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

Daniel G. Mulcahy [1]

Department of Biology, Utah State University, Logan, UT 84322-5305, USA

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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-

[1] Present address: Department of Biology, 401 WIDB (Widtsoe), Brigham Young University, Provo, UT 84602-5181, USA.
E-mail address: dmulcahy@byu.edu

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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

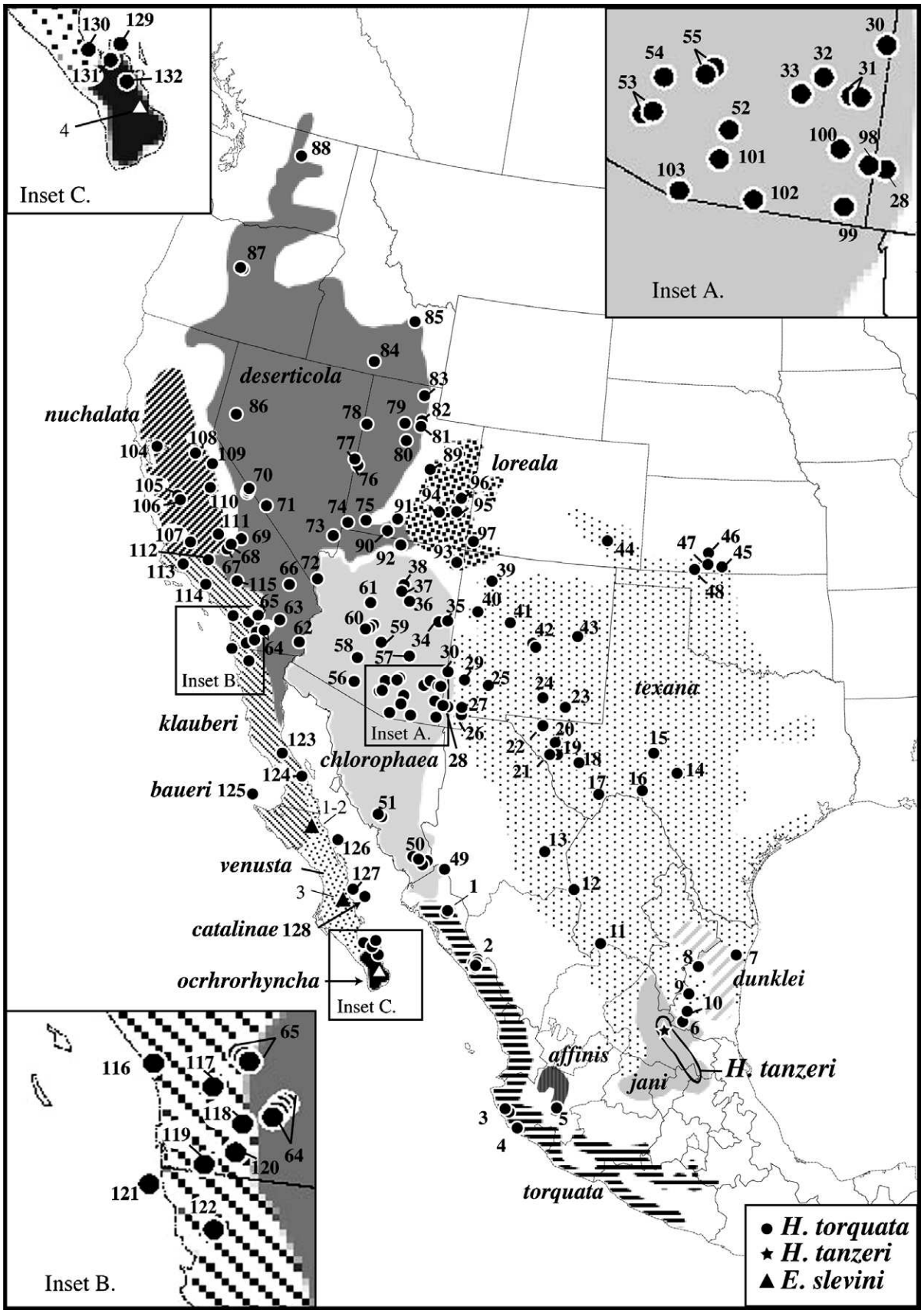

Fig. 1. The geographic distribution of *Hypsiglena torquata* is shown by shaded regions each named by subspecies. Populations of genetic samples for this study are indicated by dots and numbers (1–132); some are represented by multiple individuals (see Table 1 for specific numbers, a brief description of each locality, and subspecific designations). Samples of *Eridiphas slevini* are shown by triangles in Baja California and numbers (1–4) correspond to haplotypes (see Section 3), while the *H. tanzeri* sample is shown by a star in mainland Mexico.

was identified as a distinct species, which has a unique combination of morphological characters. I address the relationship among the major clades in another study using complete mtDNA genome data (Mulcahy, 2006); whereas, here I focus on the geographic and taxonomic boundaries of these clades. This study not only demonstrates the benefit of fine-scale sampling while providing a phylogeographic assessment of the North America deserts, but also shows the need to maintain subspecific taxonomies to provisionally identify incipient lineage diversity. Additionally, several areas of secondary contact between lineages were found—critical for future analyses to investigate the veracity of such recently diverged lineages.

2. Materials and methods

2.1. Geographic sampling

I collected sequence data from 178 individuals (Table 1) from 132 unique localities, throughout the geographic distribution of *H. torquata* (Fig. 1), representing every described mainland subspecies, with most represented by multiple individuals. Attempts were made to include multiple samples per locality, particularly near presumed contact zones between subspecies, but because of the discreet nature of snakes, obtaining such dense sampling is challenging. Specimens were classified according to subspecific designations based on geographic location and morphology. During this investigation a unique lineage was identified by the mtDNA data that also is fixed for a particular morphology, which makes it distinct from other forms, and is hereafter referred to as the "Cochise" form (Table 1; see Section 3). In addition to the 178 individual *H. torquata*, I also sampled the closest outgroup taxa possible to test for monophyly of the focal species (Wiens and Penkrot, 2002), which included one *H. tanzeri*, four individuals of *Eridiphas slevini* spanning the geographic distribution of this species, and one *P. latifasciata*. One each of *Leptodeira punctata* and *Sibon sartorii* were also included as further outgroup taxa (Mulcahy, 2007).

2.2. Laboratory and sequence protocols

Total genomic DNA was extracted from either frozen (−80 °C) or ethanol-preserved heart, liver, muscle, tail-tip tissues, or from dried shed skins. Extractions were done using standard proteinase K digestion, followed by phenol-chloroform extractions (Palumbi, 1996). Polymerase chain reaction (PCR) was performed on the genomic DNA extractions for the mtDNA *nad4* gene and three associated transfer ribonucleic acid (tRNA) genes ($tRNA^{His}$, $tRNA^{Ser}$, $tRNA^{Leu}$) using the primers ND4 and Leu from Arévalo et al. (1994), and primers designed specifically for *Hypsiglena: HypNad4f.1* 5′-TGC CTA GCA GCC TTY ATA GCT A-3′ and *HypLeu2r.1* 5′-TAC CAC TTG GAT TTG CAC CA -3′ based on complete mt-genome data (Mulcahy, 2006). The profiles for PCR were: initial denature for 5 min of 92–94 °C, followed by 30 cycles of 1 min melt at 92–94 °C, 1 min annealing at 51–55 °C, elongation of 2 min at 72 °C, with a final elongation of 5 min at 72 °C. The PCRs were conducted in 50 μl reactions, with 2 μl of primers (5 μM), 0.1–0.5 μl Taq (Promega; Madison, WI), 5 μl of buffer, 5 μl MgCl2 (25μM; buffer and MgCl2 supplied with Promega Taq), 8 μl of dNTPs (5 μM) and 2–15 μl of DNA template, based on concentration. The PCR products were purified using Wizardprep™ kits (Promega) and sequences were obtained for both directions using PCR primers and BigDye™ version 2.0 in reactions of 10–12 μl total volume following manufacturer's protocols. Sequence reaction products were purified with Sephadex (Sigma; St. Louis, MO) and run on an ABI 377 automated sequencer. Heavy and light strand sequences of DNA were examined and complimentary strands were combined and initially aligned in Sequencher™ 3.1.1. Sequences were then translated for the protein-coding regions and inspected for stop codons in MacClade 4.08 (Maddison and Maddison, 2005), and compared with the other sequences available in GenBank: *nad4* region for *Heterodon*, *Farancia*, and *Helicops* (Kraus and Brown, 1998). Secondary structures for the tRNAs were compared with other vertebrate taxa to identify stem and loop structures, which were used to further align these regions manually (Macey and Verma, 1997). PAUP* 4.0b10 (Swofford, 2000) was used to calculate an average, uncorrected pair-wise sequence divergence between unique haplotypes for the major clades revealed in *Hypsiglena* (below), and Modeltest 3.06 (Posada and Crandall, 1998) was used to select a nucleotide substitution model (GTR + I + G) under the hierarchical likelihood ratio test (hLRT) criteria, which was then employed in PAUP* to calculate corrected pair-wise sequence divergence for the entire dataset.

2.3. Phylogeographic analyses

A statistical parsimony analysis (Templeton et al., 1992) was conducted with all individual sequences of *H. torquata* using TCS 1.16 (Clement et al., 2000) to determine the number of unique haplotypes, the distribution of shared haplotypes, and to generate the haplotype networks. As a heuristic exercise, after the initial connection limit of 95% was used, the connection limit was set to calculate at 90% confidence limit in TCS to see if fewer networks could be recovered. Duplicate haplotypes were removed and all unique haplotypes of *H. torquata* were combined with sequence data from *H. tanzeri*, *E. slevini*, *P. latifasciata*, *S. sartorii*, and *L. punctata* for phylogenetic analyses, with only *L. punctata* designated as the outgroup, based on a more inclusive phylogeny (Mulcahy, 2007). This dataset was then examined under maximum parsimony criteria in PAUP* using heuristic search options with 100 random, stepwise additions, and a tree-bisection-reconnection branch swapping algorithm. Because of the low level of

