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Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (Serpentes: Viperidae)

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Species in the genus *Bothrops s. l.* are extraordinarily variable in ecology and geography, compared with other genera in the subfamily Crotalinae. In contrast to the trend of splitting large and variable groups into smaller, more ecologically and phenotypically cohesive genera, the genus *Bothrops* has remained speciose. In addition, previous phylogenetic analyses have found *Bothrops* to be paraphyletic with respect to the genus *Bothriopsis*. Taxonomic arguments exist for synonymizing *Bothriopsis* with *Bothrops*, and for splitting *Bothrops* into smaller genera, but the greatest hindrance to taxonomic revision has been incomplete phylogenetic information. We present a phylogeny of *Bothrops*, *Bothriopsis*, and *Bothrocophias* based on 85 characters of morphology and 2343 bp of four mitochondrial gene regions, and with significantly greater taxonomic coverage than previous studies. The combined data provide improved support over independent datasets, and support the existence of discrete species groups within *Bothrops*. The monophyly and distinctness of these groups warrant recognition at the generic level, and we propose a new taxonomic arrangement to reflect these findings. *Zoological Journal of the Linnean Society*, 2009, **156**, 617–640.

ADDITIONAL KEYWORDS: Bayesian – *Bothropoides* – cytochrome *b* – morphology – ND4 – parsimony – *Rhinocerophis* – ribosomal RNA – taxonomy.

INTRODUCTION

The South American pitviper clade of *Bothrops*, *Bothriopsis*, and *Bothrocophias* is distributed throughout South America and the associated continental islands, and includes species that range into Central America, Mexico, and the Caribbean (Campbell & Lamar, 2004). The monophyly of these bothropoids has been supported by several phylogenetic analyses (e.g. Gutberlet & Campbell, 2001; Parkinson, Campbell & Chippindale, 2002; Castoe, Sasa & Parkinson, 2005; Castoe & Parkinson, 2006). The clade contains 47 species: five toad-headed pitvipers (*Bothrocophias*), six forest pitvipers (*Bothriopsis*), and 36 lanceheads (*Bothrops*) (Campbell & Lamar, 2004). Among the phylogenetic hypotheses for the group, common relationships appear (see Table 1 and references therein). For example, *Bothrocophias* is generally found to be monophyletic (Gutberlet & Campbell, 2001; Gutberlet & Harvey, 2002; Castoe & Parkinson, 2006; but see Wüster *et al.*, 2002) and a sister group to *Bothrops* + *Bothriopsis*. *Bothriopsis* is also supported as monophyletic (Wüster *et al.*, 1999b; Wüster *et al.*, 2002), but *Bothrops* is paraphyletic with respect to the forest pitvipers (Campbell & Lamar, 1992;

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Table 1. Content of clades recovered by phylogenetic studies of *Bothrops*, *Bothriopsis*, and *Bothrocophias* species

Werman (1992)	Salomão *et al.* (1999)	Wüster *et al.* (2002)	Gutberlet & Harvey (2002)	Castoe & Parkinson (2006)
		Bothrocophias hyoprora	*Bothrocophias campbelli*	*Bothrocophias hyoprora*
		B. microphthalmus	*B. hyoprora*	*B. microphthalmus*
		Bothrocophias campbelli	*B. microphthalmus*	
Bothrops atrox	***Bothrops atrox***	***Bothrops atrox***	*Bothrops asper*	*Bothrops asper*
B. brazili	*B. brazili*	*B. asper*	*B. atrox*	*B. atrox*
B. jararacussu	*B. colombiensis*	*B. brazili*		*B. jararacussu*
B. leucurus	*B. isabelae*	*B. caribbaeus*		
B. moojeni	*B. jararacussu*	*B. colombiensis*		
	B. leucurus	*B. isabelae*		
	B. marajoensis	*B. jararacussu*		
	B. moojeni	*B. lanceolatus*		
	Bothriopsis bilineata	*B. leucurus*		
	Bothriopsis taeniata	*B. marajoensis*		
	Bothrops caribbaeus	*B. moojeni*		
	B. lanceolatus	*B. punctatus*		
Bothriopsis taeniata		*Bothriopsis bilineata*	*Bothriopsis bilineata*	*Bothriopsis bilineata*
		B. pulchra		*B. chloromelas*
		B. taeniata		*B. taeniata*
Bothrops jararaca	***Bothrops jararaca***	***Bothrops neuwiedi***	*B. neuwiedi*	*Bothrops diporus*
	B. insularis	(*sensu* Silva 2004)	*B. alternatus*	*B. erythromelas*
Bothrops neuwiedi	***Bothrops alternatus***	*B. erythromelas*		*B. insularis*
(*s. l.*)	*B. cotiara*	*B. jararaca*		
B. alternatus	*B. fonsecai*	*B. insularis*		*Bothrops alternatus*
B. erythromelas		***Bothrops alternatus***		*B. ammodytoides*
B. itapetiningae		*B. cotiara*		*B. cotiara*
		B. fonsecai		
		B. itapetiningae		

Species names have been changed to reflect the current classification. Lines delineate clades recovered by the studies; names in bold are group names given by the authors.

Salomão *et al.*, 1997; Vidal *et al.*, 1997; Parkinson, 1999; Gutberlet & Harvey, 2002; Parkinson *et al.*, 2002; Wüster *et al.*, 2002; Castoe & Parkinson, 2006). Within *Bothrops*, several species groups have been repeatedly recovered and named (Table 1): a *Bothrops alternatus* group, *Bothrops neuwiedi* group, *Bothrops jararaca* group, *Bothrops atrox* group, and *Bothriopsis* (a complete species list can be found in Appendix 1). Numerous ecological and evolutionary studies (e.g. Martins *et al.*, 2001; Martins, Marques & Sazima, 2002; Araújo & Martins, 2006) have traditionally used these species groups as well, recognizing *alternatus*, *neuwiedi*, *jararaca*, *atrox*, *jararacussu* (part of the *atrox* group in Table 1), and *taeniatus* (= *Bothriopsis*) groups.

Although the clade contains 47 species, the most comprehensive studies to date included eight (morphology: Gutberlet & Harvey, 2002), eleven (morphology and allozymes: Werman, 1992), and 28 species (mitochondrial DNA: Wüster *et al.*, 2002). While these studies have generally recovered the same clades within the South American pitviper complex, the different species included in these phylogenies may lead to confusion about the content of the clades (compare Salomão *et al.*, 1999 with Castoe & Parkinson, 2006). In addition, species in certain sparsely sampled regions, like the Pacific versant of the Andes, have rarely been included in phylogenetic hypotheses [*Bothrops pictus* (Tschudi, 1845), included in Wüster *et al.*, 2002; *Bothrops roedingeri* Mertens, 1942, *Both-*

rops andianus Amaral, 1923, and *Bothrops lojanus* Parker, 1930, not included in the phylogenetic analysis], making it difficult to evaluate the classification of these species.

The knowledge that *Bothrops* is paraphyletic has led to taxonomic arguments about how to revise the content of this genus. Some suggest synonymizing *Bothriopsis* with *Bothrops*, and also mention the possibility of synonymizing the small, cohesive sister genus *Bothrocophias* with *Bothrops* (Salomão *et al.*, 1997; Wüster *et al.*, 2002). Others propose dividing *Bothrops* into smaller monophyletic genera (Parkinson, 1999; Gutberlet & Campbell, 2001; Harvey, Aparicio & Gonzales, 2005; Castoe & Parkinson, 2006). There is no completely objective criterion for distinguishing between these options, but a comprehensive phylogeny provides the best information for evaluating taxonomic alternatives.

An accurate and stable taxonomy for South American pitvipers is critical, as all species are venomous, and several are known to cause human fatalities (Russell, 1980; Warrell, 2004). Venom composition generally has a phylogenetic component (Wüster, 1996; Wüster, Golay & Warrell, 1997), and because most biologists primarily receive phylogenetic information through classification (Frost *et al.*, 2006), a naming system based on a well-supported hypothesis of evolutionary relationships can benefit antivenom production and treatment of envenomation. In addition, the taxonomy will enlighten research in comparative biology, trait evolution, historical biogeography, and other fields.

We believe the current taxonomy has persisted because, as mentioned above, no phylogenetic hypothesis of South American pitvipers has yet considered a significant array of taxa. In this study, we achieve almost complete taxon sampling through the use of both morphological and molecular data. Most taxa are included on the basis of morphological characters as well as one or more gene fragments, and a few are included on the basis of morphology only. In the case of South American pitvipers, as well as in many other clades, some rare taxa are available only as formalin-preserved museum specimens, and acquiring samples for DNA analyses has been prohibitively difficult. Morphological characters can be observed for almost all taxa, and can be united with available molecular characters in a combined evidence analysis. In addition, we applied as much DNA sequence data as possible to the analysis to achieve a robust combined evidence phylogeny. Therefore, the primary goal of the present work is a phylogenetic analysis of 90% of the currently recognized taxa in the genera *Bothrops*, *Bothriopsis*, and *Bothrocophias*, using a morphological and multigene mitochondrial data set. This is the most taxon- and character-comprehensive study to date on this group of venomous snakes. The phylogeny recovered allows us to identify the major evolutionary lineages in this speciose group, and to determine the species composition of each major lineage. We evaluate previous taxonomic suggestions, and propose a systematic revision of the group that recognizes evolutionarily, ecologically, and morphologically distinct lineages as genera.

MATERIAL AND METHODS

Morphological data

Forty-three taxa of *Bothrops* (31 species), *Bothriopsis* (seven taxa of six species), and *Bothrocophias* (five species) were examined: slightly over 90% of the currently recognized species. In addition, the *Bothriopsis* subspecies *Bothriopsis bilineata bilineata* (Wied-Neuwied, 1821) and *Bothriopsis bilineata smaragdina* (Hoge, 1966) were treated as separate terminal taxa. The species of the South American pitviper clade that were unavailable to this study were: *Bothrops lutzi* (Miranda-Ribeiro, 1915), *Bothrops muriciencis* Ferrarezzi & Freire, 2001, *Bothrops pirajai* Amaral, 1923, and *B. roedingeri*. Species were included in the phylogenetic estimation if: (1) we had sequence data for at least one individual, (2) we had data from more than one type of morphological character, or (3) we had scalation data for at least eight individuals (which was the average number of individuals examined). Five species failed these criteria, and were therefore excluded from all analyses: *Bothrocophias colombianus* (Rendahl & Vestergren, 1940), *Bothriopsis medusa* (Sternfeld, 1920), *Bothriopsis oligolepis* (Werner, 1901), *Bothrops lojanus*, and *Bothrops pubescens* (Cope, 1870) (Appendix 2). In accordance with current hypotheses of crotaline phylogeny (Castoe & Parkinson, 2006), *Atropoides picadoi* Dunn, 1939 and *Cerrophidion godmani* (Günther, 1863) were used as near out-groups, and *Agkistrodon contortrix* (Linnaeus, 1766) was chosen as a far out-group.

