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Research Article

Molecular systematics of the genus *Sonora* (Squamata: Colubridae) in central and western Mexico

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Mexico possesses high levels of endemic biodiversity, especially for squamate reptiles. However, the evolutionary relationships among many reptiles in this region are not well known. The closely related genera of *Sonora* Baird and Girard 1853 and *Procinura* Cope 1879 are coralsnake mimics found from the central and western United States to southwestern Mexico and Baja California. Although species delimitation in this group has historically relied upon colour pattern and other morphological characters, many populations of these species display colour pattern polymorphism, which may confound taxonomy. We used molecular phylogenetics to assess the evolutionary relationships and delimit species within *Sonora*, focusing on the phylogenetic position of *Procinura* and the validity of *S. mutabilis* and *aequalis*. We sequenced two mitochondrial (*ND4* and *cytb*) and two nuclear (*c-mos* and *RAG-1*) genes for the single species of *Procinura* and each of the four species of *Sonora*. We analysed these sequences using maximum parsimony, maximum likelihood and Bayesian phylogenetic analyses on separately concatenated mitochondrial and nuclear datasets. Additionally, we used Bayesian coalescent methods to build a species tree (Bayesian species tree analysis) and delimit species boundaries (Bayesian species delimitation). All methods indicated that *Procinura* is deeply nested within *Sonora*, and most individual species are well supported. However, we found that one taxon (*S. aequalis*) is paraphyletic with regard to another (*S. mutabilis*). We recommend that the genus *Procinura* be synonymised with *Sonora* and that *S. aequalis* be synonymised with *S. mutabilis.* Additionally, the phylogenetic patterns that we document are broadly congruent with a Miocene or Pliocene divergence between *S. michoacanensis* and *S. mutabilis* along the Trans-Mexican Volcanic Belt. Finally, our data are consistent with the early evolution of coralsnake mimicry and colour pattern polymorphism within the genus *Sonora*.

Key words: colour pattern polymorphism, coralsnake mimicry, Mexico, *Procinura aemula*, Sonora, *S. michoacanensis*, *S. mutabilis*

Introduction

The country of Mexico is an extremely diverse region (Mittermeier *et al.*, 2005), especially for squamate reptiles (Flores-Villela & Canseco-Márquez, 2004). High endemism and species richness of this country has been explained by its complex landscape, geology, tropical latitude and ecological diversity (Peterson *et al.*, 1993; Ramamoorthy *et al.*, 1993; Flores-Villela & Gerez, 1994). Despite this diversity (or perhaps because of it), genetic relationships of many squamate species in Mexico are unknown and their taxonomy is unstable. Contributing to this taxonomic uncertainty for squamate reptiles is variable and polymorphic colour pattern, which can cause taxonomists to either assign multiple species designations within single polymorphic species or to lump geographically widespread species under a single 'polymorphic' species. This leads to the potential for cryptic biodiversity and thus the systematics of such species complexes are a matter of high taxonomic priority.

The genus *Sonora* Baird and Girard 1853 is one lineage of snakes that is relatively poorly known and displays striking colour pattern polymorphism. Members of *Sonora* are small, arthropod-consuming, semifossorial snakes that are found in the central and western United States to southwestern Mexico and Baja California (Figs 1–8; Stickel, 1943; Ernst & Ernst, 2003). These snakes are normally placed in the colubrid tribe Sonorini with the genera *Chilomensiscus*, *Chionactis*, *Conopsis*, *Ficimia*, *Gyalopion*, *Pseudoficimia*,

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Stennorrhina and *Sympholis* (Dowling, 1975; Dowling & Duellman, 1978), although some authors include *Tantilla* and *Geagras*, and by extension *Tantillita* and *Scolecophis* (Savitzky, 1983; Greene, 1997). However, some authors have questioned the traditional Sonorini based upon molecular and morphological data (Holm, 2008; Goynechea, 2009).

There are five species that have recently been included in the genus *Sonora* (Echternacht, 1973; Ernst & Ernst, 2003). *Sonora semiannulata* Baird and Girard 1853 is found in the central and western United States and northern Mexico. *Procinura aemula* Cope 1879 was until recently (Lemos-Espinal *et al.*, 2004*a*, 2004*b*, 2004*c*) included in the genus *Sonora* (Bogert & Oliver, 1945; Zweifel & Norris, 1955; Nickerson & Heringhi, 1966) and is found in western Mexico in the states of Chihuahua, Sonora and Sinaloa (Fig. 9). *Sonora mutabilis* Stickel 1943 and *S. aequalis* Smith and Taylor 1945 are found mostly sympatrically in the foothills of the Sierra Madre Occidental in Jalisco, Nayarit, Aguascalientes, southern Zacatecas and extreme southern Sinaloa (Fig. 9). *Sonora michoacanensis* Duges in Cope (1885) is currently known from the Balsas basin of Michoacan, Guerrero, Morelos, Puebla and Colima and the coastal regions of Colima and Guerrero (Fig. 9). Notably, all species possess colour pattern polymorphism, with uniform, striped, banded, bicolour and tricolour morphs known for the different species (Figs 1–8). Herein, we focus on the exclusively Mexican species of *P. aemula*, *S. mutabilis*, *S. michoacanensis* and *S. aequalis*.

Taxonomic confusion has reigned in the exclusively Mexican species of *Sonora* and *Procinura*. While the validity of the species *P. aemula* is not generally questioned, this species was recently placed in the monotypic genus *Procinura* on the basis of its unusual caudal morphology, a 'file-like' tail (Lemos-Espinal *et al.*, 2004*a*, 2004*b*, 2004*c*). However, a phylogenetic analysis was not undertaken at the time of the genus re-elevation, and so the reciprocal monophyly of *Procinura* and *Sonora* is not established. The three species of *Sonora* (*S. aequalis*, *S. michoacanensis*, *S. mutabilis*) from southern and western Mexico have been at various times considered a single species with up to two subspecies of *S. michoacanensis michoacanensis* and *S. m. mutabilis* (Stickel, 1943; Echternacht, 1973) or up to three species including *S. erythrura*, *S. mutabilis* and *S. michoacanensis* (Taylor, 1937; Smith & Taylor, 1945). Most recently, Ponce-Campos *et al*. (2004) elevated *S. michoacanensis michoacanensis* and *S. m. mutabilis* to full species based on colour pattern, and resurrected the name *S. aequalis* for bicolour ground snakes formerly included under *S. mutabilis*.

One reason for the unstable taxonomy of Mexican *Sonora* is their extreme colour pattern polymorphism (Figs 1–8). *Procinura aemula* is considered a coralsnake mimic (Echternacht, 1973; Campbell & Lamar, 2004) and possesses morphs that are uniform red or tricolour, monadal or triadal with a varying number of triads (Nickerson & Heringhi, 1966). According to current taxonomy, *S. mutabilis* is tricoloured and *S. aequalis* is bicoloured (Ponce-Campos *et al.*, 2004), with both considered coralsnake mimics (Echternacht, 1973; Campbell & Lamar, 2004). Finally, *S. michoacanensis* is also considered a coralsnake mimic (Echternacht, 1973; Campbell & Lamar, 2004) and possesses uniform red and tricolour morphs (some of the bands on tricoloured animals may appear as white dots with a black centre). These three species are currently distinguished based solely on colour pattern; *S. mutabilis* is tricoloured, *S. aequalis* is bicoloured, and *S. michoacanensis* can be distinguished from *S. aequalis* and *S. mutabilis* by the absence of banding on its tail. Given that colour pattern polymorphism is documented within all members of the genera *Sonora* and *Procinura* and is a well-known characteristic of mimicry complexes (Echternacht, 1973; Mallet & Joron, 1999; Brodie & Brodie, 2004), taxonomy based solely on colour pattern in coralsnake mimics may be deceptive.

With current taxonomy based on colour pattern, a revision of the genera *Sonora* and *Procinura* based upon more appropriate characters is necessary. Morphological characters such as scale counts and colour pattern have traditionally been used in snake systematics, but may suffer from problems of homoplasy and environmentally induced variation (e.g. Burbrink *et al.*, 2000; Devitt *et al.*, 2008) especially because many snake genera such as *Sonora* are morphologically conservative. We use a molecular approach to evaluate the phylogenetic relationships of the genera *Sonora* and *Procinura*.

Our goals were to use both mitochondrial and nuclear loci to: (1) determine the number of distinct genetic lineages of the genera *Sonora* and *Procinura* in western Mexico, (2) determine the phylogenetic relationships among the different

Figs 1–8. Snakes of the genus *Sonora* (and *Procinura*) found exclusively in Mexico. Images deposited in the University of Texas-Arlington Digital collection (UTADC). **1**. Uniform morph of *Sonora* (*Procinura*) *aemula* from near Rio Cuchojaqui, Sonora (photo by C.M. Bogert, UTADC 7405). **2**. *S. aemula* from Rio Cuchojaqui with a few bands (photo by C.M. Bogert, UTADC 7406). **3.** Tricolour morph of *S. aemula* from near Alamos, Sonora (photo by C. Rodriguez, UTADC 7407). **4**. Bicolour *S. mutabilis* from near Guadalajara, Jalisco (*aequalis*; photo by C. Grunwald, UTADC 7408). 5. Tricolour *S. mutabilis* from near Rio Blanco, Jalisco (photo by C.L. Cox, UTADC 7409). 6. Tricolour *S. mutabilis* from Rio Blanco, Jalisco (photo by J. Reyes-Velasco, UTADC 7410). 7. Tricolour *S. michoacanensis* from near Arcelia, Guerrero (photo by A. Mendoza, UTADC 7411). 8. Uniform morph of *S. michoacanensis* from near Tacambaro, Michoacan (photo by O. Medina-Aguilar, UTADC 7412).

