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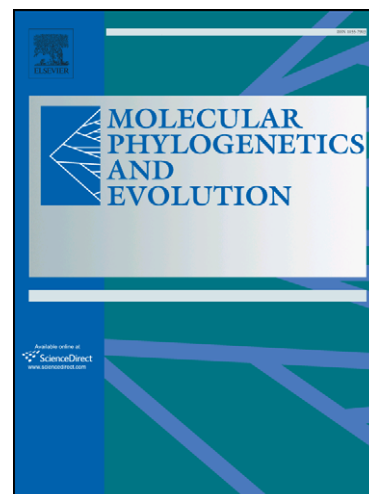
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Mitochondrial DNA analysis reveals a new member of the Asian pitviper genus *Viridovipera* (Serpentes: Viperidae: Crotalinae) .

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Introduction

Pitvipers of the former *Trimeresurus* group occur throughout southern and South East Asia. Extraordinary morphological conservativeness has been observed between many of these species (eg Malhotra and Thorpe 2000, Malhotra and Thorpe 2004a, Sanders et al 2006) and determining the systematic relationships within this group has proven to be extremely difficult, amplified by ongoing morphological and molecular studies continuing to reveal previously unknown diversity. Recent genetic studies involving high levels of sampling across all Asian pitvipers have led to radical reorganisation of genera and revealed many historically misaligned species (eg Tu et al 2000, Malhotra and Thorpe 2000, Malhotra and Thorpe 2004b). External morphology has proven misleading in many cases. Sanders et al (2004) used a combination of genetic and morphological methods to demonstrate between-clade ecological convergence of traits previously treated as phylogenetically informative in the genus *Parias*. Genetic studies are thus invaluable in resolving the taxonomic relationships in these animals, though this can leave some difficult-to-sample taxa in uncertain taxonomic positions.

This study seeks to elucidate the taxonomic position of "*Trimeresurus*" *truongsonensis*, a recently discovered species of pitviper from the karst region of central Vietnam (Orlov et al 2004). This species is currently known only from the type locality, Phong Nha – Ke Bang National Park, and adjacent communes within Quang Binh province (Ziegler et al. 2006). The original description by Orlov et al. (2004) was based on a type series consisting of males only, and Ziegler et al. (2006) provided for the first time the description of an adult female. The type specimen of this species was initially referred to *Cryptelytrops*

kanburiensis on the basis of its superficial resemblance to both *Cryptelytrops kanburiensis* and *C. venustus*, with which it shares a banded coloration, similar body proportions and scalation characters as well as habitat type (Orlov et al 2004). However, it has repeatedly been demonstrated that general morphological resemblance is not a reliable method of generic allocation in pitvipers. For example, several species assigned to *Ovophis* (Burger 1971, Hoge and Romano Hoge 1981) on the basis of morphological and ecological similarity were later revealed by genetic analysis to render the genus paraphyletic (Malhotra and Thorpe 2000, Tu et al 2000, Malhotra and Thorpe 2004b). "*Trimeresurus*" *cornutus* was recently reassigned to the genus *Protobothrops* on the basis of genetic evidence, despite possessing several unique features (small size, prehensile tail) not shared by the remaining members of the genus (Herrmann et al 2004).

While superficial morphological resemblance in characters such as colour pattern, body proportions and scale counts can prove misleading when attempting to determine relationships in Asian pitvipers, several characters have proved to be of systematic value. In *Trimeresurus sensu lato*, hemipenis type was found to coincide closely with clades in the mtDNA tree, later recognized as genera (Malhotra and Thorpe 2000, 2004b). Malhotra and Thorpe (2004b) also found that the condition of the first labial scale, which can be either fused with, or divided from, the nasal scale, appears to be a character with phylogenetic utility; their mitochondrial DNA tree indicates that the fused condition is a defining synapomorphy of the genus *Cryptelytrops*.

In "*Trimeresurus*" *truongsonensis* the first nasal scale is unfused and clearly separated from the nasal shield, unlike all currently known *Cryptelytrops* species. Additionally, the description and images of the hemipenis in the original

description of “*T.*” *truongsonensis* (Orlov et al 2004) most closely resemble the short spinose form found in *Viridovipera* species, and are unlike the long calyculate or papillose forms found in *Cryptelytrops* described by Malhotra and Thorpe (2004b). However, it is important to note that the hemipenis, as pictured in Orlov et al (2004) is clearly only partially everted, and in similar situations the hemipenes of other species have been misleadingly described based on partial hemipenis morphology eg., *Parias (Trimeresurus) schultzei* (Leviton 1964).

