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MOLECULAR SYSTEMATICS OF THE MIDDLE AMERICAN JUMPING PITVIPERS (GENUS *ATROPOIDES*) AND PHYLOGEOGRAPHY OF THE *ATROPOIDES NUMMIFER* COMPLEX

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ABSTRACT: We used 1400 bp of mitochondrial DNA sequence from two gene fragments (ND4 and cyt-b) to investigate phylogenetic relationships within *Atropoides*, with emphasis on the subspecies of *A. nummifer*. Although many relationships within the genus are strongly supported, monophyly of *Atropoides* was never supported, although it could not be rejected with statistical confidence. In most analyses, the genus was paraphyletic with respect to *Porthidium* and *Cerrophidion*, due to the problematic placement of *A. picadoi*. Our results suggest that the current taxonomy may underestimate species diversity within this group. *Atropoides nummifer* was found to comprise three distinct phylogroups, generally coinciding with the current subspecies recognized under *A. nummifer* but paraphyletic with respect to *A. olmec*. Additionally, disjunct populations previously thought to represent *A. nummifer* in Oaxaca, Mexico, and Baja Verapaz, Guatemala, appear to represent *A. olmec*. We use the phylogeny recovered for *A. nummifer* and *A. olmec* to discuss geological and climatic events that may historically have affected gene flow within this complex.

Key words: *Atropoides*; Central America; Jumping pitvipers; Middle America; *nummifer*; *olmec*; Phylogeny; Phylogeography; *picadoi*; Pitvipers; *Porthidium*; Systematics

THE CLASSIFICATION of the lineages within the subfamily Crotalinae (Viperidae) remains controversial (e.g., Campbell and Lamar, 1989; Parkinson et al., 2002; Werman, 1992; and references therein). One group that has received considerable attention has been what Burger (1971) recognized as the genus *Porthidium* Cope, which consisted of nine species that he removed from the genus *Bothrops* (*barbouri*, *godmani*, *hyoprora*, *lansbergii*, *melanurum*, *nasutum*, *nummifer*, *ophryomegas*, and *picadoi*). Werman (1992) found Burger's *Porthidium* to be paraphyletic based on analyses of allozymes and morphology and erected the genus *Atropoides* to contain *P. picadoi* (Dunn), *P. nummifer* (Ruppell), and *P. olmec* (Perez-Higareda, Smith, and Julia-Zertuche). Werman's conclusions were based on analyses that included *A. picadoi* and *A. nummifer*, but lacked *A. olmec*. The inclusion of *A. olmec* by Werman (1992) within the genus was based on the original description by Pérez-Higareda et al. (1985). Campbell and Lamar (1992) additionally removed *barbouri*, *godmani*, and *tzotzlilorum* from Burger's *Porthidium* and placed them in the new genus *Cerrophidion*. Gutberlet (1998) reallocated *P. melanurum* to the genus *Ophryacus*, and Gutberlet and Campbell (2001) allocated *P. hyoprora* (*Bothrops hypororus*, sensu McDiarmid et al., 1999) to the new genus *Bothrocophias*.

Several published hypotheses of relationships within *Porthidium* (sensu Burger, 1971) based on either morphological and/or limited molecular evidence have been generally incongruent and suggested different positions for *Atropoides* within the Crotalinae (e.g., Brattstrom, 1964; Kraus et al., 1996; Werman, 1992). Based on unspecified interpretations of morphological data, Brattstrom (1964) and Burger (1971) derived evolutionary inferences for the relationships within *Porthidium* (sensu Burger). According to Brattstrom (1964), *A. nummifer* is most closely related to the arboreal genus *Bothriechis*, whereas *A. picadoi*

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and the remaining members of the current genera *Atropoides*, *Cerrophidion*, and *Porthidium* represent another group together with taxa currently assigned to *Bothrops*. In contrast, Burger (1971) found evidence for a monophyletic lineage representing his concept of *Porthidium* and remarked on the similarities among the current members of *Atropoides*. Werman (1992) found *Atropoides* monophyletic and sister to a clade containing *Bothrops*, *Porthidium* (sensu stricto), *Ophryacus*, and *Bothriechis*, which differs from the conclusions of Brattstrom (1964) and Burger (1971). Kraus et al. (1996), however, recovered a paraphyletic *Atropoides* and a clade containing *Porthidium* (sensu stricto), *Cerrophidion*, and *Atropoides*, i.e., the "*Porthidium* group" of Parkinson et al. (2002). The most recent study to use morphological evidence to resolve phylogenetic relationships among pitvipers (Gutberlet and Harvey, 2002) found strong support for a monophyletic *Atropoides*, yet did not recover a monophyletic *Porthidium* group.

Phylogenetic analyses based on multiple genes and intensive taxon sampling within Crotalinae (Parkinson, 1999; Parkinson et al., 2002) have been used in an attempt to clarify relationships within the subfamily. Using DNA sequence data from two mitochondrial ribosomal DNA regions, Parkinson (1999) recovered *Atropoides*, as well as the *Porthidium* group, as monophyletic in all analyses (minus *P. hyoprora*, later allocated to the genus *Bothrocophias*; Gutberlet and Campbell, 2001). These clades, however, had weak bootstrap support (<50%). With two additional mitochondrial gene fragments (cyt-b and ND4), Parkinson et al. (2002) found a monophyletic *Porthidium* group with substantial bootstrap support and inferred that the genus *Porthidium* is sister to a clade containing *Cerrophidion* and a monophyletic *Atropoides*. Within *Atropoides*, they recovered *A. picadoi* as the sister taxon to a clade containing *A. nummifer* and *A. olmec*. However, Parkinson et al. (2002) included only a single representative of each of the three currently recognized species of *Atropoides*.

Snakes of the genus *Atropoides* generally inhabit humid upland forests of Middle America and, to a lesser extent, humid forests at lower elevations (Campbell and Lamar, 1989). Of the three recognized species of *Atropoides*, *A. olmec* reportedly occupies the smallest range, occurring only in the Sierra de Los Tuxtlas in southern Veracruz, Mexico (Campbell and Lamar, 1989; Pérez-Higareda et al., 1985; Fig. 1). The range of *A. picadoi* is more extensive, occurring in lower and middle elevations of Costa Rica and western Panama (Campbell and Lamar, 1989; Fig. 1). The range of *A. nummifer* is the largest of the species, extending from San Luis Potosí, Mexico, to central Panama (Campbell and Lamar, 1989; Fig. 1). Within *A. nummifer* three subspecies are currently recognized (*A. n. nummifer*, *A. n. occiduus*, and *A. n. mexicanus*), yet the relationships among these remain poorly known (Campbell and Lamar, 1989; Perez-Higareda et al., 1985). In addition to the uncertain composition of the group now recognized under the name *A. nummifer*, the status of *A. olmec* as a species distinct from *A. nummifer* is controversial (see Campbell and Lamar, 1989).

