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Modeling nucleotide evolution at the mesoscale: The phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: Crotalinae)

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Abstract

We analyzed the phylogeny of the Neotropical pitvipers within the *Porthidium* group (including intra-specific through inter-generic relationships) using 1.4 kb of DNA sequences from two mitochondrial protein-coding genes (ND4 and cyt-*b*). We investigated how Bayesian Markov chain Monte-Carlo (MCMC) phylogenetic hypotheses based on this 'mesoscale' dataset were affected by analysis under various complex models of nucleotide evolution that partition models across the dataset. We develop an approach, employing three statistics (Akaike weights, Bayes factors, and relative Bayes factors), for examining the performance of complex models in order to identify the best-fit model for data analysis. Our results suggest that: (1) model choice may have important practical effects on phylogenetic conclusions even for mesoscale datasets, (2) the use of a complex partitioned model did not produce widespread increases or decreases in nodal posterior probability support, and (3) most differences in resolution resulting from model choice were concentrated at deeper nodes. Our phylogenetic estimates of relationships among members of the *Porthidium* group (genera: *Atropoides*, *Cerrophidion*, and *Porthidium*) resolve the monophyly of the three genera. Bayesian MCMC results suggest that *Cerrophidion* and *Porthidium* form a clade that is the sister taxon to *Atropoides*. In addition to resolving the intra-specific relationships among a majority of *Porthidium* group taxa, our results highlight phylogeographic patterns across Middle and South America and suggest that each of the three genera may harbor undescribed species diversity.

Keywords: Akaike weights; *Atropoides*; Bayes factors; *Cerrophidion*; MCMC model testing; *Porthidium*; Relative Bayes factors

1. Introduction

1.1. Modeling nucleotide evolution at the mesoscale

Incorporating DNA sequence data from multiple genes to solve phylogenetic problems has essentially become a standard across contemporary molecular phylogenetic studies. Paralleling the increasing frequency of multi-locus datasets, model-based techniques have also become a standard in molecular phylogenetics. These methods are attractive because they effectively incorporate probabilistic models of DNA substitution and should, therefore, be less likely to be misled by the complexities of DNA evolution (Huelsenbeck and Crandall, 1997). Numerous empirical studies have demonstrated an array of molecular evolutionary patterns that varies across partitions of molecular datasets including mutation and base-compositional biases (e.g., Faith and Pollock, 2003; Reeder, 2003), and among-site rate variation (e.g., Castoe et al., 2004; Monclavo et al., 2000; Yang, 1996). Thus, an important concern arises when utilizing parametric model-based techniques: a single model with one set of parameters to account for molecular evolution over multiple heterogeneous partitions

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(e.g., multiple loci, codon positions, structural RNA vs. protein-coding regions, etc.) in a combined analysis may fail to portray partition-specific evolutionary patterns.

The use of a single model of evolution for a dataset that is heterogeneous forces a compromise (or averaging) in parameter estimates that may introduce a major source of systematic error and mislead phylogenetic conclusions (Brandley et al., 2005; Reeder, 2003; Wilgenbusch and de Queiroz, 2000; see also Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004). This type of systematic error may be avoided by employing independent models of evolution (and parameter estimates) for subsets of a heterogeneous dataset within a combined analysis (Nylander et al., 2004; Ronquist and Huelsenbeck, 2003; Yang, 1996). Development of robust methods for fitting appropriately complex models of evolution to data partitions, however, has only recently been addressed directly (e.g., Brandley et al., 2005; Castoe et al., 2004; Nylander et al., 2004; Pupko et al., 2002; Yang, 1996).

Model choice has been shown to affect both the phylogenetic topology (e.g., Huelsenbeck, 1995, 1997; Sullivan and Swofford, 2001) and the accurate estimation of posterior probabilities (e.g., Buckley, 2002; Castoe et al., 2004; Erixon et al., 2003; Huelsenbeck and Rannala, 2004; Suzuki et al., 2002). Because the accuracy of posterior probabilities in Bayesian phylogenetic methods relies (at least in part) on the model, models that may not affect the consensus topology may have notable effects on the posterior probability distribution of parameter estimates, and thus on the confidence regarding phylogenetic conclusions. Based on this logic, employing complex models that more accurately portray DNA evolution should produce less-biased posterior-probability estimates as long as parameters can be accurately estimated from the data (Huelsenbeck et al., 2002; Huelsenbeck and Rannala, 2004). The benefits of constructing and employing more realistic evolutionary models of DNA substitution are challenged by the potential for imprecise and inaccurate parameter estimation (including topology). This may result from overparameterization when the ratio of free parameters to data increases past a poorly characterized critical point (where parameters are no longer identifiable based on the data), beyond which a likelihood function may become unreliable (Huelsenbeck et al., 2002; Rannala, 2002; Rogers, 2001; Wald, 1949).

Fundamental differences in the process of optimization of Bayesian and maximum-likelihood methods (see reviews in Holder and Lewis, 2003; Huelsenbeck et al., 2001) have required reconsideration of methods and criteria for selection of best-fit models of evolution. Specific to Bayesian phylogenetics, analytical derivation of the marginal model likelihood is usually impossible when the number of parameters is large, although several estimators of the model likelihood have been proposed. Nylander et al. (2004) followed the proposal of Newton and Raftery (1994) by using the harmonic mean of the post-burn-in likelihood values as a reasonable estimate of the marginal model likelihood (for details and justification see Nylander et al., 2004; see also Aris-Brosou and Yang, 2002; Huelsenbeck et al., 2004; Suchard et al., 2001). Here, we take advantage of the harmonic mean estimation of Bayesian model likelihoods to employ Bayes factors (Nylander et al., 2004) and an adapted version of Akaike weights (Buckley et al., 2002; based on Akaike Information Criteria: Akaike, 1973, 1974, 1983; Sakamoto et al., 1986) to identify the best-fit model of nucleotide substitution for our combined nucleotide data comprising two mitochondrial protein-coding gene fragments.

In this study, we analyze what we believe is representative of a mid-sized molecular phylogeny that ranges in sampling scope from intra-specific to inter-generic. The nucleotide data consist of two of the more common genes used in molecular phylogenetics, the mitochondrial NADH dehydrogenase subunit 4 (ND4) and cytochrome-*b* (cyt-*b*), from 61 terminal taxa. This dataset provides a reasonably representative model of contemporary 'mesoscale' molecular phylogenetics. As such, understanding how phylogenetic hypotheses from this 'mesoscale' dataset are affected by analysis under various complex models of nucleotide evolution is an important concern relevant to a majority of contemporary analyses of similar molecular and taxon-sampling scope.

1.2. Systematics of the Neotropical pitvipers of the Porthidium group

Pitvipers (Viperidae: Crotalinae) comprise an extensive radiation of both Old and New World venomous snakes with over 180 species allocated to 29 genera (Campbell and Lamar, 2004; Malhotra and Thorpe, 2004; McDiarmid et al., 1999). This diverse radiation of highly venomous snakes has received substantial taxonomic and phylogenetic attention over the last several decades, yet many taxonomic and phylogenetic hypotheses remain unresolved. Recent studies examining molecular characters from a large number of taxa (Parkinson, 1999; Parkinson et al., 2002) have supported several higher-level relationships within Neotropical pitvipers. Within Neotropical pitvipers there appears to be: (1) several basal clades (genera: *Bothriechis*, *Lachesis*, and *Ophryacus*), (2) a primarily South American lineage (genera: *Bothrocophias*, *Bothriopsis*, and *Bothrops*), and (3) a primarily Middle American lineage (genera: *Atropoides*, *Cerrophidion*, and *Porthidium*). This study focuses on this third clade of Neotropical species, referred to as the '*Porthidium* group' (Castoe et al., 2003; Parkinson et al., 2002; see Campbell and Lamar (2004) for detailed updated distribution maps of all *Porthidium* group species).

The *Porthidium* group radiation of Neotropical pitvipers contains three genera, each of which is morphologically and ecologically distinct. *Cerrophidion* (montane pitvipers) contains four mid-sized species that inhabit mid-to-high elevation Middle American subtropical habitats. *Atropoides* (jumping pitvipers) contains five species of particularly stout-bodied pitvipers that inhabit low-to-middle elevation tropical and subtropical habitats in Middle America (ranging from rainforest and cloud forest to pine–oak forest). *Porthidium* (hognose pitvipers) contains nine more diminutive species that primarily inhabit low elevation wet and dry tropical and subtropical forests across Middle America and northern South America (Campbell and Lamar, 2004).

The *Porthidium* group has been the subject of a number of taxonomic rearrangements and specific additions over the last few decades (see detailed reviews in Campbell and Lamar, 1989, 2004; Castoe et al., 2003; Gutberlet and Harvey, 2004). Initially, all members of this group were recognized under the nominal genus *Porthidium* (Burger, 1971; Campbell and Lamar, 1989), and later were dissected into the three current genera (Campbell and Lamar, 1992; Werman, 1992). In addition to these revisions, two taxa that were once considered members of the *Porthidium* group have been subsequently reallocated to different genera (*Ophryacus melanurum*, Gutberlet, 1998; *Bothrocophias hyoprora*, Gutberlet and Campbell, 2001). At the level of alpha taxonomy, new species have been recently recognized in each of the three genera. Several of these new additions have suggested the taxonomic splitting of widely ranging species (*Atropoides* spp., Campbell and Lamar, 2004; *P. porrasi*, Lamar and Sasa, 2003), while other recently described species represent previously unknown populations only recently discovered (e.g., *C. petlalcalensis*, López-Luna et al., 2000; *P. volcanicum*, Solórzano, 1995).