Table 1
Voucher specimen information

Pop.	CT	ST	County	Locality	Voucher	Subspecies	GenBank No.
1 (2)	MX	SI	—	Hwy 24, btw Badiraguato-Perico	MZFC-JAC 24822-23	*torquata*	EU363045-6
2 (6)	MX	SI	—	Rd. to Cosala, N of Libre	UTA R-51980-82,	*torquata*	EF078548
					MZFC 16916, 16925-26		EU363047-51
3 (2)	MX	JA	—	near Melaque	UTA R-53380, MZFC-JAC 23920	*torquata*	EU363052-53
4	MX	JA	—	near Autlan de Navarro	UTA R 53379	*torquata*	EU363054
5	MX	JA	—	Rd. to Tapalpa, MX Hwy 54	LSUMZ 39533	*affinis*	EU363055
6	MX	TA	—	SW Tula Desert	MF21713	*jani*	EU363059
7	MX	TA	—	N of San Fernando	MF9597	*dunklei*	EU363056
8 (2)	MX	TA	—	SW of Valle Mainero	MF21731-32	*dunklei* + *texana*	EU363057-8
9	MX	TA	—	SE of Villa de Bustamante	MF9528	*texana*	EU363060
10	MX	TA	—	W of Ejido San Pablo	MF21696	*texana*	EU363061
11	MX	ZA	—	S of Bajio de Ahuichila	MVZ 236398	*texana*	EU363062
12	MX	DU	—	Laboratorio del Desierto	RWM 5256	*texana*	EU363063
13	MX	CH	—	Rd. to Ojinaga, MX Hwy 67	UTA R51983	*texana*	EF078551
14	US	TX	Sutton	E of Sonora, TX	UTEP 18438	*texana*	EU363064
15	US	TX	Irion	U. S. Hwy 67, E of Barnhart	TNHC 66672	*texana*	EU363065
16	US	TX	Val verde	Pandale rd., N of Langtry	UTEP 16307	*texana*	EU363066
17	US	TX	Brewster	NW of La Linda	UTA R-34835	*texana*	EU363067
18	US	TX	Jeff Davis	TX Hwy 118, W Ft. Davis	TNHC 66659	*texana*	EU363068
19	US	TX	Culberson	US Hwy 90, w of Brewster	CAS 229920	*texana*	EU363069
20	US	TX	Culberson	Hwy 54, N of Van Horn	CAS 228960	*texana*	EU363070
21	US	TX	Hudspeth	Indio Mountain Research Station	UTEP 14082	*texana*	EU363071
22	US	TX	Hudspeth	FM 1111, S of FM 2317	UTEP 18484	*texana*	EU363077
23	US	NM	Eddy	near jct. CR 409/Hwy 137	CR409137 shed only	*texana*	EU363078
24	US	NM	Otero	Hwy 506, E jct w/County rd G-5	UTEP 16309	*texana*	EU363079
25	US	NM	Sierra	Hwy 27, Mimbres Mts.	CAS 229229	*texana*	EU363072
26	US	NM	Hidalgo	Hwy 81, S jct Hwy 9	UTA R-52350	*texana*	EU363073
27	US	NM	Grant	Hwy 9, E of Animas	UTA R-52486	*texana*	EU363074
28 (3)	US	NM	Hidalgo	Hwy 9, E Hwy 80	MVZ 226235*, CAS 228934, SM 662	Cochise+*texana**	EF078552*
						Cochise	EU363168-9
29	US	NM	Grant	Hwy 180, S jct Hwy 211	UTA R-52351	*texana*	EU363096
30	US	AZ	Greenlee	NE of Three Way Jct. on Hwy 78	CAS 228952	*texana*	EU363095
31 (2)	US	AZ	Graham	Hwy 266, W of 181	CAS 228967, 229956	*texana*	EU363091-2
32	US	AZ	Graham	Stockton Pass, Hwy 266	CAS 228965	*texana*	EU363093
33	US	AZ	Graham	W of Bonita, N Fort Grant rd.	CAS 228966	*texana*	EU363094
34	US	AZ	Apache	W of Concho, on Snowflake Rd	LSUMZ 84790	*texana*	EU363084
35	US	AZ	Apache	Hwy 180, N of Jct w/Hwy 61	CAS 228936	*texana*	EU363088
36	US	AZ	Coconino	end of Meteor Crater rd.	CAS 228933	*texana*	EU363089
37	US	AZ	Coconino	Merriam Crater, NE of Flagstaff	CAS 229281	*texana*	EU363090
38	US	AZ	Coconino	Wupatki Nat'l Mon.	WUPA 24720	*texana*	EU363085
39	US	NM	San Juan	Chaco Culture Nat'l Historic Park	MSB 87812	*texana*	EU363075
40	US	NM	Cibola	El Morro Nat'l Monument	MSB 87826	*texana*	EU363086
41	US	NM	Valencia	Hwy 6, W of I-25	CAS 229231	*texana*	EU363080
42 (2)	US	NM	Torrence	Salinas Pueblo Miss. Nat'l Mon.	MSB 87874, 87876	*texana*	EU363081, 83
43	US	NM	De Baca	Sumner Lake	MSB 72615	*texana*	EU363076
44	US	CO	Otero	David Cyn, Comanche Nat'l. Gr.	SM CO1	*texana*	EU363082
45	US	KS	Barber		MHP 8260	*texana*	EU363097

(*continued on next page*)

Table 1 (*continued*)

Pop.	CT	ST	County	Locality	Voucher	Subspecies	GenBank No.
46	US	KS	Barber		MHP 12366	*texana*	EU363098
47	US	KS	Comanche		MHP 13388	*texana*	EU363099
48	US	KS	Clark		MHP 10783	*texana*	EU363100
49	MX	SI	—	Mex Hwy 32 North of Choix	UTA R 54569	?	EU363101
50 (4)	MX	SO	—	Navojoa-Alamos	BYU 42373, 42376, ROM 14944, 14932	*chlorophaea*	EU363102-05
51 (2)	MX	SO	—	5 km S of Ortiz on Hwy 48,	BYU 42382, JRO 694	*chlorophaea*	EU363108-9
52	US	AZ	Pima	Colossal Cave Rd., SE Tucson	TNHC 60066	*chlorophaea*	EU363110
53 (2)	US	AZ	Pima	W of Tuscon (I-10) on Hwy 86	MVZ 237359, CAS 228930	*chlorophaea*	EU363111, 14
54	US	AZ	Pima	Picture Rocks rd., Tucson Mts.	UTA R-52345	*chlorophaea*	EU363122
55 (2)	US	AZ	Pima	Vistoso, N Tucson	CAS 228956, UTA R-52347	*chlorophaea*	EU363115-6
56	US	AZ	Pima	Rd. to Pisinimo, Hwy 86	CAS 228929	*chlorophaea*	EU363121
57	US	AZ	Pinal		MHP 10516	*chlorophaea*	EU363113
58	US	AZ	Maricopa	Maricopa Rd., NE of Gila Bend	CAS 228918	*chlorophaea*	EU363117
59	US	AZ	Maricopa	McDowell Mts., Scottsdale	CAS 228937	*chlorophaea*	EU363120
60 (3)	US	AZ	Maricopa	E New River Rd., N Phoenix	CAS 228919-21	*chlorophaea*	EU363111, EU363112-9
61	US	AZ	Yavapai	W of Hwy 89, Chino Valley	CAS 228932	*chlorophaea*	EU363167
62	US	CA	Imperial	Black Mt. Rd.	CAS 205337	*deserticola*	EF078550
63	US	CA	Riverside	Box Canyon rd., Shavers Valley	CAS 223533	*deserticola*	EU363124
64 (4)	US	CA	San Diego	Borrego Springs-Yaqui Pass rds.	CAS 223504, 223520, 228971-2	*deserticola*	EU363125-8
65 (3)	US	CA	Riverside	Lake Hemet-Idyllwild	CAS 228968-70	*klauberi*	EU363203-5
66	US	CA	San Bern.	Granite Mts.	CAS 229917	*deserticola*	EU363143
67	US	CA	Kern	Piute Mtns, Sequoia Nat'l. Forest	CAS 219685	*deserticola*	EU363144
68	US	CA	Kern	Kelso Creek rd., Kelso Valley	MVZ 229142	*deserticola*	EU363145
69	US	CA	Inyo	Ninemile Canyon	CAS 228911	*deserticola*	EU363146
70 (3)	US	CA	Inyo	White Mts., near Westgard Pass	SDSNH 72820, MVZ 164933, CAS 206502	*deserticola*	EU363147-9
71	US	NV	Nye	Hwy 267, NE CA/NV state line	CAS 223437	*deserticola*	EU363150
72	US	NV	Clark	Hiko Springs, near Hwy 163	CAS 229952	*deserticola*	EU363151
73	US	NV	Clark	Hwy 170/E New Gold Butte rd.	CAS 223373	*deserticola*	EU363152
74	US	UT	Washington	Beaver Dam Wash, Lytle ranch	BYU 49402	*deserticola*	EU363129
75	US	UT	Washington	NE of St George off I-15	Photo/Tail-tip	*deserticola*	EU363153
76	US	NV	White Pine	Great Basin Nat'l., Snake Range	CAS 223427	*deserticola*	EU363137
77	US	UT	Millard	S of Garrison, Snake Valley	CAS 223414	*deserticola*	EU363138
78	US	UT	Tooele	Road to Ibapah	MVZ 241611	*deserticola*	EU363132
79	US	UT	Tooele	S/Grantsville, E Stansburry Mtns	MVZ 235920	*deserticola*	EU363133
80	US	UT	Tooele	Hwy 36, Tintic Mts.	MVZ 241612	*deserticola* (type)	EU363140
81	US	UT	Salt Lake	Big Cottonwood Canyon	MVZ 241609	*deserticola*	EU363134
82	US	UT	Salt Lake	Mill Creek Canyon	MVZ 241610	*deserticola*	EU363135
83	US	UT	Cache	Logan, below Dry Canyon	CAS 235907	*deserticola*	EU363139
84	US	ID	Cassia	Rock Creek, S of Twin Falls	UTA R-51097	*deserticola*	EU363136
85	US	ID	Butte	E of Howe	IMNH-DGM 1705	*deserticola*	EU363141
86	US	NV	Washoe	Pyramid Lake	UNR 07721	*deserticola*	EU363154
87 (2)	US	OR	Crook	Hwy 27, along Crooked River	CAS 228916-17	*deserticola*	EU363130-1
88	US	WA	Okanogan	Hwy 97, E of Brewster	CAS 231507	*deserticola*	EU363142
89	US	UT	Emery Co.	Hwy 57, W of Castledale	CAS 229249	*loreala* (type)	EU363155
90	US	UT	Kane	Johnson Canyon Rd., GSENP	BYU 48474	*loreala*	EU363159
91 (3)	US	UT	Kane/Garfield	S of Cannonville	MVZ 241607, 241608, 241604	*loreala*	EU363157-8, EU363160
92	US	AZ	Coconino	Hwy 89A, N Cliff Dwellers	CAS 229947	*loreala*	EU363161

