MULTIGENE PHYLOGENETIC ANALYSIS OF PITVIPERS, WITH COMMENTS ON THEIR BIOGEOGRAPHY

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ABSTRACT: Recent attempts to determine the phylogenetic relationships among pitvipers (Crotalinae) have continued to improve understanding of their evolutionary history. A robust phylogeny of pitvipers will help elucidate their biogeographical history. Evolutionary relationships of snakes in the subfamily Crotalinae were investigated using multiple mitochondrial gene regions (12S and 16S rDNA, ND4, and cyt-*b* for a total of 2,341 bp). Representatives of all but two currently recognized pitviper genera (*Ermia* and *Triceratolepidophis*) were included. Two members of the Causinae, two genera of the Viperinae, and *Azemiops feae*, were included as outgroups for a total of 61 taxa. Individual gene analyses resulted in topologies that were largely unresolved, and for which support for many nodes was weak; combining all regions for a "total mitochondrial molecular evidence" approach yielded a well-resolved phylogenetic framework with strong support for many relationships. Combined analyses strongly support monophyly of the New World taxa. A clade containing *Gloydius* and *Ovophis monticola* was found to be the sister group of the New World pitvipers in three of four analyses. These results suggest a single invasion into the New World. This emigration event (presumably via the Bering Land Bridge) probably occurred during the early Tertiary or late Cretaceous. A subsequent divergence resulted in a North temperate group and a Neotropical group with at least five subsequent dispersals into South America. All currently proposed New World genera are monophyletic except *Bothrops* and *Sistrurus*, which are paraphyletic with regard to *Bothriopsis* and *Sistrurus ravus*, respectively. The Old World genus *Trimeresurus* (*sensu lato*) is polyphyletic; recognition of *Protobothrops* and *Tropidolaemus*, along with *Trimeresurus* (*sensu stricto*), yields three monophyletic genera. *Ovophis* appears to be polyphyletic; however, a more in-depth study is required before taxonomic revisions can reliably be made. A clade containing *Calloselasma*, *Deinagkistrodon*, *Hypnale*, and *Tropidolaemus* appears to represent the sister group to all other crotalines. The geographic distribution of this group and monophyly of New World pitvipers is consistent with a Eurasian center of origin for the Crotalinae.

INTRODUCTION

The snake family Viperidae currently contains four subfamilies: Causinae, Viperinae, Azemiopinae, and Crotalinae (McDiarmid et al., 1999). The phylogenetic relationships among the subfamilies have not been determined conclusively, but the prevailing view is that Causinae is sister to a group that consists of Viperinae, Azemiopinae, and Crotalinae, and that Viperinae is sister to Azemiopinae + Crotalinae (McDiarmid et al., 1999). The Crotalinae, characterized by the presence of heat-sensory loreal pits, contains approximately 150 species (McDiarmid et al., 1999). In the last three decades, this group has been the focus of numerous systematic studies, using both morphological and molecular data (e.g., Parkinson, 1999; Salomão et al. 1999; Vidal et al., 1999; Werman, 1999; Malhotra and Thorpe, 2000). Currently, 19–22 generic names are in use, and approximately 13 of those have been recognized within the last 30 years (e.g., Hoge and Romano-Hoge, 1981, 1983; Campbell and Lamar, 1992; Werman, 1992; Zang, 1998; Ziegler et al, 2000; Gutberlet and Campbell, 2001). The recent trend in pitviper systematics has been to recognize morphologically distinct monophyletic lineages as separate genera, even if these lineages may be phylogenetically nested within larger, often unwieldy species groups. Many phylogenetic studies of the group have been based on morphological data, but morphological approaches are limited by the fact that snakes are morphologically conservative, and much of the morphological variation that is present is the result of reduction and simplification of homologous structures (Keogh, 1998). This simplification is often reflected by varying degrees of convergence, which makes determination of evolutionary relationships difficult. Until recently, few workers have addressed intergeneric relationships within this diverse group of snakes, with the exceptions of Brattstrom (1964), Burger (1971), and Werman (1992). However, with advances in molecular systematics, numerous molecular studies on generic relationships of pitvipers have been published within the last few years (e.g., Parkinson, 1999; Malhotra and Thorpe, 2000).

Even with the many recent studies, the phylogenetic relationships within the subfamily Crotalinae are still controversial (e.g., Parkinson, 1999; Malhotra and Thorpe, 2000). One reason for the inconsistency among results of the studies is that only two have included most or all of the proposed crotaline genera (Kraus et al., 1996; Parkinson, 1999). Limited taxonomic sampling can be problematic in phylogenetic

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analyses (Hillis, 1998), and when only a few representatives of a diverse group are sampled, the resulting phylogenies may reflect the taxonomic sampling rather than accurately portraying genealogical relationships. Another problem may be that the sequence data utilized are highly homoplastic for the level of divergence being studied.

Certain groupings are common among the recent molecular studies. Several studies indicate that New and Old World *Agkistrodon* do not form a clade (Knight et al., 1992; Kraus et al., 1996; Cullings et al., 1997; Parkinson et al., 1997; Parkinson, 1999). Hoge and Romano-Hoge (1981) proposed *Gloydius* for the Asiatic *Agkistrodon*, and we recommend adoption of their classification. A close relationship between *Calloselasma* and *Hypnale* was found by Kraus et al. (1996) and confirmed by Parkinson et al. (1997) and Parkinson (1999). The genus *Trimeresurus* (*sensu lato*) is not monophyletic (Kraus et al., 1996; Parkinson, 1999; Malhotra and Thorpe, 2000). However, if one recognizes *Protobothrops,* as suggested by Hoge and Romano-Hoge (1983), monophyly is established for the remaining species of *Trimeresurus* (but see discussion below regarding *Ovophis*). Kraus et al. (1996) suggested that New World pitvipers are monophyletic; Parkinson (1999) and Vidal et al. (1999) supported their conclusions, although this clade was not strongly supported in either study. In all analyses by Parkinson (1999), a sister group relationship between the rattlesnakes (*Crotalus* and *Sistrurus*) and copperheads/moccasins (*Agkistrodon*) was established, whereas this result was supported in only one of the analyses by Kraus et al. (1996). Vidal et al. (1999) also found this relationship, but only a single species of each group was used. Few relationships within the bothropoid genera are supported by multiple studies. Exceptions are the close relationship between *Bothrops* (*sensu stricto*) and *Bothriopsis* (but see discussion in Parkinson, 1999), the reallocation of *Porthidium melanurum* into *Ophryacus* (Gutberlet, 1998; Parkinson, 1999), and the recognition of *Porthidium hyoprora* as a lineage that diverged early within *Bothrops* (Kraus et al., 1996; Parkinson, 1999; Gutberlet and Campbell, 2001). These areas of agreement represent an important first step toward an understanding of phylogenetic relationships within Crotalinae.

Recently, the trend in molecular systematic studies has been to utilize multiple gene data sets to reconstruct phylogenetic relationships (e.g., Soltis et al., 1999; Qui et al., 1999; Parkinson et al., 1999). The philosophical and practical implications of this "total molecular evidence" method have been highly debated in the literature (e.g., Bull et al., 1993; Chippindale and Wiens, 1994; de Queiroz et al., 1995; Huelsenbeck et al., 1994), but in practice it has led to a new synthesis in numerous groups (e.g., Mindell et al., 1999; Parkinson et al., 1999; van Tuinen et al., 2000). Accordingly, we used a molecular data set based on multiple mitochondrial genes, incorporating both new data and sequences obtained from databases, to investigate the generic relationships of pitvipers and evaluate competing biogeographical hypotheses that attempt to explain the current distribution of pitvipers.

MATERIALS AND METHODS

Sampling and Laboratory Protocols

The ingroup contained 56 species of pitvipers, including all genera except *Ermia* and *Triceratolepidophis*; two recently proposed monotypic genera of the *Trimeresurus* complex. We used two species of the Causinae, two genera of Viperinae, and *Azemiops feae* (Azemiopinae) as outgroups (see Table 1 for voucher information and GenBank accession numbers). Fragments of four mitochondrial genes were sequenced for this analysis: 12S and 16S rDNA, NADH dehydrogenase subunit 4 (ND4), and cytochrome *b* (cyt-*b*). Because these genes evolve at different rates and exhibit different levels of variability, we expected that in combination they would resolve relationships at various depths in the phylogenetic tree.