No previous molecular studies of Asian pitvipers have incorporated data from “*T.*” *truongsonensis*, thus all hypothesized relationships so far have been based entirely on morphological data. In this study, phylogenetic analysis of four mitochondrial gene regions is used to resolve the position of this new species within the “*Trimeresurus*” complex. We also include a redescription of the hemipenis based on a new specimen in which the hemipenis is nearly fully everted to clarify the diagnostic utility of this character with respect to genera within the former genus *Trimeresurus*.

Materials and Methods

The specimen of “*Trimeresurus*” *truongsonensis* used in this study for genetic and morphological (hemipenis) analysis was an adult male collected by TZ together with Astrid Heidrich, Ralf Hendrix, and Vu Ngoc Thanh during the night of 19 June 2006 at Phong Nha – Ke Bang National Park. It was caught on the ground inside a narrow limestone karst forest cave crevice in the region of Hang E. The specimen is now deposited in the Zoological Museum, Vietnam National University, Hanoi under VNUH 190606. Other sequences were selected based on the hypothesised relationships of “*T.*” *truongsonensis* to other Asian

pitvipers. As preliminary data suggested “*T.*” *truongsonensis* was most likely to be a species of *Viridovipera*, all known species from this genus were represented using as many samples as were available. In addition four species of *Cryptelytrops*, two *Parias* (sister clade to the clade containing both *Viridovipera* and *Cryptelytrops*), two *Trimeresurus* (as redefined in Malhotra and Thorpe 2004b) and several Asian pitvipers from genera outside the former *Trimeresurus* group were included, since it is possible that this species’ affinities lie outside the former *Trimeresurus* group.

DNA isolation, amplification and sequencing

The following protocols apply only to the “*T.*” *truongsonensis* sample. Other novel sequences presented in this study were obtained as described in Malhotra and Thorpe (2000, 2004b).

Whole genomic DNA was extracted from muscle tissue stored in 80% ethanol using Sigma GenElute mammalian Genomic DNA miniprep kits. Four regions of the mitochondrial genome were amplified i.e., those encoding portions of 12S rRNA (12S), 16S rRNA (16S), Cytochrome b (*cytb*) and NADH dehydrogenase subunit 4 (*ND4*).

Cytb from “*T.*” *truongsonensis* was amplified using the primers Gludgmod2 (5'- CTGAAAACCCACCGTTGT -3') (Wüster et al, in press) and H16064mod (5'- GGTTTACAAGAACAAYGCT-3') (modified from H16064, Burbrink et al 2000).

The reaction conditions were 35 cycles of 94°C for 30 seconds, 60°C for 1 minute and 72°C for 2 minutes, with an initial denaturation phase at 94°C for 3 minutes and a final extension phase of 72°C for 5 minutes. *ND4* was amplified using primers *ND4* and LEU as described in Arevalo et al (1994). It is worth noting that the primer LEU described in Parkinson (2000) which is frequently

referred to in pitviper studies and cites Arevalo (1994) is incorrect; it appears to be a simple reversal of the original sequence, and did not match any *ND4* sequence in a Blast search, or yield any successful amplifications. The primer following the original sequence from Arevalo (1994) is used here. *ND4* reaction conditions were: 5 minute denaturation at 94°C, followed by 30 cycles of 1 minute denaturation at 94°C, 2 minute annealing at 60°C, 2 minute extension at 72°C, with a single final extension at 72°C for 7 minutes. 12S from "*T.*" *truongsonensis* was amplified using the primers detailed in Knight and Mindell (1993) under the following conditions: 2 minute denaturation at 94°C, followed by 30 cycles of 30 second denaturation at 94°C, 30 second annealing at 40°C, 1 minute extension at 72°C, with a single final extension at 72°C for 5 minutes. 16S from "*T.*" *truongsonensis* was amplified using the same reaction conditions, with the primers L2510 and H3059 as outlined in Parkinson et al (1997). Prior to sequencing PCR products were cleaned using shrimp alkaline phosphatase (SAP) to dephosphorylate residual deoxynucleotides and Exonuclease I to degrade excess primers (Werle et al 1994). Sequencing reactions were performed by MacroGen inc. (www.macrogen.com).