93	US	AZ	Apache	Rte N12, SSE jct. Hwy 191	ASU 34818	*loreala*	EU363156
94 (3)	US	UT	Garfield	Hwy. 95, btw Hwy 276-Hite	CAS 228912-14	*loreala*	EU363162-4
95	US	UT	Grand	Hwy 279, N of Potash	MVZ 241606	*loreala*	EU363165
96	US	UT	San Juan	Salt Creek, Canyonlands NP	USGS-725	*loreala*	EU363166
97	US	CO	Montezuma	Hwy 41, E of Utah state line	MVZ 180265	*loreala*	EU36308
98 (4)	US	AZ	Cochise	Portal Rd., near Portal	CAS 228951, FMNH 259910, SM 660, 828*	Cochise Cochise + *texana**	EU363170-2 EU363186
99 (8)	US	AZ	Cochise	San Bernardino Valley	ASU 3335, CAS 174417, CAS 228924-28, UTEP 17673	Cochise Cochise	EU363173-80
100	US	AZ	Cochise	Chiricahua Nat'l Monument	UAZ 55852	Cochise	EU363181
101	US	AZ	Pima	Hwy 83, Pima/Santa Cruz Co.	CAS 228935	Cochise	EU363182
102 (2)	US	AZ	Cochise	Ramsey Rd., Sierra Vista	CAS 228958-59	Cochise	EU363183-4
103	US	AZ	Santa Cruz	Hwy 289, Pajarito Mts.	CAS 228938	Cochise	EU363185
104	US	CA	Contra Costa	Alhambra Valley rd.	CAS 228915	*nuchalata*	EU363187
105	US	CA	Fresno	Panoche Hills	MVZ 229141	*nuchalata*	EU363188
106	US	CA	Fresno	Tumey Hills	MVZ 230713	*nuchalata*	EU363189
107	US	CA	Kern	Hwy 58, Temblor Range	CAS 223543	*nuchalata*	EU363190
108	US	CA	Calaveras	Dogtown rd., N of Hwy 4	MVZ 180363	*nuchalata*	EU363191
109	US	CA	Tuolumne	Hetch Hetchy, Yosemite Nat'l P,	MVZ 241094	*nuchalata*	EU363192
110	US	CA	Madera	Coarsegold Creek off Hwy 41	MVZ 229213	*nuchalata*	EF078549
111	US	CA	Tulare	along Arrastre River	CAS 205784	*nuchalata*	EU363193
112	US	CA	Kern	Frazier Park, San Emigdio Mts.	CAS 205790	*klauberi*	EU363206
113	US	CA	Santa Barbara	Camino Cielo, Santa Ynez Mts.	CAS 223549	*klauberi*	EU363200
114	US	CA	Los Angeles	Malibu Creek, Santa Monica Mts.	CAS 229918	*klauberi*	EU363201
115	US	CA	Los Angeles	Largo Vista rd,, San Gabriel Mts.	DGM 1706 (tail-tip only)	*klauberi*	EU363202
116	US	CA	San Diego	San Onofre State Beach	MVZ 229143	*klauberi*	EU363194
117	US	CA	San Diego	Palomar Mtn., W of Observatory	CAS 223622	*klauberi*	EU363199
118	US	CA	San Diego	on Hwy 78, near San Felipe rd.	CAS 228973	*klauberi*	EU363195
119	US	CA	San Diego	SE/El Cajon, Honey Springs rd.	SDSNH 72821	*klauberi*	EU363196
120	US	CA	San Diego	near Cuyamaca Reservoir	MHP 11238	*klauberi*	EU363209
121	MX	BCN	—	Isla South Coronados	IBUNAM-GP 460	*klauberi* (type)	EU363198
122	MX	BCN	—	S of Tecate	MVZ 236390	*klauberi*	EU363197
123	MX	BCN	—	Catavina	MVZ 236389	*klauberi*	EU363207
124	MX	BCN	—	Bahia de los Angeles	MVZ 236391	*klauberi*	EU363208
125	MX	BCN	—	Isla Cedros	RWM 1859	*baueri*	EU363210
126	MX	BCS	—	Isla San Marcos	ROM 14478	*venusta*	EU363211
127	MX	BCS	—	Isla Danzante	RWM 1694	*venusta*	EU363212
128 (2)	MX	BCS	—	Isla Santa Catalina	MVZ 164935, RWM 1553	*catalinae*	EU363106-7
129	MX	BCS	—	NE of La Paz, Punta Coyote	MVZ 236397	*ochrorhyncha*	EU363213
130	MX	BCS	—	NW of La Paz, SJ de la Costa	MVZ 236396	*ochrorhyncha* (type)	EU363214
131 (2)	MX	BCS	—	La Paz	MVZ 236392, 236395	*ochrorhyncha*	EU363215-6
132 (2)	MX	BCS	—	S of La Paz, SJ de los Planes	MVZ 236393-94	*ochrorhyncha*	EU363217-8

Populations listed in the first column correspond with Fig. 1, country (CT), state (ST), and county (for US) are listed, followed by a more precise description of the locality, voucher specimen number, subspecies designation including the newly discovered "Cochise" form, and GenBank Accession numbers. Specimens that were collected from near the type locality for particular subspecies are indicated in the 'subspecies' column. Museum acronyms follow Leviton et al. (1985), with the addition of MHP = Museum of the High Plains, Sternberg Museum of Natural History (Hays, KS), IMNH = Idaho Museum of Natural History, MZFC = Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), IBUNAM = Instituto de Biología at UNAM, and 'WUPA' is housed at Flagstaff Area National Monuments Collection in Flagstaff, AZ, and the following abbreviations are used for field numbers: MF Mike Forstner, SM Steve Mackessy, DGM Dan Mulcahy, RWM Robert Murphy, JRO John Ottley.

sequence variation in the tRNAs, gaps in the loop-regions were treated as a 5th character state. Parsimony bootstrap analyses were conducted with 1000 "fast" stepwise-additions. The number of mutational steps required to join individual networks were estimated under the parsimony criterion in PAUP*.

Bayesian analyses were conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and were run four times to ensure searches did not become fixed on local optima (Leaché and Reeder, 2002). Log-likelihood scores were plotted against generations to assess stationarity using the program Tracer v1.3 (Rambaut and Drummond, 2004). Trees sampled during the burn-in period were discarded, the remaining trees were used to construct a 50% majority-rules consensus tree, and posterior-probabilities were calculated for each node. Clades were considered significantly supported when posterior-probabilities were 95% or greater. Elsewhere (Mulcahy, 2006), I explored several partition strategies for the data (sensu Brandley et al., 2005). The preferred strategy, presented here, consisted of two partitions, one for the protein-coding *nad4* region and another for the combined tRNAs. Gaps were removed and MrModeltest (Nylander, 2004) was used for each gene region to select the GTR + I + G model for *nad4* and the GTR + G model for the tRNAs, both under the hLRT criteria. Bayesian analyses of this strategy were run with parameters unlinked, with six substitution types, and 10 gamma rate categories (invariable gamma for *nad4*). Four analyses were run for 10×10^6 generations, using four chains, sampling trees every 1000 generations. Runs appeared to reach stationarity after the first 1 million generations; to be conservative, the first 1800 trees were discarded as the burn-in from each run, and the remaining trees were combined to obtain a 50% majority-rule consensus topology.

2.4. Species concept and criteria

A clearly defined species concept is important to any taxonomic study, but perhaps more importantly is the distinction of the criteria that are used to evaluate the group of interest (Sites and Crandall, 1997; de Queiroz, 2005). The 'general lineage concept of species' proposed by de Queiroz (1998) unifies the more traditional species concepts because they share a common factor—that species are separately evolving metapopulation lineages (de Queiroz, 2007). de Queiroz (1998, 2005, 2007) asserts that the definitions of most contemporary species concepts are merely criteria, that speciation is a gradual, continual process, and as diverging lineages undergo this transition, they pass through various stages exhibiting some of these characteristics that are used for criteria. Instead, the definitions of species concepts should remain separate from the criteria used to define them (de Queiroz, 2007). Therefore, I follow the definition of the general lineage concept and here outline the operational criteria I used to delimit species boundaries. Specifically, I used a variation of the Wiens and Penkrot (2002) method that employs sequence data in combination with Nested-Clade Phylogeographic Analysis (NCPA, Templeton et al., 1992; Templeton, 2004), phylogenetic analyses (Farris, 1977; Felsenstein, 1981), and taxonomic designations of haplotypes from specified geographic regions (i.e. subspecies). In this method, a dichotomous key is provided to interpret the phylogeny of the focal species, such as well-supported basal clades (based on the mtDNA data) of named taxa (subspecies based on morphology) that are geographically concordant, which are then recognized as species (Wiens and Penkrot, 2002). Here, I used only the statistical parsimony analysis (Templeton et al., 1992) step of NCPA because of the relatively few localities with more than one sample and the large number of discrete haplotype networks recovered (see Section 3). This method uses three independent lines of evidence to assess species boundaries, which include the phylogeny based on molecular data, morphology, and geography.

3. Results

3.1. Sequence variation

A sequence alignment of 802 bp was obtained from a total of 186 snakes, consisting of 178 *H. torquata* (Table 2), four *Eridiphas*, and one each of *H. tanzeri*, *P. latifasciata*, *L. punctata*, and *S. sartorii* (Table 3). The uncorrected sequence divergence within *H. torquata* ranged from 0% to 10.95%, with an average of 7.5% (Table 4). The first 663 nucleotides were from the 3′ end of *nad4*, and translate into 220 amino acids, corresponding to positions 11,737–12,399 of *Dinodon* (GenBank No. AB008539; Kumazawa et al., 1998). The remaining 139 nucleotide positions came from the transfer RNAs *trnH*, *trnS1*, and a portion of the *trnL2*. Length variation in the *trnH* was observed in the d-loop: most individuals contained 4 bp, fewer had 5 bp; the T-stem consisted of only three base-pairs in all *Hypsiglena*, *Eridiphas*, and *Sibon* sequences, while *Pseudoleptodeira* and *Leptodeira* contained the usual five. Shortening of the T-stem of the *trnH* has also been observed in vipers (Macey and Verma, 1997). *Hypsiglena* sequences contained seven to eight bp on the t-loop and had eight bp between the *trnH* and *trnS1* with the exception of *Leptodeira* that had only seven; there was no length variation on the *trnS1* or the portion of the *trnL2* that was included.

3.2. Phylogeographic analyses

3.2.1. Haplotype networks

The 178 individual *H. torquata* samples contained 110 unique haplotypes, which were placed into 26 separate networks using TCS under a 95% confidence limit of 12 steps (Figs. 2 and 3; Table 2). Haplotype networks are shown on a simplified parsimony phylogeny (see below) by circles and the minimum number of steps connecting networks

Table 2
Haplotype networks of *H. torquata* are presented in networks designated by TCS

