

REDESCRIPTION AND SYSTEMATICS OF *TRIMERESURUS* *CORNUTUS* (SERPENTES: VIPERIDAE) BASED ON MORPHOLOGY AND MOLECULAR DATA

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ABSTRACT: We redescribe *Trimeresurus cornutus*, a species that has not been reported for more than half a century, based on a recently collected live specimen from the Annamite Mountains of Central Vietnam. Besides new data on the distribution and habitat, we compare coloration and morphological data of the first known male of the species with the type specimen and a third known specimen from North Vietnam. We describe male genital morphology and analyze the systematic relationships of the species using external morphology, hemipenial morphology and molecular data. A phylogenetic hypothesis, based on four mitochondrial genes, suggests that the species is most closely related to *Protobothrops jerdonii*. The external morphology and hemipenial morphology are consistent with this placement. Based on the few specimens available, *Protobothrops cornutus* comb. nov. exhibits a very patchy distribution, with an altitudinal range reaching from 150–2000 m.

Key words: Distribution; Habitat; Hemipenial morphology; Molecular systematics; *Protobothrops*; Redescription; Serpentes; *Trimeresurus cornutus*; Vietnam; Viperidae

TRIMERESURUS (sensu lato), a species-rich genus of pitvipers with some 40 species, is widely distributed throughout Asia (David and Ineich, 1999; McDiarmid et al., 1999). This group of venomous snakes includes a variety of different ecological types; some species are mainly terrestrial, some semi-arboreal, and others exclusively arboreal (Malhotra and Thorpe, 1997). The morphological conservatism observed across *Trimeresurus* (sensu lato) has led to a rather poor understanding of the relationships and, consequently, the taxonomy of this group. Both morphological and molecular studies (Burger, 1971; Hoge and Romano-Hoge, 1983; Malhotra and Thorpe, 2000; Parkinson et al., 2002; Tu et al., 2000) have clarified some of the relationships within *Trimeresurus* (sensu lato). Several new genera (*Ermia*, Zhang, 1993; *Ovophis*, Burger in Hoge and Romano-Hoge, 1981; *Protobothrops*, Hoge and Romano Hoge, 1983; and *Tropidolaemus*, Wagler, 1830), representing distinct evolutionary lineages, have been pro-

posed within *Trimeresurus* (sensu lato). However, opinions vary as to which of these genera should be recognized (David and Ineich, 1999; McDiarmid et al., 1999). The monotypic genus *Triceratolepidophis* represents another taxon within this systematic group that has been described (Herrmann et al., 2002; Mebs et al., 2003; Ziegler et al., 2000). In addition to the continuing debate over valid genera, recent molecular studies have shown that some species placed within *Trimeresurus* (sensu stricto) have been misclassified (Malhotra and Thorpe, 2000; Tu et al., 2000). Malhotra and Thorpe (2000) presented a phylogenetic analysis of several morphological characters of potential taxonomic value within *Trimeresurus* (sensu stricto) and determined that hemipenial morphology was the most informative. However, data from the hemipenis is lacking for several rare species, including *Trimeresurus cornutus* (Orlow, 1998; Ziegler and Herrmann, 2002).

Trimeresurus cornutus, named for its horn-like supraocular scales, has been known only from the type specimen, which was collected in the 1920's from Mt. Fan Si Pan, Lai Châu Province, Northern Vietnam (Smith, 1930). A second specimen was collected in 1937 (Smith,

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1943) from North Vietnam ("Tonkin" without precise locality data), while a third was reported by Campden-Main (1970) from Bach Ma, Thua Thien-Hue Province in South Vietnam, unfortunately the voucher was subsequently lost (see Ziegler et al., 2000:206).

In 2001, the third verifiable specimen of *T. cornutus* was collected in the Central Annamite Mountains of the Quang Binh Province in the Phong Nha-Ke Bang National Park, Central Vietnam by two of us (H-W. Herrmann and T. Ziegler) during faunal surveys (Ziegler and Herrmann, 2002). These surveys were part of the Cologne Zoo's conservation activities in the area (Ziegler and Herrmann, 2000). This recent collection represents the first live specimen for which coloration is documented, and it is the first known record of a male *T. cornutus*.

In this paper, we review the coloration, morphology, and distribution of *T. cornutus* and provide information on its habitat; we redescribe the species based on known specimens, including the newly documented specimen from 2001. We employ external morphology, hemipenial morphology, and molecular data to infer the phylogenetic position of *T. cornutus* within *Trimeresurus* (sensu lato).

MATERIALS AND METHODS

Field and Morphological Methods

The male specimen, ZFMK 75067, was fixed in 98% ethanol and then transferred into 70% ethanol. Locality coordinates for ZFMK 75067 were obtained by handheld Garmin[®] GPS instruments. Climate data in Vietnam were collected by a Hobo[®] 08 data logger that recorded temperature and relative humidity every hour. The data logger was mounted on a tree trunk at 2 m height within a closed forest in close proximity to the locality of specimen ZFMK 75067. Logger data were processed with the computer program BoxCar version 4.0.

Snout-vent length (SVL) and tail length (TL) were measured in the preserved specimens to the nearest millimeter using a meter ruler. Other standard measurements were taken to the nearest millimeter using a digital slide-caliper. External features were observed under a dissecting microscope. Scale termi-

nology follows Klauber (1956) except for circumoculars, which are defined here as all scales around and in contact with the eye. Ventral scales were counted following Dowling (1951), in which scales wider than long but not in contact with the first row of dorsals are defined as preventrals. Anterior dorsal scale row counts were made approximately one head-length posterior to the head. Posterior dorsal scale row counts were made approximately one head-length anterior to the vent. Values for symmetric head characters are presented as left/right. Coloration is based on photographs of living and preserved specimens. For description of hemipenes, we largely follow the terminology of Dowling and Savage (1960) and, where appropriate, use the terms and modifications proposed by Ziegler and Böhme (1997). Hemipenial description was based on both everted hemipenes. Hemipenes were everted immediately postmortem, following the method described in Ziegler and Böhme (1997). Museum acronyms are BMNH (The Natural History Museum, London), MNHN (Muséum National d'Histoire Naturelle, Paris), and ZFMK (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn).