Although no molecular phylogenetic analyses have inclusively examined relationships across the entire *Porthidium* group, several studies have provided insight into the phylogeny and systematics of the group. The most comprehensively sampled inter-generic molecular phylogenetic study of pitvipers to date (Parkinson et al., 2002) resolved a monophyletic *Porthidium* group and the genus *Porthidium* as the sister taxon to *Atropoides* plus *Cerrophidion*. Castoe et al. (2003) did not find support for the monophyly of *Atropoides* and demonstrated the paraphyly of *A. nummifer* (later rectified by raising each subspecies to species status by Campbell and Lamar, 2004). Castoe et al. (2003) also demonstrated large divergences among populations of the widespread species *Cerrophidion godmani*. Similarly, Wüster et al. (2002) demonstrated paraphyly of the species *Porthidium nasutum* and *P. lansbergi* (each of which have also recently been taxonomically subdivided; Campbell and Lamar, 2004; Lamar and Sasa, 2003). In summary, results of previous systematic work leave several remaining questions regarding the evolutionary relationships and taxonomy of the *Porthidium* group due to weakly resolved phylogenetic hypotheses or limited taxonomic sampling. In this study, we try to rectify these problems by reconstructing the phylogenetic relationships within this group including samples representing nearly all species, with many species represented by multiple samples from geographically distinct or isolated populations.

1.3. Theoretical and empirical scope of this study

The goals of this study incorporate a number of theoretical and empirical questions. We employ two different objective methods (Bayes factors and an adapted version of AIC) for identifying complex best-fit models of nucleotide evolution in a Bayesian phylogenetic context. In doing so, we address the question, "Is it practically important to consider complex models of evolution for 'mesoscale' phylogenetic analyses?" Given careful consideration of appropriate model choice, we apply the resulting phylogenetic hypotheses to outstanding questions regarding systematics of the *Porthidium* group. Specifically, we sought to address the following empirical questions: (1) Do we find evidence for the monophyly of *Atropoides*? (2) What are the relationships among the three *Porthidium* group genera? (3) Is there evidence of undescribed or non-monophyletic *Porthidium* group taxa?

2. Materials and methods

2.1. Taxon sampling

In total, 61 terminal taxa (OTUs) were included in this study. The ingroup (members of the genera *Atropoides*, or *Cerrophidion*, and *Porthidium*) included 52 samples representing 15 of 18 nominal species. When sampling ingroup taxa, we attempted to include multiple representatives of nominal species where possible, particularly samples from geographically distant or isolated portions of their respective ranges. Details of terminal-taxon sampling (along with voucher information) are provided in Table 1. Our sampling of recognized species included 5/5 *Atropoides* species, 3/4 *Cerrophidion* species (lacking *C. barbouri*), and 7/9 *Porthidium* species (lacking *P. hespere* and *P. volcanicum*). Outgroup taxa were chosen based on results from recent large-scale pitviper phylogenetic studies (Parkinson, 1999; Parkinson et al., 2002; Castoe and Parkinson, unpublished). Additionally, we intentionally included two taxa (*Ophryacus melanurum* and *Bothrocophias hyoprora*) that were at one time considered members of the *Porthidium* group and later removed (Gutberlet, 1998; Gutberlet and Campbell, 2001). Our outgroup-sampling strategy

Table 1
Specimens used in this study including taxa represented, reference identifiers, vouchers, localities, and GenBank accession numbers

Taxon	Specimen reference ID	Voucher	Locality	ND4	cyt-*b*
Outgroups					
Lachesis stenophrys	*Lachesis stenophrys*		Costa Rica: Limón	U41885	AY223603
Ophryacus melanurus	*Ophryacus melanurus*	UTA-R-34605	Mexico	AY223634	AY223587
Ophryacus undulatus	*Ophryacus undulatus*	CLP-73	Mexico	AY223633	AY223586
Bothriechis schlegelii	*Bothriechis schlegelii*	MZUCR-11149	Costa Rica	AY223636	AY223590
Bothriechis nigroviridis	*Bothriechis nigroviridis*	MZUCR-11151	Costa Rica	AY223635	AY223589
Bothriechis lateralis	*Bothriechis lateralis*	MZUCR-11155	Costa Rica	U41873	AY223588
Bothrocophias hyoprora	*Bothrocophias hyoprora*		Colombia: Leticia	U41886	AY223593
Bothriopsis taeniata	*Bothriopsis taeniata*		Surinam	AY223637	AY223592
Bothrops ammodytoides	*Bothrops ammodytoides*	MVZ-223514	Argentina: Neuquén	AY223639	AY223595
Ingroup					
Atropoides mexicanus	*A. mexicanus* Costa Rica1	UTA-R-12943	Costa Rica: Cartago: Pavones de Turrialba	AY220335	AY220312
	A. mexicanus Costa Rica2	MSM	Costa Rica: Puntarenas: San Vito	AY220336	AY220313
	A. mexicanus Costa Rica3	CLP-168	Costa Rica: San José	U41871	AY223584
	A. mexicanus Guatemala1	UTA-R-35942	Guatemala: Baja Verapaz: Nino Perdido	AY220330	AY220037
	A. mexicanus Guatemala2	UTA-R-32746	Guatemala: Huehetanango: Finca Chiblac	AY220331	AY220308
	A. mexicanus Guatemala3	UTA-R-35944	Guatemala: Izabal: Puerto Barrios	AY220332	AY220309
	A. mexicanus Guatemala4	UTA-R-43592	Guatemala: Quiché: Mountains West of El Soch	AY220334	AY220311
	A. mexicanus Guatemala5	UTA-R-46616	Guatemala: Alta Verapaz: Finca San Juan	AY220329	AY220306
	A. mexicanus Guatemala6	UTA-R-32419	Guatemala: Petén: San José El Espinero	AY220333	AY220310
Atropoides nummifer	*A. nummifer* Mexico1	UTA-R-24842	Mexico: Hidalgo: vic. Huejutla	AY220337	AY220314
	A. nummifer Mexico2	ENS-10515	Mexico: Puebla: San Andres Tziaulan	DQ061220	DQ061195
Atropoides occiduus	*A. occiduus* Guatemala1	UTA-R-29680	Guatemala: Escuintla: S. slope Volcán de Agua	AY220338	AY220315
	A. occiduus Guatemala2	UTA-R-46719	Guatemala: Sololá: San Lucas Tolimán	AY220340	AY220317
	A. occiduus Guatemala3	UTA-R-24763	Guatemala: Guatemala: Villa Nueva	AY220339	AY220316
	A. occiduus Honduras	ENS-10630	Honduras: Olancho: Sierra de Botaderos	DQ061219	DQ061194
Atropoides olmec	*A. olmec* Guatemala	UTA-R-34158	Guatemala: Baja Verapaz: Niño Perdido	AY220342	AY220319
	A. olmec Mexico1	ENS-10510	Mexico: Chiapas: Mapastepec	DQ061221	DQ061196
	A. olmec Mexico2	JAC-9745	Mexico: Oaxaca: Cerro El Baúl	AY220343	AY220320
	A. olmec Mexico3	UTA-R-25113	Mexico: Veracruz: Sierra de los Tuxtlas	AY220344	AY220321
	A. olmec Mexico4	UTA-R-14233	Mexico: Veracruz: Sierra de los Tuxtlas	AY220345	AY220322
Atropoides picadoi	*A. picadoi* Costa Rica1	CLP-45	Costa Rica: Alajuela: Varablanca	U41872	AY223593
	A. picadoi Costa Rica2	UTA-R-23837	Costa Rica: San José: Bajo la Hondura	AY220347	AY220324
	A. picadoi Costa Rica3	MSM-10350	Costa Rica: San José: Bajo la Hondura	DQ061222	DQ061197
Cerrophidion godmani	*C. godmani* Costa Rica1	MSM	Costa Rica: San José	AY220351	AY220328
	C. godmani Costa Rica2	MSM	Costa Rica: San José: Goicochea	DQ061224	DQ061199
	C. godmani Costa Rica3	MSM	Costa Rica: San José: Goicochea	DQ061225	DQ061200
	C. godmani Guatemala1	UTA-R-40008	Guatemala: Baja Verapaz: La Unión Barrios	AY220348	AY220325
	C. godmani Guatemala2	ENS-8195	Guatemala: Quiché	DQ061223	DQ061198
	C. godmani Honduras	ENS-10631	Honduras: Ocotepéque: Güisayote	DQ061226	DQ061201
	C. godmani Mexico	JAC-15709	Mexico: Oaxaca: Cerro El Baúl	AY220349	AY220326
Cerrophidion petlalcalensis	*C. petlalcalensis* Mexico	ENS-10528	Mexico: Veracruz: Orizaba	DQ061227	DQ061202
Cerrophidion tzotzilorum	*C. tzotzilorum* Mexico1	ENS-10529	Mexico: Chiapas: Las Rosas	DQ061228	DQ061203
	C. tzotzilorum Mexico2	ENS-10530	Mexico: Chiapas: Zinacantán	DQ061229	DQ061204
Porthidium arcoase	*P. arcosae* Ecuador	WWW-750	Ecuador: Manabí: Salango	AY223631	AY223582

