

ORIGINS AND EVOLUTION OF THE SOUTH AMERICAN PITVIPER FAUNA: EVIDENCE FROM MITOCHONDRIAL DNA SEQUENCE ANALYSIS

WOLFGANG WÜSTER¹, MARIA DA GRAÇA SALOMÃO², J. ADRIÁN QUIJADA-MASCAREÑAS¹,
ROGER S. THORPE¹, AND BBBSP³

ABSTRACT: We use published and new mitochondrial DNA sequence data to investigate the origins and evolution of the South American pitviper fauna. South America was invaded by at least four independent lineages of pitvipers: *Bothriechis schlegelii*, *Crotalus durissus*, *Porthidium*, and the ancestor of *Bothrops*. *Bothrops* diversified in South America, but the continent's remaining genera of pitvipers are more recent colonizers. The origin of *Lachesis* remains unresolved. *Bothrops* shows the greatest amount of diversity with respect to species numbers, morphology, and natural history. Sequence divergence within *Bothrops*, however, is no greater than in other clades or genera of New World pitvipers. The diversity of morphological and natural history traits in *Bothrops* compared with clades of similar age may have resulted from colonization of a continent devoid of viperids, followed by rapid adaptive radiation. More recent lineages that colonized South America are highly restricted in distribution and typically lack morphological and/or natural history traits present in *Bothrops*. We propose that further partitioning of the genus *Bothrops*, by recognition of the genus *Bothriopsis*, obscures the biogeographical and evolutionary pattern we present.

INTRODUCTION

Pitvipers are a conspicuous component of most South American snake faunas. They show tremendous morphological and ecological diversity, including extremes in body size (maximum adult total length 45–300 cm), body shape (e.g., slender, *Bothrops taeniatus*; stout, *Bothrocophias hyoprora*), macrohabitat (deserts to montane forests), microhabitat (subterranean to arboreal), and diet (generalists to highly specialized) (see Martins et al., this volume). Approximately 44–47 species of pitvipers in 5–6 genera (David and Ineich, 1999; McDiarmid et al., 1999) are currently recognized from continental South America and nearby islands. The genera differ considerably in terms of species numbers, as well as in morphological and ecological diversity. Several genera are widespread, but others are more restricted in distribution. We present and compare morphological diversity and habitats occupied by the genera in Table 1.

Bothriechis is a genus consisting of small, arboreal or semi-arboreal species that are widespread in Central America, where nine species are currently recognized (Campbell and Lamar, 1989; Solórzano et al., 1998; Campbell and Smith, 2000); in South America *Bothriechis* is represented by a single species, *B. schlegelii*, which is restricted to the northwestern part of the continent. Recent studies by Werman (1992), Kraus et al. (1996), and Parkinson et al. (this volume) support the monophyly of *Bothriechis*.

The genus *Porthidium*, commonly known as Hog-nosed Pitvipers, is comprised of seven small terrestrial species that are widespread in Central America. Two species (*P. lansbergii* and *P. nasutum*) occur in South America, where their distribution is restricted to the northern and northwestern edge of the continent (Ecuador to Venezuela). Although species of *Porthidium* are relatively uniform in general appearance, they differ in body size and habitat preference. The genus *Porthidium* has undergone several taxonomic revisions. Burger (1971) included most small terrestrial Central American pitvipers in *Porthidium*, but several recent phylogenetic analyses cast doubt on the monophyly of this assemblage (Werman, 1992; Gutberlet, 1998a, b). These analyses led to the description of the genera *Atropoides* for the Jumping Pitvipers (Werman, 1992), *Cerrophidion* for the Montane Pitvipers (Campbell and Lamar, 1992), and *P. melanurum* was later placed in the genus *Ophryacus* (Gutberlet, 1998a; Parkinson, 1999). In addition, several phylogenetic studies (Kraus et al., 1996; Gutberlet, 1998b; Parkinson, 1999; Parkinson et al., this volume) found that one species, *P. hyoprora*, is more closely related to *Bothrops* than to *Porthidium* (see below). Studies by Kraus et al. (1996), Parkinson (1999), and Parkinson et al. (this volume), however, agree that *Atropoides*, *Cerrophidion*, and *Porthidium* (excluding *hyoprora*), form a monophyletic group. In this paper, we explore the consequences of our interpretation of New World pitviper evolution regarding *Atropoides*, *Cerrophidion*, and *Porthidium* as a single clade of small- to medium-sized terrestrial pitvipers, hereafter referred to as *Porthidium (sensu lato)*, as opposed to *Porthidium (sensu stricto)* for the Hog-nosed Pitvipers.

¹School of Biological Sciences, University of Wales, Bangor LL57 2UW, UK. E-mail (WW): w.wuster@bangor.ac.uk

²Laboratório de Herpetologia, Instituto Butantan, Av. Vital Brazil 1500, 05503-900 São Paulo SP, Brazil

³Butantan-British *Bothrops* Systematics Project: G. J. Duckett, G. Puerto, M. G. Salomão, R. D. G. Theakston, R. S. Thorpe, D. A. Warrell, and W. Wüster

Table 1. Morphological and natural history diversity in New World pitviper clades. N = number of species assigned to the genus. TL = total length. Microhabitat: T = terrestrial; A = arboreal. Macrohabitat: L = lowlands; H = highlands; F = forests; O = open areas; and D = deserts.

Taxon	N	Maximum TL of smallest and largest species (cm)	Stature	Microhabitat	Macrohabitats
<i>Bothrops</i>	~ 36	60–250	Very slender to stout	T, A	L, H, F, O, D
<i>Bothrocophias</i>	4	80–125	Stout to very stout	T	L, H, F
<i>Porthidium (sensu lato)</i>	14	50–110	Moderate to very stout	T	L, H, F, O
<i>Porthidium (sensu stricto)</i>	7	60–90	Moderate to stout	T	L, F, O
<i>Bothriechis</i>	9	60–100	Slender to moderate	A	L, H, F
<i>Crotalus</i> + <i>Sistrurus</i>	~ 30	45–240	Moderate to stout	T	L, H, F, O, D
<i>Lachesis</i>	3	200–300	Moderate to stout	T	L, F

Lachesis is a genus of large terrestrial pitvipers represented by two species in Central America (*L. melanocephala* and *L. stenophrys*) and one (*L. muta*) in South America (Zamudio and Greene, 1997). The affinities of *Lachesis* from the northwest coast of South America, however, remain unresolved. As currently understood, populations of *Lachesis* differ little in morphology and natural history.

Crotalus is a speciose genus (Klauber, 1972) represented in South America by a single widespread species, *C. durissus*; we follow Campbell and Lamar (1989) in treating *pifanorum*, *unicolor*, and *vegrandis* as conspecific with *C. durissus*. *Crotalus* is less diverse than *Bothrops* with respect to its morphology and ecology, although North American species of *Crotalus* show considerable differentiation in body size and habitat preferences (Table 1).

Most other South American pitvipers are classified in the genus *Bothrops*. They occur throughout most of the continent except for southwestern South America, the extreme highlands of the Andes, and southernmost Patagonia. *Bothrops* shows great diversity in size, shape, and habitat preferences, which encompasses nearly the entire spectrum of variation seen in all other genera of New World pitvipers (Table 1). Three species are endemic to islands (*B. caribbaeus*, St. Lucia; *B. insularis*, Queimada Grande; *B. lanceolatus*, Martinique), and one species (*B. asper*) extends northward throughout most of Central America to northeastern Mexico. Some arboreal forms of *Bothrops* have previously been assigned to the genus *Bothriopsis* (Burger, 1971; Campbell and Lamar, 1989). However, all phylogenetic studies of this group (Cadle, 1992; Werman, 1992; Kraus et al., 1996; Salomão et al., 1997, 1999; Parkinson, 1999; Vidal et al., 1997; Parkinson et al., this volume) have suggested that *Bothrops (sensu Burger, 1971)* is paraphyletic with respect to *Bothriopsis*. Salomão et al. (1997) pro-

posed synonymizing *Bothriopsis* with *Bothrops*. Although some authors have not followed this arrangement (e.g., McDiarmid et al., 1999; Parkinson et al., this volume), we follow Salomão et al. (1997) in regarding *Bothriopsis* as a synonym of *Bothrops*.