H. jani Clade (Duges, 1866):
H. j. jani + *H. texana* (Stejneger, 1893)
Network 1:
jani 1*: 6,9-[MF21713, MF9528]
jani 2: 10-[MF21696]
H. j. dunklei (Taylor, 1938) + *H. texana*
Network 2:
dunklei 1: 7-[MF9597]
dunklei 2*: 8-[MF2173–32]
H. j. texana (Stejneger, 1893)
Network 3:
texana 1: 11-[MVZ 236398]
Network 4:
texana 2: 12-[RWM 5256]
Network 5:
texana 3: 13-[UTA R-51983]
Network 6:
texana 4: 14-[UTEP 18438]
texana 5: 15-[TNHC 66672]
texana 6: 16-[UTEP 16307]
texana 7: 17-[UTA R-34835]
texana 8: 18-[TNHC 66659]
texana 9*: 19–21, 25–27, 39-[CAS 229920, 228060, UTEP 14082, CAS 229229, UTA R-52350, R-52486, MSB 87812]
texana 10: 43-[MSB 72615]
texana 11: 22–24, 41–42, 44-[UTEP 18484, CR409137, UTEP 16309, CAS 229231, TBP 194, SM CO1]
texana 12: 39-[TBP 191]
texana 13: 34, 38, 40, 97-[LSUMZ 84790, WUPA 24720, MSB 87826, MVZ 180265]
texana 14: 35-[CAS 228936]
texana 15: 36-[CAS 228933]
texana 16: 37-[CAS 229281]
texana 17: 31–32-[CAS 229281, 228967, 228965]
texana 18: 28, 30, 33-[MVZ 226235, CAS 228952, 228966]
texana 19: 29-[UTA R-52351]
Network 7:
texana 20*: 45–47-[MHP 8260, 12366, 13388]
texana 21: 48-[MHP 10783]
torquata Clade:
H. torquata (Gunther, 1860)
Network 8:
torquata 1: 1-[MZFC-JAC 24822–23]
torquata 2: 2-[UTA R-51981–82, MZFC 16926]
torquata 3: 2-[UTA R-51980]
torquata 4*: 2- [MZFC 16925]
torquata 5: 2-[MZFC 16916]
Network 9:
torquata 6*: 3-[UTA R-53380]
torquata 7: 3-[MZFC-JAC 23920]
torquata 8: 4-[UTA R-53379]
H. affinis Boulenger, 1894
Network 10:
affinis 1: 5-[LSU 18175]
Cochise Clade (*H.* sp. nov. Mulcahy, 2006):
Network 11:
Cochise 1*: 28, 98–100-[SM 622, CAS 228934, 228951, FMNH 259910, SM 828, ASU 33335, CAS 174417, 228924–8, UTEP 17673, UAZ 55852]
Cochise 2: 101-[CAS 228935]
Cochise 3: 102-[CAS 228958–59]
Cochise 4: 103-[CAS 228938]
Cochise 5: 98-[SM 660]

Table 2 (*continued*)

Desert Clade (*H. chlorophaea* Cope, 1860):
H. c. chlorophaea + *H. c. catalinae* (Tanner, 1976)
Network 12:
chlorophaea 1: 49-[UTA R-54569]
chlorophaea 2: 50-[ROM 14932, ROM 14944, BYU 42376]
chlorophaea 3*: 50-[BYU 42373]
catalinae 1: 128-[MVZ 164935]
catalinae 2: 128-[RWM 1553]
H. c. chlorophaea
Network 13:
chlorophaea 4*: 51-[JRO 694]
chlorophaea 5: 51-[BYU 42832]
H. c. chlorophaea + *H. c. deserticola* (Tanner, 1944)
Network 14:
chlorophaea 6*: 52–53, 57, 60-[TNHC 60066, MVZ 237359, MHP 10516, CAS 228919]
chlorophaea 7: 53, 55-[CAS 228930, 228956, UTA R-52347]
chlorophaea 8: 58-[CAS 228918]
chlorophaea 9: 60-[CAS 228920]
chlorophaea 10: 60-[CAS 228921]
chlorophaea 11: 59-[CAS 228937]
chlorophaea 12: 56-[CAS 228929]
chlorophaea 13: 54-[UTA R-52345]
deserticola 1: 62-[CAS205337]
H. c. deserticola (part)
Network 15:
deserticola 2*: 63–64-[CAS223504, 223533, 228971–2]
deserticola 3: 64-[CAS 223520]
H. c. deserticola + *H. c. chlorophaea* + *H. c. loreala* (Tanner, 1944)
Network 16:
deserticola 4: 74-[BYU 49402]
deserticola 5*: 78–79, 81–82, 87-[MVZ 241611, 235920, 241609, 241610, UTA R-51097, CAS 228916–17]
deserticola 6: 76–77, 83-[CAS 223427, 223414, 235907]
deserticola 7: 80-[MVZ 241612]
deserticola 8: -85-[IMNH-DGM 1705]
deserticola 9: 88-[CAS 231507]
deserticola 10: 66-[CAS 229917]
deserticola 11: 67-[CAS 219685]
deserticola 12: 68-[MVZ 229142]
deserticola 13: 69-[CAS 228911]
deserticola 14: 70-[SDSNH 72820, MVZ 164933]
deserticola 15: 70-[CAS 206502]
deserticola 16: 71-[CAS 223437]
deserticola 17: 72-[CAS 229952]
deserticola 18: 73-[CAS 223373]
deserticola 19: 75-[photo/tail]
deserticola 20: 86-[UNR 07721]
loreala 1: 89, 93-[CAS 229249, ASU 34818]
loreala 2: 91-[MVZ 241607]
loreala 3: 90–91-[BYU 48474, MVZ 241608]
loreala 4: 91-[MVZ 241604]
loreala 5: 92-[CAS 229947]
loreala 6: 94-[CAS 228912]
loreala 7: 94-[CAS 228913]
loreala 8: 94-[CAS 228914]
loreala 9: 95-[MVZ 241606]
loreala 10: 96-[USGS 725]
chlorophaea 14: 61-[CAS 228932]
Coast Clade (*H. ochrorhyncha* Cope, 1860):
H. o. nuchalata (Tanner, 1943)
Network 17:
nuchalata 1: 104-[CAS 228915]
nuchalata 2*: 105-[MVZ 229141]
nuchalata 3: 106-[MVZ 230713]

(*continued on next page*)

Table 2 (*continued*)

H. o. nuchalata (Tanner, 1943)
Network 18:
nuchalata 4: 107-[CAS 223543]
Network 19:
nuchalata 5: 108-[MVZ 180363]
nuchalata 6: 109-[MVZ 241094]
nuchalata 7*: 110-[MVZ 229213]
Network 20:
nuchalata 8: 111-[CAS 205784]
H. o. klauberi Tanner, 1944
Network 21:
klauberi 1*: 116, 118–119, 122-[MVZ 229143, CAS 228973, SDSNH 72821, MVZ 236390]
klauberi 2: 121-[IBUNAM-GP 460]
klauberi 3: 113–14, 117-[CAS 223549, 229918, 223622]
klauberi 4: 115-[DGM 1706]
klauberi 5: 65-[CAS 228968–69]
klauberi 6: 65-[CAS 228970]
klauberi 9: 120-[MHP 11238]
Network 22:
klauberi 7: 112-[CAS 205790]
Network 23:
klauberi 8: 123–24-[MVZ 236389, 236391]
H. o. baueri (Zweifel, 1958)
Network 24:
baueri 1: 125-[RWM 1859]
H. o. venusta (Mocquard, 1899)
Network 25:
venusta 1*: 126-[ROM 14478]
venusta 2: 127-[RWM 1694]
H. o. ochrorhyncha Cope, 1860
Network 26:
ochrorhyncha 1: 129-[MVZ 236397]
ochrorhyncha 2: 130-[MVZ 236396]
ochrorhyncha 3: 131-[MVZ 236392]
ochrorhyncha 4*: 131–32-[MVZ 236393, 236395]
ochrorhyncha 5: 132-[MVZ 236394]

Networks are arranged by the major clades in which they were placed, along with suggested taxonomy. Haplotypes are arranged by networks, named and assigned numbers within respective subspecific designations, and are followed by the population number, with individual voucher number in brackets. Asterisks indicate haplotype with highest outgroup probability for networks containing multiple haplotypes.

Table 3
Additional voucher specimen information for outgroup specimens used in the phylogenetic analyses, followed by Genbank Accession numbers

Taxon:	Hap.	Voucher Number	GenBank Number
Hypsiglena tanzeri	1	TCWC A-2055	EU363044
Eridiphas slevini	1	SDSNH 68728	EU363043
Eridiphas slevini	2	SDSNH 68729	EF078545
Eridiphas slevini	3	MVZ 236388	EF078546
Eridiphas slevini	4	MVZ 234613	EF078547
Pseudoleptodeira latifasciata	—	LSUMZ 39571	EF078582
Sibon sartorii	—	KU 289806	EF078588
Leptodeira punctata	—	UTA R-51974	EF078577

Haplotypes for *H. tanzeri* and *Eridiphas* correspond to localities in Fig. 1 identified by the star and triangles, respectively.

placed close to one another under the parsimony analysis in Fig. 3 (inset). Fig. 2 shows the distribution of the haplotype networks overlaid on the geography of the region. Of the 26 networks, only 17 contained multiple haplotypes (Table 2). When the TCS parsimony confidence limit was relaxed to 90%, 21 haplotype networks were recovered with the maximum number of 18 steps joining haplotypes. For simplicity, only the 95% confidence limit results are shown in the figures and Table 2, but the discrepancies are presented below.

The first six networks included specimens from the Chihuahuan Desert, the Tamualipan floodplain, and the southeastern Colorado Plateau (Fig. 2). The largest network (6) ranged from western Texas to eastern Colorado, through New Mexico to eastern Arizona, including southwestern Colorado, with 3 haplotypes (*texana* 9, 11, 13) that were widespread (Fig. 2). In the Tamualipan region, a *jani* specimen shared an identical a haplotype with a *texana* specimen (*jani 1*), while the other *jani* haplotype differed by only a single base-pair. Similarly, a *dunklei* and a *texana* specimen found syntopically (Pop. 8) shared an identical haplotype, whereas a nearby *dunklei* (Pop. 7) differed by a single base-pair. The remaining southern Chihuahuan specimens (*texana* 1–3) were each placed in their own networks (3–5). Networks 4–5 were combined into a single network when the parsimony limits were relaxed to 90%. Networks 8–9 contained haplotypes (*torquata* 1–8) from Sinaloa (Pops. 1–2) and Jalisco (Pops. 3–4). Network 10 contained the *affinis* haplotype (Pop. 5), from upland Jalisco, Mexico. Network 11 contained 5 haplotypes (Cochise 1–5) recovered from 19 individuals collected in Pima, Santa Cruz, and Cochise counties, Arizona, and Hidalgo County, New Mexico (Pops. 28, 98–103). Network 12 contained 5 haplotypes, 3 near Alamos, Sonora, and northern Sinaloa, Mexico (Pops. 49–50) and 2 found on Isla Santa Catalina (Pop. 128). Networks 13–16 contained 41 haplotypes from 59 individuals ranging from Ortiz, Sonora, Mexico (Pop. 51) through southwestern Arizona and southeastern California (Pops. 52–64) into the Mojave (Pops. 66–75) and Great Basin (Pops. 76–88) deserts and the northern portion of the Colorado Plateau (Pops. 89–97). When parsimony limits were relaxed to 90%, haplotypes from networks 14–16 were placed into one network. The remaining 10 networks (17–26) contained 24 haplotypes from 32 individuals collected from northern California to the Cape of Baja (Fig. 3). The first four of these networks (17–20) were from individuals around the Central Valley of California (Pops. 104–111), including haplotypes from the Sierra Nevada (networks 19–20), which were placed into a single network when the parsimony limits were relaxed to 90%. The next three networks (21–23) contained 9 haplotypes, from 15 individuals occurring from the Transverse Ranges (Pops. 112–115) down the Peninsular Ranges (Pops. 65, 116–124), including South Coronado Island (Pop. 121). Networks 21–22 were recovered as a single network when the parsimony limits were relaxed to 90%. The individuals from Islas San Marcos and Danzante (Pops. 126 and 127, respectively) contained unique haplotypes that were placed in a single network and were two steps apart. Net-

Table 4
Pair-wise sequence divergences for species of "*H. torquata*" (*sensu lato*)

Taxon	*H. jani*	*H. affinis*	*H. torquata*	Cochise	Desert	Coast
H. jani	**0.0254**	0.1533	0.1465	0.1280	0.1534	0.1768
H. affinis	0.0951	—	0.0632	0.0880	0.0986	0.1059
H. torquata	0.0928	0.0492	**0.0173**	0.0874	0.1065	0.1153
Cochise	0.0854	0.0649	0.0638	**0.0081**	0.0870	0.0997
Desert	0.0959	0.0701	0.0731	0.0632	**0.0263**	0.0967
Coast	0.1050	0.0741	0.0776	0.0703	0.0688	**0.0465**

Uncorrected sequence divergences are shown below the diagonal between major clades, in the diagonal are uncorrected comparisons within major clades (shown in boldface text). Corrected (GTR + I + G) comparisons between major clades are shown above the diagonal.

work 26 contained 5 haplotypes (*ochrorhyncha* 1–5), found in six individuals from the Cape region of Baja California (Pops. 129–132) with extensive structure between haplotypes (Fig. 2).