Genomic DNA was isolated from whole blood, liver, or epidermal tissue samples by standard proteinase-K digestion followed by organic purification (Knight et al., 1992). The ND4 region was amplified as in Parkinson et al. (2000), and the ribosomal genes were amplified as in Parkinson et al. (1997) and Parkinson (1999). The cyt-*b* region was amplified using the primers Gludg (5'-TGA CTT GAA RAA CCA YCG TTG-3'; Palumbi, 1996) and ATRCB3 (5'-TGA GAA GTT TTC YGG GTC RTT-3'), following the protocol described in Parkinson et al. (2000) for the ND4 gene. In a few cases, PCR amplifications could not be sequenced satisfactorily. In these cases the PCR products were cloned using the TOPO-TA cloning kit (Invitrogen, Palo Alto, California). Subsequently, plasmid DNA was isolated using the PERFECTprep plasmid purification system (5'3' INC, Boulder, Colorado). Multiple clones for each species were sequenced. All newly amplified fragments and cloned fragments were sequenced using ABI fluorescent dye terminator chemistry on an

Table 1. Species used, voucher data, collecting locality, and GenBank accession numbers for each taxon. GenBank accession numbers for all missing cells are availableat http://biology.ucf.edu/~clp/. Field series tags: Cadle = J. Cadle, CLP = C. L. Parkinson, ENS = E. Smith, FK = F. Kraus, JAC = J. A. Campbell, Moody = S. M. Moody, and WWW = W. Wüster

	Species	Voucher	Locality	12S rDNA	16S rDNA	ND4
1	*Atheris nitschei rungwensis*		Tanzania			
2	*Bitis arietans*		Togo	AF057185	AF057232	
3	*Causus defilipii*	CLP 154	Tanzania	AF057186	AF057233	
4	*Causus resimus*	Moody 515	Africa			
5	*Azemiops feae*	CLP 157	China	AF057187	AF057234	AFU41865
6	*Agkistrodon taylori*	CLP 140	Tamaulipas, Mexico	AF057230	AF057230	AF156580
7	*Agkistrodon b. howardgloydi*	Lamar-2	Guanacaste, Costa Rica	AF156593	AF156572	AF156585
8	*Agkistrodon contortrix*	MOODY 338	Athens Co.,Ohio, USA	AF057229	AF057276	AF156576
9	*Agkistrodon piscivorus*	CLP 30	South Carolina, USA	AF057231	AF057278	AF156578
10	*Atropoides nummifer*	CLP 168	Costa Rica	AF057207	AF057254	U41871
11	*Atropoides picadoi*	CLP 45	Varablanca, Costa Rica	AF057208	AF057255	U41872
12	*Atropoides olmec*	JAC 16021	Veracruz, Mexico			
13	*Bothrops ammodytoides*	MVZ 223514	Neuguen Prov., Argentina			
14	*Bothrops asper*	MZUCR 11152	Costa Rica	AF057218	AF057265	U41876
15	*Bothrops alternatus*	DPL 2879				
16	*Bothrops atrox*	WWW-743				
17	*Bothrops cotiara*	WWW	Brazil	AF057217	AF057264	
18	*Bothrops erythromelas*	RG 829	Piranhas, Alagóas, Brazil	AF057219	AF057266	U41877
19	*Bothrocophias hyoprora*		Letícia, Colombia	AF057206	AF057253	U41886
20	*Bothrops insularis*	WWWg	Ilha Queimada Grande, São Paulo, Brazil	AF057216	AF057263	AF188705
21	*Bothrops jararacussu*	DPL 104				
22	*Bothrocophias microphthalmus*	LSUMZ H-9372	Dept. Pasco, Peru			
23	*Bothriechis lateralis*	MZUCR 11155	Acosta, Costa Rica	AF057211	AF057258	U41873
24	*Bothriechis nigroviridis*	MZUCR 11151	San Gerondo de Dota, Costa Rica	AF057212	AF057259	
25	*Bothriechis schlegelii*	MZUCR 11149	Cariblanco de Sarapique, Costa Rica	AF057213	AF057260	
26	*Bothriopsis bilineata smaragdina*		Letícia, Colombia	AF057214	AF057261	U41875
27	*Bothriopsis taeniata*		Suriname	AF057215	AF057262	
28	*Calloselasma rhodostoma*	UTA-R22247		AF057190	AF057237	U41878
29	*Cerrophidion godmani*	MZUCR 11153	Las Nubes de Coronado, Costa Rica	AF057203	AF057250	U41879
30	*Crotalus adamanteus*	CLP 4	St. Johns Co., Florida, USA	AF057222	AF057269	U41880
31	*Crotalus atrox*	CLP 64	Jeff Davis Co., Texas, USA	AF057225	AF057272	
32	*Crotalus molossus*	CLP 66	El Paso Co., Texas, USA	AF057224	AF057271	
33	*Crotalus tigris*	CLP 169	Pima Co., Arizona, USA	AF057223	AF057270	AF156574
34	*Deinagkistrodon acutus*	CLP 28	China	AF057188	AF057235	U41883
35	*Gloydius halys caraganus*		Kazakhstan	AF057191	AF057238	
36	*Gloydius shedaoensis*	ROM 20468	Liaoning, China	AF057194	AF057241	
37	*Gloydius strauchi*	ROM 20473	Waqie Sichuan, Jilin, China	AF057192	AF057239	
38	*Gloydius ussuriensis*	ROM 20452	Kouqian, Jilin, China	AF057193	AF057240	
39	*Lachesis stenophrys*		Limón, Costa Rica	AF057220	AF057267	U41885
40	*Lachesis muta*	Cadle 135	Peru	AF057221	AF057268	
41	*Hypnale hypnale*	CLP 164	Columbo, Sri Lanka	AF057189	AF057236	U41884
42	*Ophryacus undulatus*	CLP 73	Mexico	AF057209	AF057256	
43	*Ophryacus melanurus*	UTA-R 34605	Mexico	AF057210	AF057257	
44	*Ovophis okinavensis*	CLP 162	Okinawa, Japan	AF057199	AF057246	
45	*Ovophis monticola*	ROM 7798	Vietnam			
46	*Porthidium dunni*	ENS 9705	Mexico			
47	*Porthidium nasutum*	MZUCR 11150	Costa Rica	AF057204	AF057251	U41887
48	*Porthidium lansbergii*	WWW-750	Ecuador			
49	*Porthidium ophryomegas*	UMMZ 210276	Guanacaste Prov., Costa Rica	AF057205	AF057252	U41888
50	*Protobothrops mucrosquamatus*	ROM 25717	Vietnam			
51	*Protobothrops flavoviridis*	UMMZ 199973	Tokunoshima, Ryukyu Is., Japan	AF057200	AF057247	U41894
52	*Protobothrops tokarensis*	FK 1997	Takarajima, Ryukyu Is., Japan	AF057202	AF057249	
53	*Protobothrops elagans*	UMMZ 199970	Ishigaki Is., Ryukyu Is., Japan	AF057201	AF057248	U41893
54	*Sistrurus catenatus*	MOODY 502.	Haskell Co., Texas, USA	AF057227	AF057274	
55	*Sistrurus miliarus*	UTA-live	Ft. Myers, Lee Co., Florida, USA	AF057228	AF057275	U41889
56	*Sistrurus ravus*	UTA-live	Zapotitlán, Puebla, Mexico	AF057226	AF057273	
57	*Trimeresurus albolabris*	MCZR 177966	Yim Tin Tsi., Port Shelter Is., Hong Kong	AF057195	AF057242	U41890
58	*Trimeresurus popeorum*	ROMfield 7234				
59	*Trimeresurus cantori*		Kamurta, Nicobar Is., India	AF057196	AF057243	U41891
60	*Trimersurus stejnegeri*	UMMZ 190532	Taipei, Taiwan	AF057197	AF057244	U41892
61	*Tropidolaemus wagleri*	CLP 141	West Kalimantan, Indonesia	AF057198	AF057245	

ABI 377 automated sequencer (ABI BigDye: Applied Biosystems, Perkin-Elmer, Foster City, California) according to the manufacturer's protocols. The 12S and 16S regions were completely sequenced in both directions using the amplification primers. The ND4 segment was sequenced from both directions with the amplification primers and, in most cases, with one internal sequencing primer (HIS, 5'-CAC TGC CTA ATG TTT TTG T-3'; Arévalo et al., 1994) resulting in 70–100% overlap between the fragments. The cyt-*b* fragment was sequenced using amplification primers and two internal primers (CB2H, 5' -CCC CTC AGA ATG ATA TTT GTC CTC 3' and ATRCB1, 5' -CGA GGM RTH TAC TAC GGC TCC TAA-3'), generally yielding 100% coverage. Both protein-coding gene fragments were translated into their amino acid sequences to check for sequencing errors, by identification of stop codons or frameshift mutations.

Alignment and Phylogenetic Reconstruction

Positional homology was determined for the 12S and 16S rDNA gene fragments based on snake secondary structures (Parkinson, 1999). The coding regions of ND4 and cyt-*b* were aligned based on their inferred amino acid sequence. Sequence alignments can be downloaded from http://biology.ucf.edu/~clp/index.html. Pairwise sequence divergences and levels of saturation were examined for all codon positions and mutation types following Parkinson et al. (2000).