We examined the scalation of 42 species, hemipenes of 21 species, and skulls or skeletons of 13 species (Appendix 2 and Appendix S1). When possible, specimens were acquired from throughout the range of each species. Scale and hemipenial data for *Bothrops alcatraz* Marques, Martins & Sazima, 2002 were taken from the description of the holotype. Observations of colour pattern were taken from colour plates in Campbell & Lamar (2004). Males and females were treated together. Some juveniles were coded for scale characters, as scalation does not change with ontogeny, but skeletal data were only collected from presumed adults.

Eighty-five morphological characters were included in this study (Appendix S2). Sixty-seven characters

were taken from Gutberlet (1998) and Gutberlet & Harvey (2002), with additional characters from Werman (1992) and Wüster, Thorpe & Puorto (1996), and some are original to this study. The ordering of characters was taken from the maximum ordering of Gutberlet & Harvey (2002) and ordering in Werman (1992), using both intermediacy and adjacency as justification for ordering.

For parsimony analyses, characters were coded using two different methods: generalized frequency coding (GFC), as described by Smith & Gutberlet (2001), or gap weighting (Thiele, 1993) and majority coding (Johnson, Zink & Marten, 1988). The GFC was developed to extend the frequency bins method of Wiens (1995) to apply not only to binary characters, but also to multistate and meristic characters. It is thought to extract the maximal phylogenetic information available in patterns of polymorphism within terminal taxa, because it codes the entire frequency distribution of a character within a taxon. Under this method, we processed data through the program FastMorphology GFC (Chang & Smith, 2001), and used unequal subcharacter weighting, as recommended by Smith & Gutberlet (2001). This method divides the weight of one character by the number of subcharacters used, and then divides the weight of each subcharacter by the number of steps between the lowest and highest frequency bin included in it, thereby allowing rare subcharacters greater weight than common subcharacters. Smith & Gutberlet (2001) found that unequal subcharacter weighting performed better than the alternative of equal subcharacter weighting.

Bayesian methods that are currently available provide no straightforward means to include frequency-based characters, so likelihood-based analyses were conducted using gap weighting for meristic characters (Thiele, 1993), and majority coding for binary and multistate characters (Johnson *et al.*, 1988). Coding was performed using Microsoft Excel. Gap weighting assigns states to taxa according to their range-standardized means (Thiele, 1993). As MrBayes allows a maximum of six ordered character states, the range of a character was divided into six bins, and states 0–5 were assigned to each taxon. Majority coding simply assigns the character state found in the majority of samples to the terminal taxon. Gap weighting and majority coding (GW/MC) methods approximate or ignore polymorphism within species; they are therefore expected to provide less phylogenetic information than frequency methods such as GFC (Smith & Gutberlet, 2001).

Molecular data

Previously published sequence data for 12S and 16S rRNA, NADH dehydrogenase subunit 4 (ND4), and cytochrome *b* (cyt *b*) were obtained from GenBank. In addition, new sequences were obtained for eight species as described in Castoe & Parkinson (2006). This provided a molecular data set with at least one gene fragment included for each of 35 taxa, or approximately 75% of the currently recognized species (Appendix 2). All specimens and accession numbers are listed in Table S1.

All sequences were aligned by eye and by using ClustalW (Thompson, Higgins & Gibson, 1994). For conservatism in determining evolutionary relationships, when more than one sequence was available for a species, aligned sequences were combined into a majority-rule consensus sequence. When two or more nucleotides were found in equal proportions, standard IUPAC codes for uncertainty were used. The alignment of protein-coding genes was straightforward, with no insertions or deletions. No internal stop codons were found in either protein-coding fragment. The alignment of rRNA genes was based on models of secondary structure for snake mitochondrial rRNAs (Parkinson, 1999). Novel sequences were deposited in GenBank (Table S1), and the final nucleotide alignment is available by request. Gaps in the alignment were treated as missing data in the analyses.

Phylogenetic analyses

Maximum parsimony and Metropolis-Hastings coupled Markov chain Monte Carlo Bayesian methods were used to reconstruct phylogenies. Table 2 shows all of the analyses. Morphological characters were analysed separately using GFC and GW/MC methods in parsimony, only using the latter method in Bayesian methodologies. Each mitochondrial gene was also analysed separately with both methods. In general, we expect phylogenies from different mitochondrial genes to recover the same relationships because they are inherited as a single linkage unit. To verify this assumption, we looked for strongly supported incongruence among gene trees and found none. As all genes appeared to support a single phylogeny, we combined them into a single analysis. Previous studies that included many of the sequences used in this study have also supported the combinability of these four gene fragments (e.g. Parkinson, 1999; Murphy *et al.*, 2002; Parkinson *et al.*, 2002; Malhotra & Thorpe, 2004; Castoe *et al.*, 2005; Castoe & Parkinson, 2006). Mitochondrial analyses were followed by combined evidence analyses of morphological and molecular data. One set of combined evidence analyses included all taxa; a second included only those taxa with both phenotypic and sequence data.

Maximum parsimony methods were conducted with the program PAUP* v4.0b10 (Swofford, 2002). We used heuristic searching with 200 random-taxon-

Table 2. Summary of phylogenetic analyses of South American pitvipers

Analysis	Figure	Optimality criterion	Description
1	S9	Parsimony	Morphology only, GFC
2	S8	Parsimony	Morphology only, gap weighting and majority coding
3	S7	Bayesian	Morphology only, gap weighting and majority coding
4	S6	Parsimony	Mitochondrial DNA only
5	S5	Bayesian	Mitochondrial DNA only
6	S4	Parsimony	All characters included, GFC
7	2/S3	Parsimony	All characters included, gap weighting and majority coding
8	2	Bayesian	All characters included, gap weighting and majority coding
9	S2	Parsimony	All characters included, GFC, taxa without molecular data excluded
10	1/S1	Parsimony	All characters included, gap weighting and majority coding, taxa without molecular data excluded
11	1	Bayesian	All characters included, gap weighting and majority coding taxa without molecular data excluded

GFC, generalized frequency coding.

addition sequences and tree bisection reconnection (TBR) branch-swapping. Support for nodes was assessed with nonparametric bootstrapping (Felsenstein, 1985), with 1000 full heuristic pseudoreplicates and two random-taxon-addition sequence replicates per pseudoreplicate.

In the Bayesian analysis, the standard Markov (Mk) model of Lewis (2001) was used for the morphology partition. Preliminary analyses determined that there was no increase in likelihood score with the addition of the Γ-distributed rate variation parameter; therefore, we chose the simpler model. Based on the results of Castoe & Parkinson (2006), maximum partitioning of the molecular data set was done *a priori*, with all codon positions, or stem and loop positions, of each gene allocated independent models. Each partition was independently analysed using MrModelTest v2.2 (Nylander, 2004) to estimate the best-fitting models of nucleotide evolution. This program only considers models that are currently available in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). PAUP* was used to calculate model likelihoods for use in MrModelTest. The best-fitting models were implemented as partition-specific models within partitioned-model analyses of the combined dataset, as described in Castoe & Parkinson (2006). The models chosen for each partition are summarized in Table 3.

Bayesian phylogenetic inference was conducted using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). All analyses were run with vague priors. Four incrementally heated chains were used in addition to the cold chain, with the temperature set at half of the default temperature of the program in order to facilitate chain swapping. Each analysis had two different runs beginning with random trees. Chains were run for at least 4.0×10^6 generations. All were sampled every 100 generations, with the first quarter of the runs conservatively discarded as burn-in. Tracer v1.4 (Rambaut & Drummond, 2007) was used to verify that stationarity was reached within the burn-in period. Summary statistics and consensus phylograms with nodal posterior probability support were estimated from the combination of both runs per analysis.

Table 3. Results of an *Akaike* information criterion (AIC) model selection conducted in MrModelTest 2.2 (Nylander, 2004) for partitions of the data set

Partition	AIC model
12S, stems	HKY + ΓI
12S, loops	GTR + Γ
16S, stems	HKY + I
16S, loops	GTR + ΓI
cyt *b*, position 1	HKY + ΓI
cyt *b*, position 2	GTR + Γ
cyt *b*, position 3	HKY + ΓI
ND4, position 1	GTR + ΓI
ND4, position 2	HKY + Γ
ND4, position 3	HKY + Γ

HKY, Hasegawa, Kishino & Yano (1985) model; GTR, generalized time reversible model (Tavaré, 1986); Γ, gamma-distribution rate variation; I, invariant sites.

We calculated genetic distance measures for cyt *b* sequences among species groups in our data set and among polytypic genera using sequences from Castoe & Parkinson (2006). We believe genetic distances should not be used to define taxonomic rank, but that an examination of distance measures can provide a rough estimate of the level of divergence

among groups, and can allow comparisons with other groups of closely related taxa. Cytochrome *b* was chosen because its genetic distances are often reported in the literature, thereby allowing more direct comparisons of genetic distances in these groups with those reported for other snakes (e.g. Wüster *et al.*, 2002; Malhotra & Thorpe, 2004). We calculated genetic distance measures with the program MEGA (Kumar, Tamura & Nei, 2004), using a Kimura two-parameter model and Γ–distributed rate variation.

RESULTS

The final alignment of four concatenated gene fragments consisted of 2343 aligned positions: 424 from 12S, 511 from 16S, 716 from cyt *b*, and 692 from ND4. This alignment contained 599 parsimony-informative characters. The GFC of morphological characters yielded 595 subcharacters, 404 of which were parsimony informative. The GW/MC of 92 morphological characters yielded 72 that were parsimony informative.

There were no strongly supported conflicts between parsimony and Bayesian phylogenies, although minor topology differences were found (e.g. compare Fig. 1 with Figs S1 and S2, and Fig. 2 with Figs S3 and S4). Additionally, support values derived from these methods were in agreement in almost all cases. Analyses with different data sets were also topologically congruent, with the highest resolution and support values in phylogenies inferred from combined evidence (Figs 1, 2, and S1–S4), followed by those inferred from molecular evidence only (Figs S5, S6), and the lowest resolution and support values in phylogenies inferred from morphological evidence only (Figs S7–S9). Combined evidence analyses excluding taxa with morphological data only (Figs 1, S1, S2) recovered five major lineages: a *Bothrocophias* clade (labelled A, posterior probability (*Pp*) = 79, bootstrap value (*Bs*) = 57–81), a *Bothrops alternatus* clade (labelled B, *Pp* = 100, *Bs* = 71–83), a *Bothrops jararaca* + *Bothrops neuwiedi* clade (labelled C, *Pp* = 100, *Bs* = 90–95), a *Bothriopsis* clade (labelled D, *Pp*, *Bs* = 100), and a *Bothrops atrox* clade (labelled E, *Pp* = 100, *Bs* = 99–100). Alternative analyses recovered the same major lineages in almost all cases, but with lower support.

