Comments on the systematic status of specimens belonging to the genus *Viridovipera* (Serpentes: Viperidae: Crotalinae) from Sichuan and Yunnan provinces of southwestern China, with a redescription of *V. yunnanensis*

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Although the genus *Viridovipera* has been relatively well studied recently, the status and diagnosis of *V. yunnanensis* are still unclear. In this study, two samples collected from southwestern Sichuan (Huili), which are putative *V. yunnanensis*, were analysed in a molecular phylogeny for the first time. These two samples formed a well supported clade that was distinct from its congeners. Multivariate morphometric analysis (principal components analysis and canonical variate analysis) including these and 14 other new Chinese specimens showed that male specimens from Tengchong, Nujiang (Yunnan), and northern Burma are very similar to both the holotype and paratypes of *V. yunnanensis* in external morphology, and are morphologically distinct from their congeners. A similar trend was found in two new female specimens from Huili, Sichuan. Other new Chinese specimens can usually be allocated to either *V. stejnegeri* or *V. gumprechti,* but specimens from northeastern India remain ambiguous in their affinities. Several specimens were inconsistently allocated to species in the phylogenetic and the morphometric analysis; this may be a signal of introgression of mtDNA across species boundaries following hybridization between species. We conclude that although *V. yunnanensis* represents a valid taxon, which in parts of its range is sympatric with both *V. gumprechti* and *V. stejnegeri*, the exact morphological and geographical limits of this species are still not clear. Hemipenial characteristics show only subtle and inconsistent differences between species within *Viridovipera* and may not be a useful diagnostic character at the species level. We provide a redescription of the holotype of *V. yunnanensis* and a comparison with similar congeneric species, as a starting point for further investigations of hybridization among them, which ideally should be based on new collections in Sichuan and Yunnan provinces of China, the northeastern region of India and northern Burma, where at least two *Viridovipera* species seem to co-exist.

Key words: hemipenis, morphometrics, northeastern India, phylogenetics, pitvipers, *Viridovipera yunnanensis*

INTRODUCTION

The genus *Viridovipera* was proposed relatively recently for a group of green and arboreal pitvipers within the Asian *Trimeresurus* complex (Malhotra & Thorpe, 2004a), based on a combination of morphometric analysis and molecular phylogenetics. This genus is diagnosed by the possession of short, stout hemipenes with relatively few, large, basal spines (Malhotra & Thorpe, 2004a,b), and is distributed in hilly regions in northern Burma, eastern Thailand, Cambodia, Laos, central and northern Vietnam, northeastern India, Xizang and southern China (Malhotra & Thorpe, 2004b; Gumprecht et al., 2004; Vogel, 2006). Most species in the genus share a common colour pattern consisting of a uniform green coloration with a white lateral line on the first dorsal scale row, often also with red lateral lines and postocular stripes. The one known exception, the banded *V. truongsonensis*, was only recently allocated to this genus following mtDNA analysis (Dawson et al., 2008). Despite this striking similarity in external appearance among most species, there is considerable within-species geographic

variation and sexual dimorphism. This has led to substantial taxonomic uncertainty (Stejneger, 1927; Pope & Pope, 1933; Regenass & Kramer, 1981), and has effectively impeded field identification (particularly among the species formerly included within *V. stejnegeri*). The recent study on this species group (formerly referred to as the *Trimeresurus stejnegeri* group) identified a few vexing taxonomic issues that still needed to be resolved (Malhotra & Thorpe, 2004b). The most significant of these concerns the status of *V. yunnanensis* (Schmidt, 1925), the type specimens of which are from Tengyueh (Tengchong Co.) in Yunnan province, China. Although it was subsequently recognized as a subspecies of *T. stejnegeri* for a long time, Zhao (1997) raised it to specific rank based on its scalation and geographical distribution compared to *T. stejnegeri*. This species is believed to be distributed in southwestern China (Yunnan and Sichuan provinces), northeastern India and Burma (David & Ineich, 1999; Gumprecht et al., 2004; Vogel, 2006).

However, previous work showed that more than one taxon is present in southwestern China (Zhao et al., 1998; Gumprecht et al., 2004; Zhao, 2006). Malhotra & Thorpe

(2004b) showed that *V. gumprechti* (as determined by DNA analysis) is also present at the type locality of *V. yunnanensis.* Furthermore, the female paratype of *V. yunnanensis* that these authors examined (the holotype had not been examined at that time) was morphologically similar to *V. gumprechti,* although the male paratype was somewhat distinct. They therefore suggested that there was a possibility that *V. gumprechti* and *V. yunnanensis* were synonyms, but that further sampling was needed in this region before the issue could be satisfactorily resolved.

Pope (1935) described the hemipenis of *Trimeresurus stejnegeri yunnanensis* (now *Viridovipera yunnanensis*) as being very similar to that of *T. s. stejnegeri* (i.e. short, stout, and not deeply forked); Malhotra & Thorpe (2004a, b) did not comment further. Recently, a hemipenial comparison of five Asian pitvipers was conducted (Guo et al., 2006) and the hemipenial structure of a putative *V. yunnanensis* specimen collected from Sichuan was found to be different from that described by Pope (1935), as well as different from its congeners *V. stejnegeri*, *V. gumprechti* and *V. vogeli*, in that it possesses a slightly more deeply forked hemipenis, similar to that of *V. medoensis* (Guo & Zhang, 2001). This inconsistent result led us to explore whether the specimens collected from southwestern China, that have previously been assigned to *V. yunnanensis*, in fact represent this species or yet another cryptic species within this genus.

In the present work, the hemipenis of a paratype of *Viridovipera yunnanensis* from the type locality was examined in detail, and compared with those of its congeners described before (Guo & Zhang, 2001; Mao et al., 1984; Guo et al., 2006). We also added morphological data from additional specimens of *Viridovipera* from Sichuan, Hainan, Yunnan and Fujian provinces to the morphometric trait analysis conducted by Malhotra & Thorpe (2004b), these being regions which were either not included or were poorly represented in the previous work. We also included the holotypes of both *V. yunnanensis* and *V. stejnegeri.* We then reconstructed a molecular phylogeny based on two mitochondrial DNA (mtDNA) genes which included new data from two new specimens (the male examined for hemipenial morphology and a female) collected from the same locality in Sichuan Province, and two additional specimens of *Viridovipera* spp. Our aims are to explore whether the currently recognized *V. yunnanensis* is a valid species, to study its phylogenetic position, and to provide a redescription of the species to aid diagnosis.