Phylogenetic inference was carried out on the individual gene data sets using maximum parsimony (MP), while the combined data set was analyzed using both MP and maximum likelihood (ML) with the programs PAUP* beta 3a (Swofford, 1999) and Fast DNAml, ver. 1.06 (Olsen et al., 1994). For the MP analyses we employed a heuristic search algorithm with 100–1,000 random-taxon addition-sequence replicates and tree bisection and reconstruction (TBR) branch swapping. To minimize effects of saturation in the protein-encoding regions, MP analyses were performed excluding third-position transitions. A combined data set was constructed, recoding all third-position nucleotides to either R (for A or G) or Y (C or T). Due to the size of the data set, all ML analyses were carried out in Fast DNAml ver. 1.06, rewritten in Parallel Virtual Machine (PVM) language to run in a parallel environment. We utilized the F84 model of Felsenstein, with the initial ti/tv ratio estimated using PUZZLE ver. 4.02 (Strimmer and von Haeseler, 1996) under the Tamura-Nei model of evolution (Tamura and Nei, 1993) with parameter estimation set to "approximate." Ten initial ML trees were inferred by randomizing taxon input order with jumble and using "global" swapping across all nodes (equivalent to subtree pruning-regrafting). The optimal tree (best log-likelihood score) was then entered into PAUP* to reoptimize the ti/tv ratio using a model that incorporates variability in rates of change. We used the F84 evolutionary model assuming a discrete gamma distribution with four categories of site-to-site rate variability. The resulting ti/tv ratio was used to infer a new tree as above, further optimizing branch lengths. This tree and the optimized ti/tv ratio were then used to estimate evolutionary rates of change for each sequence position by partitioning the sites into 35 "rate" categories using the program DNArates (S. Pract, R. Overbeek, and G. Olsen; pers. comm.). A new ML tree, incorporating the rate categories and the reoptimized ti/tv ratio, was then inferred. This new optimal tree was then used for a second round of rate estimation and tree inference. This process was iterated until a stable topology was achieved.

Nonparametric bootstrapping (BS; Felsenstein, 1985) and relative-likelihood support (RLS; Jermiin et al., 1997) were used to determine nodal support. Parsimony bootstrapping was conducted in PAUP*. ML bootstrapping analyses were performed in Fast DNAml ver. 1.06 (Olsen et al., 1994). To generate the 100 pseudoreplicates for ML bootstrapping we used the SEQBOOT program in PHYLIP version 3.5c (Felsenstein, 1993). Each individual pseudoreplicate was analyzed; the 100 resultant topologies were then input into the CONSENSE module of PHYLIP ver. 3.5c (Felsenstein, 1993) to calculate the bootstrap values. 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. RLS scores were calculated with the program TreeCons ver. 1.0 (Jermiin et al., 1997), using a class V weighting scheme and an a value of 0.05 on 1,000 best trees determined using the "keep" option of Fast DNAml ver. 1.01 (Olsen et al., 1994) on both the ML "no-rates" and "rates" topologies.

RESULTS

Sequence Evolution

We obtained 421 base pairs (bp) of sequence for 12S rDNA, 510 bp for 16S rDNA, 693 bp for ND4, and 717 bp for the cyt-*b* region for a total of 2,341 bp of sequence. New sequences will be deposited in GenBank (Table 1). The number of parsimony-informative characters for each region was 153, 125,

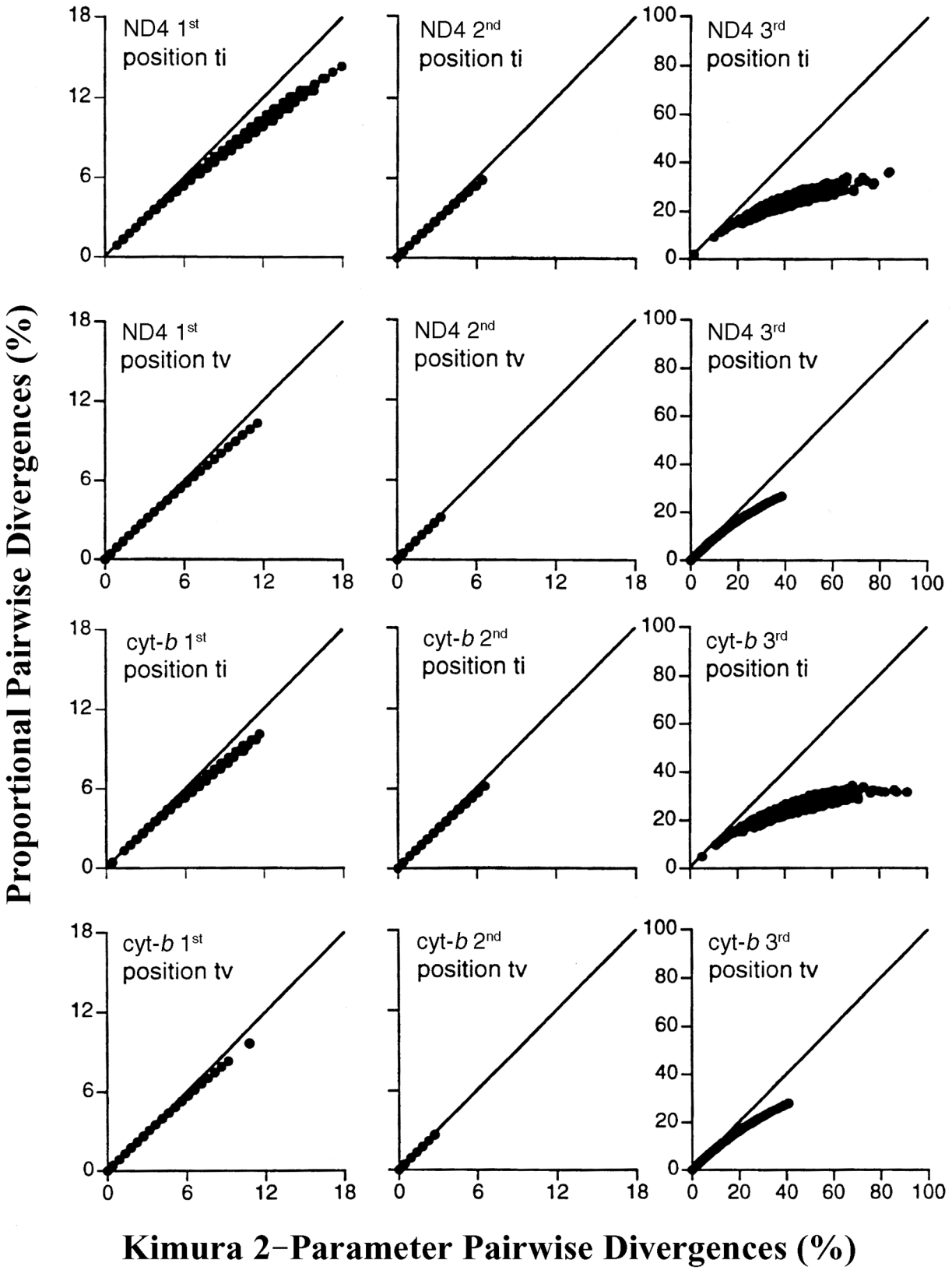


Fig. 1. Plots of uncorrected pairwise sequence divergence (*p*-distance) vs Kimura-2 parameter corrected distances for transitions (ti) and transversions (tv) at first, second, and third codon positions. Each plot represents all possible pairwise comparisons. (Top) ND4 693 bp (Bottom) Cyt-*b* 717 bp. Deviation from the X = Y line in the plots is a measure of the degree of saturation for the indicated class of substitution.