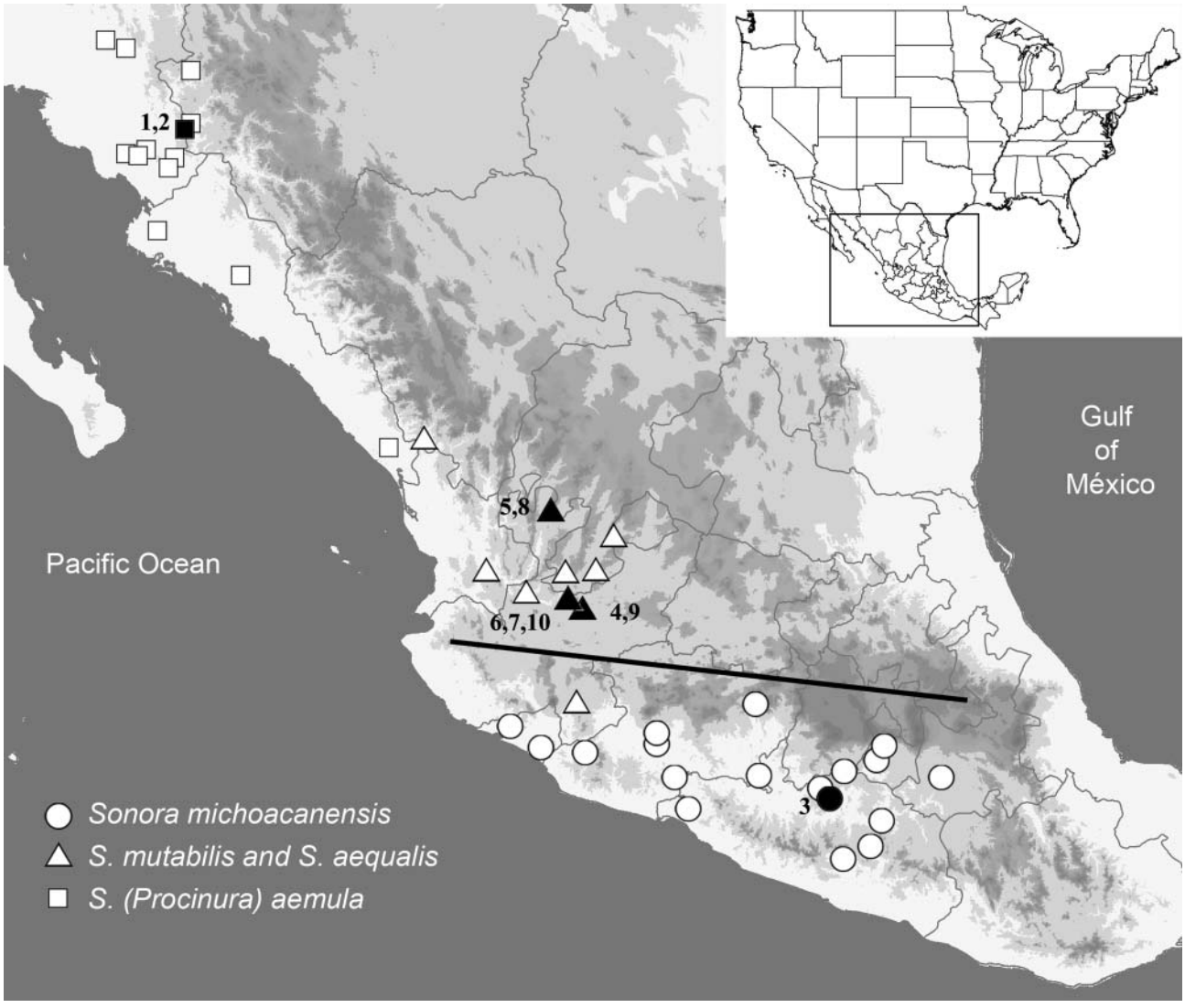


Fig. 9. Map of specimen localities for snakes of the genus *Sonora* (and *Procinura*) found exclusively in Mexico (i.e. excluding *S. semiannulata*). Inset displays the geographic context of the map. Filled symbols represent localities with the tissue samples that are used in this study, and numbers next to symbols indicate localities from Table 1. Elevation is indicated on the map using shaded areas, with sea level represented by white and shaded areas in dark grey to a maximum of 5636 m. The approximate position of the Trans-Mexican Volcanic Belt is indicated with a solid line.

species of the genera *Sonora* and *Procinura* and (3) assess the match between current taxonomy and molecular phylogeny of the genera *Sonora* and *Procinura*. Based upon the results of this analysis, we make taxonomic recommendations for this group and discuss morphology in the context of this taxonomy.

Materials and methods

Taxonomic sampling

We obtained at least one tissue for *P. aemula* and *S. aequalis*, *S. michoacanensis*, *S. mutabilis* and *S. semiannulata* during fieldwork (2001–2009) and/or from museum collections (Fig. 9; Table 1). We also obtained one sequence for *P. aemula* from an unpublished dissertation (Holm, 2008). Specimens and photos were deposited in the University of Texas at Arlington Amphibian and Reptile Diversity Research Centre and Digital Collection (UTA ARDRC and UTA ARDRC DC) and the Museo de Zoología, Facultad de Ciencias (MZFC). We chose to use a hierarchical outgroup scheme to test the monophyly of the ingroup, using *Coluber constrictor*, a closely related member of the subfamily (Colubrinae) containing *Sonora* and *Procinura* (Pyron *et al.*, 2011) and *Agkistrodon contortrix*, a member of the family Viperidae.

Table 1. Sample information and Genbank Accession numbers for the specimens included in this study.

#[a]	Voucher ID[b]	Taxon	Country: State	Locality	Lat	Long	Elevation (m)	*cyt-b*	*ND4*	*c-mos*	*RAG-1*
1	UANL 6976	*Sonora* (*Procinura*) *aemula*	Mexico: Sonora	near Alamos	27.02458	−108.9397	400	JQ265959	JQ265979	JQ265952	JQ265970
2	ASDM 21449	*S. aemula*	Mexico: Sonora	near Alamos	27.02458	−108.9397	400	NA[c]	NA	NA	NA
	CAS 206503	*S. semiannulata*	USA: California	Inyo County near Bishop	36.24532	−117.4531	907	AF471048	JQ265981	AF471164	JQ265970
3	MZFC 23956	*S. michoacanensis*	Mexico: Guerrero	Campo Morado, Canada 'El Naranjo'	18.19316	−100.1609	1072	JQ265958	JQ265980	JQ265951	JQ265969
4	UTA BTM 26[d]	*S. mutabilis* ('*aequalis*')	Mexico: Jalisco	Barranca del Rio Santiago	20.79239	−103.3297	107	JQ265954	JQ265975	JQ265945	(a) JQ265967; (b) JQ265968[f]
5	UTA R-53488	*S. mutabilis* ('*aequalis*')	Mexico: Jalisco	near Bolanos	21.87539	−103.8207	1633	JQ265953	JQ265973	JQ265947	JQ265962
6	UTA JRV 127[d]	*S. mutabilis* ('*aequalis*')	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	−103.6567	1450	JQ265955	JQ265976	JQ265950	NA
7	UTA JRV 129[d]	*S. mutabilis* ('*aequalis*')	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	−103.6567	1450	JQ265956	JQ265978	NA	(a) JQ265960; (b) JQ265951[f]
8	UTA R-53487	*S. mutabilis*	Mexico: Jalisco	near Bolanos	21.87539	−103.8207	1633	NA	JQ265972	JQ265946	(a) JQ265965; (b) JQ265966[f]
9	UTA R-59762	*S. mutabilis*	Mexico: Jalisco	Road to Pueblitos near Barranca del Rio Santiago	21.02544	−103.4607	1350	JQ265957	JQ265974	JQ265948	JQ265963
10	UTA JRV 128[d]	*S. mutabilis*	Mexico: Jalisco	Huaxtla: canyon below town	20.72845	−103.6567	1450	NA	JQ265977	JQ265949	JQ265964
	CAS 212760[e]	*Coluber constrictor*	USA: California	Mendocino National Forest	39.16058	−122.6681	597	EU180467	AY487041	AY486938	NA
	SDSU 3929[e]	*Coluber constrictor*	–	–	–	–	–	NA	NA	NA	EU402841
	Moody338[e]	*Agkistrodon contortix*	–	–	–	–	–	NA	AF156576	NA	NA
	LSU H0607[e]	*Agkistrodon contortix*	–	–	–	–	–	EU483403	NA	NA	EU402833
	CAS 214406[e]	*Agkistrodon piscivorous*	–	–	–	–	–	NA	NA	AF471096	NA

[a]Numbers correspond to localities in Fig. 2. [b]Voucher IDs are either museum numbers or field numbers. [c]This sequence is published in Holm (2008). [d]Field notes and tissues for UTA BTM and UTA JRV specimens are deposited at the UTA ARDRC. [e]Genes for all outgroup taxa were downloaded from Genbank. [f]Accession numbers for phased RAG-1 sequences are indicated with a and b and correspond to identifiers in Fig. 3.

Table 2. Primer name and primer sequence for the amplification and sequencing of gene fragments analysed in this study.