Gutberlet and Campbell (2001) assigned the taxa *campbelli*, *hyoprora*, *microphthalmus*, and *myersi* to the genus *Bothrocophias*. The taxa concerned have been grouped as members of *Bothrops* in mtDNA-based studies (Kraus et al., 1996; Parkinson, 1999; Parkinson et al., this volume; this study), but many of the morphological analyses of Gutberlet (1998b) did not support this hypothesis. We have chosen to follow the results of the previous mtDNA studies and treat *Bothrocophias* as a synonym of *Bothrops*. The morphological and natural history diversity seen in *Bothrops* is greater than in other clades of New World pitvipers, which provides an interesting opportunity to investigate causes of diversification. Causal hypotheses may include the relative age of these clades (e.g., older clades have had more time to evolve greater differences in phenotype and natural history), or the possibility of rapid adaptive radiation in response to open niches (e.g., the colonization of regions devoid of pitvipers with similar natural history characteristics).

Pitviper diversity in South America is linked to the biotic interchange between North and Central America throughout the Tertiary, particularly after emergence of the Isthmus of Panama. Emergence of the Isthmus 3.5 mya (Coates and Obando, 1996) does not appear to have been a key factor in the biotic interchange of ectothermic vertebrates, unlike with mammals. Among pitvipers, Vanzolini and Heyer (1985) identified only *C. durissus* as a likely example of a post-Isthmian dispersal event beyond northern South America. Vanzolini and Heyer (1985), however, were unable to provide resolution to the origin and timing of dispersal for *Lachesis* or the “bothropoid” pitvipers,

due to the lack of phylogenetic understanding of the group at that time. Crother et al. (1992) assumed South American *B. schlegelii* to be the result of post-Isthmian colonization.

Resolution of biogeographical problems of this nature requires a robust phylogenetic hypothesis for the taxa concerned. In recent years, the phylogeny of pitvipers has become a subject of intense interest (e.g., Crother et al., 1992; Knight et al., 1992; Werman, 1992, 1997; Kraus et al., 1996; Salomão et al., 1997, 1999; Parkinson et al., 1997, this volume; Vidal et al., 1997, 1999; Gutberlet, 1998a, b; Vidal and Lecoindre, 1998; Parkinson, 1999). Studies using mtDNA sequences have been prominent in pitviper systematics (e.g., Kraus et al., 1996; Salomão et al., 1997, 1999; Vidal et al., 1997, 1999; Wüster et al., 1997, 1999; Zamudio and Greene, 1997; Parkinson, 1999; Puerto et al., 2001; papers in this volume). Although many finer points of pitviper systematics remain to be clarified, these studies have made considerable progress toward our understanding of their relationships.

Molecular markers are particularly promising for this kind of research, since they are unlikely to be affected by the same selection pressures as morphology, thus providing an independent assessment of relationships unaffected by convergent selection for similar natural history traits (Avice, 1994, 2000). Furthermore, DNA sequences can be used to estimate the age of different clades, and provide a temporal basis for historical biogeography and the evolution of morphological diversification (e.g., Zamudio and Greene, 1997). Existing databases (e.g., GenBank) of mitochondrial DNA sequences are particularly useful for future studies, as new sequences can be added to existing databases.

In this study, we used our own mtDNA sequence data, as well as published databases (Kraus et al., 1996; Parkinson, 1999) to assess the origin and biogeography of the South American pitvipers, as well as the ecological correlates of diversification within this group.

MATERIALS AND METHODS

Sampling and Molecular Techniques

Individuals of a variety of taxa of *Bothriechis*, *Bothrops*, *C. durissus*, and *Porthidium* were obtained through fieldwork or from captive collections. For DNA analysis, we collected tissue (ventral scale clippings or liver tissue) and/or blood (from the caudal vein) from living or recently dead specimens. Tissue samples were stored in 70–100% ethanol, whereas

blood samples were collected into 0.5M EDTA and stored in a solution of 0.1M EDTA, 0.1M Tris, 2% sodium dodecyl sulphate (SDS). All samples were kept refrigerated as soon as possible after collection.

Genomic DNA was extracted from tissue and blood samples using standard molecular laboratory protocols (Hillis et al., 1996b). Two regions of the mtDNA molecule were amplified using the polymerase chain reaction (PCR): a 758 base pair (bp) section of the gene for cytochrome *b* (*cyt-b*), and/or a 890 bp region of the gene for NADH dehydrogenase subunit 4 (ND4). Details of primers, PCR conditions, and sequencing protocols are described in Pook et al. (2000). Sequencing was carried out on an ABI377 automated sequencer using the manufacturer's recommended protocols. The sequences were checked against the chromatogram output of the automated sequencer and aligned by eye using WordPerfect 5.1 (WordPerfect Corporation). Samples, vouchers, and GenBank accession numbers are listed in Appendix I.

Information on mtDNA Sequence Divergences

We estimated levels of mtDNA sequence divergence for the *cyt-b*, ND4 and 12s and 16s rDNA gene regions in a number of clades of pitvipers, depending on sequence availability. We generated ND4 and/or *cyt-b* sequences for various *Bothriechis*, *Bothrops*, *Crotalus*, and South American *Porthidium* samples and combined our ND4 data with that reported by Kraus et al. (1996); 12s and 16s rDNA data were those of Parkinson (1999).

Since the more divergent sequences are subject to saturation of transition sites, we used Kimura 2-parameter distances in all calculations involving distance measures. To estimate the levels of sequence divergence within genera or major clades, we selected the two most-basal clades within each group and calculated the average distance between them using the program PHYLTEST (Kumar, 1996).

We did not have access to sequence data of all genes for all taxa; therefore, it was important to compare rates of divergence among genes in order to determine if estimates of divergence in different genes are comparable. While it is well known that 12s and 16s rDNA show a slower rate of sequence evolution than *cyt-b* and ND4 (e.g., Thorpe et al., 1994), the relative rate of evolution between the latter two is less clearly established. In order to compare the rate of sequence divergence of ND4 and *cyt-b* for our pitviper data, we calculated a pairwise p-distance matrix for a subset of crotaline taxa for ND4 and *cyt-b*, and plotted

ND4 distances against the corresponding *cyt-b* distances. We then calculated the regression slope to estimate the relative rate of divergence of the two genes.

Lachesis sequences were from Zamudio and Greene (1997). Because the individual fragments of *cyt-b* and ND4 used in that study were short, and the genes appear to evolve at a similar rate in pitvipers (see below), we found joined sequences preferable to a separate analysis. To assess sequence divergence within *Lachesis*, we calculated the average distance between the Central and South American clades.

Sequence divergences between species of *Bothrops* were calculated separately from our own *cyt-b* and ND4 data. We included representatives of numerous species, including several major radiations within a species complex (e.g. the *B. atrox* complex; Wüster et al., 1997, 1999). Within-group divergence was calculated from the average distance between *B. pictus* and the remainder of the genus.

For *Crotalus*, we calculated Kimura 2-parameter distances for *cyt-b*, based on our data on *C. basiliscus*, *C. durissus*, *C. molossus*, *C. scutulatus*, *C. viridis*, and a published sequence of *C. cerastes*, using *C. cerastes* as the sister group to other species of *Crotalus*, based on preliminary analyses (not shown). For ND4, we combined the published sequences of Kraus et al. (1996) with our own data on *C. durissus*, *C. scutulatus*, and *C. viridis*. As *C. durissus* appears to be the sister species to other *Crotalus* species included (not shown), we calculated distances between this taxon and the remainder of the genus. We also calculated average levels of divergence in ND4 and 12s and 16s sequences between *Crotalus* and *Sistrurus*. Since the sampling of *Crotalus* species was limited for these datasets, *Sistrurus* increased the likelihood of including both branches of what is likely one of the most basal dichotomies of the rattlesnake phylogenetic tree (Parkinson et al., this volume). Divergences of South American *C. durissus* were calculated from our own *cyt-b* data.

For *Porthidium*, we calculated ND4 divergences for three subsets of taxa: one for *Porthidium (sensu stricto)*, one for South American samples of *Porthidium*, and one for *Porthidium (sensu lato)*. We calculated *cyt-b* divergences for the South American populations of *Porthidium* from our own data.