Porthidium dunni	*P. dunni* Mexico1	MS	Mexico: Chiapas: Guardiania	DQ061243	DQ061217
	P. dunni Mexico2	ENS-9705	Mexico: Oaxaca: near San Pedro Pochutla	AY223630	AY223581
Porthidium lansbergii	*P. lansbergii* Panama	MSM	Panama: Darién	DQ061231	DQ061206
	P. lansbergii Venezuela	WES	Venezuela: Isla Margarita	DQ061230	DQ061205
Porthidium nasutum	*P. nasutum* Costa Rica1	MSM	Costa Rica: Alajuela: Río Cuarto de Grecia	DQ061235	DQ061210
	P. nasutum Costa Rica2	MSM	Costa Rica: Cartago: Guayacán de Turrialba	DQ061233	DQ061208
	P. nasutum Costa Rica3	MSM	Costa Rica: Cartago: Guayacán de Turrialba	DQ061234	DQ061209
	P. nasutum Costa Rica4	MZUCR-11150	Costa Rica	U41887	AY223579
	P. nasutum Ecuador	FGO-live-517	Ecuador: Esmeraldas: Zapallo Grande	AF29574	AF292612
	P. nasutum Guatemala	UTA-R-44749	Guatemala: Alta Verapaz: Cobán	DQ061232	DQ061207
Porthidium ophryomegas	*P. ophryomegas* Costa Rica	UMMZ-210276	Costa Rica: Guanacaste	U41888	AY223580
	P. ophryomegas Guatemala	MSM-23	Guatemala: Zacapa	DQ061241	DQ061216
	P. ophryomegas Honduras	UTA-R-52580	Honduras: Gracias a Dios: Mocorón	DQ061240	
Porthidium porrasi	*P. porrasi* Costa Rica1	MSM	Costa Rica: Puntarenas	DQ061239	DQ061214
	P. porrasi Costa Rica2	MSM	Costa Rica: Puntarenas: Sierpe	DQ061236	DQ061211
	P. porrasi Costa Rica3	MSM	Costa Rica: Puntarenas: San Pedrillo	DQ061237	DQ061212
	P. porrasi Costa Rica4	MSM	Costa Rica: Puntarenas: Golfito	DQ061238	DQ061213
Porthidium yucatanicum	*P. yucatanicum* Mexico	JAC-24438	Mexico: Yucatán: Car. Yaxcabá-Tahdzibichen	DQ061244	DQ061215

Voucher acronyms follow Leviton et al. (1985) except the following: CLP = Christopher L. Parkinson; ENS = Eric N. Smith; FGO = Fundacion Herpetologica Gustavo Orces, Quito, Ecuador; JAC = Jonathan A. Campbell; MSM = Mahmood Sasa; WWW = Wolfgang W. Wüster.

included multiple successive outgroups (Smith, 1994) based on the expectation that this approach would reduce potential biases imposed by rooting phylogenies with a single outgroup.

2.2. DNA sequencing and sequence alignment

In addition to the novel sequences generated from this study, several sequences used in this study have been previously published (Castoe et al., 2003; Parkinson, 1999; Parkinson et al., 2002; Wüster et al., 2002; see Table 1 for details). Laboratory methods for obtaining novel sequences used in this study are as follows. Genomic DNA was isolated from tissue samples (liver or skin preserved in ethanol) using the Qiagen DNeasy extraction kit and protocol (Qiagen, Hilden, Germany). Two protein-coding mitochondrial gene fragments were amplified and sequenced per sample: the ND4 fragment (including the 3′ region of the NADH dehydrogenase subunit 4 gene) and the cyt-*b* fragment (including the 3′ region of the cytochrome-*b* gene).

The ND4 fragment was amplified via PCR using the primers ND4 and LEU or ND4 and HIS (Arévalo et al., 1994). The cyt-*b* fragment was PCR amplified using the primers Gludg and AtrCB3 (Parkinson et al., 2002). Genechoice or Sigma brand PCR reagents were used to conduct PCR in the following final concentrations: 1× standard PCR buffer, 1.5 U *Taq* polymerase, 0.1 μM per primer, 1.0 mM dNTPs, 2.0 mM $MgCl_2$, and 0.004% DMSO. Thermocycling conditions included initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. Positive PCR products were excised from agarose electrophoretic gels and purified using the GeneCleanIII kit (BIO101). Purified PCR products were sequenced in both the directions with the amplification primers (and for ND4, an additional internal primer HIS; Arévalo et al., 1994). Purified PCR products were sequenced using the CEQ D Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman-Coulter) and run on a Beckman CEQ8000 automated sequencer according to the manufacturer's protocols. Raw sequence chromatographs for sequences generated in this study were edited using Sequencher 4.1 (Gene Codes Corp., 2002). Sequences of each fragment were aligned manually in GeneDoc (Nicholas and Nicholas, 1997). Alignment was unambiguous and contained no inferred indels within the ingroup but included the absence of a complete codon in the cyt-*b* fragment in several outgroup specimens. No internal stop codons were found in either fragment. The final alignment of both gene fragments concatenated comprised a total of 1405 aligned positions: 693 from ND4 and 712 from cyt-*b*. Novel sequences were deposited in GenBank (GenBank accession numbers for all the sequences used are given in Table 1).

2.3. Phylogenetic reconstruction

Throughout all phylogenetic reconstructions, gaps in alignment were treated as missing data. Maximum parsimony (MP) and Bayesian Metropolis-Hastings coupled Markov chain Monte-Carlo (MCMC) phylogenetic methods were used to reconstruct phylogenies. Both methods were initially used to compare phylogenetic reconstructions based on each gene fragment independently to identify any instances where different gene fragments demonstrated strongly supported alternative phylogenetic arrangements. We expect that mitochondrial loci should all contain phylogenetic signal supporting a common phylogeny because mitochondrial haplotypes are inherited maternally as a single linkage unit. We tested this assumption (prior to combining data) by estimating individual gene fragment phylogenies and checking for bipartitions that differed between gene fragments and were well supported (e.g., Weins, 1998) using both maximum parsimony and Bayesian MCMC analyses.

All MP phylogenetic analyses were conducted using PAUP* version 4.0b10 (Swofford, 2002). All characters were treated as equally weighted in MP searches. We used the heuristic search option with inactive steepest descent option, tree bisection reconnection (TBR) branch-swapping option, and 10,000 random-taxon-addition sequences to search for optimal trees. Support for nodes in MP reconstructions was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1000 full heuristic pseudo-replicates (10 random-taxon-addition sequence replicates per bootstrap pseudo-replicate).

ModelTest version 3.0 (Posada and Crandall, 1998, 2001) was used to select an appropriate model of evolution for MCMC analyses based on consideration of both available criteria, hLRT and AIC (with likelihoods for models estimated in PAUP*). In addition to the combined dataset, all putative partitions of the dataset were independently analyzed using ModelTest to determine best-fit models of nucleotide evolution. These estimates were used as a partial justification for partition-specific model choice during the construction of partitioned MCMC models, similar to the suggestions of Brandley et al. (2005).

All MCMC phylogenetic analyses were conducted in MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) with vague priors and three heated chains in addition to the cold chain (as per the program's defaults). Each MCMC analysis was conducted in triplicate, with three independent runs initiated with random trees, and run for a total of 4.0×10^7 generations (sampling trees every 100 generations). Conservatively, the first 1.0×10^7 generations from each run were discarded as burn-in. Summary statistics and consensus phylograms with nodal posterior probability support were estimated from the combination of the triplicate set of runs per analysis.

An initial set of MCMC (for the individual and combined datasets) was run using the model estimated by ModelTest (considering both AIC and hLRT criteria) to fit each individual gene or combined dataset (or nearest model available in MrBayes 3.0, as explained below). In addition to the model selected by ModelTest, the combined dataset was subjected to five additional MCMC analyses under alternative evolutionary models. These five additional MCMC analyses were designed to allow independent models of evolution to be used for partitions of the combined dataset. This was accomplished by partitioning the dataset into what we assumed were biologically relevant partitions and specifying that an independent GTR + Γ + I model, with independent base frequencies, be used for each identified partition (using the "unlink" command in MrBayes 3.0). For these complex models, only branch lengths and topology remained linked between partitions. The names and details of all models used to analyze the combined dataset are summarized in Table 2. These models partitioned the combined dataset based on combinations of codon position and/or gene fragment (ND4 vs. cyt-*b*).

Several methods are available for model selection in a Bayesian context. In this study, we employ three statistics for the purposes of model selection: (1) Bayes factors (B_{10}), (2) relative Bayes factors (RBF), and (3) Akaike weights (A*w*) to choose a best-fit model from among the alternative models outlined above. Each of these criteria allows testing of non-nested models [not allowed by hierarchical log-likelihood ratio tests (hLRTs)], which is important here because two alternative models are non-nested ("2x-gene" and "2x-pos12,3" models). Also, each criteria allows accommodation of marginal model likelihoods (rather than maximum likelihoods) derived from Bayesian MCMC analyses (accommodation of marginal model likelihoods for AIC is described below).