Molecular Methods

Whole genomic DNA was extracted from 0.01–0.02 g of ethanol-preserved muscle tissue using standard protocols (Sambrook et al., 1989). Four different regions of the mitochondrial genome were amplified, using primers and reaction conditions described in Malhotra and Thorpe (2000) for cytochrome b (cytb). The 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 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.

The phylogenetic analysis was based on 2414 aligned nucleotide positions of combined data from the four gene regions, comprising 668 bp of ND4, 806 bp of cytb, 426 bp of 12S, and 514 bp of 16S. Alignment of cytb 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 certain new sequences. A total of 11 bp in regions of uncertain alignment was excluded from the analysis, including positions 227–228 and 291–292 of 12S and positions 286–287, 294–297 and 310 of 16S sequence. The cytb 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 (mt genome) and were found to evolve similarly (Parkinson et al., 2002), the sequences were concatenated and analyzed as a single matrix.

The dataset analyzed included 63 OTUs representing all putative genera within *Trimeresurus* (sensu lato, [*Ermia*, *Ovophis*, *Protobothrops*, *Triceratolepidophis* and *Tropidolaemus*]), all four species groups of *Trimeresurus* (sensu stricto) (Malhotra and Thorpe, 2000), and representatives of all other Asian pitviper genera and several New World taxa. This diverse sampling strategy was employed to determine, in an unbiased manner, the phylogenetic position of *T. cornutus* with a minimum of a priori assumptions. The presence of phylogenetic signal in the data matrix was tested using the g_1 statistic (Hillis and Huelsenbeck, 1992) for the skewness of tree length distributions, estimated from 10^6 random trees. The critical values of g_1 were obtained from the table published in Hillis and Huelsenbeck (1992), and a significant result indicates that the length of the actual tree is significantly shorter than expected from random data (i.e., data without any phylogenetic structure). The possibility of nonneutral 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), and Tajima's D (Tajima, 1989).

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 unweighted which follows the substantial body of literature that 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 pseudo replicates using the same settings, except that the number of random taxon additions was reduced to 10.

Bayesian (MCMC) analyses were 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 vague 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, as per the program's 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

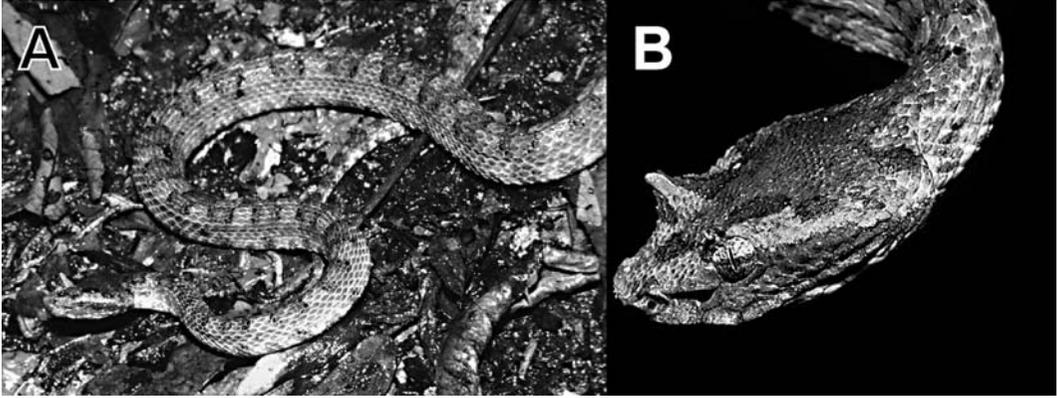


FIG. 1.—Adult male *Trimeresurus cornutus* (ZFMK 75067), a) in natural habitat; b) close up of head. Photos by T. Ziegler.

million generations, 400,000 of which were discarded as burn-in, yielding 1 million post burn-in generations.

RESULTS

The new specimen of *T. cornutus* (ZFMK 75067; Fig. 1) was collected on 1 September 2001 by H-W. Herrmann and T. Ziegler at Phong Nha–Ke Bang National Park (17° 30' N, 106° 16' E), Quang Binh Province, Central Vietnam, near the Laotian border at an elevation of approximately 150 m. This adult male snake was collected at night (2230 h) in dry, semi evergreen forest (<50% deciduous) in a limestone karst area. It was found on the side of a small path while crawling on leaf litter. During the day on which it was collected, slight rainfall had occurred in the morning, followed by sunny hot weather in the afternoon. That night, the sky was overcast with steady rain the next day. The temperature and relative humidity at the collection site within the Phong Nha–Ke Bang National Park was recorded between 13 May 2000 and 20 June 2001 using a data logger. The absolute minimum temperature was 12 C; the absolute maximum during this time period was 35 C; relative humidity ranged from 54–100% (for detailed climatic and habitat data see Herrmann et al., 2002).

Systematic Account

Trimeresurus cornutus Smith, 1930

Holotype.—BMNH 1946.1.19.25 (formerly 1930.11.16.2); Type-locality: “Fan-si-pan Mts.,

Tong-king.” [= Mt. Fan Si Pan, Lai Châu Province, Northern Vietnam].

Referred specimens.—MNHN 1937.35 and ZFMK 75067.

