

A contribution to the systematics of two commonly confused pitvipers from the Sunda Region: *Trimeresurus hageni* and *T. sumatranus*

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SYNOPSIS. The systematics of two Southeast Asian green pitviper species, *Trimeresurus hageni* and *T. sumatranus*, are investigated by canonical variate analysis. Preliminary results reveal two morphological forms corresponding to mainly *T. hageni* in West Malaysia, Thailand and Singapore and *T. sumatranus* in Borneo. Allopatric populations of both taxa are examined from Sumatra. Geographic variation is present in both species, which are distinguished mainly by head scalation, but also by colour and pattern.

INTRODUCTION

Trimeresurus sumatranus (Raffles, 1822) and *T. hageni* (Lidth de Jeude, 1886) are closely related species, occupying low elevations in undisturbed forests and having largely overlapping ranges. The systematics of these species and their precise distribution is an area of long-standing confusion. Many workers assign both species to *T. sumatranus* by default (Tweedie, 1983; Lim, 1991; Jintakune, 1995; David and Vogel, 1996) and the status of *T. hageni* has been in dispute since its initial description (Lidth de Jeude, 1886; Lidth de Jeude, 1890; Boulenger, 1896; Brongersma, 1933).

T. hageni was described as a separate species from *T. sumatranus* on the basis that only one or two supralabial scales are in contact with the subocular (compared with three in *T. sumatranus*), and the dark edges on head and body scales and dorsal cross-bands that are characteristic of *T. sumatranus* are not present (Lidth de Jeude, 1886). The species' distribution is widely debated, but specimens from south Thailand, West Malaysia and Singapore are normally assigned to *T. hageni*, and specimens from Borneo are normally assigned to *T. sumatranus* (David and Vogel, 1996; Cox *et al.*, 1998; Stuebing and Inger, 1999), but see Dring (1979) who placed specimens in the NHM collections from West Malaysia, southern Thailand and Sarawak in *T. sumatranus*. Both species are thought to occur on Sumatra and surrounding islands (Brongersma, 1933; Dring *et al.*, 1989; Cox *et al.*, 1998).

There have been few attempts to resolve the systematics of *T. hageni* and *T. sumatranus* since their initial description; these have been based on small sample sizes and a traditional character-by-character approach (Boulenger, 1896; Brongersma, 1933). Given the levels of geographic, ontogenetic and sexual variation usually present in viper species (Wüster *et al.*, 1992; Malhotra and Thorpe, 1997), the systematics of these taxa is best approached using modern statistical methods based on a broad range of morphological characters. In this paper, we present preliminary results from an ongoing investigation of the systematics and interrelationships of *T. hageni* and *T. sumatranus*.

MATERIALS AND METHODS

We examined 78 specimens from museum collections in the United States, Europe and Malaysia (Figure 1). A total of 93 characters

relating to scalation, colour and pattern were recorded for each specimen. Ventral scales were counted from head to vent, with the first ventral identified according to the method of Dowling (1951). The positions of scale reductions along the body (recorded as the number of the ventral or subcaudal scale opposite which it was situated) were transformed to percentage ventral scale (%VS) or caudal scale (%CS) position, in order to compensate for variation in ventral and subcaudal scale number. Male and female specimens were treated separately in all analyses to avoid bias caused by sexual dimorphism.

Specimens were grouped by locality into operational taxonomic units (OTUs). Two groups dominated the analysis, one was comprised of specimens from Thailand, West Malaysia and Singapore, and another was comprised of specimens from Borneo (Sabah and Sarawak). These groups were shown to be monophyletic by molecular analysis (unpublished data), which revealed a clear distinction between western specimens that lacked dorsal cross-bands and had at most two supralabials connected to the subocular scale, and eastern specimens that had dorsal cross-bands and had three supralabials in contact with the subocular scale. Molecular data was not available for specimens from Sumatra, and these were grouped individually to avoid combining sympatric species in one OTU.

Each OTU was checked prior to further analysis using Principal Component Analysis, which does not require that individuals be assigned groups prior to the analysis. The integrity of the OTUs was confirmed with the exception of one specimen from Betong (south Thailand), which had dark banding and in the PCA ordination was closest to the Borneo OTU. In subsequent analysis this specimen was grouped separately from the other western specimens. The OTUs used and their sample size for each sex is listed in Table 1.