In order to clarify the relationships within *Atropoides* and within the *Porthidium* group, we investigated the following: (1) is the genus *Atropoides* monophyletic? (2) are the currently recognized subspecies of *A. nummifer* monophyletic? (3) is *A. olmec* a valid species? and (4) what geological or climatic events may have historically contributed to the diversification of phylogroups within *Atropoides*? To address these issues, the present study includes all recognized subspecies of *Atropoides* represented by multiple localities where possible. Additionally, outgroup taxa including members of the *Porthidium* group were included to investigate monophyly of *Atropoides*. We used DNA sequences obtained from two mitochondrial gene fragments to reconstruct the evolutionary history of *Atropoides*. We present both separate and combined analyses of these two molecular data sets to examine hypotheses relative to the evolution of *Atropoides*. With the resulting phylogenetic hypothesis for the group, we consider geological and climatic phenomena that may have historically shaped patterns of gene flow in these snakes.

Methods

Taxon Sampling

Eighteen samples of the currently recognized species and subspecies of *Atropoides*

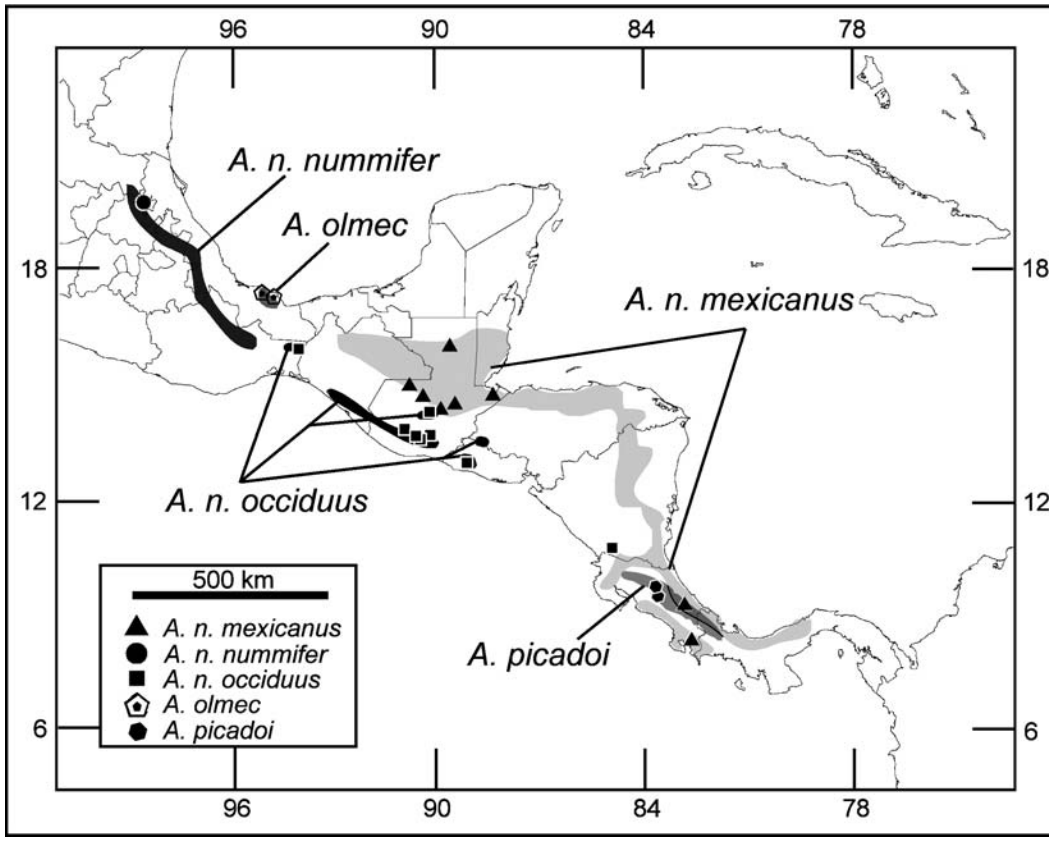

FIG. 1.—Map of Middle America showing sampled localities overlaid on reported distributions for species and subspecies of *Atropoides* (based largely on Campbell and Lamar, 1989).

(sensu McDiarmid et al., 1999), represented by multiple localities (when possible), were examined (Table 1). Additionally, *Bothriechis lateralis*, *B. nigroviridis*, *B. schlegelii*, three samples of *C. godmani* (representing multiple localities), *P. dunni*, *P. lansbergii*, *P. nasutum*, and *P. ophryomegas* were included to investigate the monophyly of *Atropoides* and were left as members of the ingroup (i.e., not forced as an outgroup) for all analyses. *Ophryacus melanurus* and *O. undulatus* were used to root the trees and were designated as outgroup taxa in all analyses, based on previous phylogenetic hypotheses (Parkinson, 1999; Parkinson et al., 2002).

DNA Isolation, Amplification and Sequencing

Fragments of two protein-coding mitochondrial genes were analyzed: NADH dehydrogenase subunit 4 (ND4, 689 bp) and cytochrome b (cyt-b, 711 bp). We chose these genes because they have been used successfully in both higher level and intrageneric studies in pitvipers (e.g., Parkinson et al., 2000, 2002; Wüster et al., 2002; Zamudio and Greene, 1997), thus their rates of evolution seemed appropriate for the objectives of this study.

Genomic DNA was isolated from tissue samples (liver, heart, or scale fragments preserved in 95% ethanol or stored frozen at –80 C, or shed skins) by standard proteinase-K digestion, followed by purification using the DNeasy extraction kit and protocol (Qiagen). The ND4 and cyt-b regions were amplified as described in Parkinson et al. (2002) using the primer pairs: ND4 + ND4His (for ND4), and Gludg + ATRCB3 (for cyt-b). Positive PCR products were cloned using the TOPO-TA cloning kit (Invitrogen). Plasmid DNA was

TABLE 1.—Specimens examined in molecular analyses. Reference ID refers to the names in Figure 3. Institutional acronyms follow Leviton et al. (1985) except: CLP = Christopher L. Parkinson, ENS = Eric N. Smith, JAC = Jonathan A. Campbell, MS = Mahmood Sasa, WWW = Wolfgang W. Wüster. Genbank accession numbers for ND4 and cyt-b gene fragment sequences are given under respective columns.

