A multilocus perspective on the speciation history of a North American aridland toad (*Anaxyrus punctatus*)

Robert W. Bryson Jr. a,*, Jef R. Jaeger a, Julio A. Lemos-Espinal b, David Lazcano c

a School of Life Sciences, University of Nevada, Las Vegas, 4505 S. Maryland Parkway, Las Vegas, NV 89154-4004, USA

b Laboratorio de Ecología, UBIPRO, Facultad de Estudios Superiores, Iztacala UNAM, Av. de los Barrios No. 1, Los Reyes Iztacala, Tlalnepantla, Ed. de México 54090, Mexico

c Laboratorio de Herpetología, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León 66440, Mexico

ARTICLE INFO

Article history:
Received 20 January 2012
Revised 19 April 2012
Accepted 21 April 2012
Available online 3 May 2012

Keywords:
Amphibia
Baja California peninsula
*Bufo punctatus*
Chihuahuan Desert
Gene trees
Sonoran Desert
Species trees

ABSTRACT

Interpretations of phylogeographic patterns can change when analyses shift from single gene-tree to multilocus coalescent analyses. Using multilocus coalescent approaches, a species tree and divergence times can be estimated from a set of gene trees while accounting for gene-tree stochasticity. We utilized the conceptual strengths of a multilocus coalescent approach coupled with complete range-wide sampling to examine the speciation history of a broadly distributed, North American warm-desert toad, *Anaxyrus punctatus*. Phylogenetic analyses provided strong support for three major lineages within *A. punctatus*. Each lineage broadly corresponded to one of three desert regions. Early speciation in *A. punctatus* appeared linked to late Miocene–Pliocene development of the Baja California peninsula. This event was likely followed by a Pleistocene divergence associated with the separation of the Chihuahuan and Sonoran Deserts. Our multilocus coalescent-based reconstruction provides an informative contrast to previous single gene-tree estimates of the evolutionary history of *A. punctatus*.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Phylogenetic power from the accumulated signal of many gene trees has been long recognized (Avise and Ball, 1990; Avise, 2000), yet only recently have robust methods for reconstructing evolutionary history from these gene trees been developed (see Edwards, 2009). Among these methods are approaches using multisequence coalescent theory designed to model gene-tree stochasticity that can mislead single gene-tree estimates of evolutionary history (Liu and Pearl, 2007; Liu et al., 2008; Heled and Drummond, 2010). Gene trees are ‘embedded’ inside a species tree by following the stochastic coalescent process back in time along each branch to a most recent common ancestor (Rannala and Yang, 2003; Heled and Drummond, 2010). Using this approach, a species-tree topology, divergence times, and ancestral population sizes can be estimated from a set of gene trees while accounting for gene-tree stochasticity caused by heterogeneous processes such as incomplete lineage sorting.

Multilocus species-tree reconstructions using coalescent theory represent a powerful approach to estimating biogeographically informative demographic parameters (Hickerson et al., 2010). Instead of using gene trees directly to infer demographic history, coalescent methods use genealogies as a transitional parameter to obtain estimates of phylogeographic parameters (e.g., ancestral population sizes, divergence times, and migration rates) given the stochastic timing of coalescent events (Hey and Machado, 2003; Wakeley, 2008; Hickerson et al., 2010). Because phylogeography is rooted in understanding causal relationships between geography, species distributions, and the mechanisms driving speciation (Avise et al., 1987), robust estimates of these parameters are important. As evidenced in recent studies, interpretations of phylogeographic patterns can change when analyses shift from a single gene tree to multilocus coalescent analyses (e.g., Gifford and Larson, 2008; Galbraith et al., 2010; Kubatko et al., 2011).

The red-spotted toad (*Anaxyrus punctatus*, also known as *Bufo punctatus*) is a widespread denizen of the warm deserts of North America (Fig. 1). Because of its broad distribution, *A. punctatus* has been used as a model organism to explore hypothesized vicariant events associated with the early formation of North American deserts (Riddle et al., 2000; Jaeger et al., 2005; Pyron and Burbrink, 2010). Three mitochondrial DNA (mtDNA) haplotype clades of *A. punctatus*, corresponding to the general boundaries of three warm desert regions (Chihuahuan Desert, Sonoran Desert, and Baja California peninsula), diverged during the late Neogene about 5 million years ago (Jaeger et al., 2005). This divergence was attributed to two vicariant events: the early development of the Baja California peninsula, and orogeny associated with secondary uplifting of the Sierra Madre Occidental. In the previous studies...
of *A. punctatus*, however, geographic sampling across central Mexico was lacking, and phylogeographic patterns in co-distributed taxa across this region (Neiswenter and Riddle, 2010; Bryson et al., 2011) indicate the possibility that additional geographic structure may be present in this toad.

