



## **Phylogeny, Taxonomy, and Biogeography of the Oriental Pitvipers of the Genus *Trimeresurus* (Reptilia: Viperidae: Crotalinae): A Molecular Perspective**

Authors: Tu, Ming-Chung, Wang, Hurng-Yi, Tsai, Mung-Pei, Toda, Mamoru, Lee, Wen-Jen, et al.

Source: Zoological Science, 17(8) : 1147-1157

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.17.1147>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Phylogeny, Taxonomy, and Biogeography of the Oriental Pitvipers of the Genus *Trimeresurus* (Reptilia: Viperidae: Crotalinae): A Molecular Perspective

Ming-Chung Tu<sup>1</sup>, Hurng-Yi Wang<sup>1</sup>, Mung-Pei Tsai<sup>2</sup>, Mamoru Toda<sup>3</sup>,  
Wen-Jen Lee<sup>1</sup>, Fu-Ji Zhang<sup>4</sup>, and Hidetoshi Ota<sup>5\*</sup>

<sup>1</sup>Department of Biology, National Taiwan Normal University, Taipei, 117 Taiwan

<sup>2</sup>Institute of Zoology, Academia Sinica, Taipei, 115 Taiwan

<sup>3</sup>Department of Zoology, Graduate School of Science, Kyoto University,  
Sakyo, Kyoto, 606-8502 Japan

<sup>4</sup>Chengdu Institute of Biology, The Chinese Academy of Sciences,  
Chengdu, 610041 People's Republic of China

<sup>5</sup>Tropical Biosphere Research Center, University of the  
Ryukyus, Nishihara, Okinawa, 903-0213 Japan

---

**ABSTRACT**—Based on sequence variation in 806 bp of the mitochondrial 12S rRNA gene, phylogenetic relationships were inferred for 14 species of *Trimeresurus* (sensu lato) including all East Asian members. Samples analyzed also included representatives of all assemblages of species that are frequently treated as separate genera except for *T. mangshanensis*, a type species of the recently described monotypic genus *Ermia*. Results support some previous accounts chiefly from morphological studies, such as distinct divergence of *T. wagleri* from the remainder, and monophyly of *T. mucrosquamatus*, *T. flavoviridis*, *T. jerdonii*, *T. elegans* and *T. tokarensis*. On the other hand, our results negated a putative close affinity of *T. monticola* and *T. okinavensis*, and indicated the sister relationship of the latter with *T. gracilis*. Phylogenetic relationships revealed in this study suggested that the genus *Trimeresurus* dispersed into the Ryukyu region at least three times, and that *T. flavoviridis* and *T. tokarensis* from the central Ryukyus constitute a relict clade.

---

## INTRODUCTION

*Trimeresurus* Lacepede, as defined by Brattstrom (1964) (henceforth referred to as *Trimeresurus* [sensu lato]), is the largest group of venomous snakes in tropical-subtropical Asia, consisting of some 40 species (David and Tong, 1997; McDiarmid *et al.*, 1999). A few species of the genus are especially dangerous and thus are of great concern to public-health authorities and medical workers in some areas (Gopalakrishnakone and Chou, 1990). Distribution of *Trimeresurus* (sensu lato), covering not only a broad part of the Asian continent but also a number of adjacent archipelagos with complex geohistories, makes the genus a particularly suitable model to investigate biological consequences of past geological events as expressed in a phylogeographical pattern of extant species.

A number of authors have addressed phylogenetic relationships within *Trimeresurus* (sensu lato) based on morphological characters, some hypothesizing scenarios for the divergence process of the genus (Brattstrom, 1964; Zhang, 1995), others proposing its taxonomic subdivisions (Burger, 1971; Hoge and Romano-Hoge, 1981, 1983; Zhang, 1993). Nevertheless, very little consensus has yet been attained to date for almost every relevant issue (e.g., see comments by Groombridge [1986], Toriba [1989] and McDiarmid *et al.* [1999] on the taxonomic changes proposed by Burger [1971], Hoge and Romano-Hoge [1983] and Zhang [1993], respectively).

Recently a few preliminary molecular studies, while confirming great divergences within *Trimeresurus* (sensu lato) predicted by previous morphological studies (see above) (Kraus *et al.*, 1996; Knight *et al.*, 1992), elucidated prominent convergences in some of the traditionally used taxonomic characters of this and other crotalinae genera (Cullings *et al.*, 1997; Malhotra and Thorpe, 1997; Vidal *et al.*, 1997; Parkinson, 1999). These findings suggest the presence of severe constraints in the use of morphological characters for the recon-

---

\* Corresponding author: Tel. 098-895-8937;  
FAX. 098-895-8966.  
E-mail: ota@sci.u-ryukyu.ac.jp

struction of phylogenetic relationships in this and related genera.

For the purpose of testing some previous hypotheses regarding the phylogeny and biogeography of *Trimeresurus* (sensu lato), we sequenced a fragment of the mitochondrial 12S ribosomal RNA gene (12S rRNA) for representatives of the genus. Sequence variation in this domain is usually used to resolve higher level phylogenetic relationships in reptiles (Heise *et al.*, 1995; Honda *et al.*, 1999; Ota *et al.*, 1999), but has also been shown to be effective to infer the infrageneric phylogeny of viperid snakes (e.g., Knight *et al.*, 1993). Analyses of sequence data suggest relationships which substantially differ from those hypothesized for *Trimeresurus* (sensu lato) based on previous morphological studies. Also, our results yield new insights into the historical biogeographical analysis of terrestrial organisms in the subtropical East Asian islands.

## MATERIALS AND METHODS

Eighteen specimens of 14 species and one subspecies of *Trimeresurus* (sensu lato) were used in this study (Table 1: Appendix 1). Of these taxa, four species and one subspecies (*T. monticola monticola*, *T. monticola makazayazaya*, *T. jerdonii*, *T. gracilis*, and *T. puniceus*) were examined by the molecular technique for the first time. The taxa examined include all eight species of the genus occurring in Taiwan and the Ryukyu Archipelago, as well as representatives of all species assemblages of *Trimeresurus* (sensu lato) but one (*Ernia Zhang*) that are frequently treated as separate genera (i.e., *Ovophis* Burger, *Protobothrops* Hoge et Romano-Hoge, *Tropidolaemus* Wagler, and *Trimeresurus* sensu stricto) (Hoge and Romano-Hoge, 1983; Toriba, 1993; Zhang, 1993; Wüster *et al.*, 1997; McDiarmid *et al.*, 1999). Specimens of *Deinagkistrodon acutus* and *Vipera russelii* that, respectively, represent another crotaline genus and a different viperid subfamily, Viperinae, were also included in the analyses as outgroups. Each specimen was designated as constituting an independent operational taxonomic unit (OTU) by itself for the phylogenetic analyses.

Crude DNA was extracted from fresh or 95% ethanol-preserved muscle samples following Kocher *et al.* (1989), but with incubation

extended for approximately ten hours. After proteinase K digestion, DNA was purified by two times of phenol extraction, once or twice of phenol/chloroform/isoamyl alcohol (25:24:1) extraction, and once of chloroform/isoamyl alcohol (24:1) extraction, each followed by precipitation in ethanol with 1/10 volume NaOAc.

A portion of the 12S rRNA gene of the mitochondrial genome was amplified by the polymerase chain reaction (PCR) using two primers— SN1: 5'-AGTCTGCTCAAAAAGATTAATGTTAA-3'; and SN2: 5'-TCTTGGTCTGAAACCTCAGTTACCTA-3' (Wang and Tu, 1997). PCR reactions were performed in 50  $\mu$ l volumes consisting of 10 mM Tris-HCl (pH 9.0), 50mM KCl, 15mM MgCl<sub>2</sub>, 0.1% [w/v] gelatin, 1% Triton X-100, 0.4 pM primer, 0.2 mM dNTP, 50–100 ng of crude DNA, and 1 U Taq polymerase (InVITaq, Germany). The temperature regimen of 35 cycles, subsequent to the two minutes of initial denaturation at 94°C, was 1 min at 94°C, 1 min at 50°C, and 1.5 min at 72°C.