Analysis 11, a Bayesian combined evidence analysis excluding taxa with morphological data only, is our preferred hypothesis for delineating species groups as it had the highest support values overall and was based on the largest data set, while avoiding the possible complications of adding taxa with 90% or more missing data to the analysis (Wiens, 2003, 2006). Analysis 8 is our preferred taxon-comprehensive hypothesis, and is also a Bayesian combined evidence analysis. Like analysis 11, it has the benefits of evolutionary models for DNA data that may be more biologically realistic than parsimony, and a method known to outperform other types of analysis under a range of conditions (Huelsenbeck *et al.*, 2002; Holder & Lewis, 2003). Analysis 8 recovered the same species groups as analysis 11, although with lower support values. We attribute this to the inclusion of taxa based on morphology only (i.e. taxa with extensive missing data), and so we prefer to use this analysis for the placement of taxa in species groups defined by analysis 11.

In our preferred phylogenetic hypotheses, the *Bothrocophias* clade (labelled A) consisted of *Bothrocophias campbelli* (Freire-Lascano, 1991), *Bothrocophias hyoprora* (Amaral, 1935), and *Bothrocophias microphthalmus* (Cope, 1875), and included *Bothrocophias myersi* Gutberlet & Campbell, 2001 on the basis of morphological data (*Pp* = 73). The *Bothrops alternatus* clade (labelled B) consisted of that species Duméril, Bibron & Duméril, 1854, *Bothrops ammodytoides* Leybold, 1873, *Bothrops itapetiningae* (Boulenger, 1907), *Bothrops cotiara* (Gomes, 1913), and *Bothrops fonsecai* Hoge & Belluomini, 1959. Analysis 8 (*Pp* = 79) also included *Bothrops jonathani* Harvey, 1994. The *Bothrops jararaca* + *Bothrops neuwiedi* clade (labelled C) consisted of those species (Wied-Neuwied, 1824), Wagler, 1824, *Bothrops diporus* Cope, 1862, *Bothrops erythromelas* Amaral, 1923, *Bothrops pauloensis* Amaral, 1925, *Bothrops insularis* (Amaral, 1922), and *B. alcatraz*. The *Bothriopsis* clade (labelled D) consisted of *Bothriopsis chloromelas* (Boulenger, 1912), *Bothriopsis taeniata* (Wagler, 1824), *Bothriopsis pulchra* (Peters, 1863), and both subspecies of *B. bilineata*. Sister to the *Bothriopsis* clade was a *Bothrops atrox* clade (labelled E), consisting of that species (Linnaeus, 1758), *Bothrops leucurus* Wagler, 1824, *Bothrops isabelae* Sandner-Montilla, 1979, *Bothrops moojeni* Hoge, 1966, *Bothrops marajoensis* Hoge, 1966, *Bothrops asper* (Garman, 1884), *Bothrops lanceolatus* (Bonnaterre, 1790), *Bothrops caribbaeus* (Garman, 1887), *Bothrops punctatus* (García, 1896), *Bothrops osbornei* Freire-Lascano, 1991, *Bothrops jararacussu* Lacerda, 1884, and *Bothrops brazili* Hoge, 1954. The positions of the taxa included in the phylogeny on the basis of morphological characters alone were generally poorly supported.

Certain species were recovered in different positions in different analyses. *Bothrops pictus* was the only species not recovered in a species group in analysis 11: it was sister to the remainder of the *Bothrops* + *Bothriopsis* clade (*Pp* = 97). In parsimony analysis 10, however, a sister relationship between *B. pictus* and the *B. alternatus* clade was supported by a bootstrap value of 56; this relationship was not

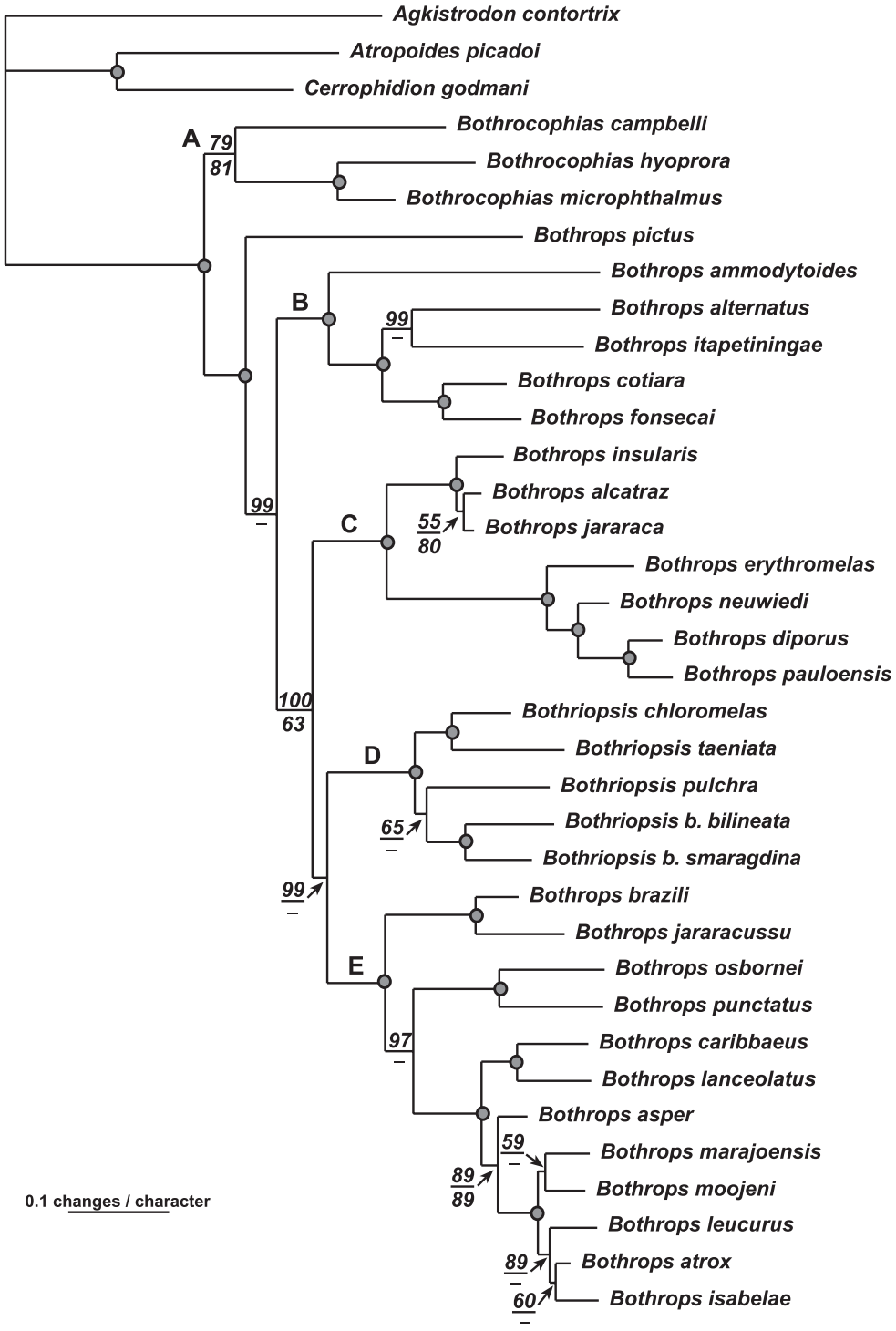

Figure 1. Bayesian Markov Chain Monte Carlo (MCMC) 50% majority-rule consensus phylogram, excluding taxa with morphological data only (analysis 11). The phylogram is derived from an analysis of 2343 bp of mitochondrial DNA and 85 gap-weighted or majority-coded morphological characters. The posterior probabilities are shown above nodes; bootstrap values from parsimony analysis of the same data set are shown below nodes (analysis 10). The parsimony analysis shows minor topological differences from Bayesian analysis; refer to Figure S1 for parsimony cladogram. Grey circles indicate posterior probabilities of 95 or greater and bootstrap values of 70 or greater. Letters correspond to major lineages: A, *Bothrocophias* clade; B, *Bothrops alternatus* clade; C, *Bothrops neuwiedi* + *Bothrops jararaca* clade; D, *Bothriopsis* clade; E, *Bothrops atrox* clade.

