

## REASSESSMENT OF THE VALIDITY AND DIAGNOSIS OF THE PITVIPER *TRIMERESURUS VENUSTUS* VOGEL, 1991

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*Trimeresurus venustus* Vogel, 1991 was described from southern Thailand in 1991, and distinguished from the similar *T. kanburiensis* primarily by the following characters: 21 scale rows at midbody rather than 19 and less irregular and indented supraoculars. However, very few specimens of *T. kanburiensis* were known at the time of this description, and the name *T. venustus* has not been universally accepted. Recently, live specimens from the type locality of *T. kanburiensis* in western Thailand have become available, allowing a reassessment of the status of the southern Thai population. Phylogenetic analysis of two mitochondrial gene regions indicated that specimens from south Thailand are genetically quite distinct from the specimen from the type locality, and the former are more closely related to *T. macrops* than to *T. kanburiensis*. We present a multivariate morphometric analysis of the six specimens of *T. kanburiensis* from the type locality that are now known and twenty specimens from southern Thailand. Despite the small sample size, it is clear that some of the diagnostic characteristics used to define *T. venustus* are invalid. We conclude that the current evidence indicates that *T. venustus* is a valid species, and present new diagnostic characters to separate it from *T. kanburiensis*.

*Key words:* Crotalinae, systematics, Thailand, *Trimeresurus kanburiensis*, Viperidae

### INTRODUCTION

The genus *Trimeresurus* (Serpentes: Viperidae: Crotalinae) contains many taxonomically vexing issues that are still awaiting resolution (Malhotra & Thorpe, 1997, 2000). Recently, molecular data have promised to resolve some of these issues. One example is the status of the taxa *T. kanburiensis* Smith 1943 and *T. venustus* Vogel 1991. The holotype of *T. kanburiensis* (a female) was collected in 1938 from Kanchanaburi (then known as Kanburi) province in western Thailand (Fig. 1). It was at first identified as *T. puniceus* and only described as a new species in 1943 (Smith, 1943). It remained the only specimen available for the species until the late 1980s. In the interim, confusion developed over the identity of this species following specimens of another species *T. purpureomaculatus*, that occurs in the same region, being mistakenly labelled *T. kanburiensis* in books and by dealers in the captive trade (see Warrell *et al.*, 1992, for details). In the late 1980s, two additional specimens, also females, were found in Kanchanaburi Province (Warrell *et al.*, 1992). Specimens apparently referable to the species were also found in southern Thailand, in Nakhon Si Thammarat and Krabi provinces. Ironically, some of these specimens found their way into the captive trade labelled "*T. purpureomaculatus*" (Warrell *et al.*, 1992). Vogel (1991) described this southern population as *T. venustus*, citing the following diagnostic characters to separate it from *T. kanburiensis*: 21 scale rows at midbody rather than 19; narrower, less indented and divided, supraoculars; slighter body and a distinctive brownish-red banded colour pattern.

However, the name *T. venustus* has not been widely accepted. Among recent checklists of venomous snakes, it has been listed by Golay *et al.* (1993) and David & Ineich (1999) but not by McDiarmid *et al.* (1999) or the EMBL taxonomy database (<http://www.embl-heidelberg.de/~uetz/families/Viperidae.html>).

Specimens of *T. kanburiensis* that were available to Vogel for comparison were in poor condition. The holotype of *T. kanburiensis* is in two pieces and clearly has a section of body and the tail tip missing (also noted by Warrell *et al.*, 1992). The head is very distorted and squashed, with torn skin on one side, and any pattern has entirely faded. One of the two additional specimens available is also almost in three pieces and the colour pattern has faded considerably in both (Warrell *et al.*, 1992). Warrell *et al.* (1992) doubted that *T. venustus* was a different species to *T. kanburiensis*, after finding a fourth specimen of *T. kanburiensis* from Kanchanaburi province in 1991 (the only male), in which the colour pattern was well preserved and appeared to be identical to that of the southern population.

Further doubt was cast on the validity of *T. venustus* when, during fieldwork in Thailand in the 1990s, we found several specimens in the vicinity of the Khao Luang massif, near Nakhon Si Thammarat (Fig. 1), that also had 19 scale rows at mid-body. One of these, a roadkill, has since been presented to the Natural History Museum, London (BMNH 2002.52). A specimen from Surat Thani with the same character state was also seen by the senior author at the Queen Savoabha Memorial Institute. Therefore, the status of *T. venustus* required further verification (Malhotra quoted in Gumprecht, 2002a). Recently, a number of live specimens of *T. kanburiensis* have become available from the type locality. One of these (a female) was sent to the authors by the

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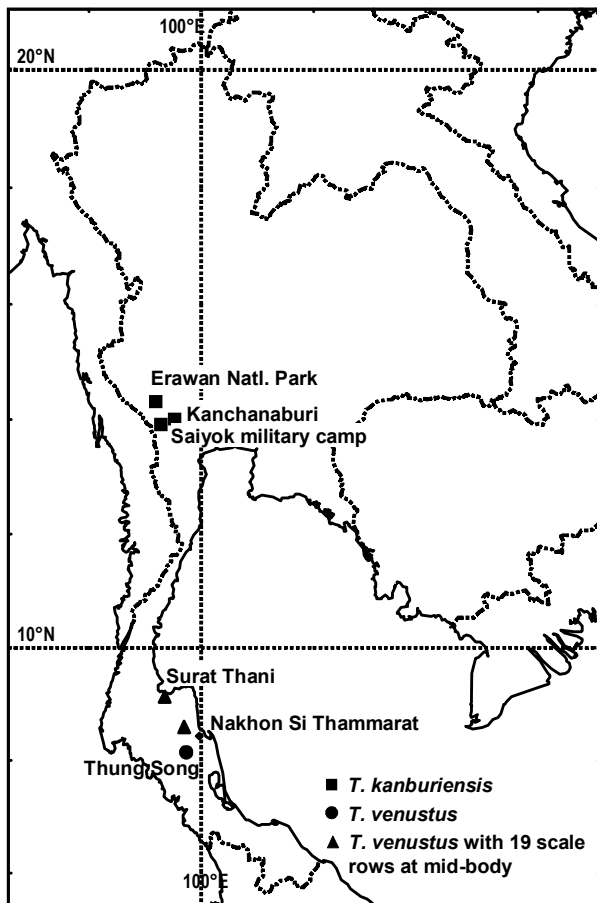


FIG. 1. Map of Thailand, showing the location of specimens. Circles represent specimens referable to *T. venustus* (with 21 scale rows at mid-body); triangles indicate the specimens from the south with 19 scale rows; squares indicate specimens referred to *T. kanburiensis*. The type locality was not precise, referring only to limestone hills in the vicinity of Kanchanaburi near the Tennaserim border. Note that the position of the Saiyok military camp where two specimens of *T. kanburiensis* were located, is different from that in Fig. 1 of Warrell *et al.* (1992), which showed the position of Saiyok village rather than the military camp (which is, confusingly, not near Saiyok village).

owner and is the first specimen available for DNA analysis. It has since been presented to the Natural History Museum, London (BMNH 2002.51). In this paper, we present a phylogenetic analysis of mitochondrial sequences to evaluate the relationship of populations referable to *T. venustus* to that from the type locality of *T. kanburiensis*. We also evaluate their morphological similarity by conducting a multivariate morphometric analysis of scalation, head shape and colour pattern, along with an analysis of internal characters (tooth numbers and position of the major internal organs) to evaluate the diagnostic characters proposed by Vogel (1991).