PCR products were purified with the Gene Clean III elution kit (BIO 101, CA). Both DNA strands were sequenced using dye terminator cycle-sequencing reactions that were subsequently loaded on an Applied Biosystems 377A automatic sequencer. The numbering system followed Anderson *et al.* (1981).

DNA sequences were aligned by using the default parameters of CLUSTAL W (Thompson *et al.*, 1994). Adjustments were made visually on the basis of maximum nucleotide similarity. Gaps and insertions (indels) were excluded from the subsequent analyses.

Based on the aligned sequences, phylogenetic relationships among OTUs were inferred using neighbor-joining (NJ: Saitou and Nei, 1987) and maximum parsimony (MP) methods. For the former, three distance models (i.e., Jukes and Cantor's [1969] one parameter model, Kimura's [1980] two parameter model, and Tamura and Nei's [1993] model) were used to correct pairwise distances for multiple hits. Resultant distance matrices were subjected to NJ analyses using PHYLIP 3.5c (Felsenstein, 1993). Degrees of supports for internal branches of the resultant tree were assessed by 1,000 bootstrap replications (Felsenstein, 1985) for each weighting scheme used. By using PAUP version 4.0 (Swofford, 1998), we further examined the content of phylogenetic information in our data set by checking the skewness of the tree distribution and the g1 values (Hillis and Huelsenbeck, 1992) for 10,000 random trees in each weighting scheme.

MP analysis was performed using PAUP\*, in which each nucleotide base was regarded as an unordered character, and the four kinds of salts as different character states. A total of 1,000 bootstrap pseudoreplications (Felsenstein, 1985) were conducted using the

**Table 1.** Species and subspecies of *Trimeresurus* (sensu lato), and two outgroup species examined in this study. Information regarding their distributions was taken from Toriba (1993) and McDiarmid *et al.* (1999). See Appendix 1 for locality data for materials used in our analyses.

Species/subspecies	Sample size	Distribution
<i>Trimeresurus albolabris</i>	1	Northern India through Southeast Asia to southern China
<i>T. elegans</i>	1	The Yaeyama Group of the southern Ryukyus, Japan
<i>T. flavoviridis</i>	1	The Okinawa and Amami Groups of the central Ryukyus, Japan
<i>T. gracilis</i>	1	Taiwan
<i>T. jerdonii</i>	1	Northern India, northern Indochina, and southern China
<i>T. monticola monticola</i>	1	Nepal, northern India, Myanmar, and southern China
<i>T. monticola makazayazaya</i>	2	Eastern China including Taiwan
<i>T. mucrosquamatus</i>	2	Northern India through southern China to Taiwan
<i>T. okinavensis</i>	1	The Okinawa and Amami Groups of the central Ryukyus, Japan
<i>T. popeiorum</i>	1	Northern India and Southeast Asia
<i>T. puniceus</i>	1	Southern Thailand, Malay Peninsula, and adjacent islands
<i>T. stejnegeri</i>	2	Northern India through Indochina and southern China to Taiwan
<i>T. tokarensis</i>	1	Southern Tokara islands of the central Ryukyus, Japan
<i>T. trigonocephalus</i>	1	Sri Lanka
<i>T. wagleri</i>	1	Southeast Asia including the Philippines
<i>Deinagkistrodon acutus</i>	1	Northern Indochina through southern China to Taiwan
<i>Vipera russelii</i>	1	South Asia through Southeast Asia and southern China to Taiwan

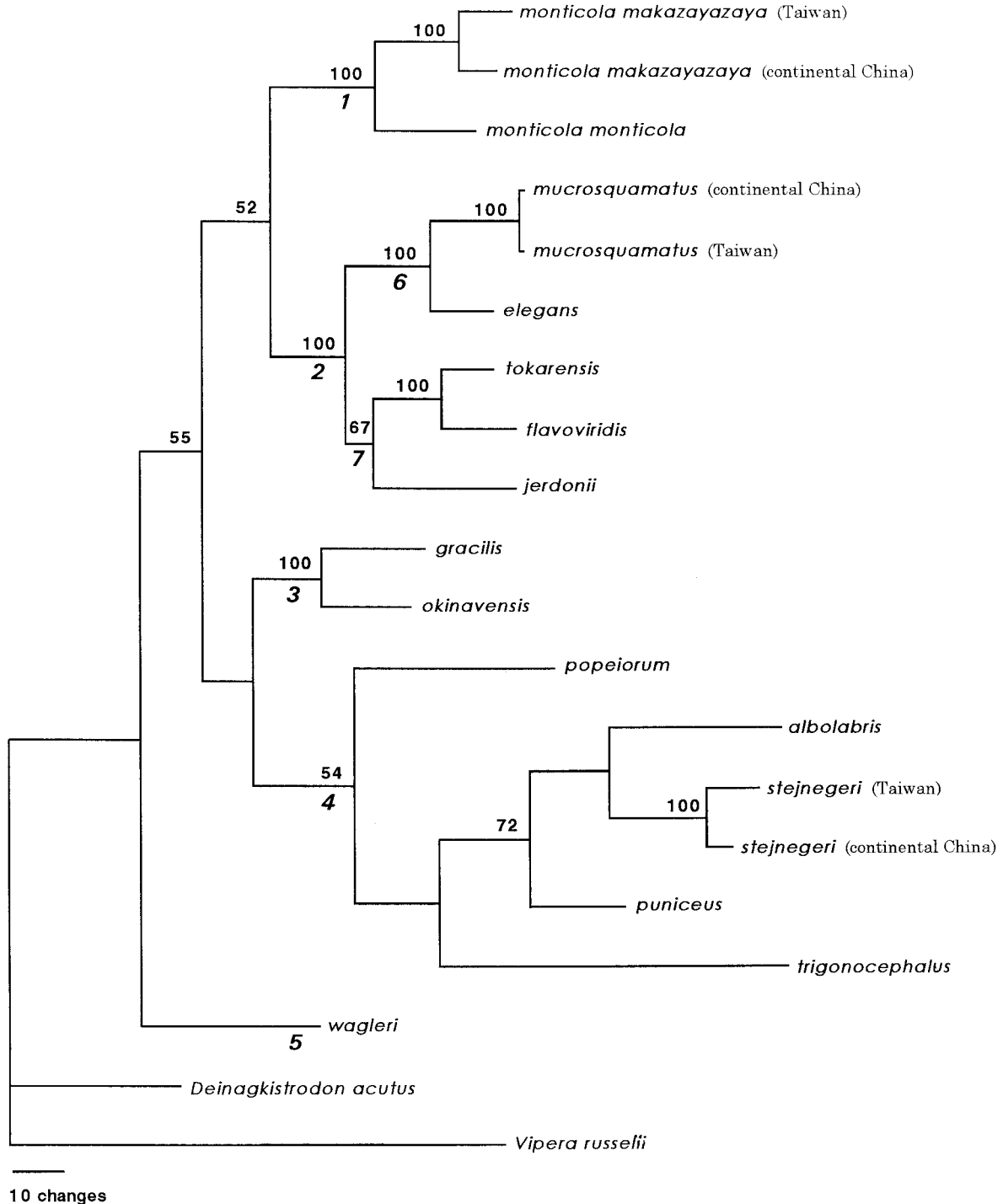
heuristic algorithm of PAUP\*. The result of this analysis was expressed as a 50% majority-rule consensus bootstrap tree. Skewness of the tree distribution and the g1 values for 10,000 random trees were also calculated.

bp including indels (Appendix 2). The first 230 and 200 sites of *T. wagleri* and *D. acutus*, respectively, could not be sequenced despite our several attempts. These portions were treated as gaps, and were excluded from the analyses.