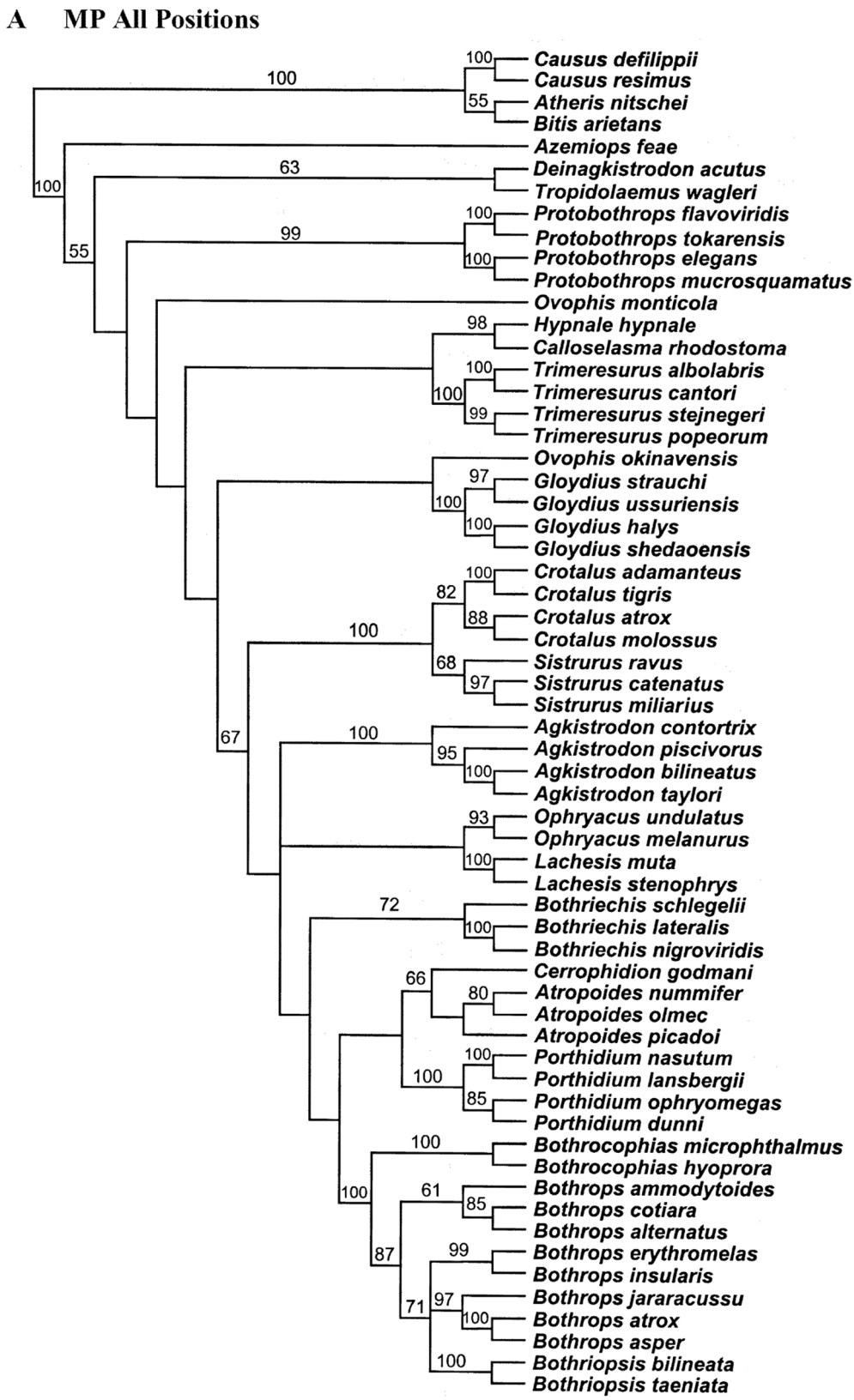
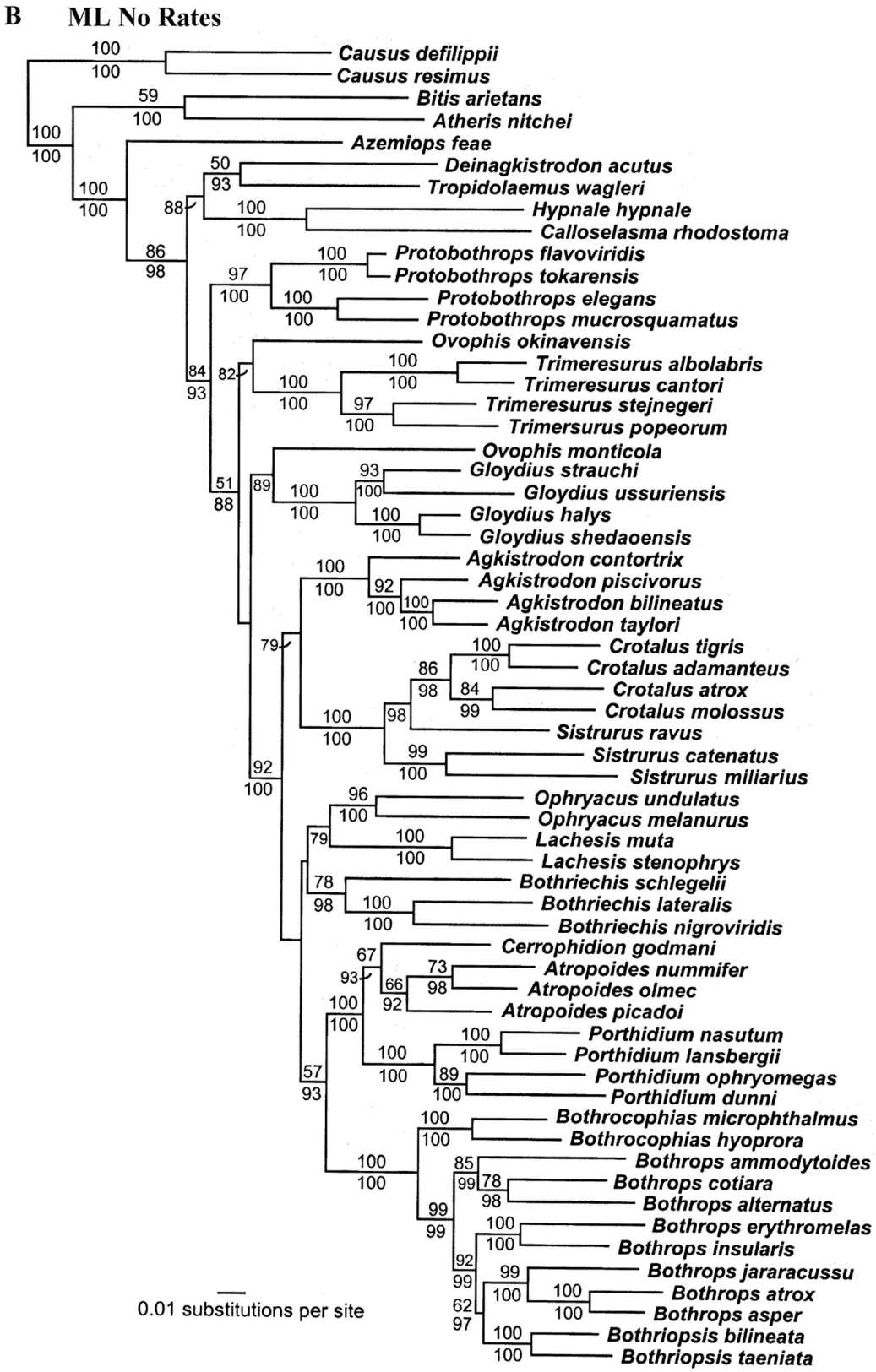


Fig. 2. Phylogenetic analyses of combined data (421 bp, 510 bp, 693 bp, 717 bp for 12S and 16S rDNA, ND4, and cyt-*b*, respectively, for a total of 2,341 bp). (A) Strict consensus of the four MP trees (TL = 8261, CI = 0.25, RC = 0.11, RI = 0.43) treating all characters equally.



(B) ML "no-rates" log-likelihood = – 43793.41, ti/tv ratio = 3.1. Nonparametric BS values > 50% are shown above and RLS values > 70 are shown below (ML topology).

