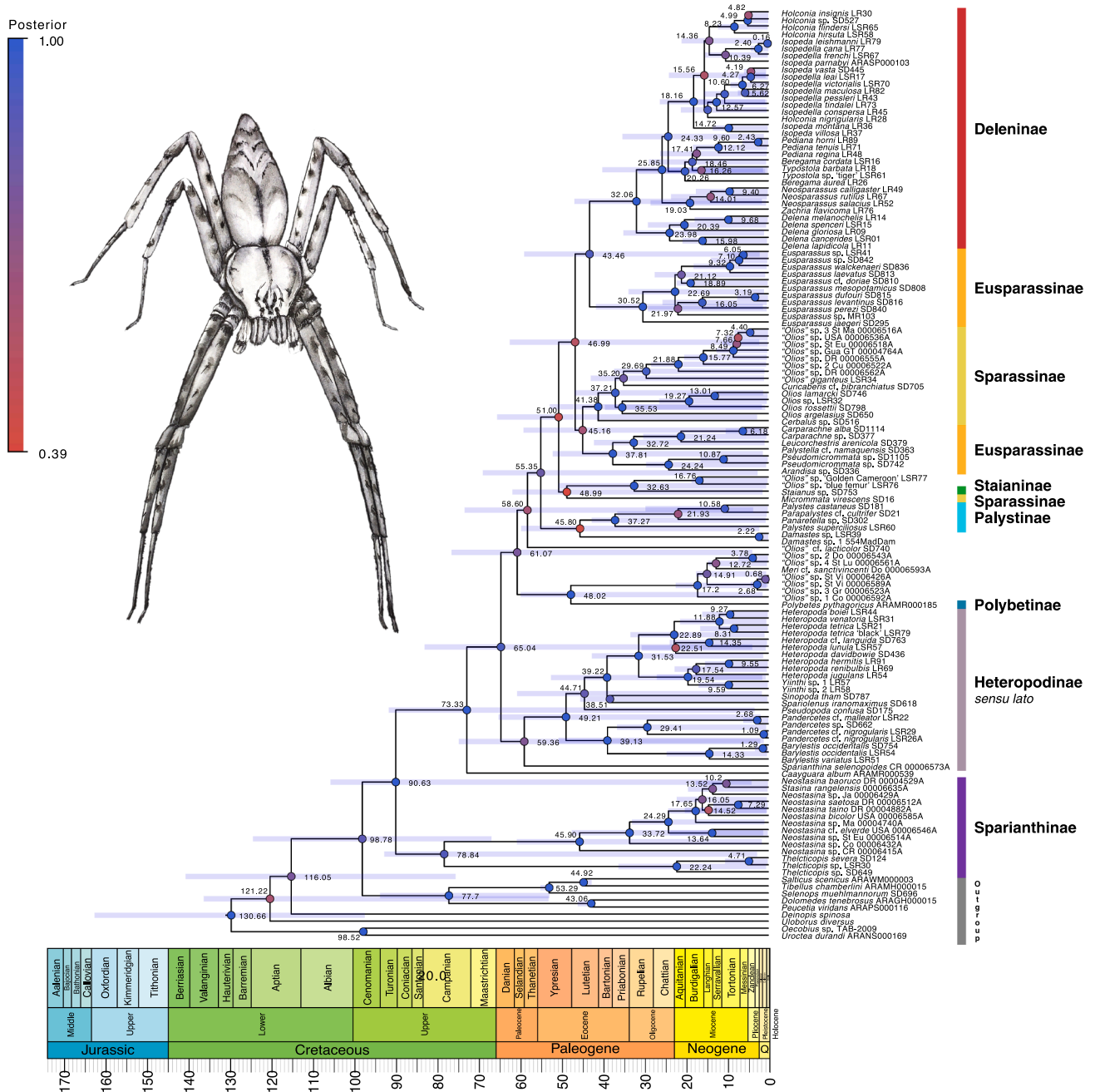
#### 3.4. Stochastic character mapping and D-test

A solitary lifestyle with transient parental care limited to maternal guarding of the egg sac and newly emerged young is the ancestral state for sparassids. The ancestral state reconstruction supports four independent origins of social behavior, with two origins of prolonged subsociality, one in *Delena* and one in *Damastes*, and two origins of subsociality in *Isopedella* (Fig. 4). The final two origins of sociality are in *Isopedella*, with *Isopedella leai* and *Isopedella pessleri* as the only huntsman species with a brief period of subsocial behavior, but not prolonged subsocial behavior. The ancestral state for the subfamily Deleninae indicates solitary origins, except in the genus *Delena*. The ancestral state of social behavior in the genus *Delena* is prolonged subsocial with one reversal to solitary behavior in *Delena gloriosa* (Rainbow, 1917). With only one representative for *Damastes*, an ancestral condition could not be inferred for the genus.

The ancestral egg sac structure for the Sparassidae excluding *Thelcticopis* Karsch 1884 (Sparianthinae) is inferred as plastered (Fig. 4, see Fig. 1B, C, D, I, and J). Lenticular egg sacs are the ancestral state for the



**Fig. 2.** Maximum likelihood phylogeny of the huntsman spiders with UFBoot support values inferred using IQ-Tree. Triangles represent collapsed nodes of multiple specimens representing a single taxon and the length corresponds to the branch length range for those collapsed specimens. Sample size is indicated in parentheses for these nodes. Colors correspond to different subfamilies. Scale of 0.08 refers to branch lengths.