Comparisons of aligned sequence revealed 362 variable sites, of which 200 were phylogenetically informative. The base composition was slightly biased, with the average nucleotide

**RESULTS**

Aligned sequence of 12S rRNA gene consisted of 806



**Fig. 1.** Neighbor-joining (NJ) tree derived from Kimura's (1980) two parameter distance matrix for 12S rRNA sequence data. Numerals above nodes indicate proportion of support in 1000 bootstrap replications (BP). Branches without BP values were not supported in  $\geq 50\%$  of the replicates. Numerals below nodes are reference numbers used in the text.

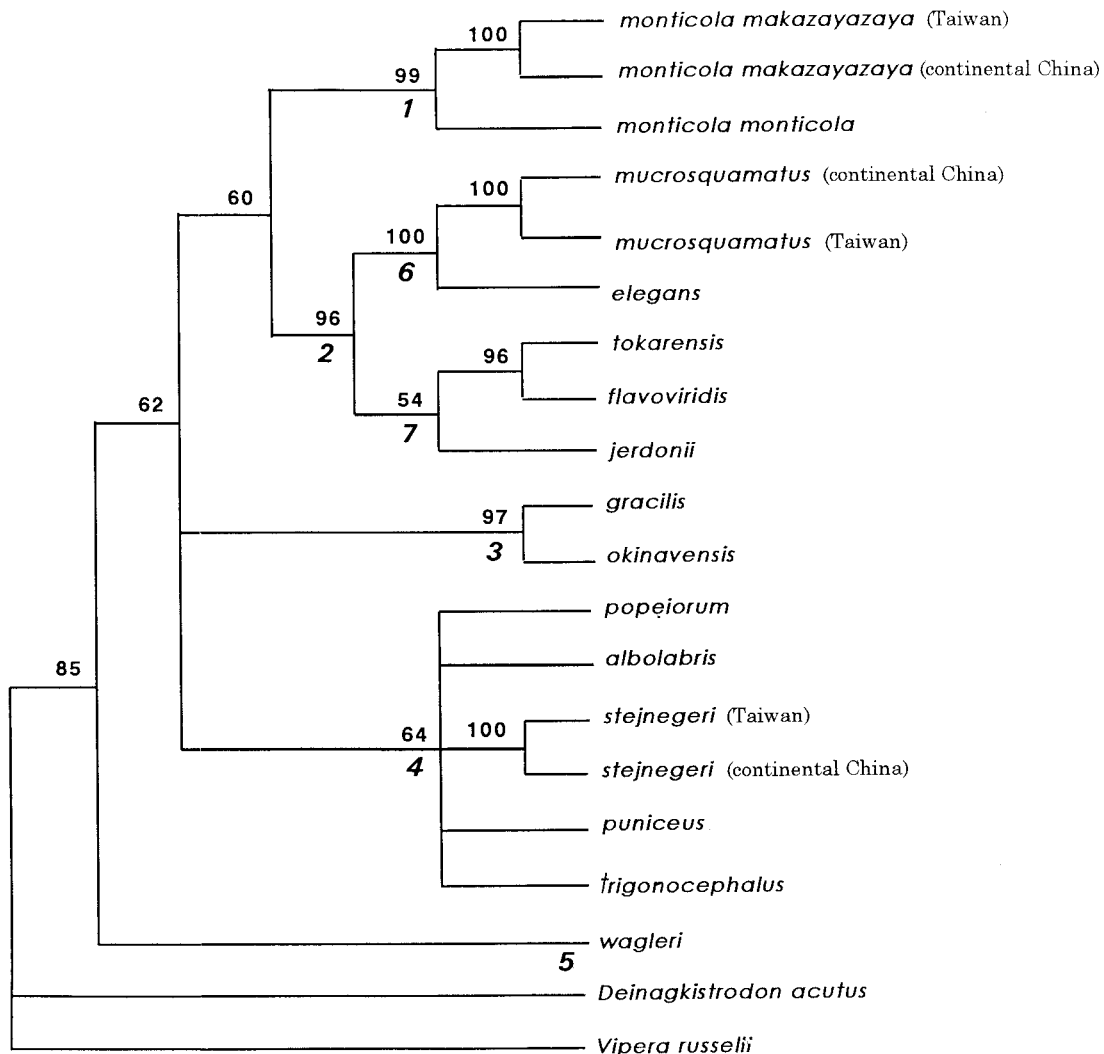
frequencies of A, T, C and G being 37.6%, 19.8%, 25.1% and 17.5%, respectively. The estimated  $g1$  values for trees resulting from our analyses were lower than critical values ( $p=0.01$ : Hillis and Huelsenbeck, 1992), indicating that the region sequenced contained phylogenetic information.

Distance values resulting from the three different models were very similar to each other. Likewise, resultant NJ trees showed no distinct differences in branching topology or relative branch length among the three models, either. Therefore, we provide results of NJ analysis using Kimura's distance matrix only (Fig. 1) (distance matrices and results of NJ using the remaining models are available from H.-Y. Wang upon request).

In the NJ analysis, monophyly of the genus *Trimeresurus* sensu lato was supported only by a low bootstrap proportion (BP:<50%), whereas the monophyly of conspecific and consubspecific OTUs invariably received a complete bootstrap support. Five major nodes (nodes 1–5) were recognized in

NJ tree, of which two consisted of single species, *T. monticola* (node 1) and *T. wagleri* (node 5), whereas another one with a complete bootstrap support (node 3) consisted of *T. gracilis* and *T. okinavensis*. Of the remaining two major nodes, one with a rather poor BP support (node 4) consisted of exclusively arboreal species, with *T. popeiorum* possibly initially diverging from the remainder, followed by *T. trigonocephalus* and *T. puniceus* in order. The remaining major node with a complete bootstrap support (node 2) was further divided into two nodes, one consisting of *T. mucrosquamatus* and *T. elegans* (node 6), and the other of *T. jerdonii*, *T. flavoviridis* and *T. tokarensis* (node 7). The last two species received a 100% bootstrap support for their sister-group relationship, whereas the sister-group relationship of these species and *T. jerdonii* was supported with a lower, but still substantial bootstrap proportion (67%).

The 50% majority-rule consensus tree resulting from the MP analysis did not contradict with NJ tree in branching topol-



**Fig. 2.** Majority-rule consensus cladogram derived from maximum parsimony analysis (MP) of 12S rRNA sequence data. Numerals above nodes indicate proportion of support in 1000 bootstrap pseudoreplications. Numerals below nodes are reference numbers used in the text. Consistency index excluding uninformative sites (Kluge and Farris, 1969) = 0.457; retention index (Farris, 1989) = 0.530.

ogy (Fig. 2), but differs from the latter in the degree of supports for a few nodes. For example, monophyly of the genus *Trimeresurus* sensu lato was supported with a much higher BP value (85%), whereas the value in support for the node 7 (54%) was lower than the corresponding value in NJ tree (see above).

## DISCUSSION

### Phylogeny

Our results corroborate the monophyly of *T. mucrosquamatus*, *T. flavoviridis*, *T. jerdonii*, *T. elegans*, and *T. tokarensis*, as was explicitly or implicitly predicted by a few previous authors (Hoge and Romano-Hoge, 1981; Kraus *et al.*, 1996; David and Ineich, 1999; Parkinson, 1999). Moreover, initial divergence of *T. wagleri* from the remaining members of *Trimeresurus* (sensu lato) in our analyses (Figs. 1 and 2) agrees well with the relationships inferred by Brattstrom (1964) on the basis of morphological analysis. However, such relationships of *T. wagleri* and other species contradict with the hypothesis resulting from molecular analyses by Parkinson (1999), in which *T. wagleri* is considered to be closer to *T. albolabris* and *T. stejnegeri* than are some other species common to our study (i.e., *T. elegans*, *T. flavoviridis*, *T. tokarensis*, and *T. okinavensis*). Moreover, *T. wagleri* is shown to be closest to *D. acutus* in Parkinson's (1999) trees. Because either his trees or ours failed to receive sufficiently high bootstrap supports (> 90%: Shaffer *et al.*, 1997) for these relationships, it is obvious that unambiguous solutions of these inconsistencies on the molecular ground require additional sequence data. Nevertheless, considering their congruence with results of morphological analysis (see above), the relationships depicted in our trees seem to be more likely at present.

