Molecular Phylogenetics and Evolution 101 (2016) 176–193

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Two DNA barcodes and morphology for multi-method species delimitation in *Bonnetina* tarantulas (Araneae: Theraphosidae)

David Ortiz *, Oscar F. Francke

Colección Nacional de Arácnidos, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

ARTICLE INFO

Article history:
Received 1 April 2015
Revised 7 April 2016
Accepted 1 May 2016
Available online 03 May 2016

Keywords:
Species delimitation
DNA barcoding
Gap coding
Genetic polymorphism
Molecular systematics
Cryptic species

ABSTRACT

Determining species boundaries is a central debate in biology. Several recently developed molecular delimitation methods have highlighted extensive inconsistency in classical morphological taxonomy. However, choosing between them is contentious. Molecular studies on theraphosid spiders have found considerable cryptic diversity and many species redundantly described. Most of these studies have relied only on COI, a mitochondrial marker that has proven its efficacy in animal studies, but which also might lead to an over-estimation of diversity.

Here we present an integrative approach to species delimitation in *Bonnetina*, a poorly known group of tarantulas endemic to Mexico. We employed morphological evidence, as well as different setups with distance-based (Hard-Gap barcoding and ABGD) and tree-based (GMYC, PTP and BPP) molecular barcoding approaches, using one mitochondrial (COI) and one nuclear (ITS1) rapidly evolving loci. BPP is also used as a multi-locus method. We also explored the influence of ambiguous alignment choice and of coding gaps as characters in phylogenetic inference and in species delimitation with that marker.

Different delimitation methods with COI gave moderately variable results and this gene exhibited a universal barcode gap. The ITS1 gene tree was well supported and robust to alignment choice; with this locus, coding gaps improved branch support and species delimitation with PTP. No universal barcode gap was found with ITS1, and single locus delimitations returned disparate results. However, this locus helped to highlight cases of under- and overestimations by COI. BPP gave solutions with many lineages, in single locus and combined analyses, especially with the recently implemented unguided methodology. We recognize 12 robustly supported species in our data set, of which seven remain undescribed, and three are morphologically cryptic. For COI *Bonnetina* species identification, we propose intra- and inter-specific thresholds of 2% and 6% sequence divergence, respectively.

We conclude that morphological signal for species delimitation in *Bonnetina* is higher than for other studied tarantulas, but it fails to recognize several lineages in the genus. COI is a functional barcoding marker, and the most reliable source of evidence that we used, but it may also lead to inaccurate delimitations. ITS1 is a highly informative locus for species delimitation and species-level phylogeny, but it performs poorly as a barcoding marker. Due to variability between delimitation methods, we suggest combining evidence from multiple approaches to get better-supported results.

1. Introduction

Species delimitation is a key issue in biology and of crucial importance in systematics, ecology and conservation biology. Although there is not a universally accepted species concept (De Queiroz, 2007), widely embraced modern views incorporate the idea that the species definition should reflect genealogy. However, this paradigm can rarely be tested using historically preponderant species delimitation based only on morphology.

1.1. Morphology-based species delimitation

The implementation of the morphological species concept has several practical advantages, as it is usually inexpensive, it can make use of the extensive information bank in the literature and traditional biological collections, and it is applicable to fossil taxa. Likewise, this approach often incorporates information directly involved in reproductive isolation mechanisms (e.g. sexual structures), and usually allows to perform field identifications, even by non-specialists (Hillis, 1987; Will and Rubinoff, 2004). However, morphology is generally uninformative below the species level. It is also constrained by selection, which can either result in

* Corresponding author.
E-mail address: davidomartinez@yahoo.es (D. Ortiz).

http://dx.doi.org/10.1016/j.ympev.2016.05.003
1055-7903/

phenotypic conservation across independent lineages or polymorphism within lineages (Bickford et al., 2007; Hebert et al., 2004). In practice, the use of morphology for species delimitation is hindered by the need for long training periods to educate specialists, subjectivity, and a dependence on the availability of specific life stages (adult males and females or juveniles) (Hebert et al., 2003; Lee, 2004; Tautz et al., 2003).

1.2. DNA-based species delimitation

Development of molecular methods has provided a new way to investigate the species delimitation problem, because using the infra-specific genealogical information in DNA markers allows an objective implementation of modern species concepts (e.g. biological, phylogenetic, genotypic cluster). These methods are less dependent on the availability of specific material and the researcher's experience with the group of study, and are becoming increasingly accessible as sequencing costs decrease. Nevertheless, their application is strongly dependent on the availability of appropriate markers, which continue to be a serious problem for those working with many groups of organisms.

The most widely used methods implement DNA barcoding, which employs a single or a few linked, highly variable and easily amplified DNA fragments for species identification and/or delimitation. Although it was initially proposed as an identification method (Hebert et al., 2003), it has been subsequently used for species discovery (Fujita et al., 2012; Pons et al., 2006; Zhang et al., 2013). Barcoding methods have been used to detect cryptic diversity in many taxonomic groups and have been proposed for large scale discovery strategies, especially in poorly studied groups (Čandek and Kuntner, 2014; Frézal and Leblois, 2008; Hebert et al., 2003; Riedel et al., 2013; Tautz et al., 2003). Nevertheless, the strong sensitivity of barcoding methods to potential marker bias can lead to erroneous results (Collins and Cruickshank, 2013; DeSalle et al., 2005; Ebach and Holdrege, 2005; Frézal and Leblois, 2008; Lee, 2004; Lipscomb et al., 2003; Taylor and Harris, 2012). Mitochondrial Cytochrome C Oxidase subunit I (COI), nuclear Internal Transcribed Spacers (ITS) and three plastid regions are the more widely used barcoding markers for animals, fungi and plants, respectively (Kress and Erickson, 2012).