Primer name	Fragment	Sequence (5′-3′)
ATRCB3	*cyt-b*	TGA GAA GTT TTC YGG GTC RTT
GLUDG	*cyt-b*	TGA CTT GAA RAA CCA YCG TTG
ND4F	*ND4*	CAC CTA TGA CTA CCA AAA CCT CAT GT
LeuR	*ND4*	CAT TAC TTT TAC TTG GAT TTG CAC CA
RAG1_f1a	*RAG-1*	CAG CTG YAG CCA RTA CCA TAA AAT
RAG1_r2	*RAG-1*	CTT TCT AGC AAA ATT TCC ATT CAT
S77cmos	*c-mos*	CAT GGA CTG GGA TCA GTT ATG
S78cmos	*c-mos*	CCT TGG GTG TGA TTT TCT CAC CT

Molecular methods

Muscle, liver and skin tissue was taken from freshly killed specimens and stored in 95% ethanol or tissue lysis buffer at −80 °C. Genomic DNA was extracted from tissues using the DNAeasy Blood and Tissue Kit (Qiagen) using standard protocol. We chose to amplify two separate mitochondrial loci, a partial fragment (639 bp) of cytochrome b (*cyt-b*) and a fragment (777 bp) containing part of NADH dehydrogenase subunit 4 (*ND4*) including complete RNA^{His} and complete and partial $tRNA^{Ser(AGY)}$ (Table 2) using primers modified from previous studies (Arevalo *et al.*, 1994; Harvey *et al.*, 2000). We also amplified two nuclear genes, a partial fragment (997 bp) of the recombination activating gene 1 (*RAG-1*) and a fragment (546 bp) of the oocyte maturation factor (*c-mos*; Table 2). *Cyt-b* and *ND4* were both amplified using polymerase chain reaction (PCR) under the following thermocycling protocol: initial denaturation at 94 °C for 3 min, then 35 cycles of denaturation for 30 s at 94 °C, annealing for 45 s at 55 °C, and extension for 90 s at 72 °C, followed by a final extension at 72 °C for 10 min. *RAG-1* and *cmos* were amplified using the same PCR protocol as the mitochondrial genes, except that the annealing temperature was 58 °C. Successful amplification was determined by gel electrophoresis of the PCR product along a 1% agarose gel, and PCR products were prepared for the sequencing reaction by using the ExoSAP-IT kit (United States Biochemical). We used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) following the manufacturer's protocol. The sequenced products were precipitated using an ethanol/sodium acetate method and rehydrated in HPLC purified formamide (Hi-Di). The sample was then analysed either on a ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas-Arlington or on a ABI 3730 Genetic Analyzer at the Museum of Vertebrate Zoology at the University of California, Berkeley. Sequences were edited and assembled using Sequencher (Genes Code Corps., Inc.). Individual sequences were exported to MEGA (Tamura *et al.*, 2011), aligned in MEGA using the CLUSTAL algorithm (Larkin *et al.*, 2007) with default parameters and manually adjusted if necessary.

Sequence analysis

Concatenated analysis. We assembled concatenated mitochondrial (*cyt-b*, *ND4* and tRNAs) and nuclear (*cmos* and *RAG-1*) datasets for maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. Phylogenetic analysis using the MP criterion was implemented for separately concatenated mitochondrial and nuclear datasets in MEGA (Tamura *et al.*, 2011) with nodal support assessed by 1000 bootstrap replicates. For maximum likelihood and Bayesian phylogenetic analysis we used four separate partitioning schemes. Both mitochondrial and nuclear datasets were (1) unpartitioned, (2) partitioned by gene or gene region, (3) partitioned by gene region and two codon partitions for protein encoding genes (the first two codon positions partitioned separately from the last codon position) and (4) partitioned by gene and three codon partitions (one for each codon position). The best-fitting model of molecular evolution for each gene was determined using MEGA (Tamura *et al.*, 2011), with models ranked by Bayes factors. Maximum likelihood phylogenetic reconstruction was implemented in RaxML (Stamatakis, 2006) with 100 independent searches using the GTRGAMMA (GTR+G) model. Nodal support for the best scoring ML tree was bootstrap proportions from 1000 pseudoreplicates. Bayesian phylogenetic reconstruction was completed in MrBayes v 3.1 (Huelsenbeck & Ronquist, 2001). The HKY+G model of evolution was used for both nuclear and mitochondrial datasets. Excepting a variable rate prior, we used the default parameters in MrBayes (Huelsenbeck & Ronquist, 2001). Markov-chain Monte-Carlo searches were run for 1 000 000 generations sampling trees every 100 generations with 4 chains (3 heated chains and one cold chain). We considered that the Bayesian searches had converged when the average standard deviation of split frequencies declined to below 0.01 and by examining log-likelihood versus generation plots. Additionally, we used the online program AWTY (Wilgenbusch *et al.*, 2004) to confirm that our analyses reached stationarity. When the runs were completed, we discarded the first 25% of trees as burnin. Bayesian posterior probabilities were used to assess nodal support in the Bayesian analysis. Trees from all analyses were visualised and manipulated using FigTree v1.3.1 (Rambaut, 2007).

Species tree analysis and Bayesian species delimitation. We conducted a species tree analysis to provide a guide tree for species delimitation analyses. Although species-tree coalescent methodology is most appropriate when applied to datasets with multiple individuals for each species, the focus of these analyses is the genetic distinctness of *S. aequalis* and *S. mutabilis* for which we have

multiple samples. We used the program *BEAST (Heled & Drummond, 2010) in the BEAST software package (Drummond & Rambaut, 2007) to estimate a species tree from our four separate loci (*ND4*+tRNAs, *cyt-b*, *c-mos* and *RAG-1*). For the species tree we initially assigned taxa to *P. aemula*, *S. aequalis*, *S. michoacanensis*, *S. mutabilis* and *S. semiannulata*. We generated species trees with unpartitioned data and the first two codon positions partitioned separately from the last, with separate models of molecular evolution for each gene (*cmos* = HKY, *cyt-b* = HKY+G, *ND4* = HKY+I, *RAG-1* = HKY+G) determined by model selection using the Bayesian Information criterion in MEGA (Tamura *et al.*, 2011). The approximately 125 bp of tRNAs in *ND4* was trimmed prior to analysis. We considered the default priors in *BEAST (Heled & Drummond, 2010) to be appropriate for our analysis, although for each partitioning scheme we varied the tree prior (Yule process or birth–death process). We used searches of 10 million generations (with trees sampled every 1000 generations) for two independent runs, and burned in 50% of runs. Data were combined using LogCombiner. Nodal support for the resulting species tree was posterior probabilities and was mapped onto the tree using TreeAnnotator.

We used the species tree from the species tree analysis as a guide tree for Bayesian species delimitation (focused on *S. aequalis* and *S. mutabilis*). We used the program BPP v2.1 (Yang & Rannala, 2010), which uses reverse jump Markov-Chain Monte Carlo (rjMCMC) to infer the posterior probabilities of a fully resolved guide tree and each partially or completely collapsed version of the guide tree, but see Leache & Fujita (2010) and Yang & Rannala (2010) for details. For our guide tree, we used the species tree generated by *BEAST (all partitioning schemes and prior sets yielded the same topology). Initially, we varied the fine-tuning parameter and starting seeds, and conducted analyses for 100 000–500 000 generations to ensure homogeneity of results. Final analyses were conducted for 100 000 generations, sampled every 10 and burned in the first 50% of trees. The fine-tuning parameters and algorithms for rjMCMC mixing were set to give consistent results and were similar to those in Leache & Fujita (2010), with all speciation models given equal priors. Additionally, we used the same three prior sets as in Leache & Fujita (2010) for ancestral population size (θ) and root age (τ). We set both θ and τ to a gamma distribution, initially with (1) G (α, β) $\sim$ G (1, 10) for both θ and τ. Two other prior combinations were also used, (2) G (2,2000) for both θ and τ and (3) $\theta \sim$ G (1,10) and $\tau \sim$ G (2,2000). Acceptance proportions for each parameter were within the recommended range (0.3–0.7) for Bayesian species delimitation (Yang & Rannala, 2010). Support for species was assessed as Bayesian speciation probabilities for each node, which is different from Bayesian posterior probability nodal support which indicates the probability a clade is true and presumably monophyletic (Huelsenbeck *et al.*, 2002) in that it indicates a probability ('Bayesian speciation probability, BSP') that a node is fully resolved or fully bifurcated.

Morphological analysis

We collated morphological data from Echternacht (1973) including data originally from Stickel (1943) for one *S. aequalis*, 18 *S. michoacanensis* and eight *S. mutabilis* and measured the same traits on eight additional specimens (Table 3). We also collected additional colour pattern data for species diagnosis information from museum specimens that were mentioned but not illustrated in Echternacht (1973) or Stickel (1943). Length measurements were taken to the nearest mm using digital callipers, and the same author (JRV) conducted all morphological measurements. We also studied the hemipenial morphology of three specimens of *S. mutabilis*, and compare it to that of *S. michoacanensis*. We followed the standard procedures to prepare hemipenes as suggested by Myers & Cadle (2003) and Zaher & Prudente (2003). Morphological definitions are based on Dowling & Savage (1960).