MATERIALS AND METHODS

Morphometric trait analysis and hemipenial description

Sixteen new specimens were examined (Appendix 1); the data from these were added to the dataset analysed in Malhotra & Thorpe (2004b). As data for internal anatomy were missing for many museum specimens where permission had not been granted for the necessary dissections, our analyses included only external characters such as scalation, colour pattern and shape. Males and females

were analysed separately due to the presence of sexual dimorphism. We first ran a principal components analysis (PCA) using exactly the same procedure as described in Malhotra & Thorpe (2004b). Briefly, this involved first screening all characters for significant between-locality differences using one-way analysis of variance and covariance (ANOVA/ANCOVA). Non-significant characters were not used in subsequent PCA analyses. Prior to PCA, all size-correlated characters were adjusted using the pooled within-group regression coefficient against either snout–vent length (SVL) or head length (LHEAD) to remove size-related bias. A few characters were highly correlated with each other ($r>0.7$), indicating that they may result in over-emphasis of the correlated variables in PCA (Thorpe, 1976). Thus, only one of the characters from the correlated character sets was used in PCA.

Subsequently, we carried out a canonical variate analysis (CVA), which has greater discriminatory power. However, it requires specimens to be assigned to groups *a priori*, which is inappropriate in the case of specimens with uncertain affinities. In this case, the species *V. stejnegeri*, *V. gumprechti* and *V. vogeli* have been well studied and their specific limits established through a combination of morphological and phylogenetic analysis. Thus, we allocated specimens assigned to these three species to groups and canonical axes were calculated based on these groups alone. All other specimens of uncertain affinity, including all new specimens and all putative *V. yunnanensis* specimens, were plotted on the resulting axes individually. CVA plots were compared to those from the PCA as a check for perturbation of the CVA by potential heteroscedasticity in the data. If present, this was controlled by using the scores of a PCA carried out on ordinal characters rather than the raw character data in the CVA. Since the CVA takes character correlation into account, and enters characters into the analysis based on their initial *F*-ratios between groups, we did not adjust or screen characters prior to the analysis. Finally, once specimens had been allocated to species based on the above procedure, we calculated the discriminant function between pairs of species to allow diagnosis of the species on the basis of external morphological characters.

The hemipenes of the holotype (AMNH 21058) and a paratype of *Viridovipera yunnanensis* (FMNH 7065) from the type locality were examined, and compared to that of a male specimen (SCUM 035114) from Huili County, Sichuan (Guo et al., 2006). The dissection methods and description terminology were based on Dowling & Savage (1960). The retracted hemipenis was dissected and internal structure was examined and recorded with the aid of a stereomicroscope. Both overall length and position of the fork are indicated by the number of the adjacent subcaudal scale, or by direct measurement by digital callipers from the base of the cloacal opening.

DNA isolation, amplification and sequencing

Whole genomic DNA was extracted from 0.01–0.02 g of liver, muscle preserved in 90% ethanol, or blood preserved in buffer with Sigma GenElute Mammalian Genomic DNA Miniprep Kits. Sections of two mitochon-

Table 1. Details of specimens included in the phylogenetic analysis. NMNS: National Museum Of Natural Science, Taiwan, China; ROM: Royal Ontario Museum, Toronto; ZMB: Museum für Naturkunde, Humboldt-Universität, Berlin; AM, RTV & GP: the authors' personal collections. The remaining abbreviations are listed in Appendix 1. Asterisks indicate specimens sequenced for this paper.

Species	Localities (province/ state, country)	Voucher nos.	GenBank accession numbers (cyt *b*, ND4)
Bothrops atrox	–	–	AF191587, AF246277
Cryptelytrops albolabris	Gia Lai, Vietnam	AM B183/ROM 34544	AY352770, AY352838
C. cantori	Nicobar Islands, India	AM A85	AF171889, AY352836
C. insularis	Java, Indonesia	AM A109	AY352767, AY352833
C. macrops	Bangkok, Thailand	AM B27	AF517184, AF517219
C. septentrionalis	Kathmandu, Nepal	AM B487	AY352755, AY352818
C. venustus	Thailand	AM A241	AF171914, AY293930
Garthius chaseni	Sabah, Malaysia	AM B306	AY352760, AY352825
Gloydius shedaoensis	Liaoning, China	–	AY223566, AY223623
Himalayophis tibetanus	Helambu, Nepal	AM B258/ZMB 65641	AY352749, AY352810
Hypnale hypnale	Tamil Nadu, India	AM A53	AY352750, AY352812
Ovophis monticola	China	AM B482	AY352748, AY352809
Parias hageni	Songhkla, Thailand	AM B33	AY059567, AY059585
P. flavomaculatus	Ryukyu Is., Japan	AM B4	AY223574, U41894
P. malcolmi	Sabah, Malaysia	AM B349	AY371832, AY371861
P. sumatranus	Bengkulu, Sumatra	AM B367	AY371824, AY371864
Popeia popeiorum	Phetburi, Thailand	AM B34	AY059572, AY059591
P. popeiorum	Phongsaly, Laos	AM B196/FMNH 258950	AY059571, AY059590
P. popeiorum	Selangor, Malaysia	AM B246	AY059570, AY059589
Protobothrops mangshanensis	Hunan, China	AM B300	AY352758, AY352821
P. sieversorum	Khammouane, Laos	AM B162/FMNH 255258	AY352753, AY352816
P. xiangchengensis	Sichuan, China	GP26/ SCUM 035042	DQ666061, DQ666058
Trimeresurus borneensis	Sabah, Malaysia	AM B301	AY352754, AY352817
T. gracilis	Taiwan, China	AM A86	AF171913, AY352823
T. gramineus	Tamil Nadu, India	AM A220	AY352761, AY352827
T. malabaricus	Tamil Nadu, India	AM A218	AY059569, AY059587
T. puniceus	Bengkulu, Sumatra	AM B392	AY352757, AY352820
Viridovipera medoensis	Kachin, Myanmar	AM B416/CAS 221528	AY352765, AY352831
V. gumprechti	Nghe An, Vietnam	AM B174/FMNH 255579	AY059573, AY059595
V. gumprechti	Yunnan, China	AM B497	AY321489, EF597530
*V. gumprechti**	Sapa, Vietnam	AM B551	EF597525, EF597531
V. gumprechti	Yunnan, China	AM B553	AY321488, EF597532
V. stejnegeri	Yunnan, China	AM B15	AY321487, AY352832
*V. stejnegeri**	Hainan, China	GP44/SCUM 035002	EF597524, EF597526
V. stejnegeri	Taiwan, China	AM A160/NMNS 2558	AF171896, AY059593
V. stejnegeri	Fujian, China	AM A222/NMNS 3651	AF277677, AY059594
V. stejnegeri	Vin Phuc, Vietnam	AM B109	AF277709, EF597529
V. vogeli	Thailand	AM B97	AY059574, AY059596
V. vogeli	Champassak, Laos	AM B125	AY059581, AF517225
*V. yunnanensis**	Sichuan, China	GP37/SCUM 035045	EF597522, EF597527

drial genes (cytochrome *b* or cyt *b*, and NADH dehydrogenase subunit 4 or ND4) were amplified and sequenced as described in Malhotra & Thorpe (2000) and Arévalo et al. (1994) respectively. PCR products were cleaned prior to sequencing using shrimp alkaline phosphatase (SAP) to dephosphorylate residual deoxynucleotides and Exonuclease I to degrade excess primers (Werle et al., 1994). PCR products were then sequenced using dye-labelled terminators (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit) and run on an ABI 3730XL automated sequencer.

