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A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*)☆

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Abstract

We present a phylogeny of the Asian pitvipers, based on 2403 bp of four mitochondrial gene regions. All but six known species of *Trimeresurus sensu stricto* (*s.s.*) as currently defined, as well as multiple populations of widespread species, which may yet be described as full species, and representatives of all other Asian pitviper genera, are included. Both the greater sampling and larger dataset provide improved resolution over previous studies and support the existence of distinct species groups within *Trimeresurus s.s.* Although all but two species currently referred to this genus form a monophyletic group, morphological and molecular analyses identify four subgroups that warrant recognition at the generic level. We propose a new generic arrangement to reflect these findings. We also highlight the non-monophyly of *Ovophis*, and propose a new genus to accommodate a species formerly assigned to *Ovophis*.

Keywords: Pitviper; *Trimeresurus*; Bayesian analysis; Phylogenetic; Taxonomy

1. Introduction

Considerable progress has been made in recent years on resolving the systematics of the Asian pitvipers (Reptilia: Serpentes: Viperidae: Crotalinae) of the *Trimeresurus* complex (Kraus et al., 1996; Malhotra and Thorpe, 2000; Parkinson, 1999; Parkinson et al., 2002; Tu et al., 2000). Originally all considered congeneric and placed in *Trimeresurus sensu lato* (*s.l.*), six genera [*Trimeresurus sensu stricto* (*s.s.*), *Ovophis*, *Protobothrops*, *Tropidolaemus* (*Tro.*), *Triceratolepidophis* (*Tric.*), and *Ermia*] are now generally recognised. The successive removal of species to these genera has left *Trimeresurus s.s.* as a large and diverse assemblage of c. 34 known species, which all molecular studies have indicated largely constitute a monophyletic group (see references above). However, some species currently assigned to *Trimeresurus* may belong in other genera. Consequently, this study is presented in the framework of a larger study on the relationships of all Asian vipers.

Another feature of the group, which has confounded systematic resolution in the past, is their remarkable morphological conservativeness, particularly well typified in the green pitvipers, or bamboo vipers. Initially considered a single species, *Trimeresurus gramineus*, Stejneger (1927) and Pope and Pope (1933) described scale and hemipenial characters that could distinguish four distinct species (*Trimeresurus albolabris*, *Trimeresurus popeiorum*, *Trimeresurus stejnegeri*, and *T. gramineus*). However, the presence of considerable geographic variation and sexual dimorphism in external colour and scalation characters (Malhotra and Thorpe, 1997) has contributed to frequent misidentification and confusion between these species. This problem has been explained in part by the recent detection and description of cryptic species (David et al., 2001, 2002; Giannasi et al., 2001; Malhotra and Thorpe, 2000; Malhotra and Thorpe, in press a, in press b; Malhotra et al., in press). Again, this study is set within a framework of a larger study that addresses the systematics of species that are particularly problematic and samples have been taken from specimens of verified identity only. We also include

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multiple samples from species that have not yet been fully revised, but are likely to be split into several species in the near future.

In an earlier report on this study, a phylogeny of 14 species of *Trimeresurus s.s.* and nine other Asian pitviper genera, based on c. 700 bp of the cytochrome *b* gene, was presented by Malhotra and Thorpe (2000). Several strongly supported conclusions of taxonomic interest could be derived from this study, including the non-monophyly of *Trimeresurus s.s.*, the non-monophyly of *Ovophis* (*Ovophis okinavensis* and *Ovophis monticola*), and the presence of several distinct species groups in remaining species of *Trimeresurus s.s.* The first two of these conclusions have since been verified by other studies using different genes (Tu et al., 2000). However, other conclusions were limited by the poor resolution and support, particularly at deeper nodes. This lack of resolution may be caused by insufficient sampling of taxa or molecular sequences. We have addressed both these issues in the present study.

2. Materials and methods

2.1. Samples and sequencing

Only specimens from known localities and in which the identification could be verified (e.g., by examining the specimen, or photograph) were used in this study. At least one sample of each of 28 recognized species of *Trimeresurus s.s.* were included. Given the strong possibility of undetected cryptic species being present, we also included multiple samples from across the range of widely distributed species wherever possible.

Samples were in the form of tail-tip biopsies preserved in 80% ethanol, liver tissue in 80% ethanol, or 100–200 μl of blood taken from the caudal vein, placed in 1 ml 5% EDTA, and preserved in 2 ml SDS–Tris buffer (100 mM Tris, 3% SDS). Whole genomic DNA was extracted from 0.01 to 0.02 g of ethanol-preserved muscle, or liver tissue, or 200–500 μl of blood/buffer, using standard protocols (Sambrook et al., 1989), or with Sigma GenElute Mammalian Genomic DNA Miniprep kits. Four different regions of the mitochondrial genome were amplified, using primers and reaction conditions described in Malhotra and Thorpe (2000) for cytochrome *b* (cyt*b*). However, in addition, since not all samples would successfully amplify with these primers, another set of primers were also used (Burbrink et al., 2000). These primers successfully overcame the problem with amplifying this gene in *Trimeresurus macrops* (Creer et al., 2003), mentioned in Malhotra and Thorpe (2000), as the 5′ primer is situated further upstream than in the previously used primer combinations. NADH dehydrogenase subunit 4 (ND4) sequences were obtained as described in Parkinson et al. (2000), 12S small subunit ribosomal RNA (12S) as described in Knight and Mindell (1993), and 16S large subunit ribosomal RNA (16S) as in Parkinson et al. (1997). Unincorporated nucleotides and primers were removed using a variety of commercially available kits, e.g., Prep-a-gene (Bio-Rad), Wizard minicolumns (Promega), or QIAquick columns (QIAGen). The double-stranded product was sequenced using dye-labelled terminators (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit), and subsequently run on an ABI Prism 377 DNA sequencer following manufacturer's protocols.

2.2. Additional sequences and outgroups

Azemiops feae (Viperidae: Azemiopinae) is well established as the sister group to the crotalines, and was used as one of the outgroups in this study. A second Viperinae outgroup, *Daboia russelii* was also used to root the tree. Because of the inadvisability of assuming that the current content, as described in current checklists (e.g., David and Ineich, 1999; McDiarmid et al., 1999; EMBL reptile database http://www.embl-heidelberg.de/~uetz/families/Viperidae.html) of *Trimeresurus s.s.* is valid, representatives of all other Asian pitviper genera, and a selection of New World taxa, were also included in the analysis.

2.3. Sequence analysis

The phylogenetic analysis was based on 2423 aligned nucleotide positions of combined data from the four gene regions, comprising 668 bp of ND4, 806 bp of cyt*b*, 426 bp of 12S, and 513 bp of 16S. Alignment of cyt*b* and ND4 was trivial as there were no indels. The 12S and 16S rDNA sequences were aligned following Parkinson (1999) with the exception of minor differences, which were required in one region of 12S and one region of 16S due to insertions found in some of the new sequences obtained. Ten base pairs in regions of uncertain alignment were excluded from the analysis: this included positions 227–228 and 291–292 of 12S, and positions 286–287, and 294–297 of 16S sequence). The cyt*b* and ND4 sequences were translated into amino acid sequences using MEGA version 2.1 (Kumar et al., 2001) to check for the unexpected occurrence of stop codons, which might indicate that pseudogenes (Zhang and Hewitt, 1996) had been amplified. Since these regions belong to a single linkage group (mitochondrial genome), and were found to evolve similarly (Parkinson et al., 2002) they were concatenated and analyzed together.

The possibility of non-neutral evolution was tested using a variety of tests implemented in the program DnaSP 3.51 (Rozas and Rozas, 1999), including McDonald and Kreitman's (1991) test, Fu and Li's D* and F*, and their modifications for use with an outgroup

sequence (Fu and Li, 1993), Tajima's D (Tajima, 1989), and the Hudson et al. (1987) test, that compares intraspecific polymorphism to interspecific divergence in two different loci. A set of nine *T. albolabris s.s.* sequences were used to provide the measure of intraspecific divergence, and the different regions used were the two coding versus the two non-coding regions.

We used both parsimony and Bayesian Markov Chain Monte Carlo (MCMC) approaches to reconstruct phylogenies. Maximum parsimony (MP) trees were inferred using the beta test version (b10) of PAUP* 4.0 (Swofford, 2003). Parsimony trees were constructed from unweighted characters following the substantial body of literature which documents the general ineffectiveness of weighting schemes in decreasing homoplasy without also decreasing useful phylogenetic information (Allard and Carpenter, 1996; Baker et al., 2001; Milinkovitch and Lyons-Weiler, 1998; Philippe et al., 1996). Searches were heuristic, with starting trees obtained by random taxon addition with 100 replicates, and tree-bisection-reconnection (TBR) branch swapping. Gaps were treated as a fifth character state. Support values for clades were calculated from 1000 bootstrap pseudoreplicates using the same settings, except that the number of random taxon additions was reduced to 10.