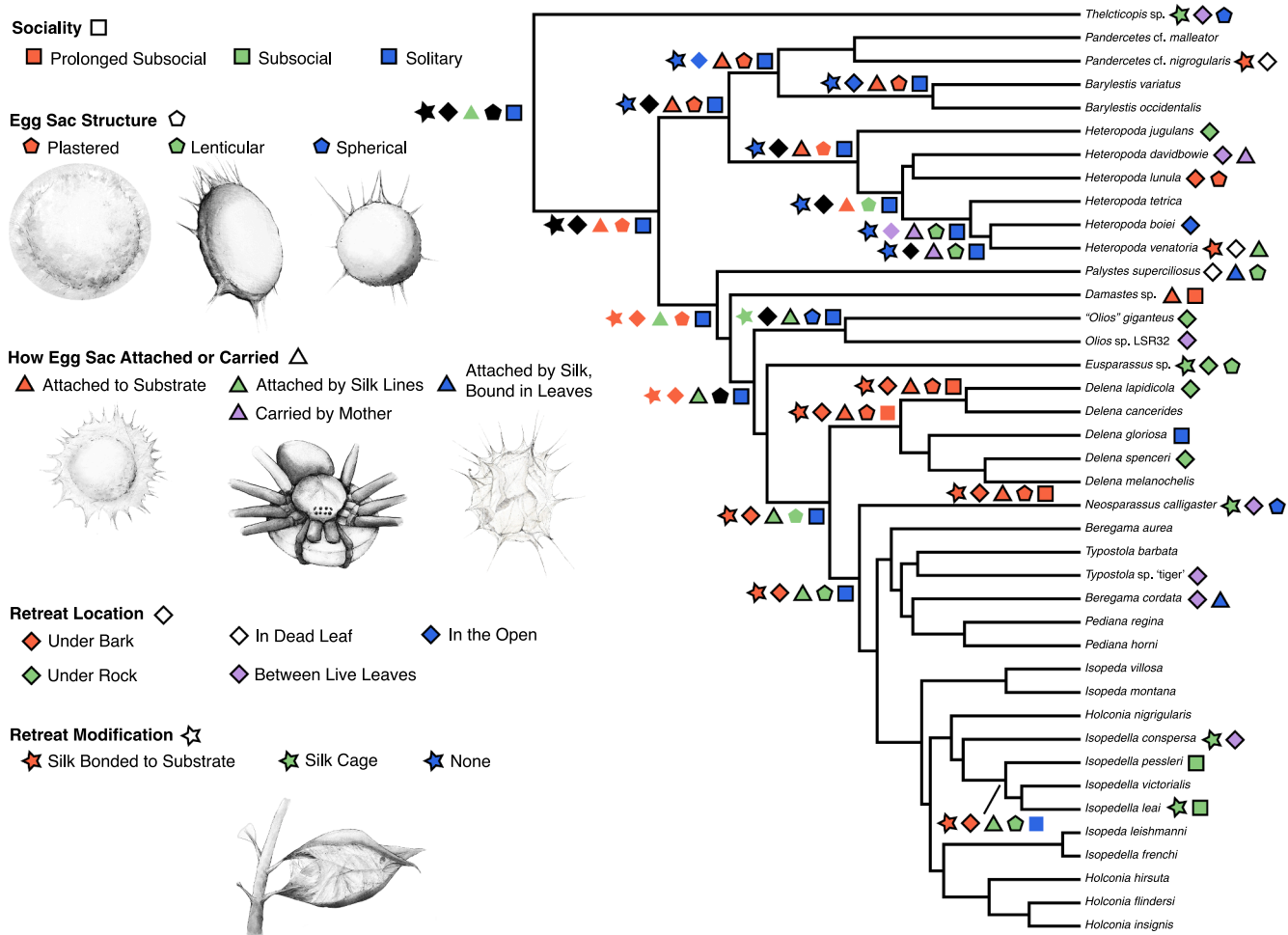


**Fig. 3.** Divergence date estimates from BEAST maximum clade credibility inference. Circles at each node correspond to posterior probabilities as indicated by color scale to the upper left of the figure. The number at each node corresponds to the age of the clade in millions of years, and the light blue rectangles represent the 95% highest posterior density (HPD). Scale of 20.0 refers to millions of years. Geologic time scale at bottom of figure, with periods, epochs, and subdivisions created using R packages *phytools*, *phyloch*, *strap*, and *coda* (Plummer et al., 2006, Heibl 2008, Revell 2012, Bell and Lloyd 2015). “Q” referring to Quaternary Period. The illustration is of *Heteropoda boiei*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Deleniinae (see Fig. 1F, L, O, P, Q, S, T, and U). However, plastered egg sacs are the ancestral condition for the social genus *Delena* and in the Heteropodinae. The genus *Damastes* also has a plastered egg sac type, but an ancestral condition could not be inferred for the genus with just one representative. While plastered egg sacs are ancestral to the Heteropodinae, including the genera *Pandercetes* L. Koch 1875, *Barylestis* Simon 1909, and *Heteropoda* Latreille 1804, lenticular egg sacs are ancestral to the genus *Heteropoda* excluding *Heteropoda jugulans* (L. Koch 1876). Spherical egg sacs are characteristic of *Olios*, *Neosparassus* Hogg 1903, and *Thelcticopis* (though note with only one representative for the latter two, an ancestral state could not be inferred for these genera; see

Fig. 1K).