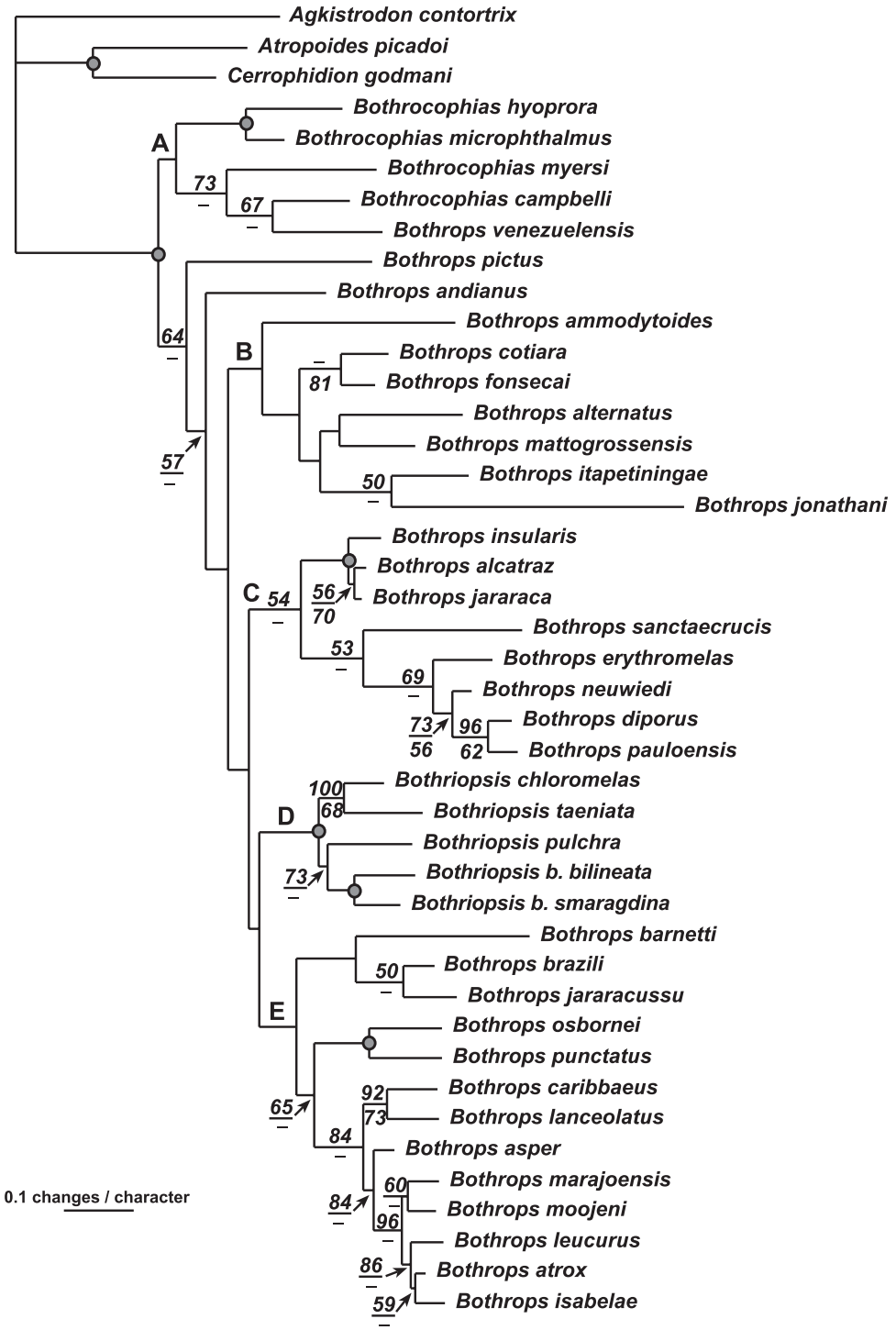

Figure 2. Bayesian Markov Chain Monte Carlo (MCMC) 50% majority-rule consensus phylogram, including taxa with morphological data only (analysis 8). The phylogram is derived from an analysis of 2343 bp mitochondrial and 85 gap-weighted or majority-coded morphological characters. The posterior probabilities are shown above nodes; bootstrap values from parsimony analysis of the same data set are shown below nodes (analysis 7). The parsimony analysis shows minor topological differences from the Bayesian analysis; refer to Figure S3 for the parsimony cladogram. Grey circles indicate posterior probabilities of 95 or greater, and bootstrap values of 70 or greater. Dashes indicate support values of less than 50. Letters correspond to the major lineages: A, *Bothrocophias* clade; B, *Bothrops alternatus* clade; C, *Bothrops neuwiedi* + *Bothrops jararaca* clade; D, *Bothriopsis* clade; E, *Bothrops atrox* clade.

recovered in the majority-rule consensus of the shortest trees. In all other cases of alternative placements, the species relationships were supported with posterior probability and bootstrap values of less than 65. Species with alternative placements were *Bothrops andianus*, *Bothrops barnetti* Parker, 1938, *Bothrops mattogrossensis* Amaral, 1925, *Bothrops sanctaecrucis* Hoge, 1966, and *Bothrops venezuelensis* Sandner-Montilla, 1952.

Genetic distance measures within South American species groups ranged from 6.5 to 11.3%, and distances between species groups within South American pitvipers ranged from 11.1 to 16.7% (Table S2). Overall, within-genus distance measures ranged from 8.5 to 21.9%.

DISCUSSION

Resolution of major lineages

Numerous studies have included species of *Bothrops*, *Bothriopsis*, and *Bothrocophias* in phylogenetic estimates, but until this study no taxon-comprehensive combined data set was available. We have recovered four major lineages in the *Bothrops* + *Bothriopsis* clade (labelled B–E, respectively): (1) *Bothrops alternatus* clade, (2) *Bothrops neuwiedi* clade + *Bothrops jararaca* clade, (3) *Bothriopsis* clade, and (4) *Bothrops atrox* clade. The resolution of these lineages is supported by several lines of evidence. In analysis 11, the species groups were supported with posterior probabilities of 100. In the corresponding parsimony analyses 9 and 10, these groups were supported with bootstrap values of 71–100. Several other taxon-comprehensive and data-limited analyses in this study had lower support, but the same groups were recovered in all phylogenies. The *Bothrops alternatus* group was supported by 27 mitochondrial and one unique morphological characters, the *Bothrops neuwiedi* + *Bothrops jararaca* group was supported by 38 mitochondrial and no unique morphological characters, *Bothriopsis* was supported by 48 mitochondrial and four unique morphological characters, and the *Bothrops atrox* group was supported by 50 mitochondrial and one unique morphological characters (Table 4). The results have been corroborated by morphological and molecular studies, including Salomão *et al.* (1997, 1999), Gutberlet & Harvey (2002), Wüster *et al.* (2002), and Castoe & Parkinson (2006).

We also recovered a monophyletic *Bothrocophias* lineage (labelled A in the figures) with strong support in mitochondrial and combined evidence phylogenies, and with lower support in other analyses. *Bothrocophias* is supported by 34 mitochondrial and three morphological synapomorphies (Table 4). Monophyly of this genus is in agreement with the morphological dataset of Gutberlet & Harvey (2002) and the molecular dataset of Castoe & Parkinson (2006).

Placement of species within lineages

In most cases, species were recovered in the same clades in multiple analyses and their phylogenetic placement was supported by prior evidence (e.g. Table 1 and references therein; Silva, 2000, 2004; Campbell & Lamar, 2004). In the case of *Bothrocophias campbelli*, two prior studies recovered alternative placements of the species: Gutberlet & Harvey's (2002) morphological analysis found it within *Bothrocophias*, thereby supporting the content of the genus as defined by Gutberlet & Campbell (2001), whereas the mitochondrial analysis of Wüster *et al.* (2002) found *B. campbelli* to be a sister to *Bothrops* + *Bothriopsis*. Combined evidence analysis 11 provided strong support for the monophyly of *Bothrocophias* including *B. campbelli* (*Pp* = 96). *Bothrocophias campbelli* did not fall within a *Bothrocophias* clade in only two cases. Analysis 2 (Fig. S8) recovered it as a sister to the rest of the in-group excluding *Bothrops erythromelas*, and analysis 5 (Fig. S5) recovered it as a sister to *Bothrops* + *Bothriopsis*. The majority of our results and most prior work strongly suggest that *B. campbelli* is part of the *Bothrocophias* lineage.

A few species were recovered in uncertain phylogenetic positions, or were unavailable to this study, but other sources of evidence allow us to make recommendations on their group placement; further phylogenetic testing of these recommendations is warranted. First, *Bothrocophias myersi* was included in the analysis on the basis of morphological data only: in Bayesian analysis 8 (Fig. 2), the species was part of *Bothrocophias*, but in parsimony analyses 1, 6, and 7, and in Bayesian morphological analysis 3 (Figs S3, S4, S7, S9) it was found within *Bothrops* (*Bs* < 50). Gutberlet & Campbell (2001) recovered *B. myersi* within *Bothrocophias* in their analysis and description of the species and genus. Based on this evidence and the results presented here, we suggest that the current generic allocation is appropriate. Second, *Bothrocophias colombianus* was included in *Bothrocophias* by Campbell & Lamar (2004) on the basis of external morphology. Too few specimens were available to include this species in phylogenetic analysis, but scale data from two specimens (FMNH 55898 and UTA R25949) support the inclusion of *B. colombianus* in *Bothrocophias*. In addition, canthorostrals were observed on FMNH 55898, which is a character state previously observed only in *Bothrocophias hyoprora* and *B. microphthalmus*.

Bothriopsis oligolepis and *B. medusa* could not be included in the final analyses because too few

Table 4. Phenotypic synapomorphies and shared natural history traits among species within major lineages of South American pitvipers

Proposed genus	Number of DNA synapomorphies	Phenotypic synapomorphies	Diet	Habitat	Geographic range
Bothrocophias	12S, 4; 16S, 5; cyt *b*, 11; ND4, 14	Keel on dorsal scales tuberculate on caudal part of body, Meckellian foramen completely or partially divided into two foramina, distinct white spots present on posterior infralabials and gulars.	Diet generalists, including a high proportion of lizards (41.7% in *B. hyoprora*), anurans, and mammals (25% each in *B. hyoprora*).	Terrestrial in rainforest, montane wet forest, and cloud forest.	Andean South America: Ecuador, Colombia, Peru, Bolivia, western Brazil.
Rhinocerophis	cyt *b*, 10; ND4, 17	One or two palatine teeth.	Diet generalists including a high proportion of mammal prey (42.8–60% in *B. ammodytoides* and *B. itapetiningae*) or mammal specialists.	Terrestrial in open areas or edges of moderate to montane broad-leaf and/or *Araucaria* forests, swamps, or cerrados.	Southern South America: southeastern Brazil, Paraguay, Uruguay, Argentina; one species found in central and southern Bolivia.
Bothropoides	12S, 6; 16S, 1; cyt *b*, 19; ND4, 12	No unique phenotypic synapomorphies, intermediate width of lateral margin of head of ectopterygoid shared with Bothrocophias.	Diet generalists, some mammal specialists (*B. pubescens*), some including a high proportion of birds (*B. insularis*), or centipedes (66.7% in *B. alcatraz*) in diet; ontogenetic shift in prey types in the larger species	Terrestrial in dry to wet habitats in caatinga vegetation, cerrados, rock outcrops, grassy areas, or broadleaf forests (*B. erythromelas* and *B. neuwiedi* complex) or semi-arboreal in Atlantic forests (*B. jararaca* complex).	Eastern South America: Brazil including continental islands, Bolivia, southeastern Peru, Paraguay, Uruguay, northern to central Argentina.
Bothriopsis	12S, 11; 16S, 4; cyt *b*, 21; ND4, 12	Pleurapophyses of midcaudal vertebrae in contact distally, choanal process of palatine positioned posteriorly, prehensile tail, green ground colour.	Diet generalists with a high proportion of mammal (40.9–50.0%) and anuran (35.7–40.9%) prey.	Semi-arboreal in lowland rainforests, Atlantic forests, wet montane forest, or cloud forests.	Amazonian South America: Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela, Guyana, French Guiana, Suriname.
Bothrops	12S, 9; 16S, 4; cyt *b*, 14; ND4, 23	Four palatine teeth (five in *B. moojeni* and *B. jararacussu*, three in *B. brazili* and *B. sanctaecrucis*).	Diet generalists with a high proportion of mammal (42.1–70.1%) and anuran (12.8–33.6%) prey.	Terrestrial to semi-arboreal in lowland rainforests to gallery forests and swamps in cerrados to Atlantic forests.	Northern South America: Pacific versant of Andes and coastal lowlands in Colombia, Ecuador, and northwestern Peru; Atlantic versant of Andes in Peru and Bolivia, Venezuelan Andes, and equatorial forests east of Andes exclusive of Uruguay, southern Paraguay, and Argentina south of Misiones. Central America: southern Mexico to Panama. Lesser Antilles: St. Lucia and Martinique.