The Bayesian MCMC approach to reconstruct phylogenies was implemented in the program MrBayes (Huelsenbeck and Ronquist, 2001). ModelTest version 3.0 (Posada and Crandall, 1998) was used to infer the simplest best-fit model of evolution for the combined data set based on hierarchical log-likelihood ratio tests comparing successively more complex models (Huelsenbeck and Crandall, 1997; Posada and Crandall, 2001). All MCMC phylogenetic reconstructions were initiated with uniform priors, model parameters estimated as part of the analyses, and the best-fit model as indicated by Modeltest. Three heated chains and a single cold chain were used in all MCMC analyses and runs were initiated with random trees, using the program defaults. Trees were sampled every 100 generations and majority rule consensus phylograms and posterior probabilities for nodes were assembled from all post burn-in sampled trees. Phylogenetic reconstructions for all data partitions were estimated using three independent runs to confirm that stationarity (or global optimality) was reached and that independent runs converged on similar stationary parameter estimates. Each of these runs was conducted with a total of 1.4 million generations, 400,000 of which were discarded as burn-in, yielding 1 million post burn-in generations.

2.4. Divergence between and within clades

Estimating time of divergence of clades requires knowledge of the rate of sequence evolution, which in turn requires calibration from the fossil record or geological evidence. The fossil record of pitvipers, and snakes in general, is poor and at best indicates that pitvipers had evolved by the Miocene, c. 18–22 million years ago (Greene, 1992). Wüster et al. (2002) calculated a combined rate for *cytb* and *ND4* evolution in pitvipers of 1.09–1.77% per million years (pMyr), based on the divergence of the genus *Porthidium* in Central and South America. Extrapolation of rates of evolution from one taxon to another is fraught with difficulties (Arbogast et al., 2002; Caccone et al., 1997; Martin et al., 1992). However, a very similar rate has been calibrated for cyt*b* for *T. stejnegeri* in Taiwan (Creer et al., 2004). In addition, as the taxon in which the rate has been calibrated is represented in the phylogenetic tree, we can explicitly test the constancy of rate evolution across the tree and in the clades of interest. This was done using the relative rate test implemented in PHYLTEST v. 2.0 (Takezaki et al., 1995). As the test is most accurate when a close outgroup rather than a distant one is used, a successive testing procedure was adopted where each pair of clades was tested using the closest related clade as the outgroup. For example, the procedure started with a test comparing *Porthidium* to the remaining New World taxa, using the Old World clade that was revealed by the analysis to be the closest relative, as the outgroup. We then compared all New World taxa to various Old World clades, using the sister group of the clades under test as the outgroup. We compared the relative rates of evolution of the cyt*b* and *ND4* sequences in our data by plotting pairwise divergence values of each gene against each other: a regression slope approximating 1.0 indicates equality of rates. Average divergence estimates (with standard errors) between and within clades of interest were calculated from all genes combined. For divergence estimates, the between-clade differences were calculated from cyt*b* and *ND4* sequences only, using the model parameters estimated by MrBayes. The above rates were used to estimate time of divergence, with the combined error rates giving a range of possible dates of divergence.

2.5. Support from morphology

Malhotra and Thorpe (2000) identified several morphological characteristics that seemed to be congruent with the species groups in *Trimeresurus s.s.* These characters were: (a) the type of hemipenis, and (b) the condition of the first upper labial and nasal scale. MacClade 4.03 (Maddison and Maddison, 2001) was used to map them onto the more complete phylogeny presented in this study, using linear parsimony.

(a) Hemipenes vary considerably throughout the group, and provide one of the main characters for distinguishing some of the green species (Pope and Pope, 1933). In *Trimeresurus s.s.*, there are two contrasting types of hemipenes present. In common with most

snake hemipenes, each organ is forked. In the first type, the lobes are elongated, slender, and lack hard spinous structures (Fig. 3). Only a few species of vipers other than species of *Trimeresurus s.s.* have a hemipenis of this type (*Calloselasma rhodostoma*, *Tropidolaemus wagleri*, and *Hypnale* species have elongated forked regions, but of these, only *Hypnale hypnale* lacks spines). Within *Trimeresurus s.s.*, this type of hemipenis shows some subtle variations. In some species, the region immediately above the fork is bare, with calyces (a type of ornamentation in which raised ridges of tissue form a reticulate pattern (Dowling and Savage, 1960)) occurring above the bare region for most of the length of the fork (referred to here as the long calyculate type). In others, soft finger-like processes (papillae), which vary in length and number, occupy the region between the fork and the calyces. This type is termed long papillose. However, in some cases, there can be a bare area above the fork, with papillae present much further along the forked region, also followed by a calyculate region. Calyces can be shallow or deep. The sulcus spermaticus (sperm groove), which runs along the inner surface of the organ, may have prominently raised edges, or less prominent edges, and the calyces may involve the edges of the sulcus or they may remain smooth. Some of these features are obvious only in mature specimens, and so may appear variable within species. The second main type is the most common type of hemipenis in vipers, in which the lobes are short, and ornamented with hard spines; the main difference is in the arrangement, number, and size of the spines. Within *Trimeresurus s.s.*, several very distinct types of spinose hemipenis are found that are not seen in any other genus of viper. In the first [termed Type 1 spinose in Malhotra and Thorpe (2000)], the region distal to the fork is very short, and the organ is ornamented with c. 10 spines per fork, some of which are rather large and stout, markedly broader at the base than the tip (Fig. 3) region. The distal region is calyculate, and this extends to the fork on the inner (sulcate) surface of the organ. In the other type [termed Type 2 spinose in Malhotra and Thorpe (2000)], the spines are long and thin and do not broaden at the base, although they may be embedded in tissue that forms a web between the bases of neighbouring spines. These spines are densely arranged in several rows (although their number varies), and tend to occur on the whole length of the forked region (although they may be absent from the extreme tip). The only calyculate regions are along the edges of the sulcus, and in some cases they also occupy the extreme tip of the organ. There are however, a few species within *Trimeresurus s.s.* that have spinose hemipenes that do not fall within these two types. This may be because their affinities actually lie outside the group, or they may represent retention of an ancestral type, or theevolution of a unique type. These will be described and discussed later.

Hemipenis type was allocated mainly on the basis of dissection of the in situ inverted hemipenis, as literature records were found to be unreliable in some cases. For example, papillae are sometimes mistaken for spines, especially when the hemipenis is only partially everted (e.g., *Trimeresurus schultzei* in Leviton (1964) and *T. albolabris* in Lazell et al. (1999)). Where specimens were not available for examination, literature records were used where they appeared to be reliable (i.e., there was good agreement between the account and our own observations where some species had been examined for this study). Table 1 lists sources of information and hemipenis type for all species of *Trimeresurus s.s.*, including those not represented in the molecular analysis and some species now allocated to different genera.

(b) The first upper labial scale can either be distinctly separated from the adjacent nasal scale by a suture, or they may be completely or partially fused (Stejneger, 1927). While the degree of fusion is variable in most species, a species was coded as having the fused state if any degree of fusion is present.

3. Results

3.1. Phylogenetic analysis

DNA extracts amplified using different primer sets always gave identical sequences, as did different PCR products amplified with the same primer sets. No deletions, insertions, or stop codons were found in the coding regions, suggesting that paralogous nuclear insertions have not been amplified. The mean base composition was not significantly different across taxa for all four regions combined. There were 973 parsimony informative characters, and a further 304 variable but parsimony uninformative characters. None of the neutrality tests showed a significant departure from neutrality. Details of specimens and the GenBank Accession Numbers for all sequences, are given in Appendix A.

Based on hierarchical ln-likelihood ratio tests (hLRT) of successively complex models of sequence evolution, Modeltest indicated the simplest best-fit model for the combined mitochondrial dataset was the GTR + I + G model. All three runs reached apparent stationarity (in estimates of substitution model parameters, as well as chain likelihood scores) prior to 350,000 generations, before the conservative burn-in period of 400,000

Table 1
Hemipenis type, and sources of information, for all species of *Trimeresurus s.s.*, including those not represented in the present molecular analysis, and some species now allocated to different genera, where this may be in doubt

