

Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils

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Abstract

We used mitochondrial DNA sequences from three gene regions and two tRNAs (ND4, tRNA-HIS-SER, 12S, and 16S rDNA) to investigate the historical ecology of the New World pitviper clade *Agkistrodon*, with emphasis on the disjunct subspecies of the cantil, *A. bilineatus*. We found strong evidence that the copperhead (*A. contortrix*) is basal to its congeners, and that the cottonmouth (*A. piscivorus*) is basal to cantils. Phylogeography and natural history of the living terminal taxa imply that *Agkistrodon* primitively occupied relatively temperate habitats, with subsequent evolution of tropicality in ancestral *A. bilineatus*. Our best supported phylogeny rejects three gulf arc scenarios for the biogeography of *A. bilineatus*. We find significant statistical support for an initial divergence between populations on the east and west coasts of México and subsequent occupancy of the Yucatán Peninsula, by way of subhumid corridors in northern Central America. Based on phylogenetic relationships, morphological and molecular divergence, and allopatry we elevate *A. b. taylori* of northeastern México to species status. Taylor's cantil is likely threatened by habitat destruction and small geographical range, and we offer recommendations for its conservation and management.

Keywords: *Agkistrodon*, biogeography, conservation, México, mitochondrial DNA, phylogeography, Serpentes

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Introduction

The pitviper clade *Agkistrodon* (copperheads, cottonmouths, and cantils) includes three widely distributed, polytypic species, collectively spanning a broad range of body sizes, diets, life history traits, and habitats. For example, Trans-Pecos copperheads (*A. contortrix pictigaster*) may reach a total length of ≈ 80 cm, whereas some cottonmouths (*A. piscivorus*) exceed 1.8 m and 4 kg (Gloyd & Conant 1990); bright tail colour is variably associated with caudal luring among and within species of *Agkistrodon* (Carpenter & Gillingham 1990; Strimple 1992), and Taylor's cantil (*A. bilineatus taylori*) exhibits sexual dichromatism, a phenomenon otherwise rare among snakes (Shine 1993).

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Copperheads and cottonmouths occur throughout much of eastern United States (Fig. 1) at sites ranging from mesic desert canyons and upland temperate deciduous forests to coastal swamps and offshore keys, whereas cantils inhabit tropical deciduous forest and thorn scrub in México and Central America (Campbell & Lamar 1989; Gloyd & Conant 1990; Lee 1996; Fig. 2). Thus, given a robust phylogeny, these pitvipers might clarify temperate and tropical components in North American biogeography (see especially Martin & Harrell 1957; Van Devender & Conant 1990), as well as elucidate adaptive divergences within that context.

Cantils, because of their relictual distribution, unusual differentiation in colour pattern, and potentially threatened status, pose interesting problems in historical ecology and conservation biology (Gloyd & Conant 1990; Greene & Campbell 1992). Van Devender & Conant (1990) portrayed them as living remnants of an early Neotropical pitviper