## MATERIALS AND METHODS

### DNA ISOLATION, AMPLIFICATION AND SEQUENCING

Whole genomic DNA was extracted from blood preserved by the addition of 5% EDTA and 2–4 ml SDS-Tris buffer (100 mM Tris, 3% SDS), or from liver or muscle tissue preserved in 80% ethanol, using stand-

ard protocols (Sambrook *et al.*, 1989). Cytochrome *b* (*cyt b*) sequences were obtained as described in Malhotra & Thorpe (2000), NADH dehydrogenase (ND4) as described in Parkinson *et al.* (2000) and 12S small subunit ribosomal RNA (12S) as described in Knight & Mindell (1993). Unincorporated nucleotides and primers were removed using a variety of commercially available kits (e.g. Prep-a-gene [Biorad], 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 automated DNA sequencer.

### PHYLOGENETIC ANALYSIS

Malhotra & Thorpe (2000) presented a phylogeny of 21 species of *Trimeresurus* (*sensu lato*) based on *cyt b* sequences, and evaluated the taxonomic value of certain morphological characteristics against this tree. On this basis, four species groups were defined within *Trimeresurus sensu stricto*, which are diagnosed by a combination of the condition of the first upper labial and nasal scale (fused or separate) and the hemipenial structure. By these criteria, the *T. kanburiensis*/*T. venustus* complex is a part of the *albolabris* species group, although it is quite genetically divergent and apparently diverged early in the history of the species group. We therefore analyse a number of species from the *albolabris* species group, as well as representatives of the other three species groups, together with one specimen of *T. kanburiensis*, and four specimens of *T. venustus*, including all three specimens from the Khao Luang area with 19 scale rows at mid-body. A full list of sequences included and their origins is listed in Table 1.

The coding sequences were first 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 amplification of pseudogenes. The possibility of non-neutral evolution was tested using a variety of tests implemented in the program DnaSP 3.51 (Rozas & 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 & Li 1993), and Tajima's  $D$  (Tajima, 1989).

We used both unweighted parsimony and Bayesian Markov Chain Monte Carlo (MCMC) approaches to reconstruct phylogenies, using PAUP\* 4.0b8 (Swofford, 2001) and MrBayes (Huelsenbeck & Ronquist, 2001) respectively. We first checked the dataset for homogeneity of base composition among taxa, to detect problems with the assumption of a similar underlying substitutional model. Parsimony searches were heuristic, with starting trees obtained by random addition with 100 replications, and tree-bisection-reconnection (TBR) branch swapping. Confidence in the inferred branches of the optimal trees was obtained by bootstrapping (1000 replication) with the search strat-

TABLE 1. List of specimens used in the phylogenetic analysis, their geographic origin and GenBank accession numbers. Cat represents the author's specimen catalogue number.

Species	Cat.	Location	GenBank accession nos. (cyt <i>b</i> , ND4, 12S)
<i>Trimeresurus venustus</i>	A74	S Thailand, Nakhon Si Thammarat Pr. (Khao Luang)	AY289224, AY289230, AY289218
<i>T. venustus</i>	A75	S Thailand, Nakhon Si Thammarat Pr. (Khao Luang)	AY289223, AY289229, AY289217
<i>T. venustus</i>	A249	S Thailand, Nakhon Si Thammarat Pr. (Khao Luang)	AY289234, AY289233, AY289235
<i>T. venustus</i>	A237	S Thailand, Nakhon Si Thammarat Pr. (Thung Song)	AY289222, AY289228, AY289216
<i>T. venustus</i>	A241	S Thailand, Nakhon Si Thammarat Pr. (Thung Song)	AF171914, to come
<i>T. kanburiensis</i>	B522	Kanchanaburi Province	AF17194, AY289231, AY289219
<i>T. macrops</i>	B27	Bangkok	AF517184, AF517219, AF517163
<i>T. macrops</i>	B161	Champassak Prov., S Laos	AY289221, AY289227, AY289215
<i>T. stejnegeri</i>	A160	Taiwan, Taipei county	AF171896, AY059593, AY059539
<i>T. gumprechtii</i>	A164	NE Thailand, Loei Province	AF171898, AF157224, AF517168
<i>T. vogeli</i>	B97	NE Thailand, Nakhon Ratchasima Pr.	AY059574, AY059596, AY059546
<i>T. flavomaculatus</i>	B3	Philippines	AF171916, AY059584, AY059535
<i>T. hageni</i>	B33	Thailand, Songkhla Province	AY059567, AY059585, AY059536
<i>T. albolabris</i>	B6	Indonesia, W Java	AF517186, AF517213, AF517158
<i>T. albolabris</i>	B22	Nonthaburi, C Thailand	AF517189, AF517221, AF517165
<i>T. albolabris</i>	B47	Phetburi Province, W Thailand	AF517187, AF517216, AF517160
<i>T. albolabris</i>	A229	N Thailand, Pha Yao Province	AY059566, AY059583, AY059544
<i>T. purpureomaculatus</i>	A83	Satun province, S Thailand	AF517188, AF517218, AF517162
<i>T. septentrionalis</i>	A100	Nepal, Mahattari District	AF171909, AY059592, AY059543
<i>T. insularis</i>	B7	Indonesia, West Timor	AY059568, AY059586, AY059534
<i>T. popeiorum</i>	A203	S Thailand, Nakhon si Thammarat Pr.	AF171904, AY059588, AY059537
<i>T. malabaricus</i>	A218	S India, Tamil Nadu State	AY059569, AY059587, AY059548
<i>T. trigonocephalus</i>	A58	SW Sri Lanka	AF171890, AY059597, AY059549
<i>Tropidolaemus wagleri</i>	B132	W Malaysia, Perak	AF517191, AF517223, AF517167
<i>Protobothrops mucrosquamatus</i>	B165	N Vietnam, Nghe An province	AY289226, AY289232, AY289220

egy modified to use only 10 replication of the start tree. In the Bayesian analysis, start trees were random, and the time reversible model with gamma distributed rates was used. Four markov chains (three heated and a single cold chain) were run for 1 378 300 generations, sampling every 100 trees. The likelihood scores of all 13 783 sampled trees were plotted against generation number and the first 215 trees, representing the burnin period before the likelihood scores approached stationarity, were discarded. A majority-rule consensus tree of the remaining 13 568 trees was produced in PAUP\* 4.0, and the percent of the time that the clade occurs among the sampled trees then represents the posterior probability of that clade existing.