The dataset analysed included 41 OTUs representing all currently recognized *Viridovipera* species and representatives of other Asian pitviper genera, which could confirm the monophyly of the new specimens within *Viridovipera*. *Hypnale hypnale* was chosen as outgroup based on more extensive analyses of pitviper phylogeny (Malhotra & Thorpe, 2004a; Castoe & Parkinson, 2006). A few sequences were retrieved from GenBank and previously unpublished sequences used in this study have been deposited in GenBank (Table 1). Alignment of sequences was straightforward as there were no indels. The
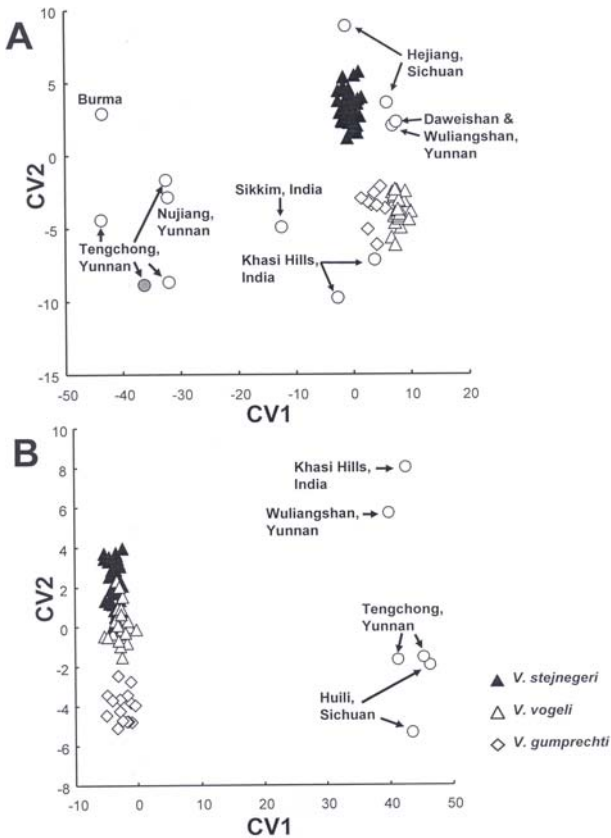
Fig. 1. Plot of first and second canonical variates from CVA on external morphology of *Viridovipera* species. A) Males, B) females. Only the well-established species *V. stejnegeri*, *V. vogeli* and *V. gumprechti* were used in the calculation of the axes. All other specimens (indicated by circles) are plotted on the axes individually. The male holotype of *V. yunnanensis* is indicated by a filled grey circle.

cyt *b* and ND4 sequences were translated into amino acid sequences using MEGA version 3.0 (Kumar et al., 2001) to check for the unexpected occurrence of stop codons, which might indicate the inadvertent amplification of pseudogenes (Zhang & Hewitt, 1996). Sequences of the two genes were concatenated and analysed as a single matrix because these regions belong to a single linkage group (mt genome) and have been shown to evolve similarly (Parkinson et al., 2002).

Both unweighted parsimony and Bayesian Markov chain Monte Carlo (MCMC) approaches were used to reconstruct phylogenies, using PAUP* 4.0b10 (Swofford, 2003) and MrBayes v3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) respectively. Parsimony searches were heuristic, with starting trees obtained by random addition with 100 replications and tree-bisection–reconnection (TBR) branch swapping. Confidence in the inferred branches of the optimal trees was obtained by bootstrapping (1000 pseudoreplicates) with the search strategy modified to use only 10 replications of the start tree.

Modeltest 3.7 (Huelsenbeck & Crandall, 1997; Posada & Crandall, 1998, 2001) was run to infer the simplest best-fit model of evolution for the combined data set prior to Bayesian analyses. Four separate runs were performed starting from a random tree, and each independent run had four Markov chains (three heated chains and a single cold chain). Each of these runs was conducted with a total of 10 million generations and sampled every 100 generations, the first 1000 of which were discarded as burn-in. Stationarity was confirmed by plotting the –ln*L* per generation in the program Tracer 1.3 (Rambaut & Drummond, 2003). After confirming that the four analyses reached stationarity at a similar likelihood score and that the topologies were similar, all the resultant trees were combined to calculate posterior probabilities for each bipartition in a 50% majority-rule consensus tree.

RESULTS

Morphometric trait analysis

Comparison of CVA and PCA indicated a problem with heteroscedasticity in the data. Consequently, we used PCA scores in the CVA rather than raw character data for ordinal characters, in both sexes. The resulting CVA plots were similar to the PCA plots, but with much clearer discrimination between putative *V. yunnanensis* and the other species (Fig. 1). Five males from Yunnan (including the holotype and two paratypes from Tengchong, and Nujiang) and another from northern Burma were all well discriminated from *V. stejnegeri*, *V. vogeli* and *V. gumprechti* as indicated by the considerable distance on CV1 separating them from these three species. Other specimens from Yunnan (Wuliangshan) and Sichuan (Hejiang) are plotted in much closer proximity to *Viridovipera stejnegeri,* while two specimens from northeastern India (Shillong, Khasi Hills) are morphologically more similar to *V. gumprechti*. However, a specimen from Sikkim (India) was plotted in a somewhat intermediate position between *V. gumprechti* and the specimens from Tengchong, Nujiang and Burma.

In females, specimens from Sichuan (Huili), Yunnan (Tengchong, Wuliangshan), as well as northeastern India (Shillong, Khasi Hills), show a close morphological similarity while being well differentiated from the three predefined species.