Diagnosis.—*Trimeresurus cornutus* is characterized by the possession of distinctly raised, horn-like, enlarged supraocular scales and dorsal scales bearing 1 unserrated keel each. The combination of these morphological characters, together with 189–193 ventrals, 21 dorsals at midbody and 3 loreals, separates this species from all other members of *Trimeresurus* (sensu lato) and from the only other known “horned” pitviper *Triceratolepidophis sieversorum* from the *Trimeresurus* group that occurs within the range of *T. cornutus* (Table 1; see also Ziegler et al., 2000).

Description of holotype.—Female with 192 ventrals plus one preventral; 71 subcaudals plus spine at tail tip; subcaudals divided; anal entire; dorsals in 19 rows anteriorly; 21 rows at midbody; 17 rows posteriorly; dorsals keeled (one unserrated keel); nine supralabials on either side of which the third is greatly enlarged; 13/14 sublabials; supralabials in contact with nasals; second supralabial in contact with loreal pit; 13/13 circumoculars; supraoculars 6/6; 13 interoculars (including supraoculars); “horns” consist of two greatly enlarged and one moderately enlarged supraocular; 2/2 scales between supralabials and suboculars; three internasals that are elongate and slightly raised at outer margins; 3/3 loreals; one chinshield on each side; 6/6 gulars; prehensile tail. SVL 476 mm; tail length 107 mm;

total length 583 mm (miscalculated in Orlov, 1998); tail length/total length 0.18; eye diameter 3/3; head length 21; head width 14; head height 7; head length/head width 1.6; head length/head height 3.0.

Color in preservative (70% ethanol).—The specimen has lost most of the color and pattern, presumably due to long-term preservation. Described aspects are only weakly visible. The dorsum is gray-brown with a series of dorsal blotches partly alternating along the dorsal midline; the blotches border on brown. These borderlines sometimes go across the median line and form crossbars. There is a lateral series of gray-brown spots without dark borders on light ground color. The ventral side is light brownish, powdered with beige. The dorsal side of the head has a dark triangular structure and the lateral side has a darker sub- and postocular stripe.

Morphological comparison with other specimens.—MNHN 1937.35 is a female with a total length of 396 mm (SVL 324 mm, TL 72 mm, SVL/TL 4.5), whereas ZFMK 75067 is a male with a total length of 696 mm (SVL 555 mm, TL 141 mm, SVL/TL 3.9). These two specimens (Table 2) differ from the holotype in the following characters (holotype characters in parentheses): MNHN 1937.35 differs from the holotype in having 7/7 supraoculars (6/6), 4 internasals (3), and 7/7 gulars (6/6). ZFMK 75067 differs from the holotype in having 189 ventrals (192) plus 1 preventral; 78 subcaudals (71) plus spine at tail tip; 21 rows of dorsals anteriorly (19); 15 rows of dorsals posteriorly (17); 16 interoculars including supraoculars (13); 5 internasals (3) and 7/7 gulars (6/6). The color and pattern of MNHN 1937.35 has faded due to long-term preservation, as noted for the holotype, and thus resembles it. The color and pattern of the male ZFMK 75067 (Fig. 1) shortly after preservation are as follows: dorsum and sides are of a dull grayish-brown with a total of 48 (left) and 51 (right) distinct brown blotches along both sides of the dorsal mid-line; 15 of them are located at the dorsum of the tail. These spots are anteriorly and posteriorly bordered by a dark brown stripe. The spots on either side of the dorsal mid-line are approximately opposite each other at midbody, whereas their position is increasingly offset towards the posterior end of the body. Corresponding with each of these dorsal

TABLE 1.—Morphological comparison between *Trimeresurus cornutus*, *Protobothrops mucrosquamatus* and *Triceratolepidophis sieversorum* (adapted from Ziegler et al., 2000).

Character	<i>Trimeresurus cornutus</i>	<i>Protobothrops mucrosquamatus</i>	<i>Triceratolepidophis sieversorum</i>
Maximal size	696	1174	1255
Tail length/ total length	0.18–0.20	0.16–0.24	0.16–0.17
Head length/ SVL ($\times 100$)	4.3–5.2	4.3–4.7	4.0–4.2
Ventrals (males)	189	198–222	228
Subcaudals (males)	78	78–100	82
Mid-body dorsals	21	23–27	21–23
Keels on dorsals	single	single	triple
Supralabials	9	9–12	8–9
Scales between nasal and loreal pit	3	2–5	2
Supraoculars	horns	flat	horns
Intersupraoculars	13–16	11–18	15–16
Keels on head	no	no	yes
Color	gray brown	gray brown	gray brown
Dorsal pattern	blotches	blotches	blotches

blotches is one dull brown lateral blotch on either body side with one additional, but smaller blotch in between on the light ground coloration of the lower lateral sides. The ventral side is mottled with brown. The tail tip is light brownish to yellowish. The dorsal aspect of the head shows a large single dark brown triangular pattern starting on the anterior neck and reaching forward between the supraocular horns with the apex ending halfway between the eyes and the rostral. The tip of this “arrowhead” marking, as well as the areas anterior and posterior to the largest supraocular scales, is characterized by large black spots. The supralabial region shows a large dark brown preorbital and postorbital stripe beginning behind the nostrils, reaching up to half of the iris diameter, and extending backwards to the posterior end of the head. The areas between these dark brown dorsal and lateral patterns are reddish-brown in the posterior region and yellowish brown around the tip of the head. The ventral side of the head is yellowish-white with sparse brown stippling. The color of the eye is ocher with a dark brown horizontal stripe.