#### MORPHOMETRIC ANALYSIS

Some specimens were obtained in the field, and morphometric measurements and macro-photographs were taken while the specimens were anaesthetised. As well as the five specimens of *T. kanburiensis* mentioned above, another female specimen (UF 61846) collected in 1985 from Prathat caves in Erawan National Park near Kanchanaburi (Fig. 1) but mistakenly identified as *T. albolabris*, was correctly identified by the senior author during studies of *T. albolabris*. This brings the final number of *T. kanburiensis* specimens from the type locality to six. All specimens used are listed in Appendix 1. Two of the three specimens collected in the Khao Luang area were roadkills; one was too badly damaged to be included in the morphological analysis, while another allowed most scalation characters but not internal or head dimension characters to be recorded. Two specimens without locality data but with 21 scale rows at mid-body, were also included in the analysis.

A list of characters used and their abbreviations can be found in Appendix 2. All morphometric analyses were carried out in BMDP Dynamic v. 7.0 (BMDP Statistical Software Inc., Cork, Ireland). Missing values were substituted with the group mean (for non-allometric characters; the south Thailand group excluded the specimens with 19 scale rows at mid-body). For allometric characters, inserted values were estimated by regression using correlated linear measurements. Missing values were only filled in for characters in which no more than 50% of specimens in that group had missing values. Characters were then checked for significant between-group variation and sexual dimorphism with a two-way analysis of variance (ANOVA) and co-variance (ANCOVA). In the case of characters measuring banding pattern, specimens with no discernible banding (i.e. BAND = 0) were excluded, since it seems likely that the absence of pattern was due to preservation effects. If no significant sexual dimorphism was present, all specimens could be analysed together; a considerable advantage given the small sample size. The assumption of homogeneity of variance was checked using Levene's test, and the Brown-Forsythe variant of the ANOVA, which relaxes this assumption, was used where it was violated (Brown & Forsythe, 1974).

A principal component analysis (PCA) was then carried out on the characters showing significant between-group differences, excluding those that showed sexual dimorphism in the case of joint analyses of both sexes. Although having less discriminatory power than methods that define *a priori* groups such as canonical variate analysis (CVA), PCA is to be preferred in this case since we do not wish to prejudge the distinctness of the groups. However, PCA does not take between-character correlations into account, so all size-related characters were first adjusted to a common size using the pooled within-group slope, with either snout-vent length (SVL) or head length (LHEAD) as the covariate. Included characters were screened for high correlations with each other ( $r > 0.7$ ), which would indicate that they do not provide independent information. In CVA, these correlations are taken into account, but in PCA they may result in over-emphasis of the correlated variables (Thorpe, 1983). If this was found, only one of the characters from the correlated character sets was used in PCA.

Adding internal characters can substantially improve the resolution of taxa (Thorpe, 1979, 1989). However, internal characters are not particularly useful for identification in many situations and also substantially reduce the sample size, as internal data were not available for many specimens. Given the generally poor condition of many of the specimens from Kanchanaburi, many head-shape characters are also missing in this group. Therefore, two sets of analyses were carried out, the first included scalation and colour pattern characters only, and the second included all types of characters. If the PCA indicated that the groups were distinct, a discriminant function analysis was carried out, using the same subsets of data as in the PCA, in order to find the characters most useful at distinguishing them.

## RESULTS

#### PHYLOGENETIC ANALYSIS

The final data set consisted of 26 taxa and 1612 base pairs of sequence data (587 bp from *cyt b*, 600 bp from ND4, and 425 bp from 12S). The coding sequences translated into amino acid sequence without the occurrence of stop codons, and were easily aligned by eye. The chi-square test for the homogeneity of base frequencies showed that base composition was not significantly different in all taxa included ( $P=0.99$ ). None of the neutrality tests showed a significant departure from neutrality. The mean likelihood score was -9419.2581.

The mean values for the parameters of the model, estimated by the program, were: base frequencies (A: 0.33704, C: 0.32727, G: 0.11341, T: 0.22228), alpha = 0.214011.

Most branches in the group of interest were highly supported. These show that while all specimens from south Thailand are closely related, regardless of whether they have 19 or 21 scale rows at mid-body, they are very distinct from the specimen from Kanchanaburi Province and indeed are more closely related to *T. macrops* (Fig.

2). *Trimeresurus venustus* is supported as a distinct species by this analysis. The relationships of these three species with the other species groups is unresolved in this analysis, however, and is addressed in a larger analysis of *Trimeresurus s.s.* that includes more species (Malhotra & Thorpe, 2004).

MULTIVARIATE MORPHOMETRICS

ANOVA and ANCOVA showed that only a few characters that were significantly different between localities were also significantly sexually dimorphic. These included SCS, TAIL, CLOPOST, VS19 to 17, HTANT, and HTPOST. Therefore, to maximise sample size, both sexes were analysed together excluding these characters. To check the effect that these characters had on the discrimination, a parallel analysis on just females, with these characters included, was conducted. In no case did they noticeably affect the results, and these analyses are not discussed further. The final scalation and colour pattern character set for PCA included VS21 to 19, DV17 to 15, BTWSUP2, LAB3, ROST, GENIAL, VENTEDGE, and SCR1. Unfortunately, one of the Khao Luang specimens (BMNH 2002.52) had some damage to the rostral scale and could not be included with this dataset. Since the position of these specimens is important, the analysis was repeated with ROST re-

moved, so that both specimens could be included. This had the effect of slightly reducing the separation between the groups, but otherwise the difference was negligible. While the resulting discrimination clearly separated the western and southern population with 21 scale rows, the intermediate position of the specimens from the south with 19 scale rows cast doubt on their distinctness (Fig. 3a). Since the number of scales at mid-body was influential in the ordination, we investigated whether the Khao Luang specimens most resembled *T. kanburiensis* or *T. venustus* in other characteristics. This was done by repeating the analysis with this character (VS21 to 19, which actually measures the position along the body where the reduction from 21 to 19 scale rows occurs) removed. The only marked effect on the discrimination was to shift the two Khao Luang specimens towards the other southern specimens (Fig. 3b), thus clearly indicating that in other respects they were clearly identifiable as *T. venustus*. Removal of VS19 to 17 also has a marked effect on the CVA, with the Khao Luang specimens grouping with the specimens from western Thailand when it was included, but with the other southern specimens when it was excluded. Finally, inclusion of internal characters (DENT, RKPOST, LKPOST) substantially improved the discrimination of the two species in the PCA (Fig. 3c) and CVA (not illustrated). In all analyses, the two specimens from an unknown locality clearly grouped with other *T. venustus* specimens as might be expected from their mid-body scale counts. These were therefore included within the southern Thailand population for the CVA.