Phylogeographic breaks across a variety of co-distributed taxa have been found to be temporally and spatially concordant with those inferred for *A. punctatus* (e.g., Riddle et al., 2000; Jaeger et al., 2005; Leavitt et al., 2007; Bryson et al., 2010, 2011). These interpretations of shared histories among the taxa were based on inferences from gene trees. Explicitly accounting for the inherent stochasticity associated with the gene-tree coalescence, however, might yield different phylogeographic interpretations. For example, coalescent tests of vicariance for 12 species distributed across the Baja California peninsula reveal two separate divergence events, whereas previous studies revealed only one (Leaché et al., 2007).

In this study, we utilize the conceptual strengths of a multilocus coalescent species tree coupled with complete range-wide sampling to expand earlier phylogeographic studies of *A. punctatus*. We first reconstruct a mtDNA gene tree to determine the maternal lineages of new samples of *A. punctatus* from mainland Mexico. We then estimate a species tree and divergence times from our multilocus dataset. We compare our multilocus reconstructions of the evolutionary history of *A. punctatus* with previous hypotheses for this species and co-distributed North American desert species, and propose several potential explanations for the phylogeographic patterns of *A. punctatus*.

### 2. Methods and materials

#### 2.1. Taxon sampling and DNA sequencing

To complete range-wide sampling of *A. punctatus*, we obtained tissues from 22 individuals from 22 localities across the Sonoran and Chihuahuan Deserts and Central Mexican Plateau of Mexico (Fig. 1; Appendix A). These samples were added to the dataset of 192 samples from 82 locations generated in a previous study by Jaeger et al. (2005). We used *Anaxyrus nelsoni* and *Incilius occidentalis* as outgroups (Pauly et al., 2004). Total genomic DNA was extracted from liver or toe clips using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following manufacturer’s recommendations.

For phylogenetic assessment of our new Mexican samples, we sequenced a 666-bp fragment of cytochrome *b* (*cyt-b*) used in the previous study (Jaeger et al., 2005). For species-tree analysis, we sequenced exemplars (*n* = 17; Table 1) representing mtDNA clades for an additional four genes: a segment of the mitochondrial ribosomal RNA 16S (16S, 852 bp), the nuclear intron beta-crystallin (*cryba*, 341 bp), and the nuclear exons proopiomelanocortin (*POMC*, 593 bp) and rhodopsin 1 (*Rho1*, 315 bp). These genes have previously been used to reconstruct bufonid (Pramuk, 2006; Maciel et al., 2010; Thomé et al., 2010) and other anuran (Bryson et al., 2010) phylogenetic relationships. Primer sequences are given in Jaeger et al. (2005; *cyt-b*), Pramuk (2006; 16S), Dolman and Philips (2004; *cryba*), Wiens et al. (2005; *POMC*), and Bossuyt and Milinkovitch (2000; *Rho1*). We generated sequence data following methods described in Bryson et al. (2010), with annealing temperatures for amplifications at 55 °C for *cyt-b* and Rho1, and 57 °C for 16S, *cryba*, and *POMC*. We edited and manually aligned the forward and reverse sequences for each individual using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI). For *cryba* data, which contained numerous indels, an additional sequence alignment was performed with MAFFT v6 using default settings and the G-INS-i algorithm (Katoh et al., 2002; Katoh and Toh, 2008). We identified heterozygous sites in nuclear segments when two different nucleotides were present at the same position in electropherograms of both strands, with the weakest peak reaching at least 50% of the strongest signal. We computationally determined the gametic phase of the variants using PHASE 2.1.1 (Stephens and Donnelly, 2000).
conducted ML analyses using RAxML 7.0.3 (Stamatakis, 2006) without support from the combination of the four runs post-burn-in. We estimated 50% generations as burn-in. We then estimated a final 50% major-convergence, and discarded trees obtained during the first one million generations.