Description of hemipenes (ZFMK 75067).—The external genital morphology is strong and forked (Fig. 2). The everted hemipenis is 19 mm long, with each lobe measuring 10 mm

TABLE 2.—Measurements (in mm) and pholidosis of the three known voucher specimens of *Trimeresurus cornutus*. Enlarged supralabials: numbers indicate position when counted towards the snout tip on the left side of the head. Values for symmetric head characters are presented as left/right.

Character	BMNH	MNHN	ZFMK
	1946.1.19.25 holotype female	1937.35 female	75067 male
Snout-vent length	476	324	555
Tail length	107	72	141
Head length	21	17	24
Head width	14	12	18
Head height	7	7	8
Eye diameter	3/3	3/3	4/4
Ventrals + preventrals	192 + 1	193 + 1	189 + 1
Subcaudals + tailtip spine	71 + 1	72 + 1	78 + 1
Anterior-body dorsals	19	19	21
Mid-body dorsals	21	21	21
Posterior-body dorsals	17	17	15
Supralabials	9/9	9/9	9/9
Enlarged supralabials	7	7	7
Sublabials	13/14	13/12	14/14
Circumoculars	13/13	13/12	13/13
Supraoculars	6/6	7/7	6/6
Interoculars	13	13	16
Enlarged supraoculars	2 + 1	2 + 1	2 + 1
Scales between			
supralabials and suboculars	2/2	2/2	2/2
Internasals	3	4	5
Chinshields	1/1	1/1	1/1
Gulars	6/6	7/7	7/7
Loreals	3/3	3/3	3/3

and extending to the eighth subcaudal. The sulcus spermaticus is centrolineal, forked at the lower truncus, and leads to the tips of each lobe. The pedicel lacks microspines. The outer surfaces of the sulcal lips are covered from the truncal region onwards with microspines, which are replaced by calyces in the upper half of the lobe. Microspines are also located at the truncus, mainly around the sulcal region, as well as on the inner surfaces and on the sulcal aspect at the base of the lobes. In the region of the fork, a large and strong spine is located on each side of the truncus. Behind and above these large and strong spines are several large to medium sized spines, decreasing in size distally and situated mainly on the back of the lobes. Fringed calyces replace these spines in the upper half of the lobes. At the tips, these calyces become wider and longer.

Distribution.—*Trimeresurus cornutus* is known from North Vietnam (type locality Fan-si-pan Mts.) and now from the Phong

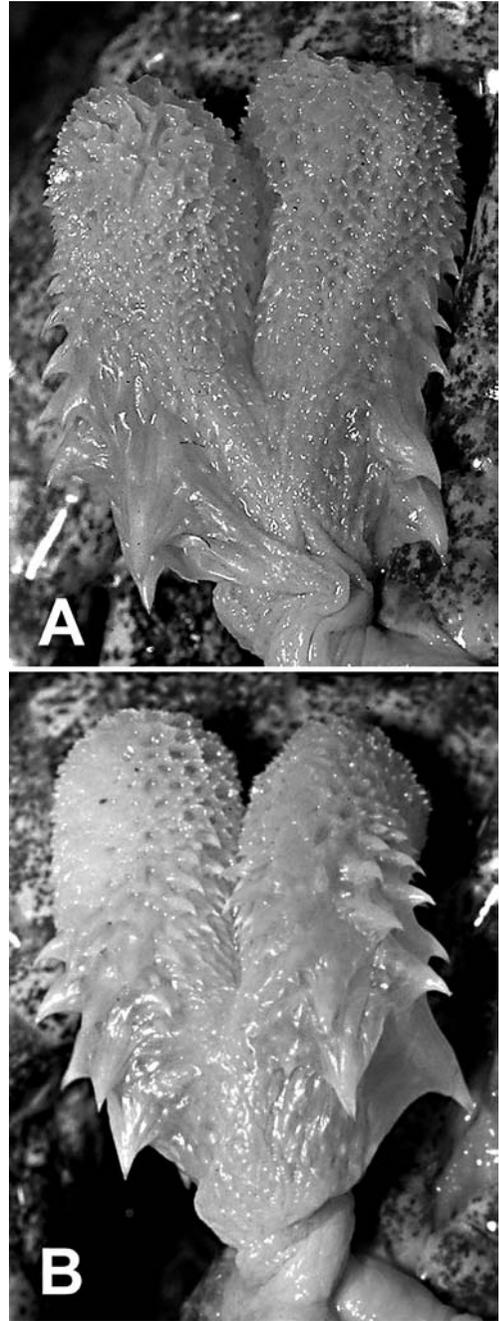


FIG. 2.—Left hemipenis a) sulcal view; b) asulcal view of *Trimeresurus cornutus* (ZFMK 75067). Photos by T. Ziegler.

Nha-Ke Bang National Park in Central Vietnam, a linear distance of approximately 650 km from the type locality (Fig. 3). Its altitudinal range is 150–2000 m. One specimen,

for which no voucher currently exists, was reported from Bach Ma, Thua Thien-Hue Province, South Vietnam, at an altitude of 1400 m (Campden-Main, 1970). Bach Ma lies about 200 km SE of the Phong Nha-Ke Bang National Park.

Phylogenetic Analyses

No stop codons were found in the two coding regions, *cytb* and *ND4*, suggesting that the sequence obtained does not represent a nonfunctional copy or pseudogene. The skewness parameter *g1* indicated that the data are significantly more structured ($P < 0.01$) than random data. The mean base composition was not significantly different across taxa for all four regions combined. There were 958 parsimony informative characters and a further 288 variable, but parsimony uninformative, characters. None of the neutrality tests suggested a significant departure from neutral sequence evolution.