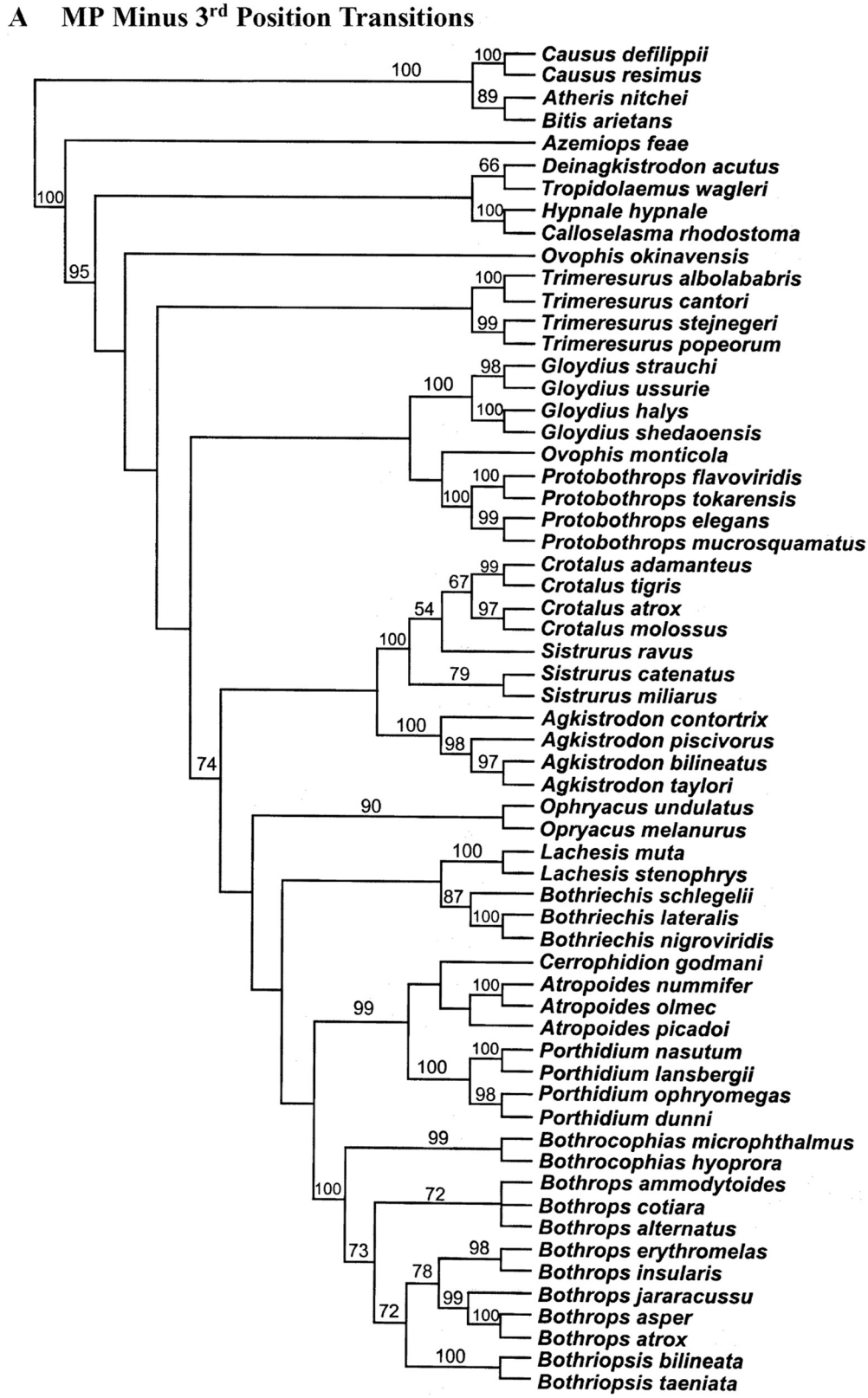
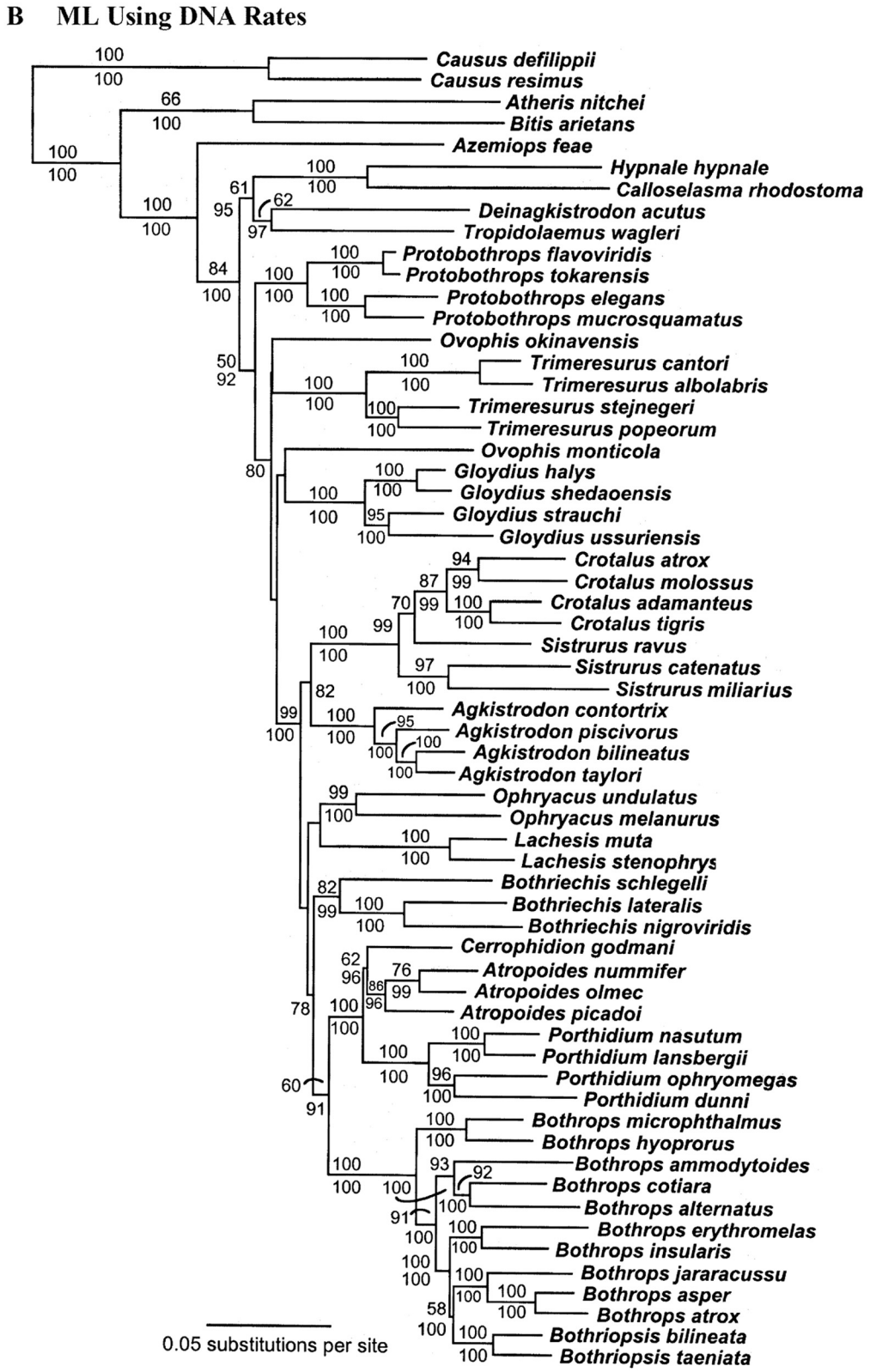


Fig. 3. Phylogenetic analyses of combined data utilizing methods to account for differential rates of substitution. (A) Strict consensus of the two MP trees excluding third position transitions (TL = 4532, CI = 0.25, RC = 0.17, RI = 0.52).



(B) Inferred ML "rates" topology (after two rounds of DNA rates), with re-optimized ti/tv ratio of 5.06, with a log-likelihood of − 34554.18. Nonparametric BS values > 50% are shown above, and RLS values > 70% are shown below (ML topology).

348, and 348, respectively. Saturation analyses suggest that third position transitions of both ND4 and cyt-*b* are potentially saturated, and thus phylogenetic analyses including these data may be biased due to multiple hits (Aquadro and Greenberg, 1983; Swofford et al., 1996: Fig. 1). Parkinson (1999) found that a slight transitional bias occurs in 12S and 16S rDNA sequence data (these analyses were not repeated for all data presented here), but not to the extent of the bias observed in the third position of the protein-encoding regions. Sequence divergence (all four regions concatenated) within and among outgroups, and ingroups ranged from 11.58% (between the two species of *Causus*) to 20% (between *Causus defilippii* and *Hypnale*). Divergences within Crotalinae ranged from 4.34% (between *A. bilineatus* and *A. taylori*) to 16.3% (between *C. rhodostoma* and *B. alternatus*).

Phylogenetic Analyses

Individual gene analyses.—An initial unweighted heuristic MP analysis of the 12S rDNA resulted in 440 MP trees (TL = 964, CI = 0.34, RC = 0.20, RI = 0.57); the 16S rDNA, 266 MP trees (TL = 744, CI = 0.41, RC = 0.21, RI = 0.52); the ND4, 8 MP trees (TL = 3082, CI = 0.23, RC = 0.09, RI = 0.41); and cyt-*b*, 2 MP trees (TL = 3314, CI = 0.21, RC = 0.09, RI = 0.42). *Azemiops feae* was nested within the Crotalinae in all but the 16S rDNA individual gene trees (MP BS ≤ 40), rendering Crotalinae paraphyletic. The strict consensus of each individual rDNA gene analysis resulted in a polytomy among recognized subfamilies, although resolution at the generic level existed. The ND4 data set supported the monophyly of New World taxa, but relationships among New World genera were not resolved. Monophyly of New World taxa was not supported in the cyt-*b* analysis, but monophyly of most genera was found. All individual gene fragment analyses showed the following genera to be monophyletic: *Agkistrodon*, *Bothriopsis*, *Gloydius*, *Lachesis*, *Ophryacus*, *Porthidium*, *Protobothrops*, *Sistrurus* (*sensu stricto*), and *Trimeresurus. Bothrops* (including *Bothriopsis*) and *Crotalus* (including *Sistrurus ravus*) were paraphyletic. *Hypnale* and *Calloselasma* are sister taxa in all individual analyses. *Ovophis*, represented by *O. okinavensis* and *O. monticola*, was not monophyletic in any of the individual gene analyses.