3.2.2. Phylogenetic analyses

The alignment of 802 nucleotide characters from 110 unique *H. torquata* haplotypes, plus four *E. slevini*, and one each of *H. tanzeri*, *Pseudoleptodeira*, *Sibon*, and *Leptodeira*, contained 267 parsimony-informative characters; 428 were constant. Parsimony analyses of these data recovered 449 trees, each 1191 steps in length. A strict consensus recovered *H. tanzeri* sister to *Eridiphas* + the remaining *Hypsiglena* (not shown), and five major clades were recovered within *H. torquata* (Fig. 3, inset). The first of these clades consisted of networks 1–7, containing 23 haplotypes (*jani* 1–2, *texana* 1–20, and *dunklei* 1–2) found in 47 individuals, ranging throughout the Chihuahuan Desert, the Tamaulipan Floodplain, Great Plains, and onto the southern portion of the Colorado Plateau. This clade is referred to hereafter as the *jani* clade (Table 2) and was the sister clade to the remaining *H. torquata* haplotypes. The relationships among the four other major clades were unresolved in the parsimony analyses, and are referred to as the *torquata*, Cochise, Coast, and Desert clades (Fig. 3, inset). Additionally, networks 16 and 21 were not recovered as monophyletic. Instead, network 22 was nested within 21, and three clades of network 16 were nested among networks 14–15 (Fig. 3, inset).

Bayesian analyses produced trees with an average likelihood score of $-\ln L = 7121.587$, which also recovered *H. tanzeri* sister to *Eridiphas* and *H. torquata* with stronger support (Fig. 3). The five major clades within the remaining *H. torquata* were also recovered, consistent with the parsimony analysis; however, the relationships among them were better resolved. The *jani* clade was again recovered as most basal, and the major difference in the Bayesian analysis was that the Cochise clade was recovered as sister to the remaining *Hypsiglena*; however, the node separating the Cochise clade from the remaining clades was not well-supported (Fig. 3). The Coast and Desert clades each received strong support and were placed sister to one another, but with low support, and the *torquata* clade was placed sister to the Coast + Desert, but again, with low support (Fig. 3).

The *torquata* clade was well-supported and contained the *affinis* haplotype that was placed sister to the two *torquata* networks (8–9). The Cochise clade (network 10) was well supported by both parsimony bootstrap and posterior-probability values and contained 5 haplotypes found in 19 individuals collected in southeastern Arizona and southwestern New Mexico (Figs. 2 and 3). Individual specimens in this clade would have been previously classified as *chlorophaea* based on geography; however, because this clade was well-supported, fixed for a unique morphology, and is geographically cohesive, it is recognized as distinct (see below). The Coast and Desert clades were both geographically widespread, and each contained samples representing multiple named subspecies (Fig. 3).

The Desert Clade, networks 12–16, contained 46 haplotypes recovered from 67 individuals consisting of the all *chlorophaea*, *deserticola*, *loreala*, and *catalinae* samples; of these only *catalinae* was monophyletic. Network 12 contained the individuals from Isla Santa Catalina (*catalinae* 1–2) and those found near Alamos, Sonora and nearby Sinaloa, Mexico (*chlorophaea* 1–3), and was basal to the rest of the Desert clade. The rest of the Desert clade (networks 13–16), consisted of the remaining *chlorophaea* haplotypes (4–14), as well as all *deserticola* (1–20), and *loreala* (1–10) haplotypes, none of which formed monophyletic groups (Fig. 3).

The Coast Clade consisted of networks 17–26 (25 haplotypes from 31 individuals), including all samples of *nuchalata*, *klauberi*, *venusta*, *baueri*, and *ochrorhyncha*, ranging from around the Central Valley of California to the cape of the Baja California peninsula. Of these samples, the only monophyletic subspecies were those that formed single networks—*ochrorhyncha* and *venusta*. Networks 24–26 (*baueri*, *venusta*, and *ochrorhyncha*) formed a clade, and networks 17–23 (all *klauberi* and *nuchalata*), formed another clade. The *nuchalata* and *klauberi* haplotypes were not recovered as reciprocally monophyletic, rather, the *nuchalata* 4 and *klauberi* 8 haplotypes, from the southern limits of each subspecies' range, were each placed sister to the remaining haplotypes of the other subspecies. Additionally, network 21 was not recovered as monophyletic, similar to the parsimony

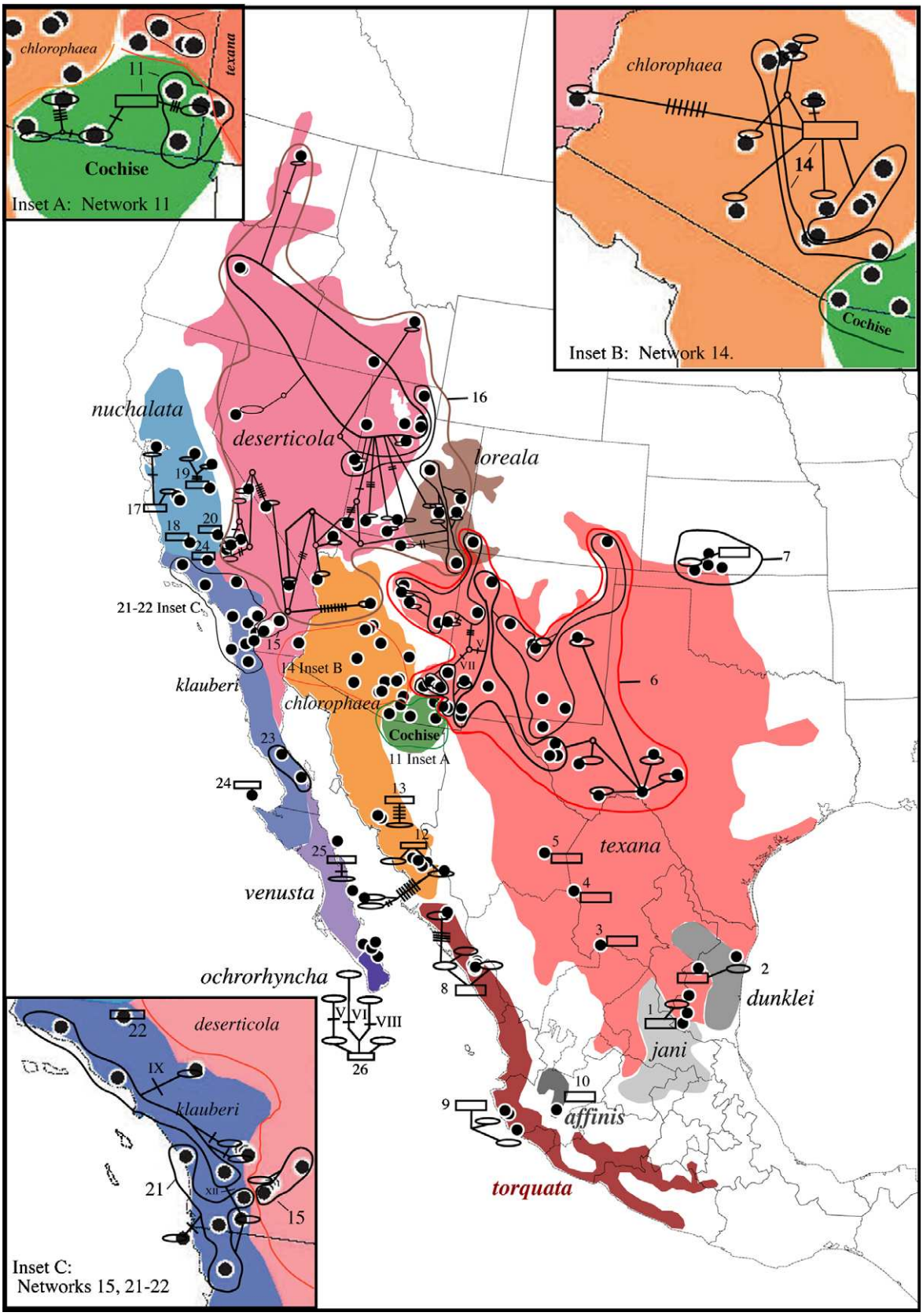

Fig. 2. Haplotype networks of *H. torquata*, generated by TCS, are shown on the geographic distribution of samples, and are numbered from 1–26 (see Table 2). Rectangles not connected to ovals represent networks composed of single individuals, ovals represent haplotypes joined to networks. Each angle, circle, or junction in a network represents one step, while additional steps are indicated by tic-marks and Roman numerals. Localities with identical haplotypes are encircled. Networks 6 and 16 are also encircled for ease of visualization, and networks from Cochise County, southwestern Arizona, and southern California, are shown in insets A–C, respectively. Colors of the shaded regions match clades recovered in phylogenetic analyses (Fig. 3).