Results

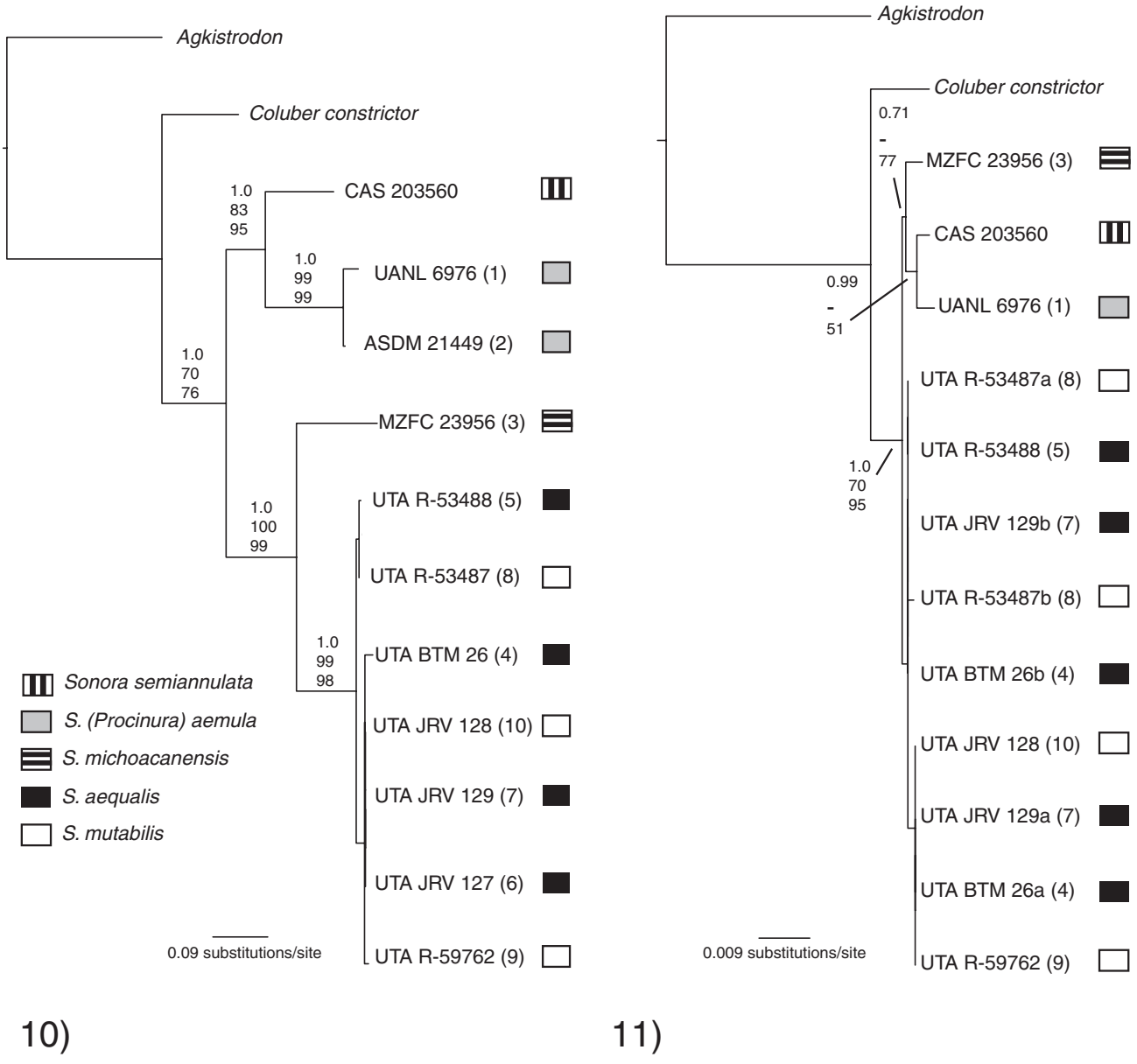
Concatenated analyses

Bayesian, maximum likelihood and maximum parsimony phylogenetic analyses all yielded similar topologies for both nuclear and mitochondrial datasets. Similarly, all gene and codon partitioning schemes yielded similar topologies in both Bayesian and maximum likelihood analyses with both datasets. Because we prefer to present an optimal tree, we elected to include the best maximum likelihood tree for both mitochondrial and nuclear datasets (partitioned by gene and first two codon positions partitioned separately from the third) with nodal support assessed as Bayesian posterior probabilities (BPP), maximum likelihood bootstrap proportions and maximum parsimony bootstrap proportions (Figs 10–11). Phylogenetic trees from both the mitochondrial and nuclear datasets recover *Sonora*+*Procinura* as a monophyletic group (BPP = 1.0), with maximum uncorrected pairwise sequence divergence of 18% and 0.8% for the mitochondrial and nuclear dataset, respectively. The mitochondrial dataset (Fig. 10) recovers a southern clade (S. *mutabilis*, *S. aequalis* and *S. michoacanensis*) and a northern clade (*S. semiannulata* and *P. aemula*) separated by 15.5% mitochondrial uncorrected sequence divergence (BPP = 1.0). In contrast, *S. michoacanensis* is recovered as sister to the *S. semiannulata/P. aemula* clade (BPP = 0.71) in the phylogenetic tree based on nuclear loci (Fig. 11). Both mitochondrial and nuclear datasets find *Procinura* nested within *Sonora* (BPPs = 1.0 and 0.99), sister to *S. semiannulata* (Figs 10–11). Additionally, both nuclear and mitochondrial phylogenetic trees indicate that *S. aequalis* is paraphyletic to *S. mutabilis* (Figs 10–11) and recover *S. michoacanensis* as being quite divergent

Table 3. Morphological measurements on *S. michoacanensis* and *S. mutabilis* from Echternacht (1973) and this study. We excluded some specimens included in Echternacht (1973) from this table because their locality is unknown.

Catalogue #	Taxon	State	Sex	TBL[a] (mm)	TL[b]	Temporals[c]	Supralabials[c]	Infralabials[c]	Ventrals	Subcaudals	Banding on tail	Source
NHMUK 1946.1.14.65	*michoacanensis*	Michoacan	M	244	56	–	–	–	165	44	no	Echternacht 1973
FMNH 37141	*michoacanensis*	Michoacan	M	205	50	3–2	7–7	7–7	152	44	no	Echternacht 1973
FMNH 39128	*michoacanensis*	Michoacan	F	169	31	2–2	7–6	8–7	173	36	no	Echternacht 1973
FMNH 39129	*michoacanensis*	Michoacan	F	201	38	2–2	7–7	8–7	171	39	no	Echternacht 1973
Holotype	*michoacanensis*	Michoacan	M	160	35	3–3	7–7	6–6	152	37	no	Echternacht 1973
HSM RS-596	*michoacanensis*	Colima	F	220	36	2–3	7–7	7–7	161	32	no	Echternacht 1973
KU 23790	*michoacanensis*	Guerrero	M	237	46	3–3	7–7	7–7	177	41	no	Echternacht 1973
KU 23791	*michoacanensis*	Guerrero	M	275	55	2–2	7–6	7–6	175	42	no	Echternacht 1973
MCZ 33650	*michoacanensis*	Guerrero	F	272	58	3–3	7–7	6–7	175	46	no	Echternacht 1973
Museo Dugés –	*michoacanensis*	Guerrero	F	–	–	–	–	–	177	43	no	Echternacht 1973
MVZ 45123	*michoacanensis*	Guerrero	F	253	54	4–3	7–7	6 - ?	175	45	no	Echternacht 1973
MVZ 76714	*michoacanensis*	Michoacan	F	228	45	2–3	8–8	7–7	170	40	no	Echternacht 1973
UIMNH 25063	*michoacanensis*	Guerrero	M	110	23	3–2	8–8	7–7	163	46	no	Echternacht 1973
UMMZ 109904	*michoacanensis*	Michoacan	F	192	34	3–3	6–7	6–5	168	37	no[d]	Echternacht 1973
UMMZ 109905	*michoacanensis*	Michoacan	F	234	41	3–3	7–7	6–7	171	38	no	Echternacht 1973
UMMZ 109906	*michoacanensis*	Michoacan	F	120	19	3–3	7–7	6–6	171	33	no	Echternacht 1973
UMMZ 119457	*michoacanensis*	Michoacan	M	211	47	3–3	7–7	7–7	157	41	no	Echternacht 1973
UIMNH 41688	*michoacanensis*	Puebla	F	257	51	3–3	6–6	7–7	177	40	no	Echternacht 1973
UTA R-38146	*michoacanensis*	Guerrero	F	205	38	5–5	7–7	6–7	171	37	no	This Study
UTA R-59760	*michoacanensis*	Colima	–	76	16	–	–	–	164	46	no	This Study
AMNH 74951	*mutabilis*	Nayarit	M	215	41	–	7–7	6–6	171	40	yes	Echternacht 1973
NHMUK 1946.1.14.63	*mutabilis*	Zacatecas	M	229	54	–	–	–	160	45	yes	Echternacht 1973
NHMUK 1946.1.14.64	*mutabilis*	Zacatecas	M	220	48	–	–	–	166	46	yes	Echternacht 1973
FMNH 105296	*mutabilis*	Jalisco	M	191	44	3–3	7–7	6–6	163	44	yes	Echternacht 1973
FMNH 105297	*mutabilis*	Jalisco	M	189	43	3–3	7–7	6–6	161	44	yes	Echternacht 1973
KU 106286	*mutabilis*	Zacatecas	F	230	45	3–3	5–7	6–6	178	43	yes	Echternacht 1973
MVZ 71356	*mutabilis*	Jalisco	M	99	15	3–3	7–7	6–6	171	34	yes	Echternacht 1973
UIMNH 18754	*mutabilis*	Jalisco	F	210	42	3–3	7–7	6–6	169	41	yes	Echternacht 1973
UTA R-7227	*mutabilis*	Sinaloa	M	225	52	6–5	7–7	7–7	174	48	yes	This Study
UTA R-53487	*mutabilis*	Jalisco	M	235	51	7–6	7–7	7–7	162	40	yes	This Study
UTA R-59762	*mutabilis*	Jalisco	–	–	–	3–3	7–7	7–7	189	41	yes	This Study
MCZ 6444	*mutabilis* (‘*aequalis*’)	–	F	225	40	3–3	7–7	6–6	174	38	yes	Echternacht 1973
UTA R-16169	*mutabilis* (‘*aequalis*’)	Jalisco	F	256	52	5–5	7–7	7–6	169	41	yes	This Study
UTA R-53488	*mutabilis* (‘*aequalis*’)	Jalisco	F	252	41	7–6	7–7	7–6	169	39	yes	This Study
UTA R-59761	*mutabilis* (‘*aequalis*’)	Jalisco	M	–	–	6–6	7–7	6–6	168	47	yes	This Study

[a]TBL = total body length. [b]TL = tail length. [c]Meristic counts are presented as left–right. [d]This specimen has a single narrow band on the tail.



Figs 10–11. Maximum likelihood phylogenetic tree of relationships among *Sonora* and *Procinura* species using (**10**) a concatenated mitochondrial dataset (*ND4* and *cyt-b*) and (**11**) a concatenated nuclear dataset (*c-mos* and *RAG-1*). Numbers in symbols next to specimen numbers correspond to localities in Table 1 and Fig. 9. In the (**11**), a lower case letter after each specimen name indicates the phase for phased heterozygous individuals. Support values for nodes are Bayesian posterior probability (top), bootstrap proportions from maximum likelihood analysis (middle) and bootstrap proportions (1000 pseudoreplicates) from maximum parsimony analysis (bottom) >50 (maximum likelihood and maximum parsimony) or 0.8 (Bayesian posterior probability). A dash (-) denotes support lower than the cut-off value for maximum likelihood or maximum parsimony. On the phylogenetic tree derived from nuclear loci, lower case letters next to specimen numbers represent gametic phases. Note that for both datasets, *Procinura* is deeply nested within *Sonora*, and that *S. aequalis* is paraphyletic with regard to *S. mutabilis.*

(12.5% in the mitochondrial data) from *S. mutabilis* and *S. aequalis* (Figs 10–11). The mitochondrial phylogenetic tree displays limited geographic structuring within clades, with *S. aequalis* and *S. mutabilis* clustering by locality (not taxonomy, Figs 10–11).