Variation between OTUs was tested for individual characters by means of one-way analysis of variance (ANOVA). Only characters showing significant between-OTU variation were used in subsequent analyses. These are presented in Table 2.

Canonical variate analysis (CVA) was used to investigate patterns of geographic variation between OTUs. This method maximises the separation between groups relative to variation within groups. It is a standard multivariate method and has been applied successfully to numerous models of geographic variation in reptiles (Wüster *et al.*, 1992; Thorpe *et al.*, 1994; Daltry *et al.*, 1996).

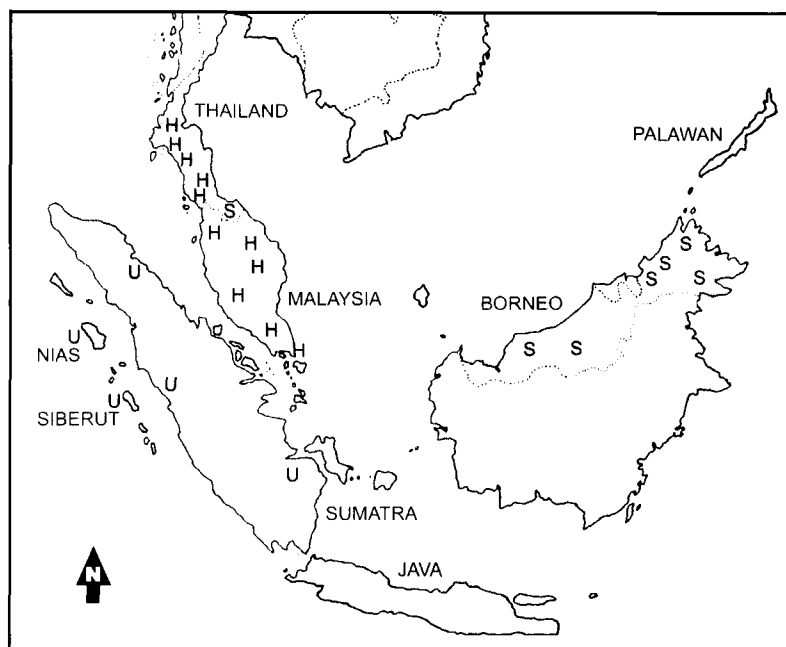


Fig. 1 Geographic origin of specimens used in multivariate analysis. S = *Trimeresurus sumatranus*; H = *Trimeresurus hageni*; U = unidentified specimens. Shading represents the known distribution of *T. hageni* and/or *T. sumatranus*.

Table 1 List of OTUs and sample size for each sex.

OTU	Sample Size	
	Males	Females
Thailand, West Malaysia, Singapore	16	15
North Sumatra 1 (Medan)	1	1
North Sumatra 2 (Medan)	0	1
Central Sumatra 1 (Padang)	1	1
Central Sumatra 2 (Padang)	0	1
South Sumatra 1 (Palembang)	0	1
Nias	1	10
Siberut	3	3
East Malaysia	4	18
Betong (south Thailand)	1	0
Total	27	51

Museum Acronyms

BMNH	The Natural History Museum, London, formerly the British Museum (Natural History), London
FMNH	Field Museum of Natural History, Chicago
IMR	Institute of Medical Research, Kuala Lumpur
KSP	Sabah Park Zoological Museum, Mount Kinabalu National Park, Sabah
MCZ	Museum of Comparative Zoology, Harvard
MHNG	Museum d'Histoire Naturelle de Geneva, Switzerland
NMBA	Naturhistorisches Museum Basel, Switzerland
NMW	Naturhistorisches Museum Vienna, Austria
QSMI	Queen Saovabha Memorial Institute, Bangkok
PH	Perhelitan, Kuala Lumpur
ZRC	Raffles Museum of Biodiversity Research, National University of Singapore, Singapore

RESULTS

The CVA of males shows clear separation along the first canonical variate of specimens normally assigned to *T. hageni* from Thailand,

West Malaysia and Singapore and those normally assigned to *T. sumatranus* from East Malaysia. The Siberut OTU and the single specimens from Nias and northern Sumatra are closest to the mainland *T. hageni* population. The specimens from Betong, Thailand and central Sumatra are closest to the Borneo OTU, but are well differentiated on CV2.