Current genus	Proposed genus	Species	DNA phylogeny	Hemipenis examined	Literature description[a]	Hemipenis type
Trimeresurus	*Cryptelytrops*	*albolabris*	Yes	Yes	1, 2, 3, *6*, *7*	Long papillose
Trimeresurus	*Cryptelytrops*	*andersonii*	Yes	No	2	Long papillose
Trimeresurus	*Trimeresurus*	*borneensis*	Yes	Yes	7[b]	Type 2 spinose
Trimeresurus	*Trimeresurus*	*brongersmai*	No	No	None known	Long calyculate
Trimeresurus	*Cryptelytrops*	*cantori*	Yes	Yes	2	Long calyculate
Trimeresurus	*Protobothrops*	*cornutus*	Yes	No	*8*	Type 3 spinose
Trimeresurus	*Cryptelytrops*	*erythrurus*	Yes	Yes	1, 2, 3	Long papillose
Trimeresurus	*Cryptelytrops*	*fasciatus*	No	No	16	Long papillose
Trimeresurus	*Parias*	*f. flavomaculatus*	Yes	Yes	5	Long papillose[c]
Trimeresurus	*Parias*	*f. mcgregori*	Yes	Yes	5	Long papillose
Trimeresurus	*?*	*gracilis*	Yes	No	15	
Trimeresurus	*Trimeresurus*	*gramineus*	Yes	Yes	1, 2	Type 1 spinose
Trimeresurus	*Viridovipera*	*gumprechti*	Yes	Yes	10	Type 1 spinose
Trimeresurus	*Parias*	*hageni*	Yes	Yes	*7*	Long papillose[c]
Trimeresurus	*Cryptelytrops*	*insularis*[d]	Yes	Yes	3	Long calyculate
Trimeresurus	*Cryptelytrops*	*kanburiensis*	Yes	Yes	None	Long papillose
Trimeresurus	*Himalayophis*	*karanshahi*[e]	No	Yes	None	Type 2 spinose
Trimeresurus	*Cryptelytrops*	*labialis*	No	No	2	Long papillose
Trimeresurus	*Peltopelor*	*macrolepis*	No	Yes	2	Long calyculate
Trimeresurus	*Cryptelytrops*	*macrops*	Yes	Yes	3, *7*, 12	Long papillose
Trimeresurus	*Trimeresurus*	*malabaricus*	Yes	Yes	2	Type 2 spinose
Trimeresurus	*Parias*	*malcolmi*	Yes	No	None known	
Trimeresurus	*Viridovipera*	*medoensis*	Yes	No	*6*	Type 1 spinose[f]
Trimeresurus	*Popeia*	*popeiorum*[g]	Yes	Yes	1, 2, 3	Long calyculate
Trimeresurus	*Trimeresurus*	*puniceus*	Yes	Yes	None known	Type 2 spinose
Trimeresurus	*Cryptelytrops*	*purpureomaculatus*	Yes	Yes	1, 2, 3, *7*	Long papillose
Trimeresurus	*Parias*	*schultzei*	Yes	Yes	5[h]	Long papillose[c]
Trimeresurus	*Cryptelytrops*	*septentrionalis*[d]	Yes	?	3	Long papillose
Trimeresurus	*Viridovipera*	*stejnegeri*	Yes	Yes	1, 2, 3, *6*, *13*, 14, *15*	Type 1 spinose
Protobothrops	*Trimeresurus*	*strigatus*				
Trimeresurus	*Parias*	*sumatranus*	Yes	Yes	3	Long papillose[c]
Trimeresurus	*Himalayophis*	*tibetanus*[e]	Yes	Yes	*11*	Unique
Trimeresurus	*Trimeresurus*	*trigonocephalus*	Yes	No	2	Type 1 spinose
Trimeresurus	*Cryptelytrops*	*venustus*[i]	Yes	Yes	*4*, *7*	Long papillose
Trimeresurus	*Viridovipera*	*vogeli*	Yes	Yes	9	Type 1 spinose
Trimeresurus	*Protobothrops*	*xiangchengensis*	No	No	*6*	Type 3 spinose
Trimeresurus	*Viridovipera*	*yunnanensis*	Yes	Yes	None known	Type 1 spinose

The table also indicates their present generic allocation according to the EMBL reptile database, and their proposed generic allocation following the present analysis. Literature sources: (1) Pope and Pope, 1933; (2) Smith, 1943; (3) Regenass and Kramer, 1981; (4) Vogel, 1991; (5) Leviton, 1964; (6) Guo and Zhang, 2000; (7) Jintakune and Chanhome, 1995; (8) Hermann et al., in press; (9) David et al., 2001; (10) David et al., 2002; (11) Orlov and Helfenberger, 1997; (12) Kramer, 1977; (13) Mao et al., 1984; (14) Pope, 1935; (15) Hidetoshi Ota, unpublished manuscript; (16) David et al., 2003.

[a] Numbers in italics indicate that the hemipenis is pictured.

[b] Listed as *T. puniceus* in this publication; however the Thai populations are referrable to *T. borneensis*.

[c] In these species, the papillae are not situated immediately above the fork, but are higher up in the forked region following a smooth region.

[d] Listed by EMBL as a subspecies of *T. albolabris* but elevated to full species status by Giannasi et al. (2001).

[e] *Trimeresurus karanshahi* was synonymised with *T. tibetanus* by Tillack et al. (2003). The specimens included in this analysis were formerly identified as *T. karanshahi*, and the decription of the hemipenis is based on these specimens. It differs from Type 3 spinose hemipenes by having realtively long lobes, with the largest spines being the most distal rather than proximal, and a substantial part of the distal end of the lobes are calyculate. Thus it appears almost as an intermediate between the spinose and non-spinose types of hemipenis found in *Trimeresurus s.s.*

[f] Similar to type 1 spiny except for length of forked region, see text for further discussion.

[g] Includes the subspecies *T. p. barati* and *T. p. sabahi*.

[h] The senior author has personally examined the specimen on which this description was based and can confirm that the hemipenis of this species was incorrectly described in this publication as it is based on a partly everted hemipenis, and the soft papillae have been described as spines.

[i] Listed by EMBL as a synonym of *T. kanburiensis* but Malhotra and Thorpe (in press) a recently confirmed it as a distinct species. Many literature references to *T. kanburiensis* are likely to refer in fact to this taxon.

generations. The phylogram with posterior probabilities based on the combined 3 million post burn-in generations (from three independent runs) is given in Fig. 1A. The parsimony analysis produced 10 most-parsimonious trees, of length 8489 steps, the 50% majority rule of which gave a fully resolved tree (Fig. 1B).

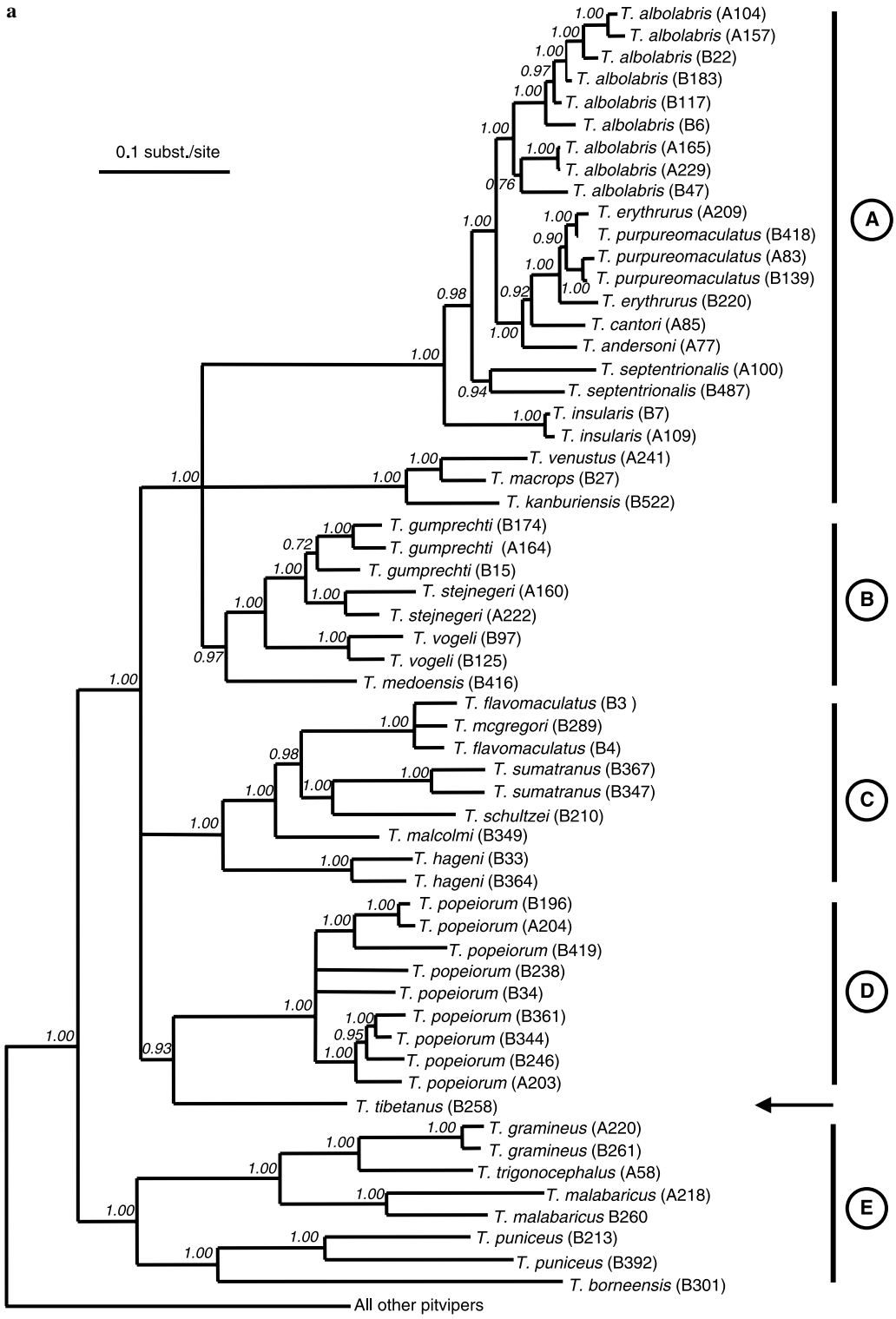

Fig. 1. (a) Bayesian phylogenetic hypothesis for *Trimeresurus s.s.*, with posterior probabilities for clades from the Bayesian MCMC analysis. The species groups referred to in the text are indicated on the tree. These are: (A) *albolabris* species group, (B) *stejnegeri* species group, (C) Indomalayan species group, (D) Popeiorum species group, (E) "Indian subcontinent" species group. An arrow indicates the position of *T. tibetanus*. The relationships of these species with other pitviper genera are illustrated in Fig. 2. (b) Fifty percentage majority rule consensus of 10 equally parsimonious trees, showing bootstrap support values (above 50%) from 1000 pseudoreplicates. Nodes that collapse in the strict consensus are indicated with a black dot. The locality of multiple samples from single species is indicated. Clade designations are as in (b).