The remaining portions of our NJ and MP trees show considerable discrepancies with previously hypothesized relationships. For example, our results do not support at all the close affinity of *Trimeresurus monticola* and *T. okinavensis*, that were combined under a separate generic name, *Ovophis* (type species: *monticola*), by Burger (1971) and Hoge and Romano-Hoge (1981) together with three other species not studied here (i.e., *T. chaseni*, *T. convictus*, and *T. tonkinensis*). Such a distant relationship of *monticola* and *okinavensis* was also implied by results of comparative studies of head musculature by Groombridge (1986). Both *monticola* and *okinavensis* are stout-bodied ground dwellers, whereas most other species examined here, including the possibly most primitive *T. wagleri* (see above), are arboreal or semi-arboreal and have thinner bodies (Koba, 1962; Zhao *et al.*, 1998; Gopalakrishnakone and Chou, 1990). It is thus possible that external morphological similarities between *monticola* and *okinavensis* actually represent convergence resulting from independent adaptations to similar, non-arboreal life-styles.

Brattstrom (1964), on the basis of descriptions by Maslin (1942), surmised that *T. gracilis* is phylogenetically closest to *T. puniceus* (and its sibling species, *T. borneensis*: see McDiarmid [1999]). Results of our analysis, however, negate

such a view, and strongly suggest a much closer affinity of *T. gracilis* to *T. okinavensis*. It is likely that similarities in some scale characters between *T. gracilis* and *T. puniceus* emphasized in a key by Maslin (1942), which obviously let Brattstrom (1964) assume their close affinity, actually represent symplesiomorphy or convergence.

Based on the allozyme analyses, Toda *et al.* (1999) surmised a relatively close relationship between *T. monticola* and *T. okinavensis*. However, materials subjected to their analyses were limited to *T. elegans*, *T. flavoviridis*, *T. mucrosquamatus*, and *T. tokarensis*, besides those two species. Moreover, subsequent allozyme reanalyses by incorporating data for a single *T. gracilis* yielded results that predict the closest affinity between this species and *okinavensis* (Toda and Ota, unpublished data). Thus, it seems unlikely that the relationships illustrated by more comprehensive allozyme analyses substantially contradict with the relationships indicated by the present analyses.

### Taxonomic implications

The relationships depicted by our analyses (Figs. 1 and 2) support the validity of *Tropidolaemus*, which had usually been regarded as synonymous with *Trimeresurus* (e.g., see a synonym list of *wagleri* in David and Vogel [1996]), and was resurrected to accommodate *wagleri* by Hoge and Romano-Hoge (1981) on the basis of Burger's (1971) morphological data. For further confirmation for the current taxonomic arrangement of *Tropidolaemus*, additional analyses are desired by incorporating sequence data for *huttoni* from India, a putative second species of the genus (David and Vogel, 1998).

Our results also support the validity of *Protobothrops* (node 2), which was originally described for *T. flavoviridis* (type species), *T. mucrosquamatus* and *T. jerdonii*, and subsequently also thought to include *T. elegans* and *T. tokarensis*, as well as *T. kaulbacki*, *T. strigatus* and *T. xiangchengensis* (Hoge and Romano-Hoge, 1983; Kraus *et al.*, 1996; David and Ineich, 1999; Parkinson, 1999). In contrast, relationships revealed here negate the validity of *Ovophis* as arranged by Hoge and Romano-Hoge (1981). Use of the generic name, *Ovophis*, thus should be avoided until more plausible delimitation and morphological redefinitions are made on the basis of comprehensive phylogenetic analyses for *Ovophis* sensu Hoge and Romano-Hoge (1981). Considering the possible close relationships of *monticola* and the node 2 species as suggested in our analyses (Figs. 1 and 2), use of the generic name, *Protobothrops*, though most likely being monophyletic by itself (see above), should be also avoided until the relationships of other species assigned to *Ovophis* by Hoge and Romano-Hoge (1981) are clarified.

It is obvious that the remaining portion of *Trimeresurus* (sensu lato) is yet highly heterogeneous, because it includes at least two distinct lineages, one represented by two strictly terrestrial species (*T. okinavensis* and *T. gracilis*) and the other by several exclusively arboreal species (*T. popeiorum*, *T. albolabris*, *T. stejnegeri*, *T. puniceus* and *T. trigonocephalus*). More comprehensive analyses incorporating data for the re-

maining species of *Trimeresurus* (sensu lato), including *T. gramineus* (type species of the genus: McDiarmid *et al.*, 1999) and *T. mangshanensis* (type species of *Ermia*: Zhang, 1993), are strongly desired to elucidate detailed process of divergence of those terrestrial and arboreal species, and to revise their classification at the generic level.

There is a remarkable sequence divergence between *T. monticola monticola* from southern continental China and the two samples of *T. m. makazayazaya*, one from eastern continental China and the other from Taiwan (Fig. 1). Because values of Kimura's (1980) distance between these supposedly conspecific subspecies (0.055–0.058) are as great as or greater than those between some different species (e.g., 0.043 between *T. mucrosquamatus* and *T. elegans*, 0.035 between *T. tokarensis* and *T. flavoviridis*, and 0.055 between *T. gracilis* and *T. okinavensis*), it is probable that *monticola* and *makazayazaya* actually represent two different species. This suggests the necessity for detailed analyses of geographic variation in the highly polytypic *T. monticola* (McDiarmid *et al.*, 1999; Zhao *et al.*, 1998).