Analysis of females also shows strong differentiation between the Thailand, West Malaysia and Singapore OTU and the Borneo OTU. The Siberut and Nias specimens are phenotypically close to *T. hageni* from Thailand, West Malaysia and Singapore. Specimens from north and south Sumatra are also closely affiliated to this mainland population. The specimens from central Sumatra are closest to the Borneo population along CV1, although are clearly differentiated on CV2.

CVA analysis can be used to identify the characters that account for most variation between groups. In both sexes scalation characters were more important in distinguishing between the taxa than were characters relating to colour and pattern. The most important character is the fifth supralabial scale, which meets the subocular scale in *T. sumatranus* and in *T. hageni* is separated from the subocular by one scale. Also important is the frequent presence of an internasal scale in *T. sumatranus*, which is usually lacking in *T. hageni*. In addition, *T. sumatranus* has fewer supralabial scales and fewer scales between supraoculars than *T. hageni*. Our work verifies two of the original diagnostic characters used by Lidth de Jeude (1886) who described *T. hageni* as a distinct species that lacks dorsal cross-bands and has fewer supralabial scales in contact with the subocular scale. However, we did not find dark edging on head and body scales to be a valid diagnostic character on the basis that *T. hageni* specimens from Nias have very strong dark edges on their head and body scales.

DISCUSSION

The results of this preliminary analysis reveal a major phenotypic

Table 2 Characters used for multivariate analysis of *T. sumatranus* & *T. hageni*.

Characters	Males	Females
1. No. of ventral scales	—	*
2. No. of subcaudal scales	—	*
3. %VS position of reduction from 21 to 19 body scale rows	*	*
4. %VS position of reduction from 19 to 17 body scale rows	—	*
5. %DV position of reduction from 19 to 17 body scale rows	—	*
6. %VS position of reduction from 17 to 15 body scale rows	*	*
7. %CS position of reduction from 14 to 12 tail scale rows	—	*
8. %DV position of reduction from 14 to 12 tail scale rows	—	*
9. %CS position of reduction from 10 to 8 tail scale rows	—	*
10. % DV position of reduction from 10 to 8 tail scale rows	—	*
11. %CS position of reduction from 8 to 6 tail scale rows	*	*
12. %CS position of reduction from 6 to 4 tail scale rows	—	*
13. No. of supralabial scales	*	*
14. No. of sublabial scales	*	*
15. No. of scales bordering the supraocular scales	*	*
16. Minimum no. of scales separating the supraocular scales	*	*
17. Maximum no. of scales separating the supraocular scales	*	*
18. No. of internasal scales	*	*
19. No. of scales separating the fourth supralabial scale from the subocular scale	*	*
20. No. of scales separating the fifth supralabial scale from the subocular scale	*	*
21. No. of scales contacting the suboculars, excluding the preoculars and postoculars	*	*
22. Average no. of scales between the first ventral scales and the anterior genial scales	*	*
23. No. of scales between the last sublabial scales and first ventral scales	*	*
24. Presence of stripe on dorsal scale row one	*	—
25. No. of scale rows involved in stripe	*	—
26. Presence of postocular stripe	—	*
27. No. of scale rows involved in postocular stripe	—	*
28. Presence of dark edging on body scales	*	*
29. No. of bands on body	*	*
30. Mean no. of scales of three half bands on body	—	*
31. Mean no. of scales between three half bands on body	—	*
32. Presence of dark edging on head scales	*	*

* indicates significance value $p < 0.05$ (ANOVA).

division in both sexes. This corresponds to *T. sumatranus* in Borneo, central Sumatra and southern Thailand and *T. hageni* in southern Thailand, West Malaysia, Singapore, north Sumatra, south Sumatra, Nias and Siberut. The species are best distinguished by head scalation, but can also be identified by colour and pattern.

Geographic variation is also present at the intra-specific level. The Siberut and Nias specimens show stronger differentiation in males than in females. Their phenotypic similarity to mainland *T. hageni* is based mainly on scalation characters. Moreover, on the basis of colour and pattern, the Nias population is quite distinct with head and body scales strongly edged in black. Nias was last connected to Sumatra in the geologically recent past (c. 18,000 years ago), whereas Siberut has been isolated for around one million years (Dring *et al.*, 1989). The extent to which these populations have diverged from the mainland population will be investigated using molecular methods and may lead to taxonomic revisions.