The ancestral state for egg sacs is ‘attached by silk lines’ in the Sparassidae, and in Sparassidae sister to *Thelcticopis*, ‘attached to substrate’ (Fig. 4). For the clade containing *Olios* + *Eusparassus* + Deleniinae, the ancestral state is an egg sac attached by silk lines (see Fig. 1L, T, Q, U). In the Heteropodinae, the ancestral state for egg sac support is an immobile egg sac attached to the substrate, but a clade in the speciose genus *Heteropoda* evolved so that the lenticular egg sac that is constructed on the substrate is subsequently pulled off and carried freely under the mother’s venter to allow mobility while guarding the egg sac (Fig. 1F). Only two species in this study have egg sacs bound in leaves



**Fig. 4.** Phylogenetic patterns of life history traits in sparassids based on stochastic character mapping for the following characters: level of sociality (square), egg sac structure (pentagon), how egg sac is attached or carried (triangle), retreat location (diamond), and retreat modification (star). The colors correspond to different character states. All red squares represent both prolonged subsociality and delayed development. All other squares represent solitary or subsocial species which have non-delayed development. An all-black shape indicates no majority ancestral state for that node. A black outline indicates an ancestral state with  $\geq 75\%$  posterior probability, while no black outline indicates  $\geq 50\%$  posterior probability of ancestral state. Along the tree, downstream ancestral nodes are the same unless otherwise indicated. Exceptions to the ancestral nodes at the tips are indicated with a corresponding icon next to the tip label. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and attached by silk: *Palystes superciliosus* L. Koch 1875 and *Beregama cordata* (L. Koch 1875).

Except for *Thelcticopsis* and Heteropodinae, the ancestral state for retreat location is under bark for all Sparassidae included in this study. The ancestral state for retreat location in the Heteropodinae is ambiguous, though the clade containing *Barylestis* and *Pandercetes* has an open retreat (Fig. 4). The ancestral state for retreats is the large, diverse genus *Heteropoda* is ambiguous.

The ancestral condition for retreat modifications for all Sparassidae, excluding *Thelcticopsis* and the Heteropodinae, is silk bonded to the substrate (Fig. 4). In the Deleninae, all members have at least some sort of retreat modification, the ancestral condition for retreat modifications being silk bonded to substrate. Retreats modified to include silken cages are found in *Neosparassus* sp., *Isopedella conspersa* (L. Koch 1875; Deleninae), *Iso. leai*, *Thelcticopsis*, *Olios*, and *Eusparassus* (Fig. 1K, R, S). Moradmand & Jäger (2012) refer to this retreat type as a “papery retreat” in *Eusparassus*. The lack of retreat modifications is ancestral and highly conserved within the Heteropodinae.

Approximate adult female body mass and adult female cephalothorax width serve as proxies for overall body size. Large variation in body size occurs throughout the sparassids, with no clear ancestral states for most major lineages and no apparent evolutionary trends associated

with body size. There is little variation in age of sexual maturity and life span among the sparassids. Most sparassid females reach sexual maturity at about 1 year and live to approximately 2.5 years. Smaller species reached sexual maturity somewhat earlier and died closer to 1.5 to 2 years. The only notable exceptions were three very large species: *Beregama aurea* (L. Koch 1875) mature at 1.7 years and commonly live to over 3.5 years old, *Damastes* sp. which matures at 17 + months and lives over 3.3 years, and a sole *Typostola barbata* (L. Koch 1875) that lived to four years.