In order to test if similar levels of sequence divergence in different clades can be interpreted as evidence of similar age, it is necessary to test if rates of nucleotide substitution differ between clades. For this purpose, we used the relative rate test. If the rate of

sequence evolution in all lineages of the ingroup is equal, then the distances of different ingroup species or clades to the outgroup should be equal. As an outgroup, we used sequences from the Malayan Pitviper (*Calloselasma rhodostoma*), which was rooted outside the New World pitvipers in all phylogenetic studies of the subfamily (e.g., Kraus et al., 1996; Vidal et al., 1997, 1999; Vidal and Lecointre, 1998; Parkinson, 1999). The New World pitvipers were grouped by genus or clade. The groups used were *Bothrops* (including *Bothriopsis* and *Bothrocophias*), *Crotalus* and *Sistrurus*, *Porthidium (sensu lato)* for *cyt-b*, these and *Bothriechis* for ND4, and all the preceding, as well as *Ophryacus* and *Lachesis* for 12s and 16s rDNA. We carried out pairwise relative rate tests, using Kimura 2-parameter distances, and the two-cluster test algorithm of Takezaki et al. (1995), implemented through the program PHYLTEST (Kumar, 1996).

Phylogenetic Analysis

We used both maximum parsimony (MP) analysis of unweighted sequence data and maximum likelihood (ML) analysis in order to infer the phylogeny of several groupings of Neotropical pitvipers. In the case of ML, we used the program MODELTEST (Posada and Crandall, 1998) to calculate the best model of sequence evolution for each dataset. A ML search using these parameters was used to construct a phylogenetic tree for the taxa concerned. The sequence evolution parameters were recalculated from that tree, and a further tree was constructed based on the recalculated parameters. This was repeated until no further changes in parameter values were found.

Porthidium.—We aligned 693 bp of ND4 sequence, corresponding to all but the first bp of Kraus et al. (1996), of the following taxa: *A. nummifer*, *P. nasutum*, and *P. ophryomegas* (Costa Rica; all from Kraus et al., 1996), *P. l. arcossae* (Ecuador), *P. l. rozeti* (NW Venezuela), and *P. nasutum* (Ecuador). Based on the results of Kraus et al. (1996) and Parkinson (1999), which support the monophyly of Hog-nosed Pitvipers, we assigned *A. nummifer* to the near outgroup and we used a sequence of *C. rhodostoma* as the far outgroup. An Old World pitviper was selected as the distant outgroup in view of the possibility that *Porthidium (sensu lato)* is not monophyletic, and includes some of the basal-most lineages of New World pitvipers (e.g., Werman, 1992). We used MP analysis of the unweighted data, implemented through the exhaustive search algorithm of PAUP* 4.0b8 (Swofford, 2001). Branch support was assessed using bootstrap analysis

(Felsenstein, 1985), with 10,000 replications using branch-and-bound searching. Bremer (1994) support for the various branches of the tree was investigated by repeating exhaustive searches while retaining successively longer trees until all nodes were collapsed. Maximum likelihood searching used branch-and-bound searching. Maximum likelihood bootstrap support was calculated over 500 replicates using heuristic searching, a neighbor-joining (NJ) starting tree, and SPR branch-swapping.

Crotalus durissus.—We aligned 701 bp of *cyt-b* sequence for 15 specimens from 10 localities, representing 8 nominal subspecies of *C. durissus* from Central and South America. Parsimony analysis of the unweighted data was implemented using PAUP* 4.0b8 (Swofford, 2001), using the branch-and-bound algorithm. Trees were outgroup-rooted using sequences of *C. scutulatus*, *C. v. viridis* (both from C. Pook, pers. comm.) and *C. molossus*. Branch support was assessed using bootstrap analysis, using 1,000 replicates and the branch-and-bound search algorithm. Bremer (1994) support for the various branches of the tree was investigated by repeating branch-and-bound searches while retaining successively longer trees until all nodes were collapsed. Maximum likelihood searching used an NJ starting tree, heuristic searching, and tree-bisection-reconnection (TBR) branch-swapping. Maximum likelihood bootstrap support was calculated over 100 replicates, using heuristic searching, an NJ starting tree and SPR branch-swapping.

Bothrops.—We used parsimony analysis of 1401 bp of the mtDNA genes for *cyt-b* and ND4. Our analysis included 28 species of *Bothrops* (*sensu stricto*), as well as two species of *Porthidium* and one of *Crotalus*. The resulting trees were outgroup-rooted using a sequence of *C. rhodostoma*. The analyses were carried out using a heuristic search algorithm, TBR branch-swapping, and 10,000 random addition sequence repeats. The levels of sequence divergence observed in the database indicated that saturation of some types of bp substitution, in particular, transitions at third bp codon positions, may be a problem. In order to investigate the effect of this potential problem on the data, we repeated the parsimony analysis under exclusion of third codon position transitions. Branch support was assessed using bootstrap analysis with 500 bootstrap replicates with five random addition sequence repeats each, using TBR branch-swapping. The initial ML search involved heuristic searching, using TBR branch-swapping and a NJ starting tree. Maximum likelihood bootstrap support was calculated

over 100 replicates, using heuristic searching, an NJ starting tree, and NNI branch-swapping.

Molecular Clock Calibration

One of the potential advantages of molecular sequence data over morphological data is that the former can, in some circumstances, allow the timing of divergences among lineages to be estimated. Time estimates for major lineage splits, independent of prevailing geological hypotheses, are particularly important for the reconstruction of biogeographical histories (Cadle, 1985). Appropriate use of a molecular clock, however, requires a number of assumptions that are unrealistic, and involves error margins that are often large compared to the times estimated (Hillis et al., 1996a). Nevertheless, the use of molecular clocks can prove illuminating, and some studies have found what appear to be highly consistent rates of sequence evolution in some groups (e.g., Macey et al., 1998). We compared two sequence evolution rate estimates at opposite ends of the spectrum for ectothermic vertebrates, and the effect of each calibration on our interpretation of the evolution of the South American pitviper fauna. Although the absolute timing of events is subject to the errors associated with clock calibration, use of these clocks allows us to estimate the relative timing of events in pitviper evolution.

The timing of cladogenic events in squamates remains uncertain, as there is no widely accepted, robust estimate of a “squamate molecular clock” for mtDNA in general, or for specific mitochondrial genes (Avice et al., 1998). Zamudio and Greene (1997) surveyed the literature and concluded that the available data were consistent with rates of between 0.47% and 1.32% my^{-1} . These estimates, however, are made either for the entire mitochondrial DNA genome, based on restriction-fragment-length polymorphism (RFLP) analysis, or for sets of sequences of several different genes. Of the latter, all included slowly-evolving 12s and 16s rDNA or cytochrome oxidase subunit I sequences. Consequently, these estimates are difficult to relate to sequence variation in specific mitochondrial genes. Based on these estimates and observed levels of sequence divergence between clades of *Lachesis*, Zamudio and Greene (1997) hypothesized that cladogenic events in *Lachesis* may have been influenced by two vicariance events—the uplifts of the northern Andes and the Cordillera de Talamanca. If one assumes the Zamudio and Greene (1997) hypotheses of causative vicariant events to be correct (which involves a certain level of circularity),

Table 2. Sequence divergence data between basal lineages of several genera and major clades of New World pitvipers. The distance measure used is the Kimura 2-parameter distance, expressed as % \pm 1 SE. The data for *Lachesis* refer to the combined ND4 + cyt-*b* dataset of Zamudio and Greene (1997).

Taxon	ND4	Cyt- <i>b</i>	12s and 16s
<i>Bothrops</i>	15.8 \pm 1.3	13.9 \pm 1.2	5.4 \pm 0.6
<i>Bothrocophias</i> (includes <i>B. campbelli</i>)	10.9 \pm 1.3	13.4 \pm 1.4	
<i>Porthidium</i> (<i>sensu stricto</i>)	13.5 \pm 1.4		5.9 \pm 0.9
<i>Porthidium</i> (South American taxa only)	5.6 \pm 0.8	4.7 \pm 0.7	
<i>Porthidium</i> (<i>sensu lato</i>)	14.9 \pm 1.2		6.4 \pm 0.7
<i>Bothriechis</i>	16.1 \pm 1.5	16.6 \pm 1.7	7.4 \pm 0.9
<i>B. schlegelii</i> (Costa Rica and Ecuador)	9.6 \pm 1.3		
<i>Crotalus</i> + <i>Sistrurus</i>	20.0 \pm 1.7		6.7 \pm 0.7
<i>Crotalus</i>	14.8 \pm 1.3	19.1 \pm 1.6	5.8 \pm 0.7
<i>C. durissus</i>		8.0 \pm 0.9	
<i>C. durissus</i> (South America only)		1.5 \pm 0.4	
<i>Ophryacus</i>			6.0 \pm 0.9
<i>Lachesis</i>	9.0 \pm 1.3		2.8 \pm 0.6
<i>Lachesis</i> (South America only)	1.1 \pm 0.3		

then correlating sequence divergences among *Lachesis* taxa with the timing of these putative vicariant events results in estimates of 0.60–0.76% my^{-1} and 0.66–1.06% my^{-1} , respectively (Pook et al., 2000), and the two estimates overlap between 0.66–0.76% my^{-1} .