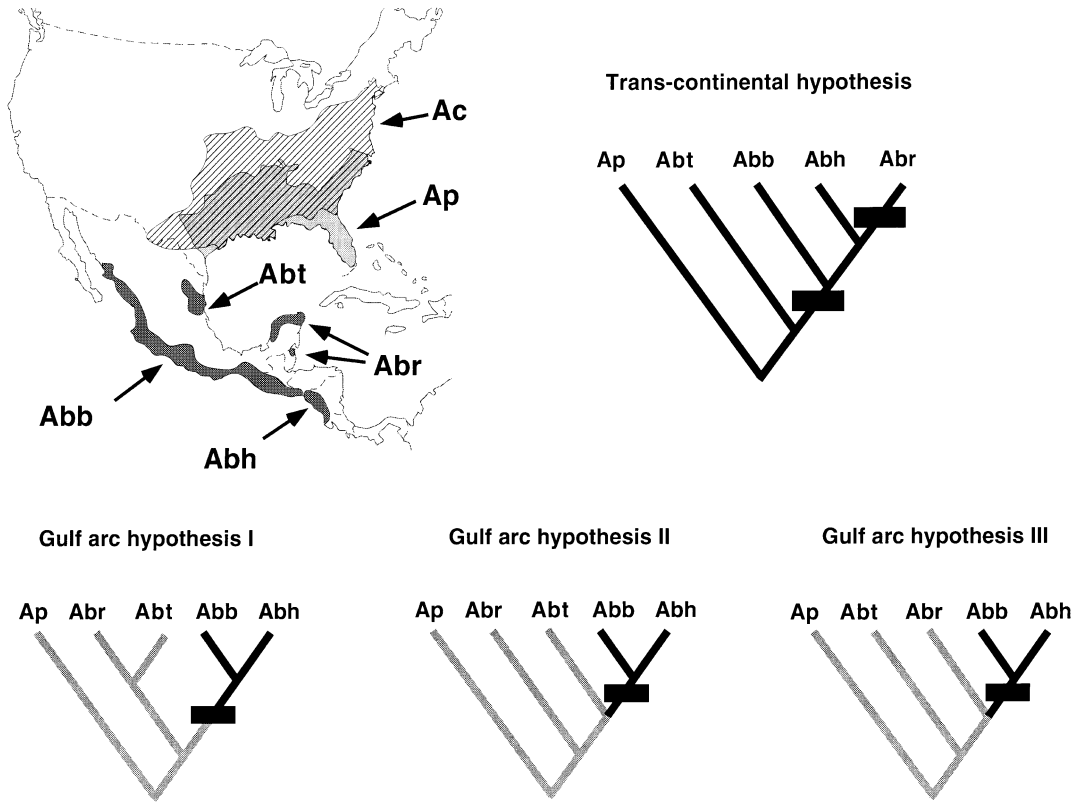


Fig. 1 Biogeographic hypotheses for divergences among subspecies of *Agkistrodon bilineatus*. The map includes the approximate range of the genus *Agkistrodon* in North America, indicating the geographical relationship among all species and subspecies included in this study (Ac = *A. contortrix*, Ap = *A. piscivorus*, Abt = *A. b. taylori*, Abr = *A. b. russeolus*, Abb = *A. b. bilineatus*, Abh = *A. b. howardgloydi*). Four predictions of phylogenetic relationships determined by different biogeographic hypotheses are shown: three involve initial divergence along the east coast of México (gulf arc hypotheses) followed by trans-continental divergence. The fourth hypothesis posits an initial trans-continental divergence, followed by differentiation of the subspecies on the Pacific Coast and Yucatán peninsula. Shaded branches on the phylogenies indicate gulf arc divergences; dark bars indicate dispersal or vicariance events leading to trans-continental divergences among subspecies of *A. bilineatus*.

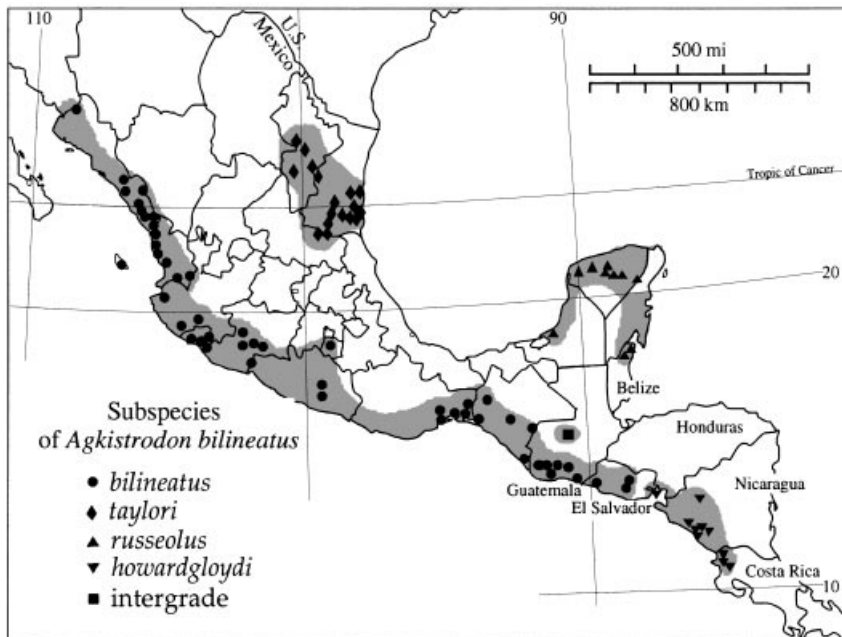


Fig. 2 Geographic range of subspecies of *Agkistrodon bilineatus* (modified from Campbell & Lamar 1989; Gloyd & Conant 1990). Symbols indicate specimens originally examined by Gloyd & Conant (1990), and thus populations that are distinguishable morphologically. The single locality record from central Guatemala represents a morphological intergrade population exhibiting characteristics of the three adjacent subspecies (*A. b. howardgloydi*, *A. b. russeolus*, and *A. b. bilineatus*).

Table 1 Specimens of the New World pitviper genus *Agkistrodon* included in this study. The voucher specimen number, collection locality, and GenBank accession numbers for all gene fragments sequenced are listed for the out-group and in-group taxa

Taxon	Voucher	Locale	12S	16S	Nd4-tRNA
<i>B. insularis</i>	WW	Ilha Queimada Grande, São Paulo, Brasil	AF057216	AF057263	AF188705
<i>C. tigris</i>	CLP169	Pima Co., AZ: US	AF057223	AF057270	AF156574
<i>A. c. contortrix</i>	Moody338	Athens Co., OH: US	AF57229	AF057276	AF156576
<i>A. c. pictigaster</i>	HWG 2218	Terrell Co., TX: US	AF156587	AF156566	AF156577
<i>A. p. piscivorus</i>	CLP30	SC; US	AF057231	AF057278	AF156578
<i>A. p. leucostoma</i>	HWG 2571	LeFlor Co., OK: US	AF156588	AF156567	AF156579
<i>A. b. taylori</i> – 140	CLP140	Tamaulipas, México	AF057230	AF057230	AF156580
<i>A. b. taylori</i> – 142	CLP142	Tamaulipas, México	AF156589	AF156568	AF156581
<i>A. b. taylori</i> – 2	EW	unknown	AF156590	AF156569	AF156582
<i>A. b. bilineatus</i>	Gladys Porter Zoo	unknown	AF156591	AF156570	AF156583
<i>A. b. russeolus</i>	WWL*	Yucatán, México	AF156594	AF156573	AF156586
<i>A. b. howardgloydi</i> – 3	WWL	Guanacaste, Costa Rica	AF156592	AF156571	AF156584
<i>A. b. howardgloydi</i> – 2	WWL	Guanacaste, Costa Rica	AF156593	AF156572	AF156585

* this specimen is pictured in Campbell & Brodie (1992: Plate 2 C and D).