2.3. Species tree reconstruction and divergence dating

We used BEAST (Heled and Drummond, 2010) to estimate a species tree and divergence times from our multilocus dataset. This program, part of the BEAST v1.6.1 package (Drummond and Rambaut, 2007), uses a multispecies coalescent with each individual gene tree embedded in a shared species-tree. We defined the exemplar samples of *A. punctatus* representing the major mtDNA lineages as 'Eastern' (*n* = 7), 'Western' (*n* = 7), or 'Peninsular' (*n* = 3) corresponding to their respective positions in the cyt-b mtDNA tree (see below). Uncorrected pairwise divergences within each of these three lineages was relatively low (less than 0.9%), and each lineage was generally separated by well-defined biogeographic barriers (as discussed below), largely satisfying operational requirements of "species" (Heled and Drummond, 2010).

We selected best-fit models of evolution using MrModeltest, and applied a Yule speciation prior and relaxed uncorrelated lognormal clocks for each gene tree. For the cyt-b and 16S mtDNA data, which represent a single locus, trees were linked in analyses but substitution and clock models were unlinked. Our clock rates were estimated relative to the bufonid cyt-b rate of 6.9 *×* 10^-3 substitutions/site/million years (Macey et al., 1998; Jaeger et al., 2005). We gave the mean rate for each nuclear gene a wide uniform prior distribution with an upper and lower bound of 1 and 0. We ran these analyses for 100 million generations, retaining samples every 1000 generations. Using TRACER, we then visually confirmed acceptable chain mixing, likelihood stationarity, burn-in, and effective sample sizes. After discarding the first 10 million generations (10%) as burn-in, we summarized the parameter values of the samples from the posterior distribution on the maximum credible tree using TreeAnnotator v1.6.1 (Drummond and Rambaut, 2007). We repeated this burn-in and visualization procedure for each of the four embedded gene trees generated by BEAST.

3. Results

3.1. DNA sequence data

The complete cyt-b dataset contained 115 parsimony informative sites. For the reduced species tree datasets, the two mtDNA datasets contained 102 (cyt-b) and 90 (16S) parsimony informative sites. Nuclear loci exhibited approximately a quarter of the variability observed in cyt-b. These genes contained 33 (cryba), 27 (POMC), and 12 (Rho1) parsimony informative sites. We were unable to obtain complete sequence data for three samples: 4446 and 4478 (cryba), and 3416 (POMC). Models of sequence evolution selected for the partitions were GTR + I + G for the cyt-b mtDNA tree dataset, and GTR + G (cyt-b), GTR + I (16S), GTR (POMC), F81 + I (cryba), and HKY + I (Rho1) for the species tree datasets. All sequences were deposited in GenBank (accession numbers JQ947821–JQ947916).

3.2. mtDNA phylogeny

From analyses of cyt-b mtDNA sequences, we inferred strong support for three major lineages of *A. punctatus* (Fig. 2). Our 21 new samples of *A. punctatus* from east of the Sierra Madre Occidental nested within the well-supported (100% PP, 95% BS) Eastern lineage, which was distributed from the Colorado Plateau south across the Chihuahuan Desert and into the Central Mexican Plateau. Our additional new sample from Sonora fell within the Western lineage (98% PP, 60% BS support), which was spread across the Mojave and Sonoran Deserts. The third Peninsular lineage (100% PP, 95% BS support) spanned the lower two-thirds of the

---

**Table 1**

Voucher data and haplotype numbers of *Anaxyrus punctatus* used in multilocus analyses. Site numbers are plotted in Fig. 1. LVT, Las Vegas Tissue Collection; RWB, Robert W. Bryson, Jr., UANL, Universidad Autónoma de Nuevo León; FMQ, Fernando Mendoza-Quijano.