Taxon	Reference ID	Voucher	Locality	ND4	cyt-b
Atropoides nummifer mexicanus	*A. n. mexicanus* AltaVerapaz GUAT	UTA-R-46616	Guatemala: Alta Verapaz: Finca San Juan	AY220329	AY220306
A. n. mexicanus	*A. n. mexicanus* BajaVerapaz GUAT	UTA-R-35942	Guatemala: Baja Verapaz: Niño Perdito	AY220330	AY220037
A. n. mexicanus	*A. n. mexicanus* Huehuetenango GUAT	UTA-R-32746	Guatemala: Huehuetenango: Finca Chiblac	AY220331	AY220308
A. n. mexicanus	*A. n. mexicanus* Izabal GUAT	UTA-R-35944	Guatemala: Izabal: Puerto Barrios	AY220332	AY220309
A. n. mexicanus	*A. n. mexicanus* Peten GUAT	UTA-R-32419	Guatemala: Peten: San Jose el Espinero	AY220333	AY220310
A. n. mexicanus	*A. n. mexicanus* Quiche GUAT	UTA-R-43592	Guatemala: Quiche	AY220334	AY220311
A. n. mexicanus	*A. n. mexicanus* CR	CLP-168	Costa Rica	U41871	AY223584
A. n. mexicanus	*A. n. mexicanus* Cartago CR	UTA-R-12943	Costa Rica: Cartago: Pavones de Turrialba	AY220335	AY220312
A. n. mexicanus	*A. n. mexicanus* Puntarenas CR	MS	Costa Rica: Puntarenas: San Vito	AY220336	AY220313
A. n. nummifer	*A. n. nummifer* Hidalgo MEX	UTA-R-24842	Mexico: Hidalgo: vicinity of Huejutla	AY220337	AY220314
A. n. occiduus	*A. n. occiduus* Escuintla GUAT	UTA-R-29680	Guatemala: Escuintla: S. of Vol. de Agua	AY220338	AY220315
A. n. occiduus	*A. n. occiduus* Guatemala GUAT	UTA-R-24763	Guatemala: Guatemala: Villa Nueva	AY220339	AY220316
A. n. occiduus	*A. n. occiduus* Solola GUAT	UTA-R-46719	Guatemala: Solola: San Lucas Toliman	AY220340	AY220317
A. n. occiduus	*A. n. occiduus* Sonsonate ES	KU-289807	El Salvador: Sonsonate	AY220341	AY220318
A. olmec (see text)	*A. "n. occiduus"* BajaVerapaz GUAT	UTA-R-34158	Guatemala: Baja Verapaz: Niño Perdito	AY220342	AY220319
A. olmec (see text)	*A. "n. occiduus"* Oaxaca MEX	JAC-9745	Mexico: Oaxaca: Cerro Baul	AY220343	AY220320
A. olmec	*A. olmec*1 Veracruz MEX	UTA-R-25113	Mexico: Veracruz: Sierra de los Tuxtlas	AY220344	AY220321
A. olmec	*A. olmec*2 Veracruz MEX	UTA-R-14233	Mexico: Veracruz: Sierra de los Tuxtlas	AY220345	AY220322
A. picadoi	*Atropoides picadoi* Alajuella CR	CLP-45	Costa Rica: Alajuella: Varablanca	U41872	AY223593
A. picadoi	*A. picadoi* Heredia CR	UTA-R-24821	Costa Rica: Heredia: Sarapiqui	AY220346	AY220323
A. picadoi	*A. picadoi* SanJose CR	UTA-R-23837	Costa Rica: San Jose: Bajo la Hondura	AY220347	AY220324
Cerrophidion godmani	*C. godmani* BajaVerapaz GUAT	UTA-R-40008	Guatemala: Baja Verapaz	AY220348	AY220325
C. godmani	*C. godmani* Oaxaca MEX	JAC-15709	Mexico: Oaxaca: Cerro Baul	AY220349	AY220326
C. godmani	*C. godmani* SanMarcos GUAT	UTA-R-42247	Guatemala: San Marcos	AY220350	AY220327
C. godmani	*Cerrophidion godmani* SanJose CR	MS	Costa Rica: San Jose	AY220351	AY220328
Porthidium nasutum	*P. nasutum*	MZUCR-11150	Costa Rica	U41887	AY223579
P. ophryomegas	*P. ophryomegas*	UMMZ-210276	Costa Rica: Guanacaste	U41888	AY223580
P. dunni	*Porthidium dunni*	ENS-9705	Mexico: Oaxaca: Carr. S. Pedro Pochulla	AY223630	AY223581
P. lansbergii	*P. lansbergii*	WWW-750	Ecuador	AY223631	AY223582
Bothriechis schlegelii	*Bothriechis schlegelii*	MZUCR-11149	Costa Rica	AY223636	AY223590
B. lateralis	*B. lateralis*	MZUCR-11155	Costa Rica	U41873	AY223588
B. nigroviridis	*B. nigroviridis*	MZUCR-11151	Costa Rica	AY223635	AY223589
Ophryacus melanurus	*Ophryacus melanurus*	UTA-R-34605	Mexico	AY223634	AY223587
O. undulatus	*O. undulatus*	CLP-73	Mexico	AY223633	AY223586

isolated using Qiagen Spin-miniprep kits. Multiple clones (at least two, or more in rare cases of single base differences between clones) for each individual were sequenced using the Thermosequenase cycle sequencing kit (USB) with dye-labeled M13 primers on a LI-COR long-read, dual-laser 4200L automated sequencer according to the manufacturer's protocols.

Phylogenetic Analyses

The DNA sequences were edited and aligned initially using the default "align automatically" algorithm in Sequencher v3.0 (Gene Codes Corporation). These alignments were translated into amino acid sequence and checked for the presence of stop codons (with none detected) and similarity in amino acid sequence using GeneDoc (Nicholas and Nicholas, 1997). No manual alignment adjustments were necessary. Sequences were submitted to Genbank (Table 1).

Phylogenies were inferred for each gene as well as their concatenated combination using unweighted maximum parsimony (MP) and maximum likelihood (ML) in PAUP* 4.0b8 (Swofford, 2001). Maximum parsimony and ML searches were conducted using the heuristic search option and employing the tree bisection reconnection (TBR) branch-swapping option. Maximum parsimony searches employed inactive steepest descent option, accelerated character transformation optimization (ACCTRAN), and 100 random-taxon-addition sequence replicates to minimize the effect of taxon addition sequence on resulting tree topology.

For ML analyses, models of sequence evolution were identified that best fit each data set based on hierarchical log likelihood ratio tests of successively complex models using ModelTest version 3.0 (Posada and Crandall, 1998, 2001). The specific model parameters employed for the individual data sets and the combined data set are given below.