Abbreviations for holdings in private collections: CLP, C. L. Parkinson; Moody, S. M. Moody; HWG, H. W. Greene; WWL, W. W. Lamar; WW, W. Wüster; and EW, E. Wozniak.

radiation, whereas molecular systematic studies (e.g. Knight *et al.* 1992; Parkinson *et al.* 1997; Parkinson 1999) imply that *A. bilineatus* arose within a temperate clade of copperheads and cottonmouths. With disjunct range segments on both versants of Middle America (Campbell 1998; Campbell & Lamar 1989), cantils also have exemplified two influential biogeographic scenarios. Martin (1958) hypothesized a 'gulf arc' pattern for taxa once widely distributed in coastal lowlands, from the Yucatán Peninsula to the southeastern United States ('gulf circumferential' pattern of Auffenberg & Milstead 1963; 'Yucatán-East México' pattern of Lee 1980). Stuart (1954) postulated 'subhumid (i.e. seasonally semi-arid) corridors' through forested southern México and northern Central America, by which xerophytic Pacific Coast species reached Atlantic drainage and even the Yucatán Peninsula ('Yucatán-West México' pattern of Lee 1980; see also Campbell & Vannini 1988; Wilson & McCranie 1998; Campbell 1999).

Inspired by Gloyd & Conant's (1990) monograph and by conflicting, preliminary molecular analyses (Knight *et al.* 1992; Minton 1990), we gathered mitochondrial DNA sequence evidence to investigate the phylogeography of *Agkistrodon*. We emphasized the disjunct subspecies of *A. bilineatus*, and interpreted the results in the context of published morphological studies. Our findings enable a strong choice among alternative biogeographic scenarios for cantils, provide an historical framework for future studies of behavioural and ecological divergence within this clade, and support recognition of *A. b. taylori* as a distinct species. Taylor's cantil is restricted to northeastern México, a region increasingly converted to agriculture, and we briefly comment on its conservation status and management.

Materials and methods

Sampling and laboratory protocols

We used 11 individuals of *Agkistrodon* in our sample, representing two subspecies each of *A. contortrix* and *A. piscivorus* and at least one representative of all described subspecies of *A. bilineatus* (Table 1). *Bothrops insularis* and *Crotalus tigris* were selected as out-group taxa (Kraus *et al.* 1996; Parkinson 1999). Fragments of three mitochondrial genes and two tRNAs were sequenced for this analysis: ND4, tRNA-HIS-SER, 16S, and 12S rDNA. Because these genes exhibit different levels of variability, we expect that in combination they will resolve relationships at various depths in the phylogenetic tree.

Genomic DNA was isolated from whole blood or from epidermal tissue samples by standard proteinase K digestion followed by organic purification (Parkinson *et al.* 1997). The ND4-tRNA region was amplified using the primers ND4 (5'-CACCTATGACTACCAAAGCTCATGTAGAAGC-3') and LEU (5'-ACCACGTTTAGGTTTCATTAC-3') under amplification conditions described in Arévalo *et al.* (1994) and Zamudio & Greene (1997). The ribosomal genes were amplified using the primers L1091F (5'-AAACTGGGA TTAGATACCCCACTAT-3') and H1557R (5'-GTACACTTA CCTTGTTACGACTT-3') for the 12S fragment, and L2510F (5'-CGCCTGTTTATCAAAA-CAT-3') and H3059R (5'-CCGGTCTGAACTCAGATCACGT-3') for the 16S fragment, under conditions described in Parkinson (1999) and Parkinson *et al.* (1997). The 12S and 16S rDNA sequences for *B. insularis*, *C. tigris*, *A. contortrix*, *A. piscivorus* and *A. b. taylori*-140 have been previously

published (Parkinson 1999). We amplified all three gene regions from all individuals included in this study. In a few cases, PCR amplifications did not yield adequate sequence (*A. b. russeolus*-12S sequence and *A. b. russeolus*, *A. b. taylora*-142 ND4 sequences). In these cases the PCR products were cloned, using the TA cloning Kit (Invitrogen, Palo Alto, CA, USA); subsequently, plasmid DNA was isolated using the PERFECTprep plasmid purification system (5' → 3' INC, Boulder, CO, USA). Multiple clones for each species were sequenced. All amplified fragments and cloned fragments were sequenced using ABI fluorescent dye terminator chemistry on an ABI 377 automated sequencer (ABI FS and ABI BigDye: Applied Biosystems, Perkin-Elmer, Foster City, CA, USA) according to manufacturer's protocols. The 12S and 16S regions were completely sequenced in both directions using the amplification primers. The ND4-tRNA segment was sequenced from both directions with the amplification primers, and in most cases with one internal sequencing primer (HIS, 5'-CACTGCCTAATGTTTTGT-3'; Arévalo *et al.* 1994) resulting in 70–100% overlap between the fragments.