<table>
<thead>
<tr>
<th>Site number</th>
<th>Haplotype</th>
<th>Voucher number</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B01</td>
<td>LVT 1780</td>
<td>Mexico: Baja California: San Francisco de la Sierra</td>
</tr>
<tr>
<td>2</td>
<td>B03</td>
<td>LVT 1789</td>
<td>Mexico: Baja California: Catavina</td>
</tr>
<tr>
<td>3</td>
<td>B07</td>
<td>LVT 4460</td>
<td>Mexico: Baja California: Agua Caliente</td>
</tr>
<tr>
<td>4</td>
<td>W01</td>
<td>LVT 3022</td>
<td>USA: La Madre Spring, Spring MTns., Clark Co., NV</td>
</tr>
<tr>
<td>5</td>
<td>W05</td>
<td>LVT 5621</td>
<td>USA: 10 miles S. of Wikieup, Mohave Co., AZ</td>
</tr>
<tr>
<td>6</td>
<td>W07</td>
<td>LVT 4446</td>
<td>USA: Arivaca Road, 2.2 mi. NW. of Arivaca, Pima Co., AZ</td>
</tr>
<tr>
<td>7</td>
<td>W08</td>
<td>LVT 4478</td>
<td>USA: Dos Cabezas Spring, Anza Borrego Desert State Park, San Diego Co., CA</td>
</tr>
<tr>
<td>8</td>
<td>W14</td>
<td>LVT 5592</td>
<td>USA: Great Falls Basin, Argus Range, Inyo Co., CA</td>
</tr>
<tr>
<td>9</td>
<td>W17</td>
<td>LVT 6338</td>
<td>Mexico: Sinaloa: Hwy 32 N. of Choix</td>
</tr>
<tr>
<td>10</td>
<td>BP4</td>
<td>RWB 7336</td>
<td>Mexico: Sonora: Puente Los Dados, Hwy 16</td>
</tr>
<tr>
<td>11</td>
<td>E01</td>
<td>LVT 5607</td>
<td>USA: Hog Spring, SE. of jct. SR55 and SR276, Garfield Co., UT</td>
</tr>
<tr>
<td>12</td>
<td>E03</td>
<td>LVT 3416</td>
<td>USA: west of Columbus, Luna Co., NM</td>
</tr>
<tr>
<td>13</td>
<td>E16</td>
<td>LVT 5636</td>
<td>USA: Rim of Palo Duro Canyon, Randall Co., TX</td>
</tr>
<tr>
<td>14</td>
<td>BP1</td>
<td>RWB 6189</td>
<td>Mexico: Zacatecas: Concepción del Oro</td>
</tr>
<tr>
<td>15</td>
<td>BP3</td>
<td>RWB 7278</td>
<td>Mexico: Chihuahua: Sierra del Nido</td>
</tr>
<tr>
<td>16</td>
<td>BP5</td>
<td>UANL uncat.</td>
<td>Mexico: Jalisco: Vaquerias</td>
</tr>
<tr>
<td>17</td>
<td>BP17</td>
<td>FMQ 4236</td>
<td>Mexico: San Luis Potosí: km 120 Carr. Valles - Río Verde</td>
</tr>
</tbody>
</table>

(2003). For each nuclear dataset, we conducted five separate runs of 400 iterations each, accepting results with a probability threshold of 0.7 or higher. All polymorphic sites with a probability <0.7 were coded in both alleles with the appropriate IUPAC ambiguity code.
Baja California peninsula. The Eastern, Western, and Peninsular lineages formed a basal polytomy in the BI analysis, but in the ML analysis the Western and Peninsular lineages showed some weak support (>50% BS) as sister taxa. Uncorrected mean pairwise sequence divergences, as calculated using MEGA 5.05 (Tamura et al., 2011), were 6.47% between the Eastern and Western lineages, 6.55% between the Eastern and Peninsular lineages, and 6.92% between the Western and Peninsular lineages. Within-group mean pairwise divergences were 0.80% (Eastern), 0.86% (Western), and 0.40% (Peninsular).

3.3. Species tree and divergence time estimation

Gene trees for each of the four loci embedded in the species tree revealed a high level of heterogeneity in tree topologies, and no individual genes produced the same tree topology (Fig. 3). Our species tree reconstruction showed an early divergence of the Peninsular lineage from a common ancestor of the Eastern and Western lineages. The Eastern and Western lineages were strongly supported (99% PP) as sister lineages (Fig. 3). We estimated the Peninsular lineage to have diverged during the late Miocene–late Pliocene (mean estimated date 4.5 Ma, 95% posterior credibility interval = 2.2–6.7 Ma). The divergence between the Eastern and Western lineages appeared to have occurred later during the Pleistocene (1.4 Ma, 0.6–2.4 Ma). Noticeably, the 95% posterior credibility intervals overlapped by only 0.2 Ma.

