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Species delimitation of the blue-spotted spiny lizard within a multilocus, multispecies coalescent framework, results in the recognition of a new *Sceloporus* species

Brenda Díaz-Cárdenas [a], Eduardo Ruiz-Sanchez [b,*], Patricia Castro-Felix [c], Gamaliel Castañeda-Gaytán [d], Sergio Ruiz-Santana [a], Héctor Gadsden [e]

[a] Instituto de Ecología, A. C., Carretera antigua a Coatepec 351, El Haya, C.P. 91070 Xalapa Enríquez, Veracruz, Mexico
[b] Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Camino Ing. Ramón Padilla Sánchez 2100, Nextipac, Zapopán, Jalisco 45510, Mexico
[c] Departamento de Biología Celular y Molecular, Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias, Camino Ing. Ramón Padilla Sánchez 2100, Nextipac, C.P. 45510 Zapopan, Jalisco, Mexico
[d] Universidad Juárez del Estado de Durango, Facultad de Ciencias Biológicas, Av. Universidad S/N col. Filadelfia, C.P. 35010 Gómez Palacio, Durango, Mexico
[e] Instituto de Ecología, A. C., Centro Regional de Bajío, Av. Lázaro Cárdenas 253, 61600 Pátzcuaro, Michoacán, Mexico

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ABSTRACT

Species delimitation is a major topic in systematics. Species delimitation methods based on molecular data have become more common since this approach provides insights about species identification via levels of gene flow, the degree of hybridization and phylogenetic relationships. Also, combining multilocus mitochondrial and nuclear DNA leads to more reliable conclusions about species limits. Coalescent-based species delimitation methods explicitly reveal separately evolving lineages using probabilistic approaches and testing the delimitation hypotheses for several species. Within a multispecies, multilocus, coalescent framework, we were able to clarify taxonomic uncertainties within *S. cyanostictus*, an endangered lizard that inhabits a narrow strip of the Chihuahuan Desert in Mexico. We included, for the first time in a phylogenetic analysis, lizards from the three populations of *S. cyanostictus* recognized so far (East Coahuila, West Coahuila and Nuevo León). Phylogenetic analysis corroborates the hypothesis of two separately evolving lineages, *i.e.* the East and West Coahuila populations, as proposed in a previous study. We also found a distant phylogenetic relationship between the lizards from Nuevo León and those of East and West Coahuila. Finally, based on the species delimitation results, we propose and describe a new species of *Sceloporus*: *S. gadsdeni* sp. nov.

1. Introduction

Several authors recognize the species as the fundamental unit in many areas in biology such as evolution, systematics, ecology and conservation (*e.g.* de Queiroz, 2007; Sites and Marshall, 2004; Wiens and Penkrot, 2002), thus species delimitation—"the process by which species' boundaries are determined and new species are discovered" (Wiens, 2007)—is a major topic in systematics. There are several ways to delimit species (see Sites and Marshall, 2003 for a review), and the most traditionally used are those based on morphological and molecular characters, now known as non-parametric analyses for delimiting species (Carstens et al., 2013).

Species delimitation methods based on molecular data have become more common with the methodological advances in DNA-based systematics approaches (Ence and Carstens, 2011; Carstens et al., 2013; Rannala, 2015). Also, molecular data provide insights into species identification by revealing things such as levels of gene flow, the degree of hybridization and phylogenetic relationships (Yang and Rannala, 2010). Even though mitochondrial DNA data (mtDNA) has been claimed to be useful when the species being studied are newly formed or difficult to delimit with morphological data (Wiens and Penkrot, 2002), some researchers realized there were inconsistencies when mtDNA is used as the only criteria and suggest combining mitochondrial and nuclear DNA (nDNA) data to arrive at more reliable conclusions about species limits (*e.g.* Leaché and McGuire, 2006; Moritz et al., 1992; Wiens et al., 2010).

* Corresponding author.
E-mail address: ruizsanchez.eduardo@gmail.com (E. Ruiz-Sanchez).

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Species delimitation methods that use data given by a single locus lose information regarding the evolutionary process (Dupuis et al., 2012; Niemiller et al., 2012). Rannala and Yang (2003) developed a Bayesian method called Multispecies Coalescent Model that uses multilocus DNA sequence data for simultaneous estimation of species divergence time (τ) and population size (θ), important parameters in evolutionary models. Based on this model, Yang and Rannala (2010, 2014) developed a Bayesian approach for species delimitation. This approach generates the posterior probabilities of species assignments taking into account the tree uncertainty inherent in delimiting species using genealogical data and can rely on a guide phylogeny (guide tree) that represents the phylogenetic relationships among the most subdivided possible delimitation of individuals into species (Yang and Rannala, 2010), or not (Yang and Rannala, 2014). Some studies addressing the species delimitation issue have highlighted the reliability of this method (*e.g.* Carstens et al., 2013; Fujita and Leaché, 2011) and it has been used in the studies of a variety of taxa such as lichens (Leavitt et al., 2011), plants (Barrett and Freudenstein, 2011; Ruiz-Sanchez, 2015), fish (Niemiller et al., 2012), birds (Fuchs et al., 2011), amphibians (Setiadi et al., 2011; Zhou et al., 2012) and reptiles (Burbrink et al., 2011; Camargo et al., 2012; Leaché and Fujita, 2010).

Sceloporus (spiny and fence lizards) is a diverse genus with up to 90 recognized species so far (Uetz and Hošek, 2016). This diversity is the result of rapid radiation (Leaché and Sites, 2009; Leaché et al., 2016), which leads to phylogenetic problems that are still being studied. As a consequence, the phylogenetic relationships between some species groups of *Sceloporus* have not yet been resolved (Leaché, 2010; Leaché et al., 2016; Wiens et al., 2010).