Bayes factors were calculated following Nylander et al. (2004) and we report the results in the form of

Table 2
Best-fit models selected by ModelTest for various partitions of the dataset based on both hLTR and AIC criteria

Partition	hLTR	AIC
Entire dataset	GTR + Γ + I	GTR + Γ + I
ND4	TVM + Γ + I	TrN + Γ + I
cyt-*b*	TVM + Γ + I	TrN + Γ + I
Codon position 1	TrN + Γ + I	TVM + Γ + I
Codon position 2	HKY + Γ + I	TIM + Γ + I
Codon position 3	TIM + Γ + I	TIM + Γ + I
P1 = (ND4,pos1)	TrNef + Γ + I	GTR + Γ + I
P2 = (ND4,pos2)	HKY + Γ	TVM + Γ + I
P3 = (ND4,pos3)	TrN + Γ	GTR + Γ + I
P4 = (cyt-*b*,pos1)	TrNef + Γ + I	HKY + Γ + I
P5 = (cyt-*b*,pos2)	HKY + Γ + I	TrN + Γ + I
P6 = (cyt-*b*,pos3)	HKY + Γ + I	TrN + Γ + I

P1–6 refer to the six independent partitions of the dataset under the 6x-gene,codon model.

$2\ln B_{10}$. To compare two competing models, M_0 and M_1, the Bayes factor supporting M_1 over M_0 is equal to the ratio of the model likelihoods. We considered $2\ln B_{10} > 10$ sufficient to support M_1 over M_0 (Kass and Raftery, 1995; see also Brandley et al., 2005; Nylander et al., 2004).

Relative Bayes factors (RBF) were used to quantify the average impact that each free model parameter had on increasing the fit of the model to the data. These values were also used qualitatively to estimate the ratio of parameters to posterior evidence (of prior modification by the data) of increasingly complex models. This statistic is a permutation of the Bayes factor between the simplest (best-fit unpartitioned) and the alternative partitioned model that is normalized to the difference in free model parameters between models. We calculated the RBF of each complex model by calculating $2\ln B_{10}$ between the base model and each complex (partitioned) model and dividing this by the difference in the number of free model parameters between the base and complex model.

We used a statistic derived from Akaike Information Criteria (AIC) in addition to statistics based on Bayes factors. Specifically, we implemented an adapted version of Akaike weights to infer the best-fit model of nucleotide evolution. Instead of using the maximum-likelihood value, we used the harmonic mean estimator of the $\ln L$ from MCMC analyses to incorporate an estimate of the marginalized likelihood of models to be compared using Akiake weights (A*w*; see also Kauermann et al., 2004; Wager et al., in press). The estimation of A*w* has been recently reviewed by Posada and Buckley (2004), and we provide a brief summary here. The AIC of each model is calculated as the $\mathrm{AIC} = -2L + 2\mathrm{K}$ where K is the number of estimatable parameters (model parameters plus branch lengths in our case; for unrooted bifurcating trees the total number of branches is equal to twice the number of taxa minus three). From this, we calculated the change in AIC across models by comparing the AIC of the ith model to the model with the highest likelihood (min AIC) using the equation $\Delta\mathrm{AIC}_i = \mathrm{AIC}_i - \min\ \mathrm{AIC}$. Akaike (1983) suggested that the relative likelihood of the models given the data may be obtained using the formula $e^{(-\Delta\mathrm{AIC}i/2)}$, which may then be normalized over all models to obtain a set of positive Akaike weights (A*w*). This is accomplished by dividing each $e^{(-\Delta\mathrm{AIC}i/2)}$ by the sum of all $e^{(-\Delta\mathrm{AIC}i/2)}$ values across all the models. Thus, the higher the A*w* for a model, the higher the relative support for that model.

In addition to employing Bayes factors and Akaike weights to identify best-fit models of nucleotide evolution, we secondarily evaluated the performance of alternative models to check for problems with mixing and convergence indicative of model over-fitting (overparameterization). Once a tentative model was chosen, this model was rigorously examined to check for evidence of parameter identifiability, failed convergence, and unreliability (which would suggest the model may be parametrically over-fit). We investigated the performance of models (using Tracer; Rambout and Drummond, 2003) by examining features of model likelihood and parameter estimate burn-in, as well as the shapes and overlap of posterior distributions of parameters. Specifically, we looked for evidence that model likelihood and parameter estimates ascended directly and relatively rapidly to a stable plateau, and that independent runs converged on similar likelihood and parameter posterior distributions (considered evidence that a model was not over-fit). We also examined the model parameter estimates to confirm that the shape of their posterior distributions reflected a substantial modification of the priors (indicating their identifiability). As a secondary validation that the partitioning of the dataset was justified, we compared posterior distributions of parameter estimates across partitions (by inspecting posterior distributions using Tracer, and by comparing 95% credibility intervals of parameters) to confirm that, in fact, different partitions demonstrated unique posterior distributions of parameter estimates.

3. Results

3.1. Dataset characteristics and individual gene phylogenies

The concatenated alignment of 1405 characters contained 538 parsimony-informative characters and 713 constant characters. Nucleotide frequencies were similar between the two loci used, and the nucleotide frequencies of the combined dataset were G = 11.57%, A = 29.79%, T = 26.46%, and C = 32.18%. Individual gene phylogenetic reconstructions showed extremely similar, yet poorly resolved, phylogenetic estimates. Based on the apparent congruence in phylogenetic signal between the two gene fragments, we proceeded with combined data analyses.

The greatest pairwise sequence divergence among terminal taxa was between *Bothrops ammodytoides* and *Porthidium yucatanicum* (uncorrected divergence of 17.4%). Within ingroup genera, the highest sequence divergence within *Atropoides* was 11.6% (between "*A. picadoi* Costa Rica2" and "*A. mexicanus* Guatemala4"), within *Cerrophidion* was 9.4% (between "*C. tzotzilorum* Mexico2" and "*C. godmani* Costa Rica1"), and within *Porthidium* was 13.7% (between "*P. dunni* Mexico2" and *P. lansbergii* Panama").

3.2. Maximum parsimony phylogenetic analysis

The MP heuristic search on the combined dataset found 144 equally parsimonious trees of 2587 steps.

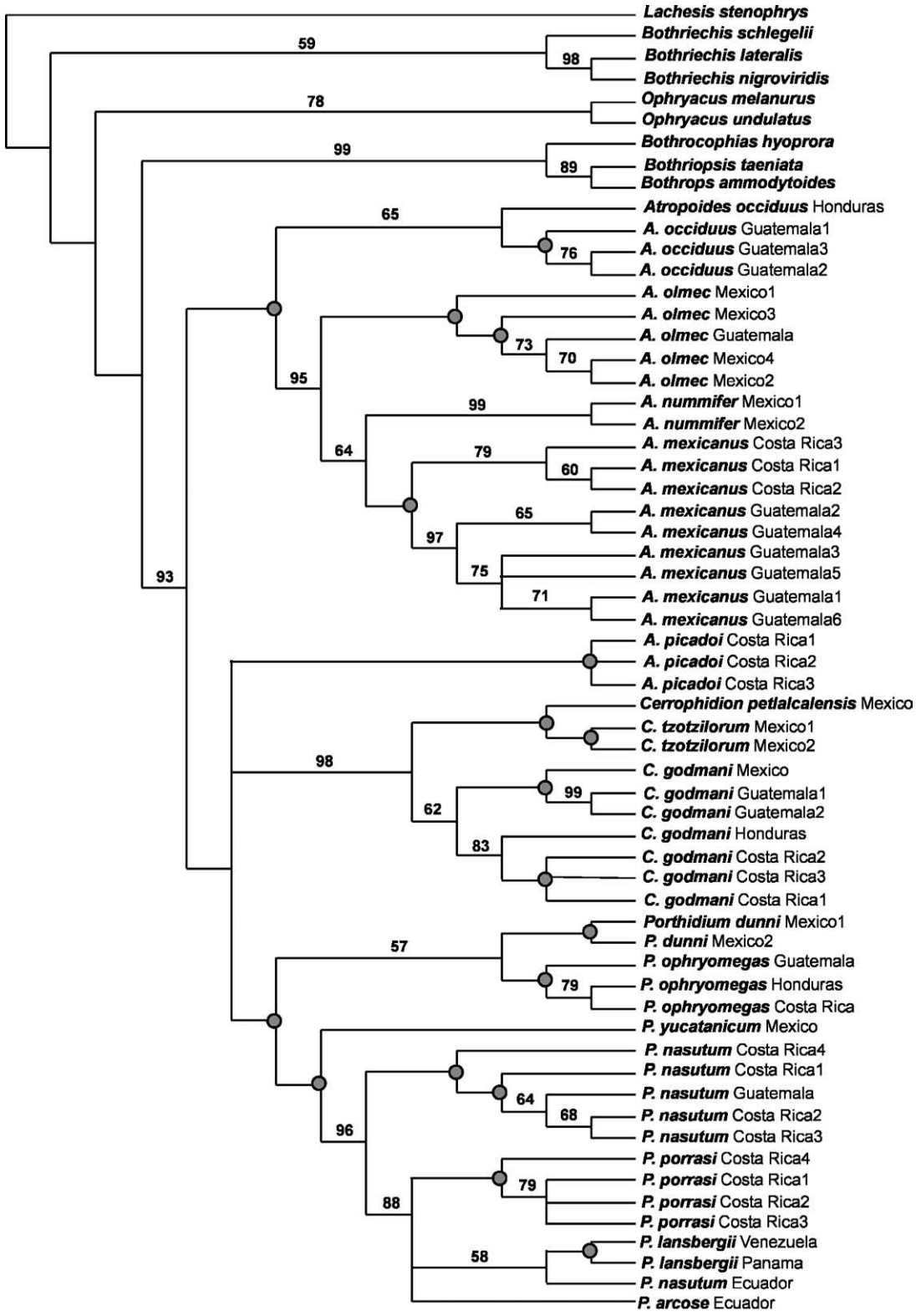

Fig. 1. Majority-rule consensus of 144 equally parsimonious trees (of 2587 steps) from heuristic maximum parsimony search based on 1405 bp. Bootstrap support for nodes is provided (values below 50% not shown). Bootstrap values of 100% are indicated with gray-filled circles.

