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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; Ramamooorthy 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 Chilomeniscus, Chionactis, Conopsis, Ficimia, Gyalopion, Pseudoficimia,
Stenorrhina and Sympholis (Dowling, 1975; Dowling & Duellman, 1978), although some authors include Tantilla and Geastris, 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 semiannullata 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., 2004a, 2004b, 2004c) 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 Dugès in Cope (1885) is currently known from the Balsas basin of Michoacán, 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., 2004a, 2004b, 2004c). 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

Molecular systematics of the genus *Sonora*
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 out-group 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.

<table>
<thead>
<tr>
<th>#</th>
<th>Voucher ID</th>
<th>Taxon</th>
<th>Country: State</th>
<th>Locality</th>
<th>Lat</th>
<th>Long</th>
<th>Elevation (m)</th>
<th>cyt-b</th>
<th>ND4</th>
<th>c-mos</th>
<th>RAG-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UANL 6976</td>
<td><em>Sonora</em> (<em>Procinctura</em>) <em>aemula</em></td>
<td>Mexico: Sonora</td>
<td>near Alamos</td>
<td>27.02458</td>
<td>−108.9397</td>
<td>400</td>
<td>JQ265959</td>
<td>JQ265979</td>
<td></td>
<td>JQ265952</td>
</tr>
<tr>
<td>2</td>
<td>ASDM 21449</td>
<td><em>S. aemula</em></td>
<td>Mexico: Sonora</td>
<td>near Alamos</td>
<td>27.02458</td>
<td>−108.9397</td>
<td>400</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>ASV 206503</td>
<td><em>S. semiamuscula</em></td>
<td>USA: California</td>
<td>Inyo County near Bishop</td>
<td>36.24532</td>
<td>−117.4531</td>
<td>907</td>
<td>AF471048</td>
<td>JQ265981</td>
<td>AF471164</td>
<td>JQ265970</td>
</tr>
<tr>
<td>4</td>
<td>MZFC 23956</td>
<td><em>S. michoacanensis</em></td>
<td>Mexico: Guerrero</td>
<td>Campo Morado, Canada ‘El Naranjo’</td>
<td>18.19316</td>
<td>−100.1609</td>
<td>1072</td>
<td>JQ265958</td>
<td>JQ265980</td>
<td>JQ265951</td>
<td>JQ265969</td>
</tr>
<tr>
<td>5</td>
<td>UTA BTM 26d</td>
<td><em>S. mutabilis</em> (<em>aequilis</em>)</td>
<td>Mexico: Jalisco</td>
<td>Barranca del Rio Santiago</td>
<td>20.79239</td>
<td>−103.3297</td>
<td>107</td>
<td>JQ265954</td>
<td>JQ265975</td>
<td>JQ265945</td>
<td>(a) JQ265967; (b) JQ265968</td>
</tr>
<tr>
<td>6</td>
<td>UTA R-53488</td>
<td><em>S. mutabilis</em> (<em>aequilis</em>)</td>
<td>Mexico: Jalisco</td>
<td>near Bolanos</td>
<td>21.87539</td>
<td>−103.8207</td>
<td>1633</td>
<td>JQ265953</td>
<td>JQ265973</td>
<td>JQ265947</td>
<td>JQ265962</td>
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<tr>
<td>7</td>
<td>UTA JRV 127</td>
<td><em>S. mutabilis</em> (<em>aequilis</em>)</td>
<td>Mexico: Jalisco</td>
<td>Huastla: canyon below town</td>
<td>20.72845</td>
<td>−103.6567</td>
<td>1450</td>
<td>JQ265955</td>
<td>JQ265976</td>
<td>JQ265950</td>
<td>NA</td>
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<tr>
<td>8</td>
<td>UTA JRV 129</td>
<td><em>S. mutabilis</em> (<em>aequilis</em>)</td>
<td>Mexico: Jalisco</td>
<td>Huastla: canyon below town</td>
<td>20.72845</td>
<td>−103.6567</td>
<td>1450</td>
<td>JQ265956</td>
<td>JQ265978</td>
<td>NA</td>
<td>(a) JQ265960; (b) JQ265951</td>
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<td><em>S. mutabilis</em></td>
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<td>near Bolanos</td>
<td>21.87539</td>
<td>−103.8207</td>
<td>1633</td>
<td>NA</td>
<td>JQ265972</td>
<td>JQ265946</td>
<td>(a) JQ265965; (b) JQ265966</td>
</tr>
<tr>
<td>10</td>
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<td><em>S. mutabilis</em></td>
<td>Mexico: Jalisco</td>
<td>Road to Pueblitos near Barranca del Rio</td>
<td>21.02544</td>
<td>−103.4607</td>
<td>1350</td>
<td>NA</td>
<td>JQ265977</td>
<td>JQ265949</td>
<td>JQ265964</td>
</tr>
<tr>
<td>11</td>
<td>CAS 212760</td>
<td><em>Coluber constrictor</em></td>
<td>USA: California</td>
<td>Mendocino National Forest</td>
<td>39.16058</td>
<td>−122.6681</td>
<td>597</td>
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<td>AY487041</td>
<td>AY486938</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>SDSU 3929e</td>
<td><em>Coluber constrictor</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>EU402841</td>
</tr>
<tr>
<td>13</td>
<td>Moody338e</td>
<td><em>Agristosodon contortix</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>AF156576</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>LSU H0607e</td>
<td><em>Agristosodon contortix</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>EU483403</td>
<td>NA</td>
<td>NA</td>
<td>EU402833</td>
</tr>
<tr>
<td>15</td>
<td>CAS 214406e</td>
<td><em>Agristosodon piscivorous</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>AF471096</td>
</tr>
</tbody>
</table>