Data were subjected to non-parametric bootstrapping (Felsenstein, 1985) with 100 full heuristic pseudoreplicates under both MP and ML criteria to assess support for nodes. For MP bootstrapping, 20 random-taxon-addition sequence replicates were employed for each bootstrap pseudoreplicate. Alternate topologies were tested for significance (at an $\alpha = 0.05$) with a one-tailed Templeton test (Templeton, 1983) following the recommendations of Goldman et al. (2000) for MP topologies and the Shimodaira-Hasegawa (S-H) test (Goldman et al., 2000; Shimodaira and Hasegawa, 1999) with 1000 bootstrap pseudoreplicates for ML topologies. Both of these tests were implemented in PAUP* 4.0b8.

Results

Sequence Data

The combined data set (ND4 + cyt-b) contained 1400 characters: 689 base pairs (bp) from the ND4 gene fragment and 711 from the cyt-b gene fragment. The ND4 data contained 223 characters that were parsimony-informative, and the cyt-b data set yielded 213 parsimony-informative characters. Alignment was unambiguous for all positions. Gaps in alignment were encountered in only one instance in the cyt-b fragment of three outgroup taxa (*O. undulatus*, *O. melanurus*, and *B. nigroviridis*) and represented the absence of a complete codon. No indels were observed in members of *Porthidium*, *Cerrophidion*, and *Atropoides*.

Both gene fragments were similar in base composition. The observed base composition for the L-strand of the combined data was: A = 34.09%; C = 33.05%; G = 10.74%; T = 25.30%. Based on the hierarchical likelihood tests conducted by ModelTest, transition:transversion (ti:tv) ratios for the ND4 and cyt-b fragments were similar, 5.87:1 and 5.19:1, respectively (both estimated under the bestfit model of evolution: HKY + gamma; Hasegawa et al., 1985; Yang, 1993). The best-fit model of evolution for the combined data set corresponded to the TVM + gamma model (Posada and Crandall, 1998; Yang, 1993) with the following parameters: [A–C] = 0.8066, [A–G] = 11.0064, [A–T] = 1.1627, [C–G] = 0.5846, [C–T] = 11.0064, [G–T] = 1.0000, gamma = 0.2627.

Uncorrected percent pairwise sequence divergence (based on the combined data set) was highest between members of *Ophryacus* and *Bothriechis* (17% between *O. melanurus* and *B. lateralis*). Within *Atropoides*, the highest observed pairwise uncorrected divergence was between *A. picadoi* (both samples have identical haplotypes) and *A. n. mexicanus* from

Quiché, Guatemala (11.5%). Within *A. nummifer*, the highest pairwise divergence was between *A. n. occiduus* from Sonsonate, El Salvador, and *A. n. mexicanus* from Quiché, Guatemala (10.1%). Within *A. nummifer*, the greatest pairwise divergence between members of the same subspecies was observed among individuals of *A. n. occiduus* from Sonsonate, El Salvador, and samples from Solola and Escuintla, Guatemala (4.8%; the latter two have identical haplotypes).

Results of Phylogenetic Analyses

Results of the phylogenetic analyses based on ND4 data are summarized in Figure 2A,B. The MP heuristic search of the ND4 data set generated 48 equally parsimonious trees, differing only slightly with respect to the terminal arrangement of Guatemalan populations of *A. n. mexicanus* and *A. n. occiduus* (sensu stricto, as discussed below). Results of phylogenetic searches based on cyt-b sequence data are summarized in Figure 2C,D. The MP analysis of the cyt-b data set (Fig. 2C) yielded 36 equally parsimonious trees, all of which showed the same intergeneric and interspecific relationships, differing only slightly in the terminal arrangement of members of *A. n. mexicanus* and *A. n. occiduus* (sensu stricto).

Topologies resulting from MP and ML phylogenetic reconstructions based on the combined gene data set were similar except for the basal relationships within the *Porthidium* group (Fig. 3). The combined-data MP heuristic search resulted in four most-parsimonious trees differing only in minor rearrangements of members of *A. n. mexicanus* from Guatemala. Tree topologies from this MP search (one of four equally most-parsimonious trees shown in Fig. 3A) were identical to the corresponding ML tree (Fig. 3B) for all major relationships within the "*nummifer* complex" (*A. nummifer* plus *A. olmec*), differing only with respect to minor rearrangements of populations of *A. n. occiduus* and *A. n. mexicanus* from Costa Rica.

The shortest trees, resulting from the unweighted MP search on the combined data set, show a paraphyletic *Atropoides* (1640 steps, one of four optimal trees shown in Fig. 3A). These trees were five steps shorter than the shortest MP trees obtained from a separate search constrained to find topologies consistent with a monophyletic *Atropoides* (1645 steps, not shown). Similarly, the optimal tree recovered from the unconstrained ML search on the combined data yielded a paraphyletic *Atropoides* (Fig. 3B). This optimal ML tree (–ln likelihood = 9277.04053) differed from the corresponding tree resulting from the constrained ML search (–ln likelihood = 9275.73364, not shown) by a –ln likelihood score of 1.30689. In both the MP and ML unconstrained searches, paraphyly of *Atropoides* was indicated by the fact that the clade containing *A. nummifer* and *A. olmec* failed to group with *A. picadoi*, exclusive of other members of the *Porthidium* group.

The Templeton test (Templeton, 1983), comparing the unconstrained and *Atropoides*-monophyly-constrained MP trees, failed to reject the null hypothesis that constraining the topology significantly added to the overall tree length ($p = 0.353$). Similarly, the S-H test (Shimodaira and Hasegawa, 1999), comparing the unconstrained and *Atropoides*-monophyly-constrained ML trees, failed to reject the null hypothesis that constraining the ML topology resulted in significantly less likely topologies ($p = 0.350$).

Discussion

Monophyly of Atropoides

The results of our analyses concur with all previously published phylogenies using only DNA sequence data (except Parkinson et al., 2002, to some extent) in failing to resolve confidently the relationships within the *Porthidium* group or support the monophyly of *Atropoides*. Both the Templeton and S-H tests indicated that the monophyly of *Atropoides* could not be rejected with statistical confidence. Furthermore, based on our limited sampling of the other members of the *Porthidium* group, we feel ancestral state reconstruction at important deeper nodes of the tree may have been inadequate, affecting assessment of basal relationships of *Atropoides* and closely related taxa. Given these considerations, we regard the monophyly of *Atropoides* as uncertain (albeit probable given the findings of Gutberlet and Harvey, 2002 and Parkinson et al., 2002) and prefer to retain the current generic taxonomy pending the results