Hemipenial description and comparison

The retracted hemipenes of specimens AMNH 21058 (holotype of *V. yunnanensis*) and FMNH 7065 (paratype) from Tengchong, Yunnan were found to be very similar. The following description is based on FMNH 7065: the hemipenis extends to the end of the 8th subcaudal plate, and forks at the level of the end of the 5th subcaudal scale (by measurement, this was determined to be approximately 70% of the length of the hemipenis measured from the base of the cloacal opening). The organ is calyculate distally, and spinous proximally. The spinous area, which contains approximately 11 large stout spines per lobe, is larger than the calyculate area. The spines closer to the calyculate area are smaller, and tend to become larger closer to the base of the organ. A few scattered minute
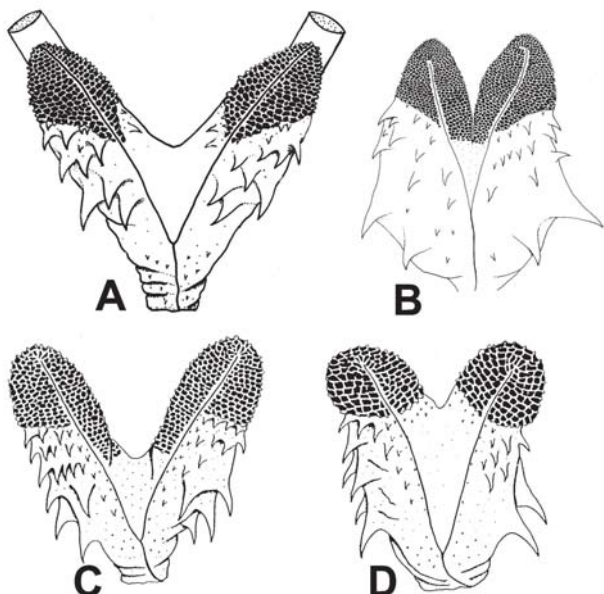
Fig. 2. The hemipenis of *Viridovipera yunnanensis*, based on FMNH 7065 paratype from Tengchong, Yunnan, China (A), compared to those of the putative *V. yunnanensis* specimen from Huili, Sichuan (B) (Guo et al., 2006), *V. medoensis* (C) (Guo & Zhang, 2001), and *V. stejnegeri* (D) (Guo & Zhang, 2001).

spines are present on the base of the organ (Fig. 2A). The demarcation between the spinous and calyculate area is distinct, but does not extend in a straight line, because the calyces adjacent to the sulcus occur closer to the base of the organ. The sulcus is divided at the level of the 2nd subcaudal, near the base of the organ. *Musculus retractor penis magnus* originates at the level of the 21st subcaudal, and is divided at the 11th subcaudal.

Hemipenes of two specimens from Huili, Sichuan described in Guo et al. (2006; also shown in Fig. 2B) are similarly calyculate distally and spinous proximally with stout spines present in the spinous area but are reported to be forked more deeply than that of *Viridovipera stejnegeri* (Fig. 2D), similar to that reported for *V. medoensis* (Guo & Zhang, 2001; Fig. 2C) and *V. truongsonensis* (Dawson et al., 2008). *V. stejnegeri* hemipenes are typically unforked for about 70% of their length (unpublished data). Thus, the hemipenes of the type material of *V. yunnanensis* resembles the condition in *V. stejnegeri* more than that of the Huili specimens. The deeper forking, however, is also present in specimens from the Khasi Hills and Sikkim in north eastern India, and Daweishan, Yunnan (where only about 50–55% of the organ is unforked).
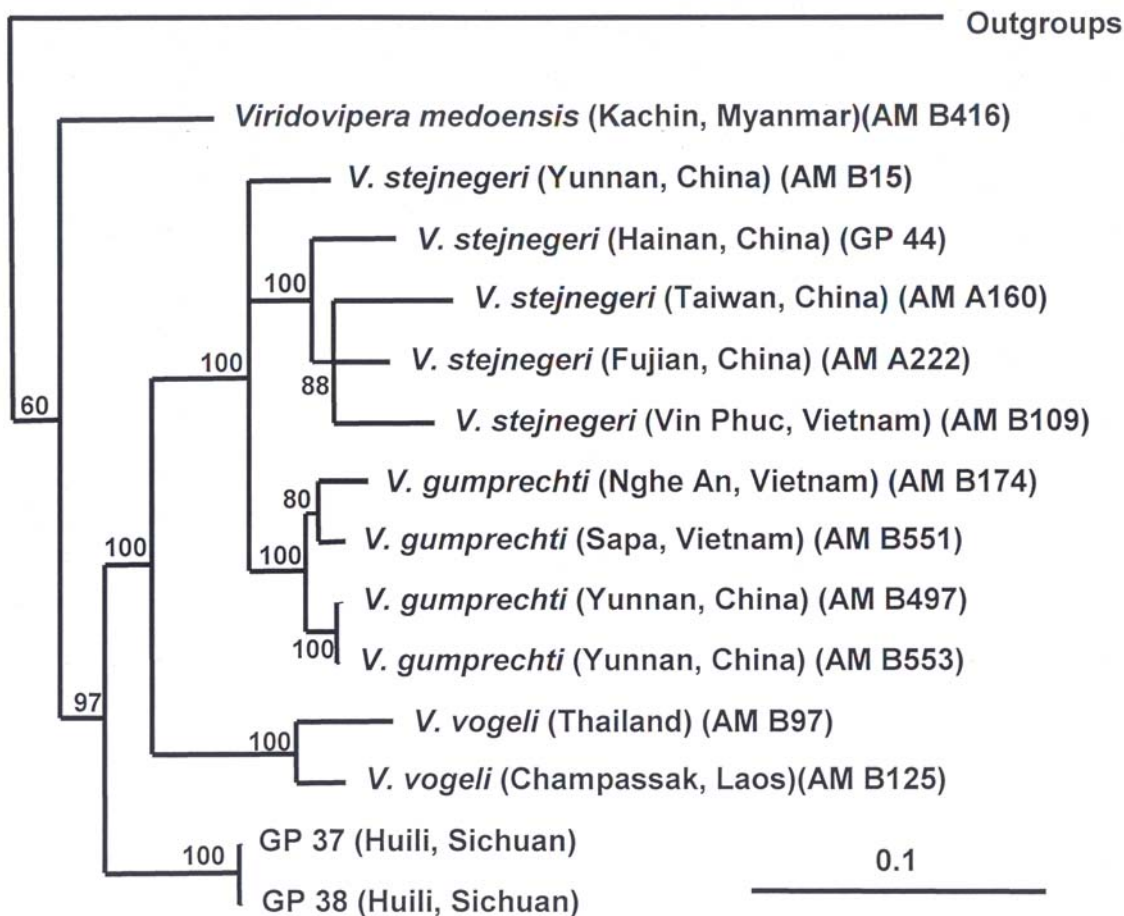

Fig. 3. 50% consensus tree from Bayesian phylogenetic inference based on two mtDNA genes. Posterior probabilities for the clades are shown adjacent to the node to which they refer.