Sumatran populations are represented by few specimens, but these exhibit the same general pattern in males and females: *T. sumatranus* from central Sumatra appear to be strongly differentiated from the Borneo OTU, whereas *T. hageni* from north and south Sumatra are only weakly differentiated from the mainland OTU. This pattern will be tested when additional data becomes available. An analysis of the phylogenetic relationships of these populations, using mitochondrial sequence data, is also underway and should help to clarify their status.

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specimens: BMNH, FMNH, IMR, KNP, MCZ, MHNG, NMBA, NMW, PH, QSMI, ZRC. This study was supported by the Natural Environment Research Council studentship to KLS (NER/S/A2000/03695), the Leverhulme Trust (F/174/I and F/174/O), the Wellcome Trust (057257/Z/99/Z and 060384/Z/00/Z), and the Darwin Initiative (162/6/65) with additional support for fieldwork from the Linnaean Society of London, Side, Bonhote, Omer-Cooper and Westwood Fund.

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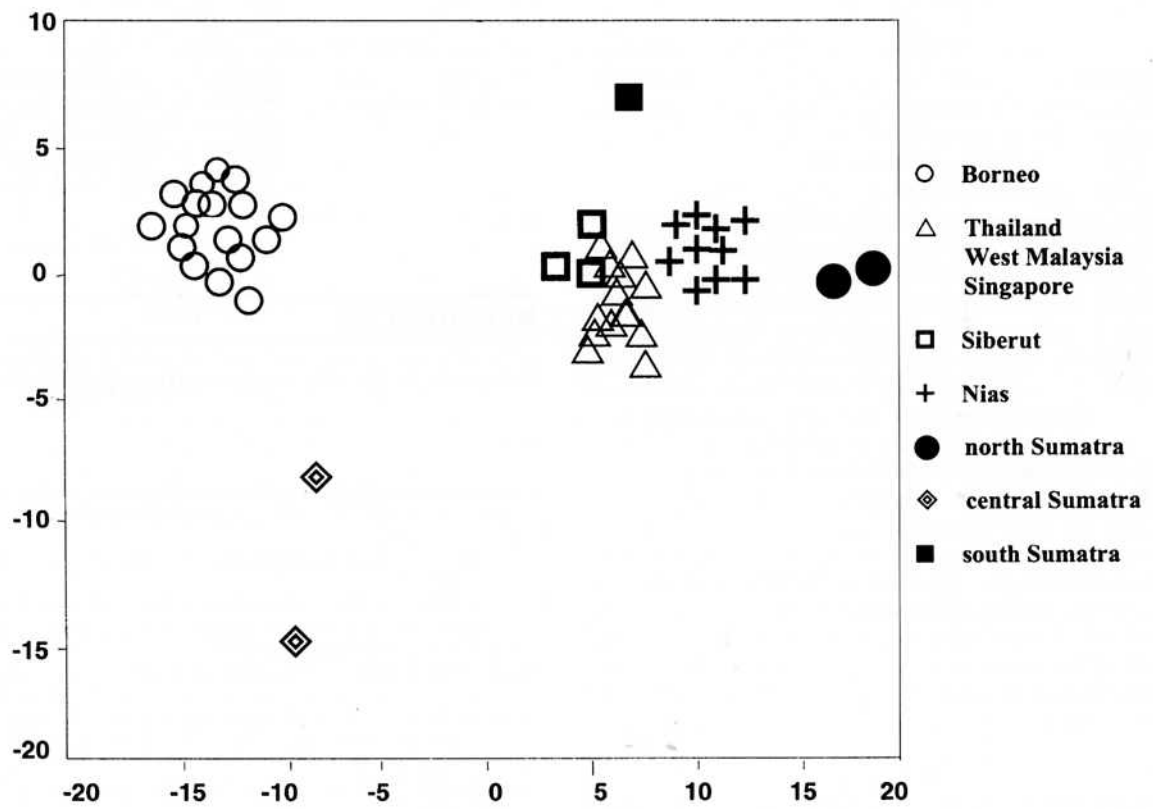
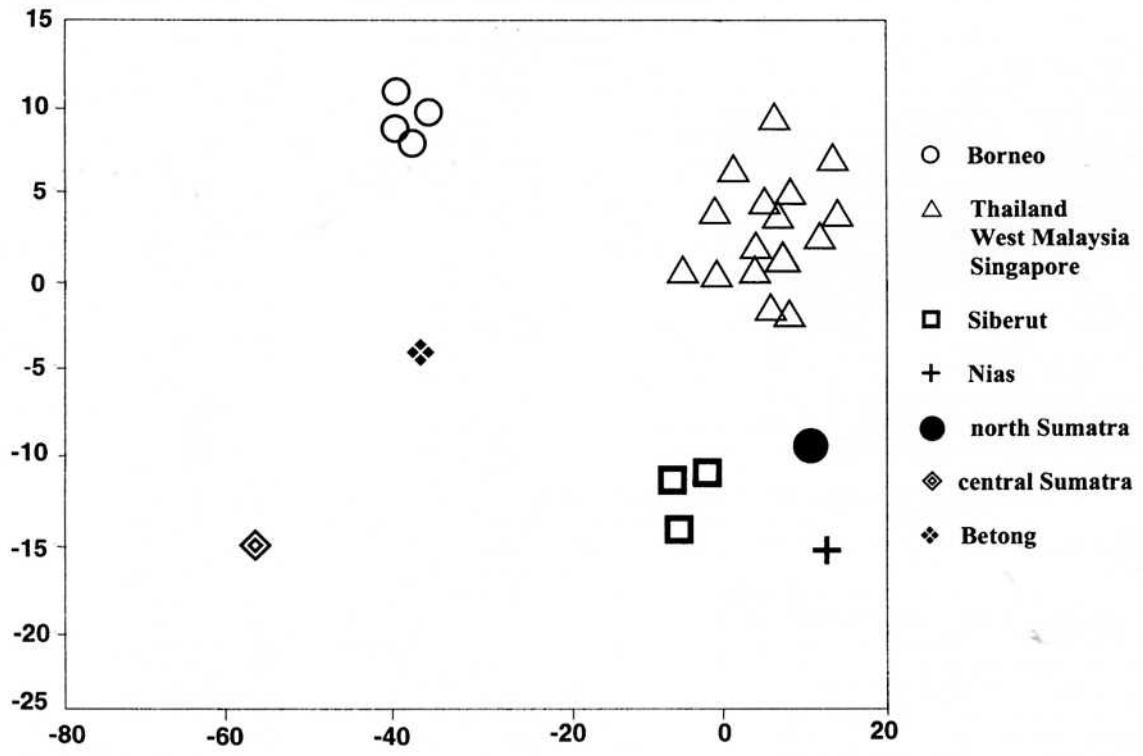