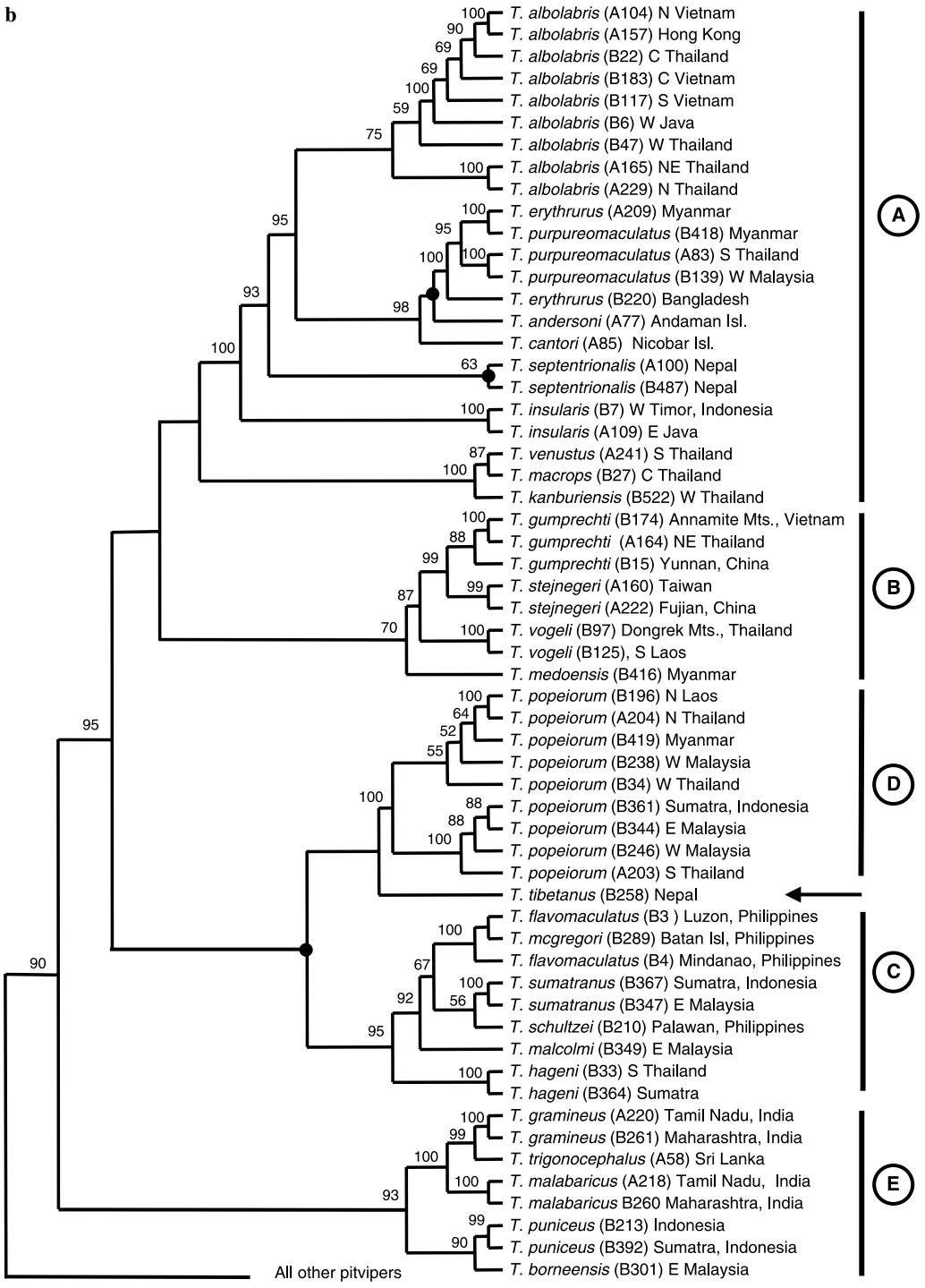

Fig. 1. (*continued*)

Parsimony and Bayesian reconstruction estimated largely identical topologies with respect to *Trimeresurus s.s.*, with no disagreement among strongly supported nodes. Although Cummings et al. (2003) have suggested that Bayesian posterior probabilities tend to be inflated relative to bootstrap percentages, all of the important inferences from this tree are supported by both high bootstrap values (>70%) and high posterior probabilities. The single exception, where a high posterior probability supports a node for which the bootstrap support in less than 50% in the MP tree, is described below.

Except for two species, *Trimeresurus s.s.* is strongly supported (bootstrap = 90%, posterior probability = 100%) as a monophyletic group, and the Bayesian

analysis places it as the sister group to all other pitvipers (posterior probability = 86%) except a few Asian species grouped by a deep lineage connecting at the base of the tree (Fig. 2). *Trimeresurus cornutus* is strongly supported as part of the genus *Protobothrops* (Herrmann et al., 2004), while *Trimeresurus gracilis* is placed as sister taxon to *O. okinavensis* (Malhotra and Thorpe, 2000; Tu et al., 2000).

The topology and arrangement of the species groups are consistent with the results of Malhotra and Thorpe (2000), although support for the species groups is much higher. The "Indian subcontinent" group is supported as the sister group to a clade containing all remaining species (95 % bootstrap and 100% posterior probability), and the relationship with the non-Indian species, *Trimeresurus borneensis* and *Trimeresurus puniceus*, is unequivocally supported (93% bootstrap and 100% posterior probability). However, despite the presence of these non-Indian species in this clade, we will continue to call it the "Indian subcontinent" group to facilitate

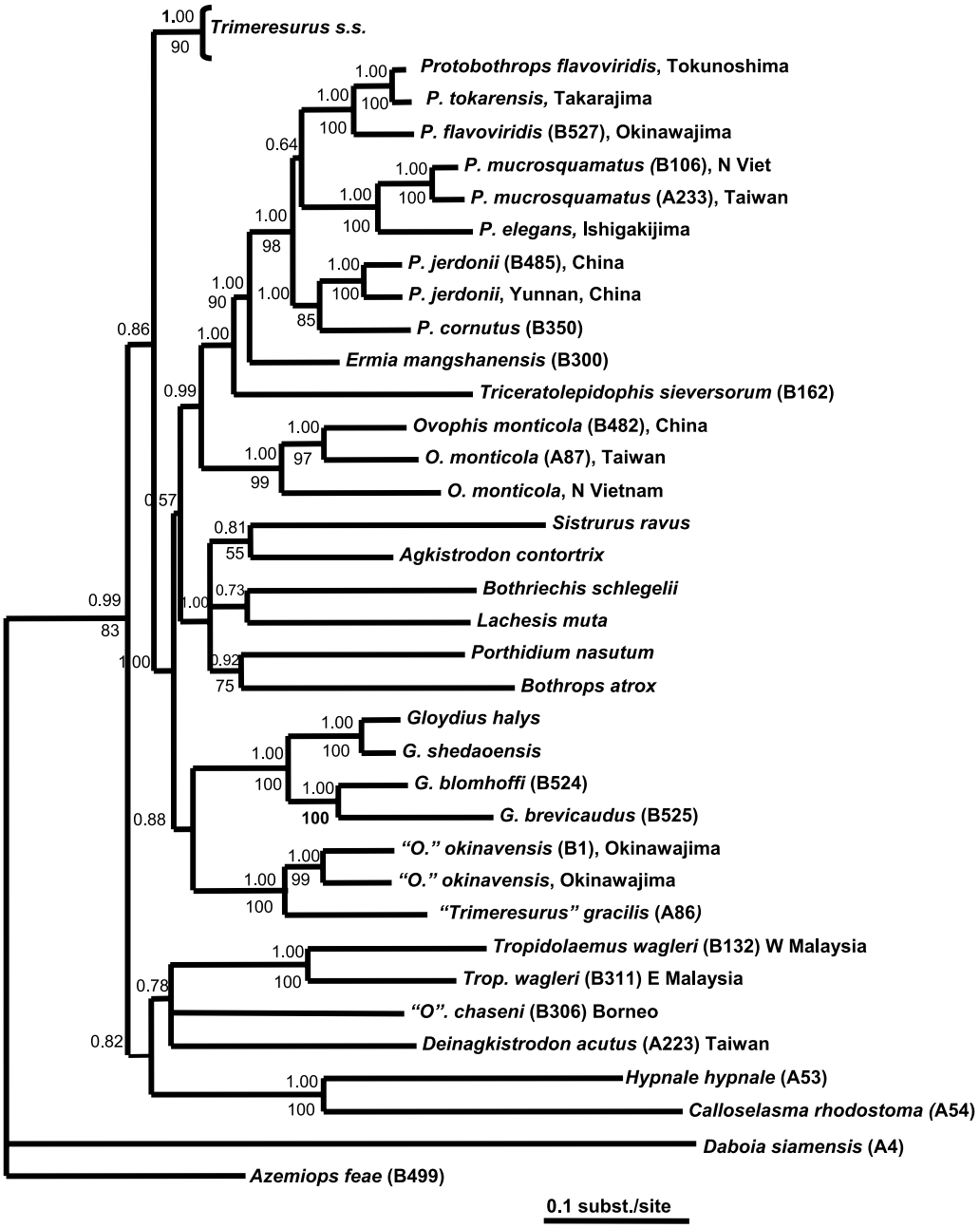

Fig. 2. Bayesian phylogenetic hypothesis for pitvipers, with posterior probabilities for clades from the Bayesian MCMC analysis above the branches and bootstrap support values from parsimony analysis below the branches. The optimal parsimony tree differs from the Bayesian tree mainly in showing the New World group as the sister group to the *Gloydius*/"*O.*"*okinavensis*/"*T.*" *gracilis* clade. The position of *Trimeresurus s.s.*, shown in detail in Fig. 1, is indicated.