Alignment and phylogenetic reconstruction

Positional homology was determined for the 12S and 16S rDNA gene fragments based on secondary structure (Parkinson 1999). The coding region of the ND4 gene was aligned based on the proposed amino acid sequence, while the alignment of the tRNA genes was accomplished using Clustal W (Thompson *et al.* 1994) using the slow/accurate option and having a gap opening penalty of 10 and a gap extension penalty of 0.05.

Pairwise sequence divergences and levels of saturation were examined for all codon positions and mutation types (transitions and transversions) for ND4, and by mutation types for the noncoding regions we sequenced. This was accomplished by plotting uncorrected per cent sequence divergences against Tamura corrected estimates for transitions (ti) and transversions (tv) separately using MEGA, version 1.01 (Kumar *et al.* 1993). Saturation graphs and sequence alignments can be downloaded from <http://sunflower.bio.indiana.edu/~cparkins>. Our results suggest that only third position transitions of the ND4 region are potentially saturated and thus phylogenetic analyses including these data may be biased due to multiple hits (Aquadro & Greenberg 1983; Swofford *et al.* 1996). We chose three a priori weighting schemes to explore the effect this bias may have on phylogenetic reconstruction. The ratio of transitions to transversions (ti:tv) within the ND4 data set as estimated using maximum likelihood (ML: under the F84 model without rate heterogeneity; Felsenstein 1993), was 3.48, thus we chose to perform three maximum parsimony (MP) analyses down weighting third position transitions by a factor of 10, 25, and 50%.

Phylogenetic inference was carried out on the individual gene data sets using MP, while the combined data set was analysed using both MP and ML, with the program PAUP* beta 2 (Swofford 1999). We employed an exhaustive search algorithm for the initial parsimony analyses of the unweighted individual data sets and on the combined data set. All other parsimony analyses consisted of Branch and Bound searches using the following options: addition sequence = furthest, initial upperbound computed via stepwise addition and 'Multrees' option in effect. For the ML analyses we used the F84 model with rate heterogeneity, estimating from the data via ML the ti:tv ratio, the gamma parameter and proportion of invariable sites. For both MP and ML, we first estimated the best phylogeny with all characters weighted equally. In MP we also reconstructed the phylogeny with the three differential weighting schemes for third position transitions of the ND4 region described above.

Bootstrapping (Felsenstein 1985), relative-likelihood support (RLS; Jermin *et al.* 1997) and decay indices were used to determine nodal support. Parsimony bootstrapping was conducted in PAUP*. ML bootstrapping analyses were performed in fastDNaml version 1.06 (Olsen *et al.* 1994). The bootstrapped data sets were generated using the SEQBOOT program in PHYLIP version 3.5c (Felsenstein 1993) and bootstraps calculated using the CONSENSE module in the same program. For these analyses, the F84 model was used, input order was jumbled, swapping across all nodes was allowed, and the ti:tv ratio was input from the previous ML analyses in PAUP*. RLS scores were calculated using the program TreeCons version 1.0 (Jermin *et al.* 1997), using a class V weighting scheme and an α value of 0.05 on 381 best trees determined using the 'keep' option of fastDNaml version 1.01 (Olsen *et al.* 1994). Decay indices were calculated using the HyperCard Stack program Autodecay Version 4.01 (Eriksson 1998) using the same branch and bound options as mentioned previously.

We tested alternative biogeographical hypotheses for this group by comparing our best tree to the topologies expected under four different scenarios for the evolution of this clade (Fig. 1). Alternative trees were generated and compared under both parsimony and likelihood criteria using Templeton's (1983) Wilcoxon signed-ranks test and the KH test (Kishino & Hasegawa 1989) in PAUP*, assuming a level of $\alpha = 0.05$ for statistical significance. The same ML model and the same parameters used in ML phylogenetic reconstruction were used to optimize the branch lengths of all topologies used in the KH tests.

Results

New 12S and 16S rDNA sequences and the ND4-tRNA sequences were deposited in GenBank (Table 1). We

Table 2 Pairwise sequence divergences among all individuals included in this study. Above diagonal: Tamura corrected divergences. Below diagonal: uncorrected sequence divergences (bp, total length 1765 bp)

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>B. insularis</i>	—	0.129	0.110	0.117	0.110	0.099	0.108	0.107	0.106	0.106	0.104	0.106	0.105
2 <i>C. tigris</i>	199	—	0.110	0.115	0.117	0.114	0.115	0.113	0.113	0.116	0.105	0.107	0.105
3 <i>A. c. contortrix</i>	173	179	—	0.017	0.060	0.063	0.064	0.062	0.062	0.060	0.060	0.062	0.063
4 <i>A. c. pictigaster</i>	183	181	30	—	0.060	0.064	0.062	0.060	0.060	0.059	0.059	0.061	0.062
5 <i>A. p. piscivorus</i>	176	187	98	99	—	0.028	0.052	0.051	0.050	0.050	0.046	0.048	0.048
6 <i>A. p. leucostoma</i>	158	183	104	104	51	—	0.042	0.040	0.040	0.044	0.040	0.042	0.041
7 <i>A. b. taylori</i> — 140	171	185	105	102	89	67	—	0.004	0.003	0.032	0.035	0.037	0.035
8 <i>A. b. taylori</i> — 142	171	185	105	102	89	67	8	—	0.002	0.031	0.034	0.035	0.032
9 <i>A. b. taylori</i> — 2	166	177	101	99	83	65	6	6	—	0.030	0.033	0.035	0.033
10 <i>A. b. bilineatus</i>	164	179	97	96	80	72	53	53	51	—	0.017	0.019	0.018
11 <i>A. b. howardgloydi</i> — 3	165	170	98	98	77	66	58	58	55	28	—	0.002	0.004
12 <i>A. b. howardgloydi</i> — 2	169	174	102	102	81	70	62	62	57	32	4	—	0.006
13 <i>A. b. russeolus</i>	166	168	102	102	81	68	58	55	55	29	7	11	—