Phylogenetic analysis

The final data set consisted of a total of 1339 base pairs (bp) of sequence data: 731 bp of cyt *b* and 608 bp of ND4. Of these, 675 characters were invariable and 523 were parsimony-informative characters. There was no indication that pseudogenes have been amplified as no deletions, insertions or stop codons were detected in the two coding regions. The chi-square test for the homogeneity of base frequencies showed that base composition was not significantly different in all taxa included (χ^2=54.9257, df=120, *P*>0.99). The mean values of parameters estimated by MrBayes were: base frequencies A= 0.357, C = 0.356, G = 0.070, T = 0.216; alpha = 1.023, pinvar = 0.448.

Modeltest indicated that the GTR+I+Γ was the simplest best-fit model for the combined mitochondrial data set as defined by the Akaike information criterion (AIC). As the focus of the present research is on the phylogenetic position of *V. yunnanensis*, only *Viridovipera* species are included in the tree and the remaining are shown as outgroups together. The resulting Bayesian inference (BI) tree (Fig. 3) had a mean likelihood score of ln *L*= –14078.681. The parsimony analysis produced 43 trees with length = 3015 steps, CI = 0.326, RI = 0.468, RC = 0.153 (not shown), which are similar to the Bayesian tree (Fig. 3) but with a slight difference in the topology. In the *Viridovipera* clade, the BI tree showed

the following relationship: (*V. medoensis*, (*V. yunnanensis*, ((*V. stejnegeri*, *V. gumprechti*), *V. vogeli*))), while the topology (*V. medoensis*, *V. yunnanensis*, (*V. stejnegeri*, *V. gumprechti*, *V. vogeli*)) was indicated in the 50% majority-rule consensus tree of 43 equally parsimonious trees. Additionally, all putative *V. stejnegeri* did not form a monophyletic group in the 50% consensus MP tree, as in the BI tree (Fig. 3). In the BI tree, all putative *Viridovipera* species formed a monophyletic group, although the inclusion of *V. medoensis* within this clade is not highly supported (PP = 60). However, the newly added specimens are all included within a very strongly supported clade containing all other *Viridovipera* species (PP = 97). The two samples representing putative *V. yunnanensis* from Huili, Sichuan (one corresponding to the male examined for hemipenial morphology, and the other to a female included in the morphometric analysis), formed a distinct clade within this group.

DISCUSSION

The validity of *Viridovipera yunnanensis* and its geographical limits

The additional male specimens of putative *Viridovipera yunnanensis* (including the holotype) from Yunnan (Nujiang and Tengchong) are shown to be very similar in
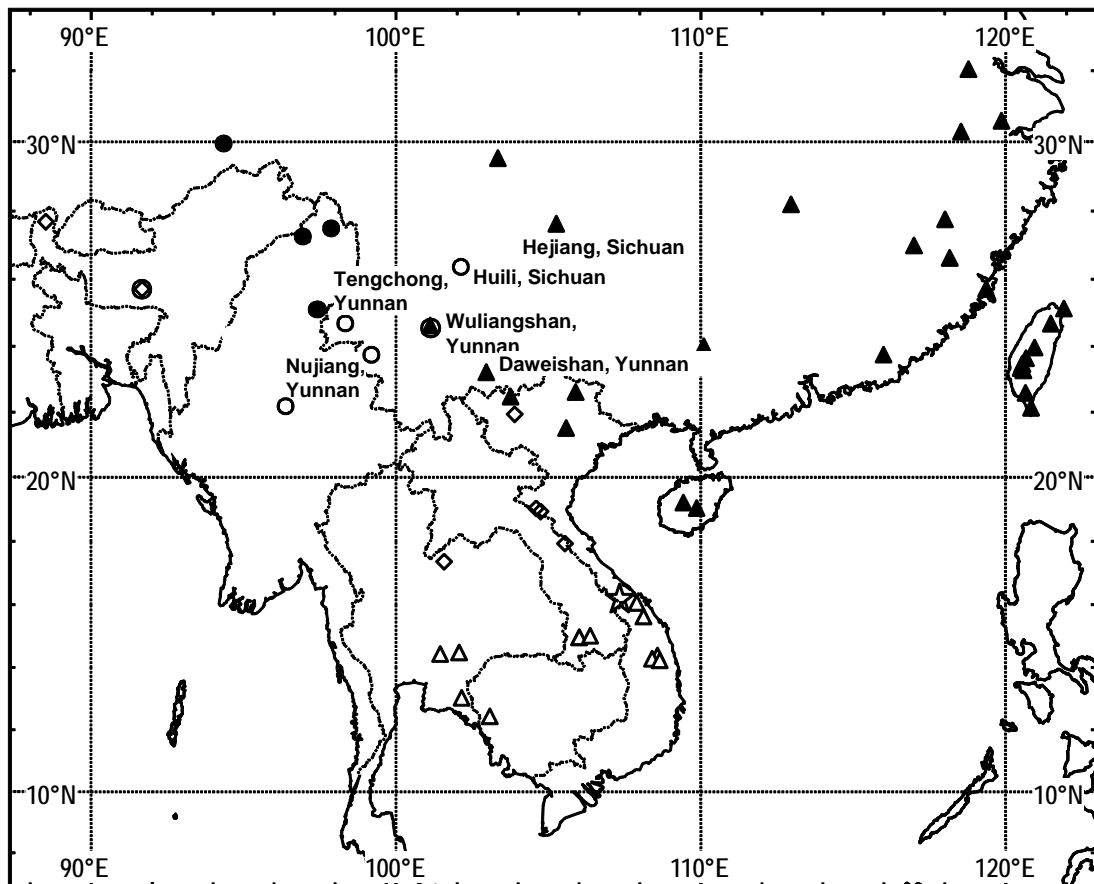

Fig. 4. The distribution of *Viridovipera* species (*V. stejnegeri* s.s.: solid triangles; *Viridovipera vogeli*: empty triangles; *V. gumprechti*: empty diamonds; *yunnanensis*: empty circles; *V. medoensis*: solid circle; *V. truongonensis*: empty star.

Table 2. Mean values, standard deviation and range (in parentheses) of morphological characters important in multivariate discrimination between *V. yunnanensis* and the other three species (see text). Size-related characters (EYE2NOS and NOS2PIT) have been adjusted to a common head length of 29.66 mm; the VS position (e.g. VS21TO19) was transformed into the percentage of the total number of ventral scales (%VS). Characters are listed in order of magnitude of their contribution to the ordination, and their abbreviations are explained in Appendix 1.