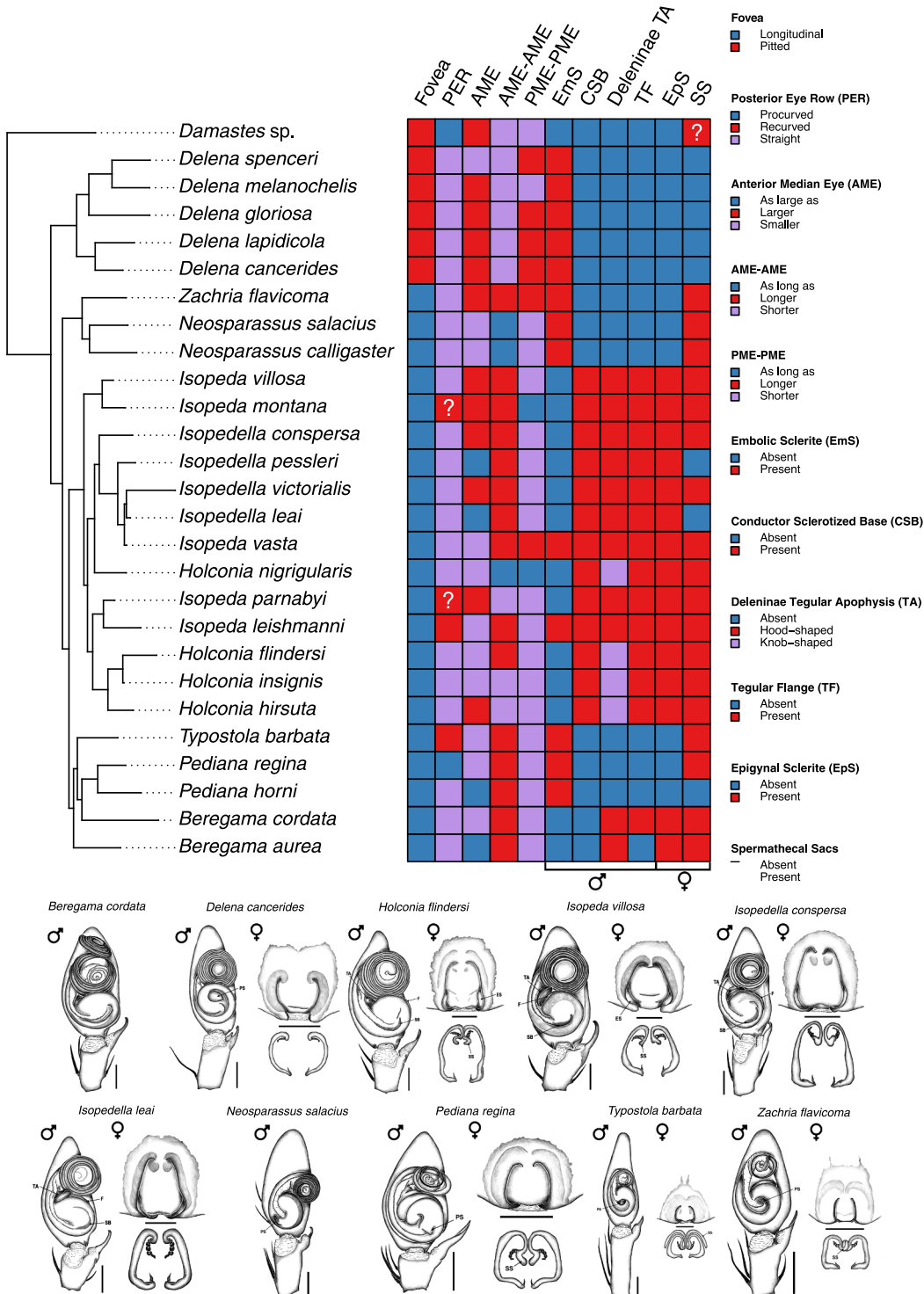
D-tests explored the relationship between patterns of group-living and life history traits. Starting to eat in the first instar out of the egg sac was significantly corrected with a solitary lifestyle ( $p = 0.006$ ), while delaying eating until the second instar was significantly associated with prolonged subsociality ( $p = 0.005$ ). Lenticular egg sacs were associated with a solitary lifestyle ( $p = 0.029$ ), while plastered egg sacs were correlated with prolonged subsociality ( $p = 0.02$ ). Immobile egg sacs attached to the retreat by silk lines were associated with being solitary ( $p = 0.026$ ), while immobile egg sacs plastered to the retreat were correlated with prolonged subsociality ( $p = 0.013$ ). A retreat under bark was correlated with prolonged subsociality ( $p = 0.035$ ). Silk bonding the substrate (whether bark, rock, or leaf) together to form a more secure, enclosed retreat is correlated with prolonged subsociality ( $p = 0.039$ ). In



terms of retreat size relative to body size, a lack of a retreat was associated with solitary behavior ( $p = 0.015$ ), and medium to large retreats were associated with prolonged subsociality ( $p = 0.011$  and  $p = 0.001$ , respectively). Solitary females with eggs or young were associated with small retreats ( $p = 0.007$ ), prolonged subsocial females with eggs or young were associated with larger retreats ( $p = 0.06$  for medium retreats and  $p = 0.029$  for large retreats). No life history traits were observed to be correlated specifically with the subsociality observed in *Isopedella leai* and *Isopedella pessleri*, nor was there a correlation between body size and level of sociality.

### 3.5. Mapping of *deleninae* and *Damastes* morphology

In the Deleninae, morphological features including eye patterns, and male and female sexual anatomy generally track phylogeny. *Delena* are clearly separated from the remaining genera by the presence of pitted fovea rather than a longitudinal fovea, the lack of spermathecal sacs in the female vulva, and posterior median eyes closer to each other than to the posterior lateral eyes (Fig. 5). Prolonged subsocial *Damastes* are most similar morphologically to the *Delena*, with seven of the eleven traits similar and differences primarily in details of the eye patterns and the



**Fig. 5.** Cladogram of subfamily Deleninae and *Damastes* with morphological traits assessed by C.A. Rheims represented to the right. Different colors indicate a different character state, and question mark indicates a degree of uncertainty to the character state for that species. The last six characters correspond to male (four characters) and female genitalia characters (two characters), respectively. Illustrations by C.A. Rheims are representative of the genitalic variation within the subfamily and with *Damastes*. Abbreviations for characters are as follows: prosoma (fovea), posterior eye row shape (PER), anterior median eye (AME) diameter, AME-AME and PME-PME interdistances, male palp (embolic sclerite (EmS), conductor sclerotized base (CSB), tegular apophysis (TA), flange (TF)) and female epigyne and vulva (epigynal sclerite (EpS), spermathecal sacs (SS)). Scale lines: 1 mm. Illustrations of other taxa, as well as a breakdown of characters and character states, can be found in the Supplementary Material of the corresponding Data in Brief paper (Gorneau et al., In Review).

absence of an embolic sclerite in the female epigyne, though this is not expected to be a direct correlate or cause of sociality in these genera.