A substantial degree of character-state homoplasy was inferred across these trees based on the homoplasy index (HI = 0.6308) and rescaled consistency index (RCI = 0.2690). The 50% majority-rule consensus of these 144 MP trees, along with bootstrap support for nodes, is shown in Fig. 1.

The MP phylogenetic reconstruction did not infer a monophyletic *Atropoides*, placing *A. picadoi* in an unresolved clade with *Cerrophidion* and *Porthidium*. *Atropoides* minus *A. picadoi*, referred to as the *nummifer* complex (Castoe et al., 2003), was resolved as monophyletic with 100% bootstrap support (BS). All *Atropoides* and *Cerrophidion* species were estimated to be monophyletic, as were all species of *Porthidium* except *P. nasutum*. Samples of Central American *P. nasutum* formed a well-supported clade (BS = 100%) distantly related to South American (Ecuadorian) *P. nasutum*. The *P. nasutum* sample from Ecuador appears to be more closely related to South American and southern Central American *P. lansbergi*. A majority of MP phylogenetic results overlap broadly with those from MCMC analyses. For this reason, and our expectation that MCMC results should produce more accurate estimates of phylogeny, we limit our discussion to these results.

3.3. Bayesian MCMC model selection and evaluation

Both AIC and hLTR model selection criteria supported the GTR + Γ + I model as the best fit for the combined dataset (Table 2). The TVM + Γ + I (under hLTR criteria) and the TrN + Γ + I (under AIC criteria) models were selected as best fitting the individual gene datasets. These models are restrictions of the GTR + Γ + I model that are not available in MrBayes 3.0; instead we used a GTR + Γ + I model as our base model for the analysis of both individual and combined data.

In addition to the GTR + Γ + I model, we analyzed the combined dataset under five additional more complex models that employed multiple GTR + Γ + I models assigned to specific partitions of the dataset (see Table 3). In MrBayes 3.0, available choices for modeling time-reversible nucleotide substitution include three possible substitution matrices including 1, 2, or 6 parameters. ModelTest results for all putative partitions indicated, in general, that there was evidence for the justification of nucleotide models including substitution matrices with greater than two parameters, as well as the parameters Γ and I (Table 2). Based on these results, we allocated independent GTR + Γ + I models, per partition, in our partitioned MCMC analyses.

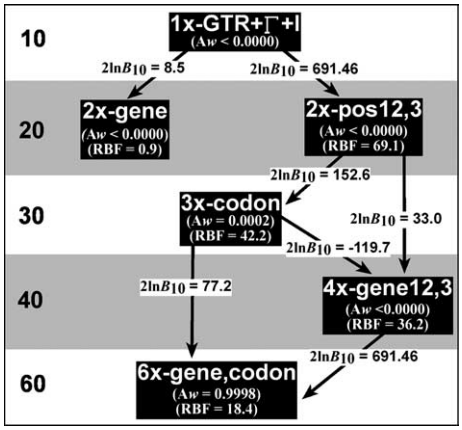

Fig. 2. Flow chart illustrating the process of model selection among complex models tested for the analysis of the combined dataset. Statistics for models are given (Aw = Akaike weights, $2\ln B_{10} = 2\ln$ Bayes factor, RBF = relative Bayes factor). For $2\ln B_{10}$ comparisons between models, M_1 is represented by the model indicated by the arrowhead. See Table 3 for definitions of models.

The evaluation of model fit for the complex models is visually depicted in Fig. 2. In comparing Bayes factors ($2\ln B_{10}$) between models, simple models were rejected in favor of more complex models that allowed parameters to be independently allocated to partitions of the dataset (Fig. 2). Ultimately, the most complex model tested, 6x-gene,codon, was supported as the best-fit model by $2\ln B_{10}$ estimates. Similarly, Akaike weights (Aw) placed nearly all relative weight (Aw = 0.9998) under the same 6x-gene,codon model as best fitting the data. Relative Bayes factors (RBF) demonstrate that, as model complexity and the number of free parameters increased, the relative improvements in model likelihood (per parameter added) decreased (Fig. 2). In summary, the RBF values suggest diminishing returns (in terms of likelihood) as more parameters were added to the model.

The best-fit complex model (6x-gene,codon) showed no evidence of parametric over-fitting based on analysis of convergence and mixing. All independent MCMC runs of this model converged on nearly identical

Table 3
Description of complex partitioned models used in the analysis of the combined dataset

Model	No. of partitions	No. of free model parameters	Description
1x-GTR + Γ + I	1	10	Base model employing a single GTR + Γ + I model for the combined data
2x-gene	2	20	Independent GTR + Γ + I models for each of the two gene fragments
2x-pos12,3	2	20	One GTR + Γ + I model for codon positions 1 and 2, and a second GTR + Γ + I for position 3
3x-codon	3	30	One GTR + Γ + I model per codon position
4x-gene12,3	4	40	Each of the two gene fragments is allocated a set of two GTR + Γ + I models, one for codon positions 1 and 2, a second for position 3
6x-gene,codon	6	60	Each codon position of each of the two gene fragments is allocated an independent GTR + Γ + I model

parameter and phylogenetic estimates. Model likelihoods and parameter estimates of all runs demonstrated effective mixing with burn-in characterized by a direct rapid ascent to a stationary plateau (for model likelihood and parameters). Across all independent runs of the 6x-gene,codon model, likelihood values reached apparent stationarity (burned-in) prior to 1.5×10^6 generations, and parameter estimates reached apparent stationary by 2.0×10^6 generations. These observations confirm that our conservative a priori choice of burn-in period at 1×10^7 effectively excluded non-stationary estimates.

Across partitions of the 6x-gene,codon model, base frequency, Γ, and I parameter estimates demonstrated posterior distributions with relatively low variance. In support of partitioning, these parameter-estimate distributions showed relatively little overlap between partitions (based on comparisons of the parameter distributions in Tracer and 95% confidence intervals; Table 4) and supported the distinctiveness of each partition. Posterior distributions of parameter estimates from the nucleotide substitution-rate matrix (i.e., GTR matrix parameters) of each partition showed higher degrees of overlap across partitions and greater variance compared with base frequencies, Γ, and I parameters (Table 4). While increasing parameter variance is expected when models are partitioned (because less data are available for estimation of each parameter), it was initially unclear if this increased variance may indicate that fitting each partition with a GTR substitution matrix over-fits the combined model. To test this, we conducted a second set of partitioned runs in which we conducted MCMC analyses under an array of partitioned models where the substitution matrices were hierarchically re-linked (thereby reducing the number of free substitution matrix parameters overall). When we examined model fitting using Aw and $2\ln B_{10}$, we found that all tested restrictions of the 6x-gene,codon model were never favored by either statistic as being a better fit to the data than the 6x-gene,codon model (data not shown). Collectively, our post hoc analyses of the 6x-gene,codon model support this model as the superior best-fit model examined for our data. Hereafter, we consider the 6x-gene,codon model as our preferred model, and results based on analyses under this model as our preferred phylogenetic hypothesis.

3.4. Effects of model choice on Bayesian phylogenetic hypotheses

We present the majority-rule consensus topology of both the chosen model (6x-gene,codon) and the unpartitioned (1x-GTR + Γ + I) model (Fig. 3) in order to compare the practical effects of model choice. No overall trend of increasing or decreasing posterior probability values for clades (*Pp* hereafter) is evident between the trees. Also, no relationships that were supported by 100% *Pp* changed more than a single percent across the two models. Instead, the majority of differences between consensus topologies and *Pp* support represented changes at weakly supported nodes ($Pp < 90\%$) that result in a change in the majority-rule consensus topology. The *Pp* support for basal relationships between *Porthidium* group genera becomes substantially stronger in the complex model (from $Pp = 64$ and 68 to $Pp = 81$ and 84, respectively). Other deep nodes, including the resolution of relationships among outgroup taxa, showed substantial changes across the two models (Fig. 3). Also, the two models produce different consensus topologies affecting the resolution of members of *Atropoides* as well as *Porthidium* (although both relationships are weakly supported under either scenario).