	V. yunnanensis	*V. stejnegeri*	*V. vogeli*	*V. gumprechti*
Males	*n*=6	*n*=51	*n*=25	*n*=10
VS	157.14±5.08 (152–168)	162.96±5.00 (152–175)	164.26±5.17 (157–173)	165.90±2.88 (161–171)
VS21TO19	0.15±0.14 (0.09–0.46)	0.64±0.03 (0.51–0.74)	0.60±0.11 (0.10–0.66)	0.61±0.03 (0.55–0.66)
SUPLAB	9.07±0.53 (8.50–10.00)	9.86±0.77 (8.50–12.00)	9.96±0.71 (8.00–11.00)	10.15±0.41 (9.50–11.00)
SCRSTR	1.29±0.49 (1.00–2.00)	1.82±0.43 (0.00–2.00)	1.80±0.41 (1.00–2.00)	1.90±0.32 (1.00–2.00)
BTWSUP1	8.00±0.58 (7.00–9.00)	10.47±1.24 (8.00–15.00)	10.28±1.02 (9.00–12.00)	10.40±0.97 (9.00–12.00)
NOS2PIT	5.37±0.33 (4.92–5.74)	5.50±0.41 (4.73–6.76)	6.02±0.71 (4.58–7.95)	5.99±0.27 (5.49–6.50)
EYE2NOS	6.03±0.20 (5.77–6.40)	5.77±0.66 (1.76–6.61)	6.42±0.64 (5.66–8.89)	6.29±0.31 (5.94–6.90)
VS19TO17	0.64±0.04 (0.56–0.68)	0.69±0.03 (0.61–0.77)	0.65±0.03 (0.51–0.70)	0.67±0.02 (0.65–0.70)
Females	*n*=4	*n*=43	*n*=23	*n*=15
VS21TO19	0.11±0.02 (0.08–0.14)	0.65±0.02 (0.59–0.70)	0.63±0.02 (0.55–0.66)	0.63±0.03 (0.57–0.69)
SC	58.35±3.03 (54.00–61.00)	63.88±3.79 (54.00–71.00)	60.91±3.41 (52.00–70.00)	58.07±3.75 (51.00–66.00)
VS19TO17	0.65±0.05 (0.61–0.71)	0.69±0.02 (0.65–0.73)	0.66±0.01 (0.64–0.69)	0.68±0.02 (0.65–0.73)
KTEMP	0.00±0.00 (0.00–0.00)	0.24±0.23 (0.00–0.50)	0.02±0.10 (0.00–0.50)	0.15±0.18 (0.00–0.50)
EYE2NOS	7.15±0.63 (6.37–7.90)	6.18±0.46 (5.20–7.66)	7.09±1.71 (5.55–11.98)	6.82±0.33 (6.18–7.34)
DV25TO23	5.85±0.24 (5.50–6.00)	7.52±3.13 (3.00–12.00)	5.76±1.67 (4.00–12.00)	5.50±0.53 (4.00–6.00)
SUBLAB	11.63±0.48 (11.00–12.00)	12.52±0.75 (11.50–14.50)	13.15±0.71 (11.50–14.00)	12.97±0.81 (11.50–14.50)
KHEADSC	0.00±0.00 (0.00–0.00)	0.21±0.28 (0.00–1.00)	0.03±0.11 (0.00–0.50)	0.07±0.15 (0.00–0.50)
SUPLAB	9.63±0.48 (9.00–10.00)	10.14±0.90 (9.00–13.50)	10.67±0.65 (10.00–12.00)	10.57±0.42 (10.00–11.50)

external morphology to the male paratype of this species already represented in Malhotra & Thorpe's (2004b) analysis. Furthermore, these specimens are all morphologically distinctly distinguishable from similar congeneric species *V. gumprechti*, *V. vogeli* and *V. stejnegeri* (Fig. 1). Female specimens from Huili, Sichuan, which have long been identified as *Viridovipera yunnanensis* (Zhao et al., 1998; Vogel, 2006), were also shown to be morphologically similar to the female paratype of *V. yunnanensis* (Tengchong, Yunnan). The molecular phylogeny indicated that the specimens from Huili are also genetically very distinct from congeners (Fig. 3). Therefore, our results based on both molecular phylogenetic and morphometric analyses support the recognition of this taxon at the specific rank as *V. yunnanensis*.

However, for other specimens the position is far less clear-cut. A single male specimen from Sikkim (India) occupies an intermediate position between *V. gumprechti* and *V. yunnanensis* (Fig. 1A). Although this specimen has 19 rows at mid-body, the reduction from 21 to 19 scale rows occurs further towards mid-body than the specimens clearly allocated to *V. yunnanensis*. Furthermore, this specimen has a rather irregular scale reduction pattern with the 21st scale row appearing and disappearing many times subsequently, within the space of a few ventral scales. Thus, it is likely to be only this equivocal characteristic that is displacing it towards the area of morphospace occupied by *V. yunnanensis*, and otherwise it may be more similar to the other male northeastern Indian specimens (from the Khasi Hills in Meghalaya State) in being placed much closer to *V. gumprechti* (Fig.

1A). In females, a specimen from the Khasi Hills clearly lies within the morphospace occupied by *V. yunnanensis* (Fig. 1B). Thus, it is likely that *V. gumprechti* and *V. yunnanensis* are sympatric, as already suggested by Malhotra & Thorpe (2004b). The present analysis also shows that male specimens from Hejiang, Sichuan (males only) and Wuliangshan, Yunnan are morphometrically more similar to *V. stejnegeri* (Fig.1A), while a female from Wuliangshan groups with *V. yunnanensis*. However, an added complication arises out of the fact that both male and female specimens from Wuliangshan are represented in the phylogenetic analysis (B497 and B553 respectively) and are grouped together with high support with other *V. gumprechti* samples from Vietnam (Fig. 3). This inconsistency in species allocation based on morphological and DNA data is hard to resolve, although it might arise out of occasional hybridization between sympatric species, leading to introgression of mtDNA across species boundaries. This scenario would require a nuclear marker that is variable enough at the species level to confirm it. If true, multivariate morphometrics are less likely to be misled than the DNA tree, as the former is determined by multiple loci in the nuclear genome, while the latter represents a single mitochondrial locus.

Thus, we tentatively conclude that *V. yunnanensis* is potentially sympatric with *V. stejnegeri* in Sichuan Province, China, while it is potentially sympatric with *V. gumprechti* in northeastern India. However, determination of the exact range of these species in this region awaits further study, preferably based on new collections that would allow DNA and morphometric data to be gathered from the same specimens. Our current

understanding of the distribution of *Viridovipera* species, which is modified from that in Malhotra & Thorpe (2004b), is illustrated in Figure 4.