The clade containing *Zachria* L. Koch 1875, *Neosparassus*, *Beregama* Hirst 1990, *Typostola* Simon 1897a, and *Pediana* Simon 1880a can be distinguished from *Isopeda*, *Isopedella*, and *Holconia* by the male palps lacking a sclerotized conductor base, a delenine tegular apophysis and a tegular flange and female epigyne lacking an epigynal sclerite (although these traits can be present in one or both *Beregama*). There are few morphological traits which are distinct within the confusing *Isopeda-Isopedella-Holconia* clade. Although all three genera have a tegular apophysis in the male palp, it is knob-shaped in *Holconia* and hood-shaped in *Isopeda* and *Isopedella*. There are no clear patterns that clearly distinguish *Isopeda* from *Isopedella*. The subsocial *Isopedella leai* and *Iso. pessleri* differ from the other *Isopedella* and *Isopeda* by the absence of spermathecal sacs and size of the AME. Of interest, the sole delenine which is regularly diurnally active, *Pediana regina* (L. Koch 1875), is the only delenine species with a procurved posterior eye row.

## 4. Discussion

### 4.1. Phylogenetic results and implications

This study represents the most comprehensive taxon sampling for a phylogeny of sparassids to date, building on previous work (Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017, Tong et al., 2019), and is the first to explore trends of life history evolution in the family. The undisputed monophyly of Sparassidae has been demonstrated previously in a variety of phylogenetic and morphological contexts (Rheims, 2007a, Ramírez, 2014, Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017), and is reinforced here. While previous work included exemplars of the Sparianthinae, multiple genera were not included, making it impossible to evaluate the monophyly of the subfamily itself (Tong et al., 2019). Evaluating the monophyly of the Sparianthinae was important as this subfamily is sister to the rest of the Sparassidae, is quite divergent as evidenced by the BEAST analysis, and has unique characteristics not present in other sparassids, including canoe-shaped eye tapeta, smaller retromarginal teeth and a median apophysis in the male palps (Jäger, 1998, Ramírez, 2014). The Sparianthinae were represented in our phylogeny by *Thelcticopis* and *Neostasina* Rheims & Alayón, 2016, and in our ancestral state reconstructions by *Thelcticopis* (Fig. 2, Fig. 4). The differences in life history and morphology in this subfamily compared to the rest of the Sparassidae as well as the ~ 20 my difference in crown ages between Sparianthinae and the Sparassidae (~80 my for Sparianthinae, ~98 my for Sparassidae Fig. 5) warrants future work.

As in previous studies, the monophyly of Deleninae and Heteropodinae are each supported (Agnarsson & Rayor, 2013, Moradmand et al., 2014, Wheeler et al., 2017). Sparassinae and Eusparassinae were recovered as not monophyletic, consistent with previous phylogenetic treatment of the group (Moradmand et al., 2014). The type genus of Eusparassinae, *Eusparassus*, arises as a monophyletic clade sister to Deleninae, while the remaining representatives of the subfamily (*Arandisa*, *Pseudomicrommata*, *Palystella*, *Leucorchestris* and *Carparachne*) arise sister to a clade including *Olios*, *Cerbalus* and several unidentified Neotropical sparassids. Similarly, the type genus of Sparassinae, *Micrommata* Latreille 1804, arises far removed from the remaining Sparassinae, here represented by the genera *Olios* and *Cerbalus*.

Previous taxonomic issues with the generic classification of *Olios* are supported by this study. *Olios*, as previously defined was paraphyletic, and the work done by Jäger (2020a) rendered this genus as monophyletic, by restricting it to 87 species distributed in Africa, southern Europe and Asia. Our results corroborate this new definition. True *Olios*, here represented by *O. argelasius* (Walckenaer, 1806), *O. rossettii* (Leardi 1901) and *O. lamarki* (Latreille, 1806) are recovered as monophyletic in a clade including an unidentified species from Singapore. “*Olios*” *giganteus*, considered misplaced in *Olios* by Rheims (2010b) and Jäger

(2020a), arises within a large clade of Neotropical sparassids distributed in North America (USA) and the Antilles. On the other hand, the two “*Olios*” in the pet trade, although originating from Cameroon (within the distribution of *Olios*), arise sister to Malagasy *Staianus* and far removed from the true *Olios*, which may provide some taxonomic insight into these Cameroonian “*Olios*” species.

This study clarified relationships within the Deleninae beyond Agnarsson and Rayor (2013). Based on both genetics and morphology the inclusion of *Beregama cordata* in our analysis demonstrated that *B. aurea* and *B. cordata* do not constitute a monophyletic *Beregama*. In this and other studies (Cardillo & Rayor in preparation), *B. aurea* is unique for having many physiological features that do not align closely with other solitary delenine sparassids and should be studied further. Additional specimens of *Typostola* sp. ‘tiger’ in this analysis were recovered with moderate support as sister to *Typostola barbata*, consistent with a previous maximum likelihood inference of the group by Agnarsson and Rayor (2013). However, a Bayesian inference by Agnarsson and Rayor (2013) was unable to recover *Typostola* sp. ‘tiger’ as sister to *Typostola barbata*. More life history data and morphological comparisons involving a greater sampling of taxa from the genus *Typostola* will be needed to place *Typostola* sp. ‘tiger’ conclusively in *Typostola*. Agnarsson and Rayor (2013) found *Zachria flavicomis* L. Koch 1875, the type species for *Zachria*, nested within *Neosparassus*, so additional taxon sampling is necessary to establish the taxonomic and phylogenetic position of *Zachria* with relation to *Neosparassus*.