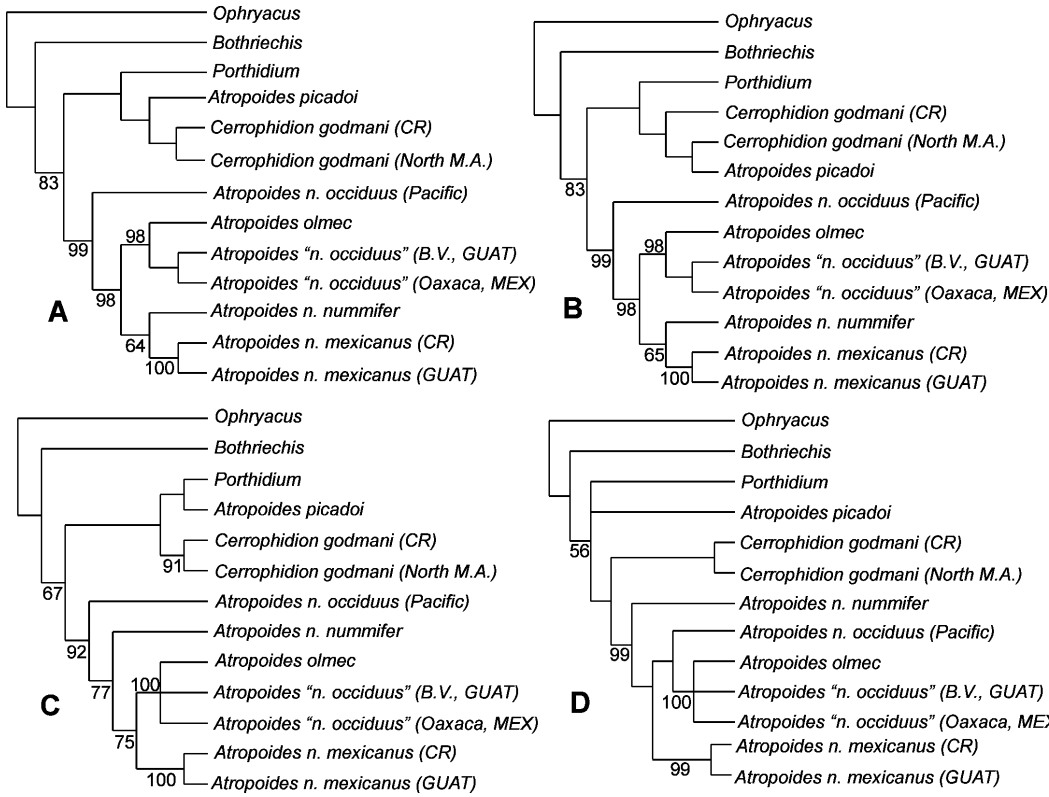

FIG. 2.—Summary trees describing the topological relationships of major clades recovered from individual gene analyses including non-parametric bootstrap values (>50%) based on separate analyses. (A) Major clades recovered from unweighted MP heuristic search on ND4 data. (B) Major clades recovered from ML heuristic search on ND4 gene data. (C) Major clades recovered from unweighted MP heuristic search on cyt-b data. (D) Major clades recovered from ML heuristic search on cyt-b gene data. Abbreviations are as follows: *Cerrophidion godmani* (North M. A.) = specimens of *C. godmani* from Mexico and Guatemala; *Atropoides n. occiduus* (Pacific) = all specimens of *A. n. occiduus* from Guatemala except specimen from Baja Verapaz; *Atropoides "n. occiduus"* (B. V., GUAT) = specimen of *A. n. occiduus* Baja Verapaz GUAT in Table 1; *Atropoides n. mexicanus* (CR) = all specimens of *A. n. mexicanus* from Costa Rica; *Atropoides n. mexicanus* (GUAT) = all specimens of *A. n. mexicanus* from Guatemala. Branches labeled with generic names only refer to clades recovered containing all of their respective members which were sampled. See Table 1 for additional detail regarding specimens and localities sampled.

of an expanded analysis of the *Porthidium* group that is in progress (T. Castoe, M. Sasa, and C. Parkinson, unpublished data).

Based on our phylograms from analyses of the combined data set (Fig. 3), in addition to those presented by Parkinson (1999) and Parkinson et al. (2002), the internodes defining relationships among *Porthidium*, *Cerrophidion*, and *Atropoides* are very short and, thus, seem to indicate that the radiation of these taxa occurred rapidly from a common ancestor. Therefore, the amount of phylogenetic signal in mitochondrial DNA is low for these early divergences. Given the rapid evolutionary rate characteristic of mitochondrial genes, the few characters that were informative about deeper relationships may have been largely obscured by continued sequence turnover (multiple hits) at these sites. This may explain why molecular investigations have repeatedly failed to robustly resolve deeper relationships within the *Porthidium* group.

Intrageneric Relationships within Atropoides

Combined and individual gene analyses indicate that the relationship of *Atropoides picadoi* to the other two species of *Atropoides* (hereafter referred to as the *nummifer* complex) and to *Cerrophidion* and *Porthidium* is uncertain. If *Atropoides* is in fact monophyletic,