Diet data from Martins *et al.* (2002), habitat data from Martins *et al.* (2001) and Campbell & Lamar (2004), and range data from Campbell & Lamar (2004).

specimens were available (Appendix 1). Preliminary analyses placed *B. oligolepis* within *Bothriopsis*, and its green coloration, prehensile tail, and arboreal lifestyle suggest that the current designation is correct. The semi-arboreal lifestyle of *B. medusa* in addition to its Venezuelan distribution (Campbell & Lamar, 2004) places it with either *Bothriopsis* or with the *Bothrops atrox* group (Table 4). The tan, brown, grey or olive coloration is unlike most *Bothriopsis* species, but the pattern of transverse bands on the dorsum is similar to *Bothriopsis* species and is unlike the spade-shaped dorsal markings on most of the *B. atrox* group specimens. We suggest retaining the current designation until more data are available.

Bothrops mattogrossensis and *B. pubescens* were elevated from subspecies of *B. neuwiedi* by Silva (2000, 2004). *Bothrops pubescens* was not included in the final analyses because of the lack of specimens, but preliminary analyses recovered it in a clade with *B. neuwiedi* and *B. diporus*. Based on this and on its membership in the *B. neuwiedi* complex, we suggest that it belongs to the *B. neuwiedi* lineage. *Bothrops mattogrossensis* was recovered in the *B. alternatus* and *B. jararaca* + *B. neuwiedi* + *B. alternatus* clades in alternative analyses (Figs 2, S3, S4, S7–S9), but the morphology that originally classified this species as *B. neuwiedi* suggests that it also belongs in the *B. neuwiedi* clade.

Bothrops sanctaecrucis was not included in prior phylogenies; it was recovered in the *Bothrops atrox* lineage in parsimony analyses (1, 2, 6, and 7), but was found in alternative placements in Bayesian analyses. Its range in Bolivia and terrestrial lifestyle in lower montane wet forests, as well as its strong resemblance to *Bothrops moojeni* (Campbell & Lamar, 2004) make it a likely member of the *Bothrops atrox* group (see Table 4). Likewise, *Bothrops andianus* was included in the analyses on the basis of morphological data only, and in analysis 8 was sister to *Bothrops* + *Bothriopsis* excluding *Bothrops pictus* and *Bothrops venezuelensis* (Fig. 2). *Bothrops andianus* was also recovered as sister to *Bothrocophias myersi* within the *Bothrops* + *Bothriopsis* clade in three parsimony analyses (1, 6, and 7; Figs S3, S4, S9). Its range in Peru and Bolivia, and its terrestrial habitat in montane wet forests, make affinities with either *Bothrocophias* or the *Bothrops atrox* group likely (Table 4). *Bothrops andianus* has a lacunolabial, like the *Bothrops atrox* group and unlike *Bothrocophias* species that have the second supralabial separate from the prelacunal scale (Campbell & Lamar, 2004). In addition, *Bothrops andianus* lacks tuberculate dorsal scales found on *Bothrocophias* individuals. We suggest a *Bothrops atrox* group placement is supported by outside evidence. Finally, *Bothrops venezuelensis* was found in or near *Bothrops*, *Bothriopsis*, and *Bothrocophias* clades in alternative analyses. Its Venezuelan range places its affinities with either the *Bothrops atrox* group or with *Bothriopsis*, but its primarily terrestrial habits, brownish coloration, and lack of a prehensile tail make it more similar to the *Bothrops atrox* group than to *Bothriopsis*. This is supported by the combined evidence analyses 6 and 7 (Figs S3, S4).

In contrast with the species discussed above, additional evidence cannot help to place four species in recovered species groups. *Bothrops barnetti* was included in the analyses on the basis of morphology only, and combined evidence analyses placed it near *Bothrops pictus*, although morphology-only analyses yielded different relationships. Similarly, the evolutionary relationships of *Bothrops lojanus* are uncertain based on scale data from six specimens (Appendix S1), although it was typically recovered as a sister taxon to most *Bothrops* + *Bothriopsis* species in pilot analyses. Based on their habitats in arid regions of Peru and southern Ecuador, respectively (Campbell & Lamar, 2004), their affinities may be with the arid Peruvian species *Bothrops pictus*. All three species may be sisters to *Bothrops* as currently defined. Until more comprehensive morphological or sequence data are available, *Bothrops barnetti*, *Bothrops lojanus* and *Bothrops pictus* cannot be definitively placed in the phylogeny. *Bothrops roedingeri* has sometimes been regarded as a synonym of *B. pictus* (see Campbell & Lamar, 2004), and because of this fact, as well as its desert habitat and range near *B. pictus*, these two species are likely to be congeners. Because of the uncertain position of *B. pictus*, we do not have a strong hypothesis for the phylogenetic placement of *B. roedingeri*.

Beta taxonomy and genetic distance

Based on evidence for the paraphyly of *Bothrops* in this and previous studies cited above, and based on the monophyly and distinctness of the species groups found in this study as well as earlier work, we suggest recognizing major lineages of *Bothrops* as distinct genera. As *Bothrops lanceolatus* is the type species of the genus, the generic name *Bothrops* is assigned to the *Bothrops atrox* group. The generic name *Rhinocerophis*, with type species *Rhinocerophis ammodytoides*, is available for the *alternatus* group. We propose the new name *Bothropoides* for the *neuwiedi–jararaca* group. As required, we define these three genera below. No taxonomic changes are necessary for *Bothriopsis* or *Bothrocophias*, as this study has found support for their monophyly.

In an overview of genetic distances among pitviper genera, the cyt *b* distances of South American pitviper species groups were similar to those in other genera,

ranging from 6.7 to 13.8% for within-group divergence and from 12.3 to 17.1% for between-group divergence (Table S2). In comparison, the clade of the Central American pitviper genera *Cerrophidion*, *Porthidium*, and *Atropoides*, closely related to the South American clade, had within-group distances of 8.3–12.7% and between-group distances of 12.1–23.4%. In Malhotra & Thorpe (2004), within-group distances ranged from 4.4 to 14.2% and between-group distances ranged from 10.3 to 26.5%. In our opinion, genetic distances alone do not provide a scheme for delimiting genera or species, but similarity of genetic distance measures may be taken as additional support for the distinctiveness of the South American groups.

Basis for systematic revision

Our taxonomy agrees with several authors who recommend dividing *Bothrops* into less speciose, and more ecologically and phenotypically cohesive, monophyletic genera (Parkinson, 1999; Gutberlet & Campbell, 2001; Harvey *et al.*, 2005; Castoe & Parkinson, 2006). We share their motivations for these changes. First, in agreement with many other studies, we find *Bothrops* to be paraphyletic with respect to *Bothriopsis*, and recommend changing the taxonomy of *Bothrops* to recognize only monophyletic groups (Campbell & Lamar, 1992; Parkinson, 1999; Gutberlet & Harvey, 2002; Parkinson *et al.*, 2002; Castoe & Parkinson, 2006). Second, we recovered evolutionarily distinct lineages in *Bothrops* formerly recognized as distinct species groups (see Table 1; Martins *et al.*, 2001, 2002; Araújo & Martins, 2006), and believe that these lineages should be named (Parkinson *et al.*, 2002). Third, we recognize the distinctiveness of *Bothriopsis*, and consider the continued recognition of that genus to be valuable (Gutberlet & Campbell, 2001). Fourth, we recognize that the major lineages not only have morphological and DNA-based synapomorphies, but they also have distinct ranges and habitats (Table 4), and these differences would be more clearly recognized through naming lineages as genera. Naming the major lineages as genera is in keeping with the recent practice in pitviper taxonomy of dividing speciose groups into smaller monophyletic genera (Burger, 1971; Campbell & Lamar, 1989, 1992; Malhotra & Thorpe, 2004).

Some authors have recommended synonymizing *Bothriopsis* with *Bothrops*, and also mention the possibility of synonymizing the small, cohesive sister genus *Bothrocophias* with *Bothrops* (Salomão *et al.*, 1997; Vidal *et al.*, 1997; Wüster *et al.*, 2002). Part of this motivation has been to avoid the problems inherent in changing the names of medically important species. Taxonomic changes are likely to result in temporary communication difficulties in the fields of research and health care (Wüster, 1996; Wüster & Harvey, 1996; Wüster *et al.*, 1997; Wüster, Golay & Warrell, 1998, 1999a; Pook & McEwing, 2005). This is a concern, but these changes will include more information on the relationships among South American pitvipers, and so are likely to be important to toxinologists and clinicians dealing with venoms and envenomations. We feel that the long-term good of a stable and evolutionarily informative taxonomy will outweigh the short-term drawbacks of proposing changes to the scientific names of venomous snake species.

Another proposed reason for synonymizing *Bothriopsis* (and possibly *Bothrocophias*) with *Bothrops* is that the clade is derived from a single invasion of South America, and splitting it could obscure this biogeographic pattern (Wüster *et al.*, 2002). This is true, but we also recognize the biogeographic pattern of South American colonization seen in the divergence of major lineages, and think it would be clarified through naming them as genera. It is likely that those studying South American biogeography using pitvipers would be familiar with their phylogeny, and therefore taxonomic changes should not greatly affect biogeographic understanding.

Wüster *et al.* (2002) also suggest that although *Bothrops* + *Bothriopsis* contains greater morphological and natural history diversity than other genera, it appears to be no older based on cyt *b* divergence levels. Our cyt *b* genetic distance results suggest that although the major lineages certainly contain less genetic divergence than *Bothrops* + *Bothriopsis*, their divergence levels are similar to those of other recognized genera.

A further motivation for synonymizing *Bothriopsis* with *Bothrops* is that because the arboreal species *Bothrops punctatus* and *Bothrops osbornei* are more closely related to the terrestrial or semiarboreal *Bothrops atrox* group than to the arboreal genus *Bothriopsis* (Table 1), there is little reason to recognize *Bothriopsis* as a separate genus (Wüster *et al.*, 2002). Arboreality has evolved several times within the Crotalinae (Gutberlet & Harvey, 2004; Malhotra & Thorpe, 2004; Castoe & Parkinson, 2006), and it can be argued that the continued recognition of *Bothriopsis* serves to cast taxonomic light on an additional instance of this phenomenon.