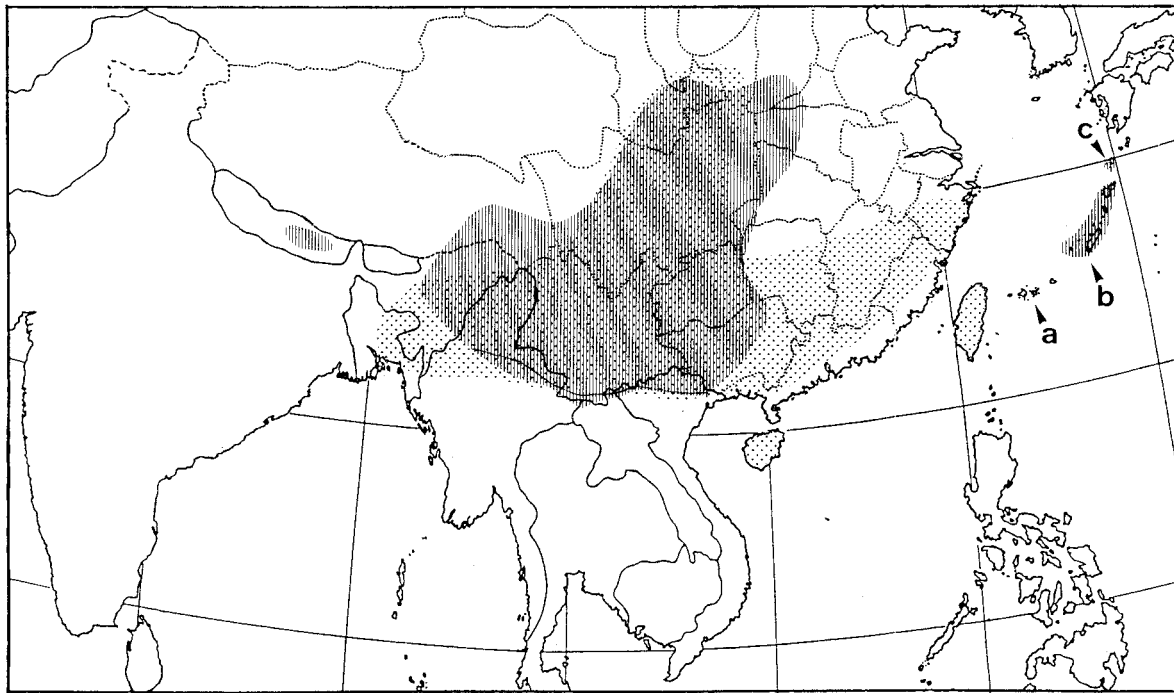
### Biogeography

It is assumed that in some vertebrate examples, taxonomic diversity in Taiwan has increased through a series of *in situ* divergences facilitated by the large size and diverse geomorphology of this island (e.g., Yu, 1995; Ota, 1997). With respect to *Trimeresurus* (sensu lato), however, the diversity within Taiwan seems to be attributable to the multiple colonizations rather than to the *in situ* divergences, because the

four Taiwanese representatives, while being distant from each other genetically, have close relatives outside Taiwan. Toda *et al.* (1999) analyzed allozyme variation in *Trimeresurus* (sensu lato), exclusive of *T. gracilis* and *T. stejnegeri* from the East Asian islands. Based on the resultant phylogeny, (*monticola, okinavensis*)(*mucrosquamatus, elegans*)(*flavoviridis, tokarensis*), they assumed a landbridge dispersal of the genus into the Ryukyu Archipelago and attributed the divergence between the *mucrosquamatus-elegans* clade and the *flavoviridis-tokarensis* clade to a vicariance event involved by the initial insularization of the central Ryukyus in the late Pliocene. They also referred to the relatively large genetic distance between those clades (Nei's [1978]  $D=0.198-0.289$ ) as evidence for long isolation of the central Ryukyus from the southern Ryukyus and Taiwan.

However, present analyses with the inclusion of *T. gracilis* and *T. jerdonii* besides others that were not included in Toda *et al.*'s (1999) analyses yielded an essentially different picture for the historical biogeography of the East Asian *Trimeresurus* (sensu lato). First of all, present results suggest that *T. okinavensis* of the central Ryukyus was derived from a lineage distinct from *T. monticola*. The closest affinity of *T. okinavensis* with the geographically disjunct *T. gracilis*, a species endemic to the high altitude area of Taiwan (Ota, 1991), suggests an extremely relictual nature of the *okinavensis-gracilis* clade (Darlington, 1957).

More surprising is the possible sister relationship of the *flavoviridis-tokarensis* clade with *T. jerdonii* from the inland area of continental China, not with the *mucrosquamatus-*



**Fig. 3.** Map of East Asia, showing geographic distributions of *T. elegans* (a: the Yaeyama Group of the southern Ryukyus), *T. mucrosquamatus* (remaining portion of stippled area), *T. flavoviridis* (b: the Okinawa and Amami Groups of the central Ryukyus), *T. tokarensis* (c: the southern Tokara islands of the central Ryukyus), and *T. jerdonii* (remaining portion of hatched area). Data for ranges were taken from Zhao and Adler (1993) and Toda *et al.* (1999).

*elegans* clade from the immediate south (Fig. 3) as was postulated by Toda *et al.* (1999). Such a relationship suggests that *T. flavoviridis* and *T. tokarensis* were derived from an ancestral form that dispersed to the Ryukyus independently from the dispersal of the *T. mucrosquamatus-elegans* clade to this archipelago. This further suggests that the sister lineage of the *T. flavoviridis-tokarensis* clade, once occurring in the southern Ryukyus, Taiwan and eastern continent, has subsequently disappeared from these broad areas.

There remain four species of *Trimeresurus* in continental China that have not yet been examined phylogenetically (*T. mangshanensis*, *T. medoensis*, *T. tibetanus*, and *T. xiangchengensis*: Zhao and Adler, 1993; Zhao *et al.*, 1998). Thus, one may argue that one or more of them may be even closer to the *flavoviridis-tokarensis* clade than *T. jerdonii* is. However, even if this is actually the case, it will not lead to any substantial changes in the above-mentioned view on the historical biogeography of *T. flavoviridis* and *T. tokarensis*, because geographic ranges of those four species were also confined to far inland of the continent like that of *T. jerdonii* (Zhao and Adler, 1993; Zhao *et al.*, 1998). It is thus likely that the *flavoviridis-tokarensis* clade of the central Ryukyus is in a relict state like several other reptiles occurring in this region (Hikida and Ota, 1997; Ota, 1998).

As such, it is likely that the current *Trimeresurus* assemblage of the Ryukyus was derived from three independent dispersals from Taiwan and the continent—one by the common ancestor of *T. gracilis* and *T. okinavensis*, another by the common ancestor of *T. jerdonii* and the *T. flavoviridis-tokarensis* clade, and the other by the common ancestor of *T. mucrosquamatus* and *T. elegans*. Present results also emphasize the importance of examining relevant inland representatives of the continent, if any, for appropriate biogeographical assessment of possibly relict organisms in the central Ryukyus.

## ACKNOWLEDGMENTS

We are grateful to Y. H. Chen, S. L. Chen, H. C. Lin, K. Y. Lue, J. J. Mao, G. Shang, G. Vogel and the staff of Field Museum of Natural History for supplying tissues for our study, and to M. Honda and H. Sato for technical advice. We also thank S.-H. Li, H. M. Smith and two anonymous reviewers for the critical reading of early versions of the manuscript, and P. David and I. Ineich for provision of relevant literature. Financial support was provided by National Science Council, R. O. C. (NSC 872311B003003B17), and Japan Ministry of Education, Science, Sports and Culture (Grant-in-aid C-11833013).