The data presented in this study offer two alternative calibration points for the squamate cyt-*b* and ND4 molecular clock. Both are based on the time of the final emergence of the Isthmus of Panama, estimated at 3.5 mya (Coates and Obando, 1996). *Crotalus durissus* almost certainly colonized South America via this landbridge (Vanzolini and Heyer, 1985). Another potential candidate for divergence after the isthmian emergence is the South American group of *Porthidium*. Species of *Porthidium* appear to be poor overwater dispersalists, since none are found on islands off the coast of Central and South America, despite the fact that several species occur in coastal areas (Porrás et al., 1981; Campbell and Lamar, 1989). The one exception to the rule is the presence of *P. lansbergii* on Isla Margarita (Venezuela), but this continental shelf island was linked to the South American mainland at various times in the Pleistocene, during times of lowered eustatic sea-levels.

In order to assess the rate of sequence divergence within these clades we assumed that the colonization of South America, and the first cladogenic event within South American taxa, occurred approximately at the same time as the emergence of the Isthmus, 3.5 mya. We used the largest measure of pairwise sequence divergence among the South American haplotype lineages to estimate rate divergence, by dividing the

p-distance by the time since colonization. We calculated 95% confidence limits for our estimates by assuming the spread of divergence values around the mean rate to correspond to a Poisson distribution (Hillis et al., 1996a).

RESULTS

mtDNA Sequence Divergence

The levels of intrageneric Kimura 2-parameter sequence divergence in cyt-*b*, ND4 and 12s and 16s rDNA are shown in Table 2. The plot of ND4 divergence against cyt-*b* divergence (not shown) reveals a regression slope of 1.04, which suggests that, in New World pitvipers these two genes evolve at approximately the same rate, so that rates of divergence across the two genes are comparable.

Several trends are apparent from the divergences. First, bearing in mind differences in species-sampling, the major clades [rattlesnakes; *Bothriechis*; *Bothrops*; *Porthidium* (*sensu lato*)] display similar levels of maximum within-group sequence divergence in the cyt-*b* and ND4 genes (~ 13.9–20.0%). Divergence levels in 12s and 16s rDNA were also similar among genera, lying between 5.4 and 7.4%. *Porthidium* (*sensu stricto*), *Bothrocophias*, and *Ophryacus* display only slightly lower levels of intrageneric divergence, and only *Lachesis* consistently shows much lower levels of variation.

Second, where information is available, and except in *Bothrops*, levels of sequence divergence within the South American representatives of each group are low, varying from 1.1 to 5.6% in ND4 and cyt-*b* (no data

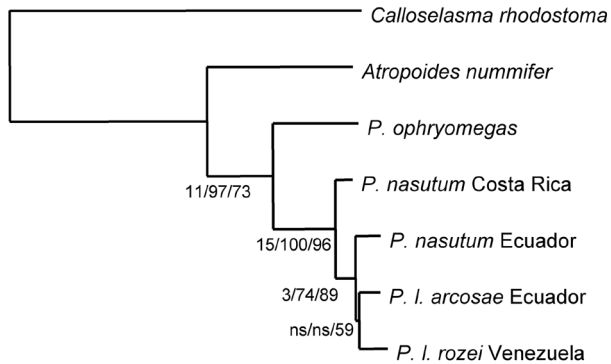


Fig. 1. Phylogenetic tree for *Porthidium* spp., based on ML analysis ($-\ln L = 2234.21490$). The consensus of two equally most-parsimonious trees was topologically identical, except that the relationships among the three South American taxa were unresolved. Numbers on nodes indicate Bremer Support / MP bootstrap support / ML bootstrap support.

are available for 12s and 16s rDNA, nor for sequence variation within South American *Bothriechis schlegelii*; however, the ND4 sequence divergence between Costa Rican and Ecuadorian *B. schlegelii* is 9.6%).

The relative rate tests show that the different genera or clades do not differ significantly in their levels of sequence divergence from *Calloselasma* in their *cyt-b* and ND4 sequence, suggesting that the rate of sequence evolution is equal across these genera. In the case of the 12s and 16s data, all pairwise comparisons were insignificant, except that *Bothriechis* differed significantly more from *Calloselasma* than did *Porthidium* (*sensu lato*). Consequently, we cannot reject the hypothesis of essentially clocklike sequence evolution in the *cyt-b* and ND4 genes, nor for the comparisons between the 12s and 16s rDNA sequences of most clades

Phylogeny

Porthidium.—The *Atropoides* and *Porthidium* database contained 90 parsimony-informative characters. Our analysis of the phylogeny of the available sequences of *Porthidium* resulted in two equally most-parsimonious trees of 307 steps (CI = 0.8143, HI = 0.1857, RI = 0.5476), which differ in arrangement of the South American taxa. Both agree on the monophyly of the three South American samples, to the exclusion of Costa Rican *P. nasutum* and *P. ophryomegas*. This monophyly is supported by reasonable bootstrap and Bremer support values. An exhaustive ML search carried out under the assumptions of the HKY85 model with gamma distribution identified as optimal by

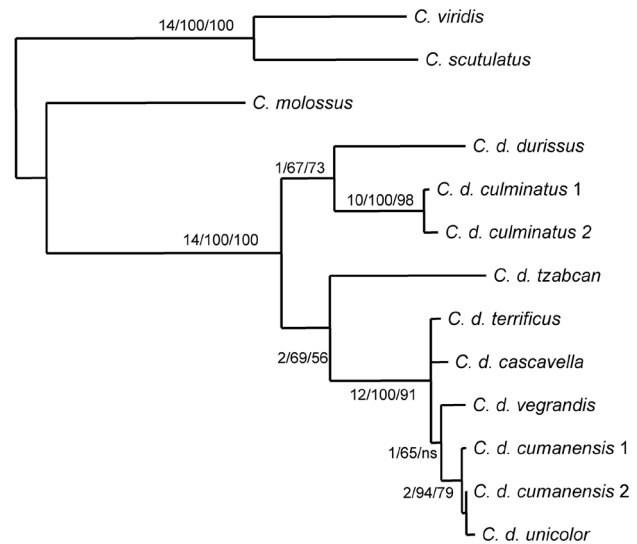


Fig. 2. Bootstrap consensus of six equally most-parsimonious phylogenetic hypotheses for populations of the *Crotalus durissus* species complex, based on parsimony analysis of 701 bp of *cyt-b* sequence information (length 272 steps, CI = 0.7426, HI = 0.2574, RI = 0.7491). Numbers above branches indicate Bremer support / MP bootstrap support / ML bootstrap support.

MODELTEST resulted in a single tree consistent with the consensus of the two MP trees (Fig. 1)

Crotalus durissus.—The *Crotalus durissus* database contained 116 parsimony-informative characters. Our parsimony analysis resulted in six equally most-parsimonious trees of 274 steps (CI = 0.7445, HI = 0.2555, RI = 0.7500). The bootstrap support for various nodes is shown in Figure 2. The ML tree generated based on the HKY85 model with gamma distribution identified as optimal by MODELTEST resulted in a single tree consistent with the MP consensus tree (Fig. 2). Bootstrap support values in the ML analysis were similar to those obtained using MP. Our analysis strongly supports the monophyly of all South American populations of the *C. durissus* complex, including *unicolor* and *vegrandis*, whereas the Central American and Mexican populations are paraphyletic. Additionally, sequence divergence among the South American *C. durissus* populations was consistently low, with a maximum pairwise divergence of 1.5%, whereas divergences among the Central American lineages ranged up to 8.0%.

Bothrops.—The *Bothrops* database contained 491 parsimony-informative characters. The analysis of the unweighted data resulted in 2 equally most parsimonious trees of 2,224 steps (CI = 0.3970; HI = 0.6030; RI = 0.5318). Bootstrap analysis of these data reveals high levels of support for many nodes (Fig. 3).