Table 3 Parsimony bootstrap support for each node in phylogenies reconstructed for each gene and weighting scheme. Comparisons across the table indicate which genes contribute most to resolution of nodes at different depths in our phylogeny

	12S	16S	12S16S	tRNA	ND4	10%	25%	50%	ND4-tRNA	All
Monophyly of <i>Agkistrodon</i>	95	84	95	63	96	58	88	96	100	100
Monophyly of <i>A. contortrix</i>	100	93	100	67	100	74	98	99	99	100
Monophyly of <i>A. piscivorus</i> + <i>A. bilineatus</i>	48	69	65	—	87	86	93	90	73	95
Monophyly of <i>A. piscivorus</i>	44	33	46	11	98	96	97	97	94	96
Monophyly of <i>A. bilineatus</i>	62	27	58	—	99	87	97	98	84	97
Monophyly of <i>A. b. taylori</i>	88	49	92	88	100	100	100	100	100	100
Monophyly of <i>A. b. bilineatus</i> + <i>A. b. russeolus</i> + <i>A. b. howardgloydi</i>	—	61	61	17	99	90	95	95	97	99

% refers to the downweighting of third position transitions in the ND4 fragment.

obtained 822 base pairs (bp) for the ND4-tRNA region (231 amino acid residues), 416 bp of 12rDNA and 500 bp of 16S rDNA, for a total of 1738 bp of sequence. Sequence divergences (Table 2) within *Agkistrodon bilineatus* subspecies ranged from 0.2% (between specimens of *A. b. howardgloydi*) to 3.7% (between *A. b. taylori*-140 and *A. b. howardgloydi*-2). Corrected sequence divergences between the subspecies of *A. bilineatus* and out-group taxa ranged from 10.4% to 11.5%.

Maximum parsimony analyses of data from individual genes (12S, 16S, ND4, and the tRNAs treated as a block) and from different weighting schemes applied to the ND4 data, resulted in very similar topologies. These analyses varied in their resolution and bootstrap support for each node (Table 3). Nodal support increases when all the gene regions are combined into a single matrix, most probably due to the increase in informative characters. Phylogenetic trees from these individual gene analyses can be found at the senior author's website (<http://sunflower.bio.indiana.edu/~cparkins>). Given the

small differences between topologies reconstructed from the various gene fragments, we focus on the results from our combined analysis.

Maximum likelihood analyses of all data combined and all characters weighted equally resulted in a single tree with a log likelihood score of $-L = 4948.87$ (Fig. 3). Our results confirm the monophyly of *A. bilineatus* (Gloyd & Conant 1990); they also indicate that *A. piscivorus* is the sister taxon of *A. bilineatus* and that *A. contortrix* is basal to those other two species. These relationships are strongly supported by all the methods used to evaluate nodal strength. Within the cantils, *A. b. taylori* diverges first from the remaining subspecies, followed by more recent divergence among *A. b. bilineatus*, *A. b. howardgloydi* and *A. b. russeolus*. Levels of differentiation (as indicated by branch lengths in ML) are substantially larger between *A. b. taylori* and the clade containing the other three subspecies than between any two of those three subspecies (*A. b. taylori* vs. *A. b. bilineatus* = 0.0373, *A. b. bilineatus* vs. *A. b. russeolus* = 0.0188, *A. b. russeolus* vs. *A. b.*

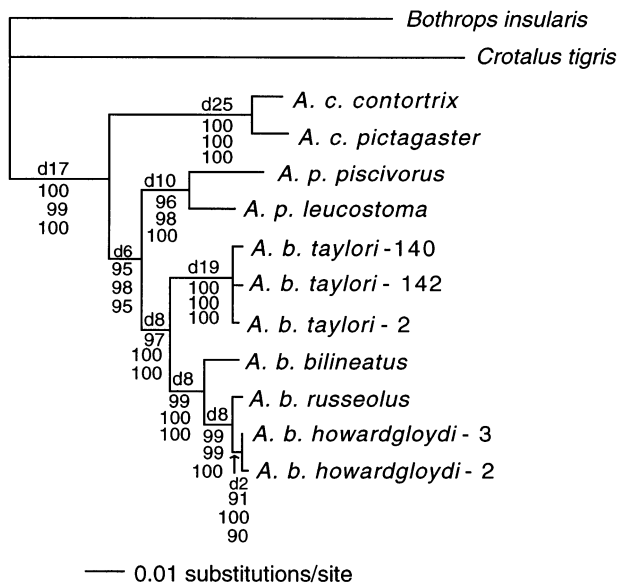


Fig. 3 Phylogenetic relationships among samples of *Agkistrodon*. The phylogeny depicted was reconstructed with maximum likelihood, incorporating rate heterogeneity. Branches with zero length were collapsed into polytomies. The strict consensus of the two shortest maximum parsimony trees yields an identical topology. Numbers above branches represent decay indices (d); numbers below branches are other indices of nodal support: top-MP bootstrap, middle-ML bootstrap, bottom-RLS score.

howardgloydi = 0.0055). Divergences between *A. b. taylori* and the other subspecies of cantils approximates those between *A. piscivorus* and *A. b. taylori* (*A. b. taylori* vs. *A. piscivorus* = 0.0565; *A. b. taylori* vs. *A. b. bilineatus* = 0.0373; *A. b. taylori* vs. *A. b. russeolus* = 0.0376; and *A. b. taylori* vs. *A. b. howardgloydi* = 0.0387).