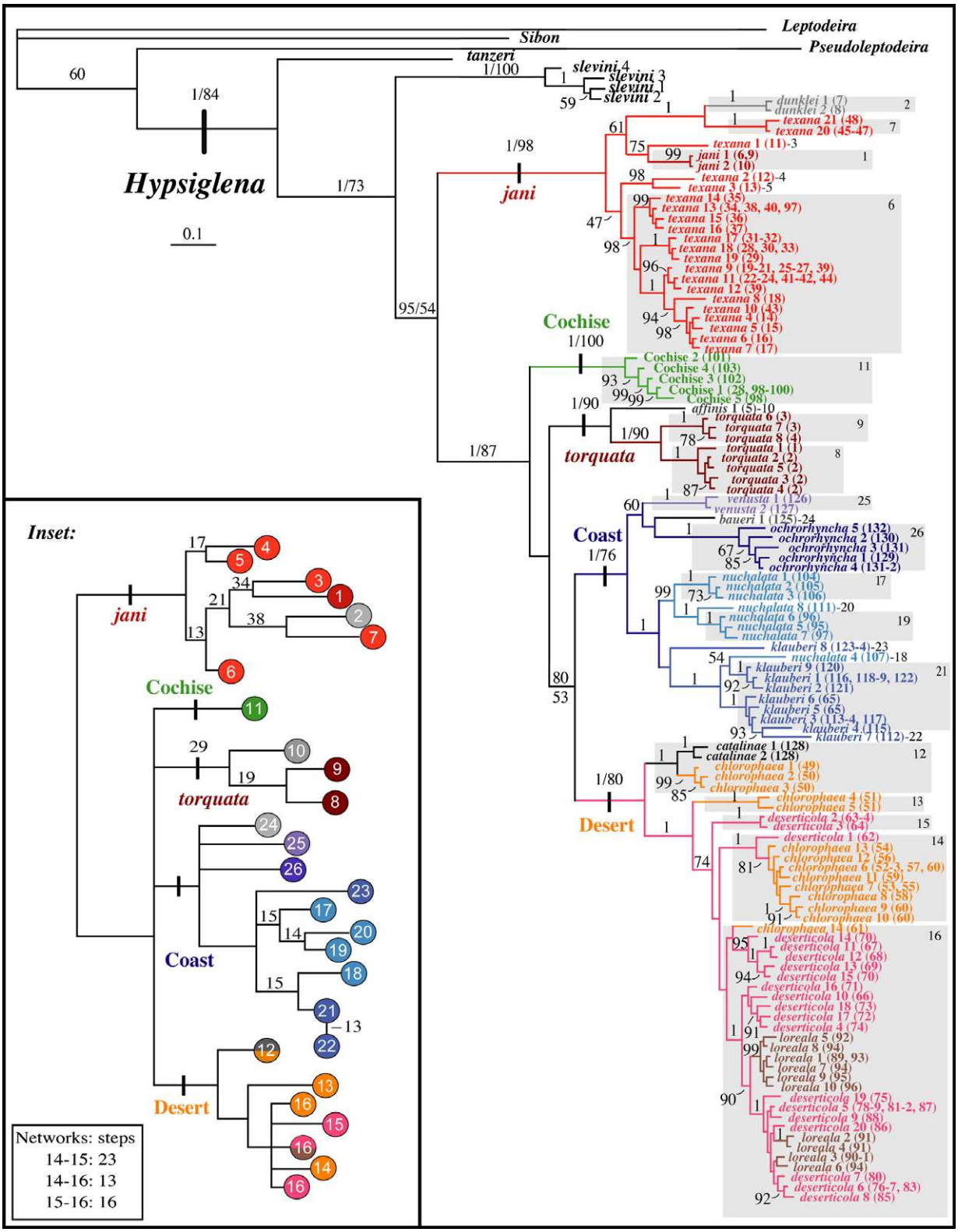

Fig. 3. Consensus Bayesian phylogram from four separate analyses, with two nucleotide substitution models (see text). The 5 major clades are labeled with vertical bars, posterior-probabilities (multiplied by 100 for values <1.0) are shown above for branches with >50 support, and parsimony bootstrap values are shown (below) for the major nodes. Terminals are haplotype numbers (Table 2) followed by population number in parentheses (Table 1). Gray boxes indicate haplotype networks; hyphenated numbers following population numbers indicate networks of single individuals. Terminal colors match shaded geographic regions of Fig. 2. Inset: Parsimony phylogeny of the haplotype networks is shown by colored circles and numbers with the number of parsimony steps required to join closely related networks indicated on the branches (*Note.* Networks 16 and 21 were paraphyletic).

analysis. Again, the *klauberi 7* haplotype, in its own network (22) from the San Emigdio Mts. (Pop. 112), was placed sister to *klauberi* 4 from the San Gabriel Mts. (Pop. 102).

4. Discussion

4.1. Phylogeography of Hypsiglena

The phylogeographic analyses of '*H. torquata*' recovered mtDNA clades largely consistent with previously described taxonomic entities, which are also concordant with the major biogeographic regions of western North America. Parsimony and Bayesian analyses both recovered *Hypsiglena* as paraphyletic with respect to *Eridiphas slevini*. In both analyses, *H. tanzeri* was placed sister to *Eridiphas* + the remaining *Hypsiglena*, and five major clades were recovered among what was previously considered *H. torquata* (Figs. 2 and 3). Three of these clades correspond with groups of subspecies confined to discrete biogeographic regions (e.g., MacMahon and Wagner, 1985): the Chihuahuan Desert (*jani*, *texana*, and *dunklei*); Sonoran–Mojave–Great Basin–Colorado Plateau (*catalinae*, *chlorophaea*, *deserticola*, and *loreala*); and Coastal/Central California–Baja California Peninsula (*nuchalata*, *klauberi*, *venusta*, *baueri*, and *ochrorhyncha*); and are referred to as the *jani*, Desert, and Coast clades, respectively. The *torquata* Clade contained individuals assigned to the nominate subspecies occurring along the Sinaloan coast and south to western Jalisco, and *affinis* from upland Jalisco. The fifth clade consisted of a previously unrecognized lineage, comprised of individuals from the Cochise Filter Barrier area of southeastern Arizona and adjacent New Mexico. Specimens from this area have a unique morphology (qualitatively described below), distinct from any other subspecies, and are here referred to as the Cochise Clade. The geographic distribution of these five clades largely corresponds with the boundaries found in other taxa (Riddle et al., 2000a; Sinclair et al., 2004; Jaeger et al., 2005; Schulte et al., 2006). Although, the relationships among the major clades were not well-supported in this study, they are resolved using complete mt-genome data and discussed elsewhere (Mulcahy, 2006). The remainder of the discussion here focuses on the geographic boundaries of the major clades and the taxonomic conclusions drawn from them.

The *jani* Clade contained individuals from the Chihuahuan Desert, Great Plains, southeastern Colorado Plateau, and the Tamaulipan Floodplain; it received strong support, and was placed as basal to the remaining four clades (Figs. 2 and 3). A basal Chihuahuan/Plains clade was also found in the western rattlesnake complex (*Crotalus viridis*; Pook et al., 2000; Ashton and de Queiroz, 2001) and side-blotched lizards (*Uta stansburiana*; Upton and Murphy, 1997), but not in desert spiny lizards (*Sceloporus magister*; Schulte et al., 2006; Leaché and Mulcahy, 2007) nor white-footed deer mice (*Peromyscus eremicus*; Riddle et al., 2000b). This clade included specimens assigned to the *jani*, *texana*, and *dunklei* subspecies, and the *dunklei* specimens from Tamaulipas showed a strong association with the *texana* specimens from Kansas, suggesting a northward expansion up the Great Plains region independent from the western Chihuahuan Desert expansion (i.e. on the Edwards Plateau and to the west). The Tamaulipan, Kansas, and southern Chihuahuan samples were recovered as basal to the remaining haplotypes, which were all placed into a single network that ranged throughout the northwestern Chihuahuan Desert, including the southern portion of the Colorado Plateau. The Chihuahuan Desert lineage of *Hypsiglena* (i.e. *texana*) was not previously thought to extend into the southern portion of the Colorado Plateau (Tanner, 1944; Dixon and Dean, 1986; see Fig. 1), but this pattern of range expansion has been documented in other taxa such as toads (*Bufo punctatus*; Jaeger et at., 2005), fence lizards (*Sceloporus undulatus*; Leaché and Reeder, 2002), western rattlesnakes (Pook et al., 2000; Ashton and de Queiroz, 2001; Douglas et al., 2002), and gopher snakes (*Pituophis catenifer*; Rodríguez-Robles and De Jesus-Escobar, 2000). The *jani* Clade was also found to occur in southern Arizona, in close proximity (~90 km) with the Cochise Clade near Mt. Graham (Pops. 31–33; Figs. 1 and 2); a distribution that was also previously unknown (Tanner, 1944; Dixon and Dean, 1986; Conant and Collins, 1991). Specimens from the southern Colorado Plateau and Mt. Graham area of southern Arizona show nape and body patterns typical of *texana*, and may have gone previously undetected in these areas because specimens from that region are scarce.

The *torquata* Clade contained three networks, two groups of the *torquata* samples from central Sinaloa and coastal Jalisco, while the third was the single *affinis* haplotype from upland Jalisco and Zacatecas. Phylogenetic analyses united the two *torquata* networks in a well-supported clade that was placed sister to *affinis*, with strong support (Fig. 3), which is suggestive of current gene-flow along the Pacific Coast of central Mexico or a more recent separation, than that with the upland form. The lowland forms cross the Trans-Mexico Neo-Volcanic Belt (TMNB)—a major geographic barrier for some fauna. The western side of TMNB has been suggested to cause a Miocene–Pliocene divergence in freshwater fish (Mateos, 2005). The uncorrected sequence divergence between haplotypes from Sinaloa and coastal Jalisco averaged ~1.7%, suggesting a more recent expansion across the TMNB in *torquata*, based on a conservative estimate of *nad*4 sequence divergence in snakes (Zamudio and Greene, 1997; Rodríguez-Robles and De Jesus-Escobar, 1999).

The discovery of the Cochise Clade in southeastern Arizona and adjacent New Mexico was unexpected, because it was not previously recognized based on morphology (Tanner, 1944; Dixon and Dean, 1986; see Fig. 1). However, samples from this clade appear to be fixed on a particular morphology discrete from the other clades (see section on taxonomy of *Hypsiglena* below). I am unaware of any other taxa endemic to this region, yet the geologic history

and floral and faunal components of this region are complex. Several amphibian and reptile species restricted to this region of the US are found in the montane areas and extend much further south along the Sierra Madre Occidental of western Mexico (see Stebbins, 2003). Nightsnakes from the Cochise Clade were collected in the low-lying valley areas surrounding these 'sky-islands,' a transitional area between the Sonoran and Chihuahuan deserts (Lowe, 1955; Morafka, 1977; MacMahon and Wagner, 1985) that has undergone extensive vegetation changes during the Pleistocene (Van Devender and Spaulding, 1983; Van Devender et al., 1987). Most lowland reptiles in this region appear to have expanded from the East, such as the massasauga (*Sisturus catenatus*), hog-nosed (*Heterodon platyrhinos*), king (*Lampropeltis getula*), and glossy snakes (*Arizona elegans*), western box turtle (*Terrepene ornata*), and Great Plains skink (*Eumeces obsoletus*; see Stebbins, 2003). The Cochise Clade of *Hypsiglena* contacts the *jani* Clade at the Arizona–New Mexico state line (Pops. 28 and 98; Fig. 1—inset A), an area that has recently been shown to be a contact zone between major clades of toads (Jaeger et al., 2005), spiny lizards (Leaché and Mulcahy, 2007), among other taxa (Riddle and Hafner, 2006). The Cochise clade of *Hypsiglena* likely extends into northern Mexico, yet its occurrence there was undetected because of a lack of sampling.