To date, 18 *Sceloporus* groups have been recognized based on morphological similarities and phylogenetic clustering (Leaché et al., 2016). Among those groups Wiens et al. (2010), based on phylogenetic analysis using mitochondrial and nuclear genes, recognized two paraphyletic groups inside the *torquatus* group and divided it into the *torquatus* and *poinsettii* groups. Leaché et al. (2016), using ultraconserved elements and protein-coding genes, detected the monophyly of the *torquatus* group and recommended assigning all the species of *torquatus* and *poinsettii* groups to the *torquatus* group. The *torquatus* group has been recognized as having a particular pattern of morphological variation in which between-species differentiation is small relative to within-species variation in some taxa (Wiens and Penkrot, 2002). Wiens and Penkrot (2002) suggested that this pattern could result from the length of time required for diagnostic morphological differences to evolve because of a rapid split between species leading to the lack of diagnostic morphological characters in some species of this group. In this regard, Wiens et al. (1999), using populations of *S. jarrovii* of the *torquatus* group as a case study, recognized that traditional species-level taxonomy (*i.e.* based on morphological data) does not always correspond to evolutionary lineages. They analyzed phylogenetic relationships between the populations corresponding to seven subspecies of *S. jarrovii*, and identified five distinct evolutionary species within *S. jarrovii* (*Sceloporus cyanostictus*, *Sceloporus jarrovii*, *Sceloporus sugillatus*, *Sceloporus oberon*, and *Sceloporus minor*).

One of the evolutionary species recognized by Wiens et al. (1999) is *Sceloporus cyanostictus* (Axtell and Axtell, 1971), an endangered species that inhabits a narrow region of the Chihuahuan Desert in Mexico (Barrows et al., 2013; Fig. 1). *S. jarrovii cyanostictus* was described from a population in an anticlinal mountain known as La Muralla (61 km south of Monclova, Coahuila, Mexico) as a subspecies of *S. jarrovii* because of its morphological similarities, *i.e.* the black neck stripe typical of *S. jarrovii* (Axtell and Axtell, 1971). In a subsequent study, Wiens and Penkrot (2002) used three different approaches of species delimitation and found support for *S. cyanostictus* as an evolutionary species and also discovered that each of the two populations of *S. cyanostictus* (East and West Coahuila; Wiens et al., 1999) included in the analysis possessed exclusive and divergent haplotypes, in addition to the diagnostic character of dorsal coloration in males, which suggested the existence of two distinct species. Although, Wiens and Penkrot (2002) suggested sampling intermediate populations of *S. cyanostictus* to distinguish between speciation or isolation-by-distance, to date there is no evidence of the presence of such intermediate populations. Gadsden et al. (2012) carried out potential distribution analysis through ecological niche modeling of this species and they did not find signals of suitable habitat between the two known populations (East and West Coahuila). Recently, Price et al. (2010) recorded a range extension for *S. cyanostictus* in the state of Nuevo León, Mexico and no specimens from this locality have been included in any phylogenetic analysis so far.

Based on the above, the main goals of this study were (1) to reconstruct the phylogenetic relationships of *Sceloporus cyanostictus* using mitochondrial and nuclear DNA, (2) to delimit species in *S. cyanostictus* applying a Bayesian species delimitation method, and (3) to investigate whether *S. cyanostictus* populations from eastern and western Coahuila and Nuevo León are separately evolving lineages within a multi-locus, multi-species coalescent framework and following the General Lineage concept of species set out by de Queiroz (1998).

2. Materials and methods

2.1. Study species

Sceloporus cyanostictus is sexually dimorphic, the average adult snout-vent length (SVL) is 72.8 mm in males and 69.6 mm in females (Axtell and Axtell, 1971). Axtell and Axtell (1971) provided a detailed description of the scale pattern and coloration of *S. cyanostictus* lizards (then known as *S. j. cyanostictus*), in which they distinguished some particular characteristics such as dark postocular stripes and pale bluish stripes bordering both sides of the postocular dark stripe. In males the gular region has a deep blue coloration, chest, and undersurfaces of forelegs are pale grayish blue with many small melanin specks, and dark ventrolateral areas. This is the observed scale and coloration pattern on the type population of *S. cyanostictus* (East Coahuila; Fig. 2A) and it is slightly different to that observed in the lizards from West Coahuila (Fig. 2B–E) and Nuevo León (Fig. 2F) populations. Sexual dimorphism is pronounced in coloration, with males having green to blue scales and females a brownish coloration with either a yellowish or very pale green tint, and no gular or ventral bluish patches.

2.2. Sample collection and DNA extraction

We sampled 38 *S. cyanostictus* lizards across its known distribution (Fig. 1, Table 1). Specifically, East Coahuila population was sampled at a site called "La Muralla", located approx. 190 km east of the West Coahuila population and approx. 70 km west of the Nuevo Leon population sampled in the locality of Mina. All these three localities are allopatrically distributed. West Coahuila population was sampled in a complex of three mountain ranges called "Sierra Texas", "Sierra Solís" and "Sierra San Lorenzo".

We sampled every individual found and captured in each site. We took body tissue by removing ~1 cm of the tail tip from each lizard and placed it in a microtube with absolute ethanol. Lizards were released in the capture place after sampling. The samples were stored at −20 °C until DNA extraction. Total genomic DNA was extracted from a ~25 mg tail fragment using a high salt protocol (Aljanabi and Martinez, 1997).