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Resolving the phylogeny of a speciose spider group, the family Linyphiidae (Araneae) ☆

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ABSTRACT

For high-level molecular phylogenies, a comprehensive sampling design is a key factor for not only improving inferential accuracy, but also for maximizing the explanatory power of the resulting phylogeny. Two standing problems in molecular phylogenies are the unstable placements of some deep and long branches, and the phylogenetic relationships shown by robust supported clades conflict with recognized knowledge. Empirical and theoretical studies suggest that increasing taxon sampling is expected to ameliorate, if not resolve, both problems; however, sometimes neither the current taxonomic system nor the established phylogeny can provide sufficient information to guide additional sampling design. We examined the phylogeny of the spider family Linyphiidae, and selected ingroup species based on epigynal morphology, which can be reconstructed in a phylogenetic context. Our analyses resulted in seven robustly supported clades within linyphiids. The placements of four deep and long branches are sensitive to variations in both outgroup and ingroup sampling, suggesting the possibility of long branch attraction artifacts. Results of ancestral state reconstruction indicate that successive state transformations of the epigynal plate are associated with early cladogenetic events in linyphiid diversification. Representatives of different subfamilies were mixed together within well supported clades and examination revealed that their defining characters, as per traditional taxonomy, are homoplastic. Furthermore, our results demonstrated that increasing taxon sampling produced a more informative framework, which in turn helps to study character evolution and interpret the relationships among linyphiid lineages. Additional defining characters are needed to revise the linyphiid taxonomic system based on our phylogenetic hypothesis.

1. Introduction

Resolving the phylogeny of a high-level taxonomic group is often challenging, particularly for speciose groups. Taxon sampling has proven to be a difficult and controversial subject, and the choice of 'more taxa or more characters' has been debated for over a decade (e.g. Graybeal, 1998; see review by Nabhan and Sarkar, 2012). Although with new sequencing technologies, attaining character dense data is becoming more easily achieved, large numbers of markers are not sufficient to guarantee accurate phylogenetic reconstruction (Boussau et al., 2014). A comprehensive sampling of taxa remains problematic for hyper-diverse groups (Heath et al., 2008; Hillis et al., 2003; Hovenkamp, 2006; Klopfstein et al., 2010; Rosenberg and Kumar, 2001). Biases from incomplete sampling have been extensively discussed using both real and simulated data, including aberrance of tree shape (Heath et al., 2008; Koentges, 2008) and negative impacts on the accuracy of phylogenetic inferences (see review by Nabhan and Sarkar, 2012). Nevertheless, the effects of taxon sampling on the interpretation of phylogenetic inference and on post-tree reconstruction applications were rarely discussed (Heath et al., 2008; Koentges, 2008).

A common phenomenon in systematics is the incongruence between a high-level molecular phylogeny and the established taxonomic system (e.g. Dabert et al., 2010; Sharma et al., 2014). Even a robust phylogeny could be significantly in conflict with the accepted morphological synapomorphies of groups and it is often difficult to interpret within an organism biology context (e.g. Arnedo et al., 2009). Several factors may cause such an uncertainty

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in phylogenetic inference. Gaps in taxon sampling result in gaps of associated character transformation, especially for lineages with long history and many accumulated morphological changes (e.g. Arnedo et al., 2009; Tu and Hormiga, 2011). Insufficient sampling may conceal homoplastic changes (Heath et al., 2008; Huelsenbeck and Lander, 2003). Long-branch attraction (LBA) is another well-recognized source of systematic errors under a biased taxon sample (Felsenstein, 1978; Pick et al., 2010; Nabhan and Sarkar, 2012; Mariadassou et al., 2012; also see review by Bergsten, 2005).

Increasing taxon sampling density is expected to resolve or reduce some of the errors that result from sampling bias (Heath et al., 2008; Klopfstein et al., 2010; Koentges, 2008). Dense taxon sampling has been suggested as an efficient way to minimize the gaps across taxa and disperse homoplastic character changes on the tree (Heath et al., 2008; Klopfstein et al., 2010; Koentges, 2008), as well as break long branches (Bergsten, 2005; Parks and Goldman, 2014). An intuitive approach is to start with a small taxon sample, and then implement an iterative taxon addition guided by interim phylogenetic results, until satisfactory results are achieved (Poe and Swofford, 1999). Nevertheless, this approach has its limitations because the potential for added taxa to break long branches largely depends on their phylogenetic placements, rather than the sample size per se (Bergsten, 2005; Mariadassou et al., 2012). Furthermore, the conflicts between established molecular phylogeny and a taxonomic system usually lead to a shortage of feasible criteria for additional sampling. Such reciprocal illumination, however, may come from other well-studied character systems with sound phylogenetic signal (Gainett et al., 2014).

