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Multiple origins of subsociality in crab spiders (Thomisidae)

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ABSTRACT

Determining factors that facilitate the transition from a solitary to a social lifestyle is a major challenge in evolutionary biology, especially in taxa that are usually aggressive towards conspecifics. Most spiders live solitarily and few species are known to be social. Nevertheless, sociality has evolved multiple times across several families and nearly all studied social lineages have originated from a periodically social (subsocial) ancestor. Group-living crab spiders (Thomisidae) are exclusively found in Australia and differ from most other social spiders because they lack a communal capture web. Three of the group-living species were placed in the genus *Diaea* and another in the genus *Xysticus*. Most Australian thomisids are, however, difficult to identify as most descriptions are old and of poor quality, and the genera *Diaea* and *Xysticus* may not correspond to monophyletic groups. Here, we clarify the phylogenetic relationships of the four group-living Australian thomisids and conclude that amongst these subsociality has evolved two to three times independently. The subsocial *Xysticus bimaculatus* is not closely related to any of the social *Diaea* and an independent origin of subsociality is likely in this case. The presented data indicates that within *Diaea* two origins of subsociality are possible. Our results help to understand the evolution of sociality in thomisids and support the hypothesis that permanent sociality in spiders has evolved multiple times relatively recently from subsocial ancestors.

1. Introduction

The evolution of sociality is puzzling considering that group living entails costs such as competition for resources and a high risk of accumulating pathogens and parasites (Hughes et al., 2002; Wilson et al., 2003). Generally, sociality may evolve when benefits outweigh the costs (Alexander, 1974). In many vertebrates, kin selection (Hamilton, 1964) and ecological constraints are thought to facilitate independent origins of sociality (Davis et al., 2011; Émlen, 1991; Faulkes et al., 1997). The same likely holds true for social spiders that form groups that usually consist of family members (Avilés, 1997; Lubin and Bilde, 2007).

Sociality is rare amongst spiders. Less than 25 of the over 44,500 described spider species are known to be permanently social or 'quasisocial' (Agnarsson et al., 2006; Avilés, 1997; Lubin and Bilde, 2007; Platnick, 2014) and about 70 are temporarily social or 'subsocial' (Yip and Rayor, 2014). Though being rare, social behaviour has been documented across various spider families (Agnarsson et al., 2006; Bilde and Lubin, 2011; Lubin and Bilde, 2007; Yip and Rayor, 2014). Quasisocial (hence 'social') spiders likely evolved from subsocial ancestors via gradual prolongation of communal activities of siblings, and elimination of the dispersal phase (Agnarsson et al., 2006; Bilde et al., 2005; Johannesen et al., 2007; Lubin and Bilde, 2007). Subsocial behaviour has evolved at least 18 times independently (Agnarsson et al., 2006; Bilde and Lubin, 2011; Yip and Rayor, 2014). Short dispersal distances in subsocial species and the lack of premating dispersal in permanently social species result in highly inbred mating systems (Bilde et al., 2005; Lubin and Bilde, 2007; Lubin et al., 2009; Ruch et al., 2009). Inbreeding, in turn, may lead to loss of genetic variability in social spiders restricting diversification (Agnarsson et al., 2013a). Extant social lineages tend to be relatively young ranging from a few hundred thousand years to about two million years (my) (Agnarsson et al., 2013a; Johannesen et al., 2007). In contrast, subsocial species are outbred and the limited available evidence suggests they may persist over much longer periods (Agnarsson et al., 2013a). Understanding the patterns of origin and persistence of subsocial and social lineages in a phylogenetic context is thus important.

There is molecular phylogenetic evidence for multiple origins of sociality within two spider families, the cobweb spiders (Theridii-

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dae) and the velvet spiders (Eresidae). In the Theridiidae, where nine independent origins of sociality in three genera are documented (Agnarsson et al., 2006; Avilés, 1997; Avilés and Bukowski, 2006), social lineages predominantly contain only a single species, indicating a lack of diversification and high extinction rates (Agnarsson et al., 2006). In the eresid genus *Stegodyphus*, three independent origins of sociality have resulted in exclusively single-species social lineages as well, although with somewhat higher intraspecific mtDNA variability than observed in social theridiids (Johannesen et al., 2007). The single origin of subsociality in the middle Miocene (16 mya) recently hypothesised for group-living huntsman spiders (*Delena*, Sparassidae) (Agnarsson and Rayor, 2013) further supports the idea that a subsocial lifestyle can be maintained over relatively long evolutionary time spans.

Group-living huntsman spiders are special among subsocial and social spiders in that Sparassidae lack a communal capture web (Agnarsson and Rayor, 2013; Avilés, 1997) and the same holds true for crab spiders (Thomisidae). More than 2100 thomisid species in 174 genera are described worldwide (Platnick, 2014). Four of them, all Australian, are group-living, with similar biology but varying social complexity. They construct nests from leaves which serve as foraging areas (Evans, 1998a; Main 1988) and protective retreats (Evans, 1998a; Unglaub et al., 2013). Subsocial crab spiders hunt by ambushing prey and females feed their offspring (Evans, 1998b; Ruch et al., 2014b). Spiderlings cooperate in nest construction, hunting and feeding for several months (Evans, 2000; Ruch et al., 2014a).