In addition to naming new genera or synonymizing *Bothriopsis* with *Bothrops*, other taxonomic options would be: (1) to delay taxonomic recommendations until complete data are available, (2) to name the major lineages and *Bothriopsis* as subgenera of *Bothrops* under the rules of the International Code of Zoological Nomenclature (ICZN), or (3) to recognize *Bothriopsis* as a clade, and name remaining clades without categorical ranks under the precepts of the

PhyloCode (de Queiroz & Gauthier, 1990, 1992, 1994). First, the paraphyly of *Bothrops* with respect to *Bothriopsis* is an ongoing taxonomic problem that will be resolved with the adoption of our proposed taxonomy. We anticipate the four species currently *incertae sedis* will be assigned to genera without requiring name changes to our proposed generic arrangement. Evidence strongly indicates that with additional data these genera will stand; therefore, we do not consider the unassigned species to be a hindrance to the adoption of our proposed taxonomy. Second, our concerns with naming subgenera are the same as the drawbacks of simply synonymizing *Bothriopsis* with *Bothrops*. Continuing to recognize the large and variable genus *Bothrops* requires disregarding a morphologically and ecologically distinct genus (*Bothriopsis*), as well as other evolutionarily distinct lineages. Within pitvipers, subgenera are rarely recognized, and so naming subgenera would not be materially different from including *Bothriopsis* within *Bothrops*. Third, as most concerns about taxonomic changes in this group are in relation to changing species names, and as the current PhyloCode (Cantino & de Queiroz, 2007) specifies that species names are to be governed under the rank-based codes such as that of the ICZN, we choose to make taxonomic recommendations under the ICZN code to avoid confusion about the correct names of species.

It is our responsibility as systematists to analyse and describe biodiversity, and to utilize nomenclature to recognize distinct evolutionary lineages. The best way to recognize the evolutionary patterns recovered in this study is to recognize the major lineages as genera. Although future biodiversity research may result in minor changes to the content of these genera, we infer – on the basis of thorough taxon and character sampling, and robust analytical methods – that the lineages themselves will continue to be supported.

Systematic account

See McDiarmid, Campbell & Touré (1999) and Campbell & Lamar (2004) for synonyms. See Gutberlet & Campbell (2001) for a description of *Bothrocophias* and Campbell & Lamar (2004) for a description of *Bothriopsis*, and for the inclusion of *Bothrocophias colombianus* in *Bothrocophias*, as the content of these genera has not changed.

BOTHROPOIDES GEN. NOV.

Type species: Bothrops neuwiedi Wagler, 1824.

Etymology: The generic name is derived from the Greek *bothros*, referring to the facial pit, and also referring to the currently named genus *Bothrops*. The term *oides* means 'similar to' or 'having the nature of', thereby recognizing the affinity of these species with other terrestrial South American pitvipers. Names ending in this suffix are masculine.

Content: Bothropoides alcatraz, *B. diporus*, *B. erythromelas*, *B. insularis*, *B. jararaca*, *B. lutzi*, *B. mattogrossensis*, *B. neuwiedi*, *B. pauloensis*, and *B. pubescens*.

Definition: Members are of moderate length and girth, and are terrestrial, lacking a prehensile tail. Dorsal colour gold (*B. insularis*) to brown or black, with spade-shaped dorsal markings, with some lacking spots between the spades (*B. alcatraz*, *B. insularis*, *B. jararaca*, *B. pauloensis*, and *B. diporus*), and with others showing them (*B. erythromelas*, *B. lutzi*, *B. mattogrossensis*, *B. neuwiedi*, and *B. pubescens*). A postorbital stripe is present (but is pale in most *B. insularis* specimens); dorsal head patterning is variable among species, and they share no other distinctive head markings.

There are 3–5 interoculabials, 7–11 supralabials, 5–12 keeled intersupraoculars (smooth in *B. erythromelas* and one specimen each of *B. insularis* and *B. alcatraz*), 4–10 scales between the first pair of postcanthals, 21–34 interrictals, 144–206 ventrals, 21–30 dorsal scale rows at midbody, and 31–66 divided or divided and entire subcaudals. The prelacunal and second supralabial are fused (in *B. jararaca*, *B. alcatraz*, and *B. insularis*) or separate, with 0–1 rows of subfoveals. Supralacunal separate from middle preocular (one *B. mattogrossensis* had scales fused). Loreal wider than high or square (one *B. neuwiedi* had loreal higher than wide), loreal pit ventral to nasoorbital line. Postnasal in contact with first supralabial in some individuals. Dorsal scales keeled with typical thin ridge.

From an examination of hemipenes of *B. diporus*, *B. alcatraz*, and *B. insularis*: many lateral spines on hemipenes with lateral calyces distal to crotch in most members of the genus, and few lateral spines with lateral calyces reaching crotch in *B. insularis*. Mesial spines present on hemipenes, except for half of the *B. insularis* specimens. Calyces spinulate, except in one *B. insularis* with smooth calyces.

From an examination of osteological samples of *B. neuwiedi* and *B. jararaca*: 3–5 palatine teeth, 10–16 pterygoid teeth, and 11–15 dentary teeth. Maxillary fang longer than height of maxilla, well-developed medial wall of maxillary pit cavity, with pit in anterolateral wall of maxillary pit cavity either simple or with a small rounded projection. Foramen absent from ventral surface of lateral process of prootic. Lateral margin of head of ectopterygoid of intermediate width, ectopterygoid shaft flat and tapering or

narrow without tapering, and ectopterygoid base with a long overlapping projection. Choanal process of palatine positioned medially, and greatly reduced (*B. neuwiedi*) or attenuate (*B. jararaca*) in shape. Meckellian foramen single; angular and splenial partially fused.

Diagnosis: Bothropoides differ from other South American pitvipers in 38 mitochondrial characters (Table 4). External characters overlap with other South American genera, with no unique synapomorphies in scalation. Distribution in eastern South America, combined with terrestrial habitat in grasslands or broad-leaf forests (*Bothropoides neuwiedi* group), or semiarboreal habitat in Atlantic forests (*B. jararaca* group), distinguishes this genus from others (see Table 4). *Bothropoides* has fewer interrictals (21–34) than the other South American genera (24–40), and some individuals have high numbers of supralabials (7–11, also seen in *Rhinocerophis*; all other South American genera have 7 or 8 supralabials). *Bothropoides* differs from *Bothrops* and *Bothriopsis* in having most species with the prelacunal separate from the second supralabial (*B. jararaca*, *B. alcatraz*, and *B. insularis* have the prelacunal fused to the second supralabial). Some specimens have both divided and entire subcaudals, a state also seen in *Bothriopsis*. *Bothropoides* differs from *Bothriopsis* in the lack of a prehensile tail and lack of green coloration. It differs from *Bothrocophias* in the lack of white spots on the gular scales, and the lack of tuberculate keels on posterior dorsal scales. *Bothropoides* differs from some *Rhinocerophis* (*R. alternatus*, *R. cotiara*, *R. fonsecai*, and *R. jonathani*) in the absence of distinctive back bars on the underside of the head.

Distribution: Eastern South America: in Brazil and associated islands, Bolivia, south-eastern Peru, Paraguay, Uruguay, and northern to central Argentina (Campbell & Lamar, 2004). See Campbell & Lamar (2004) for range maps of individual species.

Remarks: We did not examine individuals of *Bothrops lutzi*, but based on prior work that elevated this species out of the *Bothrops neuwiedi* complex (Silva, 2000, 2004), we include it in the genus *Bothropoides*.

RHINOCEROPHIS GARMAN, 1881

Type species: Rhinocerophis nasus (Garman, 1881), a junior synonym of *Bothrops ammodytoides* (Leybold, 1873).

Etymology: The generic name is derived from the Latin *Rhinoceros*, meaning 'nose-horn', referring to the strongly upturned snout of *R. ammodytoides*, and *ophis*, meaning 'snake'. Names ending in this suffix are masculine.

Content: Rhinocerophis alternatus, *R. ammodytoides*, *R. cotiara*, *R. fonsecai*, *R. itapetiningae*, and *R. jonathani*.

Definition: Members are short to elongate, of moderate girth to stout, and are terrestrial, lacking a prehensile tail. Dorsal colour brown to black, with spade-shaped dorsal markings, generally with spots between spades (*R. alternatus*, *R. fonsecai*; no spots between spades in *R. jonathani*, and sometimes missing in *R. cotiara*), or trapezoidal dorsal markings, with spots between trapezoids (*R. itapetiningae*), or with chequered pattern (*R. ammodytoides*). Spade-shaped dorsal markings and a postorbital stripe on head, with distinctive black bars on the gulars of *R. alternatus*, *R. cotiara, R. fonsecai*, and *R. jonathani*.

There are 3 or 4 interoculabials, 7–10 supralabials, 5–16 keeled intersupraoculars, 5–12 scales between the first postcanthals, 25–40 interrictals, 145–181 ventrals, 23–35 dorsal scale rows at midbody, and 25–55 divided subcaudals. Prelacunal and second supralabial are separate, with either one or no subfoveal scale row, and with supralacunal separate from middle preocular (fused in *R. jonathani* and in one specimen of *R. alternatus*). Loreal wider than high to higher than wide, and loreal pit ventral to naso-orbital line. Postnasal not in contact with first supralabial. Dorsal scales keeled with typical thin ridge.

From an examination of the hemipenes of *R. alternatus*: mesial spines on hemipenes present, spinulate calyces distal to crotch, and many (> 12) lateral spines.

From an examination of osteological samples of *R. cotiara*, *R. fonsecai*, and *R. itapetiningae*: 1 or 2 palatine teeth, 10–14 pterygoid teeth, and 11–13 dentary teeth. Maxillary fang shorter than height of maxilla, medial wall of pit cavity in maxilla well developed. Lateral margin of head of ectopterygoid narrow, single pit on posterior surface of anterior end of ectopterygoid, ectopterygoid shaft narrow and not tapered, and base with a long overlapping projection. Choanal process of palatine positioned anteriorly to medially, and moderately high to attenuate. Supratemporal thick and rounded, with a small projection. Meckellian foramen single; angular and splenial partially to completely fused.