## REFERENCES

- Anderson S, Bankier AT, Barrell BG, de Bruijin MHL, Coulson AR, Droun J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. *Nature* 290: 457–465
- Brattstrom BH (1964) Evolution of the pitvipers. *Trans San Diego Soc Nat Hist* 13: 185–268
- Burger WL (1971) Genera of Pitvipers (Serpentes: Crotalidae). Ph. D. Dissertation. University of Kansas, Lawrence
- Cullings KW, Morafka DJ, Hernandez J, Roberts JW (1997) Reassessment of phylogenetic relationships among pitviper genera based on mitochondrial cytochrome *b* gene sequences. *Copeia* 1997: 429–432
- Darlington PJ Jr (1957) *Zoogeography: The Geographic Distribution of Animals*. John Wiley, New York
- David P, Tong H (1997) Translations of recent descriptions of Chinese pitvipers of the *Trimeresurus*-complex (Serpentes, Viperidae), with a key to the complex in China and adjacent areas. *Smithsonian Herpetol Inf Serv* 112: 1–31
- David P, Ineich I (1999) Les serpents venimeux du monde: systematique et repartition. *Dumerilia* 3: 3–499
- David P, Vogel G (1996) *The Snakes of Sumatra*. Edition Chimera, Frankfurt am Main
- David P, Vogel G (1998) Redescription of *Trimeresurus huttoni* Smith, 1949 (Serpentes, Crotalinae), with a discussion of its relationships. *Hamadryad* 22: 73–87
- Farris JS (1989) The retention index and the rescaled consistency index. *Cladistics* 5: 417–419
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791
- Felsenstein J (1993) PHYLIP, 3.75c edn. Department of Genetics SK-50, University of Washington, Seattle
- Gopalakrishnakone P, Chou LM (eds) (1990) *Snakes of Medical Importance (Asia-Pacific region)*. *Venom and Toxin Research Group*, Singapore
- Groombridge B (1986) Comments on the *M. pterygoideus glandulae* of crotaline snakes (Reptilia: Viperidae). *Herpetologica* 42: 449–457
- Heise PJ, Maxon LR, Dowling HG, Hedges SB (1995) Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. *Mol Biol Evol* 12: 259–265
- Hillis DM, Huelsenbeck JP (1992) Signal, noise, and reliability in molecular phylogenetic analysis. *J Hered* 83: 189–195
- Hikida T, Ota H (1997) Biogeography of reptiles in the subtropical East Asian islands. In “*Proceedings of the Symposium on the Phylogeny, Biogeography and Conservation of Fauna and Flora of East Asian Region*” Eds by KY Lue, TH Chen, National Taiwan Normal University, Taipei, pp 11–28
- Hoge AR, Romano-Hoge SAL (1981) Poisonous snakes of the world. I. Checklist of the pitvipers: Viperioidea, Viperidae, Crotalinae. *Mem Inst Butantan* 42/43: 179–309
- Hoge AR, Romano-Hoge SAL (1983) Notes on micro and ultrastructure of “Oberhautschen” in Viperioidea. *Mem Inst Butantan* 44/45: 81–118
- Honda M, Ota H, Kobayashi M, Hikida T (1999) Phylogenetic relationships of Australian skinks of the *Mabuya* group (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. *Genes Genet Syst* 74: 135–139
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In “*Mammalian Protein Metabolism*” Ed by HN Munro, Academic Press, New York, pp 21–132
- Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 116–120
- Kluge AG, Farris JS (1969) Quantitative phyletics and the evolution of anurans. *Syst Zool* 18: 1–32
- Knight A, Densmore LD III, Rael ED (1992) Molecular systematics of the Agkistrodon complex. In “*Biology of Pitvipers*” Eds by JA Campbell, ED Brodie Jr, Selva, Tyler, pp 49–69
- Knight A, Styer D, Pelikan S, Campbell JA, Densmore LD III, Mindell DP (1993) Choosing among hypotheses of rattlesnake phylogeny: A best-fit rate test for DNA sequence data. *Syst Biol* 42: 356–367
- Koba K (1962) *Studies on the Snakes of the Genus Trimeresurus of the Amami and Tokara Islands, Japan*. Japan Society for the Promotion of Science, Tokyo (in Japanese with English summary)
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca



- FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA* 86: 6196–6200
- Kraus F, Mink DG, Brown WM (1996) Crotaline intergeneric relationships based on mitochondrial DNA sequence data. *Copeia* 1996: 763–773
- Malhotra A, Thorpe RS (1997) New perspectives on the evolution of south-east Asian pitvipers (genus *Trimeresurus*) from molecular studies. In "Venomous Snakes: Ecology, Evolution and Snakebite" Eds by RS Thorpe, W Wüster, A Malhotra, Zoological Society of London, London, pp 115–128
- Maslin P (1942) Evidence for the separation of the crotalid genera *Trimeresurus* and *Bothrops*, with a key to the genus *Trimeresurus*. *Copeia* 1942: 18–24
- McDiarmid RW, Campbell JA, Toure T (1999) Snake Species of the World. A Taxonomic and Geographic References. Vol. 1. Herpetologists' League, Washington, DC
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small sample number of individuals. *Genetics* 89: 583–590
- Ota H (1991) Systematics and biogeography of terrestrial reptiles of Taiwan. In "Proceedings of the First International Symposium on Wildlife Conservation, ROC" Eds by YS Lin, KH Chang, Council of Agriculture, Taipei, pp 47–112
- Ota H (1997) Historical biogeographical implications in the variation and diversity of amphibians and reptiles in Taiwan. In "Proceedings of the Symposium on the Phylogeny, Biogeography and Conservation of Fauna and Flora of East Asian Region" Eds by KY Lue, TH Chen, National Taiwan Normal University, Taipei, pp 75–86
- Ota H (1998) Geographic pattern of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. *Res Popul Ecol* 40: 189–204
- Ota H, Honda M, Kobayashi M, Sengoku S, Hikida T (1999) Phylogenetic relationships of eublepharid geckos (Reptilia: Squamata): A molecular approach. *Zool Sci*: 659–666
- Parkinson CL (1999) Molecular systematics and biogeographical history of pitvipers as determined by mitochondrial ribosomal DNA sequences. *Copeia* 1999: 576–586
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425
- Shaffer HB, Meylan P, McKnight ML (1997) Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. *Syst Biol* 46: 235–268
- Swofford DL (1998) PAUP: A Phylogenetic Analysis Using Parsimony, version 4.0 (preliminary release). Sinauer, Sunderland, Massachusetts
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10: 512–526
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acid Res* 22: 4673–4680
- Toda M, Nishida M, Tu MC, Hikida T, Ota H (1999) Genetic variation, phylogeny and biogeography of the pitvipers of the genus *Trimeresurus* sensu lato (Reptilia: Viperidae) in the subtropical East Asian islands. In "Tropical Island Herpetofauna: Origin, Current Diversity, and Conservation" Ed by H Ota, Elsevier Science, Amsterdam, pp 249–270
- Toriba M (1989) Review: Snakes of the Orient, a Checklist. By Kenneth R. G. Welch, 1988, Krieger, Melbourne, Florida. *Jpn J Herpetol* 13: 21–25 (in Japanese)
- Toriba M (1993) The genera *Ovophis* and *Trimeresurus*. In "Endoglyphs and Other Venomous Snakes of the World: A Checklist" Eds by P Golay, HM Smith, DG Broadley, JR Dixon, C McCarthy, JC Rage, B Schatti, M Toriba, Azemiops S. A., Geneva, pp 81–83, 94–108
- Vidal N, Lecointre G, Vie JC, Gasc JP (1997) Molecular systematics of pitvipers: paraphyly of the *Bothrops* complex. *CR Acad Sci III* 320: 95–101
- Wang HY, Tu MC (1997) Molecular evidence that the endemic snake, *Trimeresurus gracilis*, in Taiwan is not evolved in situ. In "Proceedings of the Symposium on the Phylogeny, Biogeography and Conservation of Fauna and Flora of East Asian Region" Eds by KY Lue, TH Chen, National Taiwan Normal University, Taipei, pp 101–108
- Wüster W, Golay P, Warrell DA (1997) Synopsis of recent developments in venomous snake systematics. *Toxicon* 35: 319–340
- Yu HT (1995) Patterns of diversification and genetic population structure of small mammals in Taiwan. *Biol J Linnean Soc* 55: 69–89
- Zhang FJ (1993) Division of the genus *Trimeresurus* (sensu lato) based on the morphology of their skulls. In "Proceedings of the First Asian Herpetological Meeting" Eds by EM Zhao, BH Chen, TJ Papenfuss, China Forestry Press, Beijing, pp 48–57, 373 (in Chinese with English abstract)
- Zhang FJ (1995) Study on the taxonomy and phylogeny between genera of *Trimeresurus* group. *Acta Herpetol Sinica* 4/5: 105–111 (in Chinese with English abstract)
- Zhao EM, Adler K (1993) Herpetology of China. Society for the Study of Amphibians and Reptiles, Athens, Oxford
- Zhao EM, Huang M, Zong Y (Eds) (1998) Fauna Sinica. Reptilia. Vol. 3. Squamata, Serpentes. Science Press, Beijing (in Chinese)

(Received March 1, 2000 / Accepted May 27, 2000)

**Appendix 1.** Specimens examined in this study. Catalogue numbers of voucher specimens are given in parentheses.

*Trimeresurus mucrosquamatus*: Hongya, Sichuan, continental China (uncatalogued specimen); Chishang, Kaohsiung, Taiwan (National Taiwan Normal University [NTNU] B201574).