The Desert Clade consisted of five networks (Table 2), all recovered as a monophyletic group, although support was moderate, under parsimony, and strong in the Bayesian analyses. This clade was found to be wide-spread, ranging from the Sonora–Sinaloa transition zone near Alamos (Pops. 49–50; Fig. 1), through the Sonoran and Mojave deserts to the Great Basin, including the northern section of the Colorado Plateau. Specimens from the Alamos area were found to be very similar to the two individuals sampled from Isla Santa Catalina, in the Gulf of California, and were placed in the same network (Table 2). Isla Santa Catalina was not connected with the peninsula during the Pleistocene, therefore it is not considered a land bridge island and its affinities with the mainland are unclear (Carreño and Helenes, 2002). Several Isla Santa Catalina reptile species show more morphological similarity with mainland relatives in southern Sonora than they do with their peninsular counterparts, such as king snakes (*Lampropeltis*; Blaney, 1977) and the whiptail lizards (*Aspidoscelis*; Grismer, 1994). This relationship was not detected in a genetic study of whiptails (Radtkey et al., 1997); however, their mainland sample was near Ortiz, not from Alamos. The Ortiz samples of *Hypsiglena* (*chlorophaea* 4–5) were placed more basal within the Desert Clade exclusive of the Alamos/Catalina group (Fig. 3). Others challenge the idea of a mainland source for reptiles on Isla Santa Catalina, as opposed to a peninsular source (Fu and Murphy, 1999; Murphy and Aguirre-Léon, 2002).

Dixon and Dean (1986) suggested the Alamos area to be a major contact zone for *Hypsiglena*, because the specimens were morphologically intermediate between the *torquata* and *chlorophaea*. Instead, the mtDNA boundary between the Desert and *torquata* clades appears to be approximately 150 km to the south. Future molecular studies using nuclear markers would be useful to address this issue. Samples from the northern Sonoran Desert, in southwestern Arizona and southeastern California, formed a network (14) that was sister to the most wide-spread network (16), with other southern Sonoran networks (13 and 15) being more basal. This step-wise ladder progression of more diverse haplotype clades in the southern Sonoran Desert leading up to a wide-spread network of much less diversity in the North may represent a recent range expansion in this direction (Mulcahy et al., 2006).

In the northern range of the Desert Clade, the same haplotype (*deserticola* 5) was recovered in the Salt Lake area of Utah (Pops. 78–79, 81–82), near Twin Falls, Idaho (Pop. 84), and Bend, Oregon (Pop. 87), and several other haplotypes recovered from nearby specimens differed by only a few base-pairs (Fig. 2). This genetic uniformity suggests a recent range expansion into the northern Great Basin, compared to greater genetic diversity seen in the western portion of the Great Basin and northern Mojave Desert (near the White-Inyo mountains and southeastern Sierra Nevada; Pops. 67–71), which is suggestive of an older presence. Haplotypes from the western Great Basin and northwestern Mojave Desert (*deserticola* 11–15), formed a well-supported clade within the widespread network (16), and extend the Desert Clade into the southern Sierra Nevada south of Lake Isabella along Kelso Creek (Pop. 68), the Piute Mts (Pop. 67), and Ninemile Canyon in Inyo County (Pop. 69). Network 16 spans the Mojave Desert, Great Basin, and the Colorado Plateau, yet these three regions were all connected by an intermediate haplotype from the individual at Lytle Ranch, Utah (Pop. 74), which caused a reticulation in this network (Fig. 2). The north-central Arizona sample (*chlorophaea* 14; Pop. 61) was placed in this network with other Mojave Desert haplotypes, representing a more eastern Mojave extension into Arizona as previously interpreted (e.g., MacMahon and Wagner, 1985). However, the phylogenetic placement of this haplotype was not well-supported, and additional sampling in this region is needed to provide better resolution in this area.

Most samples from the Colorado Plateau were recovered in a sub-clade within network 16. However, the three individuals from population 91, and one sample from population 94 (haplotypes *loreala* 2–4, 6) were placed outside of this sub-clade, and associated with specimens west of the Wasatch Front in Utah (Fig. 3). The presence of a haplotype from population 94 that was only one step different from the wide-ranging haplotype (*deserticola* 5) could represent insufficient time for coalescence of the plateau haplotypes, or may be the result of continued gene-flow across the Wasatch Front. Nonetheless, the presence of this and the *jani* Clade on the Colorado Plateau is suggestive of two independent expansions onto the plateau in *Hypsiglena*, a similar pattern seen in the western rattlesnake com-

plex (Pook et al., 2000; Ashton and de Queiroz, 2001; Douglas et al., 2002) and fence lizards (Leaché and Reeder, 2002). These two clades apparently overlap in geographic distribution—the presence of a *jani* haplotype within the distribution of *loreala* indicates secondary contact. In fact, a putative hybrid specimen from population 97 has a nuchal and body pattern typical of *loreala*, but possessed a *jani* Clade haplotype (*texana* 13), suggesting hybridization between these two forms. Both forms have been reported from the nearby Zuni Mts. of New Mexico, where morphologically intermediate specimens were also suspected (Gehlbach, 1965).

The Coast Clade contained 10 networks, most of which consisted of only a few individuals. Specimens from this clade were found from the Cape of Baja California to the Bay Area of San Francisco, including the western foothills of the Sierra Nevada, the Transverse, and Peninsular ranges of southern California (Figs. 2 and 3). This clade has a geographic distribution similar to several other taxa for which phylogeographic data are available (*Ensatina*, Moritz et al., 1992; *Taricha*, Tan and Wake, 1995; Kuchta and Tan, 2006; *Lampropeltis zonata*, Rodríguez-Robles et al., 1999; *Batrachoseps*, Jockusch and Wake, 2002; *Neotoma*, Matocq, 2002). A common pattern seen in all those studies, with the exception of *Ensatina* (Moritz et al., 1992), was the extension of southern coastal California clades into the southern Sierra Nevada. This was not seen in *Hypsiglena*, rather, the one sample from the southwestern Sierra Nevada (Pop. 111) grouped with those from the northern Sierra (Pops. 108–110; Fig. 2). However, additional sampling in this area may reveal the existence of haplotypes from other clades. The haplotype from the Temblor Range (*nuchalata 7*; Pop. 107) was placed basal to a southern California sub-clade, suggesting the southern form may extend into the southern Coast Ranges. This pattern is also seen in slender salamanders (Jockusch and Wake, 2002), skinks (Richmond and Reeder, 2002), and woodrats (Matocq, 2002). The Transverse Range appears to present a barrier for other taxa, such as mountain kingsnakes (Rodríguez-Robles et al., 1999), but not in *Hypsiglena*. In southern California and northern Baja California, two abundant haplotypes (*klauberi* 1 and 3) were found from San Onofre (Pop. 116) to south of Tecate (Pop. 122), and from the Transverse Range (Santa Barbara Mts., Pop. 113) to Mt. Palomar (Pop. 117). Network 21 was not recovered as monophyletic, network 22 (Pop. 112) from the Transverse Range, was nested within network 21 in the phylogenetic analyses (Fig. 3).

In Baja California, an identical haplotype was recovered in specimens near Cataviña and Bahía de los Ángeles (*klauberi* 8). This haplotype was found sister to the southern California sub-clade (Fig. 3). Individuals from the gulf islands of Danzante and San Marcos, nearly 200 km apart, were only a few base pairs different from each other, suggesting a recent ancestry between these populations. Both Danzante and San Marcos are land bridge islands that were connected to the peninsula during portions of the Pleistocene (Carreño and Helenes, 2002). Sampling on the mainland portion of the Baja California peninsula was not sufficient to detect the presence of a mid-peninsular seaway as recently suggested (Upton and Murphy, 1997; Riddle et al., 2000a; Lindell et al., 2006). Samples from the Cape of Baja (network 26), although they were all taken from the northeastern area near La Paz (Pops. 129–132), showed extensive genetic variation and structure as compared to other peninsular haplotypes (Fig. 2), indicating a long separation from the remaining peninsula and maintenance of haplotype diversity in this region.

4.2. Hypsiglena Taxonomy and the subspecies concept

The process of speciation does not occur in discrete steps, but more appropriately as a continuum (de Queiroz, 1998). In making nomenclatural decisions, we attempt to name discrete entities that are the result of this continual process, which can often present problems (Wake, 2006). Species may not always be recovered as monophyletic based on haplotype analyses because of either incomplete lineage sorting or introgression (Funk and Omland, 2003). Nevertheless, attempts are often made to name intraspecific lineages, or the stages of incipient species (Omland et al., 2006). Here, subspecies of '*H. torquata*' were either found to be reciprocally monophyletic, or formed monophyletic groups composed of several subspecies (Fig. 3). In this case, it is advantageous to recognize the basal monophyletic groups as species, but to also maintain the subspecific designations to represent putative incipient species in this diverse group. Future studies can then focus within each of these major clades to investigate these lineages. Subspecific nomenclature not only provides names for these putative species, but also provides important taxonomic resolution for wildlife management agencies. For instance, the US Fish and Wildlife Service currently lists nine subspecies of snakes as either *Threatened* or *Endangered* (www.fws.gov/endangered/wildlife.html#Species).

In this study, haplotypes of the focal species '*H. torquata*' were found to be exclusive; however, there is no apparent gene flow between basal lineages, based on strong support for their monophyly. To the contrary, several of the subspecies were not recovered as reciprocally monophyletic, suggesting incomplete lineage sorting, or limited gene flow between them. Following the species delimitation criteria of Wiens and Penkrot (2002; [strong support for basal clades with geographic concordance]), I recognize six species within what was previously considered *H. torquata* (sensu Tanner, 1985; Dixon and Dean, 1986) and maintain several subspecies within the wide-ranging species (Table 5). The *torquata* clade contains two species: *H. torquata* (Günther, 1860) and *Hypsiglena affinis* (Boulenger, 1894). *H. affinis* is recognized by having two nape bands, a complete white collar 5–6 scale rows in length, followed posteriorly by a complete dark nuchal collar 6–8 scale rows long, and 19 dorsal scale rows at mid-body (all other *Hypsiglena*

have 21), seven upper labials and one preocular, as opposed to eight upper labials and two preoculars in other *Hypsiglena* (Dixon and Dean, 1986). *H. torquata* (*sensu stricto*) is more variable: all specimens appear to have an elongate, posterior dark nuchal collar 4–5 scale rows long, and may or may not have the presence of an anterior white nuchal band (variable within populations). In both species, a dark stripe extends from the eye back to the white collar, but does not cross it, and both have one row of large dorsal spots along the body.

The *jani* Clade contained haplotypes representing *jani*, *texana* and *dunklei*. Dixon and Dean (1986) found these three taxa to be morphologically similar, and their degrees of variation were found to represent clinal patterns, therefore they placed *texana* and *dunklei* in synonymy with *H. t. jani*. The *jani* Clade of the present study was very well-supported and sister to the remaining *Hypsiglena*, and differed by as much as 10% uncorrected sequence divergence (Table 4). *Hypsiglena t. dunklei* is the only other lineage (save *H. torquata* and *H. affinis*) that has the white nuchal collar preceding a dark collar (Dixon, 1965; Dixon and Dean, 1986). The samples of *dunklei* in this study were placed among *texana* and *jani* haplotypes, suggesting this form may represent polymorphic variation within, or paraphyly among *texana*, which warrants further investigation. Therefore, the entire clade is recognized as a distinct species *Hypsiglena jani* (Dugès, 1865), but the subspecies are maintained pending further study. The species includes *H. j. jani*, in which the eye stripe extends back and widens to meet a dark nuchal band that wraps around entirely to form a complete collar (Tanner, 1944). The northern subspecies (*H. j. texana*) has a three-part nuchal pattern in which each eye stripe extends back to a large nuchal blotch that forms a saddle-like pattern on the dorsum. Where the two sides of the saddles meet, the third part of the nuchal pattern is a dark narrow stripe that extends forward to the parietals. In *H. j. jani*, the two saddles join fully to form a complete nuchal collar, which differs from *H. torquata* and *H. affinis* by being connected with the eye stripe. Whether the white nuchal collar present in the *dunklei* specimens represents a polymorphism in the *jani* Clade, or a fixed character in a discrete lineage remains to be seen, therefore it remains *H. j. dunklei* (Taylor, 1938).