The CVA allowed us to assess which characters contribute to the discrimination of *T. venustus* and *T. kanburiensis*, as well as their mean value, and range. Since the use of internal characters substantially reduces sample size, the results of the analysis of external characters are used in preference. However, internal characters that are important in the discrimination when all characters are used are also listed. It can be seen that the difference in any single character is subtle, with largely overlapping ranges (Table 2).

*T. venustus* is distinguished from *T. kanburiensis* in both sexes by a scale reduction from 17 to 15 scale rows (DV17to15) that involves scale rows higher on the body in *T. kanburiensis* than in *T. venustus* (Table 2), a smaller light blotch on the first dorsal scale row in *T. kanburiensis* than in *T. venustus* (SCR1), a rostral scale with a higher ratio (a squarer shape) in *T. venustus* than in *T. kanburiensis* (ROST). There also tends to be a higher number of gulars (GULAR), fewer scales between the rear edges of the supraoculars (BTWSUP2), and at least one scale separating the third supralabial and the subocular (LAB3) is more likely to be present in *T. kanburiensis* than in *T. venustus*. Of the internal and head dimension characters, only the length of the head (LHEAD) serves to separate the two species, with *T. kanburiensis* having a relatively longer head than *T. venustus*. Of the sexually dimorphic characters that are also significantly different between groups, only the

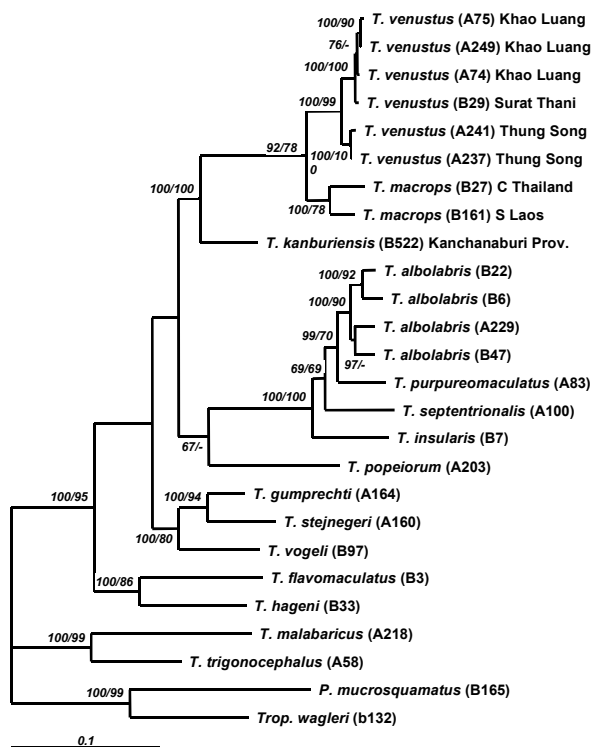


FIG. 2. Bayesian evolutionary hypothesis of the relationships between *T. kanburiensis*, *T. venustus* and *T. macrops*. Despite the close morphological similarity between the former two species, *T. venustus* is actually most closely related to *T. macrops*. Branch lengths are proportional to amount of evolution, and are averaged over all trees. Figures at nodes indicate posterior probabilities (first) of the clades from the Bayesian analysis, followed by parsimony bootstrap support values.

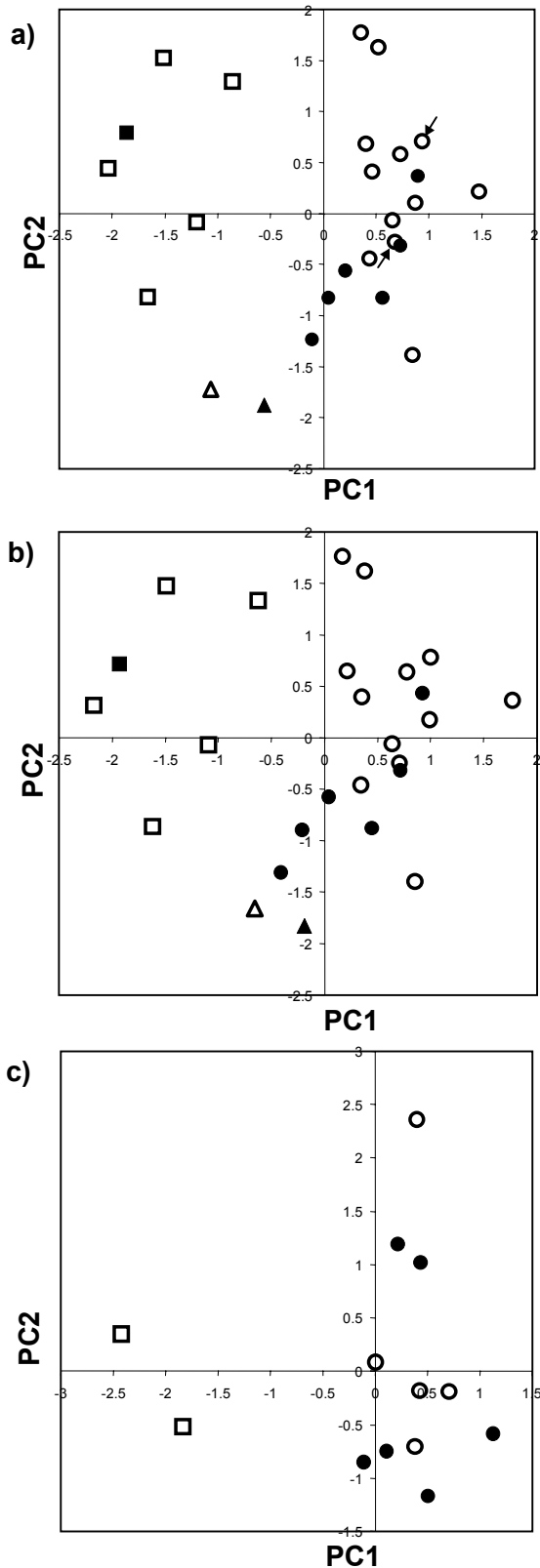


FIG. 3. Plot of first two principal components for both sexes. a) external characters only, including VS21to19; b) external characters excluding VS21to19; c) external and internal characters. Circles: *T. venustus* (with 21 scale rows at mid body); triangles: southern specimens with 19 scale rows; squares: *T. kanburiensis*. Empty symbols indicate females and filled symbols indicate males. The two specimens of unknown locality are indicated by arrows in 3a and are clearly assignable to *T. venustus*. They are therefore not highlighted in the remaining figures.

number of subcaudal scales is important, and tends to be higher in *T. venustus*.