Species tree and Bayesian species delimitation analyses

Tree prior and codon partitioning combinations for the species tree analyses resulted in very similar topologies, so we present the partitioned dataset using a Yule process

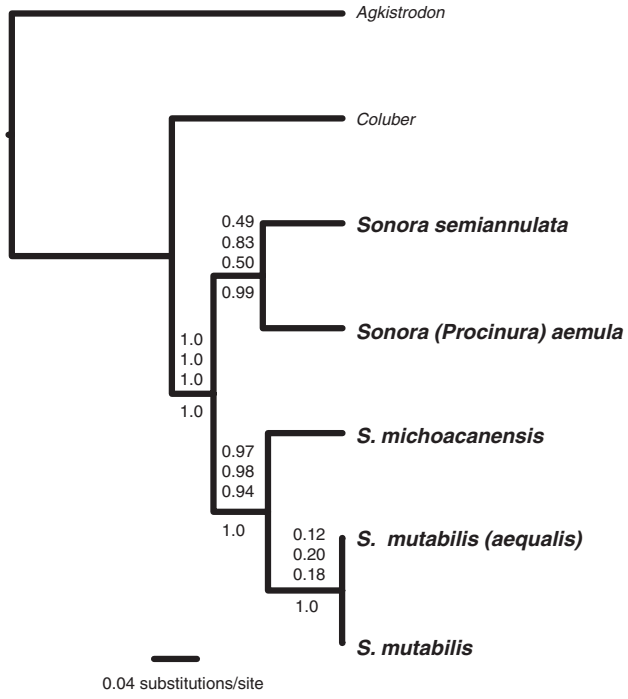


Fig. 12. Species tree of *Sonora* and *Procinura* using four genes (*ND4*, *cyt-b*, *c-mos*, *RAG-1*) with recommended taxonomic nomenclature (previous nomenclature in parentheses). Support values above the node are speciation probabilities from the Bayesian species delimitation analysis, which represents the probability that a node is fully resolved (or fully bifurcates). The top value represents the probability from prior set 1 (G [1, 10] for both θ and τ), the middle value is from prior set 2 (G [2, 2000] for both θ and τ), and the bottom value from prior set 3 (G [1, 10] for θ and G [2, 2000] for τ). The support value below the node is the Bayesian posterior probability of that node from the species tree analysis.

tree prior with nodal support of Bayesian posterior probabilities. The coalescent analysis largely agreed with the concatenated dataset analyses (Fig. 12). In agreement with the mitochondrial dataset, a southern clade (S. *mutabilis*, *S. aequalis* and *S. michoacanensis*) and a northern clade (*S. semiannulata* and *P. aemula*) are well supported (Fig. 12; BPP = 1.0). *Procinura* is deeply nested within *Sonora*, sister to *S. semiannulata*. *Sonora aequalis* and *S. mutabilis* are recovered as a monophyletic group (but with almost no sequence divergence; BPP = 1.0) and are sister to *S. michoacanensis* (Fig. 12: BPP = 1.0).

Bayesian species delimitation returned similar results for each prior set, and was mostly congruent with the other analyses (Fig. 12). Generally, this analysis supported a topology that was resolved at all nodes except the *aequalis/mutabilis* node (Fig. 12). The *P. aemula/S. semiannulata* node had mixed support (based upon prior set), perhaps as the result of limited sampling for these two species (Fig. 12). Nonetheless, these analyses demonstrate that *P. aemula* is nested within the currently recognised species of *Sonora*.

Morphological analysis

Hemipenial and meristic scale characters were mostly overlapping between *S. aequalis*, *S. michoacanensis* and *S. mutabilis* (Table 3). *Sonora aequalis* possessed overlapping but somewhat higher number of temporal scales than *S. michoacanensis* or *S. mutabilis*. The only consistent morphological difference between *S. michoacanensis* and *S. mutabilis/aequalis* is the complete banding on the tail of *S. mutabilis/aequalis* and the lack of banding on the tail of *S. michoacanensis* (Table 3).

Species diagnoses

Below we provide species accounts for *S. aemula*, *S. michoacanensis* and *S. mutabilis*. We refrain from presenting a species account for *S. semiannulata* due to our limited sampling from this geographically widespread species.

Sonora aemula (Cope, 1879)

Procinura aemula Cope (1879). Holotype: Academy of Natural Sciences in Philadelphia (ANSP) 11614 (Bogert & Oliver, 1945). Type locality: 'Batopilas, Chihuahua' (Cope, 1879).

Scolecophis aemulus – Amaral (1929)
Sonora aemula – Bogert & Oliver 1945
Sonora aemula – Zweifel & Norris 1955
Procinura aemula – Lemos-Espinal *et al*. (2004*a*)

Diagnosis: This species can be distinguished from both *S. michoacanensis* and *S. mutabilis* by the presence of distinctly raised tubercular scales or caudal spines (Fig. 13) creating a 'file-like' tail (Bogert & Oliver, 1945).

Variation: This species is extremely variable in colour pattern, ranging from a uniformly red to banded tricoloured pattern (Bogert & Oliver, 1945; Zweifel & Norris, 1955; Nickerson & Heringhi, 1966). In tricoloured animals, the number and arrangement of triads can vary greatly (Bogert & Oliver, 1945; Zweifel & Norris, 1955; Nickerson & Heringhi, 1966). A more detailed description of meristic characters and a hemipenial description are found in Bogert & Oliver (1945).

Distribution: This species is found on the Pacific versant of the Mexican states of Chihuahua, Sonora and Sinaloa (Fig. 9).

Sonora michoacanensis Duges *in* (Cope, 1885)

Contia michoacanensis Duges in Cope (1885). Holotype: Neotype British Museum of Natural History (BMNH), now the Natural History Museum, London (NHMUK) 1903.3.21, now 1946.1.14.65. The original holotype from the Museo Alfredo Dugès was lost (Stickel, 1943); a specimen collected in Michoacan with no additional locality information was designated as neotype by Stickel (1943). Type locality: None given in Duges in

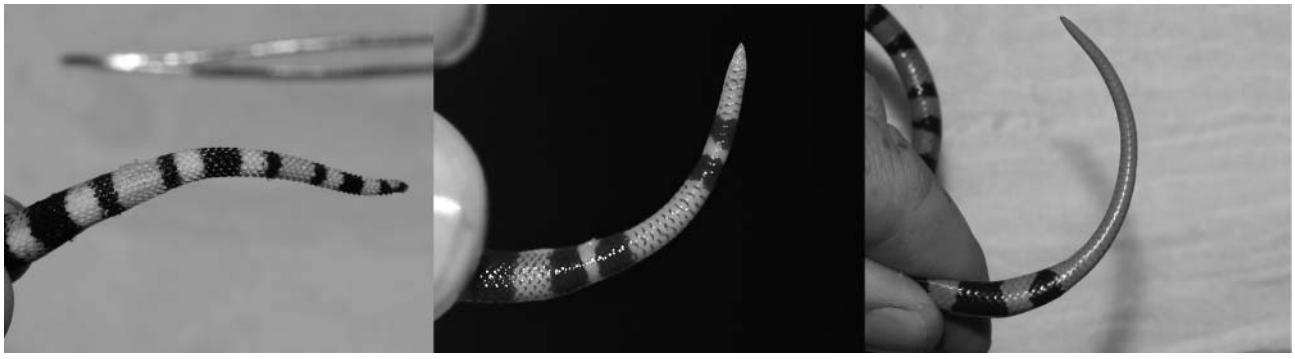


Fig. 13. Comparison of tail morphology for *Sonora aemula* (left, UAZ 45675, note caudal spines), *S. mutabilis* (centre, KU 23791, note banding on tail) and *S. michoacanensis* (right, MVZ 71356, note lack of banding on tail).

Cope (1885). Neotype locality is given as 'Michoacán' (Stickel, 1943). Restricted to 'Apatzingan, Michoacán' by Smith & Taylor (1950).

Elapomorphus michoacanensis – Cope (1895)
Homalocranium michoacanense – Gunther (1895)
Chionactis michoacanensis – Cope (1896)
Scolecophis michoacanensis – Boulenger (1896)
Sonora erythura – Taylor (1937) Holotype: University of Illinois Museum of Natural History (UIMNH) 25063. Type locality: '16 km S of Taxco, Guerrero'.
Sonora michoacanensis michoacanensis – Stickel 1943
Sonora michoacanensis – Ponce-Campos *et al*. 2004

Diagnosis: This species can be distinguished from *S. mutabilis* based on the almost invariable absence of banding on the tail, and from *S. aemula* based on the absence of a file-like tail (Fig. 13). We note that one specimen from the University of Michigan Museum of Zoology (UMMZ 109904) has a single narrow band on the tail.

Variation: This species is extremely variable in colour pattern, ranging from uniform red to banded tricoloured pattern (Echternacht, 1973). In tricoloured animals, the number of bands and shape of bands varies greatly (Echternacht, 1973). In some individuals, the black and yellow bands appear as black-bordered yellow spots (Fig. 7). Morphological measurements and meristic characters are mostly overlapping between *S. mutabilis* and *S. michoacanensis* (Table 3). The hemipenis is depicted in Cope (Cope, 1895, Plate XXIX, Fig. 6).

Distribution: This species is found on the Pacific coast and Balsas basin in the Mexican states of Colima, Guerrero, Michoacan, Morelos and Puebla (Fig. 9).