cross-reference with earlier work (Malhotra and Thorpe, 2000). The "*popeiorum*" group, however, splits into two fairly divergent and well-defined subgroups with the exception of *Trimeresurus tibetanus*. The first of these is the *T. popeiorum* complex, which comprises populations all currently assigned to the same species, but containing several as yet unnamed cryptic species. Second, there is the Indomalayan group, comprising the species *T. sumatranus*, *T. hageni*, *T. malcolmi*, *T. schultzei*, and *T. flavomaculatus* (including two subspecies, *T. f. flavomaculatus* and *T. f. mcgregori*, which are likely to prove to be distinct species) (Sanders et al., in press). *T. tibetanus* appears as the sister taxon to the *T. popeiorum* complex (supported by a posterior probability of 93%, but with a bootstrap value <50%). However, the monophyly of the "*popeiorum*" group is not supported in either analysis, and its subgroups are treated henceforth as distinct species groups. Another node lacking support involves the small but well-defined cluster of species including *T. kanburiensis*, *T. venustus* (Malhotra and Thorpe, in press a) and *T. macrops*, located near the *T. albolabris* group. Although more species and more data have been added since Malhotra and Thorpe's (2000) study, the phylogenetic position of this group remains uncertain. Conversely, the present analysis shows a large increase in support for the monophyly of *Trimeresurus s.s.*, the "Indian subcontinent" group, and the *stejnegeri* group, compared to Malhotra and Thorpe (2000). Some additional resolution may be afforded by sequencing independently evolving loci. For example, the addition of nuclear intron sequence data supports the position of *T. macrops* within the *albolabris* group (Creer et al., 2003). However, the lack of resolution of some poorly supported nodes may be a consequence of evolutionary history rather than lack of sequence information (i.e., hard rather than soft polytomy).

The second major group of pitvipers, the sister group to *Trimeresurus s.s.*, consists of genera that were formerly considered part of the *Trimeresurus* group, as well as those classically placed in the other major division of Asian pitvipers, the *Agkistrodon* group (Gloyd and Conant, 1990). *Protobothrops* continues to be supported as a monophyletic group, and is clearly allied to the monotypic genera *Ermia* and *Triceratolepidophis*. In turn, these form the sister group to *O. monticola*. *O. okinavensis* was shown by both Malhotra and Thorpe (2000) and Tu et al. (2000) to be more closely related to "*T. gracilis*" than to its congener *O. monticola.* However, the affinity of these taxa was not clear. In this tree, they are placed as the sister group to *Gloydius*. This clade may in turn be the sister group to the New World taxa, as indicated by the most parsimonious reconstruction, or the New World taxa may be more closely related to the *Protobothrops*/*Ermia*/*Triceratolepidophis*/*Ovophis s.s.* clade, as in the Bayesian hypothesis illustrated in Fig. 2. However, neither arrangement is well supported. Identifying the closest Old World relative of the New World pitvipers may possibly be resolved in the future by adding more species of *Gloydius* and New World species.

Finally, there is a group characterised by many monotypic genera (e.g., *Calloselasma*, *Deinagkistrodon*) or genera with only a few species (e.g., *Tropidolaemus*, *Hypnale* have no more than three species), and that are separated from each other by long branches, which forms the sister group to all other pitvipers. This result is in accord with the findings of Parkinson et al. (2002). The third species of *Ovophis* represented in this study, *O. chaseni*, which is currently only known from Mount Kinabalu, Sabah, East Malaysia, also falls within this group. It forms a cluster with *Tropidolaemus* and *Deinagkistrodon*, but its closest relative is not well defined, and it is separated from both by long branches.

3.2. Divergence between and within clades

Table 2 gives average divergence estimates between and within the major clades identified by the above analysis, together with distances from some other pitviper genera for comparison. Despite the large number of species in the *albolabris* group, the average divergence is lowest within this group if the basal *kanburiensis* group is not included (not shown). When the latter is included within the *albolabris* group, the *popeiorum* species complex has the lowest within-group diversity. The "Indian subcontinent" group, conversely, has the highest within-group divergence, higher than that shown by *Protobothrops*, *Ovophis* (*s.s.*) and *Gloydius* and not much lower than the average divergence between all the New World viper genera represented in this study (not shown). The largest between-group difference within *Trimeresurus s.s.* is between the "Indian subcontinent" and the Indomalayan group (0.168), closely followed by the average divergence between the "Indian subcontinent" and *albolabris* species group (0.165). This is similar to, or exceeds, the average divergence between some other pitviper genera. For example, the average distance between *Ovophis s.s.* and *Protobothrops* is 0.148, and between *Protobothrops* and *Gloydius* is 0.162 (Table 2). Rate tests show that the only clade for which rate constancy can be rejected is the *Protobothrops*/*Ermia*/*Triceratolepidophis* clade, which appears to be evolving c. 20% faster than the remaining taxa.

3.3. Support from morphology

The 50% majority-rule consensus of the set of most parsimonious trees was used for this purpose, as it is fully resolved (the Bayesian hypothesis is less suitable as it produces a less resolved consensus tree). This tree (Fig. 3) represents the most parsimonious reconstruction of the evolution of these characters (involving eight

Table 2
Average divergence estimates (Tamura-Nei distances, with gamma correction) between and within the major species groups defined by the analysis, calculated for all genes

	albolabris	*stejnegeri*	*popeiorum*	Indomalayan	*T. tibetanus*	Indian sub.	*Protoboth.*	*Gloydius*	*Ovophis s.s.*
albolabris	*0.069 ± 0.004*	0.193 ± 0.014	0.196 ± 0.015	0.216 ± 0.015	0.184 ± 0.016	0.231 ± 0.014	0.249 ± 0.016	0.265 ± 0.018	0.242 ± 0.017
stejnegeri	0.129 ± 0.007	*0.062 ± 0.004*	0.172 ± 0.014	0.179 ± 0.013	0.155 ± 0.014	0.221 ± 0.015	0.216 ± 0.016	0.254 ± 0.018	0.223 ± 0.016
popeiorum	0.130 ± 0.009	0.118 ± 0.007	*0.044 ± 0.003*	0.229 ± 0.013	0.150 ± 0.014	0.229 ± 0.017	0.230 ± 0.018	0.250 ± 0.019	0.234 ± 0.018
Indomalayan	0.150 ± 0.009	0.124 ± 0.008	0.126 ± 0.008	*0.087 ± 0.005*	0.120 ± 0.019	0.226 ± 0.015	0.226 ± 0.015	0.260 ± 0.018	0.244 ± 0.017
T. tibetanus	0.129 ± 0.009	0.110 ± 0.007	0.103 ± 0.007	0.126 ± 0.008	—	0.223 ± 0.016	0.214 ± 0.017	0.253 ± 0.016	0.206 ± 0.016
Indian sub.	0.165 ± 0.009	0.160 ± 0.009	0.163 ± 0.009	0.168 ± 0.009	0.159 ± 0.009	*0.142 ± 0.008*	0.241 ± 0.015	0.263 ± 0.017	0.242 ± 0.016
Protoboth.	0.173 ± 0.010	0.152 ± 0.009	0.163 ± 0.010	0.165 ± 0.010	0.148 ± 0.010	0.179 ± 0.009	*0.085 ± 0.005*	0.239 ± 0.016	0.207 ± 0.015
Gloydius	0.182 ± 0.011	0.172 ± 0.011	0.171 ± 0.011	0.184 ± 0.011	0.178 ± 0.012	0.192 ± 0.011	0.162 ± 0.009	*0.097 ± 0.006*	0.224 ± 0.014
Ovophis s.s.	0.178 ± 0.010	0.163 ± 0.009	0.162 ± 0.010	0.176 ± 0.010	0.145 ± 0.010	0.185 ± 0.009	0.148 ± 0.008	0.160 ± 0.009	*0.104 ± 0.007*

Distances between and within some other pitviper genera are also given for comparison. Above diagonal: distances calculated from cyt *b* and ND4 only for calculation of divergence rates; below diagonal, distances calculated from all genes; on diagonal (in italics): within-group divergence calculated from all genes.

changes, with a consistency index of 0.75, a rescaled consistency index of 0.67, and a retention index of 0.89). Alternative arrangements of taxa whose position is not strongly supported (e.g., the *T. kanburiensis*/*T. venustus*/*T. macrops* group) involve a greater number of changes and lowered indices.

The conclusions reached in Malhotra and Thorpe (2000) regarding the congruence between gross hemipenis type and species groups are corroborated and elaborated here. A Type 2 spinose hemipenis characterises all members of the Indian subcontintent group, including its Southeast Asian representatives (further examination of specimens has resolved the anomalies noted in Malhotra and Thorpe (2000) with respect to *T. borneensis*). A Type 1 spinose hemipenis is found in members of the *stejnegeri* group. Although the hemipenis of *T. medoensis* is not exactly similar, the main difference is that the forked regions are longer than in the rest of the group. However, in other aspects (e.g., the continuation of the calyculate area on the inner, sulcate, side of the forked regions all the way to the fork, the number and distribution of spines on the outer face and the forking of the sulcus near the base of the organ), the hemipenis of *T. medoensis* resembles the Type 1 spinose rather than the Type 3 spinose hemipenis.