3.5. Bayesian MCMC phylogenetic results under the best-fit model

The phylogenetic estimates for the *Porthidium* group derived from the MCMC analyses under the 6x-gene,codon model strongly support monophyly of the group ($Pp = 100\%$) and also inferred a clade comprising

Table 4
Mean and 95% credibility interval for each parameter sampled from the combined posterior distribution of three independent MCMC runs of the 6x-gene,codon model

	P1—(ND4,pos1)	P2—(ND4,pos2)	P3—(ND4,pos3)	P4—(cyt-*b*,pos1)	P5—(cyt-*b*,pos2)	P6—(cyt-*b*,pos3)
r(G–T)	1	1	1	1	1	1
r(C–T)	32.32 (9.69–82.3)	33.36 (5.04–84.36)	17.46 (5.1–43.57)	16.7 (3.81–49.34)	2.57 (1.18–5.08)	13.39 (3.37–28.14)
r(C–G)	0.32 (0.02–1.18)	24.19 (3.11–67.99)	0.17 (0.01–0.94)	1.10 (0.14–4.02)	0.33 (0.01–1.22)	4.32 (0.92–9.79)
r(A-T)	3 (0.78–8.17)	4.64 (0.35–16.35)	1.47 (0.34–3.78)	2.05 (0.39–6.45)	0.25 (0.04–0.69)	1.41 (0.28–3.14)
r(A-G)	7.51 (2.13–20.28)	44.37 (7.06–94.96)	33.40 (10.18–82.42)	17.10 (4.37–48.85)	83.59 (53.96–99.42)	52.14 (13.85–97.19)
r(A-C)	0.78 (0.15–2.32)	6.88 (0.49–23.94)	0.92 (0.24–2.27)	1.71 (0.31–5.35)	0.32 (0.05–0.88)	0.50 (0.1–1.16)
pi(A)	0.361 (0.308–0.414)	0.161 (0.118–0.208)	0.408 (0.362–0.453)	0.338 (0.281–0.399)	0.240 (0.19–0.295)	0.313 (0.269–0.358)
pi(C)	0.306 (0.256–0.359)	0.32 (0.266–0.377)	0.367 (0.326–0.409)	0.254 (0.207–0.305)	0.257 (0.208–0.309)	0.469 (0.429–0.51)
pi(G)	0.178 (0.14–0.219)	0.128 (0.089–0.172)	0.065 (0.053–0.079)	0.158 (0.11–0.205)	0.104 (0.069–0.144)	0.036 (0.028–0.044)
pi(T)	0.155 (0.122–0.194)	0.392 (0.334–0.452)	0.16 (0.137–0.185)	0.249 (0.202–0.3)	0.399 (0.342–0.456)	0.182 (0.16–0.207)
Γ	0.218 (0.181–0.266)	0.098 (0.085–0.113)	3.836 (2.35–6.333)	0.306 (0.232–0.408)	0.264 (0.161–0.471)	4.958 (2.786–9.137)
I	0.170 (0.06–0.277)	0.599 (0.481–0.705)	0.054 (0.01–0.104)	0.400 (0.276–0.506)	0.549 (0.4–0.681)	0.039 (0.009–0.08)

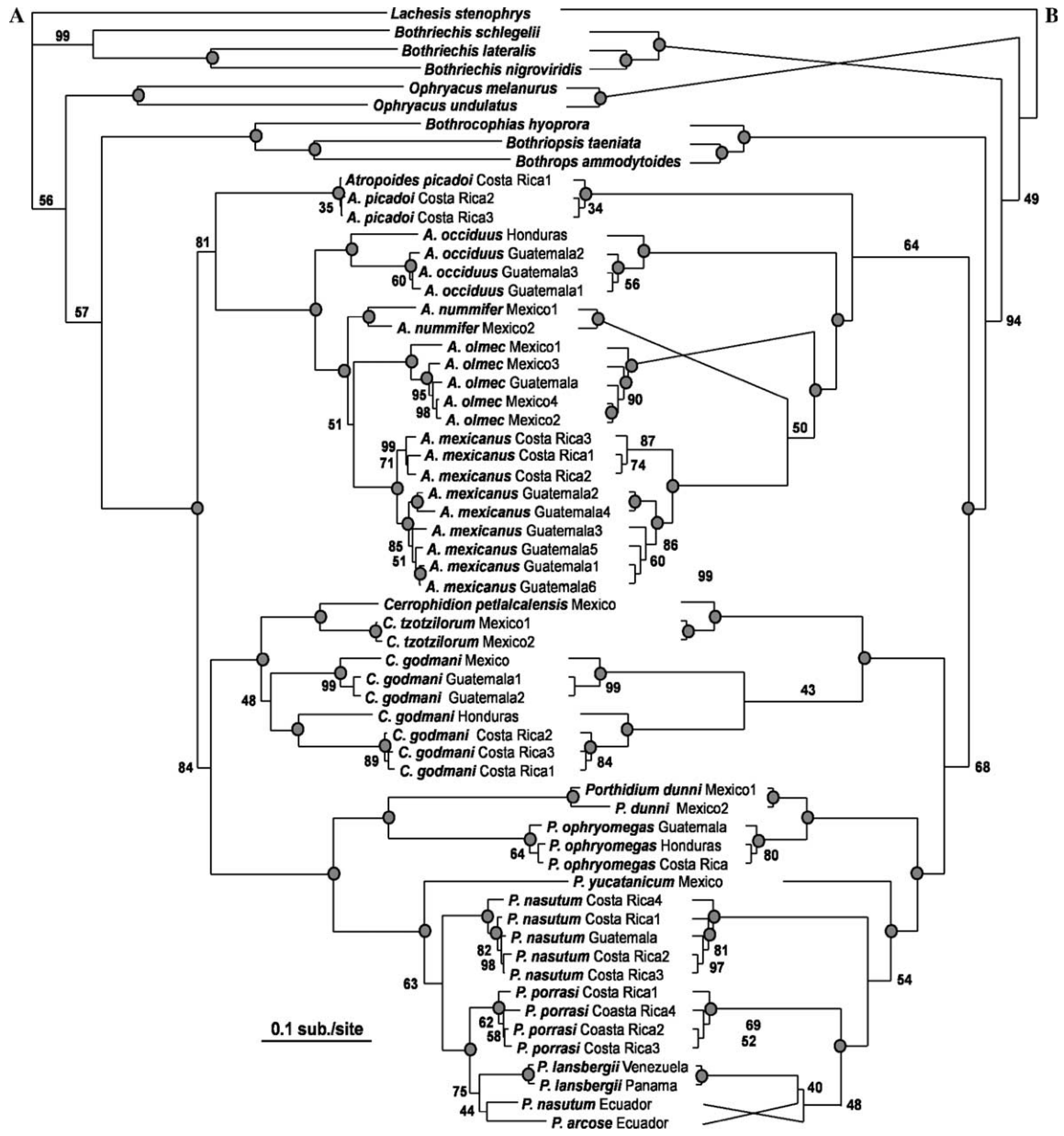

Fig. 3. Majority-rule consensus trees resulting from Bayesian MCMC phylogenetic reconstructions under two different models of nucleotide evolution (the favored partitioned model "6x-gene,codon" and the base-unpartitioned 1x-GTR + Γ + I). Nodal posterior probabilities are indicated; nodal posterior probabilities of 100% are indicated with a gray-filled circle. (A) Majority-rule consensus phylogram based on a combined 9×10^7 post-burn-in Bayesian MCMC generations of the favored "6x-gene,codon" partitioned model. (B) Majority-rule consensus cladogram based on a combined 9×10^6 post-burn-in Bayesian MCMC generations of the unpartitioned 1x-GTR + Γ + I model (note: branch lengths are not informative in B).

the primarily South American bothropoid lineages (genera *Bothrops*, *Bothriopsis*, and *Bothrocophias*). Monophyly is well supported for each of the genera *Cerrophidion* and *Porthidium* ($Pp = 100$), which are grouped ($Pp = 84$) as the sister taxon to a monophyletic *Atropoides* ($Pp = 81$). Within *Atropoides*, *A. picadoi* was inferred as the sister taxon to the remaining species ($Pp = 81\%$), which collectively form the *nummifer* complex. This group of *Atropoides* species was strongly supported as monophyletic, with a clade containing *A. mexicanus* and *A. olmec* ($Pp = 51$) forming the sister taxon to *A. nummifer*, and *A. occiduus* being the sister lineage to the remaining *nummifer* complex species ($Pp = 100$). Within *A. occiduus*, we found Honduran and Guatemalan populations to be well differentiated (~5.7%) compared to more shallow intra-specific divergences among populations of other *Atropoides* species.

Monophyly of the genus *Cerrophidion* received strong support ($Pp = 100$). The widespread species *C. godmani* was inferred with very weak support as monophyletic ($Pp = 48$), although a clade containing Honduran and Costa Rican populations received strong support ($Pp = 100$). Within this genus, we found evidence for an early phylogenetic split between a clade containing the

two species restricted to Mexico (*C. tzotzilorum* and *C. petlalcalensis*) and *C. godmani*. Our sampling of *C. godmani* populations throughout Middle America highlights several cladogenetic divisions within this species (among northern, central, and southern Middle American populations; divergences among the three lineages all >7%) that are deeper than those observed between the two other *Cerrophidion* species (<6%).

The first phylogenetic split within *Porthidium* separates a branch comprising *P. dunni* and *P. ophryomegas* ($Pp = 100$) from a branch comprising the remaining species ($Pp = 100$). All *Porthidium* species were resolved as monophyletic except *P. nasutum*. South American *P. nasutum* formed a weakly supported clade with *P. arcosae* ($Pp = 44$), the sister taxon to *P. lansbergii* ($Pp = 75$). This group of three South American lineages formed a clade with *P. porrasi* ($Pp = 100$). Central American populations of *P. nasutum* were found to represent a monophyletic group ($Pp = 100$) inferred to be the sister lineage ($Pp = 63$) to a clade comprising *P. porrasi* and the South American species.