4. Discussion

4.1. Speciation and biogeography

Previous research based on mtDNA patterns suggested that two vicariant events – the early formation of the Baja California peninsula and the secondary uplift of the Sierra Madre Occidental – were primarily responsible for the simultaneous divergence between Eastern, Western, and Peninsular lineages of *Anaxyrus punctatus* (Riddle...
Fig. 3. Multilocus species tree for *Anaxyrus punctatus* reconstructed using the ‘BEAST’ program. Bars indicate 95% highest posterior densities of divergence dates, with mean estimates in millions of years (Ma) given at nodes. Shown below the species tree are each of the four embedded gene trees used by the ‘BEAST’ program. All major nodes that received >95% Bayesian posterior probability are depicted with black dots.
et al., 2000; Jaeger et al., 2005). These purportedly concurrent separations were estimated to have occurred at about 5 Ma near the Miocene–Pliocene boundary. Our results using range-wide sampling and multilocus coalescent analyses provide a new perspective on the role that these processes may have had on driving speciation in *A. punctatus*.

### 4.1.1. Baja California peninsula

The historical development of the Baja California peninsula has been heavily debated in the literature (Carreño and Helenes, 2002; Lindell et al., 2006; Hurtado et al., 2010; Wilson and Pitts, 2010). In particular, the existence and timing of a mid-peninsular seaway has been hotly contested. Many co-distributed species exhibit seemingly congruent genetic breaks around the mid-peninsular region (Riddle et al., 2000; Lindell et al., 2006; but see Leaché et al., 2007), yet the causal mechanisms driving this pattern are disputed, ranging from a proposed mid-peninsular seaway (Upton and Murphy, 1997; Riddle et al., 2000) to a break caused by ecological turnover (Grismer, 2002). Recent studies suggest that multiple mid-peninsular vicariant events may better explain similar genetic breaks observed in co-distributed taxa (e.g., Crews and Hedin, 2006; Hurtado et al., 2010). This would in part explain why mid-peninsular genetic breaks appear spatially congruent yet temporally discordant (Leaché et al., 2007).

Based on geological and palaeontological evidence, at least three-quarters of Baja California was divided by a mid-peninsular seaway during the late Miocene–Pliocene (Carreño and Helenes, 2002; Lindell et al., 2006). The proposed timing of this vicariant event is concordant with our estimates of initial speciation of the Peninsular lineage in *A. punctatus* at around 4.5 Ma. Although the geographic distribution of the Peninsular lineage extends north of the proposed mid-Baja California break, this pattern has been shown in several species hypothesized to have been split by the mid-peninsular seaway (Murphy and Aguirre-León, 2002), and could potentially be attributed to northern dispersal. Indeed, previous research (Jaeger et al., 2005) revealed evidence of population expansion in the Peninsular lineage of *A. punctatus*.

A second biogeographic scenario may also explain our inferred divergence of the Peninsular lineage. As previously proposed (Riddle et al., 2000; Jaeger et al., 2005), an additional marine transgression near the northern end of the Baja California peninsula (‘northern gulf vicariance’) may have been responsible for the divergence of *A. punctatus* from mainland populations. As with the mid-peninsular seaway, however, the date of this event is debated (Leavitt et al., 2007). Estimated dates for a northern Baja California marine seaway range from 3 Ma (Murphy, 1983; Riddle et al., 2000) to around 7 Ma (Murphy and Aguirre-León, 2002). Geological and fossil data tend to support the latter claim (Dorsey et al., 2007), yet also suggest that the seaway may be several million years older (Lucchitta, 1979; Pacheco et al., 2006). While our mean divergence estimate (4.5 Ma) for separation of the Peninsular lineage appears insufficient to be congruent with the geology, the credibility interval is wide (2.2–6.7 Ma) and encompasses the potential dates for the northern gulf vicariance. Regardless of the exact causal mechanism – a mid-peninsular or northern peninsular seaway – early speciation in *A. punctatus* appears linked to late Miocene–Pliocene development of the Baja California peninsula.

### 4.1.2. Chihuahuan Desert/Sonoran Desert

Divergences in arid-adapted species associated with the separation of the Chihuahuan and Sonoran Deserts are well documented (Riddle and Hafner, 2006; Pyron and Burbrik, 2010), with two processes proposed to explain these ‘East–West’ splits. The oldest process implicates late Miocene–early Pliocene orogeny associated with secondary uplifting of the Sierra Madre Occidental and concurrent climate change as largely driving the development of Chihuahuan and Sonoran Desert biotas (Riddle et al., 2000; Zink et al., 2000). This Neogene vicariant event probably subdivided many taxa spanning both deserts (Riddle and Hafner, 2006; Bryson et al., 2011), with the more strongly desert-adapted taxa likely isolated in separate arid regions by the Sierra Madre Occidental (Pyron and Burbrik, 2010). Many species, however, appear to have maintained populations and connections across the narrow interface between the two deserts north of the Sierra Madre Occidental, an area termed the Cochise Filter Barrier (see Riddle and Hafner, 2006). The second process implicates ecological changes during the Pleistocene as producing unfavorable conditions in this northern area that then promoted isolation between populations within the two desert regions (see Pyron and Burbrik, 2010). These ‘hard’ and ‘soft’ vicariant events appear to have cumulatively promoted and maintained divergent lineages in the Chihuahuan and Sonoran Deserts (Riddle and Hafner, 2006; Pyron and Burbrik, 2010).