Sonora mutabilis Stickel 1943

Sonora michoacanensis mutabilis – Stickel 1943. Holotype: The holotype is in the Field Museum of Natural History (FMNH) 105257, with paratypes FMNH 105296, NHMUK 1946.1.14.63– NHMUK 1946.1.14.64 and American Museum of Natural History (AMNH) 19714–19716 (Stickel, 1943; Echternacht, 1973). Type locality: 'Magdalena, Jalisco' (Stickel, 1943).
Sonora aequalis – Smith and Taylor 1945. Holotype: Museum of Comparative Zoology (MCZ) 6444. Type Locality: Originally given as 'Matagalpa, Nicaragua' (Stickel, 1943), later concluded to be 'within or somewhat to the east of the ranges of *mutabilis* and *michoacanensis*, on the southern part of the Mexican plateau or in the surrounding mountains' (Stickel, 1943; Echternacht, 1973).
Sonora michoacanensis mutabilis – Echternacht 1973
Sonora aequalis – Ponce-Campos *et al*. 2004
Sonora mutabilis – Ponce-Campos *et al*. 2004

Diagnosis: Both bicoloured (formerly *aequalis*) and tricoloured forms of this species can be distinguished from *S. michoacanensis* based on complete banding on the tail and from *S. aemula* based on the absence of a file-like tail (Fig. 13).

Variation: *Sonora mutabilis* possesses bicoloured (red and black) and tricoloured (red, black and yellow) morphs (Echternacht, 1973). In tricolour morphs, the extent of black interspaces between bands may be quite variable, and bands may have red dorsal or lateral inclusions (e.g. Figs 4–6). Bands may be regular, irregular or absent ventrally. Morphological measurements and meristic characters are mostly overlapping between *S. mutabilis* and *S. michoacanensis* (Table 3). The hemipenis of *S. michoacanensis* was described by Stickel (1943). His description was based on one specimen of *S. michoacanensis* and one of *S. mutabilis.* Here we describe the hemipenis of *S. mutabilis* (Fig. 14) and compare it with that of *S. michoacanensis* (Cope, 1895). The hemipenis is slightly bilobed, differentiated and with a simple sulcus spermaticus. The apical lobes are covered with numerous papillated calyces; the papillae are so numerous and large that the calyces are nearly indiscernible. The papillae become enlarged towards the base of the calyces and grade into spines. The calyces cover 54% of

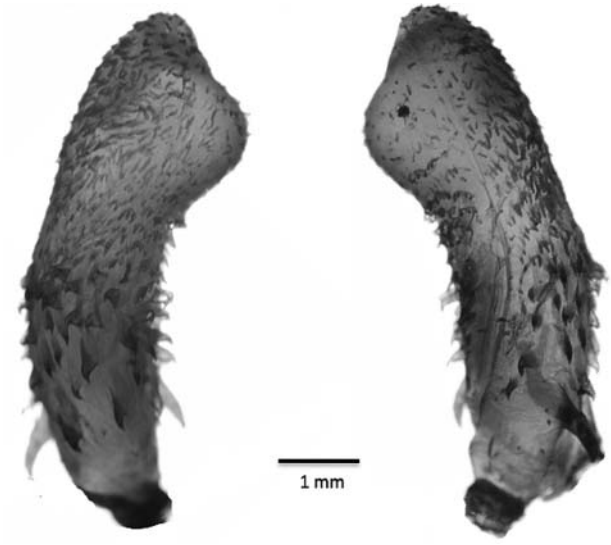


Fig. 14. Hemipenis of *Sonora mutabilis* (UTA R-53487). Right, sulcate side, left, asulcate side.

the hemipenis in a specimen from Jalisco (UTAR-53487) and 38% of the hemipenis in a specimen from Plomosas, Sinaloa (UTAR-7227), and 39% in another bicoloured specimen (formerly *S. aequalis*) from Jalisco (UTA R-59761). Approximately 45–60 hooked spines cover the surface between the base and the calyces; this area represents 28% of the hemipenis of UTA R-53487, 35% of UTA R-7227 and 31% of UTA R-59761. Two large basal hooks are found in all specimens. The basal area of the hemipenis is naked and this area comprises 19% of the hemipenis for UTA R-53487, 27% for UTA R-7227 and 29% for UTA R-59761. The everted hemipenis of UTA R-53487 is 6 subcaudals long, while that of UTA R-7227 and UTA R-59761 are 7 subcaudals *in situ*. The main difference between the hemipenis of *S. mutabilis* and *S. michoacanensis* is the size of the papillae in the apical region, being very large and abundant in *S. mutabilis*, to the point of making the calyces undistinguishable, while in *S. michoacanensis* the calyces are conspicuous.

Distribution: *Sonora mutabilis* is found in the Mexican states of Aguascalientes, Jalisco, Nayarit, southern Zacatecas and extreme southern Sinaloa.

Discussion

Taxonomic implications

We adhere to the evolutionary species (Wiley, 1978) and general lineage (de Queiroz, 1998) theoretical species concepts when evaluating the taxonomy of the genera *Sonora* and *Procinura*, and implement the focal-species approach of Schargel *et al.* (2010). We consider putative geographic barriers, and consider that ecological differentiation and morphological divergence represent additional evidence that lineages are valid species (i.e. Schargel *et al.*, 2010). Our results have implications for both generic and species-level taxonomy for the genus *Sonora*. Both nuclear and mitochondrial datasets, and combined coalescent analyses recover *P. aemula* as sister to *S. semiannulata* (the type-species of the genus *Sonora*) and nested within the other *Sonora* species, rendering *Sonora* paraphyletic (BPPs >0.99). In fact, many previous taxonomic treatments of *P. aemula* have considered this species to be within the genus *Sonora* (Bogert & Oliver, 1945; Zweifel & Norris, 1955), and it was only re-elevated to the monotypic genus *Procinura* (Lemos-Espinal *et al.*, 2004*a*, 2004*b*, 2004*c*) based on a single morphological autapomorphy (the file-like caudal anatomy). We propose that *P. aemula* be returned to the genus *Sonora*, which renders *Sonora* monophyletic and accurately reflects the evolutionary history of this genus.

Our molecular analyses also indicate that *S. aequalis* and *S. mutabilis* are paraphyletic with regard to one another (BSPs < 0.21). Specimens group genetically based upon locality, not colour pattern, and so *S. aequalis* is best considered a bicolour morph of *S. mutabilis* and not a valid species. *Sonora mutabilis* has taxonomic priority (Stickel, 1943), so we suggest that *S. aequalis* be placed in synonymy with *S. mutabilis* and that the species diagnosis for *S. mutabilis* reverts to the diagnosis by Stickel (1943), with the inclusion of a bicolour morph. In contrast, the results of this study reveal a deep genetic divergence between *S. mutabilis* and *S. michoacanensis*. This genetic divergence is reflected in discontinuity in their respective geographic distribution. We concur with previous recommendations that both *S. mutabilis* and *S. michoacanensis* should be considered separate species (Stickel, 1943; Echternacht, 1973; Ponce-Campos *et al.*, 2004) and suggest the species diagnosis for *S. michoacanensis* be as in Stickel (1943). We note that the lack of banding on the tail of *S. michoacanensis* is a reliable morphological feature that can be used to distinguish it from *S. mutabilis* (Fig. 13, Table 3). While colour pattern variation is probably an underlying factor in the taxonomy uncertainty in *Sonora*, it is also useful as a field character for distinguishing *S. michoacanensis* from *S. mutabilis*. Besides the consistent differences in tail banding, *S. michoacanensis* is either uniform red or tricoloured, with bands or saddles that vary in size and position. In contrast, *S. mutabilis* is either bicoloured or tricoloured with regularly shaped bands (e.g. Figs 1–8) and has no uniformly red morph. While colour pattern polymorphism is easier to interpret in the context of a molecular phylogeny, prior generations of herpetologists reached the same taxonomical conclusions as our study based on careful assessment of morphology, including colour pattern (Bogert & Oliver, 1945; Zweifel & Norris, 1955; Echternacht, 1973).

Although our study focused on Mexican *Sonora* (mostly *S. michoacanensis* and *S. mutabilis*), there is still great need for molecular and taxonomic reviews of some of the other *Sonora* species and related taxa. *S. semiannulata* was only represented by a single specimen in this study, and so we

cannot comment on either the biogeography or taxonomy of this taxon. Because *S. semiannulata* is (1) morphologically distinct from other *Sonora* species and (2) has a non-overlapping geographic range with other *Sonora* species, inclusion of additional *S. semiannulata* specimens should not change the conclusions of this study. Our study did not include the genera *Chionactis* and *Chilomeniscus*, which are hypothesised to be close relatives of *Sonora* (Dowling, 1975; Dowling & Duellman, 1978), with *Chionactis* at one time considered synonymous with *Sonora* (Stickel, 1938, 1943). Multiple species and subspecies have been recognised for both of these genera (Ernst & Ernst, 2003), and evaluating the taxonomy and molecular systematics of these genera was beyond the scope of this study. A complete molecular evaluation of all species and subspecies of *Chionactis*, *Chilomeniscus* and *S. semiannulata* is needed to clarify the complex biogeographic history and taxonomic nomenclature of this group.

Methodological congruence

We found marked differences in rates of molecular evolution between mitochondrial and nuclear loci. Maximum pairwise divergence within *Sonora* varied by two orders of magnitude (from 0.8% uncorrected divergence for nuclear loci compared with 18% for mitochondrial loci) for nuclear (*c-mos*, *RAG-1*) and mitochondrial loci (*cyt-b*, *ND4*) commonly used in snake systematics (Burbrink *et al.*, 2000; Townsend *et al.*, 2004; Noonan & Chippindale, 2006; Vidal & Hedges, 2009). Rate variation between nuclear and mitochondrial loci is well known (Vawter & Brown, 1986; Hare, 2001) and often causes incomplete lineage sorting in nuclear loci (Madison & Knowles, 2006; Makowsky *et al.*, 2010). Yet despite great differences in rates of evolution, separate mitochondrial and nuclear phylogenetic analyses supported very similar topologies (Figs 10–11; except for the phylogenetic position of *S. michoacanensis*). These results demonstrate the potential for rate heterogeneity between snake clades and between mitochondrial and nuclear genomes.