The Cochise Clade represents a distinct lineage separated by more than 6% uncorrected sequence divergence from other lineages (Table 4), which is morphologically discrete, and geographically cohesive. Specimens in this clade can be recognized by the presence of a complete nuchal collar, which is rounded posteriorly and narrows on the dorsum, and forms a dark line extending anteriorly. The eye stripe is prominent and tapers to a point where it meets the nuchal collar. The body contains two rows of small dorsal spots, unlike one large row in most other forms (except *loreala*). Previous morphological analyses failed to detect this distinct taxon, most likely because of the few available specimens from this region (Tanner, 1944; Dixon and Dean, 1986). As mentioned above, a few specimens collected at the contact zone with the *jani* Clade appear to represent hybrid individuals because they were found to have the mtDNA haplotype of one species and the morphology of the other. The Cochise Clade may also come into contact with the Desert Clade southeast of Tucson, AZ, between populations 52 and 101 (Figs. 1 and 2). I wait for the examination of additional specimens for a proper diagnosis and description of this species.

Uncorrected sequence divergence within the Desert Clade averaged 2.6%, and ranged from 6.3% to 9.5% between other major clades. This clade may prove to represent a multiple-species complex upon the examination of additional material and molecular markers (preferably nuclear). Nevertheless, with the data examined thus far, this clade is recognized as *Hypsiglena chlorophaea* Cope (1860). The type locality is "Ft. Buchanan, AZ" near the Santa Cruz–Pima county line (between Pops. 52 and 101; Fig. 1). There are two syntypes of *H. chlorophaea* (ANSP 3748–9), one of which has a *chlorophaea* pattern (see below), another has the Cochise pattern. Cope's (1860) description of *H. chlorophaea* and others (Tanner, 1944; Tanner, 1985; Dixon and Dean, 1986) do not adequately describe the Cochise morphology, but better represent the variation seen in the Desert Clade. However, because this clade may represent multiple lineages, the subspecies are maintained. Specimens from the northern part of the range (*H. c. deserticola*) have a three-part nuchal pattern, where

Table 5
Taxonomy of nightsnakes based on the current study

H. torquata (Günther, 1860)—Sinaloan Nightsnake

H. affinis Boulenger, 1894—Rio Grande de Santiago Nightsnake

H. slevini Tanner, 1943—Baja California Nightsnake

H. tanzeri Dixon and Lieb, 1972—Rio Verde Nightsnake

H. sp. nov. (Mulcahy, 2006)—Hooded Nightsnake (Cochise clade)

H. jani (Dugès, 1865)—Chihuahuan Nightsnake
- *H. j. texana* (Stejneger, 1893)—Texas Nightsnake
- *H. j. dunklei* (Taylor, 1938)—Tamaulipas Nightsnake
- *H. j. jani* (Duges, 1866)—San Luis Potosi Nightsnake

H. chlorophaea Cope, 1860—Desert Nightsnake
- *H. c. deserticola* (Tanner, 1944)—Great Basin Nightsnake
- *H. c. loreala* (Tanner, 1944)—Mesa Verde Nightsnake
- *H. c. catalinae* (Tanner, 1966)—Isla Santa Catalina Nightsnake
- *H. c. tiburonensis*[a](Tanner, 1981)—Isla Tiburon Nightsnake
- *H. c. chlorophaea* Cope, 1860—Sonoran Nightsnake

H. ochrorhyncha Cope, 1860—Coast Nightsnake
- *H. o. nuchalata* (Tanner, 1943)—California Nightsnake
- *H. o. klauberi* Tanner, 1944—San Diego Nightsnake
- *H. o. venusta* (Mocquard, 1899)—Magdalena Nightsnake
- *H. o. baueri* (Zweifel, 1958)—Isla Cedros Nightsnake
- *H. o. gularis*[ab] (Tanner, 1954)—Isla Partida Norte Nightsnake
- *H. o. unaocularus*[a] (Tanner, 1944)—Islas Revillagigedo Nightsnake
- *H. o. martinensis*[a] (Tanner and Banta, 1962)—Isla San Martin Nightsnake
- *H. o. ochrorhyncha* Cope, 1860—Cape Nightsnake

[a] Placement inferred (no DNA samples available).
[b] Grismer (1999) recognized as distinct species.

the eye stripe extends posteriorly to form a lateral blotch, which extends partly up the dorsum. In between the two lateral blotches is a rectangular-shaped, wide central blotch that has a spine extending anteriorly. In some areas, the median spot is irregular and wedge-shape (near Phoenix, AZ), or the lateral blotches fuse to the dorsal blotch, forming a partial collar (near Tucson, AZ). The dorsal body blotches are typically small and are generally in one or two rows, but two rows are present in most *loreala*.

The Coast Clade may also prove to represent multiple species, but for now is recognized as one—*Hypsiglena ochrorhyncha* Cope (1860). This species shows more morphological variation than any other species of *Hypsiglena*, which may be a result of the diverse habitats it occupies (e.g., Klauber, 1941). In the northern region, surrounding the Central Valley of California, the name *H. o. nuchalata* (Tanner, 1943) is applied, which is characterized by large nuchal blotches on the sides that often come together to form a collar, and one row of large dorsal body blotches; the eye stripe comes to a point, just contacting the lateral blotches or collar. This subspecies is closely related to *klauberi* and the area between these forms should be surveyed for additional material for future investigation into these putative species. In southern California, *H. o. klauberi* Tanner (1944) is characterized by a three-part nuchal collar formed by two lateral blotches, not in contact with the eye stripe, and an elongate, irregular median nape spot. Klauber (1938) documented extensive variation in scalation and color pattern in specimens of *Hypsiglena* from the San Diego area; however, he also recognized a sharp change in scalation and color pattern when comparing specimens from the nearby desert. Although he later found some of the variation in scalation to be correlated with habitat (Klauber, 1941), this abrupt change in morphology is also consistent with the Coast and Desert clades in this study. *Hypsiglena o. klauberi* appears to extend onto the Baja California peninsula to at least the area near Bahía de los Ángeles. Grismer (2002) observed the *klauberi* type only on the northwestern portion of the peninsula, and inferred the *deserticola* type to extend to the Cataviña–Bahía de los Ángeles region. This was not observed in the mtDNA data, and may reflect convergence in color patterns between the continental and peninsular deserts.

Specimens on the remaining portion of the peninsula formed three groups (Figs. 2 and 3), each of which corresponds to previously described taxa. The individual from Isla Cedros on the Pacific side of the Peninsula (Pop. 125) represents *H. o. baueri* (Zweifel, 1958). The individuals from the land bridge islands (San Marcos and Danzante; Pops. 126–127) were recovered together in a well-supported clade and differed by only two base-pairs (Figs. 2 and 3), but were quite different from those on the Cape of Baja. *Hypsiglena* occurs on many islands associated with the Baja peninsula, several of which have been described as distinct forms; however, genetic material was not available for this study. Grismer (1999) evaluated all forms on gulf islands based on morphologically discrete characters under an evolutionary species concept, and found only one (*H. gularis*) to represent a distinct species. However, he maintained pattern classes for the various island forms because they are useful for representing particular morphologies (Grismer, 2002). These may prove to represent distinct species upon the examination of additional material; rather than using pattern classes that may reflect convergence, I maintain the subspecific designations for future investigations and the possibility that these groups may represent incipient species. *Hypsiglena o. venusta* (Mocquard, 1899) and *H. o. ochrorhyncha* Cope (1860) have similar nape patterns, convergent with *H. c. chlorophaea* (as mentioned above). However, *H. o. venusta* has two rows of small dorsal body blotches (convergent with Cochise and *H. c. loreala*), whereas *H. o. ochrorhyncha* has one row of larger dorsal blotches (Cope, 1860; Tanner, 1944).

The phylogenetic placement of *H. tanzeri* basal to *Eridiphas slevini* plus all other *Hypsiglena* warrants the synonymy of *Eridiphas* with *Hypsiglena*. This position was supported by both parsimony (not shown) and Bayesian analyses (Fig. 3), and is additionally supported by nearly 5 kb of mtDNA data (Mulcahy, unpubl. data). *Eridaphis slevini* was initially described as a species of *Hypsiglena* (Tanner, 1943), but was placed in its own genus because it was thought to be intermediate between *Hypsiglena* and *Leptodeira* (Leviton and Tanner, 1960). However, molecular data support its close relationship with *Hypsiglena* (Mulcahy, 2007; this study) and it is here placed in back in synonymy with *Hypsiglena*. Additionally, *P. latifasciata* was also initially described as a species of *Hypsiglena* (Günther, 1894), which may also be placed back in synonymy with *Hypsiglena*; however, the status of *Pseudoleptodeira* will be treated elsewhere.

4.3. Conclusions

I collected ~800 bp of *nad4* (and 2 tRNAs) sequence data from 178 individual nightsnakes (*Hypsiglena torquata*) to examine genetic associations with geography and evaluate species boundaries under a generalized lineage species concept. I included other closely related species: *H. tanzeri*, *Eridiphas slevini*, and *P. latifasciata* in the parsimony and Bayesian phylogenetic analyses. *Eridiphas slevini*, previously described as a *Hypsiglena*, was found to be nested within *Hypsiglena* and is here placed in its synonymy. Using the Wiens and Penkrot (2002) DNA tree-based method to delineate species boundaries, I identified six species (*H. torquata*, *H. affinis*, *H. jani*, *H. chlorophaea*, *H. ochrorhyncha*, and an undescribed form) all from within what was previously considered to be one '*H. torquata.*' The newly discovered species revealed by the mtDNA has a unique morphology and is restricted to the Cochise Filter Barrier area, in southeastern Arizona, associated New Mexico, and presumably parts of Mexico. Two previously described subspecies of *H. torquata* were identified as species (*H. torquata* and *H. affinis*), while three widespread clades, here recognized as independent species (*H. jani*,

H. chlorophaea, and *H. ochrorhyncha*), were each recovered as groups of multiple subspecies. The subspecies within these wide-ranging species were maintained pending further evaluation. These subspecies may represent incipient species that may not yet have achieved reciprocal monophyly, but possess unique morphologies, and are geographically discrete. The species and subspecies distributions are congruent with biogeographic regions of western North America; the relationships among them were not well-resolved in this study. Instead, the relationships among these species are fully resolved in a related study based on complete mtDNA genome data (Mulcahy, 2006), which support a ring-species hypothesis for this complex around the Gulf of California.

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