Alignment of *cytb* and *ND4* was trivial as no indels were detected. Sequence for these genes was translated into amino acid sequence using CodonCode aligner to check for stop codons, which might indicate that a pseudogene had been amplified (Zhang and Hewitt 1996). 12S and 16S rRNA sequence was aligned following Parkinson (1999) and Malhotra and Thorpe (2004). The uniformity of base composition across all samples was tested in PAUP*4.0.

Sequence analysis

Phylogenetic analysis was performed using Bayesian Markov Chain Monte Carlo (MCMC) using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). *Azemiops feae* was selected as the outgroup. Bayesian analysis was preceded by application of MrModeltest 2.2, a program based on Modeltest 3.6 (Posada and Crandall, 1998) and modified to work with MrBayes, to obtain the most appropriate model of evolution prior to analysis. Prior to further analysis, the data were subjected to various tests for non-neutral evolution including Tajima's D statistic (Tajima, 1989), Fu and Li's D* and F* statistics (Fu and Li, 1993), using the program DnaSp 4.10.9 (Rozas et al, 2003).

For Bayesian analysis, the sequence data was partitioned by gene for rRNA genes, and by gene and codon position for protein-coding genes, to give a total of eight partitions (12S, 16S, *cytb* codon position 1, *cytb* pos 2, *cytb* pos 3, *ND4* pos 1, *ND4* pos 2 and *ND4* pos 3). The optimal model of evolution for each partition was selected using MrModeltest. Monte Carlo Markov Chain analysis was run using one cold chain and three heated chains using the combination of best-fit models selected by MrModeltest. Two independent iterations were run for 2,000,000 generations, and sampled every 1000 generations, with the first 200,000 generations (10%) discarded as burn-in. Parameters were plotted against generations to check that convergence had been reached well before the post burn-in portion of the data. The 1800 remaining trees were used to construct a 50% majority rule consensus tree.

As a check for the appropriateness of the chosen partitioning scheme, other possible partitions were investigated (including partitioning by gene only, partitioning by rRNA/protein coding regions) and an alternative method, in which

no a priori partitioning is done, but the n partitions are defined as part of the optimisation process (where n is a user-defined number) was implemented using BayesPhylogenies (Pagel and Meade 2004). The effect of different partitioning schemes were compared through examination of topologies and PP support values for significant nodes between the resulting trees.

Results

Phylogenetic analysis

The final data set consisted of 2387 base pairs of sequence data: 414 bp of 12S, 506 bp of 16S, 663 bp of *ND4* and 804 bp of *cytb*. No insertions, deletions or stop codons were detected in the protein-coding genes, so there was no indication that pseudogenes had been amplified. Indels were observed in the rRNA data (12S, 16S), which were aligned following Malhotra and Thorpe (2004b). Several base positions in 16S could not be aligned with any confidence due to the presence of multiple insertions/deletions in the sequences, so 25 bases were excluded from the analysis, comprising positions 283-307 in the 16S alignment. Thus the final analysis is based on 2362 aligned nucleotides. Table 1 lists GenBank accession numbers for all sequences used in this study.

No significant difference in base-pair frequency was detected among sequences (chi-squared = 37.98, $P=1.00$), and none of the tests for non-neutral evolution showed a significant departure from neutrality. The best fit model indicated by MrModeltest varied among data partitions. In 12S and 16S, GTR+I+ Γ was indicated as the optimal model of sequence evolution. For *cytb*

pos1, *cytb* pos3, *ND4* pos1 and *ND4* pos 3, GTR + Γ was selected, and for *cytb* pos 2 and *ND4* pos 2 MrModeltest found HKY + Γ to be optimal.

The resultant Bayesian inference tree (partitioned by gene and codon position) had a mean likelihood score of $\ln L = -14011.9$. "*Trimeresurus*" *truongsonensis* was placed within *Viridovipera*, with strong support for the monophyly of the genus (Bayesian posterior probability [PP] = 0.96). Although there were some differences in tree topology at deeper levels of the tree between this study and others (eg Malhotra and Thorpe 2000, 2004b), this is likely due to the low density of sampling from these groups and is of little relevance to the aim of this study. This lack of resolution does not affect the conclusions with regard to *Viridovipera* and the position of *truongsonensis* within it. Placement of *V. yunnanensis* as the sister group of all other *Viridovipera* species apart from *V. medoensis* was also well supported in the Bayesian analysis (PP=0.98). However, the position of *V. truongsonensis* with respect to *V. vogeli* and a clade comprising the sister species *V. gumprechtii* and *V. stejnegeri* is less clear. While clearly part of this larger clade, its exact position within it as sister group to the *V. gumprechtii/V. stejnegeri* clade is not well supported (PP =0.93). The exact partitioning scheme applied does not seem to have a large effect on the analysis as topologies of trees based on different methods of data partitioning were essentially identical to that illustrated in Fig 1. for *Viridovipera*, but gave slightly poorer support values for critical nodes and some collapsed nodes outside this clade.