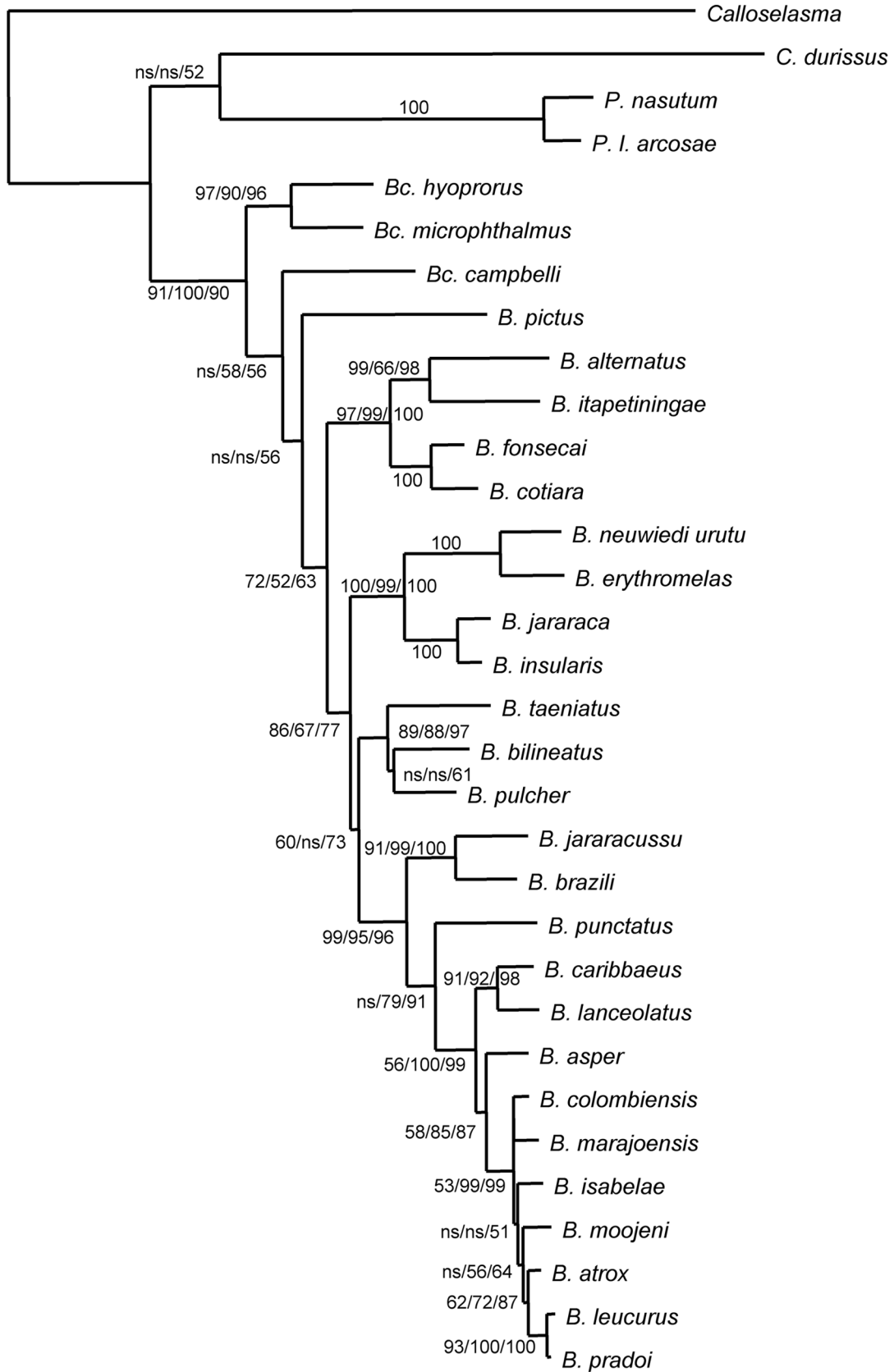


Fig. 3. Phylogenetic tree for the included species of *Bothrops* and *Bothrocophias*, based on ML analysis ($-\ln L = 11310.48163$). Numbers on nodes indicate MP unweighted bootstrap support / MP no 3rd codon position transition bootstrap support / ML bootstrap support. NS = bootstrap support < 50% or contradicted by relevant analysis.

Eliminating third codon position transitions from the analysis results in eight equally most parsimonious trees of 875 steps (CI = 0.5234; HI = 0.4766; RI = 0.6099). The eight trees differ only in the arrangement of the branches within the *B. atrox* complex. Bootstrap support levels are shown in Figure 3. Although levels of bootstrap support differ for some nodes, there are no strong contradictions with respect to the unweighted tree, and none of the main conclusions enumerated below are affected in any way. The ML search, run using the GTR + I + G model of sequence evolution identified as optimal by MODELTEST, yielded the tree shown in Figure 3. Although some nodes differ from those supported by the MP analyses, the major points noted below are supported by this tree.

All analyses support several nodes which substantially affect our understanding of phylogenetic relationships within *Bothrops* and related genera: (1) the taxa *hyoprora*, *microphthalmus*, and *campbelli* share a more recent ancestor with other *Bothrops*, not *Porthidium*, contrary to Schätti and Kramer (1993); the association between *hyoprora* and other *Bothrops* was previously noted by Gutberlet (1998b), Kraus et al. (1996) and Parkinson (1999); (2) the monophyly of *Bothrocophias* is not supported, as *B. campbelli* does not group with *B. microphthalmus* and *B. hyoprora*; (3) all included species of *Bothriopsis* are rooted within *Bothrops*, making the latter paraphyletic if *Bothriopsis* is recognized; (4) *Bothriopsis* is polyphyletic, as *Bothrops punctatus* shares a more recent common ancestor with species such as *B. jararacussu* and *B. atrox* than with the Amazonian species of *Bothriopsis*; and (5) the Central American and Caribbean taxa (*B. asper*, *B. caribbaeus*, *B. lanceolatus*) are rooted deep within the South American taxa. Bootstrap support for these important nodes is consistently high.

Molecular Clock Calibration

The various South American lineages of *C. durissus* differ by a maximum of 10 bp positions (1.4%) in their *cyt-b* sequences. Assuming that colonization of South America and splitting of haplotype lineages of South American *Crotalus* occurred immediately after the uplift of the Isthmus leads to an estimate of *cyt-b* sequence evolution of 0.4% my^{-1} . Taking the highest pairwise measures of divergence (10 bp positions) and assuming a Poisson distribution for the accumulation rate of substitutions, the 95% confidence limits for the rate of sequence evolution lie between 0.2–0.73% my^{-1} . There is no reason, however, why the first cladogenic split could not have occurred long after emergence of

the Isthmus. Since this estimate lies at the lower end of substitution rate estimates for squamates and any faster rate is consistent with the evidence, this estimate cannot be regarded as useful, as it does not constrain the possible rates of substitution beyond what has been estimated elsewhere.

The South American populations of *Porthidium*, when assessed across 1,388 bp of combined *cyt-b* and ND4 sequences, differ from each other at 66–70 bp positions (*p*-distance 4.76–5.04%). If the date of emergence of the Isthmus of Panama of 3.5 mya, as proposed by Coates and Obando (1996), is taken as the earliest date of divergence of the three haplotype lineages involved, this leads to an estimated rate of sequence divergence for these two genes of 1.36–1.44% my^{-1} . Since the phylogenetic relationships among the three South American *Porthidium* sequences are unresolved, taking the mean of the three pairwise measures of divergence (68 bp positions) and assuming a Poisson distribution for the accumulation rate of substitutions, the 95% confidence limits for the rate of sequence evolution lie between 1.09–1.77% my^{-1} . This estimate for the mean rate is higher than most previous estimates of a squamate mtDNA molecular clock. Given the apparently limited overwater dispersal capability of *Porthidium*, however, we see little or no a priori reason to dismiss this calibration for a squamate *cyt-b* and ND4 molecular clock. A more detailed mtDNA phylogeny for a greater number of South American and Isthmian Central American populations of *Porthidium* could further test this hypothesis. Acceptance of a “*Porthidium*-clock” clearly leads to a later estimate than a “*Lachesis*-clock” for major events in New World pitviper phylogeny and biogeography.