Maximum parsimony analyses with all characters weighted equally resulted in two most parsimonious trees (CI = 0.7917, HI = 0.2083, L = 509), the strict consensus of which is identical to the topology inferred from the ML analyses (Fig. 3). The degree of support for most nodes, measured by bootstrap values and decay indices is high. The most parsimonious tree suggests significant structure and differentiation among *A. b. taylori* and the other three subspecies in this clade (number of unique character changes for *A. piscivorus* vs. *A. b. taylori* = 85, or *A. b. taylori* vs. *A. b. bilineatus* = 56, or *A. b. bilineatus* vs. *A. b. russeolus* = 29, for *A. b. bilineatus* and *A. b. howardgloydi* = 30, and for *A. b. russeolus* vs. *A. b. howardgloydi* = 9). Downweighting third position transitions for the ND4 sequences (by 10, 25, and 50%) yields two most parsimonious trees for each separate analysis. These inferred trees are the same two topologies recovered in the analyses with all characters weighted equally.

We compared our best topology (Fig. 3) with four possible biogeographical scenarios for the radiation of *A.*

bilineatus (Fig. 1). Templeton and KH tests yield the same results and suggest significant statistical differences between the optimally inferred phylogeny (trans-continental topology: $-L$ 4948.77; total length (tl) = 511) and the three gulf arc topologies (gulf arc I: $-L$ 4998.95, tl = 531, $P < 0.0001$; gulf arc II: $-L$ 4998.95, tl = 532, $P < 0.0001$; gulf arc III: $-L$ 4966.95, tl = 520; $P < 0.003$). Our robust phylogenetic results and the tree comparisons imply that divergences within *A. bilineatus* are best explained by the trans-continental biogeographical hypothesis (Fig. 1).

Discussion

Evolution and biogeography

Van Devender & Conant (1990) asserted that *Agkistrodon b. bilineatus* is the 'surviving ancestor' of its extant congeners, and that the cantil once occupied tropical deciduous forest over much of North America, during the Oligocene or early Miocene (≈ 24 Ma). They speculated that *A. piscivorus* and then *A. contortrix* branched off from *A. b. bilineatus* prior to divergence among the remaining cantil subspecies, during the middle to late Miocene (≈ 12 Ma), when the region, now occupied by the former two species, became increasingly temperate. Van Devender & Conant (1990) thus implied that *A. bilineatus* is paraphyletic, in that the other two species of *Agkistrodon* arose within it.

Subsequent studies suggest instead that the deep history of cantils included a single New World invasion by ancestral Asian pitvipers, followed by divergence into a predominantly Nearctic clade and a mainly Neotropical clade; the former differentiated into common ancestors of *Agkistrodon* and of rattlesnakes (Fig. 4, top; Kraus *et al.* 1996; Parkinson 1999). Interspecific DNA sequence differences might imply a relatively recent origin of *Agkistrodon* (Knight *et al.* 1992), and the oldest fossils conclusively identified as *Agkistrodon* or its immediate out-group (rattlesnakes) date that divergence to minimally late Miocene (≈ 10 – 12 Ma; Conant 1990). Previous analyses of restriction fragment length polymorphism and DNA sequences implied the successive origin of *A. contortrix*, *A. piscivorus*, and *A. bilineatus* from common ancestral stocks (Knight *et al.* 1992; Parkinson *et al.* 1997; Parkinson 1999), and our more extensive study strongly supports this hypothesis.

Regardless of the habitat of the earliest North American pitvipers (subtropical according to Van Devender & Conant 1990), our phylogenetic conclusions imply that ancestral habitats of *Agkistrodon* were temperate, as are those of its most basal living lineages (*A. contortrix* and *A. piscivorus*) and of most rattlesnakes (Klauber 1972). It follows that *A. bilineatus* subsequently occupied increasingly tropical regions, following its divergence from a common ancestor with *A. piscivorus* (Fig. 4, top). One should be aware that