Of the characters that help to distinguish between the sexes of both species (Table 3), the most important is relative tail length (TAIL), which is longer in males than females. The number of teeth on the pterygoid bone (PTERY) is also sexually dimorphic with higher numbers in males than females. The scale reduction from 8 to 6 rows on the tail (SC8to6) occurs further down the tail in males than females, which is associated with the length of the tail, the ventral surface tends to be more heavily blotched and speckled in males than females, and the position of the front and rear edges of the liver (LVANT, LVPOST) occur more posteriorly in males than females, as does the anterior edge of the left kidney (LKANT).

## DISCUSSION

### EVALUATION OF DIAGNOSTIC CHARACTERS.

Of the characters stated in the description (Vogel, 1991) to distinguish *T. venustus* from *T. kanburiensis*, we have shown that the number of scale rows at mid-body is not diagnostic. It is also stated to be slighter in body shape than *T. kanburiensis*. The analysis shows that this is only discernible in the slightly longer head of *T. kanburiensis*, which in practise may not be a very useful diagnostic character. Vogel (1991) also states that *T. venustus* lacks the indented, divided and very broad supraoculars found in *T. kanburiensis*. However, in none of the specimens analysed were the supraoculars actually divided. The width (and length) of the supraoculars was also not significantly different between the two species. The irregularity of the inner edge of the supraoculars was not included in the analysis because it is difficult to measure in an objective manner. While it does appear to be more irregular in the specimens of *T. kanburiensis* examined than in *T. venustus*, this is not always true. For example, the *T. kanburiensis* specimen BMHN 1987.943 has broad supraoculars with a smooth margin, while *T. kanburiensis* specimens BMNH 1988.385 and BMNH 2002.51 have only slightly indented supraoculars on one side. Many of the *T. venustus* specimens examined had at least slightly indented and irregular inner edges to the supraoculars, and so this character may only be useful when expressed in its extreme form in each species. Finally, the species were said to differ in colour pattern, in the presence of bands and in the ventral surface being more blotched in *T. venustus*, whereas *T. kanburiensis* was unbanded and had a plain or speckled ventral surface. However, these aspects of colour pattern can now be shown to be an artefact of small sample size. All the specimens of *T. kanburiensis* from which Vogel obtained this information have a very faded colour pattern and are females. The new specimens indicate that banding is the norm in *T. kanburiensis* as well, and although pictures of the live specimens suggest that the colour of the bands may be slightly different in the two species, this cannot be confirmed by analysis at present. The difference in ventral

TABLE 2. Mean values and range of morphological characters important in multivariate discrimination between *T. venustus* and *T. kanburiensis*. Size-related characters are adjusted to a common size of SVL (47.0 cm). Characters are listed in order of magnitude of their contribution to the discriminant function, and their abbreviations are explained in Appendix 2.

Character	<i>T. venustus</i>	<i>n</i>	<i>T. kanburiensis</i>	<i>n</i>
DV17to15	3.38 (3.0 – 5.0)	20	4.0 (3.5 – 4.5)	6
SCR1	0.39 (0.1 – 0.8)	20	0.17 (0.0 – 0.2)	6
ROST	0.45 (0.33 – 0.58)	17	0.33 (0.22 – 0.47)	6
GULAR	6.9 (5.5 – 9.5)	20	8.6 (7 – 9.5)	6
BTWSUP2	12.85 (11.0 – 16.0)	20	10.33 (9.0 – 12.0)	6
LAB3	0.15 (0 – 1)	20	0.58 (0 – 1)	6
LHEAD	19.74 (17.9 – 23.6)	19	21.62 (19.0 – 22.6)	5
SCS ( females only)	56.08 (50 – 66)	13	51.25 (50 – 52)	4

coloration appears to be due to sexual dimorphism rather than being a diagnostic difference. Thus, it seems that the diagnosis and characteristics of the two species requires redefinition.

REDESCRIPTION OF VARIATION WITHIN *T.*

*KANBURIENSIS*

*Colour in preservative.* The banding pattern seems to be easily lost especially after some time in formalin (Warrell *et al.*, 1992), leaving a uniformly brown dorsal and lateral coloration. In these specimens, the ventral surface appears white, although it is still possible to detect a pale blotch on the first dorsal scale row, and some darker pigment extending onto the edge of the ventral scales. The darker colour also extends onto the sublabial scales and some of the scale rows between these and the genial scales, although it may be patchy. In better preserved specimens, darker blotches are visible on the head and form irregular bands on the body.

*Colour in life.* This description is based on a specimen freshly preserved in alcohol and on pictures of two living males and one female specimen from the same population. The ground colour of the body is a shade of olive or greyish green. The head is heavily blotched with dull brown or orange brown, and bands of the same colour occur on the body. The ventral surface is creamy white. Brown pigment encroaches onto the edges of

some of the ventrals. This is more extensive in males than females, in which the encroachment is just a sprinkling of brown spots. Males and females both have white or bluish spots on the first dorsal scale row, the overall effect being a blurred margin between dorsal and ventral scales. Labial scales are also scattered with white or bluish pigment, and in males are boldly marked with two or more patches of dark pigment. A broad and irregular dark stripe extends from the rear edge of the eye, again this is bolder in males than females. The eye is light orange heavily speckled with brown. The tail is similarly patterned to the body but the colour of the bands is brighter than on the rest of the body. One of the males has a series of white vertebral spots.

*Morphometric and meristic characters.* Females have relatively shorter tails than males, and this is also reflected in the number of subcaudals (ranging in females between 50-52, compared to 61 in the male. Maximum recorded size for females is 58.2 cm SVL compared to 41.5 cm for the male. The number of ventral scales varies between 172 and 177 in both sexes. Body scales are keeled, although this may vary from weak to strong. Temporal scales and scales on the side of the head between the temporals and supralabials are also usually keeled, as are scales on the rear of the head. The ratio of the upper and lower edges of the rostral scale varies between 0.2 and 0.5 (note that this character is not noticeably allometric as it is not correlated with any linear measurement on the head or body). Supralabials vary between 10 and 11 and sublabials between 11-13. The minimum number of scales between supraoculars varies between 7-9 and there are 9-12 scales between the posterior edges of the supraoculars. The number of scales between the nasals and shield bordering the anterior of the pit varies between 0 - 1 and there may be up to one scale between the internasals. The third supralabial may be separated from the subocular by up to one scale, but there is always one scale between the fourth and fifth supralabials and the subocular. The supraoculars may be heavily indented on the inner margin with the adjacent head scales, making the edge appear very uneven and irregular. However, a few specimens have regular supraoculars, and they may also be very broad. A few specimens have a suture run-

TABLE 3. Mean values and range of morphological characters in *T. venustus* and *T. kanburiensis* which do not discriminate among species but distinguish the sexes. Data are for the maximum number of specimens available for that character. Size-related characters are adjusted to a common size of SVL (42.0 cm). Characters are listed in order of magnitude of the F-ratio in the ANOVA/ANCOVA.