Hirst (1990) split the genus *Isopeda* into *Isopeda*, *Isopedella* and *Holconia*. The phylogenetic relationships inferred here are consistent with Agnarsson and Rayor (2013), in that while the three form a clade, the three genera, as currently defined, are not monophyletic. Based on these results we could synonymize all three genera. However, *Holconia* becomes monophyletic by removing *Holconia nigrigularis* Simon 1908 from the genus, and this is corroborated by a number of distinctive morphological traits (e.g. large body size, dorsoventrally flattened carapace, bold black-white-grey coloration) that distinguish the genus from both *Isopeda* and *Isopedella*. But, maintaining *Holconia* as a valid genus also means sinking *Isopedella* into *Isopeda* (including at least, *H. nigrigularis*, *Isopeda* (*I.*) *vasta* (L. Koch 1867), *Isopedella* (*Iso.*) *conspersa*, *Iso. tindalei* Hirst 1993, *Iso. pessleri*, *Iso. maculosa* Hirst 1993, *Iso. victorialis* Hirst 1993, and *Iso. leai*) and proposing at least two new genera (one, including at least: *I. villosa* and *I. montana* Hogg 1903; and the other, including at least: *I. leishmanni* Hogg 1903, *I. parnabyi* Hirst 1992, *Iso. cana* (Simon 1908), and *Iso. frenchi* (Hogg, 1903)). Thus, at this point, we refrain from making any major taxonomical changes, since this goes beyond the scope of the current paper. We believe that the study of the complex *Isopeda-Isopedella* requires further molecular and morphological investigation, with broader species representation, to determine the limits of possible groups and genera as well as their relationship with *Holconia*.

Some subfamilies lack specimens that have yielded successful DNA extractions, including genera from monotypic subfamilies: *Clastes* Walckenaer 1837, *Chrosioderma* Simon 1897a, and *Tibellomma* Simon 1903a. Consistent with Agnarsson and Rayor (2013) and Moradmand et al. (2014), we were also unable to successfully extract molecular data from older *Clastes* or from *Chrosioderma* specimens. As *Tibellomatinae* exists based on a single description from an immature specimen, and as the type is lost, it is currently impossible to confirm this suspected case of *nomen dubium* (Simon, 1898b, Jäger & Rheims, 2008).

We anticipate some remaining uncertainties, particularly in the backbone of the sparassid tree of life, to be due in part to some of the alignment issues encountered with rRNA loci, first discussed by Moradmand et al. (2014), and encountered here. This affected our ability to include multiple outgroups from the RTA clade as well as affecting some of our alignments of ingroup taxa. Future molecular work on the group will likely employ a genomic approach, which will lessen the impact of alignment issues with single genes on phylogenetic inferences.

#### 4.2. BEAST fossil-calibrated estimated divergence of Sparassidae

The taxonomy and phylogenetic utility of the few known sparassid fossils are disputed (Agnarsson & Rayor, 2013; Magalhães et al., 2020). For example, one species, *Zachria desiderabilis* Petrunkevitch 1950, a fossil species tentatively placed by Petrunkevitch (1950) in the extant Australian endemic genus *Zachria* was designated *genus indeterminate* by Wunderlich (2012). To estimate the origins of sparassids without fossil sparassids, Agnarsson and Rayor (2013) used base pair substitution rates, and focused on the Deleninae, inferring a median age of 23 million years for the subfamily. Moradmand et al. (2014) and Tong et al. (2019) employed the use of an *Eusparassus crassipes* (C.L. Koch & Berendt, 1854) fossil to calibrate their inferences, but this use was defined as problematic by Magalhães et al. (2020) and credited as the reason for older divergence estimates. Consequently, Magalhães et al. (2020) proposed a median age of ~ 130 million years for the node leading to sparassids (*Caayguara* + marronoid clade) by using fossils elsewhere in the spider tree of life. The results of the BEAST fossil-calibrated divergence time in this study resulted in a considerably younger estimation than the age inferred by Magalhães et al. (2020), and instead demonstrated a crown age for the sparassids of about 90 million years. Divergence estimates made with BEAST resulted in low ESS values for some non- nuisance parameters that were not resolved after combining three independent chains of 100,000,000 generations and by using a starting tree, but it is not expected to greatly impact divergence estimates.

Obtaining well-resolved, time-calibrated phylogenetic inferences is essential to evaluate the monophyly of described taxa, resolve taxonomic issues, and to better understand evolutionary trends within the huntsman spiders, particularly with respect to life history. Having a more well-calibrated divergence inference will assist in exploring any potential associations of life history evolution to relevant geological events.

#### 4.3. Stochastic character mapping and D-test

Our hypothesis that prolonged subsociality is associated with a suite of life history traits that better accommodate longer-term associations between adult females and multiple cohorts of offspring was largely supported, even though some of the individual traits are shared by solitary species (Fig. 4). Prolonged subsocial species are found in relatively large, sturdy, well-protected and long-lasting retreats under bark or rocks, with silk modifications to better affix the retreat components and limit access to the retreat from potential predators. They provide space for multiple large cohorts of developing spiders to remain near their mother. Long tenure in the retreat is associated with egg sacs that are permanently plastered in place. Delayed development is associated a period in which relatively immobile first instar spiderlings remain in an interactive social situation (Cardillo and Rayor, in preparation). When they begin to feed in the second instar, social species are considerably larger than comparable second instar spiderlings of all other solitary or subsocial sparassid species studied. However, our predictions about an association of sociality with patterns of earlier sexual maturity, longer lifespan, or greater body size were not supported.