Combined analyses.—All individual gene analyses, in general, yielded similar results. Monophyly of most genera was supported, but not all intergeneric relationships were resolved. Because these genes are all mitochondrial (and thus part of a single linkage group thought to be subject to little or no recombination), and the trees resulting from individual analyses of each were similar, we combined them for a total mtDNA analysis. Because the combined analyses will serve as the basis for most of the *Discussion*, we will provide little detail of the results of these analyses here. An initial unweighted MP analysis on the combined data set yielded four MP trees (TL = 8261, CI = 0.25, RC = 0.11, RI = 0.43), and the strict consensus is shown in Figure 2a. Recoding the third positions of the protein-encoding regions resulted in 2 MP trees (TL = 4532, CI = 0.25, RC = 0.17, RI = 0.52); the strict consensus is shown in Figure 3a. All 10 of the initial ML analyses, using a ti/tv ratio of 3.1, converged on the same topology (log-likelihood = – 43793.41; Fig. 2b). A topology was estimated utilizing the DNArates categories to try to account for differential substitution rates (log-likelihood = – 34554.18; Fig. 3b).

DISCUSSION

In all trees based on combined analyses, *Azemiops feae* is the sister taxon of the Crotalinae (MP, BS = 55; MP-RY, BS = 95; ML "no-rates," BS = 86; ML "rates," BS = 84; ML, RLS "no-rates" = 98, RLS "rates" = 100). To date, all DNA sequence-based evidence and certain morphological characters indicate that *Azemiops* is the sister group of pitvipers (Liem et al., 1971; Knight and Mindell, 1993; Heise et al., 1995; Parkinson, 1999). Our results support the conclusions of these earlier studies.

The clade ((*Calloselasma*, *Hypnale*) (*Deinagkistrodon*, *Tropidolaemus*)) was sister to all other pitvipers in three of four combined analyses (MP-RY, ML "no-rates," and "rates"), whereas the unweighted MP analyses placed (*Deinagkistrodon*, *Tropidolaemus*) as sister to all other crotalines. Nodal support for all of these placements is relatively low (BS ≤ 66, RLS = ≤ 94). Brattstrom (1964) considered either *Trimeresurus* (including *Tropidolaemus*) or *Agkistrodon* to be sister to all other pitvipers because they have more osteological characters in common with true vipers than any other pitviper. He indicated that the palatine of *Tropidolaemus* is similar to that of Viperinae. Kraus et al. (1996) inferred that a clade containing *Deinagkistrodon* and *Tropidolaemus* was sister to other pitvipers, but did not comment on the evidence supporting this arrangement. Using *Thamnophis* as the outgroup (with six ingroup pitvipers), Cullings et al. (1997) found *Calloselasma* to be the sister taxon to other crotalines in their analyses. Vidal and Lecointre

(1998) and Vidal et al. (1999) suggested that *Calloselasma* and *Hypnale* are sister to all other pitvipers (although *Hypnale* was not included in their analyses). Parkinson (1999) found *Protobothrops* to be the sister group to the remaining pitvipers using MP, and found *Gloydius* to be sister using ML, although neither of these placements was strongly supported. Thus, among these previous studies, there is little agreement regarding the basal-most divergences in the group. Strong support for any single group as sister to the others is not found, although numerous studies suggest that *Calloselasma* represents a very early divergence in the pitviper tree. Although the molecular data are inconclusive as to which group is sister to the others, it seems probable that it is one or more of the following genera: *Calloselasma*, *Deinagkistrodon*, *Hypnale*, or *Tropidolaemus*.

Old World Genera

Hoge and Romano-Hoge (1981) proposed the genus *Gloydius* for the Asiatic members of the genus *Agkistrodon*. Gloyd and Conant (1990, and appended articles therein), however, argued that *Agkistrodon* (*sensu lato*) was monophyletic and did not follow Hoge and Romano-Hoge's taxonomic proposal. Numerous molecular studies since then have investigated whether recognition of New and Old World taxa as *Agkistrodon* is valid; all studies support the polyphyly of *Agkistrodon* (*sensu lato*) (Knight et al., 1992; Kraus et al., 1996; Cullings et al., 1997; Parkinson et al., 1997; Vidal and Lecointre, 1998; Parkinson 1999). A main characteristic used to unite the New and Old World *Agkistrodon* is the presence of large head shields, but this character is presumably homoplastic because molecular data indicate polyphyly of the genus.

The *Trimeresurus* complex is widely distributed across southern Asia and the Indo-Malayan archipelago. McDiarmid et al. (1999) recognized only three of the five (at that time) proposed genera (*Ovophis*, *Trimeresurus*, and *Tropidolaemus*); they did not recognize *Ermia* or *Protobothrops.* Recently, the monotypic genus *Triceratolepidophis* was described from Vietnam (Ziegler et al., 2000); thus there are six proposed genera, with about 43 species in the complex.

Members of this group occupy a diverse set of habitats (low tropical wet forest to high elevation mountains) and have varying lifestyles (arboreal live bearers compared to terrestrial egg layers). Our data, and those of Kraus et al. (1996), Parkinson (1999), and Malhotra and Thorpe (2000) indicate that *Trimeresurus* (*sensu lato*) is paraphyletic. Recognition of *Ovophis*, *Protobothrops*, *Trimeresurus*, and *Tropidolaemus*, seems to rectify the situation (although see discussion below on *Ovophis*). The result that these four genera of the *Trimeresurus* complex do not form a monophyletic group was unexpected. For many years all these taxa were grouped under *Trimeresurus*, and it has not been until recently that workers in the field erected new genera for perceived clades within the genus. Morphologically these snakes are very similar, and, based on phenetic analyses of morphology, a close relationship was proposed (Maslin, 1942; Brattstrom, 1964).

Trimeresurus (*sensu stricto*) is monophyletic in all combined analyses. This group contains about 30 species (McDiarmid et al., 1999), with new taxa still being discovered (Orlov and Helfenberger, 1997). Many of these taxa are endemic to islands and other areas difficult to access. The most comprehensive study to date on this group is by Malhotra and Thorpe (2000). They included numerous individuals of this group and related genera, and their results indicate that *Trimeresurus* (*sensu stricto*) is monophyletic (although *T. gracilis* forms a clade with *Ovophis okanavensis* in all of their analyses). We included only four species; a better sampling representing as many species as possible with multiple genes is required before exact limits of this genus can be reliably determined.

Our analyses suggest that *Ovophis* is polyphyletic; this supports the findings of Malhotra and Thorpe (2000). This stout, terrestrial, egg-laying genus currently contains three species (*chaseni*, *monticola* [type for genus], and *okinavensis*; McDiarmid et al., 1999). Malhotra and Thorpe (2000), however, found that *Trimeresurus gracilis* is sister to *O. okinavensis* in all analyses, although they did not make taxonomic changes. Burger (1971) proposed this genus in his dissertation, although it was not formally recognized until Hoge and Romano-Hoge (1981) included it in their treatise on pitvipers. We included two species in our analyses, *okinavensis* and *monticola*; they did not form a clade. In fact, these two taxa held different positions in the different analyses, none of which were highly supported. Until a more robust phylogeny is obtained, including all species of this group, we do not think it appropriate to suggest taxonomic modifications.

The monotypic genus *Ermia* was proposed for the species *Trimeresurus mangshanensis* (Zang, 1998). Because this taxon was not included in our study, we

cannot comment on its validity. McDiarmid et al. (1999) did not recognize this genus.

Hoge and Romano-Hoge (1983) erected the genus *Protobothrops* for several species of gracile terrestrial snakes formerly placed in *Trimeresurus* (*sensu lato*). Our data support monophyly of this group. However, only four members were included in our analyses. A better sampling is necessary to verify the status of this group. Although McDiarmid et al. (1999) did not recognize the genus, our data, as well as those of Kraus et al. (1996), Parkinson (1999) and Malhotra and Thorpe (2000) support its validity.

Ziegler et al. (2000) erected the genus *Triceratolepidophis* based on morphological characters and SEM photographs of microdermatoglyphic patterns of dorsal scales, for a single species found in Vietnam and Laos. This taxon is phenotypically similar to *Protobothrops mucrosquamatus*. At this time we cannot comment on its validity as we did not include it in our analyses.