*T. elegans*: Yaeyama Group, Okinawa, Japan (NTNU B201580).

*T. tokarensis*: Kodakurajima Island, Tokara Group, Kagoshima, Japan (Kyoto University Zoological Collection [KUZ] 21104).

*T. flavoviridis*: Kumejima Island, Okinawa Group, Okinawa, Japan (KUZ 45840).

*T. jerdonii*: Hongya, Sichuan, continental China (uncatalogued specimen).

*T. monticola makazayazaya*: Yangmingshang, Taipei, Taiwan (NTNU B200800; Yizhang, Hunan, continental China (uncatalogued specimen).

*T. m. monticola*: Kunming, Yunnan, continental China (NTNU B201401).

*T. stejnegeri*: Fushan, Ilan, Taiwan (NTNU B201588); Kunming, Yunnan, continental China (uncatalogued specimen).

*T. albolabris*: Foochow, Fujian, continental China (NTNU B201408).

*T. puniceus*: Sumatra, Indonesia (private collection of G. Vogel).

*T. gracilis*: Alishan, Chiayi, Taiwan (uncatalogued specimen).

*T. okinavensis*: Tokunoshima Island, Amami Group, Kagoshima, Japan (KUZ 45871).

*T. popeiorum*: Sakaerat, Thailand (NTNU B200511).

*T. trigonocephalus*: Sri Lanka (private collection of G. Vogel).

*Tropidolaemus wagleri*: Kuching, Sarawak, Malaysia (NTNU B200512).

*Deinagkistrodon acutus*: Hoping, Taichung, Taiwan (uncatalogued specimen)

*Vipera russelii*: Fanshan, Pintung, Taiwan (NTNU B201587).

**Appendix 2.** Aligned sequences of a part of the mitochondrial DNA 12S rRNA gene investigated. Dot indicates an identity with the first sequence; dash denotes an indel. Abbreviations are: MA, *Trimeresurus monticola makazayazaya* from Taiwan; MO, *T. m. monticola*; MU, *T. mucrosquamatus* from continental China; EL, *T. elegans*; TO, *T. tokarensis*; FL, *T. flavoviridis*; JE, *T. jerdoni*; GR, *T. gracilis*; OK, *T. okinavensis*; PO, *T. popeiorum*; AL, *T. albolabris*; ST, *T. stejnegeri* from Taiwan; PU, *T. puniceus*; TR, *T. trigonocephalus*, WA, *T. wagleri*; DA, *Deinagkistrodon acutus*; VR, *Vipera russelii*.

	1		100
MA	GCCACACCCCA-CGGGCAACACAGCAGTAATTAA-CATTAGGCC-ATAAGTGAAACTTGACCAAGCAAATGAAT-AAA-GGGCCGGTTAATCTCGTG		
MO	.....A.....		
MU	.....G.....G.....C.....		
EL	.....A.....G.....A.....C.....G.....		
TO	.....G.....G.....G.....C.....A.....		
FL	.....T.....G.....GA.....G.....C.....CA.....		
JE	.....G.....T.....G.....G.....C.....GA.....		
GR	.....G.....T.....G.....C.....C.....		
OK	.....T.....C.....C.....		
PO	.....T.....T.....C.....G.....C.....		
AL	.....T.....G.....C.....		
ST	.....T.....G.....G.....G.....		
PU	.....T.....G.....G.....C.....A.....		
TR	.....G.....G.....CT.....TA.....T.....		
WA	-----		
DA	-----		
VR	.....G.....T.....T.....T.....AT.....CT.....A.....		
	101		200
MA	CCAGCGACCCGGTTACAGTAGACCCAAGGTAATATTAGCGCGTAAAAG-TGACTAAA-A-CAACAGTC-CCAAC-TTAGGGCGGAACCCGGACTGG		
MO	.....G.....A.....A.....A.....A.....T.TTT.....G.....A.....A.....		
MU	.....G.....A.....A.....A.....A.....CA.....T.TA.....CC.....TAA.....A.TAA.....		
EL	.....G.....A.....A.....A.....A.....TA.....T.A.....C.....TAA.....A.TAA.....		
TO	.....G.T.....A.....A.....A.....A.....G.C.....T.AT.....T.....TAA.....A.AC.....A.....		
FL	.....G.....AC.....A.....A.....A.....G.C.....T.AT.....T.....TAA.....A.AA.....A.....		
JE	.....G.....A.....A.....A.....A.....CT.....T.ATC.....C.....TTA.....A.AA.....A.....		
GR	.....A.....A.....A.....A.....T.TT.....TA.....TA.....A.AA.....A.....		
OK	.....G.....AC.....A.....A.....A.....T.CT.....T.G.G.....A.....A.....A.....		
PO	.....G.....A.....C.A.....A.....A.....T.T.....TATAC.....TC.....G.....GGT.AA.....A.....		
AL	.....T.....A.....A.....A.....A.....TT.....TAT.T.AAC.....TAA.GA.TAC.....		
ST	.....G.....A.....A.....A.....A.....T.T.....TA.T.ACC.....ATAA.....A.TACC.....		
PU	.....G.....A.....A.....A.....A.....CT.....TA.T.GTC.....TAA.....A.AC.....		
TR	.....T.....G.....A.....A.....A.....TTTA.GTT.T.....A.....G.AAC.C.....		
WA	-----		
DA	-----		
VR	.....T.....G.T.....G.AC.....A.A.....T.GCAC.....A-T.....-A.A.C.C.....ATA.....A.AAC.CA.....		
	201		300
MA	GCTGTAAAAAGCCATAAGTCACATTAACACCCCCCC-TAACACCACACAACCTTTGACT-CATGAAAGCTAGGACACAACCTAAGATTAGATACCCTA-C		
MO	.....C.....C.....A.....A.....A.....C.....T.....		
MU	.....T-T.T.....C.....T.....A.....G.....		
EL	.....C.....T-T.TT.....C.....A.....CG.....T.....		
TO	.....TTT.....CG.....		
FL	.....TTTTTT.....C.G.....T.....		
JE	.....GC.....C.....TC-.....T.....C.....G.....		
GR	.....CT.....TCG.TA.....T.....A.....A.....T.....T.....		
OK	.....CT.T.....TCA.A.....T.T.T.....A.....A.....T.....T.....		
PO	.....G.....CA.....CC.....CA.TA.....T.....		
AL	.....C.....AC-.....TT.....C.G.....T.....A.....T.....		
ST	.....C.....C.....GCA.A.....C-G.....A.....T.....		
PU	.....T.....C.....GCA.AA.....C-G.....A.....A.....		
TR	.....C.....CC.....TCA.A.....G.....A.....T.....		
WA	-----		
DA	.....CCAC.....TTCA.....A.....C.A.....C.A.....T.....C.....GT.....T.A.....		
VR	.....CITT.A.....TT-AT.TAC.....-T.....TCT.....A.....CG.....CG.....T.....T.....		
	301		400
MA	TATGCCTAGCCGTAACACAC-AATTAATTAACCAATFGTTCGCAAAACAACCTACGAGTCC-TACTTAAAACTTAAAA-GACTTGACGGTACTTCCACACA		
MO	.....A.....A.....C.....T.....C.....T.....C.....T.....G.....		
MU	.....C.....T.....C.....C.....T-AC.....		
EL	.....C.....T.....C.....C.....T-A.....G.....G.....		
TO	.....A.....T.....C.....C.....A.....G.....G.....		
FL	.....A.....T.....G.....C.....C.....AA.....A.....G.....		
JE	.....A.A.....T.....C.....C.....C.....A.....C.....GA.....G.....		
GR	.....C.TA.....C.....C.....AA.....G.....		
OK	.....C.TA.....C.....C.....AG.....		
PO	.....C.....AC.....C.....T.....-AA.....C.....T.C.....		
AL	.....A.....C.....A.....-AA.....C.....C.G.....		
ST	.....C.....G.....-AGC.....C.G.....		
PU	.....A.....C.....CC.....G.....-AA.....C.....		
TR	.....AC.....C.....CC.....T.....-AA.....T.....C.G.....		
WA	.....A.....T.....C.....C.....G.....AATA.....C.G.....		
DA	.....T.....C.....GATA.....T.....		
VR	.....A.C.....AC.....G.....T.....A.....G.....AGTA.....G.....T.....		
	401		500
MA	GCCT-AGAGGAGCCTGTCTAATAACCGACAATCCACGATTAACCCACCCCTTCTCGCCTACAGTCTATATACCGCCGTCGCCAGCTTACCTTGTAATAA		
MO	.....C.....T.....C.....T.....CA.....		
MU	.....A.....T.....T.....T.....T.....CA.....		
EL	.....G.....T.....T.....T.....CA.....		
TO	.....A.....T.....CA.....		
FL	.....A.....CA.....		
JE	.....A.....A.....G.....A.....CA.....		
GR	.....C.....G.....C.....CA.....		
OK	.....A.....C.....T.A.....C.....CA.....		
PO	.....A.....TC.....T.A.....AC.....A.....A.....T.....		
AL	.....A.....T.....C.....A.....AC.....T.....C.....		
ST	.....A.....A.....A.....AC.....T.....CA.....		
PU	.....A.....C.....T.....C.....A.....AC.....CA.....T.....		
TR	.....A.....G.....C.....A.....A.....CAC.....CC.....		
WA	.....A.....C.....AG.....A.....CA.....		
DA	.....T.....C.....G.....T.....A.....T.....C.....		
VR	.....A.....G.....C.....G.....C.....C.....ACG.....G.....G.....A.....ATA.....		