Character	Male	<i>n</i>	Female	<i>n</i>
TAIL	9.15 (± 0.23)	7	6.83 (± 0.22)	15
PTERY	14.8 (14–16)	6	13.2 (12–14)	9
SC8to6	16.4 (14.0–18.5)	8	9.9 (5.0–15.5)	18
DARKVENT	64.4 (0–100)	8	43.1 (0–100)	18
LVANT	63.25 (62– 64)	4	61.6 (58–66)	9
LKANT	138.75 (133–142)	4	135.5 (125–145)	8
LVPOST	96.5 (91– 99)	4	95.1 (93–103)	9

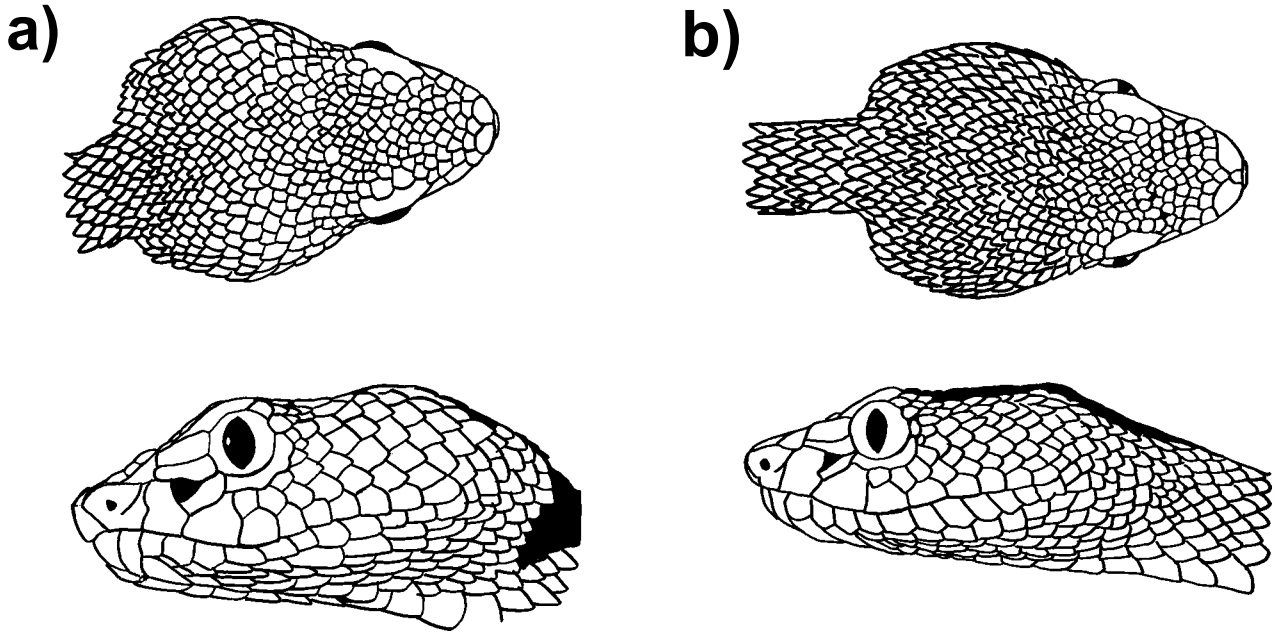


FIG. 4. Comparison of scalation features of the head in a) *Trimeresurus kanburiensis* and b) *Trimeresurus venustus*. There tend to be fewer scales between the rear edges of the supraoculars, and there is more likely to be at least one scale between the third supralabial and the subocular, in *T. kanburiensis* than in *T. venustus*. Although the latter is not apparent in the specimen pictured, there is a distinctly narrower portion of the first labial in contact with the subocular than in *T. venustus*. In addition, note that the inner edges of the supraoculars are not necessarily more indented in *T. kanburiensis* than in *T. venustus*, contrary to the original description of *T. venustus*.

ning partially across the scale, but the supraoculars are never completely divided. There are always 19 scale rows at mid-body, with the reduction from 21 to 19 scale rows occurring between 10 and 28% of the distance between the first ventral scale and the anal scale. Some of these scalation patterns are illustrated in Fig. 4a. There are 2-3 postocular scales, 5-8 scales bordering the subocular scale (not counting the pre- and post-oculars) and 12-14 teeth on the pterygoid and 12-15 on the dentary bone.

#### REDESCRIPTION OF VARIATION WITHIN *T. VENUSTUS*

*Colour in preservative.* Some specimens have a uniformly dark dorsal and lateral coloration. This is almost certainly a preservation effect since no specimen of *T. venustus* without bands has ever been found (although one aberrant striped individual has been recorded: Gumprecht, 2002b). The ventral surface appears white, with a pale blotch extending onto some scales of the first dorsal scale row, and some darker pigment extending from the dorsal surface onto the edge of the ventral scales. In better preserved specimens, darker blotches are visible on the head and form irregular bands on the body. Supralabials are lighter than the rest of the head, but may have a number of darker blotches (usually no more than one). Sublabial scales are generally white and unmarked, although in some specimens they appear darker.

*Colour in life.* This description is based on macrophotographs of five specimens (one male, four females) captured alive in south Thailand. The ground colour of the body is dull olive or bluish-green (male) to grass-

green (female). The head is heavily blotched with dull brown, and bands of the same colour occur on the body. The ventral surface is a similar, slightly lighter colour, than the dorsal surface. Brown pigment encroaches onto the edges of some of the ventrals, although the extent of the encroachment is variable and some females have virtually immaculate ventral scales. Males and females both have a white spot on the first dorsal scale row, which is more regular than in *T. kanburiensis* and therefore forms a clear margin between the dorsal and ventral scales although in the male there are also white flecks on some ventral scales. The labials are the same green colour as the rest of the dorsal surface, and in both sexes are boldly marked with at least one brown patch. A broad and irregular dark stripe may extend from the rear edge of the eye, not appearing to be different in intensity in males and females. The eye is light orange heavily speckled with brown. The tail is patterned and coloured identically to the body.