Many solitary genera either do not use retreats or utilize more ephemeral retreats in leaves, or rely, in part, on a silken cage to help define the retreat space and protect the eggs (Fig. 4). Other groups, such as most Deleninae, use smaller, long-lasting retreats under loose bark that could contain large clutches of early instar spiderlings, but are not large enough to accommodate spiders to near-maturity. In all solitary species, individuals in the first instar were mobile foragers that dispersed rapidly by the second or third instars. In summary, the associated life history traits of the prolonged subsocial species are suitable for facilitating large interactive groups of growing spiderlings that remain in their natal retreat for many months, while those of the solitary species are not.

Despite independent origins of prolonged subsociality, *Delena* and

*Damastes* share many life history traits: all *Delena* and the *Damastes* in this study have delayed development with a non-feeding first instar, plastered egg sacs that adhere to the substrate and modify their retreat with short silk bonds to bind the bark or rocks together and limit access to the retreat. All prolonged subsocial retreats are extremely narrow crevices, except for *D. melanochelis* which live in long narrow tubes of rolled bark. The most dorsoventrally flattened and large-bodied prolonged subsocial species, *D. cancerides*, *D. lapidicola*, and *Damastes*, are all found in retreats less than 1 cm deep. We propose that plastered egg sacs may be an adaptation to minimize space in these narrow retreats, and possibly offer a single face for the adult female to guard from potential colony invaders. The single solitary *Delena* species whose behavior has been studied, *Delena gloriosa*, shares most of these ancestral traits (except they feed in the first instar) and our study and Agnarsson and Rayor (2013) indicate that it has secondarily lost sociality. There are other members of the genus *Delena* that have not been investigated in a behavioral or molecular context which may have implications for future inferences of the social evolution of this genus. Except for the solitary *Delena gloriosa*, each time another *Delena* species was found in the wild by LSR they were unexpectedly discovered to live in prolonged subsocial matrilineal groups (*D. lapidicola*, *D. melanochelis*, *D. spenceri*), suggesting that more members of the genus may be prolonged subsocial. It also suggests that further basic behavioral, developmental, and molecular research into the genus is recommended, particularly in the little known and recently described species (*Delena convexa* Hirst 1991a; *D. loftiensis* (Hirst, 1991b), *D. tasmaniensis* (Hirst, 1991b), *D. kosciuskoensis*, Hirst 1991b). Additionally, with the type of *D. craboides* Walckenaer 1837 lost, the species represents a *nomen dubium* and may in fact be *D. cancerides* (see Simon, 1880a). The convergent life history similarities in *Delena* and *Damastes* provide a blueprint of characteristics that should be explored when examining other sparassids for prolonged subsocial behavior. However, there were not obvious life history traits that were characteristic of the two subsocial species, *Isopedella leai* and *Iso. pessleri*, that differed from those of solitary species. Unlike the extensive field observations in the prolonged subsocial *Delena*, these subsocial *Isopedella* species have not been extensively observed in the field, only in the laboratory, and the extent of their association with their mother and siblings may differ in the field. The paucity of field observations is due to the anthropophilic tendencies of both species which are more often collected in (or removed from) homes than from the field and the short ≤ five-week period of association making observations during the period of association less common.

Plastered egg sacs are found in a few other solitary Heteropodinae; *Heteropoda jugulans*, *H. lunula* (Doleschall, 1857), *H. dagmarae* Jäger & Vedel, 2005 (not included in this phylogenetic analysis), *Pandercetes*, and *Barylestis*. The plastered egg sacs of *Pandercetes* and some *Heteropoda* (*H. jugulans*, *H. dagmarae*) species are like those built by *Delena* and *Damastes* with a flat sheet fully adhered to the substrate and an outer sheet that fits around the egg mass. All lenticular egg sacs are constructed as a distinct disk that is initially lightly plastered to the substrate during egg laying and then pulled off to be attached with guy-lines of silk or carried by the female. Unlike the construction of the other species plastered sacs, the plastered egg sacs of *Barylestis* and *H. lunula* are constructed like the lenticular sacs but with more extensive silk around the edges of the disk that tightly affixes it to the substrate through development of the eggs. When the eggs hatch and the post-embryo ‘eggs with legs’ swell the sac, the sides of the sac split open so the young emerge through the fissure (Fig. 1C). In contrast, *Delena* and *Damastes* mothers actively chew a hole in the egg sac through which the young emerge. All of the delenine females studied chewed holes in the egg sac for the young to emerge from (Fig. 1O, 1Q). The plastered sacs of *Pandercetes* and *Barylestis* are often camouflaged on the exterior of trees or dead leaves, and the plastered sac likely blends more effectively into the background without a shadow than would a lenticular egg sac. Lenticular sacs are the ancestral state in a clade of *Heteropoda*, as well as the clade of Deleninae including *Beregama*, *Pediana*, *Typostola*, *Holconia*,

*Isopeda*, and *Isopedella*. Spherical sacs were represented by our sole exemplars of *Olios*, *Thelcticopis*, *Neosparassus*, and *Eusparassus*. While we lacked multiple exemplars for *Olios* and *Neosparassus*, generally *Olios* (including species now formerly *Olios* following Jäger, 2020a) and *Neosparassus* build silken cages (Fig. 1K, Rayor, personal observations; Hirst, personal communication). At least some species of *Isopedella*, including *Iso. conspersa* and *Iso. leai*, build silk cages (Fig. 1R, 1S). The egg sacs of *Palystes* L. Koch 1875 are unique in that the shape of the egg sac itself appears to vary from lenticular to spherical depending on the egg mass, and females incorporate leaves and other vegetation into the outer structure of the sac with silk irregularly pulled off the surface of the sac where silk lines attach to the substrate (Hamilton-Attwell & Dippenaar-Schoeman, 2020). *Beregama aurea* sacs also vary from lenticular to spherical with the egg mass (Fig. 1L).