501 600  
 MA GAAAAAAGTGAAGCAAAACAGTACTACGCTAAAACGACAGGTCGAGGTGTAACCCATGAAAGGGACTACGATGGGCTACATTCTTC-AACCAAGAATAC  
 MO .....T.....T.....A.....T.....GA.....  
 MU .....T.....A.....C.....A.....G.....A.....C.....G.....  
 EL .....T.....T.....A.....G.....A.....CT.....G.....  
 TO .....T.....C.....G.....A.....A.....C.....A.....C.....G.....G.....  
 FL .....T.....C.....G.....A.....A.....C.....A.....C.....C.....G.....G.....  
 JE .....T.....C.....G.....A.....GT.....A.....C.....G.....G.....  
 GR .....T.....T.....T.....A.....T.....G.....-.....A.....C.....C.....  
 OK .....T.....TT.....A.....T.....G.....-.....A.....C.....C.....  
 PO .....T.....A.....T.....T.....AT.....A.....T.....GT.....AC.....A.....T.....G.....  
 AL .....T.....C.....T.....AC.....A.....T.....GA.....-.....A.....CT.....AG.....-.....  
 ST .....T.....T.....AC.....A.....TT.....GA.....-.....C.....A.....C.....AG.....  
 PU .....T.....T.....AC.....A.....GT.....C.....A.....C.....AG.....C.....C.....  
 TR .....T.....C.....TC.....TC.....A.....T.....TG.....T.....G.....A.....-.....A.....A.....T.....CT.....  
 WA .....T.....T.....C.....C.....-.....A.....C.....A.....C.....A.....C.....C.....G.....C.....  
 DA .....C.....T.....AG.....TC.....T.....CG.....C.....A.....A.....A.....A.....CA.....G.....G.....C.....  
 VR .....C.....-.....A.....G.....T.....T.....C.....A.....G.....-.....A.....A.....C.....T.....AG.....C.....G.....

601 700  
 MA GAATAACAC-TATGAAATTAATGCTTGAAGACGGATTAGCAGTAAGATAAAGAATAAAATGCCTAAGTGAACATAACCGCAATGAAGTGCCTACACACCGC  
 MU .....G.....C.....G.....CA.....C.....  
 MO .....C.....G.....CA.....G.....CCA.....G.....CA.....T.....C.....  
 EL .....C.....G.....C.....G.....CC.....G.....CA.....T.....C.....  
 TO .....G.....C.....G.....C.....G.....CA.....C.....  
 FL .....G.....C.....G.....C.....G.....CA.....C.....C.....  
 JE .....G.....C.....G.....C.....G.....CA.....C.....  
 GR .....C.....G.....T.....A.....G.....CA.....TA.....  
 OK .....C.....G.....C.....G.....T.....A.....G.....CA.....T.....  
 PO .....C.....T.....T.....G.....CA.....T.....  
 AL .....C.....G.....T.....C.....G.....A.....A.....C.....  
 ST .....C.....T.....G.....C.....CA.....TC.....C.....  
 PU .....C.....G.....T.....C.....GA.....A.....T.....C.....  
 TR .....G.....C.....CG.....C.....G.....T.....A.....G.....G.....A.....T.....TC.....C.....T.....  
 WA .....C.....CG.....CAGG.....T.....G.....C.....G.....A.....C.....  
 DA .....G.....T.....A.....GCAT.....G.....T.....A.....  
 VR .....G.....TA.....G.....AC.....GT.....T.....G.....A.....A.....

701 800  
 MA CCGTCCACCC- TGTCACCACA-ACATCAAACCTCAATAAATTAACCAGATAAACTAAACAGGGCAAGTCGTAAACATGGTAAGTGTACTGGAAAGTGCACCT  
 MO .....T.....C.....T.....T.....C.....T.....A.....AG.....TC.....  
 MU .....T.....C.....A.....C.....TA.....T.....CC.....-.....GA.....AG.....C.....G.....A.....  
 EL .....T.....T.....A.....T.....TA.....T.....CC.....-.....A.....A.....C.....G.....T.....G.....  
 TO .....T.....C.....T.....T.....TT.....CTC.....-.....TA.....CC.....T.....A.....A.....TC.....T.....  
 FL .....T.....C.....CTT.....-.....TA.....CC.....T.....A.....A.....TC.....T.....  
 JE .....T.....C.....-.....T.....T.....T.....CC.....-.....A.....A.....G.....C.....T.....  
 GR .....T.....C.....T.....T.....-.....T.....CC.....TAA.....AGG.....C.....T.....  
 OK .....T.....C.....T.....-.....T.....CC.....T.....A.....A.....G.....TC.....T.....  
 PO .....CT.....T.....ATAT.....T.....C.....A.....AGA.....TTA.....A.....G.....T.....C.....  
 AL .....T.....C.....T.....T.....G.....A.....C.....CA.....C.....C.....G.....G.....T.....C.....  
 ST .....T.....C.....T.....T.....T.....T.....C.....TTA.....-.....GTC.....C.....G.....T.....C.....  
 PU .....T.....C.....A.....TT.....A.....CA.....C.....T.....A.....-.....AC.....C.....G.....T.....G.....  
 TR .....T.....C.....T.....TCT.....T.....-.....CTT.....T.....CCT.....T.....A.....G.....CTTC.....G.....  
 WA .....T.....C.....-.....A.....CC.....C.....-.....T.....AC.....ACAGC.....C.....  
 DA .....T.....C.....A.....A.....CAACAA.....CGA.....C.....T.....  
 VR .....T.....C.....CAT.....T.....T.....AC.....A.....TCTACAAA.....CCCCT.....CC.....AT.....CT.....CC.....

801 806  
 MA TAGAAA  
 MO .....  
 MU .....  
 EL .....  
 TO .....  
 FL .....  
 JE .....  
 GR .....  
 OK .....  
 PO .....  
 AL .....  
 ST .....  
 PU .....  
 TR .....  
 WA .....  
 DA .....  
 VR .....