Tropidolaemus has generally been considered monotypic (Burger, 1971; Hoge and Romano-Hoge, 1981). A second species, *Trimeresurus huttoni*, was described by Smith (1949) and allocated to *Tropidolaemus* by David and Vogel (1998). McDiarmid et al. (1999) included *huttoni* in this genus. Unfortunately, we did not have a sample of this taxon in our study but we included *T. wagleri*. *Tropidolaemus wagleri* grouped with *Deinagkistrodon acutus* in all analyses, and nodal support for this relationship was generally low. Kraus et al. (1996), Vidal and Lecointre (1998), and Parkinson (1999) also came to this conclusion. Malhotra and Thorpe (2000), using MP analysis of cyt-*b* sequence, found that *Tropidolaemus* is the basal-most pitviper, and their ML analyses grouped this taxon as the basal lineage of a clade containing *Calloselasma*, *Deinagkistrodon*, and four New World species. Parkinson (1999) commented that the association between *Tropidolaemus* and *Deinagkistrodon* is problematic, noting the morphological differences between the taxa and suggesting that more individuals and data are needed to understand the nature of this putative relationship. We added more data (ND4, cyt-*b*, 12S and 16S rDNA), and included three individuals of each genus, from different localities, in preliminary analyses (data not shown); *Tropidolaemus* always formed a clade with *Deinagkistrodon*. Nodal support is low in the basal area of the topologies presented here; thus, this hypothesized relationship may be spurious. More sequence data, especially from more conserved regions, are necessary to test this relationship.

The monotypic genera *Calloselasma* and *Deinagkistrodon* are each very distinct, and we recommend continued recognition of these genera. Only a single species of *Hypnale* was included in our analyses, but our results are consistent with continued recognition of this genus. *Calloselasma* and *Hypnale* form a highly supported sister group relationship in all analyses. This is consistent with the morphologically-based conclusions of Gloyd and Conant (1990) and the molecular-based conclusions of Kraus et al. (1996), Parkinson et al. (1997), and Parkinson (1999).

New World Genera

Monophyly of New World crotalines is strongly supported in all of the combined analyses (MP BS = 67; MP-RY BS = 74; ML "no-rates" BS = 92; ML "rates" BS = 99; ML RLS "no-rates" = 100; ML RLS "rates" = 100). Kraus et al. (1996) first presented this hypothesis based on analyses of ND4 sequence data, and the results of Vidal and Lecointre (1998) and Parkinson (1999) supported this hypothesis, although support for the node uniting the New World taxa was weak in all studies. The results presented here (based on use of additional taxa and sequence data) strongly support monophyly of New World pitvipers. The topologies of all trees indicate an ancient, successive series of divergences in the Old World and a relatively recent origin for the New World group. This pattern supports the hypothesis that pitvipers evolved in the Old World (presumably Eurasia).

All combined analyses supported a monophyletic temperate group (*Agkistrodon*, *Crotalus*, and *Sistrurus*), and a monophyletic Neotropical group (bothropoid genera + *Lachesis*). These results supported the conclusions of Parkinson (1999) that a temperate versus tropical cladogenetic event occurred early in the evolution of New World pitvipers. This finding also was supported in the morphological analyses of Gutberlet and Harvey (this volume).

All four species of *Agkistrodon* were included and formed a well-supported monophyletic group in all analyses. These results are consistent with those of Parkinson et al. (2000). Rattlesnakes formed a monophyletic group in all combined analyses. In the unweighted MP analysis both *Crotalus* (BS = 82) and *Sistrurus* (BS = 68) were monophyletic, but in the RY and ML analyses, *S. ravus* was sister to *Crotalus* (MP-RY BS = 54; ML "no-rates" BS =38; ML "rates" BS = 70; ML RLS "no-rates" = 98; ML RLS "rates" = 99).

These results are contrary to those of Parkinson (1999), who found *Crotalus* paraphyletic with regard to *Sistrurus*. However, the relationships among species currently assigned to *Crotalus* and *Sistrurus* cannot be resolved with the small number of taxa included in this study (see Murphy et al., this volume).

The Neotropical group was monophyletic in all analyses, but support for this group was weak. Taxa that were formerly placed in *Bothrops* (before Burger, 1971) did not form a monophyletic group in our analyses. In all analyses, *Lachesis* fell within the bothropoid group, but subsets of this group were monophyletic.

The genus *Bothriechis* formed a monophyletic group in all of our analyses (BS $\geq$ 72, RLS $\geq$ 98). Parkinson (1999) found that *B. schlegelii* grouped as the sister taxon of *Bothrops* in analyses of rDNA sequence, although he commented that this relationship was probably due to homoplasy within the data set. Crother et al. (1992) studied phylogenetic relationships and speciation patterns within *Bothriechis* and found *B. schlegelii* to be sister to other members of the genus. Results of a phylogeographic study on this genus using sequence data indicate that this genus is monophyletic and that *B. schlegelii* and *B. supraciliaris* are the earliest branching lineages of this clade (C. Parkinson, unpublished).

Our data support Gutberlet's (1998) taxonomic revision of *Ophryacus*, involving the transfer of *Porthidium melanurum* into *Ophryacus*. In all but the minus-third-position-transition analyses, *Ophryacus* was found to be the sister group of *Lachesis*. *Ophryacus* was found to be sister to members of the Neotropical clade in the minus-third-position transition analyses. Neither of these relationships was strongly supported. These results are problematic, as *Lachesis* and *Ophryacus* are morphologically very different. *Lachesis* is a large, terrestrial, whereas *Ophryacus* is smaller and terrestrial to semi-arboreal; *Ophryacus* has raised superciliary scales whereas *Lachesis* does not, and *Lachesis* is oviparous whereas *Ophryacus* is viviparous. Werman (1992) and Gutberlet and Harvey (this volume) found strong support for *Ophryacus* being sister to *Bothriechis*; however, this relationship was not found in any of our sequence-based analyses.

Three closely allied genera (*Atropoides*, *Cerrophidion*, and *Porthidium*) form a monophyletic group that we refer to as the *Porthidium* complex, because all have been included in this genus at one time or another (Campbell and Lamar, 1989). The jumping vipers (*Atropoides*) consist of three currently recognized species, all of which are included in our analyses. They form a monophyletic group that is weakly to moderately well supported (MP BS = 49; MP-RY BS = 32; ML "no-rates," BS = 66; ML "rates," BS = 86; ML RLS "no-rates" = 92; ML RLS "rates" = 96). Kraus et al. (1996), using two of the three described species, did not find *Atropoides* to be monophyletic, although the data presented herein and those of Parkinson (1999) and Gutberlet and Harvey (this volume) support Werman's (1992) hypothesis that this group is monophyletic. In all cases *Cerrophidion* is the sister group of *Atropoides*. Monophyly of *Cerrophidion* was not tested, as only a single species of the four comprising the genus was included. Seven species of hognosed vipers (*Porthidium*) are currently recognized; we included four species, and they form a strongly supported monophyletic group (MP BS = 100; MP-RY, BS = 100; ML "no-rates," BS = 100; ML "rates," BS = 100; ML RLS "no-rates" and "rates" = 100). The phylogenies presented here and those of Kraus et al. (1996), Parkinson (1999), and Gutberlet and Harvey (this volume) indicate that the South American species *P. hyoprora* (= *Bothrops* / *Bothrocophias hyoprora*) is sister to the genus *Bothrops*. The *Porthidium* complex formed the sister group of *Bothrops* (including *Bothriopsis*) in all analyses, although this relationship was not well supported (BS $\leq$ 57, RLS $\leq$ 91). Kraus et al. (1996), using transversion parsimony, also found this relationship. Werman (1992) suggested that *Porthidium* (*sensu stricto*) is the sister group of *Bothrops*, with *Atropoides* and *Cerrophidion* representing lineages that diverged near the base of the tree. Werman (1999) discussed the morphological features that unite *Porthidium* and *Bothrops* and contrasted this with the published molecular-based topologies. He suggested that if the molecular data were correct, then his findings (*Bothrops* sister to *Porthidium*) were based on morphological convergence. The monophyly of the *Porthidium* complex is strongly supported by our data. It will be interesting to see if more molecular and morphological data, and a combination of the two, will bear out the hypothesis of a sister group relationship.