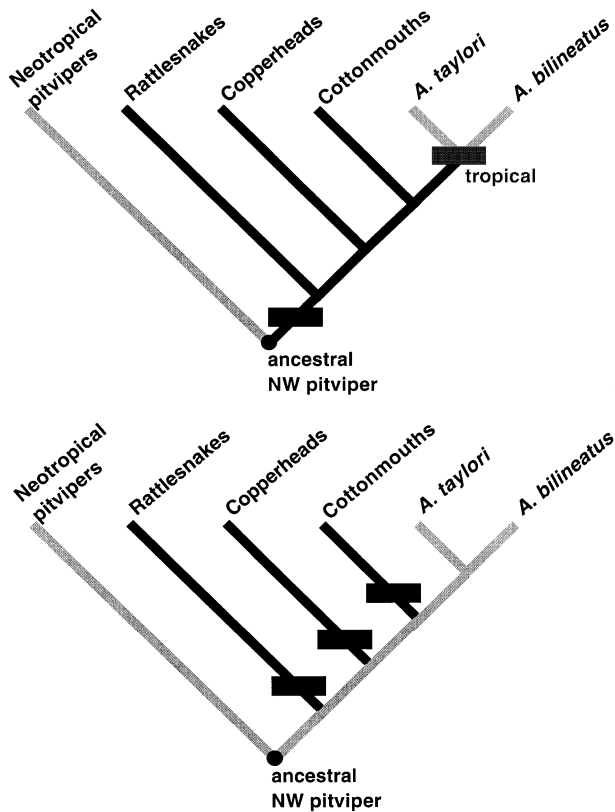


Fig. 4 Phylogenetic perspectives on hypothetical historical shifts in habitat by Nearctic pitvipers; light bars and branches represent tropical habitats, whereas dark bars and branches indicate temperate habitats. Top scenario: common ancestor of *Agkistrodon* and rattlesnakes occurred in temperate areas, with subsequent occupancy of tropical habitats by cantils (*A. taylori* and *A. bilineatus*). Bottom scenario: common ancestor of New World pitvipers is tropical, with subsequent independent occupancy of temperate habitats by ancestral rattlesnakes, copperheads, and cottonmouths (Van Devender & Conant 1990).

New World out-groups to the *Agkistrodon*-rattlesnake clade are tropical (Parkinson 1999), so that even granting our preferred tree, Van Devender & Conant's (1990) hypothesis of tropical ancestry for cantils could prove valid. Phylogenetic evidence that more basal Old World pitvipers were tropical, coupled with fossil evidence that the ancestral landscape of *Agkistrodon* was early Tertiary and could not have been temperate, would be consistent with independent occupancy of temperate habitats by rattlesnakes, copperheads, and cottonmouths (Fig. 4, bottom).

Two nonexclusive scenarios have been proposed for the more recent history of Mexican and Central American *Agkistrodon*: (i) Martin (1958) and Lee (1980) both used cantils as examples of a 'gulf arc' or 'Yucatán-East México' pattern of relictual distributions; and (ii) Lee (1980), Campbell & Vannini (1988), and Gloyd & Conant (1990) interpreted cantil biogeography in terms of Stuart's (1954)

trans-continental corridors across southern México and northern Central America. These scenarios inspire four alternative biogeographic hypotheses, each dependent on a particular cladistic topology and thus potentially falsifiable (Fig. 1). Gulf arc hypotheses I, II, and III require that the immediate common ancestor of *A. b. taylori* and *A. b. russeolus* occupied coastal southeastern México, as would be implied if they were sister taxa or successively basal to other subspecies; tree-comparison analyses conclusively reject the cladograms necessitated by all three gulf arc propositions. The trans-continental alternative requires that *A. b. bilineatus*, *A. b. howardgloydi*, and *A. b. russeolus* are more closely related to each other than any one of them is to *A. b. taylori*, and that *A. b. russeolus* is not basal to the first two subspecies; our phylogeny (Fig. 3) corroborates this hypothesis.

The phylogeography of *A. bilineatus* indicates a former trans-continental distribution that did not initially include the Yucatán Peninsula. Given the restriction of *A. b. taylori*, *A. piscivorus*, and *A. contortrix* to eastern North America, a common ancestor of all other cantils must have dispersed to the Pacific lowlands of Middle America. Our findings do not imply a particular route for that connection, but mountainous habitats in northern México are perhaps too ancient for Martin's (1958) trans-plateau corridor to be relevant for these relatively low elevation snakes (cf. Campbell 1999). Conversely, the distributions of several trans-continental species of pairs of amphibians and reptiles suggest that their common ancestors once occurred across the Isthmus of Tehuantepec and/or northern Central America (Lee 1996; Campbell 1999), as perhaps did ancestral cantils. Our findings also support Gloyd & Conant's (1990) suggestion that the ancestor of *A. b. russeolus* must once have been in contact with Pacific Coast populations of *A. b. bilineatus* and *A. b. howardgloydi*. The disjunct range of the former taxon could result from dispersal to the north through Stuart's (1954) subhumid corridors, or imply that all three subspecies are remnants of a past distribution that extended from Pacific coastal Middle America to the Yucatán Peninsula. Cantils have recently been found in northern Guatemala (Campbell 1998), and clearly more refined phylogeographic studies of that species and other Middle American taxa with disjunct distributions have great potential for elucidating general biogeographic patterns (Campbell 1999; cf. Schneider *et al.* 1998; Walker & Avise 1998).

Species limits and taxonomy

Although Knight *et al.* (1992) remarked on the distinctiveness of Taylor's cantil, there have been no departures from traditional nomenclature for North American *Agkistrodon* in recent decades. Burger & Robertson (1951) described *A. bilineatus taylori* as 'completely distinct'

from other cantils in scalation and colour pattern, and explicitly justified treating it as a subspecies. They expected that intermediates would be found along the Atlantic Coast of México, between the state of Tamaulipas and populations of *A. bilineatus* on the Yucatán Peninsula, and reasoned that even in the absence of intergrades, differences between *A. b. taylori* and other cantils were less than those between sympatric, congeneric *Agkistrodon* elsewhere.