DISCUSSION

Origins of the South American Pitviper Fauna

The results presented here allow for a number of conclusions regarding origins, evolution, and biogeography of the South American herpetofauna. We present alternative timing for the various events, based on joint *cyt-b* and ND 4 data (where sequences for both genes are available) and both the *Lachesis*-clock of 0.66–0.76% my^{-1} calculated from the data of Zamudio and Greene (1997) and the *Porthidium*-clock of 1.36–1.44% my^{-1} calculated for that group from our data. In view of the many caveats surrounding the use and interpretation of molecular clocks (Hillis et al. 1996a), we suggest that the two clocks could be interpreted as upper and lower boundaries of the timeframe

Table 3. Approximate chronology of the colonization of South America by pitvipers, based on two different estimates of rates of sequence divergence for *cyt b* and ND4, assuming a clock-like rate of sequence divergence: 0.66–0.76% my⁻¹ is derived from the *Lachesis* data of Zamudio and Greene (1997); 1.36–1.44% my⁻¹ is derived from South American *Porthidium* used in this study.

Timing based on <i>Lachesis</i> -clock (mya)	Timing based on <i>Porthidium</i> -clock (mya)	Event
30–16	15–8.5	First divergence within major New World pitviper clades
23–20	11–10	Ancestor of <i>Bothrops</i> diversifies in South America
14–12	6.6–6.2	Split between Central and South American <i>Lachesis</i>
14–12	7–6.6	Split between Costa Rican and Ecuadorian <i>Bothriechis schlegelii</i> lineages
12–10.5	5.9–5.6	First cladogenesis within <i>C. durissus</i>
7.7–6.7	3.5	First cladogenesis in South American <i>Porthidium</i>
2.2–1.9	1	First cladogenesis in South American <i>C. durissus</i>

when these events may have occurred. Nonetheless, it is worth noting that the estimate of the oldest divergences in *Bothrops* at 13 mya, calculated by Hedges (1996) based on the data of Cadle (1992), is more consistent with the faster end of the spectrum of pitviper *cyt-b* and ND4 rates suggested here. The possible timetable of the colonization of South America by pitvipers is summarized in Table 3. The absolute timing of those events should be regarded as of lesser interest than their relative timing.

Among New World pitvipers, Parkinson et al. (this volume) suggest a basal dichotomy (albeit weakly supported) between a Nearctic clade, including rattlesnakes and *Agkistrodon*, and a Neotropical pitviper clade, including the remaining New World pitviper genera. This basal division may correspond to the Tertiary Vicariance II event (dispersal of northern taxa into Central America, followed by vicariance between central and northern clades; proposed by Savage, 1982). The latter clade gave rise to the ancestral stocks of today's Neotropical pitviper genera at some point during the Tertiary, presumably in Central America. At least four separate colonizations of the South American mainland must have taken place from within the Neotropical clade: an early colonization by the ancestor of all *Bothrops*, and, much later, one by *Porthidium*, one by *Bothriechis schlegelii*, and one by *Crotalus durissus* (see Parkinson, 1999; Vidal et al., 1999; Parkinson et al., this volume). The origins of *Lachesis* remain uncertain. Zamudio and Greene (1997) showed reciprocal monophyly of Central and South American *Lachesis*, and other studies of mtDNA sequences have failed to converge on a robust estimate of the sister group of *Lachesis*. Different hypotheses have variously placed *Lachesis* as unresolved (Vidal et al., 1999), or as the sister group of all bothropoid genera except *Cerrophidion* (Werman,

1992), of all New World pitvipers (Kraus et al., 1996, transversion parsimony), of *Bothrops* (Kraus et al., 1996, third codon position transitions excluded), of *Bothriechis* + *Bothrops* (Vidal et al., 1997), of *Bothriechis* + *Porthidium* (Werman et al., 1999), of *Bothriechis* (Parkinson, 1999), or of *Ophryacus* and/or *Bothriechis* (Parkinson et al., this volume). Most of the analyses of Gutberlet (1998b) found *Lachesis* to be the sister taxon of *Bothrops*. But even if *Lachesis* is the sister taxon of all *Bothrops*, this does not preclude a Central American origin, as *Lachesis* may be the sister to the ancestor of the first pitviper to colonize South America. The origin of *Lachesis* is insufficiently resolved, although most published pitviper phylogenies support a Central American origin.

Although the levels of sequence divergence found within the major clades are similar, the genus *Bothrops* contains considerably more sequence divergence than the South American representatives of other clades. The South American *Bothrops* are paraphyletic with respect to species also found in Central America, and diversification of the genus appears to have taken place in South America. This suggests that the common ancestor of all *Bothrops* was the first viperid to colonize South America, sometime during the Miocene, 10–23 mya. A single species, *B. asper*, reinvaded Central America much later, and remains the only widespread species of *Bothrops* there.

More extensive sampling of species and populations of *Porthidium* is needed, but the present data suggest that South American populations of *P. lansbergii* and *P. nasutum* form a monophyletic group that represent a single invasion from Central America to South America. The *Lachesis*-clock places this event in the late Miocene, 7.7–6.6 mya, whereas the *Porthidium*-clock takes an invasion immediately after the emer-

gence of the Isthmus of Panama (3.5 mya) as its calibration point.

We do not have sequence data from sufficient localities to shed light on the history of colonization of South America by *Bothriechis*, but Crother et al. (1992) hypothesized that this event followed emergence of the Isthmus of Panama. The high levels of sequence divergence between Ecuadorian and Costa Rican *B. schlegelii* identified in this study are compatible with an invasion as long as 14–6.6 mya, pre-dating the emergence of the Isthmus by a considerable margin, but a more detailed phylogeographic study of this wide-ranging species is required to elucidate this problem.

Crotalus durissus is clearly a recent occupant of the South American continent, as noted by Vanzolini and Heyer (1985). The low levels of sequence divergence among South American populations of *C. durissus* are consistent with the hypothesis that this species invaded the South America during the Pleistocene, 1–2 mya, after the uplift of the Panama landbridge. On the other hand, the Central American lineages (*C. d. durissus*, *C. d. culminatus* and *C. d. tzabcan*) are clearly much older.

In summary, our sequence data suggest that only the colonization of South America by *C. durissus* can be unambiguously attributed to overland colonization after final emergence of the Isthmus of Panama. Colonization by *Porthidium*, however, may also post-date this emergence. The ancestor of all *Bothrops* clearly occupied South America long before the emergence of the Isthmus, and the available data for *Lachesis* and *B. schlegelii* are consistent with pre-Isthmian divergence between Central and South American populations.

Faunistic exchange between Central and South America prior to final emergence of the Isthmus of Panama appears to be a common pattern among ectothermic vertebrates, including amphibians (see Hanken and Wake, 1982) and snakes (see Cadle, 1985). Iturralde-Vinent and MacPhee (1999) note the possibility of a land connection between Central and South America in the late Middle Miocene, 12.9–11.8 mya. Our data can be regarded as consistent with this hypothesis. Depending on rates of sequence divergence, the time of this land connection would correspond either to the first cladogenesis of *Bothrops* in South America (assuming fast rates of sequence evolution), or the split between Central and South American *Lachesis* and *B. schlegelii* (assuming slow rates of sequence evolution) (Table 3).

Morphological Diversification of Pitvipers in Central and South America

A comparison of the levels of mtDNA sequence divergence and morphological diversity in the different clades of New World pitvipers reveals an interesting pattern. Several clades [rattlesnakes, *Bothriechis*, *Bothrops* and *Porthidium* (*sensu lato*) and (*sensu stricto*)] contain broadly similar levels of sequence divergence in the genes examined here. The same applies to *Ophryacus*, at least as far as 12s and 16srDNA data are concerned.

The relative rate tests generally revealed approximately equal rates of mtDNA sequence divergence from the outgroup taxon *Calloselasma*, suggesting that rates of sequence evolution do not differ significantly between these clades. Consequently, the similar levels of DNA sequence divergence within each clade suggest that they are of approximately equal age, dating back to the late Oligocene or the Miocene. Our sampling of *Bothrops* species was more comprehensive than that for other genera. Consequently, compared to *Bothrops*, our sequence divergence data are likely to underestimate the pairwise divergence present in other clades.