The parsimony analysis produced a single most parsimonious tree, of length 7830 steps. Based on hierarchical log 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. All three runs reached apparent stationarity (in estimates of substitution model parameters, as well as chain likelihood scores) prior to 50,000 generations, well before the conservative burn-in period of 400,000 generations. Posterior probabilities from the Bayesian analyses were based on the combined 3 million post burn-in generations (from the three independent runs). Both parsimony and Bayesian reconstructions inferred identical topologies with respect to the position of *T. cornutus* (Fig. 4). In both reconstructions, this taxon is strongly supported (bootstrap = 85%, posterior probability = 100%) as being a member of the genus *Protobothrops*, with its sister species being *P. jerdonii* (supported 100% in both analyses). These data also indicate that *Protobothrops* is a monophyletic lineage distinct from all other proposed genera, including *Ermia*, *Ovophis*, *Triceratolepidophis*, *Trimeresurus* (sensu lato), and *Tropidolaemus*. As the focus of the present research is on the phylogenetic position of *T. cornutus*, the whole tree will be published elsewhere. Details of

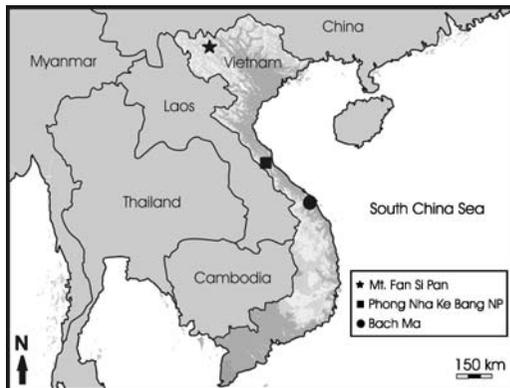


FIG. 3.—Locality records for *Trimeresurus cornutus*.

specimens belonging to *Protobothrops*, including GenBank accession numbers, are listed in Table 3.

DISCUSSION

Systematics Based on External Morphology

Trimeresurus cornutus and *Protobothrops mucrosquamatus* share the following three characters relative to *Triceratolepidophis sieversorum* (Table 1; see also Ziegler et al., 2000): (1) a longer head (*T. cornutus* 4.3–5.2 [mean 4.6] % of SVL and *P. mucrosquamatus* 4.3–4.7 % of SVL versus *T. sieversorum* 4.0–4.2 [mean 4.1] % of SVL); (2) a single dorsal scale keel which is not longitudinally divided; and (3) smooth cephalic scales.

The only shared character between *T. cornutus* and *Triceratolepidophis sieversorum* not also present in *P. mucrosquamatus* is horn-like raised supraoculars. However, this character differs in the two species with the “horns” being long and almost upright in *T. cornutus* while being short and raised at an outward angle of approximately 45° in *Triceratolepidophis sieversorum*. Horn-like raised supraoculars exist in a number of species of all major viperid groups. Their morphology can vary widely within one species and even within one population (i.e., *Cerastes cerastes*) thus making them generally problematic in systematic analyses (H-W. Herrmann, personal observation).

Trimeresurus cornutus shows similarities with *P. mucrosquamatus* in its external morphology thus supporting a reclassification with *Protobothrops*, however there are distinct morphological differences between members

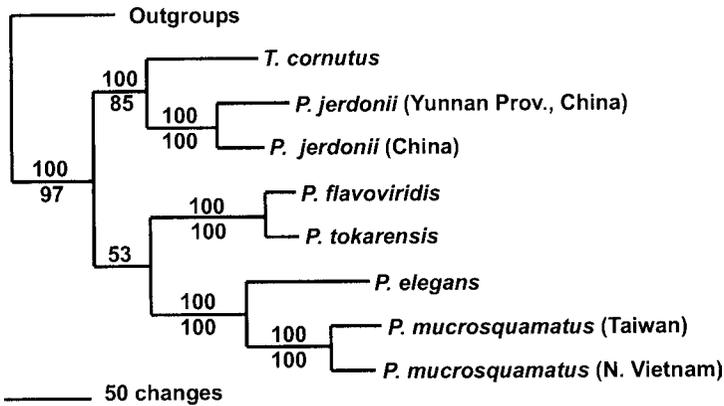


FIG. 4.—Bayesian phylogenetic hypothesis for the evolutionary position of *Trimeresurus cornutus*. The mean and 95% credibility intervals for the Bayesian Markov Chain log likelihood scores were -34761.51 (-34745.76 to -34778.73). Posterior probabilities indicated above internodes as estimated from 3 million post burn-in generations. Bootstrap percentages from MP analyses $>50\%$ are shown below internodes.

in the genus *Protobothrops* and *Triceratolepidophis* (Ziegler et al., 2000).

Systematics Based on Hemipenial Morphology

The hemipenial morphology of *T. cornutus* distinctly resembles that of *Protobothrops mucrosquamatus* (compare Maki, 1931; Mao et al., 1984; Pope, 1935), *P. xiangchengensis* (compare Guo and Zhang, 2001), and *P. elegans* (H. Ota, personal communication). Malhotra and Thorpe (2000) showed that the hemipenis was of systematic value in at least *Trimeresurus* (sensu stricto), with the three main types being congruent with the main clades defined by the molecular evidence. One of these types lacks spines altogether (see figure 1a in Guo and Zhang, 2001 for an example) and will not be discussed further. The remaining types that bear spines are clearly different from *T. cornutus*. Some Indian subcontinental species such as *T. gramineus* have a hemipenis that is covered in many long thin spines from base to tip, with the calyculate area confined to the immediate vicinity of the sulcus spermaticus (described in Pope and Pope, 1933). The hemipenis of the related *T. borneensis* and *T. puniceus* are similar. The *T. stejnegeri* group differs from *T. cornutus* in that in most species (with the exception of *T. medoensis*) the hemipenis appears bilobed rather than forked (pictured in figure 1b and 1c in Guo and Zhang, 2001), and the inner face of the lobes are calyced