Thomisid taxonomy is poorly understood and many genera need revision (Benjamin et al., 2008; Garb and Gillespie, 2006; Szymkowiak, 2007). According to the current classification, three of the group-living thomisids belong to the genus *Diaea* (Evans, 1995). The only permanently social thomisid is *D. socialis* Main, 1988; Rowell and Main, 1992). Only little is known about the biology of *D. inornata*, but like *D. socialis* it has a female-based sex-ratio and like *D. ergandros* (but different from *D. socialis*) it has an annual life-cycle (Evans, 1995). The subsocial lifestyle of the fourth species, *Xysticus bimaculatus* Koch, 1867, was only recently discovered (Ruch et al. 2014c) and its lifestyle seems very similar to that of *D. ergandros*. Offspring disperse relatively late and the presence of an adult living female seems beneficial for spiderling survival (Ruch et al. 2014c). The discovery of a subsocial thomisid species outside the genus *Diaea* indicates that sociality may have evolved more than once in Thomisidae. Alternatively, taxonomic and classificatory uncertainties might disguise a common origin of sociality in Thomisidae (see e.g. Agnarsson and Rayor (2013) for Sparassidae). Considering the male pedipalp morphology, *X. bimaculatus* can be excluded from "*Xysticus* s. str." sensu Jantscher (2002) and a revision of the Australian thomisids may place this species into a distinct monophyletic group (Ruch et al. 2014c). The morphology of the *X. bimaculatus* pedipalps closely resembles the group-living *Diaea* species (Evans, 1995; Ruch et al., 2014c; Szymkowiak and Dymek, 2012). Such morphological evidence as well as the shared derived behaviour may be taken as evidence that amongst the Australian thomisids, these group-living species comprise a separate genus (Benjamin, pers. comm.).

In order to infer the phylogenetic relationships among the group-living crab spiders a molecular approach may provide clarification. However, only few molecular phylogenetic studies have been performed on crab spiders (Benjamin et al., 2008; Garb and Gillespie, 2006, 2009) and none of these included group-living thomisids.

Here, we explore the phylogenetic relationship of the group-living Australian thomisids based on four loci. We test whether the permanently social *D. socialis* has evolved from subsocial *Diaea* and estimate the age of sociality in thomisids. Further, we aim to test the hypothesis that subsociality evolved multiple times independently in this clade.

2. Material and methods

2.1. Data collection and phylogenetics

We collected living thomisids (Fig. 1) in 2012 and 2013 in NSW, QLD, TAS and WA, Australia. Whole spiders were preserved in 90% ethanol. In addition, specimens which have been collected in 2008 in NSW, QLD and WA and preserved in 70% Ethanol were used for DNA extraction. In total, we extracted DNA from 93 crab spiders belonging to 26 species (Table 1). Specimens were identified based on original taxonomic descriptions and comparisons with type material. Species were categorised as having a non-social, subsocial, or social behaviour according to previous behavioural classifications (Avilés and Harwood, 2012; Lubin and Bilde, 2007). Because social or subsocial behaviour may have been overlooked in certain species (see Ruch et al. 2014c), we also took into account whether spiders occurred solitarily or in groups at the collections sites. Three undescribed species that require separate mentioning are *Diaea* sp. 2, *Diaea* sp. 3, and *Diaea* ID35, because these cluster amongst the social/subsocial *Diaea* species and their behavioural classification is critical to the inference of the origins of social behaviour in thomisids. *Diaea* sp. 2 and *Diaea* sp. 3 were collected in heath land as single juvenile individuals using a sweep net. Due to their occurrence as singletons and the absence of communal nests in the area we consider a non-social lifestyle very likely for these otherwise unknown species. *Diaea* ID35 was collected as adult female and produced an egg sac in the laboratory. The spiderlings of this species started cannibalizing each other a few days after hatching. This observation is a clear indication for a non-social behaviour.

DNA was extracted from one or two legs of each specimen or whole carapace of small spiders using a modified Proteinase K-extraction protocol. We amplified partial fragments for two mitochondrial genes (16S rRNA (16S) and cytochrome c oxidase subunit I (COI)) and two nuclear genes (Histone H3 (H3) and the Internal Transcribed Spacer 2 (ITS2)). For the loci COI, H3, and ITS2 we used primers and protocols as described in (Agnarsson, 2010, 2012; Agnarsson et al., 2013b, 2007). For 16S we used the forward primer 16SA/12261 CGCCTGTTTACCAAAAACAT (Hedin, 1997) and the reverse primer SPID-ND1/13398 TCRTAAGAAATTATTTGAGC at an annealing temperature of 48 °C (Simon et al., 1994). Amplified fragments were sequenced in both directions by the University of Arizona Genetic Core and then assembled and proofread using the Chromaseq module (Maddison and Maddison, 2011a) in Mesquite (Maddison and Maddison, 2011b) employing Phred (Ewing and Green, 1998; Ewing et al., 1998) and Phrap.

We augmented the taxon sampling with 121 species from GenBank (Table 3 appendix) (Benson et al., 2007). Short 16S fragments (shorter than 500 bases) were not included.

We aligned sequences using MAFFT (Katoh et al., 2005) through the EMBL-EBI online portal with 100 tree rebuilding replications and 100 max iterations for a thorough search otherwise using default settings. Protein coding gene sequences were translated and confirmed to contain no stop codons. For all analyses, gaps and ambiguous bases were treated as missing data. The gene matrices were concatenated in Mesquite (Maddison and Maddison, 2011b). We created several different matrices to test for the effects of missing data, including 'all data', '2 genes' (including only taxa for which sequences from at least 2/4 genes were available), and '4 genes' (including only taxa for which sequences from all 4 genes were available, Table 2). Summary statistics were calculated using Geneious (Drummond et al., 2011).

We partitioned the data by gene, and partitions were exported from Mesquite for model choice. The appropriate models for each gene were chosen using jModeltest v0.1.1 employing the Akaike