Comparisons of hemipenial characters between species

The hemipenes of the Huili specimens allocated by both phylogenetic analysis of mtDNA and morphometric analysis in this study to *V. yunnanensis* were described in Guo et al. (2006) as calyculate distally, spinous proximally, and relatively deeply forked. In the last characteristic, it appears to shows a closer similarity to *V. medoensis* and *V. truongsonensis* than to *V. vogeli*, *V. gumprechti* and *V. stejnegeri* (Mao et al., 1984; Guo & Zhang, 2001; David et al., 2001, 2002). However, this characteristic does not seem consistent among all specimens allocated to *V. yunnanensis* in our analysis, with specimens from northern Burma and the holotype and paratype from Tengchong falling within the range measured in *V. stejnegeri* (forking occurring at about 70% of the total length of the hemipenis). On the other hand, specimens from northeastern India, which more closely resemble *V. gumprechti* in the morphometric analysis and for which no mtDNA data are available, do appear to have more deeply forked organs. This inconsistency may at least partly be due to preservation effects and the difference between examining the inverted compared to the everted hemipenes. It is common, for example, for only partial eversion of the forked regions to occur during preparation of the organ, and this is known to have led to many misunderstandings in the past (Malhotra & Thorpe, 2004a). Thus, at the moment, the depth of forking of the hemipenis does not seem to be a reliable diagnostic character at the species level. However, all known *Viridovipera* species share several characters, e.g. the base of the organ bears minute spines, and the demarcation between spinous area and calyculate area is distinct, but not in a straight line.

Redescription of *Viridovipera yunnanensis* Schmidt, 1925

Trimeresurus yunnanensis Schmidt Amer. Mus. Novitates 157:1–5 [4]. Type locality: Tengyueh [= Tengchong County], Yunnan Province, China. Holotype: AMNH 21058, male.

Description of the holotype. Total length 716 mm (SVL: 571 mm, tail: 145 mm). Ventral scales and subcaudals number 155 and 66 respectively, nine supralabials and 10/11 sublabials. The fourth and fifth supralabials are separated from the subocular by one and two scales respectively. There are three preoculars and two postoculars; six scales directly border the supraocular scales. The supraocular scales are separated by a minimum of eight scales, while the posterior edges of the supraoculars are separated by 14 scales. Six or seven scales contact the subocular (not counting the scales immediately before and after it). The number of scales between the edge of the mouth and the ventral scales (starting at, and including, the last sublabial) is 6/7. The rostral scale is roughly trapezoid in shape, with the top edge approximately one-third the length of the bottom edge. There are 19 scale rows at mid-body, and the reduction from 21 to 19 dorsal scale rows occurs at the level of the 13th and 14th ventral scale positions on right and left sides respectively, while the reduction to 17 dorsal scale rows occurs at the level of the 94th and 95th ventral scales. The head is somewhat rounded in appearance and is 29.15 mm long and 14.55 mm wide at the level of the posterior edge of the supraoculars, while it is 23.5 mm at its widest point at the rear of the jaws. The diameter of the eye is 4.25 mm, and the distance between the anterior edge of the eye and the posterior angle of the pit is 2.00 mm. The right supraocular is 5.50 mm long and 2.30 mm wide. Mid-body scales are weekly keeled, while scales on the head are smooth. The lower 50% of the first scale row (at mid-body) is covered by traces of red pigment, while above this another 20% of the scale is covered by the white patch. The lateral bicoloured stripe extends as far as the head and on to the tail until around the tenth subcaudal scale. Neither bands nor spots are apparent on the body. There is no trace of an ocular stripe or lighter coloration on the lip scales. There are 11 teeth on the pterygoid and 13 on the dentary.

Variation within V. yunnanensis. Females have a relatively longer tail and larger body than males (the maximum recorded SVL and tail length for females is 778 mm and 173 mm vs 637 mm and 138 mm for males). The females also have slightly more ventral scales (average 157.14 vs 156.13) and subcaudals (62 vs 58 on average). Head scales are always smooth. Supralabials vary between 10 and 12, and sublabials between eight and 10. The minimum number of scales between supraoculars varies between seven and 11 and there are between 12 and 16 scales between the posterior edges of the supraoculars. There are between two and four postocular scales and 6–8 scales border the subocular scale (not counting the pre- and post-oculars). The number of small scales between the nasal scale and the shield bordering the anterior of the pit varies between one and two, and there may be 0–2 scales separating the internasal scales. There are 1–2 scales between the 5th supralabial and the subocular scale. There are 19 scale rows at mid-body, with the reduction from 21 to 19 dorsal scale rows occurring between the level of the 13th and 21st ventral scales (the average being 15.9).

Comparison with the other species

Viridovipera yunnanensis was diagnosed mainly on the basis of having only 19 scale rows at mid-body (Schmidt, 1925) vs 21 in *V. stejnegeri* (the only other related species known at the time). On the basis of our present analysis, this character does play a vital role in distinguishing it from its congeners (Table 2), but is more reliably measured as the distance along the body that the reduction from 21 to 19 scale rows occurs. In both sexes, *V. yunnanensis* has a more anterior occurrence of the reduction of dorsal scale rows from 21 to 19 (VS21TO19); its occurrence does not exceed the 20th ventral scale in any of the specimens examined here (except BMNH 53.8.12.14 from Sikkim, discussed in more detail above). A similar tendency also occurs in VS19TO17. Our analysis also shows that there are several additional characters that are of importance in

discriminating *V. yunnanensis* from its relatives. In males, *V. yunnanensis* has a lower number of ventral scales, fewer supralabials (SUPLAB), fewer scales between the supraoculars (BTWSUP1), at most an indistinct postocular stripe (OCSTR), and a shorter snout, as measured by the distance between the posterior edge of the pit and the nostril (NOS2PIT). In females, *V. yunnanensis* could also be differentiated from the other species by having a lower number of sublabial (SUBLAB) and supralabial (SUPLAB) scales, no keeling on head scales (KTEMP and KHEADSC), and a longer snout, as measured by the distance between the anterior edge of the eye and the nostril (EYE2NOS). The mean value and ranges for these characters, which in combination help to distinguish *V. yunnanensis* from the other three species, are listed in Table 2.

ACKNOWLEDGEMENTS

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APPENDIX 1

Specimens added for morphometric analysis

Abbreviations

AFS & AM: author's personal collection, field numbers; AMNH: American Museum of Natural History, New York; BMNH: Natural History Museum, London; CAS: California Academy of Sciences, San Francisco; CIB: Chengdu Institute of Biology, Chinese Academy of Sciences; FMNH: Field Museum of Natural History, Chicago; HNU: Hainan Normal University, China; MCZ: Museum of Comparative Zoology, Harvard; MHNG: Museum d'Histoire Naturelle, Geneva; NMNS: National Museum of Natural Science, Taiwan; NMW: Naturhistorisches Museum Wien; PLWRS: Phu Luang Wildlife Research Station, Thailand; RNHM: Rijksmuseum Van Natuurlijke Historie, Leiden; ROM: Royal Ontario Museum, Toronto; RTV: author's live collection; SCUM: Sichuan University Museum, China; SNHM: Shanghai Natural History Museum, China; USNM: United States National Museum of Natural History, Smithsonian Institute, Washington; YBU: Yibin University, China.