Hemipenis of *Viridovipera truongsonensis*.

This description is based on the everted hemipenes of VNUH 190606. Both hemipenes are nearly fully everted, with the right hand lobe of the right

hand hemipenis appearing to be fully everted. There are about 9-11 stout spines on the outside (sulcal) side of the each hemipenis, with the remainder of the hemipenis being covered with calyces or microspines apart from a short region at the base of the sulcal side which is smooth. The calyces are also edged with microspines. The lobes of the forked region are at least as long as the distance from the base of the organ to the fork (and might possibly be longer, depending on how much of the lobes remain inverted). The sulcus forks near the base of the organ, and on the sulcal side this demarcates the boundary between the smooth (below) and area covered with microspines (above). This description is similar to that of Orlov et al. (2004) except that the hemipenes of the type specimen pictured are clearly only partially everted and therefore the lobes appear much shorter than they actually are. As stated in that publication, the hemipenis of *V. truongsoneensis* corresponds most closely to the Type 1 spinose of Malhotra and Thorpe (2004b). It differs from some other species of *Viridovipera*, which have very short lobes (*V. stejnegeri*, *V. gumprechtii*, *V. vogeli*) but is similar in its proportions to *V. medoensis* and *V. yunnanensis*, and like these two species, differs from the Type III spinose hemipenis in the number and distribution of spines (Malhotra and Thorpe 2004b). The hemipenis morphology as a whole supports the position of this species in the genus *Viridovipera*.

Discussion

Phylogenetic analysis of mitochondrial DNA sequence indicates that the examined specimen of "*Trimeresurus*" *truongsoneensis* is a member of the genus *Viridovipera*, a conclusion well supported in the Bayesian analysis. While its inclusion within the clade consisting of *V. gumprechtii*, *V. stejnegeri* and *V. vogeli* seems clear, its sister-group relationships are less clear cut, with relatively poor

support for its position as the sister group to a clade consisting of *V. gumprechtii* and *V. stejnegeri*.

In *V. truongsoneis* the overall structure of the hemipenis more closely resembles that of other *Viridovipera* species than that of any other genus in this group, further demonstrating the utility of general hemipenis morphology in determining generic allocation within the former *Trimeresurus* group (Malhotra and Thorpe 2004b). Within the genus, although differences were observed in the occurrence of calyces edged with microspines, the hemipenial morphology of *V. truongsoneis* most closely resembles that of *V. medoensis* and *V. yunnanensis* in proportions. However, in the Bayesian tree, the species with short-lobed hemipenes (*V. stejnegeri*, *V. gumprechtii* and *V. vogeli*) form a paraphyletic group, with respect to the long-lobed *V. truongsoneis*. This might imply that it is not appropriate to treat fine details of hemipenis morphology as reliable indicators of sister group relationships within genera. This is supported by the situation, in *Cryptelytrops*, where the apomorphic long calyculate hemipenis is seen in two non-sister species, while all other congeners possess the ancestral long papillose type (Malhotra and Thorpe 2004b). However, given that support for the sister group relationship of the long-lobed *V. truongsoneis* with the clade consisting of short-lobed species *V. stejnegeri/V. gumprechtii* is only 93%, and that in simulations using artificially generated data, posterior probabilities assigned in the Bayesian analysis have been known to overestimate the support that a particular arrangement should receive (Kolaczkowski and Thornton 2007), the possibility that *V. truongsoneis* has been incorrectly placed, and that hemipenis morphology is an accurate indicator of relationships within the genus, cannot be ignored.

The systematic position of *V. truongsoneensis* is further supported by biogeographic considerations, as it occurs within the previously known range of *Viridovipera* (North-eastern India, Xizang, northern Burma, southern China, central Vietnam, Laos, and eastern Thailand, mapped in Malhotra and Thorpe [2004a]). It co-occurs with *V. vogeli* in Phong Nha – Ke Bang National Park (Ziegler et al 2006) and the range of *V. gumprechtii* also extends close to this locality though it has not been recorded in the Park itself.