from the point of forking, rather than being ornamented with small spines as in *T. cornutus*. Only one other hemipenis type that bears spines is known from *Trimeresurus* (sensu stricto), that of *T. tibetanus* (pictured in Orlov and Helfenberger, 1997; although referred to as *T. karanshahi* in that publication it has since been synonymised with *T. tibetanus* [Tillack et al., 2003]). However, this is quite different from the hemipenis of *T. cornutus*, with the spines occurring in the proximal part of the organ, with a sharply defined change to deeply frilled calyces just above the point of forking, and having relatively long lobes. More detailed descriptions of the hemipenes of *Trimeresurus* (sensu stricto) can be found in Malhotra and Thorpe, in press. The hemipenis of *T. cornutus* is shared by other members of *Protobothrops* (e.g., *P. mucrosquamatus*), and with only minor differences, is similar to other pitvipers such as *Deinagkistrodon* (Guo and Zhang, 2001; Malnate, 1990; Mao et al., 2000), *Triceratolepidophis* (Ziegler et al., 2000) and *Gloydus* (Malnate, 1990) species, and may represent an ancestral hemipenis type. Thus, hemipenial characteristics alone confirm that *cornutus* has been misclassified in *Trimeresurus* (sensu stricto), but do not necessarily support its reclassification as *Protobothrops*.

Systematics Based on Molecular Data

The results of the molecular analyses provide evidence that *cornutus* should be reassigned to *Protobothrops*. The validity of *Protobothrops*

TABLE 3.—Specimen details and GenBank accession numbers for the *Protobothrops* species used in this analysis. UMMZ = University of Michigan Museum of Zoology; ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; CAS = California Academy of Science, San Francisco; FK = Fred Kraus, field tag; AM = Anita Malhotra, catalogue number.

Species	Specimen number	Locality	GenBank accession no. (cytb, ND4, 12S, 16S)
<i>P. flavoviridis</i>	UMMZ 199973	Tokunoshima, Ryukyu Island, Japan	AY223574, U41894, AF057200, AF057247
<i>P. elegans</i>	UMMZ 199970	Ishigaki Island, Ryukyu Island, Japan	AY223575, U41893, AF057201, AF057248
<i>P. cornutus</i>	ZFMK 75067	Phong Nha–Ke Bang NP, C Vietnam	AY294272, AY294262, AY294276, AY294267
<i>P. jerdonii</i>	CAS 215051	Nu Jiang Pref., Yunnan Province, China	AY294274, AY294264, AY294278, AY294269
<i>P. jerdonii</i>	AM B485	China	AY294273, AY294263, AY294277, AY294268
<i>P. mucrosquamatus</i>	AM A233	Hualien Province, Taiwan	AF171897, AY294265, AY294279, AY294270
<i>P. mucrosquamatus</i>	AM B106	Vin Phuc Province, N Vietnam	AY294275, AY294266, AY294280, AY294271
<i>P. tokarensis</i>	FK 1997	Takarajima, Ryukyu Island, Japan	AY223576, AY223628, AF057202, AF057249

has been supported in a number of other molecular studies (Kraus et al., 1996; Malhotra and Thorpe, 2000; Parkinson, 1999; Parkinson et al., 2002; Tu et al., 2000). Species tentatively assigned to this genus that have not yet been verified by molecular analysis, but whose hemipenes have been studied, include *P. kaulbacki* and *P. xiangchengensis*. These two species have hemipenes that resemble other *Protobothrops* species (Guo and Zhang, 2001; Smith, 1943). On the other hand, *T. strigatus*, which has also been placed in *Protobothrops* by some authors (Kraus et al., 1994; David and Ineich, 1999), possesses a derived hemipenis most similar to those found in other members of *Trimeresurus* (sensu stricto) from South India and does not appear referable to *Protobothrops* (Malhotra and Thorpe, 2000).

The position of *cornutus* within *Protobothrops* is somewhat surprising for a number of reasons. First, all other species of *Protobothrops* are relatively large animals (among the largest of the *Trimeresurus* (sensu lato) complex) usually exceeding 1 m in total length, and include the longest Asian pitviper (*P. flavoviridis*, recorded at 2.2 m total length [Koba, 1971]). Currently, the largest known specimen of *T. cornutus* is only 696 mm in total length. However, this specimen is male, and females of most other pitvipers are usually larger than males (e.g., *P. mucrosquamatus* [Zhao et al., 1998]). Second, it has a prehensile tail, possibly indicating that it is adapted to arboreal habitats; it is the only member of the

genus to have this adaptation (Smith, 1943). Nothing further is known about the biology of the species. Its sister species, *P. jerdonii*, is the only member of *Protobothrops* that is viviparous (Liu, 1939), and it will be of interest in determining the reproductive mode of *T. cornutus*.

Taken together, the external morphology, hemipenial morphology and the results of the molecular analyses suggest that *T. cornutus* should be reclassified as *Protobothrops cornutus* comb. nov. The molecular data indicate as well that the genus *Protobothrops* is a monophyletic lineage distinct from all other proposed genera.

Distribution and Habitat

The altitude at which the new specimen was found (150 m) is markedly lower than the former records (2000 m for two vouchers in North Vietnam and 1400 m for the record from South Vietnam). The Phong Nha–Ke Bang area in Central Vietnam, together with the Hin Namno National Biodiversity Conservation Area in Laos, form a natural unit within the central Annamite Mountain range along the Vietnam–Laos border and ascend to approximately 1000 m altitude. With only three known *P. cornutus* specimens, the extent and continuity of this species range is still unclear. Its distribution could be restricted to glacial mountain forest refugia (Brandon-Jones, 1996) in widely separated areas in Vietnam and possibly Laos, or the species

may have a wider geographical range than currently known but be naturally very rare. Pope (1935: 404) wrote “. . . it is certain to turn up in the mountains of southeastern Yunnan.”