The ancestral state for retreats in the Deleninae is under bark or in bark rolls. The exceptions, *D. lapidicola* and *D. spenceri*, live under exfoliating rocks on granite headlands or dolerite boulders, respectively, in rocky habitats with no trees and only small shrubs that do not have retreat spaces under their bark. The genus *Heteropoda* represents about 15% of all described sparassids, which are found in a wide range of habitats with equally varied retreat use including the lack of a retreat, in leaves, under rocks, and under bark.

#### 4.4. Final remarks

To resolve the taxonomic issues presented in this study, more basic research and taxonomy are needed on huntsman spiders. It will be particularly useful to acquire recent specimens viable for DNA extraction, as well as information about the life history and behavior of understudied lineages such as *Clastes* Walckenaer 1837 (Clastinae) and *Chrosioderma* Simon 1897a (Chrosiodermatinae). *Clastes freycineti* Walckenaer 1837 is especially of interest regarding both placement and life history as morphological inferences recover the group as sister to Deleninae, which may have implications for social evolution of the family (Rheims, 2007a, Rheims personal communication). Like the other prolonged subsocial species studied here, *C. freycineti* produces a plastered egg sac and has been observed in putative social groups with the mother sharing prey with older offspring (L. Higgins, personal communication). Future phylogenetic work should center on resolving higher relationships of the genera currently designated *incertae sedis*, which represent almost one-third of all described sparassid species.

## 5. Conclusions

We provide the most synthesized inference of Sparassidae by increasing the depth of delenine sampling, including taxa from previously published work on the family, and by contributing new sequence data on key taxa. This inference further confirms that extensive phylogenetic work remains to be done to further elucidate relationships and resolve taxonomic issues within the family. Results of nearly two decades of field and lab study by LSR analyzed via ancestral state reconstruction have revealed life history traits that have evolved in association with social behavior. This work provides a basis to study more social and life history traits in the Sparassidae to further understand predictors of prolonged subsocial behavior in the family.

#### CRediT authorship contribution statement

**Jacob A. Gorneau:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Cristina A. Rheims:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Corrie S. Moreau:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing –

review & editing. **Linda S. Rayor:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

We are indebted to Ivan Magalhaes, Dave Rowell, Ingi Agnarsson, Manuela Oliveira Ramalho and an anonymous reviewer for advice on the project, manuscript, and/or methodology. With gratitude we thank David Hirst, Frank Somma, Alan Henderson, D. Rowell, Nicky Bay, Joseph Koh, Linden Higgins, and Charles Haddad for extensive biological information and the first three for live specimens. Melbourne Museum Bugs Alive! and Lachlan Manning, Australian Museum, contributed live specimens. We gratefully thank Cole Gilbert, D. Rowell, Eric Yip, Francesca VanDenBerg, Jacob Hurst, David Hirst, Daiqin Li, and David Court for assistance in the field. Extensive institutional and intellectual support was provided by both D. Rowell and the Australian National University and D. Li, National University of Singapore. Liam Revell (University of Massachusetts Boston) provided input on use of the D-test in his *phytools* package to determine correlations between sociality and life history. Sabrina Celis (Cornell University) illustrated the egg sacs and retreats. Funding for JAG includes the Jane E. Brody Undergraduate Research Award, the Fredric N. Gabler '93 Memorial Research Endowment, and the CALS Alumni Association Academic Enrichment Program. Additionally, this work was supported by an NSF IOS grant [1916995] to CSM; Monster Bug Wars, Women's International Science Collaboration (AAAS/ NSF) and the President's Council of Cornell Women (Affinito-Stewart Grant) to LSR; and FAPESP grants [11/18694-3 and 15/18982-0] to CAR.

#### Appendix A. Supplementary material

**Voucher information.xlsx** – Excel file with information on vouchers representing exemplars for the molecular data contributed in this study. Specimens deposited in the Smithsonian arachnology collections. **New\_data\_generated\_GenBank.xlsx** – Excel file with information for all individuals contributed for the molecular component of data collection study, including locality and date information where known, and GenBank accession numbers. Red cells indicate no sequence data for that gene, and gray cells indicate sequence data but only from a single sequencing direction (forward or reverse). Location/soil and date information also provided, where known. **GenBank\_sequences.xlsx** – Excel file with accession numbers for sequences downloaded from GenBank. **Figure\_S1\_IQ\_TREE\_Phylogeny\_Uncollapsed.pdf** – Maximum likelihood phylogeny of the huntsman spiders with UFBoot support values inferred using IQ-Tree. Colors correspond to different subfamilies. Scale of 0.08 refers to branch lengths. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmpev.2022.107530>.

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