Whereas these various clades have approximately equal levels of mtDNA sequence divergence, and thus may be inferred to be of similar age, they differ profoundly in levels of morphological divergence. The Central American clades *Bothriechis* and *Porthidium* (*sensu lato*) are relatively conserved in their size, body shape, and microhabitat use, and this trend occurs to an even greater extent in *Porthidium* (*sensu stricto*). The rattlesnakes (*Crotalus* and *Sistrurus*) are relatively conserved with respect to body characteristics and microhabitat use, but diverse in terms of body size and macrohabitat use. *Bothrops*, however, is even more variable and contains representatives of extremes in all of these categories. Furthermore, *Bothrops* is conspicuously more speciose than the other genera/clades, except the rattlesnakes. If *Porthidium* (*sensu lato*) is indeed the sister clade of *Bothrops*, as suggested by Parkinson et al. (this volume), then the contrast is even more marked (see Table 1).

Our sequence divergence data suggest that greater age is an unlikely explanation for greater diversity of species, body plans, and natural history in *Bothrops*. Instead, the available data suggest that the common ancestor of all *Bothrops* was the first viperid to colonize South America, at least 4–10 my before any further pitvipers. Populations of this common ancestor would have been exposed to environments devoid of viperid

faunas and with open niches to occupy. The mid-Tertiary macrostomatan snake fauna of South America likely consisted primarily of boines and xenodontines (Cadle, 1985; Cadle and Greene, 1993), which do not show the trophic specializations of viperids. It is therefore likely that the niches occupied by viperid snakes elsewhere were at least partially unoccupied in mid-Tertiary South America, as were many niches presently occupied by a variety of Central American xenodontines, particularly the “goo-eaters” (Cadle and Greene, 1993). Consequently, the cause of the relatively rapid diversification of *Bothrops* in South America may have been adaptive radiations into niches largely unoccupied. Clades of the same age from Central America would have lacked similar opportunities for diversification, perhaps due to the presence of other pitviper lineages already occupying relevant niches.

The ecological structure of Neotropical colubrid snake assemblages appears to be heavily influenced by historical contingency (i.e., which clades with specific, relatively conserved natural history parameters occupy a given area; Cadle and Greene, 1993). The ecological structure of pitviper communities, however, may follow a more deterministic pathway with relatively similar ecomorphs evolving repeatedly in different clades (= convergence). This would parallel the situation seen in Greater Antillean *Anolis* lizards, which have repeatedly evolved a series of comparable ecomorphs after independent colonization of different, previously unoccupied islands (Losos et al., 1998). A quantitative approach to the definition of ecomorphs and niche partitioning in pitvipers (Martins et al., 2001, this volume) across their entire distribution and taxonomic breadth could be a fruitful field of research in this context.

The hypothesis of an adaptive radiation in *Bothrops* in South America, in contradistinction to constraint by competition among other pitviper genera in Central America, is also supported by the biogeographical distribution of later invaders to South America. Both *Porthidium* and *Bothriechis* are restricted to the northwestern regions of South America, where there are no species of *Bothrops* of similar size and habitat use. Where such species approach the range of *Porthidium* or *Bothriechis*, they tend to be found in different habitats. *Bothrocophias campbelli* occupies higher elevations than *P. lansbergii* or *P. nasutum* (and attains a considerably larger maximum size), and *B. medusa* occupies higher and moister habitats than *P. lansbergii* in northern Venezuela

(Roze, 1966; Campbell and Lamar, 1989). *Bothrops punctatus* overlaps macrogeographically with *B. schlegelii*, but grows much larger (Campbell and Lamar, 1989) and the extent of its arboreality and ecological overlap with *B. schlegelii* is unclear. The range of *B. schlegelii* does not overlap with that of more clearly arboreal species of *Bothrops*, which are confined to South America east of the Andes.

Of the more recent colonists of South America, only *C. durissus* has achieved a wide distribution. Although *C. durissus* is broadly sympatric with several species of *Bothrops*, it is different in size, morphology, behavior, habitat choice, and other ecological factors. These differences may reduce ecological competition between these two groups. In many parts of its range (e.g., northeastern Brazil), *C. durissus* is the only large pitviper, and in areas where it is broadly sympatric with species of *Bothrops* of similar size, it often occupies drier, more open microhabitats. Finally, *Lachesis* is considerably larger than any *Bothrops*, and differs in various aspects of its natural history (Greene, 1997).

Systematic Implications

Porthidium.—Our analysis found *P. nasutum* to be paraphyletic with respect to *P. lansbergii*. This suggests that these two taxa form part of an incompletely understood species complex. More detailed systematic studies of these snakes are required for resolution.

Bothriechis schlegelii.—Our analysis revealed a surprisingly high level of ND4 sequence divergence (9.6 %) between Ecuadorian and Costa Rican *B. schlegelii*. This level of divergence is almost identical to that found between the most divergent species of *Lachesis* (Zamudio and Greene, 1997), or between unquestionably distinct species of *Bothrops* (e.g., between *B. jararaca* and *B. neuwiedi*, or between *B. asper* and *B. jararacussu*). It is also higher than all but one out of the 47 examples of mtDNA sequence divergence among major intraspecific phylogroups in amphibians and reptiles considered by Avise et al. (1998). This suggests that the systematics of various populations of *B. schlegelii* should be studied, and that this form is likely to be polyspecific, a view supported by the work of Solórzano et al. (1998).

Crotalus durissus.—Our analyses revealed high levels of sequence divergence (up to 8.3 %) between the Central American lineages of this species complex, suggesting the existence of lineages with long and independent evolutionary histories. As in *Bothriechis*, these high divergence values suggest that some of

these taxa may merit elevation to specific status. On the other hand, mtDNA divergence values of the South American taxa are extremely low. This includes *C. d. unicolor* and *C. d. vegrandis*, which differ from other South American populations with respect to color pattern and body size. These taxa have been regarded as separate species in the past (e.g., Klauber, 1972). They were treated as subspecies of *C. durissus* by Campbell and Lamar (1989) on the basis that *C. durissus* would be paraphyletic if they were excluded, a situation confirmed by this study. Several other South American subspecies of *C. durissus* have been recognized by various workers, but appear to be poorly defined. The low levels of sequence divergence of South American *C. durissus* suggest that these populations share a recent common past, and do not constitute lineages with long and independent evolutionary histories. Further studies of the *C. durissus* complex are in progress by J. Quijada-Mascareñas and others.

The status of *Bothriopsis*.—There is growing evidence that *Bothrops* (*sensu* Campbell and Lamar, 1989) is paraphyletic with respect to *Bothriopsis* (Cadle, 1992; Kraus et al., 1996; Parkinson, 1999; Parkinson et al., this volume; Salomão et al., 1997, 1999; Werman, 1992; this study). Salomão et al. (1997) acknowledged this parphyly and synonymized *Bothriopsis* with *Bothrops*.

Despite the evidence for rooting *Bothriopsis* within *Bothrops*, a number of workers have been reluctant to accept this change (e.g., McDiarmid et al., 1999; Parkinson, 1999; Parkinson et al., this volume). Their reluctance appears to be largely based on the untested assumption that *Bothriopsis* is a monophyletic group worthy of recognition. This implies that further splits of the genus *Bothrops* are required to avoid parphyly of this genus relative to *Bothriopsis*. As stated above, we prefer to retain *Bothrops* as a single, large genus that includes *Bothriopsis* (see Salomão et al., 1997; Wüster et al., 1998).

In our opinion, the results presented in this paper provide further reasons to consider *Bothriopsis* as a synonym of *Bothrops*, and to retain the latter as a single, highly diverse genus. Several of these arguments could also be applied to recognition of *Bothrocophias*. However, uncertainty about the relationships between the species assigned to this genus and the rest of *Bothrops* (Gutberlet, 1998b) suggest that recognition of *Bothrocophias* may be a more conservative approach. Such changes in nomenclature in this group of snakes will undoubtedly cause temporary confusion in the medical community.

Regarding the status of *Bothriopsis*, our data suggest that *Bothriopsis* (*sensu* Burger, 1971) is polyphyletic, as *B. punctata* shares a more recent common ancestor with various species of *Bothrops* (e.g., *B. atrox*, *B. jararacussu*) than with the Amazonian arboreal forms. The case for recognizing only a subset of the arboreal South American pitvipers as a separate genus seems weak at best.

Second, the genus *Bothrops*, including *Bothriopsis*, comprises a single monophyletic group, resulting from a single radiation in South America. This is in contradistinction to other South American pitviper genera that represent later invasions by Central American groups, and splitting *Bothrops* would obscure this biogeographic pattern. Highlighting interesting biogeographic patterns has been used to justify synonymizing established genera in other groups (e.g., Kluge, 1991).