In recent years, methods that integrate information from multiple molecular markers have been developed (Camargo et al., 2012; Ence and Carstens, 2011; Fujita et al., 2012; Grummer et al., 2014; Jones et al., 2015; O'Meara et al., 2006; Solís-Lemus et al., 2015; Yang and Rannala, 2010). They have the potential to overcome single marker biases by applying more inclusive evidence to the species delimitation problem. According to some studies, robustness in the results increases with the number of incorporated loci to some point at which stabilization is achieved (Camargo et al., 2012; Ence and Carstens, 2011; Jones et al., 2015; Olave et al., 2014; Yang and Rannala, 2010; Zhang et al., 2011). Although those methods are mainly of validation of previously hypothesized groupings, a new development (Jones et al., 2015) includes the possibility of performing specimens clustering and delimitation hypotheses by itself.

1.3. Study group

The spider family Theraphosidae (tarantulas) includes nearly 1000 mostly tropical and sub-tropical species (World Spider Catalog, 2016) that are among the largest terrestrial arthropods. They are strongly linked to human culture, because of their imposing looks, remarkable visual attractiveness and high abundances close to human populated areas. However, our knowledge on the diversity of theraphosids is still poor and our understanding of their phylogeny remains rudimentary.

Unlike other spiders, tarantulas are not known to exhibit ballooning (Hendrixson et al., 2013), a behavior that allows spiderlings to disperse by wind. This limitation is likely to reduce their capability for dispersal, increasing genetic structure, diversification and local endemicity. Conversely, their large size and the nomadic nature of adult males (Janowski-Bell and Horner, 1999; Shillington, 2005; Stoltey and Shillington, 2009) could explain the relatively wider distributions and lower genetic structure found in theraphosids (Graham et al., 2015; Hamilton et al., 2016, 2014, 2011; Hendrixson et al., 2015, 2013; Longhorn et al., 2007; Montes de Oca et al., 2016; Petersen et al., 2007; Wilson et al., 2013) when compared to deeply structured smaller mygalomorphs (Arnedo and Ferrández, 2007; Bond et al., 2001; Castalanelli et al., 2014; Cooper et al., 2011; Opatova and Arnedo, 2014; Satler et al., 2013; Starrett and Hedin, 2007).

The systematics of tarantulas has essentially been based on morphological characters. Sexual features have played a dominant role in species delimitation. In general, males have more informative features than females, whereas juveniles are rarely of utility. As most mygalomorphs, theraphosids commonly exhibit high homoplasy and a combination of high intra- and low interspecific morphological variability. This has hampered the taxonomy of the group (Bertani, 2001; Prentice, 1997; Raven, 1985).

To our knowledge, DNA-based theraphosid delimitation studies have been primarily done with U.S. species of the taxonomically contentious *Aphonopelma* Pocock 1901 (Graham et al., 2015; Hamilton et al., 2016, 2014, 2011; Hendrixson et al., 2015, 2013; Wilson et al., 2013), except for single works on CITES protected Central and North American *Brachypelma* 1891 (Petersen et al., 2007), and South American *Grammostola* Simon 1892 (Montes de Oca et al., 2016). These studies showed strong differences between the current morphology-based taxonomy, and the evidence from molecular and ecological data. In the most comprehensive of these works, using evidence from 455 loci, morphology and geospatial data, Hamilton et al. (2016) synonymized 33 of the 55 previously recognized U.S. *Aphonopelma* species, and also described 14 new taxa. Therefore, expanding the usage of molecular data is likely to deeply change our understanding of the diversity and biogeography of Theraphosidae.

Here we carry out a multiple evidence exploration of species delimitation in tarantulas of the genus *Bonnetina* Vol 2000, a poorly studied group with nine previously known species from only 11 localities in central-southern Mexico (Ortiz and Francke, 2015). *Bonnetina* is known to occur mainly in the Balsas Basin, the Pacific Lowlands, the Trans-Mexican Volcanic Belt and the Sierra Madre del Sur, which are connected regions with complex orography and high biodiversity (Espinosa and Ocegueda, 2008; Morrone, 2014). Apart from the original taxonomic descriptions, COI sequences from one of the species and some ecological and behavioral notes, no additional data exist for the genus (Mendoza-Marroquín, 2012; Ortiz and Francke, 2015). We evaluate the results obtained by morphology and several molecular methods, using two rapidly evolving markers: the mitochondrial standard animal barcode COI, and the nuclear ITS. Additionally, with the strongly divergent ITS locus, the effects of alignment method and of gaps-coding choice in both phylogenetic reconstruction and species delimitation are evaluated.

2. Material and methods

2.1. Specimens sampling

We conducted extensive fieldwork for fresh *Bonnetina* material, focusing our collecting efforts on the type localities of nominal species, localities known from scientific collections, and other places