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Phylogeography and species boundaries of the western North American Nightsnake (*Hypsiglena torquata*): Revisiting the subspecies concept

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Abstract

The subspecies concept has received considerable debate throughout the past century. Subspecies were originally used to delineate potential incipient species, but were later employed to simply capture geographical variation. There is a recent trend to eliminate the trinomial in light of new evidence. Discrete, diagnosable lineages are elevated to specific status, while those that show clinal variation and/or appear to represent ecological pattern classes are placed in synonymy with the parent species and the subspecific epithets are disregarded. Here, I examine the species boundaries of nightsnakes (*Hypsiglena torquata*) using standard phylogeographic methods and mtDNA data from 178 individuals. Previously, seventeen subspecies of *H. torquata* were described. In this study, I recognize six species in what was previously considered *H. torquata*: one is novel, two were previously recognized subspecies, while the remaining three are wide-spread, polymorphic lineages, composed of multiple subspecies. I make the case to maintain the subspecific lineages in these wide-ranging species because they are geographically cohesive, morphologically discrete, and may represent incipient species within each complex, which have not yet achieved speciation. These subspecies are maintained, not only pending future investigations, but because they provide a useful identity for the taxonomy of this diverse lineage.

Keywords: Baja California; Biogeography; Deserts; *Eridiphas*; *tanzeri*; *Pseudoleptodeira*

1. Introduction

The process of speciation is a fundamental evolutionary concept inspiring extensive deliberation (Darwin, 1859; Dobzhansky, 1937; Mayr, 1942; Moritz et al., 1992; Coyne and Orr, 2004; Wake, 1997, 2006). Identifying the point at which diverging lineages have achieved speciation has often proven to be a challenging task. Part of this task is choosing a widely accepted species concept, while another is selecting appropriate criteria to delimit species boundaries (Sites and Marshall, 2004). Recently, these challenges have to a certain extent been reconciled. de Queiroz (1998, 2005, 2007) proposed that most contemporary species concepts share a common element in the conceptualization of what constitutes a species and their incompatibilities are often in the criteria used to determine species boundaries. Most contemporary species concepts are consistent with the notion that species are segments of separately evolving metapopulation lineages, which de Queiroz (1998) coined as the ‘general lineage concept of species.’ Challenges remain in determining at what point in this gradual process of a diverging lineage has speciation been achieved. From a taxonomic perspective, the interface of diverging lineages and secondary contact is often at the subspecific level, an area that has long been controversial among systematic biologists (Darwin, 1859; Wilson and Brown, 1953; Frost and Hillis, 1990).

Historically, many vertebrate lineages at the species–subspecies boundary have been described based on minor differences in morphology, including color patterns. Reptiles are no exception, and many subspecies described on the basis of color patterns and scalation were typically con-

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fined to non-overlapping geographical areas with respect to conspecifics. The subspecific rank now represents an issue of concern in systematic biology, particularly amongst herpetologists (Frost and Hillis, 1990; Burbrink et al., 2000; Manier, 2004). Often, these subspecies represented morphological extremes in characters that were later shown to have clinal variation. As part of a recent movement from a traditionally rank-based taxonomy to a phylogenetically-based taxonomy, there has been a general consensus to eliminate the trinomial designation in species names (Frost and Hillis, 1990; Collins, 1991; Grismer, 1999; for a review see Manier, 2004). Morphologically discrete, geographically-isolated groups were considered to have achieved speciation (Frost et al., 1992). To the contrary, if morphological variation is shown to be clinal, or associated with particular ecologies, the subspecific designations are placed in synonymy of the species (e.g., Manier, 2004).

Phylogeographic studies based on mtDNA are more commonly used to evaluate subspecific designations in many reptilian species groups (e.g., Zamudio et al., 1997; Wiens et al., 1999; Rodríguez-Robles and De Jesus-Escobar, 2000; Burbrink et al., 2000), reveal clinal patterns of geographic variation (e.g., Ashton, 2001) or ecologically associated pattern classes (e.g., Richmond and Reeder, 2002; Leaché and Reeder, 2002), and identify areas of conservation (e.g., Moritz and Faith, 1998; Mulcahy et al., 2006). Recently, more rigorous methods for delimiting species boundaries using mtDNA sequence data have been proposed (Templeton, 2001; Davis and Nixon, 1992; Wiens and Penkrot, 2002; Cardosos and Vogler, 2005). A combined approach of applying network-based methods for similar haplotypes (e.g., Templeton et al., 1992, 1995) with standard phylogenetic-based analyses (e.g., Farris, 1977; Felsenstein, 1981) for more divergent haplotypes, capitalizes on the statistical power at both levels (Crandall and Fitzpatrick, 1996), and has proven to be widely successful (Wiens and Penkrot, 2002; Morando et al., 2003; Cardosos and Vogler, 2005).