Diagnosis: Rhinocerophis differs from other South American pitvipers in 27 mitochondrial characters, and in having few (1 or 2) palatine teeth (versus 3–6 teeth), which is a morphological synapomorphy (Table 4). Distribution in southern South America, combined with terrestrial habitat in open areas, grasslands, swamps, or broad-leaf and *Araucaria*

forests, distinguishes this genus from others (see Table 4). *Rhinocerophis* individuals have the maxillary fang shorter than the height of the maxilla, and show black bars on the gular scales of some species (*R. alternatus*, *R. cotiara*, *R. fonsecai*, and *R. jonathani*). *Rhinocerophis* have fewer subcaudals (25–55) than the other genera (31–86), and some specimens have high numbers of supralabials (7–10, also seen in *Bothropoides*; other South American genera have 7–8). *Rhinocerophis* differs from *Bothrops* and *Bothriopsis* in having the prelacunal scale separated from the second supralabial. It differs from *Bothriopsis* in the lack of green coloration, and in the lack of a prehensile tail. It differs from *Bothrocophias* in the lack of tuberculate keels on posterior dorsal scales. Almost all species differ from *Bothrocophias* in colour pattern: whereas *Bothrocophias* species have spade-shaped dorsal markings lacking spots between the spades, *Rhinocerophis* species have spots between the spades (*R. alternatus*, *R. cotiara*, and *R. fonsecai*), have trapezoidal markings with spots between them (*R. itapetiningae*), or have a checkered pattern (*R. ammodytoides*). Only *R. jonathani* lacks spots between spades, but it can be distinguished by the presence of black bars on the gular scales, as mentioned above.

Distribution: Southern South America: in south-eastern Brazil, central and southern Bolivia, Paraguay, Uruguay, and Argentina (Campbell & Lamar, 2004). See Campbell & Lamar (2004) for range maps of individual species.

BOTHROPS WAGLER, 1824

Type species: Bothrops lanceolatus (Bonnaterre, 1790)

Etymology: The generic name is derived from the Greek *bothros*, referring to the facial pit, and *ops*, meaning either 'eye' or 'face'. It refers to the loreal pit between the nostril and eye, and names ending in this suffix are masculine.

Content: Bothrops andianus, *B. asper*, *B. atrox*, *B. brazili*, *B. caribbaeus*, *B. isabelae*, *B. jararacussu*, *B. lanceolatus*, *B. leucurus*, *B. marajoensis*, *B. moojeni*, *B. muriciencis*, *B. osbornei*, *B. pirajai*, *B. punctatus*, *B. sanctaecrucis*, and *B. venezuelensis*.

Definition: Members are of moderate length to elongate, are thin to moderately stout, and are terrestrial, lacking a prehensile tail. Dorsal colour brown to black, with trapezoidal to spade-shaped markings on most species (*B. lanceolatus* with spots, *B. osbornei* and *B. punctatus* with vertical bands). Head pattern variable, from patternless, to speckled, to paired spots, to spade-shaped pattern, showing a postorbital stripe in most species (faint to absent in *B. brazili* and *B. sanctaecrucis*, absent in some *B. moojeni*); there are no other distinctive head markings.

There are 3 or 4 interoculabials, 7 or 8 supralabials, 3–13 smooth or keeled intersupraoculars, 3–11 scales between the first pair of postcanthals, 24–36 interrictals, 153–227 ventrals, 22–33 dorsal scale rows at midbody, and 38–72 divided subcaudals (one *B. atrox* and two *B. jararacussu* specimens with both divided and entire subcaudals). Prelacunal and second supralabial fused (one *B. brazili* specimen with scales divided), supralacunal separate from middle preocular (one *B. asper* and one *B. atrox* with scales fused). Sublacunal entire, loreal pit ventral to naso-orbital line (one *B. caribbaeus* and one *B. venezuelensis* with pit crossed by line). Dorsal scales keeled with typical thin ridge.

From an examination of the hemipenes of *B. atrox*, *B. asper*, *B. brazili*, *B. jararacussu*, *B. leucurus*, *B. moojeni*, *B. punctatus*, and *B. venezuelensis*: many lateral spines, lateral calyces distal to crotch (one quarter of *B. brazili* specimens with lateral calyces reaching crotch).

From an examination of osteological samples of *B. asper*, *B. atrox*, *B. brazili*, *B. jararacussu*, *B. moojeni*, and *B. punctatus*: pleurapophyses of midcaudal vertebrae long and slender (one-quarter of *B. brazili* specimens with short and slender pleurapophyses), 3–5 palatine teeth, 12–21 pterygoid teeth, and 8–18 dentary teeth. Maxillary fang longer than height of maxilla, well-developed medial wall of pit cavity in maxilla, pit in anterolateral wall of maxillary pit cavity simple or with a small rounded projection. Lateral margin of head of ectopterygoid intermediate to narrow, shaft of ectopterygoid flat and tapering or narrow without tapering, pits on posterior surface of anterior end of ectopterygoid single or paired, ectopterygoid base long and overlapping, base of ectopterygoid longer than base of pterygoid. Choanal process of palatine positioned medially, moderate to attenuate in shape. Medial margin of dorsal portion of prefrontal moderately to weakly concave, dorsal surface of frontals with elevated margins (one specimen of *B. asper* and one of *B. atrox* with flat dorsal surface). Supratemporal with a small projection (one *B. asper* with expanded supratemporals lacking projections); supratemporal thick and rounded. Single Meckellian foramen.

Diagnosis: Bothrops differs from other South American pitvipers in 50 mitichondrial characters (Table 4). In addition, *Bothrops* species generally have four palatine teeth, which is a morphological synapomorphy of the genus (*B. moojeni* and *B. jararacussu* have five; *B. brazili* and *B. sanctaecrucis* have three). *Bothriopsis* and *Bothrops* are distinguished from other

KEY TO SOUTH AMERICAN BOTHROPOID GENERA

1. Dorsal ground colour green, grey, or brown, dorsal head colour black or matching dorsum, tail prehensile, prelacunal and second supralabial fused.....2
   Dorsal ground colour and dorsal head colour gold or brown to black, tail not prehensile, prelacunal and second supralabial fused or separate, with no or one row of subfoveals.....3
2. Found east of the Andes, dorsal colour usually green (lavender grey to green in *Bothriopsis taeniata*, tan, brown, grey, or olive in *Bothriopsis medusa*).....*Bothriopsis*
   Found west of the Andes, dorsal colour brown to greenish tan.....*Bothrops*
3. Keel on dorsal scales tuberculate on caudal part of body, rostral higher than broad or square, distinct white spots on posterior infralabials and gulars may be present, canthorostrals may be present, 125–169 ventral scales (one specimen with 192 scales),.....*Bothrocophias*
   Keel on dorsal scales typical thin ridge, rostral broader than high to square, or higher than broad in species lacking tuberculate dorsal scales, distinct white spots and canthorostrals absent, 145–227 ventral scales.....4
4. Black bars on gular scales may be present; if absent, species has dorsal pattern of spots or parallel bands, and nonprehensile tail. Dark patterning on head generally spade-shaped; head has a pattern of paired spots in species that have black bars on gular scales, have a dorsal pattern of parallel bands, or lack a nasal pore. Prelacunal and second supralabial separate with no or one subfoveal, loreal scale longer than high to higher than long, 25–40 interrictals, 25–55 subcaudals.....*Rhinocerophis*
   Black bars on gular scales absent, markings on dorsum trapezoidal to spade shaped, except in species with prehensile tails. Dark patterning on head absent, speckled, as paired spots, or spade shaped. Prelacunal and second supralabial fused or separate, with either no or one subfoveal, loreal scale longer than high to square, 21–34 interrictals, 31–72 subcaudals.....5
5. Prelacunal and second supralabial separate, with no or one subfoveal scale; if fused, species is a Brazilian island endemic (*B. alcatraz* or *B. insularis*) or a coastal mainland species in southern Brazil, northeastern Paraguay, and northern Argentina, generally having 8 supralabials and 170–216 ventrals (*B. jararaca*). Subcaudals both divided and entire, or all divided, 7–11 supralabial scales, 144–206 ventral scales, mesial spines on hemipenes present .....*Bothropoides*
   Prelacunal and second supralabial fused. Species sympatric with *B. jararaca* either have fewer supralabials or fewer ventrals, or both. In all species, subcaudal scales divided, 7 or 8 supralabial scales, 153–227 ventral scales, mesial spines on hemipenes absent or present.....*Bothrops*

South American genera by having higher numbers of ventrals (157–236 and 153–227, respectively, compared with 125–206), and by having the prelacunal fused to the second supralabial (also seen in *Bothropoides jararaca*, *B. alcatraz*, *B. insularis*, and in some *Bothrocophias*). *Bothrops* is distinguished from *Bothriopsis* in its brown to black coloration and lack of a prehensile tail, except for *Bothrops osbornei* and *Bothrops punctatus* with prehensile tails. These two *Bothrops* species occur west of the Andes, as opposed to *Bothriopsis* species that all range east of the Andes.

Distribution: Most species are found in South America east of the Andes, exclusive of Uruguay, southern Paraguay, and central to southern Argentina (Campbell & Lamar, 2004). *Bothrops caribbaeus* and *B. lanceolatus* are found on the Caribbean islands of Saint Lucia and Martinique. *Bothrops osbornei*, *B. punctatus*, and *B. asper* range through Peru, Ecuador, and portions of Colombia west of the Andes, and *B. asper* ranges northwards in Middle America through the countries of Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Belize, and Mexico. See Campbell & Lamar (2004) for range maps of individual species.

Remarks: According to Ferrarezzi & Freire (2001), in Campbell & Lamar (2004), *Bothrops muriciencis* is most similar in overall appearance to *Bothrops pirajai*, *B. brazili*, *B. jararacussu*, and *B. sanctaecrucis*, with *B. pirajai* suggested as the closest relative. *Bothrops pirajai* is poorly known, and specimens were unavailable, but it is very similar to some specimens of *B. brazili* and *B. jararacussu* (Campbell & Lamar, 2004). As the aforementioned species included in the study all are found in *Bothrops*, as described in this paper, we assign *B. muriciencis* and *B. pirajai* to the genus as well.