In addition to traditional analytical methods (maximum parsimony, maximum likelihood and Bayesian phylogenetic analysis), we used coalescent-based species tree analyses within a Bayesian framework and Bayesian species delimitation. Generally, each method supported the same taxonomy and evolutionary relationships among focal taxa. All methods supported the monophyly of *Sonora* + *Procinura*, the nesting of *Sonora* (formerly *Procinura*) *aemula* within the genus *Sonora*, and the distinctness of *S. michoacanensis* (BPPs >0.98). None of the methods supported the genetic distinctness of *S. mutabilis* (formerly *aequalis*) and *S. mutabilis* (BSPs < 0.21). We obtained inconsistent results for one relationship (between *S. aemula* and *S. semiannulata*) with Bayesian species delimitation analysis (BSPs from 0.49–0.83), which is sensitive to prior conditions (Yang & Rannala, 2010). The resolution of this node received some support with high θ and τ parameters, but was not supported with the other two prior conditions with lower θ and τ parameters. Given that the validity of *S. aemula* and *S. semiannulata* is well supported by multiple lines of evidence (e.g. Stickel, 1938; Bogert & Oliver, 1945, this study), we suspect that this mixed support was due to our very limited sampling of both of these species. In fact, both species tree analyses and Bayesian species delimitation use coalescent methodology that are more appropriate for studies with greater molecular and specimen sampling (i.e. Knowles & Kubatko, 2010; Leache & Fujita, 2010; Yang & Rannala, 2010). Nonetheless, all methodologies consistently recover key relationships among focal taxa, suggesting that coalescent methods may be somewhat robust to limited sampling (Burbrink *et al.*, 2011; Leache & Rannala, 2011), at least if focal taxa are very genetically distinct.

Historical biogeography

Phylogenetic relationships among Mexican *Sonora* species are generally consistent with the biogeographic patterns documented in many other Mexican vertebrates. In the south, *S. michoacanensis* and *S. mutabilis* are separated by the Trans-Mexican Volcanic Belt, which has been implicated in biogeographic breaks in other snakes (Devitt *et al.*, 2008; Bryson *et al.*, 2011), anurans (Mulcahy & Mendelson, 2000; Greenbaum *et al.*, 2011), fish (Mateos, 2005) and many other taxa (Ferrusquia-Villafranca, 2007). We note that although the uplift of the Trans-Mexican Volcanic Belt has been implicated in these biogeographic patterns, they could also arise from geographic features associated with this uplift, including the closing and aridification of the Balsas Basin (Gómez-Tuena & Carrasco-Núñez, 2000; Ruiz-Martinez *et al.*, 2000). Although we lacked appropriate data for detailed divergence analyses, our results (12.5% uncorrected mitochondrial sequence divergence between *S. mutabilis* and *S. michoacanensis*) are consistent with a Pliocene or Miocene divergence between these two species given the potential for an accelerated rate of mitochondrial evolution in snakes (Mateos, 2005; Jiang *et al.*, 2007; Bryson *et al.*, 2011). This temporal framework is broadly consistent with the diversification in other Mexican fauna (Mulcahy & Mendelson, 2000; Mateos, 2005; Devitt *et al.*, 2008; Greenbaum *et al.*, 2011). Highland diversification is thought to be a major driver of species richness of vertebrates in Mexico (Demastes *et al.*, 2002; Jaeger *et al.*, 2005; Riddle & Hafner, 2006; Bryson *et al.*, 2011). Our data may support that hypothesis within *S. mutabilis*, with the specimens from Bolaños, Jalisco forming a moderately (1.8% uncorrected sequence distance) divergent mitochondrial clade. Finally, our data are structured latitudinally, with most analyses (BPPs >0.98) supporting a southern clade (*S. mutabilis* and *S. michoacanensis*) and a northern clade (*S. aemula* and *S. semiannulata*). While greater

geographic sampling is necessary for *S. aemula* and *S. semiannulata*, many other Mexican species with latitudinally structured phylogenies show evidence for northern range expansion from the southern and central highlands of Mexico into central North America (e.g. Savage, 1982; Mulcahy & Mendelson, 2000; Mateos, 2005) and a similar pattern in *Sonora* would be unsurprising.

Evolution of colour pattern in the genus *Sonora*

All Mexican *Sonora* are thought to be coralsnake mimics (Campbell & Lamar, 2004), and it is likely that red and black colouration in *S. semiannulata* has evolved in the context of mimicry given the probable Mesoamerican origin of the genus (Savage, 1982). Additionally, each of the currently recognized species of *Sonora* contains populations that have colour pattern polymorphism (Figs 1–8). Both *S. michoacanensis* and *S. aemula* are either uniform red or tricoloured, with variation in the shape, arrangement and number of bands (Echternacht, 1973; Figs 1–8). In contrast, *S. mutabilis* has bicolour (red/orange and black banded) or tricolour morphs. The most northern distributed member of the genus (*S. semiannulata*) displays the most extreme colour pattern polymorphism, with individuals that are plain, red-striped, darkly banded or both banded and red-striped (Ernst & Ernst, 2003). The phylogenetic distribution of colour pattern polymorphism in these coralsnake mimics may support the ubiquity of colour pattern polymorphism in mimicry complexes (Mallet & Joron, 1999; Brodie & Brodie, 2004; Kunte, 2009).

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References

Amaral, A.D. 1929. Estudos sobre ophidios neotropicos XVIII. Lista remissiva dos ophidios da regiao neotropica. *Memoir Institut Butantan* **4**, 126–271.

Arevalo, E., Davis, S.K. & Sites, J.W.J. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology* **43**, 387–418.

Bogert, C.M. & Oliver, J.A. 1945. A preliminary analysis of the herpetofauna of Sonora. *Bulletin of the American Museum of Natural History* **83**, 297–426.

Boulenger, G.A. 1896. *Catalogue of the Snakes in the British Museum* (*Natural History*). British Museum (Natural History), London.

Brodie, E.D. & Brodie, E.D. 2004. Venomous snake mimicry. In: Campbell, J.A. & Lamar, W.W., Eds., *The Venomous Reptiles of the Western Hemisphere*. Comstock Publishing Associates, Ithaca, NY, vii + 870 pp.

Bryson, R.W., Murphy, R.W., Lathrop, A. & Lazcano-Villareal, D. 2011. Evolutionary drivers of phylogeographic diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of montane rattlesnakes. *Journal of Biogeography* **38**, 697–710.

Burbrink, F.T., Lawson, R. & Slowinski, J.B. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* **54**, 2107–2118.

Burbrink, F.T., Yao, H., Ingrasci, M., Bryson, R.W., Guiher, T.J. & Ruane, S. 2011. Speciation at the Mogollon Rim in the Arizona mountain kingsnake (*Lampropeltis pyromelana*). *Molecular Phylogenetics and Evolution* **60**, 445–454.

Campbell, J.A. & Lamar, W.W. 2004. *The Venomous Reptiles of the Western Hemisphere*. Cornell University Press, Ithaca, NY.

Cope, E.D. 1879. Eleventh contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society* **18**, 261–277.

Cope, E.D. 1885. Twelfth contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society* **22**, 167–194.

Cope, E.D. 1895. The classification of the Ophidia. *Transactions of the American Philosophical Society* **18**, 186–219.

Cope, E.D. 1896. The geographical distribution of Batrachia and Reptilia in North America (continued). *American Naturalist* **30**, 1003–1026.

DE Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: Howard, D.J. & Berlocher, S.H., Eds., *Endless Forms: Species and Speciation*. Oxford University Press, Oxford, pp. 57–75.

Demastes, J.W., Spradlin, T.A., Hafner, M.S., Hafner, D.J. & Reed, D.L. 2002. Systematics and phylogeography of pocket gophers in the genera *Cratogeomy* and *Pappogeomys*. *Molecular Phylogenetics and Evolution* **22**, 144–154.

Devitt, T.J., Laduc, T.J. & Mcguire, J.A. 2008. The *Trimorphodon biscutatus* (Squamata: Colubridae) species complex revisited: A multivariate statistical analysis of geographic variation. *Copeia* **2008**, 370–387.

Dowling, H.G. 1975. A provisional classification of snakes. In: Dowling, H.G., Ed., *Yearbook of Herpetology*. Hiss Publications, New York.

Dowling, H.G. & Duellman, W.E. 1978. *Systematic Herpetology: A Synopsis of Families and Higher Categories*. Hiss Publications, New York.

Dowling, H.G. & Savage, J.M. 1960. A guide to the snakes hemipenes: A survey of basic structure and systematic characteristics. *Zoologica* **45**, 17–28.

DRUMMOND, A.J. & RAMBAUT, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 214.

ECHTERNACHT, A.C. 1973. The color pattern of *Sonora michoacanensis* (Dugés) (Serpentes, Colubridae) and its bearing on the origin of the species. *Breviora of the Museum of Comparative Zoology* **410**, 1–18.

ERNST, C.H. & ERNST, E.M. 2003. *Snakes of the United States and Canada*. Smithsonian Books, Washington.

FERRUSQUIA-VILLAFRANCA, I. 2007. En sayo sobre la caracterización y significancia biológica. In: LUNA, I., MORRONE, J.J. & ESPINOSA, D., Eds., *Biodiversidad de la Faja Volcánica Transmexicana*. UNAM, Distrito Federal, Mexico, pp. 7–23.

FLORES-VILLELA, O. & CANSECO-MÁRQUEZ, L. 2004. Nuevas especies y cambios taxonómicos para la Herpetofauna de México. *Acta Zoológica Mexicana* (*Nueva Serie*) **20**, 115–144.

FLORES-VILLELA, O. & GEREZ, P. 1994. *Biodiversidad y conservación en México: vertebrados, vegetación y uso del suelo*. CONABIO/UNAM, Distrito Federal, México.

GÓMEZ-TUENA, A. & CARRASCO-NÚÑEZ, G. 2000. Cerro Grande volcano: the evolution of a Miocene stratocone in the early Trans-Mexican Volcanic Belt. *Tectonophysics* **318**, 249–280.