The spinose hemipenis type of *T. tibetanus* appears unique to this species. While it resembles the Type 2 spinose hemipenis in many respects, there are also substantial differences, and to some extent it appears intermediate between the ancestral spinose type and the derived long calyculate type. All other *Trimeresurus* species represented have the long hemipenis type. Although only the long calyculate type is found in the *popeiorum* species group, and the long papillose type in the Indomalayan species group, both long papillose and long calyculate hemipenes are found in the *albolabris* species group. However, all members of the *albolabris*/*kanburiensis* species group are characterised by a nasal scale that is fully, or partially, fused with the first upper labial.

4. Discussion

4.1. Implications for the taxonomy of Trimeresurus s.s.

Trimeresurus s.s. (as currently defined) appears to be sufficiently structured and genetically diverse to warrant further subdivision into genera. While it could be argued that retaining all the species groups within *Trimeresurus s.s.* promotes taxonomic stability, *Trimeresurus s.l.* has been subject to considerable taxonomic rearrangement in recent years and has yet to achieve stability. The presence of diagnostic morphological characters is important since a workable taxonomy cannot rely entirely on molecular data, as that would leave some species

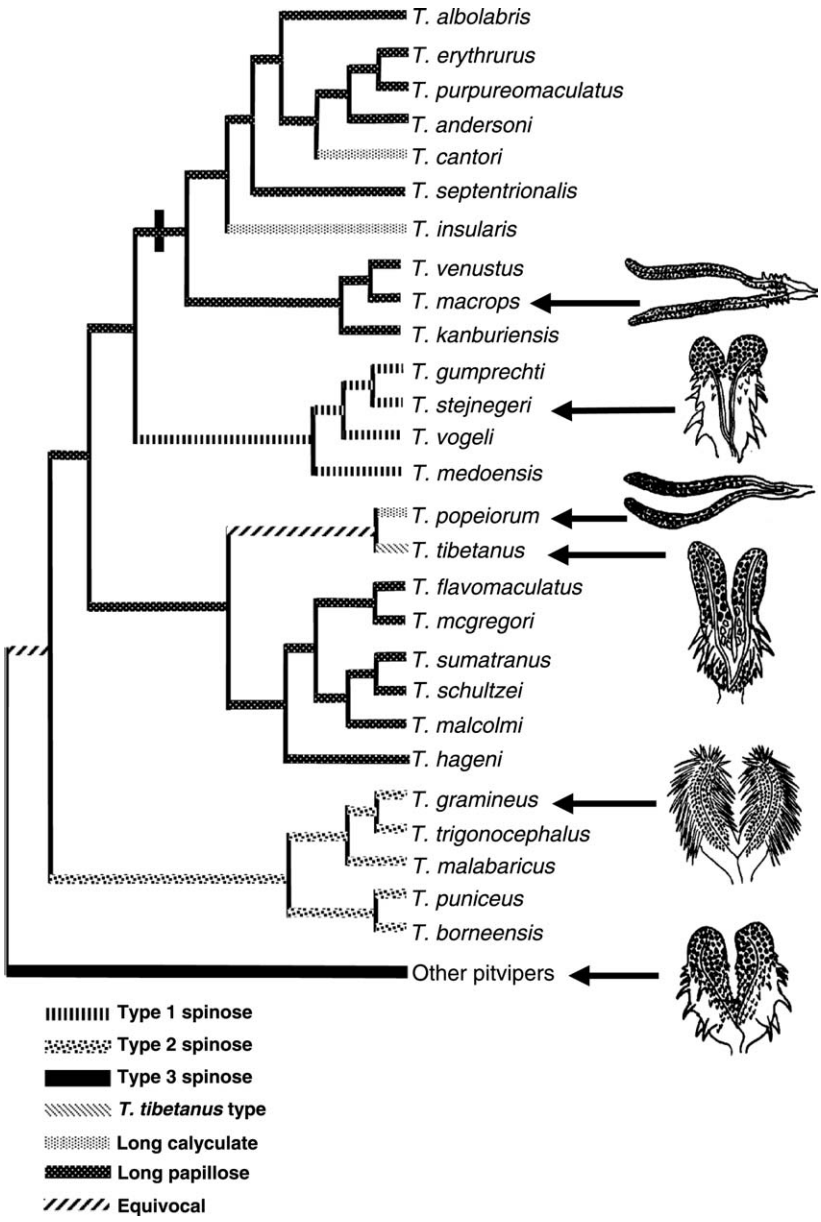

Fig. 3. Morphological characters mapped onto the parsimony tree represented in Fig. 1b (with morphologically conservative species condensed to a single tip for clarity). The proposed genera (see text) can be characterised by a combination of hemipenial morphology and fusion of first upper labial scale and nasal scale. The bold vertical line indicates the position of the change from an unfused first supralabial and nasal scale to the fused condition. Diagrammatic representations of the different hemipenis types are included, based upon the author's own observations and the sources given in Table 1 (note however that no everted hemipenis from the "Indian subcontinent" group has been examined and the diagram is based on the author's extrapolation from a dissected hemipenis).

(even in the best-studied groups), in an uncertain taxonomic position. Based on the characters analyzed here, six genera could be diagnosed within what is currently described as *Trimeresurus s.s.*, each diagnosed by at least one change in character state along the branch leading to it.

We can use these morphological characteristics to infer the relationships of the few species for which genetic data are not currently available (some of which may remain unobtainable for the forseeable future due to their rarity or inaccessibility). These include *Trimeresurus strigatus* and *Trimeresurus macrolepis*, both "Indian subcontinent" species. *T. strigatus*, although placed in *Protobothrops* after the suggestion of Kraus et al. (1996), has a Type 1 spinose hemipenis in common with the other "Indian subcontinent" species, and its affinities are almost certainly with this group rather than with *Protobothrops*. *T. macrolepis*, on the other hand, has a hemipenis most similar to that of the *popeiorum* complex, being spineless and lacking papillae, with the

calyculate area beginning at some distance from the fork and continuing until the tip. *T. macrolepis* has the very distinctive feature (unique within *Trimeresurus s.s.*) of possessing enlarged head scales, which otherwise are found only in the species formerly allocated to the "Agkistrodon" group (Gloyd and Conant, 1990). However, this character does not seem to have any value in determining relationships as it has now been shown that these species are not closely related. Presently, the *popeiorum* complex is not represented in the Indian peninsula, although it is present in northeast India and the Himalayan region. As many other Indian peninsular species also have affinities in Southeast Asia (Hora, 1953), a relationship between *T. macrolepis* and *T. popeiorum* is not unlikely on distributional grounds alone. However, at least in the pitvipers, the taxa showing this distributional pattern are early offshoots of the radiation, while the divergence in the *popeiorum* group is relatively recent (see further discussion of this point below). It seems likely therefore that this species is placed outside *Trimeresurus s.s.* altogether.

Trimeresurus labialis and *T. fasciatus* are clearly assignable to the *albolabris* group as they both have a fused first upper labial and nasal scale and long papillose hemipenes. *Trimeresurus brongersmai* is clearly closely related to *T. puniceus* and *T. borneensis* and assignable to the "Indian subcontinent" clade. Similarly, *T. yunnanensis*, although of uncertain taxonomic validity (Malhotra and Thorpe, in press b), would clearly be part of the *stejnegeri* group. *Trimeresurus xiangchengensis* (listed by the EMBL reptile database under *Trimeresurus* and therefore considered here) is apparently closely related to *Protobothrops mucrosquamatus* [indeed some consider it a synonym of the latter species (McDiarmid et al., 1999)]. The removal of *T. cornutus* from *Trimeresurus* to *Protobothrops* and *T. gracilis* to an unnamed genus is supported, or at least not contradicted, by their hemipenis types.

Another South Indian species, *T. huttoni*, has been placed in *Tropidolaemus* by David and Vogel (1998). Unfortunately, the hemipenis cannot be used to assist in this case, as the only male specimen known is a neonate and the hemipenis is too undeveloped to allow an assessment of its basic type. Certain features of its head scalation are shared by *Tro. wagleri*, but also by members of the "Indian subcontinent" group (e.g., the separation of the second supralabial scale from the scale that forms the anterior edge of the pit). However, other features such as the extreme keeling of the head scales, the distinctive colour pattern, and head shape, are all in common with *Tro. wagleri* alone.

4.2. Implications for the taxonomy of Ovophis

Another genus clearly needing major revision is *Ovophis*. Originally proposed by Burger (1971) in an unpublished Ph.D. Dissertation, the name was made valid by Hoge and Romano-Hoge (1981). Burger did not make clear which species he actually examined (although he comments that he did not examine many Asian species). However, the content of the genus *Ovophis* and the *Trimeresurus* species groups he lists corresponds to the species groups proposed by Maslin (1942). Although the species placed in *Ovophis* do share some superficial similarities (they are all terrestrial egg layers, and tend to have stout bodies, compact heads and smooth shiny scales), these similarities can now be seen not to be indicative of a close relationship. The position of *O. okinavensis* may warrant its inclusion within *Gloydius*, together with *T. gracilis*. However, morphological characters, including hemipenes, have not been extensively studied in these species, and further information is desirable before a decision is made. Studies are currently in progress. *Ovophis chaseni* represents a deep lineage and is not closely related to any other extant pitviper, occupying a restricted range in the mountains of north Borneo.