*Morphometric and meristic characters.* Females have relatively shorter tails than males, also reflected in the number of subcaudals (ranging in females between 50-66, compared to 68-72 in males). Females reach a slightly larger size than males but this is not a marked difference (maximum recorded 48.6 cm SVL compared to 43.4 cm for males). The number of ventral scales varies between 166 and 183 in both sexes. Body scales are always keeled, with a range from weak keeling to strong keeling. Temporal scales, scales between the temporals and supralabials, and scales on the rear of the head are also weakly to strongly keeled. The ratio of the upper and lower edges of the rostral scale varies between 0.3 and 0.6 (note that this character is not noticeably allom-

etric as it is not correlated with any linear measurement on the head or body). Supralabials vary between 9 and 11 and sublabials between 10-13. The minimum number of scales between supraoculars varies between 6-10 and there are 11-16 scales between the posterior edges of the supraoculars. The number of scales between the nasals and shield bordering the anterior of the pit varies between 0 and 1, and there may be 0-1 internasal scale. There may be up to 1 scale between the third and fourth supralabial and the subocular, and 1-2 scales between the fifth supralabials and the subocular scale. The inner edge of the supraoculars may be smooth or slightly indented by the adjacent head scales (especially towards the rear of the scale). There may be 21 or 19 scale rows at mid-body, with the reduction from 21 to 19 scale rows occurring between 59 and 67% of the distance between the first ventral scale and the anal scale in the former case, or between 4.5 – 23% of this distance in the latter. There are 1-4 postocular scales, 5-8 scales bordering the subocular scale (not counting the pre- and post-oculars) and 12-16 pterygoid and 13-16 dentary teeth. Some of the head scalation patterns are illustrated in Fig 4b.

#### DIAGNOSIS

*T. kanburiensis* can best be distinguished from *T. venustus* by its coloration, which is always less saturated in the former (Fig. 5). The ventral colour is white or cream rather than a shade of green. Males also have more boldly marked labial scales in *T. kanburiensis*. The white lateral stripe is less obvious in *T. kanburiensis*, appearing rather as a blurring of the boundary between dorsal and ventral coloration. There is no single scalation character that can unequivocally distinguish between the two species. However, the rostral tends to be more triangular in shape, there tends to be fewer scales between the rear edges of the



FIG. 5. Top: Male *T. kanburiensis* from Kanchanaburi province, Thailand. Photo: Kamphol Udomritthiruj (AquariCORP, Thailand). Bottom: female *T. venustus*, Nakhon si Thammarat province, Thailand (A. Malhotra).

supraoculars, and more likely to be at least one scale between the third supralabial and the subocular, in *T. kanburiensis* than in *T. venustus*. The scale reduction from 17 to 15 scale rows (the most posterior on the body) is more likely to involve higher scale rows in *T. venustus* than in *T. kanburiensis*.

#### COMPARISONS

Although this paper is focused primarily on the species *T. kanburiensis* and *T. venustus*, Vogel (1991) has provided diagnostic characters to separate *T. venustus* from some other species with which it co-occurs and/or has been confused in the past. These include *T. purpureomaculatus*, *T. erythrurus*, *T. macrops* and *T. albolabaris*, *T. popeiorum*, *T. stejnegeri* and *T. sumatranus*. The first four of these are all members of the *albolabris* group (*sensu* Malhotra & Thorpe, 2000). Vogel (1991) stated that *T. venustus* could be separated from members of the *albolabris* group by separation of the first supralabial shield from the nasal shield. However, this is incorrect. In fact it is very rare that these scales are not fused to some extent in both *T. venustus* and *T. kanburiensis*. Only one out of the 26 specimens examined had a completely divided nasal and first labial scale, and even this only showed this character state on one side. Thus this character actually serves to diagnose *T. venustus*/*T. kanburiensis* from all other *Trimeresurus* species except members of the *albolabris* group. *Trimeresurus purpureomaculatus* is very distinctive and Warrell *et al.* (1992) have provided a table of characters that serves to distinguish this species from *T. venustus*/*T. kanburiensis*. *Trimeresurus erythrurus* can be distinguished by a higher number of scales at mid body (23 and above), and is not currently known to overlap in range with *T. kanburiensis*, although this is possible. However, confusion with *T. albolabris* and *T. macrops* is still likely, especially in preserved specimens of *T. venustus* which have 21 scale rows at mid-body and in which the distinctive banding pattern has faded. *Trimeresurus albolabris* may be distinguished by its narrow supraoculars and larger head, but *T. macrops* is similar to *T. venustus* in head shape and in having broad supraoculars. *Trimeresurus macrops* can be distinguished by a number of scalation differences (Table 4) such as the scale reduction from 10 to 8 rows on the tail (SC10to8) and from 6 to 4 rows (SC6to4) occurring further towards the tip and a lower ventral scale count (VSC). Although the range of *T. macrops* does not appear to overlap with that of either *T. kanburiensis* or *T. venustus* according to Regenass & Kramer (1981), in fact it has a much wider distribution (Viravan *et al.*, 1992; personal observation).

#### DISTRIBUTION AND NATURAL HISTORY

Based on verifiable records, *T. venustus* and *T. kanburiensis* are presently known only from Thailand. *Trimeresurus kanburiensis* is known from the western province of Kanchanaburi, which is on the border with Myanmar, and it seems likely that it will also be found in

TABLE 4. Mean values and range of scalation characters distinguishing between *T. venustus* (with 21 scale rows at mid-body) and *T. macrops* (from central and northeastern Thailand), but are not sexually dimorphic. Data are for the maximum number of specimens available for that character. Characters are listed in order of magnitude of the F-ratio in the ANOVA/ANCOVA.

Character	<i>T. venustus</i>	<i>n</i>	<i>T. macrops</i>	<i>n</i>
SC10to8	5.15 (2.9–8.3)	17	9.17 (5.8–13.6)	15
DV17to15	3.28 (3–4)	17	4.26 (3.5–4.5)	15
VSC	174.4 (166–183)	17	166.1 (159–173)	15
BSCK	0.9 (0.5–1)	17	0.6 (0.5–1)	12
VENTEDGE	8.2 (7–9)	17	7.4 (7–8.5)	14
SC6to4	62.7 (50.9–71.8)	17	73.0 (60.1–85.9)	15
SOCBORD	6.4 (5.5–8)	17	7.2 (5.5–9)	15
Dv21TO19	3.9 (3.5–5)	17	4.3 (3–5)	15

Myanmar eventually. Although precise localities are not available for some specimens, all specimens with known localities come from a relatively short distance around the provincial capital Kanchanaburi. In the course of searching for the species, we found very few villagers who recognised pictures of them; those who did were engaged in bamboo cutting in the limestone hills. This is in accord with the details in Warrell *et al.* (1992) which describes the case history of a woman who was bitten by this species while cutting bamboo. The scarcity of the species until recently is more likely to be due to the fact that they are confined to higher parts of these hills, and may also have a limited activity period since these hills get extremely arid during the dry season.