The spider family Linyphiidae provides an excellent system for studying some of the uncertainties associated with phylogenetic inference. It is the second largest spider family and the most speciose family-level lineage of Araneoidea (the ecribellate orb-weavers), including 4503 described species (World Spider Catalog, 2014), currently classified into seven subfamilies (Tanasevitch, 2015). Most linyphiid subfamilies have been proposed without explicit phylogenetic justification, and are based largely on overall similarity and/or single character system. There are several linyphiid phylogenetic studies based on morphological data, all focused on subgroups of linyphiids with modest and often admittedly biased taxonomic sampling (Hormiga, 1994, 2000; Hormiga and Scharff, 2005; Miller and Hormiga, 2004; Tu and Hormiga, 2011). Arnedo et al. (2009) presented the first and so far the most comprehensive molecular and morphological phylogeny of the family, which included 35 ingroup species representing six of the seven subfamilies. Their results support the monophyly of Linyphiidae and 'linyphioids' (see also Hormiga and Tu, 2008), the early-branching position of *Stemonyphantes,* and the monophyly of Erigoninae and Mynogleninae. All ingroup taxa, except the unstable placements of *Stemonyphantes* and four single-taxon branches, were divided into four well supported clades. Nevertheless, several issues arose from their results. First, the monophyly of Linyphiidae was not recovered when only the molecular data were analyzed, despite the fact that the monophyly of this family has been robustly supported by multiple morphological synapomorphies in several analyses. Second, the placements of several deep branches varied among the results from different data partitions and different data treatments, and were always weakly supported. Lastly and perhaps most importantly, taxa from different subfamilies nested within the four well-supported main clades and did not form respective clades. Such an unexpected topology of linyphiids greatly conflicts with the established knowledge of their systematics. It refutes the validity of several linyphiid subfamilies, and the overall results are also difficult to interpret.

The uncertainties of the linyphiid phylogeny of Arnedo et al. (2009) likely come from the unstable placements of some deep branches and the unclear phylogenetic relationships among the four main clades. Each of these unstable deep branches includes only a single taxon or two congeners and all have long branch lengths, therefore long branch attraction (LBA) is a possible cause for the instability of these deep branches. The four well supported clades were formed by taxa sampled from different subfamilies and hence imply large morphological gaps across taxa. This is largely responsible for the difficulty of interpreting relationships among clades within a morphological context. Furthermore, in their phylogenies exclusively based on molecular data, the "micronetines" of scaped epigyna and the erigonines of desmitracheate tracheal system nested together forming a "micronetines-erigonines" clade (clade ME hereafter). This suggests that homoplasy is possibly involved in the epigynal and tracheal evolution.

To guide an increase in taxon sampling and to better understand the intrafamilial relationships in light of unexpected topologies, we propose to study a character system that captures morphological variation across the ingroup taxa. Compared to other araneoid groups, such as araneids and theridiids, linyphiids have a relatively uniform somatic morphology; however, their genitalia are among the most complex known for spiders, and the species-specific genital characters provide a rich source of information for phylogenetic reconstruction (Hormiga, 2000; Hormiga and Scharff, 2005; Miller and Hormiga, 2004; Tu and Hormiga, 2011; Gavish-Regev et al., 2013). In contrast to the male copulatory organ, the pedipalp, which is comprised by several sclerites with multiple gain/loss events, the epigynum has only a single structural element, the epigynal plate. The complex epigynal morphology is derived from a series of modifications on this plate (Tu and Hormiga, 2010). Accordingly, epigynal character variation not only covers all ingroup taxa, but is also continuous (in the sense that the plate is always there) and traceable on a phylogeny. These likely minimize the difficulties inherent with homologizing structural elements across taxa (e.g., Arnedo et al., 2009; Gavish-Regev et al., 2013). Furthermore, various epigynal "morphotypes" are the results of sets of modifications, accumulated in different evolutionary paths. Taxon sampling according to epigynal types means sampling from diverse linyphiid lineages, and thus it may help to better understand the intrafamilial relationships in this family.

The main objective of this study is to reconstruct the intrafamilial phylogenetic relationships of linyphiid spiders based on DNA sequence data. Our starting point is the data of Arnedo et al. (2009); we then include a large number of additional taxa using epigynal morphology as sampling guide. By significantly increasing the ingroup and outgroup taxa, we attempt to resolve key uncertainties in linyphiid phylogenetic reconstruction: the ambiguous placements of some deep and poorly sampled branches, as well as to better understand the conflict between molecular phylogenies and the current taxonomic system.

2. Materials and methods

2.1. Taxon sampling

A total of 211 taxa were sampled, including 134 ingroup and 77 outgroup taxa. For the ingroup, 34 of the 35 taxa used by Arnedo et al. (2009) were included in this study; *Erigone dentipalpis* was excluded because it had data from only one gene (COI). An additional 100 linyphiid species were selected. Taxon selection for representing the seven linyphiid subfamilies was guided in part by epigynal morphology. We emphasize sampling of the large ME clade (Micronetinae–Erigoninae), with special focus on those species with transitional characters between the typical "micronetine"