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LITERATURE CITED

- ALLARD, M. W., AND J. M. CARPENTER. 1996. On weighting and congruence. *Cladistics* 12:183–198.
- BAKER, R. H., G. S. WILKINSON, AND R. DESALLE. 2001. Phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diptera: Diopsidae). *Systematic Biology* 50:87–105.
- BRANDON-JONES, D. 1996. The Asian Colobinae (Mammalia: Cercopithecidae) as indicators of quaternary climatic change. *Biological Journal of the Linnean Society* 59:327–350.
- BURGER, W. L. 1971. Genera of Pitvipers. Ph.D. Dissertation, University of Kansas, Lawrence, Kansas, U.S.A.
- CAMPDEN-MAIN, S. M. 1970. A Field Guide to the Snakes of South Vietnam. Smithsonian Institution, Washington, D. C., U.S.A.
- DAVID, P., AND I. INEICH. 1999. Les serpents venimeux du monde: systématique et repartition. Dumerilia, Paris 3:3–499.
- DOWLING, H. G. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1:97–99.
- DOWLING, H. G., AND J. M. SAVAGE. 1960. A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. *Zoologica* 45:17–30.
- FU, Y.-X., AND W.-H. LI. 1993. Statistical tests of neutrality of mutations. *Genetics* 133:693–709.
- GUO, P., AND F. ZHANG. 2001. Comparative studies on hemipenes of four species of *Trimeresurus* (sensu stricto) (Serpentes: Crotalinae). *Amphibia-Reptilia* 22:113–117.
- HERRMANN, H.-W., T. ZIEGLER, B. L. STUART, AND N. L. ORLOV. 2002. New findings on the distribution, morphology and natural history of *Triceratolepidophis sieversorum* (Serpentes: Viperidae). *Herpetological Natural History* 9:89–94.
- HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analysis. *Journal of Heredity* 83:189–195.
- HOGUE, A. R., AND S. A. R. W. ROMANO-HOGE. 1981. Notes on micro and ultrastructure of “Oberhäutchen” in viperioidea. *Memorias do Instituto Butantan* 44/45:81–118.
- HUELSENBECK, J. P., AND K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* 28:437–466.
- HUELSENBECK, J. P., AND F. R. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755.
- KOBA, K. 1971. Natural history of the Habu, *Trimeresurus flavoviridis* (Hallowell). *The Snake* 3:75–96.
- KLAUBER, L. M. 1956. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind, Vol. 1. University of California Press, Berkeley, California, U.S.A.
- KNIGHT, A., AND D. P. MINDELL. 1993. Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea’s viper. *Systematic Biology* 42:18–31.
- KRAUS, F., D. G. MINK, AND W. M. BROWN. 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. *Copeia* 1996:763–773.
- KUMAR, S., K. TAMURA, I. B. JAKOBSEN, AND M. NEI. 2001. MEGA2: Molecular Evolutionary Genetics Analysis Software. Arizona State University, Tempe, Arizona, U.S.A.
- LIU, C. 1939–40. Life history of *Trimeresurus jerdonii*. *Peking Natural History Bulletin* 14:245–253.
- MAKI, M. 1931–33. A Monograph of the Snakes of Japan. Dai-Ichi Shobo, Tokyo, Vol. I (1931), No. 8, pp. 1–240 (English text); Vol. II (1931), plates 1–39; Vol. III (1933), No. 5, pp. 1–57 (Japanese text), pl. 40–81.
- MALHOTRA, A., AND R. S. THORPE. 1997. New perspectives on the evolution of south-east Asian pitvipers (genus *Trimeresurus*) from molecular studies. Pp. 115–128. In R. S. Thorpe, W. Wüster, and A. Malhotra (Eds.), *Venomous Snakes: Ecology, Evolution and Snakebite*. Symposia of the Zoological Society of London 70, Clarendon Press, Oxford, England.
- . 2000. A phylogeny of the *Trimeresurus* group of pit vipers: new evidence from a mitochondrial gene tree. *Molecular Phylogenetics and Evolution* 16:199–211.
- . In press. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pit vipers (*Trimeresurus* and *Ovophis*). *Molecular Phylogenetics and Evolution*.
- MALNATE, E. V. 1990. A review and comparison of hemipenial structure in the genus *Agkistrodon* (sensu lato). Pp. 583–588. In H. K. Gloyd and R. Conant (Eds.), *Snakes of the Agkistrodon Complex: A Monographic Review*. Society for the Study of Amphibians and Reptiles, Oxford, Ohio, U.S.A.
- MAO, S. H., F. Y. YIN, AND Y. W. GUO. 1984. The hemipenes of common Taiwanese venomous snakes. *Herpetologica* 40:406–410.
- MCDIARMID, R. W., J. A. CAMPBELL, AND T. TOURÉ. 1999. *Snake Species of the World. A Taxonomic and Geo-*