V. truongsoneensis is the first described member of *Viridovipera* to depart from the typical coloration of uniform green with a white lateral line (Sanders et al 2006, Malhotra and Thorpe 2004b). While its distinctly banded pattern differs from the known remaining members of the genus, it resembles that of three species in the sister genus *Cryptelytrops* (Orlov 2004, Gumprecht et al 2004, Grismer et al 2008). Hence, in *Viridovipera* as in many other Asian pitviper clades (eg Sanders et al 2006, Malhotra and Thorpe 2000), superficial similarities in colour pattern and body proportions are not reliable indicators of interspecific relationships. At present, no conclusions can be drawn concerning the evolutionary explanation for the observed pattern. Sanders et al (2006) found no compelling evidence for a Müllerian mimetic explanation for the green-plus-banding colour patterns in *Trimeresurus*-group pitvipers. However, their study did not include *V. truongsoneensis* or the newly described *Cryptelytrops honsonensis* currently known from an island off the coast of southern Vietnam. The four banded species (*V. truongsoneensis*, *C. kanburiensis*, *C. venustus*, *C. honsonensis*) share habitat preferences, all inhabiting forests of the karst massifs (Orlov et al 2004, Grismer et al 2008), and *V. truongsoneensis* was observed to be well camouflaged in this habitat. Although this makes a simple adaptive explanation for the observed similarity a tempting idea, the sympatry of several

closely related non-banded species must be considered; the green species *Cryptelytrops albolabris* and *Viridovipera vogeli* both occur in Phong Nha – Ke Bang National Park alongside *V. truongsonensis* (Ziegler et al 2006). Data on the ecology and behaviour of these species and most other Asian pitvipers is rather sparse, largely limited to captive specimens, scattered observations made during field collection and dietary analyses based on faeces and stomach contents, so currently no serious investigation of the ecological correlates of morphological variation is feasible. Further work regarding the evolution of colour pattern in Asian pitvipers will depend on the ability to discriminate ecologically among co-occurring species and to model the disparate selection pressures that they may experience.

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Figure 1. The Bayesian inference tree based on four mtDNA genes. Posterior probabilities for clades are shown adjacent to appropriate node. The species under consideration, *Viridovipera truongsonensis*, is marked with an arrow.

Sample numbers are given in Table 1.

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Table 1: Details of specimens used in this analysis.

Species	Specimen no.	Locality	Genbank Accession No. cytb, ND4, 12S, 16S			
<i>Azemiops feae</i>	AM B499	China	AY352747	AY352808	AY352774	Ay352713
<i>"Trimeresurus" gracilis</i>	AM A86	Taiwan	AF171913	AY352823	AY352789	AY352728
<i>Calloselasma rhodostoma</i>	AM A54	Satun Prov, Thailand	AF171918	AY352813	AY352779	AY352718
<i>Protobothrops flavoviridis</i>	AM B527	Okinawajima, Ryukyu Is., Japan	=	AY352826	AY352792	AY352730
<i>Popeia popeiorum</i>	AM A203	Nakhon si Thammarat prov., S Thailand	AY371796	AY059588	AY059537	AY059553
<i>Himalayophis tibetanus</i>	AM B258 (ZMB 65641)	Helambu province, Nepal	AY352749	AY352810	AY352776	AY352715
<i>Cryptelytrops insularis</i>	AM A109	E Java, Indonesia	AY352767	AY352833	AY352799	AY352738
<i>Cryptelytrops albolabris</i>	AM A157	Shek Kwu Chan, Hong Kong	AF171884	AY352839	AY352805	AY352744
<i>Cryptelytrops venustus</i>	AM A241	Nakhon si Thammarat prov., S Thailand	AF171914	AY293930	AY293931	AY352723
<i>Cryptelytrops kanburiensis</i>	AM B522	Kanchanaburi province, W Thailand	AY289225	AY289231	AY289219	AY352737
<i>Viridovipera medoensis</i>	AM B416 (CAS 221528)	Kachin state, Myanmar	AY352765	AY352831	AY352797	AY352735
<i>Viridovipera truongsongensis</i>	AM B659 (VNUH 190606)	Quang Binh, Central Vietnam	EU443815	EU443816	EU443817	EU443818
<i>Viridovipera gumprechtii</i>	AM B15 (NMNS 3113)	Yunnan province, China	AY321487	AY352832	AY352798	AY352736
<i>Viridovipera gumprechtii</i>	AM B128	Annamites, Laos	AY059579	EU443787	EU443788	EU443789
<i>Viridovipera gumprechtii</i>	AM B174 (FMNH 255579)	Nghe An province, N Vietnam	AY059573	AY059595	AY059547	AY059563
<i>Viridovipera gumprechtii</i>	AM A164	Loei province, NE Thailand	AY352766	AF157224	AF517168	AF517181
<i>Viridovipera gumprechtii</i>	AM B497	S.W. Yunnan	AY321489	EU443790	EU443791	EU443792
<i>Viridovipera stejnegeri</i>	AM B181	Cao Bang, Vietnam	AF278711	EU443793	EU443794	EU443795
<i>Viridovipera stejnegeri</i>	AM TST148	Hualien co., Taiwan	AF277690	EU443796	EU443797	EU443798
<i>Viridovipera stejnegeri</i>	AM A160	Taipei county, Taiwan	AF171896	AY059593	AY059539	AY059555
<i>Viridovipera stejnegeri</i>	AM TST4	Taipei county, Taiwan	AF277700/ AF171896	AY059593	AY059539	AY059555