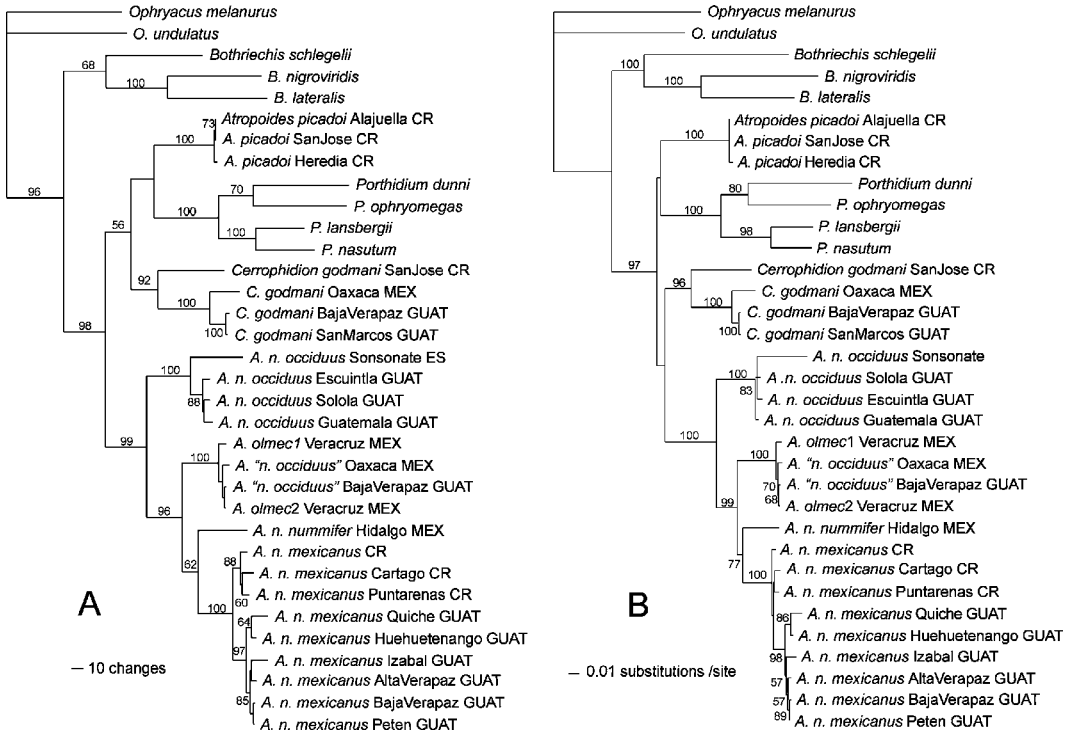

FIG. 3.—(A) One of four equally parsimonious trees resulting from the heuristic MP search (with 100 random-taxon-addition replicates) on the 1401 bp combined gene data (all four optimal MP trees differed only slightly in terminal taxa arrangements); (B) Phylogram resulting from the heuristic ML search on the 1401 bp combined gene data (employing the TVM+G model of evolution). Non-parametric bootstrap values for nodes >50%, from separate analyses, are shown. Abbreviations correspond to individual sample names given in Table 1.

A. picadoi probably represents the sister lineage to the rest of the members of the genus. In analyses in which monophyly of *Atropoides* was constrained (not shown), *A. picadoi* consistently appeared as the sister taxon to a clade containing all other species of *Atropoides*, as was found by Parkinson et al. (2002) in analyses with more limited sampling within *Atropoides*.

Within the *nummifer* complex, *A. n. occiduus* appears to be sister to the rest of the complex in all analyses except the ML cyt-b tree (Fig. 2D). Although the individual gene analyses show varying degrees of support for the relationships among the remaining members of the *nummifer* complex, both MP and ML combined-gene analyses clearly resolved haplotypes of *A. olmec* as nested within those of *A. nummifer*, sister to a clade containing *A. n. nummifer* and *A. n. mexicanus*. Based on the results obtained from the combined and individual gene analyses, haplotypes of *A. nummifer* (as currently recognized) appear paraphyletic with respect to haplotypes of *A. olmec*. This suggests two taxonomic possibilities. The first is to consider *A. olmec* a junior synonym of *A. nummifer*. The alternative is to elevate the currently recognized subspecies of *A. nummifer* (*A. n. nummifer*, *A. n. mexicanus*, and *A. n. occiduus*) to full species. The latter alternative seems most appropriate with respect to the results of the current study. Species recognition, in this case, would be logically consistent with the evolutionary and phylogenetic species concepts (sensu Frost and Hillis, 1990; Mayr, 1982; Wiley, 1978). The evolutionary species concept identifies species as distinct lineages, which will continue to remain so until they either go extinct or undergo additional speciation (Chippindale et al., 2000; Frost and Hillis, 1990; Wiley, 1978), while phylogenetic species concepts (e.g., Cracraft, 1989) simply require species to be diagnosable monophyletic units.

Sequence divergence among major groups of the *nummifer* complex is fairly high (ranging from about 5.5%–10.5% for the combined data set), which suggests millions of years of reproductive isolation. These levels of sequence divergence are also comparable to those between pitviper lineages currently recognized as distinct species (e.g., 6.5%–12.5% among the four species of *Porthidium* sampled). Furthermore, the occurrence of specimens representing *A. n. mexicanus* and what appears to be *A. olmec* (based on our analyses) within the Department of Baja Verapaz, Guatemala, suggests that these species may maintain their identity by some means of reproductive isolation other than allopatry. If this is the case, the biological species concept (sensu Frost and Hillis, 1990) may also be applicable to this situation. Based on these considerations, it is apparent that the taxonomic status of the members of the *nummifer* complex is in need of critical review. A more extensive geographical analysis, including additional specimens of *A. n. nummifer* from southern portions of their range and additional material from disjunct populations, is necessary, in combination with morphological analyses and re-descriptions of the taxa within the *nummifer* complex.

In the original description of *A. olmec* (Pérez-Higareda et al., 1985) and the species account provided by Campbell and Lamar (1989), the range of this species was thought to be confined to the Sierra de Los Tuxtlas of Veracruz, Mexico. In our molecular analyses, a disjunct population that was assigned to *A. n. occiduus* (Campbell and Lamar, 1989) occurring in the Cerro Baúl region of extreme eastern Oaxaca, Mexico, is sister to, and nearly identical in sequence to, specimens of *A. olmec* from the type locality. An additional disjunct population assigned to *A. n. occiduus* occurring in Baja Verapaz, Guatemala, also appears very closely related (nearly identical in DNA sequence) to specimens of *A. olmec*. Furthermore, individuals from these three disjunct populations form a tight clade in every analysis (separate and combined genes) under all methods of phylogenetic reconstruction employed. No published accounts have specifically examined the taxonomic status and morphological characteristics of any of these populations since the recognition of *A. olmec*.

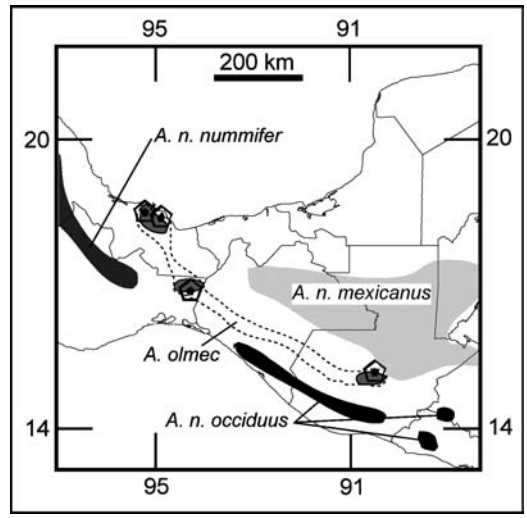

FIG. 4.—Distribution of the members of the *Atropoides nummifer* complex in northern Middle America, including sampled localities for populations referred to *A. olmec* (symbols and shading follow Fig. 1). The currently known disjunct range of *A. olmec* is indicated with dark shading (as in Fig. 1), and the hypothesized historical range of this species is indicated with a dashed border surrounding sampled localities.

Although Pérez-Higareda et al. (1985) made the claim that *A. olmec* is distinct from *A. nummifer* in adjacent regions, all *A. nummifer* they examined were from the Sierra Madre Oriental (the Mexican states of Hidalgo, Querétaro, and Veracruz); they did not examine specimens to the south, in eastern Oaxaca and Chiapas, Mexico, or in Baja Verapaz, Guatemala. Our molecular analyses suggest that these disjunct populations of *Atropoides* inhabiting eastern Oaxaca and Baja Verapaz are in fact *A. olmec* (Fig. 4).