Third, although *Bothrops* contains greater morphological and natural historical diversity than other pitviper genera (e.g., New World crotalines such as *Bothriechis* and *Crotalus*), it appears to be no older. The *cyt-b* divergence levels in our data for *Bothrops* *cyt-b* are similar to those observed in many other genera of reptiles and amphibians (Johns and Avise, 1998), and thus do not in themselves present grounds for splitting the genus.

Finally, the greater diversity of body forms and natural history traits found in *Bothrops* reflect the unique historical circumstances of the evolution of this clade (i.e., adaptive radiation on a continent previously devoid of viperid snakes), in contrast to other New World pitviper clades not exposed to these circumstances.

The ecological and morphological diversity of *Bothrops* may be seen as a justification to split this genus into several entities representing more homogeneous groupings (Parkinson, 1999; Gutberlet and Campbell, 2001). However, sister clades often differ in the number and diversity of species they contain, sometimes as a result of taxonomic bias but often for other reasons (Minelli, 1993). *Bothrops* clearly falls into this latter category. Further splitting of the genus, to create smaller or more homogeneous genera would obscure the evolutionary history of the clades concerned and the pattern of unequal diversity resulting from this history.

In conclusion, we feel that synonymizing *Bothriopsis* with *Bothrops* is supported by conceptual, theoretical, and pragmatic considerations, such as treatment of snakebite and use of venom in medical

research. Recognition of *Bothrops* as a single, diverse genus is thus the most appropriate reflection of the unique evolutionary and biogeographical history of this clade.

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APPENDIX I

Origin, vouchers, and GenBank accession numbers of samples sequenced in this study.

Institutional Codes for vouchers: IB = Instituto Butantan, São Paulo, Brazil, Herpetological Collection. FHGO = Fundación Herpetológica Gustavo Orcés, Quito, Ecuador. INHMT = Instituto de Higiene y Medicina Tropical “L. Izquieta Pérez”, Guayaquil, Ecuador. UTEP = University of Texas, El Paso. Collection numbers refer to preserved specimens unless otherwise stated. Photographs and/or morphological data for many unvouchered specimens are available from the first author. The first accession number represents the cytochrome *b* sequence, the second ND4. n/a = sequence not available. For 12s and 16s GenBank accession numbers, see Parkinson (1999).

Bothriechis: *B. lateralis*: Costa Rica. No voucher. AF292572, AF292610 *B. schlegelii*: Ecuador: Pichincha: Pedro Vicente Maldonado. FHGO live coll. 2170. AF292573, AF292611.

Bothrocophias: *B. campbelli*: Ecuador: Chimborazo: Pallatanga. INHMT, unnumbered. AF292584, AF292622. *B. hyoprora*: Ecuador: Morona Santiago: Macuma. FHGO 4005. AF292576, AF292614. *B. microphthalmus*: Ecuador: Zamora Chinchipe: Cuenca del Río Jamboe: Pumbami. FHGO 2566. AF292577, AF292615.

Bothrops: *B. alternatus*: Brazil: Paraná: Pinhão. IB 55314. AF292579, AF292617. *B. asper*: Belize: Mile 38, Western Highway. Belize Zoo, live collection. AF292600, AF292638. *B. atrox*: Suriname: Coronie District. Released after sampling. AF246267, AF246277. *B. bilineatus*: Ecuador: Morona Santiago: Macuma. FHGO 983. AF292592, AF292630. *B. brazili*: Ecuador: Morona Santiago: Macuma. FHGO 982. AF292597, AF292635. *B. caribbaeus*: Saint Lucia: released after sampling. AF292598, AF292636. *B. colombiensis*: Venezuela: Guárico: Altigracia de Orituco. Roadkill, not collected. AF292602, AF292640. *B. cotiara*: Brazil: Santa Catarina: Herval d'Oeste. IB live coll. 3829. AF292581, AF292619. *B. erythromelas*: Brazil: Bahia: Guanambi. IB 55541. AF292588, AF292626. *B. fonsecai*: Brazil: São Paulo: Campos do Jordão. IB 55543. AF292580, AF292618. *B. insularis*: Brazil: São Paulo: Ilha da Queimada Grande. Released after sampling. AF292590, AF292628. *B. isabellae*: Venezuela: Portuguesa: Guanare. Released after sampling. AF292603, AF292641. *B. itapetiningae*: Brazil: Distrito Federal: Brasília. IB live coll. 4982. AF292582, AF292620. *B. jararaca*: Brazil: Paraná: Piracuara. Not vouchered. AF292589, AF292627. *B. jararacussu*: Brazil: São Paulo: Cananéia. IB 55313. AF292596, AF292634. *B. lanceolatus*: Martinique. Not vouchered. AF292599, AF292637. *B. leucurus*: Brazil: Bahia: Porto Seguro. IB 55480. AF246272, AF246284. *B. marajoensis*: Brazil: Pará: Ilha de Marajó: 10 km NW Camará. Released after sampling. AF292605, AF292643. *B. moojeni*: Brazil: Distrito Federal: Brasília. IB 56558. AF292606, AF292644. *B. neuwiedi urutu*: Brazil: São Paulo: Angatuba. IB 55555. AF292585, AF292623. *B. pictus*: Peru: Ayacucho: Pullo. O. Pesantes, personal collection AF292583, AF292621. *B. pradoi*: Brazil: Espírito Santo: Domingos Martins. IB 55557. AF246269, AF246282. *B. pulcher*: Ecuador: Zamora Chinchipe: Estación Científica San Francisco. FHGO live coll. 2142. AF292593, AF292622. *B. punctatus**: Ecuador: Pichincha: Pedro Vicente Maldonado. FHGO live coll. 2166. AF292595, AF292633. *B. taeniatus*: Ecuador: Morona Santiago: Macuma. FHGO 195. AF292591, AF292629.

* The specimen here assigned to *Bothrops punctatus* resembled specimens described as *B. osbornei* by Freire-Lascano (1991) in color pattern, and showed scale counts intermediate between Colombian *B. punctatus* and southwestern Ecuadorian *B. osbornei*. The status of *B. osbornei* is uncertain, and it has been regarded as conspecific with *B. punctatus* by some authors (David and Ineich, 1999; McDiarmid et al., 1999).

Crotalus: *C. cerastes*: Unknown. U69773, n/a. *C. durissus cascavella*: Brazil: Pernambuco: Belém do São Francisco. IB live coll. 4112. AF393633, n/a. *C. d. culminates*, Haplotype I: Unknown. London Zoo live coll. AF393626, n/a. *C. d. culminatus*, Haplotype II: Mexico: Puebla: El Aguacate. No voucher. AF393627, n/a. *C. d. cumanensis*, Haplotype I: Venezuela: Falcón: Curimagua. J. L. Yrausquin, live coll. AF393629, n/a. *C. d. cumanensis*, Haplotype II: Venezuela: Falcón: Las Ventosas. J. L. Yrausquin, live coll. AF393630, n/a. *C. d. durissus*: Mexico: Chiapas: Selva Lancandona. No voucher. AF393628, n/a. *C. d. terrificus*: Brazil: São Paulo: Pindamonhangaba. IB 55601. AF292570, AF292608. *C. d. tzabcan*: Belize: Corozal District: Xaibe Village. P. Singfield, live coll. AF393625, n/a. *C. d. unicolor*: Unknown. London Zoo, live coll. AF393631, n/a. *C. d. vegrandis*: Unknown. London Zoo, live coll. AF393632, n/a. *C. molossus*: USA: New Mexico: Las Uvas Mts. UTEP live coll. AF393624, n/a. *C. scutulatus*: USA. UTEP CRH153. AF292571, n/a. *C. viridis viridis*: USA: Colorado: Moffat Co. K. Ashton live coll. AF147867, n/a.

Porthidium: *P. lansbergii arcossae*: Ecuador: Manabí: Salango. FHGO live coll. 738. AF292575, AF292613. *P. l. rozei*: Venezuela: Falcón: San Antonio. J. L. Yrausquin, live coll. n/a, AF393623. *P. nasutum*: Ecuador Esmeraldas: Zapallo Grande. FHGO live coll. 517. AF292574, AF292612.

Calloselasma: *C. rhodostoma*: Unknown. Cyt-*b* - no voucher; ND4 - UMMZ 184314. AF292569, U41787.