GOYNECHEA, I. 2009. Relaciones filogenéticas de las serpientes del género *Conopsis* con base en la morfología. *Revista Mexican de Biodiversidad* **80**, 721–725.

GREENBAUM, E., SMITH, E.N. & SÁ, R.O.D. 2011. Molecular systematics of the Middle American genus *Hypopachus* (Anura: Microhylidae). *Molecular Phylogenetics and Evolution* **61**, 265–277.

GREENE, H.W. 1997. *Snakes: The Evolution of Mystery in Nature*. University of California Press, Berkeley, CA.

GUNTHER, A. 1895. *Reptiles and Batrachia*. Dulau and Co., London.

HARE, M.P. 2001. Prospects for nuclear gene phylogeography. *Trends in Ecology and Evolution* **12**, 700–706.

HARVEY, M.B., BARKER, D.G., AMMERMAN, L.K. & CHIPPINDALE, P.T. 2000. Systematics of the pythons of the *Morelia amethistina* complex (Serpentes:Boidae) with the description of three new species. *Herpetological Monographs* **2000**, 139–185.

HELED, J. & DRUMMOND, A.J. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27**, 570–580.

HOLM, P.A. 2008. *Phylogenetic biology of the burrowing snake tribe Sonorini (Colubridae).* Unpublished dissertation. University of Arizona, Tucson, AZ, 240 pp.

HUELSENBECK, J.P., LARGET, B., MILLER, R.E. & RONQUIST, F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* **51**, 673–688.

HUELSENBECK, J.P. & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755.

JAEGER, J.R., RIDDLE, B.R. & BRADFORD, D.F. 2005. Cryptic Neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas. *Molecular Ecology* **14**, 3033–3048.

JIANG, Z.J., CASTOE, T.A., AUSTIN, C.C., BURBRINK, F.T., HERRON, M.D., MCGUIRE, J.A., PARKINSON, C.L. & POLLOCK, D.D. 2007. Comparative mitochondrial genomics of snakes: extraordinary sustitution rate dynamics and functionality of the duplicate control region. *BMC Evolutionary Biology* **7**, 123.

KNOWLES, L.L. & KUBATKO, L.S. 2010. *Estimating Species Trees: Practical and Theoretical Aspects*. Wiley-Blackwell, Hoboken, NJ.

KUNTE, K. 2009. The diversity and evolution of Batesian mimicry in *Papilio* swallowtail butterflies. *Evolution* **63**, 2707–2716.

LARKIN, M.A., BLACKSHIELDS, G., BROWN, N.P., CHENNA, R., MCGETTIGAN, P.A., MCWILLIAM, H., VALENTIN, F., WALLACE, I.M., WILM, A., LOPEZ, R., THOPSON, J.D., GIBSON, T.J. & HIGGINS, D.G. 2007. ClustalW and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

LEACHE, A.D. & FUJITA, M.K. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings of the Royal Society of London B* **277**, 3071–3077.

LEACHE, A.D. & RANNALA, B. 2011. The accuracy of species tree estimation under simulation: a comparison of methods. *Systematic Biology* **60**, 126–137.

LEMOS-ESPINAL, J.A., CHISZAR, D., INGRASCI, M.J. & SMITH, H.M. 2004a. Year 2002 turtles and snakes from Chihuahua, Mexico. *Bulletin of the Chicago Herpetological Society* **39**, 82–87.

LEMOS-ESPINAL, J.A., CHISZAR, D. & SMITH, H.M. 2004b. Variation in *Procinura aemula*, the File-tailed Groundsnake of Mexico. *Bulletin of the Maryland Herpetological Society* **40**, 61–69.

LEMOS-ESPINAL, J.A., SMITH, H.M. & CHISZAR, D. 2004c. *Introduccíon a los anfidios y reptiles del estado de Chihuahua, México*. Universidad Nacional Autonoma de México y Conabio, D.F. México.

MADISON, W.P. & KNOWLES, L.L. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* **55**, 21–30.

MAKOWSKY, R., COX, C.L., ROELKE, C. & CHIPPINDALE, P.T. 2010. Analyzing the relationship between sequence divergence and nodal support using Bayesian phylogenetic analyses. *Molecular Phylogenetics and Evolution* **57**, 485–494.

MALLET, J. & JORON, M. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. *Annual Reviews of Ecology and Systematics* **30**, 201–233.

MATEOS, M. 2005. Comparative phylogeography of livebearing fishes in the genera *Poeciliopsis* and *Poecilia* (Poeciliidae: Cyprinodontiformes) in central Mexico. *Journal of Biogeography* **32**, 775–780.

MITTERMEIER, R.A., GIL, P.R., HOFFMAN, M., PILGRIM, J., MITTERMEIER, C.G., LAMOREUX, J. & FONSECA, G.A.B.D. 2005. *Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions*. Conservation International, Washington, DC.

MULCAHY, D.G. & MENDELSON, J.R. 2000. Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. *Molecular Phylogenetics and Evolution* **17**, 173–189.

MYERS, C.W. & CADLE, J.E. 2003. On the snake hemipenis, with notes of *Psomophis* and techniques of eversion: a response to Dowling. *Herpetological Review* **34**, 295–302.

NICKERSON, M.A. & HERINGHI, H.L. 1966. Three noteworthy colubrids from southern Sonora, Mexico. *Great Basin Naturalist* **26**, 136–140.

NOONAN, B.P. & CHIPPINDALE, P.T. 2006. Dispersal and vicariance: the complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution* **40**, 347–358.

PETERSON, A.T., FLORES-VILLELA, O.A., LEON-PANIAGUA, L.S., LLORENTE-BOUSQUETS, J.E., LUIS-MARTINEZ, M.A., NAVARRO-SIGUENZA, A.G., TORRES-CHAVEZ, M.G. & VARGAS-FERNANDEZ, I. 1993. Conservation priorities in northern Middle America: moving up in the world. *Biodiversity Letters* **1**, 33–38.

Ponce-Campos, P., Smith, H.M., Harris, H.S. & Chiszar, D. 2004. A review of the taxonomic status of the members of the *Sonora michoacanensis* group (Serpentes: Colubridae). *Bulletin of the Maryland Herpetological Society* **40**, 144–151.

Pyron, R.A., Burbrink, F.T., Colli, G.R., Oca, A.N.M.D., Vitt, L.J., Kuczynski, C.A. & Wiens, J.J. 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. *Molecular Phylogenetics and Evolution* **58**, 329–342.

Ramamoorthy, T., Bye, R., Lot, A. & Fa, J. 1993. *Biological Diversity of Mexico: Origins and Distribution*. Oxford University Press, Oxford.

Rambaut, A. 2007. FigTree, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree/, accessed 9 February 2012.

Riddle, B.R. & Hafner, D.J. 2006. A step-wise approach to integrating phylogeographic and phylogenetic biogeographic perspectives on the history of core North American warm deserts biota. *Journal of Arid Environments* **66**, 435–461.

Ruiz-Martinez, V.C., Osete, M.L., Núñez-Aguilar, J.I., Urrutia-Facugauchi, J. & Tarling, D.H. 2000. Paleomagnetism of late Miocene to Quaternary volcanics from the eastern segment of the Trans-Mexican Volcanic Belt. *Tectonophysics* **318**, 217–233.

Savage, J.M. 1982. The enigma of the Central American herpetofauna: dispersals or vicariance. *Annals of the Missouri Botanical Garden* **69**, 464–547.

Savitzky, A.H. 1983. Coadapted character complexes among snakes: fossoriality, piscivory, and durophagy. *American Zoologist* **23**, 397–409.

Schargel, W.E., Rivas, G.A., Makowsky, R., Señaris, J.C., Natera, M.A., Barros, T.R., Molina, C.R. & Barrio-Amorós, C.L. 2010. Phylogenetic systematics of the genus *Gonatodes* (Squamata: Sphaerodactylidae) in the Guyana region, with description of a new species from Venezuela. *Systematics and Biodiversity* **8**, 321–339.

Smith, H.M. & Taylor, E.H. 1945. An annotated checklist and key to the snakes of Mexico. *U.S. National Museum Bulletin* **187**, iv + 239 pp.

Smith, H.M. & Taylor, E.H. 1950. Type localities of Mexican reptiles and amphibians. *University of Kansas Science Bulletin* **33**, 313–380.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Stickel, W.H. 1938. The snakes of the genus *Sonora* in the United States and Lower California. *Copeia* **1938**, 182–190.

Stickel, W.H. 1943. The Mexican snakes of the genera *Sonora* and *Chionactis* with notes on the status of other colubrid genera. *Proceedings of the Biological Society of Washington* **56**, 109–128.

Tamura, K., Peterson, D., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–3739.

Taylor, E.H. 1937. A new snake of the genus *Sonora* from Mexico, with comments on *S. michoacanensis*. *Herpetologica* **1**, 69–74.

Townsend, T.M., Larson, A., Louis, E. & Macey, J.R. 2004. Molecular phylogenetics of squamata: the position of snakes, amphisbaenids and dibamids and the root of the squamate tree. *Systematic Biology* **53**, 735–757.

Vawter, L. & Brown, W.M. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* **234**, 194–196.

Vidal, N. & Hedges, S.B. 2009. The molecular evolutionary tree of lizards, snakes, and amphisbaenians. *Comptes Rendus Biologies* **332**, 129–139.

Wiley, E.O. 1978. The evolutionary species concept reconsidered. *Systematic Biology* **27**, 88–92.

Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. 2004. A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. http://ceb.csit.fsu.edu/awty, accessed 9 February 2012.

Yang, Z. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Science USA* **107**, 9264–9269.

Zaher, H. & Prudente, A.L.C. 2003. Hemipenes of *Siphlophis* (Serpentes, Xerodontinae) and techniques of hemipenial preparation in snakes: a response to Dowling. *Herpetologial Review* **34**, 302–307.

Zweifel, R.G. & Norris, K.S. 1955. Contributions to the herpetology of Sonora, Mexico. *American Midland Naturalist* **54**, 230–249.

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