<i>Viridovipera stejnegeri</i>	AM A222	Fujian province, China	<u>AF277677</u>	<u>AY059594</u>	<u>AY059541</u>	<u>AY059557</u>
<i>Viridovipera stejnegeri</i>	AM T23	Taichung county, Taiwan	<u>AF277689</u>	<u>EU443799</u>	<u>EU443800</u>	<u>EU443801</u>
<i>Viridovipera stejnegeri</i>	AM T60	Taitung county, Taiwan	<u>AF277676/ AF171880</u>	<u>EU443802</u>	<u>EU443803</u>	<u>EU443804</u>
<i>Viridovipera vogeli</i>	AM B182	Central Vietnam	<u>AY059578</u>	<u>EU443805</u>	<u>EU443806</u>	<u>EU443807</u>
<i>Viridovipera vogeli</i>	AM B97	Nakhon si Ratchasima prov., Thailand	<u>AY059574</u>	<u>AY059596</u>	<u>AY059546</u>	<u>AY059562</u>
<i>Viridovipera vogeli</i>	AM B124	Lao PDR, Champasak Province	<u>AY059580</u>	<u>EU443808</u>	<u>EU443809</u>	<u>EU443810</u>
<i>Viridovipera vogeli</i>	AM B125	Lao PDR, Champasak Province	<u>AY059581</u>	<u>AY059581</u>	<u>AF517225</u>	<u>AF517170</u>
<i>Viridovipera yunnanensis</i>	GP 37	Huili, Sichuan	<u>EF597522</u>	<u>EF597527</u>	<u>EU443811</u>	<u>EU443812</u>
<i>Viridovipera yunnanensis</i>	GP 38	Huili, Sichuan	<u>EF597523</u>	<u>EF597528</u>	<u>EU443813</u>	<u>EU443814</u>
<i>Parias flavomaculatus</i>	AM B3	Luzon, Philippines	<u>AF171916</u>	<u>AY059584</u>	<u>AY059535</u>	<u>AY059551</u>
<i>Parias hageni</i>	AM B364	Bengkulu province, Sumatra	<u>AY371825</u>	<u>AY371863</u>	<u>AY371763</u>	<u>AY371790</u>
<i>Trimeresurus gramineus</i>	AM A220	Tamil Nadu state, India	<u>AY352761</u>	<u>AY352827</u>	<u>AY352793</u>	<u>AY352731</u>

Abbreviations used as follows: AM, GP: Authors' catalogue numbers; ZMB: Zoologisches Museum für Naturkunde der Humboldt-Universität, Berlin; CAS: California Academy of Sciences, San Francisco; NMNS: National Museum of Natural Science, Taiwan; FMNH: Field Museum of Natural History, Chicago.

Abstract: In morphologically conservative groups, such as the former *Trimeresurus* radiation of Asian pitvipers, it can be difficult to allocate new species to the correct genus. "*Trimeresurus*" *truongsonensis*, a recently discovered pitviper, was of uncertain phylogenetic position at the time of description. A mitochondrial DNA phylogeny, in conjunction with the study of key morphological features, indicates that this species belongs to the genus *Viridovipera*. While the new species is well within the previously known range of this species, it is the first known member of this genus to deviate from uniform green colouration, bearing superficial resemblance to several species of *Cryptelytrops*.

ACCEPTED MANUSCRIPT