Based on our multilocus coalescent analyses, the Eastern and Western lineages of *A. punctatus* diverged in the Pleistocene (Fig. 3). This estimate is temporally concordant with phylogeographic breaks observed along the Cochise Filter Barrier in many other co-distributed taxa (e.g., Castoe et al., 2007; Pyron and Burbrik, 2009), but our estimate is almost several million years later than the approximate 5 Ma divergence date proposed by Jaeger et al. (2005). Gene-tree stochasticity is one plausible explanation for this discrepancy between divergence date estimates. Gene trees necessarily overestimate divergence times since gene-tree estimates do not correct for ancestral polymorphisms (Edwards and Beerli, 2000). On average, gene divergences occur prior to speciation by a factor of 2 Ne generations (Edwards and Beerli, 2000; Arborgast et al., 2002), and divergence times inferred from gene trees can become increasingly unreliable as effective population size increases and time since the species divergence decreases. If ancestral population sizes in the Eastern and Western lineages were large in comparison to the timing of lineage divergence, then the discrepancy between gene-tree and species-tree estimates could be large (Edwards and Beerli, 2000).

Conversely, violations of the assumptions of multilocus species-tree approaches may have skewed our estimated divergence dates. »BEAST interprets discordance between the species and gene trees, as we observed in our data (Fig. 3), to be a result of incomplete lineage sorting. Sharing of alleles between lineages, however, might also be a result of gene flow. Incomplete lineage sorting and gene flow may cause similar genetic patterns, yet operationally these processes each make different predictions regarding the topologies and branch lengths of gene trees that evolve in accordance with the underlying species phylogeny (Holder et al., 2001). We attempted to guard against the possible influence of ongoing gene flow on our species-tree estimates by sequencing numerous exemplar samples from throughout the core distributions (based on geography) of the lineages, and away from the potential contact zones along the Cochise Filter Barrier and Continental Divide (Jaeger et al., 2005).

Our exploration of the coalescent-based species tree provided an informative contrast to the previous single-gene estimates for *A. punctatus* (Riddle et al., 2000; Jaeger et al., 2005). The incorporation of a coalescent model to estimate divergence times likely improved divergence estimates; however, our inferences of the timing of molecular divergences in *A. punctatus*, like the earlier studies, was based on a molecular clock derived from a broad historical vicariance within bionuids (Macey et al., 1998; Jaeger et al., 2005). While the estimated rate appears reasonable, future assessments of molecular evolutionary rates within this group would be useful. Furthermore, it would be interesting to use a larger multilocus dataset and incorporate coalescent models (e.g., Hey and Nielsen, 2007) that can account for bouts of limited gene flow during the transition between a single ancestral species and two
Acknowledgments

We thank the following people, curators, and institutions for providing tissue samples: O. Flores-Villela and A. Nieto-Montes (MZFC, Universidad Nacional Autónoma de México), J.A. Campbell, C. Franklin, and E.N. Smith (University of Texas at Arlington), W. Farr, U.O. García-Vazquez, C. Grünwald, J. Jones, F.R. Mendoza-Paz, the late F. Mendoza-Quijano, and R. Tracy. We thank the numerous people who assisted in the field, including E. Enderson, E. García-Padilla, M.R. Graham, G. Quijano-Manila, F.R. Mendoza-Paz, F. Mendoza-Quijano, and M. Torocco. Collecting was conducted under permits granted by SEMARNAT to R.W.B., D. Lazcano, and J.L.E. For additional support and advice, we thank J. Chaves, R.H. Hansen, A. Nieto-Montes, R. Schell (for use of A. punctatus photo), and B.T. Smith. We thank two anonymous reviewers and A. Larson for providing comments that improved the final manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.04.014.

References