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APPENDIX 1

Habitat, distribution, and proposed genera for all species of *Bothrops* (*sensu* Campbell & Lamar, 2004), including those not represented in the present analysis

Proposed genus	Specific name	Original Describer	Distribution	Habitat
Rhinocerophis	*alternatus*	Duméril, Bibron, & Duméril, 1854	South-eastern Brazil, Paraguay, Uruguay, northern Argentina	Humid habitats in tropical, subtropical, and temperate deciduous forests
Rhinocerophis	*ammodytoides*	Leybold, 1873	along eastern versant of Andes in Argentina	Temperate to subtropical savannas and steppes; arid, sandy, rocky areas
Rhinocerophis	*cotiara*	Gomes, 1913	South-eastern Brazil and northern Argentina	Humid temperate *Araucaria* forest and associated savannas
Rhinocerophis	*fonsecai*	Hoge & Belluomini, 1959	South-eastern Brazil	Mixed forest dominated by *Araucaria*, *Podocarpus*, and broad-leaved trees
Rhinocerophis	*itapetiningae*	Boulenger, 1907	South-eastern Brazil	Open fields and bushy areas
Rhinocerophis	*jonathani*	Harvey, 1994	Eastern slopes of Altiplano, central and southern Bolivia	Dry, rocky grassland
Bothropoides	*alcatraz*	Marques, Martins & Sazima, 2002	Ilha Alcatrazes, Brazil	Low Atlantic forest vegetation
Bothropoides	*diporus*	Cope, 1862	Argentina, Paraguay, south-western Brazil	Chaco, wet palm-grasslands, semitropical deciduous forest, *Araucaria* forest, pampas
Bothropoides	*erythromelas*	Amaral, 1923	North-eastern Brazil	Xeric and semiarid thornforest, dry tropical deciduous forest, open rocky areas
Bothropoides	*insularis*	Amaral, 1922	Ilha Queimada Grande, Brazil	Dry, rocky island habitat with scrubby forest, clearings, and shrubs
Bothropoides	*jararaca*	Wied-Neuwied, 1824	Southern Brazil, north-eastern Paraguay, northern Argentina	Tropical deciduous forests and savanna, semitropical upland forests
Bothropoides	*lutzi*	Miranda-Ribeiro, 1915	North-western Brazil	Savanna (cerrado) and thornscrub
Bothropoides	*mattogrossensis*	Amaral, 1925	Southern Peru, Bolivia, Paraguay, northern Argentina, southern to central Brazil	Savanna (cerrado), Pantanal, Chaco, wet palm-grasslands

APPENDIX 1 *Continued*

Proposed genus	Specific name	Original Describer	Distribution	Habitat
Bothropoides	*neuwiedi*	Wagler, 1824	Eastern Brazil	Tropical and semitropical deciduous forest, temperate forest, Atlantic coast sand ridges
Bothropoides	*pauloensis*	Amaral, 1925	Southern Brazil	Seasonally dry savanna (cerrado) and Atlantic forest associated with open areas
Bothropoides	*pubescens*	Cope, 1870	Uruguay and extreme southern Brazil	Pampas and grasslands
Bothrops	*andianus*	Amaral, 1923	Central Andes in Peru and Bolivia	Montane and lower montane wet forests
Bothrops	*asper*	Garman, 1884	Atlantic versant of Mexico from Tamaulipas southward, northern Guatemala and Honduras, Atlantic lowlands of Nicaragua, Costa Rica and Panama, Pacific versant of Colombian and Ecuadorian Andes, northern Venezuela	Principally tropical rainforest and tropical evergreen forest, or edges of savannas
Bothrops	*atrox*	Linnaeus, 1758	Tropical lowlands east of Andes, exclusive of Paraguay, Uruguay, and Argentina	Lower montane wet forest, savanna/ gallery forest, tropical deciduous forest, rainforest
Bothrops	*brazili*	Hoge, 1954	East of Andes in equatorial forests of Colombia, Ecuador, Peru, Bolivia, southern and eastern Venezuela, Guyana, Suriname, French Guiana, and north-western Brazil	Elevated Amazonian primary forest
Bothrops	*caribbaeus*	Garman, 1887	Saint Lucia Island, Lesser Antilles	Lowland tropical forest, including coastal plains with low humidity
Bothrops	*jararacussu*	Lacerda, 1884	Brazil, Paraguay, southern Bolivia, north-eastern Argentina	Tropical rainforest, tropical semideciduous forest, broad-leaved evergreen forest, paraná pine forest

APPENDIX 1 *Continued*

Proposed genus	Specific name	Original Describer	Distribution	Habitat
Bothrops	*lanceolatus*	Bonnaterre, 1790	Martinique, Lesser Antilles	Humid upland regions and wetter portions of northern windward coast
Bothrops	*leucurus*	Wagler, 1824	Eastern Brazil	Atlantic forest remnants, tropical deciduous forest
Bothrops	*marajoensis*	Hoge, 1966	Northern Brazil	Lowland savanna
Bothrops	*moojeni*	Hoge, 1966	Central and southeastern Brazil, eastern Paraguay, northern Argentina, eastern Bolivia	Semi-arid or seasonally dry tropical savannas
Bothrops	*muriciensis*	Ferrarezzi & Freire, 2001	Eastern Brazil	Mesic Murici Forest, in Atlantic forest
Bothrops	*osbornei*	Freire-Lascano, 1991	Western slopes of Andes in Ecuador and extreme northwestern Peru	Subtropical moist and wet forest and montane wet forest
Bothrops	*pirajai*	Amaral, 1923	Eastern Brazil	Atlantic lowland wet forest and lower montane wet forest
Bothrops	*punctatus*	García, 1896	Pacific foothills and coastal plain in Panama, Colombia, Ecuador	Subtropical and tropical moist and wet forest and montane wet forest
Bothrops	*sanctaecrucis*	Hoge, 1966	Amazonian lowlands of Bolivia	Lower montane wet forest
Bothrops	*venezuelensis*	Sandner-Montilla, 1952	Northern and central Venezuela	Lower montane wet forest and cloud forest, including temparate areas
–	*barnetti*	Parker, 1938	Pacific coast of Peru	Arid desert scrub
–	*lojanus*	Parker, 1930	Southern Ecuador	Arid temperate regions, primarily montane dry forest
–	*pictus*	Tschudi, 1845	Peru	Arid to semiarid coastal foothills, river valleys, and lower Andean slopes; dry rocky regions
–	*roedingeri*	Mertens, 1942	Peru, on Pacific coastal plain and foothills	Desert, low deciduous thickets, lower montane dry forest

Distribution and habitat data from Campbell & Lamar (2004).

APPENDIX 2

Numbers of individuals examined/sequenced for the data used in this study

Species	Scalation	Hemipene Morphology	Osteology	12S	16S	Cyt *b*	ND4
Agkistrodon contortrix	10	1	3	3	3	4	4
Atropoides picadoi	4	3	2	1	1	3	5
Cerrophidion godmani	10	–	1	1	1	1	1
Bothrops alcatraz	1	1	–	–	–	5	–
Bothrops alternatus	11	4	1	4	4	6	5
Bothrops ammodytoides	9	4	–	1	1	1	1
Bothrops andianus	10	2	–	–	–	–	–
Bothrops asper	21	2	4	1	1	2	2
Bothrops atrox	23	6	6	1	1	5	4
Bothrops barnetti	10	1	–	–	–	–	–
Bothrops brazili	7	1	5	1	1	2	2
Bothrops caribbaeus	10	–	–	–	–	1	1
Bothrops cotiara	10	–	1	1	1	2	2
Bothrops diporus	10	5	–	1	1	1	1
Bothrops erythromelas	1	–	–	1	1	3	2
Bothrops fonsecai	10	–	–	–	–	1	1
Bothrops insularis	10	2	–	1	1	3	2
Bothrops isabelae	–	–	–	–	–	1	1
Bothrops itapetiningae	13	–	–	1	1	2	2
Bothrops jararaca	9	–	1	1	1	10	9
Bothrops jararacussu	10	3	2	1	1	3	2
Bothrops jonathani	1	1	–	–	–	–	–
Bothrops lanceolatus	10	–	–	–	–	1	1
Bothrops leucurus	10	2	–	1	1	1	1
*Bothrops lojanus**	6	–	–	–	–	–	–
Bothrops marajoensis	–	–	–	–	–	1	1
Bothrops mattogrossensis	14	2	–	–	–	–	–
Bothrops moojeni	10	1	1	4	4	6	5
Bothrops neuwiedi	10	–	–	–	–	2	2
Bothrops osbornei	2	–	–	–	–	1	1
Bothrops pauloensis	5	–	–	1	1	1	1
Bothrops pictus	10	1	–	–	–	1	1
*Bothrops pubescens**	4	–	–	–	–	–	–
Bothrops punctatus	9	1	–	–	–	1	1
Bothrops sanctaecrucis	9	–	–	–	–	–	–
Bothrops venezuelensis	5	2	–	–	–	–	–
Bothrocophias campbelli	2	–	–	–	–	1	1
*Bothrocophias colombianus**	2	–	–	–	–	–	–
Bothrocophias hyoprora	14	1	1	1	1	2	2
Bothrocophias microphthalmus	8	–	1	1	1	2	2
Bothrocophias myersi	12	1	1	–	–	–	–
Bothriopsis b.bilineata	7	1	–	–	1	1	–
Bothriopsis b.smaragdina	10	–	–	1	1	2	2
Bothriopsis chloromelas	3	–	–	1	1	1	1
*Bothriopsis medusa**	1	–	–	–	–	–	–
*Bothriopsis oligolepis**	1	–	–	–	–	–	–
Bothriopsis pulchra	8	–	1	–	–	1	1
Bothriopsis taeniata	7	1	1	1	1	2	2

*Species not included in phylogenetic estimation.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Majority-rule consensus cladogram of six most parsimonious trees from an analysis excluding taxa with morphological data only (analysis 10).

Figure S2. Phylogram of single most parsimonious tree from an analysis excluding taxa with morphological data only (analysis 9).

Figure S3. Majority-rule consensus cladogram of ten most parsimonious trees from an analysis including taxa with morphological data only (analysis 7).

Figure S4. Phylogram of a single most parsimonious tree from an analysis including taxa with morphological data only (analysis 6).

Figure S5. Bayesian Markov Chain Monte Carlo (MCMC) 50% majority-rule consensus phylogram derived from an analysis of 2343 bp of mitochondrial DNA (analysis 5).

Figure S6. Majority-rule consensus cladogram of 11 most parsimonious trees derived from an analysis of 2343 bp of mitochondrial DNA (analysis 4).

Figure S7. Bayesian Markov Chain Monte Carlo (MCMC) 50% majority-rule consensus phylogram derived from an analysis of 85 gap-weighted or majority-coded morphological characters (analysis 3).

Figure S8. Parsimony 50% majority-rule consensus cladogram of the 107 shortest trees derived from analysis of 85 gap-weighted or majority-coded morphological characters.

Figure S9. Phylogram of the single most parsimonious tree derived from an analysis of 85 generalized frequency coded morphological characters.

Table S1. Species used, voucher data, collecting locality, and GenBank accession numbers for each taxon.

Table S2. Cytochrome *b* distances within and among selected genera recovered with the Kimura two-parameter model with Γ-distributed rate variation.

Appendix S1. Specimens examined for morphological data.

Appendix S2. List of characters used in this study.

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