4. Discussion

4.1. Model partitioning in Bayesian MCMC analyses

Our results support three important conclusions relevant to the use of complex partition-specific models in combined MCMC analyses. 1) Model choice may have important practical effects on phylogenetic conclusions even for mesoscale datasets such as the one used here. 2) The use of a complex partitioned model did not produce widespread increases or decreases in *Pp* nodal support. 3) A majority of differences in resolution resulting from model choice was concentrated at deeper nodes. Also, a majority of these deeper nodes increased substantially in resolution (as measured by nodal *Pp*) with increasing model complexity.

Several studies have supported a direct relationship between accuracy of posterior probabilities and model complexity. In these studies, Bayesian analyses conducted with underparameterized models appear to experience elevated error rates, compared with parameter-rich models (Erixon et al., 2003; Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004; Suzuki et al., 2002). Also, simpler models have been shown to exhibit signs of poor mixing when compared to more complex partitioned models, based on the variance in *Pp* estimates through MCMC generations (Castoe et al., 2004). In addition to the overall accuracy of results, this study (and Brandley et al., 2005) found that complex partitioned models may have important effects in the resolution of deeper nodes, a majority of which receive increased support under complex models. These results suggest that more complex models may be more effective in estimating the patterns of molecular evolution when sequences are more divergent and phylogenetic signal is otherwise obscured by multiple substitutions or by homoplasy (see also discussion below). While not a panacea for resolving deep nodes, complex models that account for natural heterogeneity of molecular evolution within combined datasets appear to extract more phylogenetic signal than would a non-partitioned "compromise" model (see also Brandley et al., 2005; and analogous studies: Pupko et al., 2002; Voelker and Edwards, 1998; Yang, 1996).

Despite considerations favoring complex models, benefits of constructing and implementing more realistic evolutionary models of DNA substitution are challenged by the potential for imprecise and inaccurate model parameter and phylogeny estimation that may result from excess model complexity. Expanding computational power, increasing genomic resources, and advances allowing broad flexibility in modeling evolutionary patterns in a Bayesian MCMC context collectively underscore the importance of developing accurate models and objective strategies for model testing.

As the implementation of complex models becomes more widespread in molecular phylogenetics, it may be useful to identify how reliant phylogenetic conclusions are on model specification. Reporting such details would provide an assessment of how much phylogenetic signal seems readily extracted from the data compared to that extracted through the implementation of more complex models (which may or may not ultimately contribute to the accuracy of phylogenetic results). In part, this is analogous to the common practice of providing results based on MP and likelihood-based phylogenetic methods. Also, advances with incorporating model averaging in phylogenetics (including reverse-jump Bayesian MCMC methods: Green, 1995; Huelsenbeck et al., 2004; Suchard et al., 2001) represent an attractive alternative to the common reliance on a single model for phylogeny estimation (see also Posada, 2003; Posada and Buckley, 2004).

4.2. Suggestions and prospects for complex Bayesian MCMC modeling and model testing

In accordance with previous empirical studies (e.g., Brandley et al., 2005; Castoe et al., 2004; Pupko et al., 2002), our results support the hypothesis that more complex models of evolution may have practical effects on phylogenetic inference. Furthermore, such models may more accurately portray heterogeneous patterns of evolution within a dataset, facilitating the extraction of more phylogenetic signal (i.e., at deep nodes) compared with simpler or non-partitioned models. Support for the use of complex models has also been reiterated by simulation studies. With simulated data, Bayesian phylogenetic analyses conducted with oversimplified models

suffer from inaccurate bipartition posterior probability estimates, whereas overly complex models do not appear to experience the same magnitude of inaccuracy (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004). The potential utility of complex models, however, is balanced by potentially inaccurate or unreliable results that may be obtained from employing overly complex models. Resolving these opposing points requires robust and objective strategies for testing and evaluating such models.

In this study, we exploited a three-part strategy for identifying, testing, and evaluating candidate complex models in a Bayesian MCMC context. We used standard methods implemented in ModelTest to examine potential models for biologically intuitive potential partitions of the dataset (as in Brandley et al., 2005), three statistics (Aw, $2\ln B_{10}$, and RBF) to examine model fit across partitioned Bayesian MCMC models, and post-hoc evaluation of model performance to check for proper mixing and convergence (including model parameter identifiability). We believe that these three steps represent a thorough strategy for the identification of best-fit models for partitioned Bayesian MCMC analyses that satisfy concerns (positive and negative) associated with employing complex models.

Several authors (Brandley et al., 2005; Nylander et al., 2004) have argued the efficacy of $2\ln B_{10}$ in Bayesian phylogenetic model selection. Here, we find the results of Aw to support the same conclusions (picking the same model) as $2\ln B_{10}$, which is not entirely surprising given the suggestions that AIC and Bayes factors are asymptotically equivalent (Akaike, 1983; see also Huelsenbeck et al., 2004). Using of the harmonic mean estimate of margin model likelihood, both methods attractively incorporate parameter uncertainty into model choice (rather than maximum likelihood point estimates of model parameters and phylogeny). In terms of convenience, $2\ln B_{10}$ allows ready comparisons between two models, while Aw provides a useful perspective on model choice simultaneously over all models. Although the results of these two criteria were similar, they provide unique information and approaches to model selection (with different assumptions), and thus represent a desirable confirmatory approach to model selection when used together.

Although many interpretations exist, Bayes factors may be interpreted as the posterior evidence provided by the data for one model versus another being true (under uniform model priors) or as a comparison of the predictive likelihoods of the models (Gelfand and Dey, 1994; Kass and Raftery, 1995; Wasserman, 2000). Alternatively, Lavine and Schervish (1999) suggested that Bayes factors should be interpreted as measuring "the change in evidence in the odds in favor of the hypothesis when going from the prior to the posterior," thus placing emphasis on the data modifying the priors as playing a primary role in determining the Bayes factor (see also Huelsenbeck et al., 2004; Wasserman, 2000). Unlike Bayes factors, AIC does not imply that the true model is contained in the set of candidate models (although the importance of this assumption for Bayes factors has been debated: e.g., Kass and Raftery, 1995; Posada and Buckley, 2004). Instead, AIC attempts to identify which model is most likely to be closest to the true model, or has the highest predictive accuracy, based on the Kullback–Liebler distance (Akaike, 1973; Forster, 2002; Sober, 2002). In comparing methods, some have suggested that Bayes factors may tend to favor simpler models than AIC (e.g., Bartlett, 1957; Kass and Raftery, 1995; Lindley, 1957; Shibata, 1976). The AIC may also be less biased by specification of priors (e.g., prior variance) whereas Bayes factors may become inaccurate if priors are too vague (diffuse and uninformative; Raftery and Zheng, 2003; see also Findley, 1991). However, AIC may only perform well when the dataset is large and when only 'good' models are compared (Burnham and Anderson, 1998). Neither method is clearly superior, but both have strengths, weaknesses, and potential biases. If methods agree, one can be more confident that biases or weaknesses of any one method have not misled model choice. If methods were to disagree regarding model choice, an investigator should weigh carefully the potential biases of each method in order to identify a preferred model; alternatively, one could evaluate multiple models and select the most complex that appears to not suffer from identifiability, mixing, and convergence problems (e.g., Huelsenbeck and Rannala, 2004).

In addition to Bayes factors and Aw, we also employed RBF (a rescaling of the Bayes factor) as a simple way to quantify the relative contribution of each added free parameter towards increasing overall model likelihood (starting from the base-unpartitioned model). As such, RBFs represent a simple post-hoc means of comparing the relative explanatory power of the added free parameters simultaneously across models. In general, as the number of free model parameters increase, we expect the RBF to decrease as the data to parameter ratio decrease. Thus, RBF values should generally decrease asymptotically with increasing model complexity. The rate of RBF decline should also be proportional to the size and heterogeneity of a dataset (assuming models are effectively portraying data heterogeneity).

These properties of the RBF make it a useful indicator that may help in deciding if model complexity is approaching the maximum justifiable complexity, or if the array of models tested still fall well below the maximum model complexity that may be warranted (e.g., through AIC or Bayes factor model choice). If RBF values steadily decrease with model complexity, an investigator may be more convinced that they are approaching the higher end of model complexity justifiable by the

data, as observed in this study. Contrastingly, if RBF values remain relatively constant across increasingly complex models, one may assume that the proportion of data to model parameters is high, which may suggest that even more complex models should be explored if possible. This later pattern has been observed with large and more heterogeneous datasets (Castoe and Parkinson, unpublished manuscript).

4.3. Relationships and taxonomy of the Porthidium group

The inter-generic relationships among pitvipers have been investigated by numerous authors using either morphological or molecular data (recently reviewed by Gutberlet and Harvey, 2004). Despite this intensive systematic effort, a cohesive and robust hypothesis of relationships among genera has yet to be achieved. Many studies have supported a sister group relationship between the *Porthidium* group and South American bothropoid genera (*Bothrops*, *Bothriopsis*, *Bothrocophias*; e.g., Gutberlet and Harvey, 2002; Kraus et al., 1996; Parkinson, 1999; Parkinson et al., 2002). This relationship was supported in all our analyses, including MP and MCMC. As in previous molecular phylogenetic studies, we found strong support for the monophyly of the *Porthidium* group; this contrasts with previous studies based on morphology or morphology plus allozymes (Gutberlet and Harvey, 2002; Werman, 1992). Also, in accordance with previous studies (Gutberlet and Harvey, 2002; Parkinson, 1999; Parkinson et al., 2002), we found strong phylogenetic evidence supporting the previous removal of *Ophryacus melanurus* and *Bothrocophias hyoproras* from the *Porthidium* group (Gutberlet, 1998; Gutberlet and Campbell, 2001).