The name *Ovophis* should now be restricted to the type species, *O. monticola*. However, the large divergence between specimens from various parts of the range of this widespread and polytypic species suggests that it consists of more than one species. The boundaries of these species would require further investigation as there is much disagreement about which subspecies are valid, and indeed subspecies have not proved a reliable guide to species boundaries in other pitvipers (Malhotra and Thorpe, in press b).

4.3. A new taxonomy

If the argument that the species groups within *Trimeresurus s.s.* deserve recognition at the generic level is accepted, then the name *Trimeresurus* Lacépède, 1804 will be further restricted to apply to members of the "Indian subcontinent" group alone, as *T. gramineus* is the type species. While some discussion regarding whether this name really applied to the South Indian species rather than *T. popeiorum* or *T. albolabris* took place some years ago, this issue has since been clarified (see McDiarmid et al., 1999). This genus is diagnosed by the possession of a Type 2 spinose hemipenis, and is distributed in the "Indian subcontinent" (including Sri Lanka), and the Indomalayan region. *Parias* Gray, 1849 is available for the Indomalayan group (type species, *flavomaculatus*). It is diagnosed by the possession of a long papillose hemipenis, and a separated first supralabial and nasal scale, and is distributed in the Indomalayan region and the Philippines. *Cryptelytrops* Cope, 1860 is available for the *albolabris* group (type species *carinatus*, now *purpureomaculatus*), diagnosed by a combination of a long papillose or calyculate hemipenis and a fused first supralabial and nasal scale. It is widely

distributed, occurring in parts of the Indomalayan region (Lesser Sunda islands, Java, Sumatra), throughout Southeast Asia, Nepal and northeast India, and China up to c. 33° N. *Peltopelor* Günther, 1864 is resurrected for the species *macrolepis*, and is diagnosed by a combination of a long calyculate hemipenis and enlarged head scales. It is restricted to the Western Ghats of the "Indian subcontinent."

There are no available names for the remaining species groups. We propose *Viridovipera* gen. nov. for the *stejnegeri* group (type species *V. stejnegeri s.s.*), the name deriving from the fact that this is the only species group of the former *Trimeresurus* in which all species are "green" pitvipers. It is diagnosed by the possession of a Type 1 spinose hemipenis, and is distributed in hilly regions in northern Myanmar and eastern Thailand, hill regions of Cambodia, Laos, and central and northern Vietnam, north-eastern India, Tibet, and much of China up to c. 33° N. We propose the generic name *Popeia* gen.nov. for the *popeiorum* complex (type species *P. popeiorum*), in recognition of the fact that Clifford and Sarah Pope were the first to point out the value of the hemipenis in separating the green species (Pope and Pope, 1933), which has now proved very important in the context of the systematics of the whole group. It is distributed in hilly regions of Sumatra and Borneo, West Malaysia, western regions of Thailand and Laos, Myanmar, and north-east India. Finally, the morphological characteristics of *T. tibetanus* do not allow it to be placed in any of the groups mentioned above. We therefore propose *Himalayophis* gen. nov. for this species, as it is known only from a small region of the Himalayas spanning Nepal and Tibet (Tillack et al., 2003). Table 1 summarises the proposed content of these new genera.

For former members of *Ovophis*, we defer a decision on *O. okinavensis* until further studies involving morphological characters are complete. We propose a new genus, *Garthius* gen. nov. for the species *chaseni*, in memory of Garth Underwood, who contributed significantly to the field of reptile systematics for more than 50 years until his death in 2002.

4.4. Evolution of Asian pitvipers

Applying the rates of divergence calculated by Wüster et al. (2002) to the divergence distances calculated between clades shows that the early pitvipers diverged from their closest viperid relatives between 16.44 and 30.18 million years ago (mya), i.e., between the early Miocene to mid Oligocene. It should be noted that the following discussion depends upon the assumptions made in calculating the rate by Wüster et al. (2002). The date calculated using this rate fits well with the earliest known fossil evidence of vipers in Asia (Greene, 1992), but conflicts with other interpretations of the historical biogeography of pitvipers. For example, Vidal and Lecointre (1998) proposed that pitvipers had dispersed into the New World by the late Cretaceous/early Tertiary. Parkinson et al. (2002) supported this timeframe although they did point out that there was no hard evidence supporting this hypothesis.

The Miocene was clearly an extremely important period not only for the origin but also for the diversification of pitvipers in Asia. The two major sister clades of pitvipers (i.e., the clade now consisting of *Trimeresurus*, *Cryptelytrops*, *Parias*, *Popeia*, and *Himalayophis*, and its sister clade consisting of *Protobothrops*, *Ermia*, *Triceratolepidophis*, *Gloydius*, *Ovophis*, and all new World pitviper genera) diverged in the early to mid Miocene (13.12–23.85 mya). The divergence between the major genera within the "*Trimeresurus*" radiation took place only a few million years after this event, with the split between *Trimeresurus s.s.* and the remainder occurring between 19.9 and 22.01 mya, while the *Parias*/*Popeia*/*Himalayophis* clade split from *Viridovipera* and *Cryptelytrops* clade between 10.34 and 19.17 mya. The divergence between the Indomalayan *Parias* and *Popeia*/*Himalayophis* took place almost simultaneously at between 9.56 and 18.07 mya.

The split between the Southeast Asian and "Indian subcontinent" relatives among the pitvipers also took place in the early to mid Miocene. The split between *Calloselasma* and *Hypnale* dates to 13.56–25.88 mya, and the split between *T. puniceus*/*T. borneensis* and the Indian species of this genus occurred between 11.99 and 22.01 mya. This would fit with the hypothesis that the Garo-Rajmahal gap, which presently forms a serious barrier to the dispersal of moist-forest organisms between the two regions, was closed by uplift some time in the Miocene (references in Gloyd and Conant, 1990). No pitviper genus with an origin more recent than the mid-Miocene seems to have invaded the Indian subcontinent further than the mountains of north-east India, suggesting that this gap has been in existence since the mid-Miocene. If *Peltopelor macrolepis* is closely related to *Popeia* on the basis of its similar hemipenis (long calyculate) and unfused first labial/nasal, these taxa must have diverged later than the divergence between *Popeia* and *Himalayophis* (between 7.69 and 15.04 mya). While this is not unfeasible, it perhaps is less likely and may indicate that *Peltopelor* diverged early in the pitviper tree.

5. Conclusions

This study goes a long way towards resolving the confusion surrounding the systematics of Asian pitvipers that has reigned for many decades. Although it is based on a single locus, at least some elements of the phylogeny have been corroborated using additional,

nuclear, loci (Creer et al., 2003), and further work is in progress in this area. Moreover, there are theoretical grounds for considering that the mitochondrial gene tree is likely to be representative of the species tree (Moore, 1995). Remaining issues will require the collection of material from species that are currently unavailable.

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Appendix A

Details of specimens used in this analysis

Species	Specimen No.	Locality	GenBank Accession No. (cyt*b*, ND4, 12S, and 16S)
(A) *albolabris* group			
T. albolabris	ROM 30854	Vin Phuc province, N Vietnam	AY352769, AY352837, AY352803, AY352742
T. albolabris	AM A157	Shek Kwu Chan, Hong Kong	AF171884, AY352839, AY352805, AY352744
T. albolabris	AM B22	Nonthaburi, C Thailand	AF517189, AF517221, AF517165, AF517178
T. albolabris	ROM 34544	Gia Lai province, C Vietnam	AY352770, AY352838, AY352804, AY352743
T. albolabris	AM B117	Ho Chi Minh City, S Vietnam	AF517190, AF517222, AF517166, AF517179
T. albolabris	AM B6	Cilacap, W Java	AF517186, AF517213, AF517158, AF517171
T. albolabris	AM B47	Phetburi province, Thailand	AF517187, AF517216, AF517160, AF517173
T. albolabris	AM A165	Loei province, NE Thailand	AF517185, AF517214, AF517169, AF517182
T. albolabris	AM A229	Pha Yao province, N Thailand	AY059566, AY059583, AY059544, AY059560
T. erythrurus	AM A209	Rangoon, Myanmar	AF171900, AF517217, AF517161, AF517174
T. erythrurus	AM B220	Chittagong district, Bangladesh	AY352768, AY352834, AY352800, AY352739
T. purpureomaculatus	CAS 212246	Ayeyarwade division, Myanmar	AY352772, AY352841, AY352807, AY352746
T. purpureomaculatus	AM A83	Satun province, S Thailand	AF517188, AF517218, AF517162, AF517175
T. purpureomaculatus	AM B139	Perak state, W Malaysia	AY352771, AY352840, AY352806, AY352745
T. andersoni	AM A77	Andaman Islands, India	AF171922, AY352835, AY352801, AY352740
T. cantori	AM A85	Nicobar Islands, India	AF171889, AY352836, AY352802, AY352741
T. septentrionalis	AM A100	Mahattari district, Nepal	AF171909, AY059592, AY059543, AY059559
T. septentrionalis	AM B487	Kathmandu district, Nepal	AY352755, AY352818, AY352784, AY352724
T. insularis	AM B7	W Timor, Indonesia	AY059568, AY059586, AY059534, AY059550
T. insularis	AM A109	E Java, Indonesia	AY352767, AY352833, AY352799, AY352738
T. venustus	AM A241	Nakhon si Thammarat prov., S Thailand	AF171914, AY293930, AY293931, AY352723
T. macrops	AM B27	Bangkok, C Thailand	AF517184, AF517219, AF517163, AF517176
T. kanburiensis	AM B522	Kanchanaburi province, W Thailand	AY289225, AY289231, AY289219, AY352737
(B) *stejnegeri* group			
T. gumprechti	FMNH 255579	Nghe An province, N Vietnam	AY059573, AY059595, AY059547, AY059563
T. gumprechti	AM A164	Loei province, NE Thailand	AY352766, AF157224, AF517168, AF517181
T. gumprechti	NMNS 3113	Yunnan province, China	AY321487, AY352832, AY352798, AY352736
T. stejnegeri	AM A160	Taipei county, Taiwan	AF171896, AY059593, AY059539, AY059555
T. stejnegeri	NMNS 3651	Fujian province, China	AF277677, AY059594, AY059541, AY059557
T. vogeli	AM B97	Nakhon si Ratchasima prov., Thailand	AY059574, AY059596, AY059546, AY059562
T. vogeli	FMNH 258945	Champassak province, S Laos	AY059581, AF517225, AF517170, AF517183
T. medoensis	CAS 221528	Kachin state, Myanmar	AY352765, AY352831, AY352797, AY352735