- graphic Reference, Vol. 1. The Herpetologist's League, Washington, D.C., U.S.A.
- MCDONALD, J. H., AND M. KREITMAN. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* 351:652–654.
- MEBS, D., U. KUCH, H.-W. HERRMANN, AND T. ZIEGLER. 2003. Biochemical and biological activities of the venom of a new genus and species of pitviper from Vietnam, *Triceratolepidophis sieversorum*. *Toxicon* 41:139–143.
- MILINKOVITCH, M. C., AND J. LYONS-WEILER. 1998. Finding optimal ingroup topologies and convexities when the choice of outgroups is not obvious. *Molecular Phylogeny and Evolution* 9:348–357.
- ORLOV, N., AND N. HELFENBERGER. 1997. New mountain species of *Trimeresurus* (Serpentes, Viperidae, Crotalinae) of the “green” pitvipers group from the Himalayas. *Russian Journal of Herpetology* 4:195–197.
- ORLOW, N. L. 1998. Die Giftschlangen der vietnamesischen Bergwälder. Teil 2: Familie Viperidae. *Herpetofauna, Weinstadt* 20:5–14.
- PARKINSON, C. L. 1999. The molecular systematics and biogeography of the subfamily Crotalinae as determined by mtDNA sequences. *Copeia* 1999:576–586.
- PARKINSON, C. L., S. M. MOODY, AND J. E. AHLQUIST. 1997. Phylogenetic relationships of the “*Agkistrodon* Complex” based on mitochondrial DNA sequence data. Pp. 63–78. *In* R. S. Thorpe, W. Wüster, and A. Malhotra (Eds.), *Venomous Snakes: Ecology, Evolution and Snakebite*. Symposium of the Zoological Society of London 70, Clarendon Press, Oxford, England.
- PARKINSON, C. L., K. R. ZAMUDIO, AND H. W. GREENE. 2000. Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils. *Molecular Ecology* 9:411–420.
- PARKINSON, C. L., J. A. CAMPBELL, AND P. T. CHIPPINDALE. 2002. Multigene phylogenetic analysis of pitvipers, with comments on the biogeography of the group. Pp. 93–110. *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), *Biology of the Vipers*. Eagle Mountain Publishing, Eagle Mountain, Utah, U.S.A.
- PHILIPPE, H., G. LECOINTRE, H. L. VAN LE, AND H. LE GUYADER. 1996. A critical study of homoplasy in molecular data with the use of a morphologically based cladogram, and its consequences for character weighting. *Molecular Biology* 13:1174–1186.
- POPE, C. H. 1935. The Reptiles of China. Turtles, Crocodylians, Snakes, Lizards. *Natural history of Central Asia*, Vol. X. American Museum of Natural History, New York.
- POPE, C. H., AND S. H. POPE. 1933. A study of the green pit-vipers of southeastern Asia and Malaysia, commonly identified as *Trimeresurus gramineus* (Shaw), with description of a new species from peninsular India. *American Museum Novitates* 620:1–12.
- POSADA, D. A., AND K. A. CRANDALL. 1998. Model-Test: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- . 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50:580–601.
- ROZAS, J., AND R. ROZAS. 1999. DNASP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* 15: 174–175.
- SAMBROOK, J., E. F. FRISCH, AND T. E. MANIATIS. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbour Press, New York, New York, U.S.A.
- SMITH, M. A. 1930. Two new snakes from Tonkin, Indo-China. *Annals and Magazine of Natural History* (Ser. 10) 6:681–683.
- . 1943. *The Fauna of British India, Ceylon and Burma Including the Whole of the Indo-chinese Sub-region. Reptilia and Amphibia, III. Serpentes*. Taylor and Francis, London, England.
- SWOFFORD, D. L. 2003. PAUP*: Phylogenetic Analysis using Parsimony and Other Methods, version 4.0. Sinauer, Sunderland, Massachusetts, U.S.A.
- TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595.
- TILLACK, F., M. LORENZ, N. L. ORLOV, N. HELFENBERGER, K. B. SHAH, AND W. ECKERT. 2003. Shah's Grubenotter *Trimeresurus karanshahi* Orlov & Helfenberger, 1997—ein Junior synonym von *Trimeresurus tibetanus* Huang, 1982 (Serpentes: Viperidae: Crotalinae), mit Angaben zur Verbreitung, Biologie und der Vorstellung neuer Farbvarianten aus Zentral-Nepal. *Sauria* 25:2–15.
- TU, M.-C., H.-Y. WANG, M.-P. TSAI, M. TODA, W.-J. LEE, F.-J. ZHANG, AND H. OTA. 2000. Phylogeny, taxonomy and biogeography of the Oriental pitvipers of the genus *Trimeresurus* (Reptilia: Viperidae: Crotalinae): a molecular perspective. *Zoological Science* 17:1147–1157.
- ZHANG, D.-X., AND G. HEWITT. 1996. Nuclear integrations—challenges for mitochondrial—DNA markers. *Trends in Ecology and Evolution* 11:247–251.
- ZHAO, E., M. HUANG, AND Y. ZONG. 1998. *Fauna Sinica: Reptilia, Vol. 3. Squamata Serpentes*. Science Press, Beijing, China.
- ZIEGLER, T., AND W. BÖHME. 1997. Genitalstrukturen und Paarungsbiologie bei squamaten Reptilien, speziell den Platynta, mit Bemerkungen zur Systematik. *Mertensiella* 8:1–207.
- ZIEGLER, T., AND H.-W. HERRMANN. 2000. Preliminary list of the herpetofauna of the Phong Nha–Ke Bang area in Quang Binh province, Vietnam. *Biogeographica* 76: 49–62.
- . 2002. Wiederentdeckung zweier gehörnter Grubenotterarten in Vietnam. *Mitteilungen Zoologische Gesellschaft für Arten- und Populationsschutz e.V.* 18:24–26.
- ZIEGLER, T., H.-W. HERRMANN, P. DAVID, N. L. ORLOV, AND O. S. G. PAUWELS. 2000. *Triceratolepidophis sieversorum*, a new genus and species of pitviper (Reptilia: Serpentes: Viperidae: Crotalinae) from Vietnam. *Russian Journal of Herpetology* 7:199–214.

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