Recent conceptual advances in systematic biology suggest that distinctive, allopatric lineages are best regarded as evolutionary species, and that significant diversity may be hidden by more traditional taxonomic practices (Frost *et al.* 1992; Sites & Crandall 1997; but see Van Devender *et al.* 1992 for a contrary view). Given all available evidence, in particular our DNA sequence data and Gloyd & Conant's (1990) study of far more specimens than were available to Burger & Robertson (1951), we recognize two species among the populations heretofore assigned to *A. bilineatus*. Snakes from western México to northern Central America (*A. b. bilineatus*, *A. b. howardgloydi*) and from the Yucatán Peninsula (*A. b. russeolus*) are characterized by similar and distinctive colour patterns, certain scale character states, and a longer tail than other *Agkistrodon*. Those subspecies are weakly differentiated morphologically, and a geographically intermediate Guatemalan population (see Fig. 2) exhibits morphological features of each of them (Gloyd & Conant 1990), so we agree with previous authors in treating all of those snakes as *A. bilineatus*. Gloyd & Conant (1990) concluded that *A. b. russeolus* and *A. b. howardgloydi* are sister taxa, with *A. b. bilineatus* and *A. b. taylori* successively basal to them, and our molecular analysis agrees with this hypothesis.

Burger & Robertson (1951) distinguished their new subspecies in part on the basis of fewer subcaudal scales than in other *A. bilineatus*; however, that character state is also present in *A. contortrix*, *A. piscivorus*, and most other pitvipers (Gloyd & Conant 1990), and thus is plesiomorphic rather than diagnostic for *A. b. taylori* (cf. Figure 3). Taylor's cantil, nevertheless, clearly is basal to other subspecies of *A. bilineatus*; it diverges in DNA sequences from them to an extent approaching that found among the three other species of *Agkistrodon*. Taylor's Cantil also is diagnosable from all other *Agkistrodon* by unique aspects of head and body colour pattern; it has the lower light line on each side of the head extending downward to the commissure (rather than passing above it, as in *A. bilineatus* [*sensu stricto*]), bright orange or yellow dorsal body markings, and sexual dichromatism in adults (Gloyd & Conant 1990). Reproductive isolation is moot with regard to taxonomy of these snakes, because sympatric *A. contortrix* and *A. piscivorus* can hybridize (Gloyd & Conant 1990), and because *A. b. taylori* is allopatric from other subspecies of *A. bilineatus*. We, therefore, regard Taylor's cantil as a distinct species, *A. taylori* (*comb. nov.*).

We emphasize that recognition of *A. taylori* as a distinct species is based on re-evaluation of the comprehensive morphological and distributional analysis provided by Gloyd & Conant (1990), rather than only on mtDNA sequence comparisons. Our subspecies sampling for the molecular comparisons was limited by the availability of tissues from Mexican populations, but the resulting conclusions are robust, and cantils are morphologically homogeneous among populations of each subspecies (Gloyd & Conant 1990). Additional geographical sampling and the use of multiple mitochondrial and nuclear markers will likely clarify further the evolutionary history of these snakes.

Conservation and management

Cantils are threatened with extinction throughout much of their collective range due to conversion of tropical deciduous forest and thorn scrub for agriculture and other human purposes (Gloyd & Conant 1990; Conant 1992; Greene & Campbell 1992). *A. taylori* occurs only in southern Tamaulipas and adjacent parts of the states of Nuevo León and San Luis Potosí (Fig. 2), and almost two decades ago Burchfield (1982) expressed particular concern for its conservation status. Habitat destruction in that region is accelerating (e.g. for Tamaulipas there has been $\approx 20\%$ reduction in vegetative cover and 13% increase in agriculture during one recent decade), and as yet there are no protected areas that might encompass substantial populations of *A. taylori* (Flores-Villela & Gerez 1994). This species breeds readily in captivity and is now maintained in a number of zoos and private collections (Gloyd & Conant 1990), but as yet there is no strategic programme for captive breeding.

Our conclusion that *A. taylori* is a distinct species with a highly restricted geographical distribution underscores the need for increased emphasis on its conservation. We recommend: (i) a survey for the continued persistence of *A. taylori* at known localities (Gloyd & Conant 1990) and in nearby suitable habitat; (ii) an assessment of factors in addition to habitat destruction that might threaten its survival; (iii) a field study of its behavioural ecology, in the context of conservation needs, with special reference to seasonal movements, diet, and habitat utilization (see Greene 1994; Reinert & Rupert 1999); (iv) a strategy for the genetic management of captive individuals; (v) a management plan that would ensure persistence of sufficient and appropriate habitat; and (vi) educational programmes that would inspire appreciation of and tolerance for this potentially very dangerous snake (Greene & Campbell 1992; Brown 1993). Urgent measures may be required to ensure continued existence of the biogeographically important and spectacularly coloured Taylor's cantil.

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This paper is an outgrowth of our collaborative and separate interests in the evolution, behavioural ecology, and conservation of terrestrial vertebrates. We are especially interested in the application of molecular phylogenetic techniques to those disciplines, and each of us focuses, to some extent, on the biology of vipers.