Geographically widespread and morphologically variable taxa are ideal candidates to use this combination of methods to study speciation, particularly if the variation has already been described. The common nightsnake (*Hypsiglena torquata*)—the focal species of this study—provides a model system because of its broad distribution and extensive morphological variation. This is a small (~30 cm), rear-fanged, mildly venomous colubrid snake within which 20 "morphological forms" (species and/or subspecies) have been described—based largely on the nuchal patterns that often take the form of a collar, small dorsal body spots in one to two rows, and differences in dorsal, ventral, and caudal scale counts. The common nightsnake is geographically wide-spread and several of the mainland forms are congruent with major biogeographic regions of western North America (Fig. 1). Systematists have recognized from one (Dunn, 1936) to five (Tanner, 1944) species within *H. torquata*, with many additional classification schemes proposed (Taylor, 1938; Dixon, 1965; Tanner, 1943, 1966; Dixon and Lieb, 1972; Dixon and Dean, 1986; Grismer, 1999, 2002; Lemos-Espinal et al., 2004); currently there are 17 subspecies recognized (Tanner, 1944, 1954, 1966, 1981; Tanner and Banta, 1962; Zweifel, 1958). These subspecies were based on scalation, nuchal patterns, and number of body-blotches, and many are endemic to islands associated with the Baja California peninsula (Murphy and Ottley, 1984; Grismer, 1999, 2002). Taxonomists have made efforts to portray this diversity by species recognition (Taylor, 1938; Tanner, 1944; Dixon, 1965), yet these early proposals have been continuously regarded with skepticism (Bogert and Oliver, 1945; Tanner, 1966; Hardy and McDiarmid, 1969; Dixon and Dean, 1986), to the point where most taxonomists have surrendered to recognizing only one species (Tanner, 1985; Dixon and Dean, 1986). Further doubts about the validity of some of the wide-ranging subspecies have been raised by reports of clinal variation in scalation (Tanner, 1944; Tanner, 1985; Dixon and Dean, 1986).

Hypsiglena is one of three genera of nightsnakes, which form a sub-clade within the neotropical Dipsadinae (Mulcahy, 2007). The banded nightsnake (*Pseudoleptodeira latifasciata*) is endemic to the Balsas Basin and associated Pacific versant of southwestern, mainland Mexico (Günther, 1894; Duellman, 1958; Dowling and Jenner, 1987). The Baja California nightsnake (*Eridiphas slevini*) is endemic to the mid-to-lower half of the peninsula (Tanner, 1943; Leviton and Tanner, 1960), and the Rio Verde nightsnake (*Hypsiglena tanzeri* [Dixon and Lieb, 1972]) occurs from near Rio Verde to Jalpan, in the Mexican states of San Luis Potosi and Queretaro, respectively (Fig. 1). The latter species is generally considered distinct (Dixon and Dean, 1986; Liner, 1994); however, some have argued that it is just another variant of *H. torquata* (Tanner, 1981).

Here, I used a combined approach of haplotype networks and phylogenetic analyses to evaluate the subspecies of *H. torquata* using ~800 base-pairs (bp) of mtDNA sequence data (*nad4* + 2 tRNAs) from 178 individuals sampled from throughout the geographic distribution of this species. Similar haplotypes were grouped into networks (Templeton et al., 1995) and phylogenetic analyses using parsimony and Bayesian methods were conducted on the unique haplotypes to join disparate networks. Morphologically based subspecies were evaluated by the molecular based phylogeny, using a method of species delimitation proposed by Wiens and Penkrot (2002). I included representatives of the other two genera of nightsnakes and *H. tanzeri* to test the exclusivity of the focal species. Two lineages recognized at the species level were congruent with previously described subspecies of *H. torquata*, another was a novel lineage initially identified by the mtDNA analyses, while others consisted of monophyletic groups containing several subspecies. The subspecies in these wide-ranging, polytypic species are retained for future evaluation, because they are characterized by distinct morphologies and increased sampling may prove them to be independent lineages. The previously un-recognized form