Phylogeography of the Atropoides nummifer *Complex*

Given the current wide distribution of the *A. nummifer* complex, it seems likely that the common ancestor of this group was broadly distributed across major portions of northern Middle America. The earliest divergence within the complex, as indicated by the combined analyses (and all separate analyses except the cyt-b ML analysis), appears to be that of the ancestor of *A. n. occiduus* (Fig. 3) from the remainder of the group. This may

have occurred via the separation of the Pacific flank populations from those to the east and north by uplift and volcanism along the Pacific versant of Guatemala, beginning in the Tertiary, climaxing in the Pliocene, and continuing to the present (Kuenzi et al., 1979; Newhall, 1987). A comparable vicariant event was suggested by Crother et al. (1992) in their account of palm pitviper biogeography, which restricted the ancestor of *Bothriechis bicolor* and *B. marchi* to the Pacific slopes of nuclear Central America.

The common ancestor of *A. n. nummifer* + *A. n. mexicanus* + *A. olmec* appears to have been fragmented in the northern portions of Middle America. Based on our phylograms (Fig. 3), the vicariance of these groups appears to have occurred in rapid succession. A true highland corridor across the Isthmus of Tehuantepec has likely not existed, if at all, since the down-dropping of regions of the Isthmus during the middle Pliocene (Barrier et al., 1998), which may predate the splitting of *A. n. nummifer*, *A. n. mexicanus*, and *A. olmec*. Later, temporary connections between these regions likely occurred during glacial periods of the Pleistocene and subsequently waned during drying and warming cycles accompanying Pleistocene progression (Potts and Behrensmeyer, 1992). These Pleistocene climatic effects may have been responsible for the establishment and subsequent fragmenting of these three groups.

Campbell (1984) pointed out the close relationships of several taxa of reptiles and amphibians occurring in both the Sierra de Los Tuxtlas and the eastern Oaxacan highlands. Based on our phylogenetic analyses, *Atropoides* seems to follow a similar pattern. Our conclusion that *A. olmec* occurs in the interior Guatemalan highlands of Baja Verapaz as well as the Sierra de los Tuxtlas and the highlands of Southeast Oaxaca suggests a recent connection between these currently disjunct highlands. Thus, a highland dispersal corridor linking regions of nuclear Central America (Sierra de los Tuxtlas–Southeast Oaxacan highlands–interior Guatemalan highlands) seems to have recently existed. A similar pattern suggesting a recent corridor for cloud forest species is evident in the phylogeny of *Abronia* lizards, which shows two sister species pairs inhabiting the Sierra de los Tuxtlas and Southeastern Oaxacan highlands (Campbell, 1984; Campbell and Frost, 1993; Chippindale et al., 1998).

Campbell and Lamar (1989) showed the current range of *Atropoides n. mexicanus* (represented as *A. nummifer*) as being continuous from nuclear Central America, through Nicaragua, and into lower Central America (as in Fig. 1). The extent of the range of this taxon in Nicaragua, however, is unknown. Regardless of its current range in Nicaragua, it is likely that in recent (Quaternary) times the range of *A. n. mexicanus* was continuous, allowing at least moderate levels of gene flow between nuclear and lower Central American populations. Evidence for this includes the minor differentiation in DNA sequences (uncorrected pairwise divergence for the combined gene data 2.0%–2.6%, see also Fig. 3) observed between individuals from nuclear Central America and those from lower Central America.

Conclusions

Many authors have reviewed the systematics of neotropical pitvipers, yet hypotheses for relationships among these snakes continue to remain dynamic. Based on this study, the monophyly and the status of the genus *Atropoides* remain largely unresolved. Our sampling of populations currently allocated to *A. nummifer* and *A. olmec* demonstrate that these taxa appear to be members of a species complex, rather than distinct homogenous lineages, as suggested by the current taxonomy. We conclude that *A. nummifer* appears to comprise three distinct phylogroups, generally coinciding with the current subspecies recognized under *A. nummifer* (*A. n. mexicanus*, *A. n. nummifer*, and *A. n. occiduus*) but paraphyletic with respect to *A. olmec*. We find strong support for the disjunct populations previously assigned to *A. nummifer* in Oaxaca, Mexico, and Baja Verapaz, Guatemala, to be re-allocated to *A. olmec*. Although it is difficult to speculate with certainty about the effects of past geological and climatic forces on speciation, the phylogeny of the *A. nummifer* complex does appear to align with the known tectonic and climatic history of Middle America.

Although species diversity present within *Atropoides* does not appear to be sufficiently

represented in the current taxonomy, we do not make any major taxonomic changes here. Additional data from nuclear genes and/or morphology are needed to test whether the mitochondrially-based phylogeny presented here does, in fact, represent the species tree and determine whether *Atropoides* as currently recognized is monophyletic. Mitochondrial data potentially can yield phylogenies incongruent with the species tree under some circumstances (hybridization or introgression of lineages, e.g., Ferris et al., 1983; incomplete lineage sorting, e.g., Neigel and Avise, 1986; or parallel fixation of polymorphic haplotypes in small populations, Wiens and Servedio, 1998). However, theoretical and empirical studies have demonstrated that phylogenies reconstructed from mitochondrial data are more likely to recover the species tree than those based on small numbers of nuclear genes (Moore, 1995) or morphology (e.g., Givnish and Sytsma, 1997; Hedges and Maxson, 1996; Wiens and Penkrot, 2002). Until additional data are available, the evidence supporting four diagnosable, well differentiated monophyletic haplotype groupings within the *nummifer* complex is compelling and will likely form the base for future taxonomic action.

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Literature Cited

Barrier, E., L. Velasquillo, M. Chavez, and R. Gaulon. 1998. Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). Tectonophysics 287: 77–96.

Brattstrom, B. H. 1964. Evolution of pit vipers. Transactions of the San Diego Society of Natural History 13:185–268.

Burger, W. L. 1971. Genera of pitvipers. Ph.D. Dissertation, University of Kansas, Lawrence, Kansas, U.S.A.

Campbell, J. A. 1984. A new species of *Abronia* (Sauria: Anguidae) with comments on the herpetogeography of the highlands of southern Mexico. Herpetologica 40:373–381.

Campbell, J. A., and D. H. Frost. 1993. Anguid lizards of the genus *Abronia*: revisionary notes, descriptions of four new species, a phylogenetic analysis, and key. Bulletin of the American Museum of Natural History 216:1–121.

Campbell, J. A., and W. W. Lamar. 1989. The Venomous Reptiles of Latin America. Cornell Univiversity Press, Ithica, New York, U.S.A.

———. 1992. Taxonomic status of miscellaneous neotropical viperids with the description of a new genus. Occasional Papers of Texas Tech University 153:1–31.