Appendix A (*continued*)

Species	Specimen No.	Locality	GenBank Accession No. (cyt*b*, ND4, 12S, and 16S)
(C) *popeiorum* group			
T. popeiorum	FMNH 258950	Phongsaly province, N Laos	AY059571, AY059590, AY059538, AY059554
T. popeiorum	AM A204	Chiang Rai province, N Thailand	AF171902, AY371843, AY371742, AY371784
T. popeiorum	CAS 222195	Mon State, Myanmar	AY371806, AY371841, AY371738, AY371777
T. popeiorum	AM B238	Pahang state, W Malaysia	AY371814, AY371839, AY371737, AY371774
T. popeiorum	AM B34	Phetburi province, Thailand	AY059572, AY059591, AY059542, AY059558
T. popeiorum	AM B361	Bengkulu province, Sumatra	AY371801, AY371837, AY371753, AY371769
T. popeiorum	AM B344	Mt Kinabalu, Sabah, E Malaysia	AY371815, AY371842, AY371736, AY371771
T. popeiorum	AM B246	Selangor state, W Malaysia	AY059570, AY059589, AY059540, AY059556
T. popeiorum	AM A203	Nakhon si Thammarat prov., S Thailand	AY371796, AY059588, AY059537, AY059553
T. tibetanus	ZMB 65641	Helambu province, Nepal	AY352749, AY352810, AY352776, AY352715
(D) Indomalayan group			
T. flavomaculatus	AM B3	Luzon, Philippines	AF171916, AY059584, AY059535, AY059551
T. flavomaculatus	AM B4	Mindanao, Philippines	AY352764, AY352830, AY352796, AY352734
T. mcgregori	AM B289	Batan islands, Philippines	AY371831, AY371858, AY371756, AY371795
T. sumatranus	AM B367	Bengkulu province, Sumatra	AY371824, AY371864, AY371765, AY371791
T. sumatranus	AM B347	Sabah, E Malaysia	AY371823, AY371859, AY371759, AY371788
T. schultzei	AM B210	Palawan, Philippines	AY352756, AY352819, AY352785, AY352725
T. malcolmi	AM B349	Mt. Kinabalu, Sabah, E Malaysia	AY371832, AY371861, AY371757, AY371786
T. hageni	AM B33	Songhkla province, S Thailand	AY059567, AY059585, AY059536, AY059552
T. hageni	AM B364	Bengkulu province, Sumatra	AY371825, AY371863, AY371763, AY371790
(E) Indian subcontinent group			
T. gramineus	AM A220	Tamil Nadu state, India	AY352761, AY352827, AY352793, AY352731
T. gramineus	AM B261	Maharashtra state, India	AY352762, AY352828, AY352794, AY352732
T. trigonocephalus	AM A58	Balangoda, Sri Lanka	AF171890, AY059597, AY059549, AY059565
T. malabaricus	AM A218	Tamil Nadu state, India	AY059569, AY059587, AY059548, AY059564
T. malabaricus	AM B260	Maharashtra state, India	AY352763, AY352829, AY352795, AY352733
T. puniceus	AM B213	Indonesia	AF517192, AF517220, AF517164, AF517177
T. puniceus	AM B392	Bengkulu province, Sumatra	AY352757, AY352820, AY352786,–
T. borneensis	AM B301	Sabah, E Malaysia	AY352754, AY352817, AY352783, AY352722
(F) *Protobothrops* and related taxa			
Ermia mangshanensis	AM B300	Hunan Province, China	AY352758, AY352821, AY352787, AY352726
Protobothrops flavoviridis	UMMZ 199973	Tokunoshima, Ryukyu Is., Japan	AY223574, U41894, AF057200, AF057247
P. flavoviridis	AM B527	Okinawajima, Ryukyu Is., Japan	–, AY352826, AY352792, AY352730
P. elegans	UMMZ 199970	Ishigakijima, Ryukyu Is., Japan	AY223575, U41893, AF057201, AF057248
P. cornutus	ZFMK 75067	Central Vietnam	AY294272, AY294262, AY294276, AY294267
P. jerdonii	CAS 215051	Nu Jiang, Yunnan Prov., China	AY294274, AY294264, AY294278, AY294269
P. jerdonii	AM B485	China	AY294273, AY294263, AY294277, AY294268
P. mucrosquamatus	AM A233	Hualien county, Taiwan	AF171897, AY294265, AY294279, AY294270
P. mucrosquamatus	AM B106	Vin Phuc Province, N Vietnam	AY294275, AY294266, AY294280, AY294271
P. tokarensis	FK 1997	Takarajima, Ryukyu Is., Japan	AY223576, AY223628, AF057202, AF057249
Triceratolepidophis sieversorum	AM B162	Central Vietnam	AY352753, AY352816, AY352782, AY352721
(G) *Ovophis*			
Ovophis monticola	AM A87	Taiwan	AF171907, AY059582, AY059545, AY059561
O. monticola	AM B482	China	AY352748, AY352809, AY352775, AY352714
O. monticola	ROM 7798	Vietnam	AY223572, AY223626, AY223652, AY223665
(H) New World			
Agkistrodon contortrix	—	—	AF039268, AF156576, AF057229, AF057276
Bothriechis schlegelii	—	—	AF039270, U41874, AF157213, AF057260
Bothrops atrox	—	—	AF191587, AF246277, AY223659, AY223672
Lachesis muta	—	—	AF039262, U96030, AF057221, AF057268
Porthidium nasutum	—	—	AF191580, U41887, AF057204, AF057251
Sistrurus ravus	—	—	AF259158, AY223647, AF057226, AF057273
(I) *Gloydius* and related taxa			
Gloydius blomhoffi	AM B524	Teuri Isl., Hokkaido, Japan	AY352751, AY352814, AY352780, AY352719
G. brevicaudus	AM B525	China	AY352752, AY352815, AY352781, AY352720
G. halys	—	Khazakstan	AY223564, AY223621, AF057191, AF057238

Appendix A (*continued*)

Species	Specimen No.	Locality	GenBank Accession No. (cyt*b*, ND4, 12S, and 16S)
G. shedaoensis	ROM 20468	Liaoning, China	AY223566, AY223623, AF057194, AF057241
''Trimeresurus'' gracilis	AM A86	Taiwan	AF171913, AY352823, AY352789, AY352728
''O''. okinavensis	AM B1	Okinawajima, Ryukyu Is., Japan	AF171915, AY352824, AY352790, –
''O''. okinavensis	CLP 162	Okinawajima, Ryukyu Is., Japan	AY223573, U41895, AF057199, AF057246
(J) Other pitvipers			
Calloselasma rhodostoma	AM A54	Satun Province, Thailand	AF171918, AY352813, AY352779, AY352718
Deinagkistrodon acutus	AM A223	Taitung County, Taiwan	AF171919, AY352811, AY352777, AY352716
Hypnale hypnale	AM A53	Tamil Nadu State, India	AY352750, AY352812, AY352778, AY352717
''O''. chaseni	AM B306	Mt. Kinabalu, Sabah, E Malaysia	AY352760, AY352825, AY352791, AY352729
Tropidolaemus wagleri	AM B132	Perak State, W Malaysia	AF517191, AF517223, AF517167, AF517180
Tro. wagleri	AM B311	Crocker range, Sabah, E Malaysia	AY352759, AY352822, AY352788, AY352727
(K) Outgroups			
Azemiops feae	AM B499	China	AY352747, AY352808, AY352774, AY352713
Daboia russelii	AM A4	C Thailand	AY165090, AY165065, AY352773, AY352712

ROM: Royal Ontario Musuem; CAS: California Academy of Science, San Francisco; NMNS: National Museum of Natural Science, Taiwan ROC; UMMZ: University of Michigan Museum of Zoology; ZFMK: Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; AM: authors' catalogue number; FK: Fred Kraus, field tag; CLP: CL Parkinson, field tag.

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