*T. venustus* is currently only known from the southern provinces of Nakhon Si Thammarat, Surat Thani and Krabi. Considering the popularity of *T. venustus* in captivity, it is surprising how little has been written about its natural history, possibly because almost all captive specimens have been bought from dealers. In December 1997, the authors were taken to a limestone outcrop near Thung Song, where local people often find the snakes while collecting forest produce. After a short but difficult vertical climb, four specimens were found in a very small, slightly more level, area. All were coiled in an alert posture on rocks, and did not seem alarmed by our arrival. A final specimen was found under a rock near the base of the outcrop on our descent. This suggests that the species are primarily ground-living ambush hunters, and that they are not high altitude species as suggested by Vogel (1991). Although some specimens have been found on the outskirts of the Khao Luang massif, these were not at any great elevation. One was found at the base of a tall limestone cliff, the others (roadkills) were found on narrow roads passing through rubber plantations. Bulian (2001) describe his observations of the species, which largely agree with our observations above. He also adds that they can be found up to 700 m above sea level, although much more common at lower altitudes, and can be found in high densities in particular localities, often narrow shaded and humid valleys with rocky substrate. He mentions that they are mostly diurnal, although they may also be active during the night, and are particularly active after rain.

It has been speculated that the presence of limestone hills almost throughout the peninsula between the locations of the known distributions of the two species suggests that they may also be present here. In this case, it would be interesting to see where the distributions of the two species meet. However, increasing numbers of herpetological surveys in the region (Grossman & Tillack, 2002; Pauwels *et al.*, 2000) have so far failed to find any evidence of these species in other southern provinces.

*Note added in proof.* Since this paper was accepted, changes to the taxonomy of the species discussed within have been made. The new generic names can be found in Malhotra & Thorpe (2004).

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## APPENDIX 1

## SPECIMENS USED IN THE MORPHOLOGICAL ANALYSIS

Museum abbreviations are as follows: UF: State University of Florida at Gainesville; BMNH: the Natural History Museum, London; PSGV: Gernot Vogel's private collection; AFS: author's field collection number. Figures in parentheses are the number of males and females respectively. The holotype of *T. kanburiensis* is indicated in italics.

## KANCHANABURI PROVINCE

No precise locality: *BMNH 194.6.1.8.91* (F); BMNH 2002.51; Erawan National Park (Prathat Caves): UF61846 (1F); SaiYok Military Camp: BMNH 1987.943 (F), BMNH 1992.535; Tanousri, 25 km NW of Kanchanaburi: BMNH 1988.383 (F).

## NAKHON SI THAMMARAT PROVINCE

Thung Song: PSGV 220-222 (2M, 1F), BMNH 1988.384-386 (1M, 2F); BMNH 1987.944 (1F); AFS97B.8-12 (2M, 3F); Khao Luang area: BMNH 2002.52 (M), AFS4 (F).

## KRABI PROVINCE

BMNH 1992.536-539 (1M, 3F).

## NO LOCALITY

UF (uncatalogued) (2F).

## APPENDIX 2

## MORPHOLOGICAL CHARACTERS USED, AND THEIR ABBREVIATIONS.

## (A) SCALATION

Scale counts recordable on both sides of the body were averages of right and left counts.

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

SCS: the number of pairs of subcaudal scales. Any unpaired scales are treated as a pair.

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

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

POSTOC: number of postocular scales.

PREOC: number of preocular scales.

BORSUPOC: the number of scales bordering the supraocular scales, not counting pre- or post-oculars.

BTWSUPOC1: the minimum number of scales between the supraoculars.

BTWSUPOC2: the number of scales between the posterior edge of the supraoculars.

INTNAS: the number of scales separating the internasal scales.

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

LABNAS: the degree of fusion of the first supralabial and nasal scale (1: fully fused, 0: not fused).

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

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

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

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

SOCBORD: the number of scales bordering the subocular scale (not including pre- or post-oculars).

BSCK: the keeling of scales at mid-body.

KTEMP: the keeling of the temporal scales.

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

VENTEDGE: the number of scales between the edge of the mouth and the ventral scales, starting at and including the last sublabial.

GULAR: the number of scales between the first ventral (see above) and second pair of sublabials (which meet ventrally).

## (B) SCALE REDUCTION FORMULA

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

VS23to21: ventral scale position of the reduction from 23 to 21 scale rows.

DV23to21: dorsoventral position of reduction from 23 to 21 scale rows.

VS21to19: ventral scale position of the reduction from 21 to 19 scale rows.

DV21to19: dorsoventral position of reduction from 21 to 19 scale rows.

VS19to17: ventral scale position of the reduction from 19 to 17 scale rows.

DV19to17: dorsoventral position of reduction from 19 to 17 scale rows.

VS17to15: ventral position of the reduction from 17 to 15 scale rows.

DV17to15: dorsoventral position of reduction from 17 to 15 scale rows.

SC10to8: subcaudal scale position of the reduction from 10 to 8 scale rows.

DV10to8: dorsoventral position of reduction from 10 to 8 scale rows.

SC8to6: subcaudal scale position of the reduction from 8 to 6 scale rows.

SC6to4: subcaudal scale position of the reduction from 6 to 4 scale rows.

#### (C) BODY DIMENSIONS

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

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

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

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

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

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

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

WSUPOC: the width of the supraoculars measured in mm, at the widest part.

LSUPOC: the length of the supraoculars measured in mm.

WINTNAS: the width of the internasals (in mm).

#### (D) INTERNAL CHARACTERS

VS positions are transformed to % VS before analysis (see scale reductions).

PTERY: the number of pterygoid teeth.

DENT: the number of dentary teeth.

HTANT: VS position of the thyroid gland.

HTPOST: VS position of the rear edge of the ventricle.

LVANT: VS position of the anterior tip of the liver.

LVPOST: VS position of the tip of the superficial lobe of the liver.

RKANT: VS position of the anterior tip of the right kidney.

CLOPOST: SC position of the posterior tip of the cloacal gland in the tail base (females only).

#### (E) COLOUR PATTERN

STRIPE: presence of a lateral stripe (0, absent; 1, indistinct; 2, distinct).

SCRSTR: number of scale rows involved in stripe.

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

SCROC: number of scale rows involved in postocular stripe.

BAND: the number of bands (counted on the right side) between the head and vent.

WBAND: the average width of three bands at mid-body, counted in numbers of scales covered.

WGAP: the average width of the gap between three bands at mid-body, counted in numbers of scales covered.

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

DARKVENT: the percentage of ventral scales with dark pigment.