Chippindale, P. T., L. K. Ammerman, and J. A. Campbell. 1998. Molecular approaches to phylogeny of *Abronia* (Anguidae: Gerrhonotinae), with emphasis on relationships in subgenus *Auriculabronia*. Copeia 1998:883–892.

Chippindale, P. T., A. H. Price, J. J. Wiens, and D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. Herpetological Monographs 14:1–80.

Cracraft, J. 1989. Speciation and its ontogeny: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. Pp. 28–59. *In* D. Otte and J. A. Endler (Eds.), Speciation and Its Consequences. Sinaur Associates, Sunderland, Massachusetts, U.S.A.

Crother, B. J., J. A. Campbell, and D. M. Hillis. 1992. Phylogeny and historical biogeography of the palm-pitvipers, genus *Bothriechis*: biochemical and morphological evidence. Pp. 1–20. *In* J. A. Campbell and E. D. Brodie, Jr. (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas, U.S.A.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Ferris, S. D., R. D. Sage, C. M. Huang, J. T. Nielsen, J. T. Ritte, and A. C. Wilson. 1983. Flow of mitochondrial DNA across a species boundary. Proceedings of the National Academy of Science USA 80:2290–2294.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practice: herpetological applications. Herpetologica 46:87–104.

Givnish, T. J., and K. J. Sytsma. 1997. Homoplasy in molecular vs. morphological data: the likelihood of correct phylogenetic inference. Pp. 55–101. *In* T. J. Givnish, and K. J. Sytsma (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, U.K.

Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. Systematic Biology 49:652–670.

Gutberlet, R. L., Jr. 1998. The phylogenetic position of the Mexican black-tailed pitviper (Squamata: Viperidae: Crotalinae). Herpetologica 54:184–206.

Gutberlet, R. L., Jr., and J. A. Campbell. 2001. Generic recognition for a neglected lineage of South American pitvipers (Squamata: Viperidae: Crotalinae), with the description of a new species from the Colombian Chocó. American Museum Novitates 3316:1–15.

Gutberlet, R. L., Jr., and M. Harvey. 2002. Phylogenetic relationships of New World pitvipers as inferred from anatomical evidence. Pp. 51–68. *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, Utah, U.S.A.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

HEDGES, S. B., AND L. R. MAXSON. 1996. Re: Molecules and Morphology in Amniote Phylogeny. Molecular Phylogenetics and Evolution 6:312–319.

KRAUS, F., D. G. MINK, AND W. M. BROWN. 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. Copeia 1996:763–773.

KUENZI, W. D., O. H. HORST, AND R. V. MCGEHEE. 1979. Effect of volcanic activity on fluvial-deltaic sedimentation in a modern arc-trench gap, southwestern Guatemala. Geological Society of America Bulletin 90:827–838.

LEVITON, A. E., R. H. GIBBS, JR., E. HEAL, AND C. E. DAWSON. 1985. Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.

MAYR, E. 1982. The Growth of Biological Thought. Harvard University Press, Cambridge, Massachussetts, U.S.A.

MCDIARMID, R. W., J. A. CAMPBELL, AND T. A. TOURÉ. 1999. Snake Species of the World: A Taxonomic and Geographical Reference, Vol. 1. The Herpetologists' League, Washington, D.C., U.S.A.

MOORE, W. S. 1995. Inferring Phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.

NEWHALL, C. G. 1987. Geology of the Lake Atitlan region, western Guatemala. Journal of Volcanology and Geothermal Research 33:23–55.

NICHOLAS, K. B., AND H. B. NICHOLAS, JR. 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the author at: www.cris.com/~Ketchup/genedoc.shtml.

NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 90–128. *In* M. Miyamoto, and J. Cracraft (Eds.), Phylogenetic Analysis of DNA Sequences. Oxford University Press, New York, New York, U.S.A.

PARKINSON, C. L. 1999. Molecular systematics and biogeographical history of pitvipers as determined by mitochondrial ribosomal DNA sequences. Copeia 1999:576–586.

PARKINSON, C. L., K. R. ZAMUDIO, AND H. W. GREENE. 2000. Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of the cantils. Molecular Ecology 9:411–420.

PARKINSON, C. L., J. A. CAMPBELL, AND P. T. CHIPPINDALE. 2002. Multigene phylogenetic analyses of pitvipers; with comments on the biogeographical history of the group. Pp. 93–110. *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, Utah, U.S.A.

PÉREZ-HIGAREDA, G., H. M. SMITH, AND J. JULIA-ZERTUCHE. 1985. A new jumping viper, *Porthidium olmec*, from southern Veracruz, Mexico. Bulletin of the Maryland Herpetological Society 21:97–106.

POSADA, D., AND K. A. CRANDALL. 1998. Model-test: testing the model of DNA substitution. Bioinformatics 14: 817–818.

———. 2001. Selecting the best-fit model of nucleotide substitution. Systematic Biology 50:580–601.

POTTS, R., AND A. K. BEHRENSMEYER. 1992. Late Cenozoic terrestrial ecosystems. Pp. 419–541. *In* A. K. Brehrensmeyer, J. D. Damuth, W. A. DiMichele, R. Potts, H-D. Sues, and S. L. Wings (Eds.), Terrestrial Ecosystems Through Time—Evolutionary Paleoecology of Terrestrial Plants and Animals. Chicago University Press, Chicago, Illinois, U.S.A.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16:1114–1116.

SWOFFORD, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b7. Sinauer Associates, Sunderland, Massachusetts, U.S.A.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

WERMAN, S. 1992. Phylogenetic relationships of Central and South American pitvipers of the genus *Bothrops* (sensu lato): cladistic analyses of biochemical and anatomical characters. Pp. 21–40. *In* J. A. Campbell, and E. D. Brodie, Jr. (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas, U.S.A.

WIENS, J. J., AND T. L. PENKROT. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Systematic Biology 51:69–91.

WIENS, J. J., AND M. R. SERVEDIO. 1998. Phylogenetic analysis and intraspecific variation: performance of parsimony, likelihood, and distance methods. Systematic Biology 47:228–253.

WILEY, E. O. 1978. The evolutionary species concept reconsidered. Systematic Biology 27:17–26.

WÜSTER, W., M. DA GRACA SALOMÃO, J. A. QUIJADA-MASCAREÑAS, R. S. THORPE, AND BUTANTAN-BRITISH *BOTHROPS* SYSTEMATICS PROJECT. 2002. Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence data. Pp. 111–128. *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, Utah, U.S.A.

YANG, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rate differs over sites. Molecular Biology and Evolution 10:1396–1401.

ZAMUDIO, K. R., AND H. W. GREENE. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. Biological Journal of the Linnaean Society 62:421–442.

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