Numbers correspond to localities in Fig. 2. Voucher IDs are either museum numbers or field numbers. This sequence is published in Holm (2008). Field notes and tissues for UTA BTM and UTA JRV specimens are deposited at the UTA ARDRC. Genes for all outgroup taxa were downloaded from Genbank. Accession numbers for phased RAG-1 sequences are indicated with a and b and correspond to identifiers in Fig. 3.
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 RNAHis and complete and partial tRNASer(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 c-mos 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 an ABI PRISM 3100xl Genetic Analyzer in the Genetics Core Facility at the University of Texas-Arlington or on an 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-I) 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 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

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’)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRCB3</td>
<td>cyt-b</td>
<td>TGA GAA GTT TTC YGG GTC RTT</td>
</tr>
<tr>
<td>GLUDG</td>
<td>cyt-b</td>
<td>TGA CTT GAA RAA CCA YCG TGG</td>
</tr>
<tr>
<td>ND4F</td>
<td>ND4</td>
<td>CAC CTA TGA CTA CCA AAA</td>
</tr>
<tr>
<td>LeuR</td>
<td>ND4</td>
<td>CAT TAC TTT TAC TTG GAT TTG CAC CA</td>
</tr>
<tr>
<td>RAG1_F1a</td>
<td>RAG-1</td>
<td>CAG CTG YAG CCA RTA CCA TAA AAT</td>
</tr>
<tr>
<td>RAG1_R2</td>
<td>RAG-1</td>
<td>CTT TCT AGC AAA ATT TCC ATT CAT</td>
</tr>
<tr>
<td>S77cmos</td>
<td>c-mos</td>
<td>CAT GGA CTG GGA TCA GTT ATG</td>
</tr>
<tr>
<td>S78cmos</td>
<td>c-mos</td>
<td>CCT TGG GTG TGA TTT TCT CAC CT</td>
</tr>
</tbody>
</table>
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+4 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 (cemos = HKY, cyt-b = HKY+G, ND4 = HKY+I, RAG-I = 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) ∼ 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) θ ∼ G (1,10) and τ ∼ 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 measured 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.

<table>
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<th>Catalogue #</th>
<th>Taxon</th>
<th>State</th>
<th>Sex</th>
<th>TBL(^a) (mm)</th>
<th>TL(^b)</th>
<th>Temporals(^c)</th>
<th>Supralabials(^c)</th>
<th>Infralabials(^c)</th>
<th>Ventralis</th>
<th>Subcaudalis</th>
<th>Banding on tail</th>
<th>Source</th>
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\(^a\)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.
Molecular systematics of the genus *Sonora*

**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.
Morphological analysis

Hemipenal 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).

*Scoleophas aemulus* – Amaral (1929)

*S. aemula* – Bogert & Oliver 1945

*Sonora aemula* – Zweifel & Norris 1955

*Procinura aemula* – Lemos-Espinal et al. (2004a)

**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 & Her inghi, 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
Molecular systematics of the genus Sonora

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 ‘Apatzingán, Michoacán’ by Smith & Taylor (1950).

Elapomorphus michoacanensis – Cope (1895)
Homalocranium michoacanense – Gunther (1895)
Chionactis michoacanensis – Cope (1896)
Scolecephis michoacanensis – Boulenger (1896)

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 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
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., 2004a, 2004b, 2004c) 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 taxonomic 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

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-
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 (Burbrik 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; Leach & 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 (Burbrik et al., 2011; Leach & Rannala, 2011), at least if focal taxa are very genetically distinct.

**Historogenetic 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-Martínez 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 redbanded (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**


Molecular systematics of the genus Sonora


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