Specimens

Specimens in italics have not previously been analysed.

Sikkim, India: BMNH 53.8.12.14.

Khasi Hills, NE India: BMNH 60.3.19.1121, BMNH 1907.12.16.27, NMW 23805.

N Burma: BMNH 1901.4.26.7.

Yunnan, China: *AMNH 21057–58,* BMNH 2002.53–54, *CAS 215141, CIB 14361, CIB 14363*, FMNH 7064–65, NMNS 3113.

Sichuan, China: FMNH 170642, *CIB 13875–76, YBU 061003–04.*

SE China: SNHM 112, SNHM 720068, SNHM 729159, MCZ 163259, AMNH 33222–9, BMNH 99.4.24.61, BMNH 54.2.10.18, FMNH 25196–204, USNM 73140, NMNS 3651: 12347, 12349, 12351, 12354, NMW 23913:1, CAS 71957, USNM 64022–23, *AMNH 21053–54.*

Taiwan: FMNH 96807–11, FMNH 96816, FMNH 120772–75, NMNS 01882–87, NMNS 01889, NMNS 01334:1–3, NMNS 01549, NMNS 01584, NMNS 01434, NMNS 01479, NMNS 01722, NMNS 01841, NMNS 01845.

Hainan, China: SNHM 500128–29, SNHM 720065, SNHM 720069–72, *SCUM 035003; AFS 08.09, HNU R0120, HNU R0049–50.*

N Vietnam: NMW 23913:3, ROM 35312–15, ROM 35318, ROM 35320–22, ROM 31066, ROM 31068, ROM 31072, AM 99.15, AM 99.17–18.

Annamite mountains, Vietnam: FMNH 255579–80, FMNH 256419.

Central Vietnam: NMW 23913:2, FMNH 11538, FMNH 252076, FMNH 252097, FMNH 252099, ROM 25403, ROM 30781–82, ROM 30785–6, ROM 30788, ROM 34559–61, ROM 35465, USNM 163967, ROM 30791.

Bolovens Plateau, Laos: FMNH 258940–46.

Cardamom mountains, Cambodia and SE Thailand: BMNH 2000.71, RNHM 16716:1–2.

Dongraek mountains, C. Thailand: RTV9–10, AM99.5, FMNH 180242–44, FMNH 180247, FMNH 180256–61, FMNH 180263–65, FMNH 180269, FMNH 180272–74, FMNH 180277.

NE Thailand: PLWRS 3–5, PLWRS 920503, AFS 94.1, AFS 94.3–18, AFS 94.20.

APPENDIX 2

Morphological characters used in the morphometric analysis, and their abbreviations

A) Scalation

BORSUPOC: number of scales bordering the supraocular scales (average of right and left), not counting pre- or post-oculars.

BSCK: keeling of body scales (mid body). Recorded as 0: none; 0.5: weak; 1: strong.

BTWSUP1: minimum number of scales between the supraoculars.

BTWSUP2: number of scales between the posterior edge of the supraoculars.

NASPIT: number of scales between the nasal and the scale bordering the anterior edge of the pit (formed by the fused second supralabial and loreal scale).

LAB3: minimum number of scales separating 3rd supralabial and subocular.

LAB4: minimum number of scales separating 4th supralabial and subocular.

LAB5: minimum number of scales separating 5th supralabial and subocular.

INTNAS: number of scales separating the internasal scales.

KHEADSC: keeling of the scales on the back of the head.

KTEMP: keeling of the temporal scales.

ROST: ratio of the anterior margin of the rostral scale to the posterior margin.

SC: number of pairs of subcaudal scales (any unpaired scales are treated as a pair).

SOCBORD: number of scales contacting the subocular, not counting the scales immediately before and after it.

SUBLAB: average number of sublabials on the left and right hand side.

SUPLAB: average number of supralabials on the left and right hand side.

VENTEDGE: number of scales between the edge of the

mouth and the ventral scales, starting at and including the last sublabial.

VS: number of ventral scales (VS), not including anal scale, recorded by the Dowling (1951) method (i.e. the first VS is the one which contacts the first dorsal scale row on both sides).

PREOC: number of preocular scales.

POSTOC: number of postocular scales.

B) Scale reduction formula

These are recorded as a series of characters, each referring to a specific reduction. Each position will have two characters, the dorso-ventral (DV) position of the reduction (the lowest of the two merging scale rows), and the ventral scale (VS) position (counted from the head), which is the ventral scale to which the scale reduction traces diagonally. Before analysis, the VS position was transformed into the percentage of the total number of ventral scales (%VS), to control for variation. (Subcaudal scale position: SC).

VS31–29: ventral scale position of the reduction from 31 to 29 scale rows. VS29–27: ventral scale position of the reduction from 29 to 27 scale rows. Then: VS27–25, VS25–23, VS23–21, VS21–19, VS19–17, VS17–15, SC6–4, SC12–10, SC10–8, DV27–25, DV25–23, DV19–17, DV17–15, DV12–10.

C) Body dimensions

All measurements are made on the right side of the head only unless this was damaged, in which case they were done on the left.

DEYE: diameter of the eye measured between the edges of the surrounding scales.

EYE2NOS: distance between the eye and the nostril, measured between the suture between the second and third preocular (from the bottom) and the inner edge of the nostril.

LHEAD: length of the head, between the tip of the snout to the posterior edge of the lower jawbone.

LSUPOC: the length of the supraoculars.

NOS2PIT: distance between the outer edges of the pit and the nostril.

PIT2EYE: distance between the eye and the pit, measured between the inner edges, along the suture between the first and second preocular scales.

SVL: distance between the tip of the snout and the cloaca.

TAIL: distance between the anterior edge of the first subcaudal scale and the tail tip.

WHEAD: width of the head measured between the outer edges of the supraoculars.

WINTNAS: the width of the internasals.

WSUPOC: the width of the supraoculars measured at the widest part.

D) Colour pattern

LIPCOL: number of scales above lip covered by ventral colour.

OCSTRIPE: presence of postocular stripe (0: absent; 1: indistinct; 2, distinct).

SCR1: the proportion of the first scale row covered by the light area.

SCRSTR: number of scale rows involved in stripe.

STRIPE: presence of stripe covering dorsal scale row one (0: absent; 1: indistinct; 2: distinct).