Resolution of the basal relationships between the three genera of the *Porthidium* group appears to be a difficult phylogenetic problem to solve with either morphological or molecular data, as can be seen in our MP analyses (Fig. 2). Several molecular phylogenetic studies have either failed to resolve the relationships altogether or failed to resolve them with any substantial support (e.g., Castoe et al., 2003; Parkinson, 1999; Parkinson et al., 2002). In all cases, molecular phylogenies have inferred very short internodes connecting the three genera, implying a rapid radiation from a common ancestor and a difficult phylogenetic problem to solve. Parkinson et al. (2002) found weak support (BS = 68) for a clade containing *Cerrophidion* and *Atropoides*, as the sister taxon to *Porthidium*. Here, our partitioned MCMC analyses instead group *Cerrophidion* and *Porthidium* as a clade ($Pp = 84$) that is the sister lineage to *Atropoides*. It is important to note that resolution of these relationships appeared particularly dependent on MCMC model choice, with increasingly complex models recovering higher Pp for these relationships (Fig. 3). These different results across MCMC models would be reconciled under the hypothesis that complex models are, in fact, doing a better job extracting phylogenetic signal from the dataset which clearly does contain substantial homoplasy.

Despite the fact that species of *Atropoides* constitute a distinctive group of morphologically similar snakes, monophyly of this genus has not been well resolved based on molecular studies (Castoe et al., 2003; Parkinson, 1999; Parkinson et al., 2002). Our MP results also fail to resolve the question of monophyly. Similar to the resolution of the *Porthidium* group, our MCMC analyses under the 6x-gene,codon model resolved monophyly of *Atropoides* with $Pp = 81$, compared to $Pp = 64$ in unpartitioned MCMC analyses.

Within the genus *Atropoides*, slight changes in the posterior distribution of trees under different MCMC models produced different majority-rule consensus trees of relationships among *Atropoides* species (in which *A. olmec* and *A. nummifer* exchanged positions). It is interesting to note that *A. olmec* and *A. mexicanus* share a presumed derived morphological feature (in having two or more subfoveal rows; Campbell and Lamar, 2004). Across all MP and MCMC analyses, *A. olmec* appears as the sister taxon to *A. mexicanus* only in the complex MCMC analysis (albeit with $Pp = 51$). These two species were resolved as the sister lineage to *A. nummifer*. These three species also all have nasorostral scales not present in the remaining species of *Atropoides*. Previous molecular and morphological studies have supported *A. picadoi* as the sister lineage to all other *Atropoides*, and *A. occiduus* as the sister taxon to the remaining '*nummifer* complex' species (Campbell and Lamar, 2004; Castoe et al., 2003; Parkinson et al., 2002). We also find strong evidence for these relationships based on MP (in part) and MCMC analyses.

Although not extensive, our intra-specific sampling within *Atropoides* illuminates several interesting patterns of phylogeography and undescribed taxonomic diversity. Castoe et al. (2003) demonstrated that the range of *A. olmec* included three closely related disjunct populations in Veracruz and Oaxaca, Mexico, and Baja Verapaz, Guatemala. They concluded that in recent evolutionary time, the range of *A. olmec* may have been more continuous between these three known populations. Additional samples in this study include newly discovered populations in Chiapas, Mexico, that further support the historical existence of a dispersal corridor spanning the Mexican Isthmus of Tehuantepec that facilitated relatively recent gene flow among these populations. *Atropoides mexicanus* is the widest-ranging species in the genus and spans a majority of Middle America, although the occurrence of this species has not been confirmed throughout a large portion of Central America (in parts of Honduras and Nicaragua; Campbell and Lamar, 2004). We found evidence for phylogenetic structure within *A. mexicanus* whereby populations in northern Middle America form a clade,

as do populations from Costa Rica. Shallow divergences between these clades indicate that gene flow across the large range of *A. mexicanus* has been prevalent at least within recent evolutionary time. These data support assertions that the ' *nummifer* complex' diversified in northern Middle America, and *A. mexicanus* later expanded its range southward (Castoe et al., 2003; Werman, 2005). Within *A. occiduus*, we found a Honduran sample to be substantially diverged from other Guatemalan populations. This and associated Honduran populations of *A. occiduus* may be candidates for species recognition if additional data support this distinction.

The genus *Cerrophidion* comprises four species, three of which occupy small isolated rages in Mexico. The two of these three range-restricted species sampled in this study, *C. tzotzilorum* and *C. petlalcalensis*, were recovered as a well-supported clade forming the sister lineage to the wide-ranging *C. godmani*. Although not sampled, the fourth *Cerrophidion* species, *C. barbouri*, shares several presumably derived characters (low numbers of teeth and low numbers of middorsal scale rows) with *C. petlacalensis*, suggesting these taxa may be sister species (Gutberlet and Harvey, 2004; although see Campbell, 1988).

The range of *C. godmani* extends from southern Mexico to northern Panama, although populations are patchily distributed across disjunct highland masses. Our results support for the existence of multiple divergent lineages within *C. godmani* that correspond to disjunct groups of populations. We found strong support for three *C. godmani* lineages including: (1) populations in Mexico and Guatemala (BS = 100, *Pp* = 100); (2) populations in Honduras; (3) populations in Costa Rica (supported with BS = 83 and *Pp* = 100 as the sister lineage to Honduran *C. godmani*). These three lineages appear associated with three discrete geographic and geologic montane complexes that have been recognized as distinct biogeographic units in a number of studies (e.g., Campbell, 1999; Savage, 1966, 1982; Stuart, 1966). Based on molecular evidence presented here, and on the allopatric distributions of these three lineages, additional work has been initiated to investigate the potential taxonomic recognition of these lineages of *C. godmani*.

Our results suggest a basal split within *Porthidium* between a clade including *P. dunni* and *P. ophryomegas* (both of which are restricted exclusively to tropical and subtropical dry habitats), and a clade comprising the remaining species, hereafter called the "*nasutum* group" (similar to Castoe et al., 2003; Parkinson, 1999; Parkinson et al., 2002). This basal split within *Porthidium* species is also supported by differences between clades in a dorsal-scale microstructural pattern (Estol, 1981; although not all *Porthidium* species were examined). The unsampled species *P. hespere* (of southwestern Mexico), like *P. ophryomegas* and *P. dunni*, is restricted to tropical dry forests and occurs geographically closest to *P. dunni*. While these facts suggest that *P. hespere* may be a member of the *P. ophryomegas*/*P. dunni* clade (see also Werman, 2005), no specific phylogenetic evidence is currently available to test this hypothesis. Within the widespread species *P. ophryomegas*, we observed shallow genetic structure across geographically distant populations, suggesting recent evolutionary genetic continuity across populations (Fig. 3, as inferred by Werman, 2005).

Porthidium yucatanicum has been hypothesized as being the sister taxon to all *Porthidium* species based on morphological data (Gutberlet and Harvey, 2002). We found strong support for this species to instead be the sister taxon to the remaining *nasutum* group species. This implies that early vicariance within the *nasutum*-group may have been centered in northern Middle America, which is not intuitive based on the lower Middle American and South American distribution of a majority of *nasutum* group taxa. We resolved *P. porrasi* as the sister lineage to this clade of South American lineages (*P. lansbergii*, *P. arcoase*, and Ecuadorian "*P. nasutum*"). *Porthidium porrasi* is restricted to the Osa Peninsula of southwestern Costa Rica (and immediately adjacent mainland), and was considered *P. nasutum* until recently (Lamar and Sasa, 2003). The close phylogenetic relationship of *P. porrasi* and South American *Porthidium* (rather than Central American lineages) seems to support a historical pattern of reticulating dispersal into and out of South America (see also Wüster et al., 2002).

We found strong evidence for paraphyly of *P. nasutum*, as reported by Wüster et al. (2002; see also Gutberlet and Harvey, 2004). Sampled populations of *P. nasutum* from Central America formed an evolutionarily shallow clade, distantly related to South American (Ecuadorian) "*P. nasutum*." These results suggest that some taxonomic action may be required to rectify the phylogenetic relationships of South American "*P. nasutum*," although the affinities of other populations allocated to *P. lansbergii* require further attention. We found Ecuadoran "*P. nasutum*" closely related to *P. lansbergii* and *P. arcosae* (both of which are geographically proximal and morphologically similar to South American populations of "*P. nasutum*"). Thus, decisive taxonomic treatment of *P. nasutum* may require a larger-scale reevaluation of the taxonomic status of *P. lansbergii* and *P. arcosae* (formerly considered a subspecies of *P. lansbergii*; Campbell and Lamar, 2004). The unsampled species *P. volcanicum* (restricted to southwestern Costa Rica) has been suggested as a close relative of *P. lansbergii* by Solórzano (1995), which implies the potential for additional complications in clarifying the phylogeny and taxonomy of species related to *P. lansbergii*. *Porthidium* has historically been plagued with difficulties regarding taxonomic stability and correct species identification (reviewed by Campbell and Lamar, 2004). The taxonomic problems discussed here and the likelihood of additional cryptic diversity among South American *Porthidium* populations (Campbell and Lamar, 2004) highlight future taxonomic activity for the genus.

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