The South American genus *Bothrops* (*sensu stricto*) currently contains about 32 recognized species (McDiarmid et al., 1999). We included 10 species in our analyses and found the genus to be paraphyletic with respect to *Bothriopsis* (BS = 100 all analyses, RLS "rates" and "no-rates" = 100). Our data indicate several evolutionarily distinct lineages within this

large and cumbersome genus. A basal *hyoprora-microphthalmus* clade is strongly supported in all analyses (BS ≥ 73, RLS ≥ 99). Gutberlet and Campbell (2001) described the new genus *Bothrocophias* for this lineage. They included *Bothrops campbelli*, *B. hyoprora*, *B. microphthalmus*, and a new species, *B. myersi*. Our data support their findings; however, we did not include *campbelli* or *myersi*. Gutberlet and Harvey's (this volume) morphological analyses also support this relationship and the inclusion of *campbelli* in the clade. However, the only molecular results to date do not support inclusion of *campbelli* in this group (Wüster et al., this volume)

A *B. alternatus* clade is strongly supported, including *B. ammodytoides, B. alternatus,* and *B. cotiara* (BS ≥ 61, RLS "no-rates" = 98 and "rates" = 99). Salomão et al. (1997, 1999) found that the *alternatus* group was sister to a group containing other members of the genus *Bothrops,* but they did not include any members of the *hyoprora* group. A morphological character that separates the *hyoprora* and *alternatus* groups from the rest of the *Bothrops* species is the presence of a divided versus an undivided lacunolabial scale in the former (the prelacunal scale and second supralabial are discrete scales) versus the latter. *Bothrops erythromelas* (of the *neuwiedi* group) and *B. insularis* (of the *jararaca* group) formed a clade in all analyses (BS ≥ 98 and RLS "no-rates" and "rates" = 100); this group is sister to a clade containing the *atrox* group and *Bothriopsis*. Strong support (all nodal values = 100) for an *atrox* group was found, although only two species were included (*B. asper* and *B. atrox*). *Bothrops jararacussu* was sister to the *atrox* clade in all analyses. Our results and the findings of numerous other studies indicated that *Bothrops* as currently recognized is paraphyletic with regard to *Bothriopsis*. More data are needed to determine the generic composition of *Bothriopsis*, and a robust phylogeny of the genus *Bothrops* should reveal what further taxonomic revisions should be made to rectify paraphyly in *Bothrops* (for opposing views see Salomão et al., 1997; Parkinson, 1999). Our phylogenetic hypotheses support those of Salomão et al. (1997) and Salomão et al. (1999), but until detailed geographical, morphological, and molecular analyses are conducted on this speciose group, results should be viewed as tenuous. Relationships within *Bothrops* (*sensu stricto*) are highly complex and controversial; we suggest that the genus *Bothrops* eventually should be separated into several smaller distinct monophyletic groups, as has been the case within other speciose genera (Burger, 1971; Campbell and Lamar, 1989, 1992; Werman, 1992).

Many of the intergeneric relationships of New World pitvipers are not well resolved. Our data support a sister group relationship between the *Bothrops* and the *Porthidium* complex. Relationships among *Bothriechis*, *Lachesis*, and *Ophryacus* are variable depending on the analysis performed. Werman (1992) and Gutberlet and Harvey (this volume) found support for a sister group relationship between *Bothriechis* and *Ophryacus*; however, this has not been found in any of the molecular studies to date. The phylogenetic position of *Lachesis* is problematic**,** and our data do not resolve its position. Data from the nuclear genome may help resolve inconsistencies. Also, a combined study including both morphology and molecular data may help resolve intergeneric relationships within the New World pitvipers (R. Gutberlet and C. Parkinson, unpublished).

Historical Biogeography

One of the many reasons for determining the evolutionary relationships within a group of organisms is to reconstruct the history of present distributions. Pitvipers are thought to have evolved in Eurasia, and their sister group, *Azemiops*, is restricted to the Old World. Our results support monophyly of New World pitvipers; these findings corroborate those of Kraus et al. (1996), Vidal and Lecointre (1998), Parkinson (1999) and Vidal et al. (1999). Kraus et al. (1996) proposed a single emigration event across the Bering Land Bridge to account for New World monophyly. The key question is: when did this dispersal event occur? The first opening of the Bering Strait since the middle Cretaceous period (Albian Stage: 105 million years ago [mya]) was postulated to have occurred between 4.8 and 7.3–7.4 mya (Marincovich and Gladenkov, 1999). Geological records indicate that Beringia was a dispersal corridor for mammals during the Cenozoic (70–0 mya; Woodburne and Swisher, 1995). Beard (1998) postulated that mammal dispersal across Beringia occurred in both directions during periods of favorable climate. Vidal and Lecointre (1998) postulated a late Cretaceous or early Cenozoic invasion of the New World. With the current distributional patterns of pitvipers in the New World, we agree that an early Tertiary or late Cretaceous crossing of the Bering Land Bridge is probable; however, at this time there is no hard evidence supporting this hypothesis.

Our best estimate as to the sister group of New World pitvipers is *Gloydius* (an Old World taxon) and

a member of *Ovophis*; however, support for this relationship (*Gloydius*-*Ovophis* clade) is low. Members of the genus *Gloydius* are known to inhabit mountainous regions at or above 2,500 m in Eurasia (Gloyd and Conant, 1990). A *Gloydius*-like ancestor might be an appropriate candidate for emigration into the New World via Beringia.

All phylogenies presented here indicate an early cladogenetic event splitting pitvipers into two groups: temperate and Neotropical. This is also supported in the morphological analyses of Gutberlet and Harvey (this volume). In addition, at least five dispersals into South America are likely to have taken place: (1) ancestor of *Bothrops*, (2) ancestor of *Lachesis*, (3) *Crotalus durissus*, (4) *Bothriechis schlegelii*, and (5) ancestor of *Porthidium nasutum* / *Porthidium lansbergii*. The phylogenetic relationships suggest that the ancestor of modern *Bothrops* dispersed into South America from the north. A single species of *Bothrops* (*B. asper*) is currently distributed throughout much of Central America and Mexico; we postulate that it dispersed northward into Central America and Mexico from South America fairly recently after formation of the Isthmus of Panama.

Lachesis has been attributed to a South American faunal assemblage using vicariance biogeography (Savage, 1966), but our data suggest that *Lachesis* is associated with a Central American assemblage. These data concur with those of Zamudio and Greene (1997); Central American *Lachesis* could be a member of the initial tropical assemblage that colonized from the north. However, until a better understanding of the phylogenetic position of *Lachesis* is gained, this hypothesis is tenuous.

Crotalus durissus is currently distributed throughout South America east of the Andes, and phylogenetic evidence suggests that it is a member of the temperate pitviper clade. It is probable that a single dispersal event from the north gave rise to its current distributional pattern, probably at about the same time that *B. asper* dispersed northward. *Bothriechis schlegelii* is part of the Middle American faunal assemblage; phylogenetic evidence indicates that a single emigration event into South America from the north across the Isthmus of Panama could account for its current distributional pattern. These data concur with those of Crother et al. (1992), who suggested that this event must have occurred during the Pliocene.

In all phylogenies presented here, *Porthidium lansbergii* and *P nasutum* are sister taxa. *Porthidium nasutum* is currently distributed in Central America, northwestern Colombia, and northwestern Ecuador, whereas *P. lansbergii* is found in southern Panama, northeastern Colombia, and northern Venezuela (Campbell and Lamar, 1989). We propose two hypotheses to account for their present distribution: (1) *P. nasutum* and *P. lansbergii* evolved in Central America and dispersed into South America via two independent events; or (2) the progenitor of *P. nasutum* and *P. lansbergii* invaded South America once, the populations became isolated with *P. nasutum* evolving in Central America and *P. lansbergii* evolving in South America, with subsequent dispersal by both species. However, until a phylogeographic study with intensive sampling from all parts of their range is completed, their biogeographical history will be uncertain (see Wüster et al. this volume).

Conclusions

Pitvipers are a monophyletic group found in Eurasia and the New World. Phylogenetic evidence implies a Eurasian center of origin with a single emigration event into the New World via the Bering Land Bridge. Progress is being made regarding the intergeneric relationships within this diverse group of snakes. Two different hypotheses are proposed regarding the deepest phylogenetic splits in the group: (1) a clade of *Calloselasma*, *Deinagkistrodon*, *Hypnale*, and *Tropidolaemus* is sister to the remaining pitvipers or (2) a clade of *Deinagkistrodon* and *Tropidolaemus* is sister to the remaining pitvipers. Relationships among Old World taxa indicate that *Trimeresurus* (*sensu lato*) is not monophyletic; recognition of *Ovophis*, *Protobothrops* and *Tropidolaemus* allows restriction of the name *Trimeresurus* to a reduced set of species that is more likely to form a monophyletic group. However, *Ovophis* is not monophyletic in our analyses, thus more data are needed to fully understand the relationships of the *Trimeresurus* complex.

Depending on the type of phylogenetic reconstruction, these data suggest that the sister group to New World pitvipers is either 1) *Gloydius* and *O. monticola*, or 2) *Gloydius*, *O. monticola*, and *Protobothrops*. New World pitvipers are a strongly supported monophyletic group. Data indicate that an early cladogenetic event gave rise to a temperate group (*Agkistrodon, Crotalus*, and *Sistrurus*) and a Neotropical group (bothropoids plus *Lachesis*). The *Porthidium* complex appears to be sister to *Bothrops*, while the relationships within most of the remaining clades of Middle America pitvipers are tenuous and more data are needed to clarify their phylogenetic relationships. Knowledge of phylogenetic

relationships among Middle America pitvipers is important because this will shed light on both the biogeographic history of Central America, and the evolution of South American groups. Species-level (interspecific) phylogenies will help clarify local biogeographical events, and lead to meaningful hypotheses for the current distributions of pitvipers (and perhaps other organisms) in Central and South America.

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