Fig. 2 Canonical Variate Analysis of *T. hageni* and *T. sumatranus* populations (top = males; bottom = females).

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Appendix 1 Specimens used in morphological analysis

MUSEUM/FIELD REF	LOCALITY	SEX
QSMI	Pangna, Thailand	M
QSMI 11190	Krabi, Thailand	M
MHNG 2072.87	Surat Thani, Thailand	M
MHNG 2072.89	Surat Thani, Thailand	M
MHNG 1403.95	Singapore	M
MCZ 132799	Kuala Lumpur, W. Malaysia	F
PH	Krau WR, W. Malaysia	F
PH	Tapa, W. Malaysia	F
PH	Selangor, W. Malaysia	F
PH no.79	Kuala Jasin, W. Malaysia	F
PH no.134	Ulu Gombak, W. Malaysia	F
FMNH 183787	Janda Baik, W. Malaysia	F
FMNH 183788	Ulu Gombak, W. Malaysia	M
FMNH 143948	Selangor, W. Malaysia	M
FMNH 138690	Kapit District, Sarawak	F
FMNH 138689	Kapit District, Sarawak	F
FMNH 148829	Kapit District, Sarawak	F
FMNH 148830	Kapit District, Sarawak	F
FMNH 138687	Kapit District, Sarawak	F
FMNH 239948	Kota Mardu, Sabah	F
FMNH 239959	Mendolong, Sabah	F
FMNH 243943	Mendolong, Sabah	F
FMNH 230064	Danum Valley, Sabah	F
FMNH 230063	Danum Valley, Sabah	F

FMNH 239952

FMNH 239950

FMNH 239958

FMNH 239957

FMNH 239947

FMNH 138688

NMBA 9179

NMBA 5108

NMBA 22401

NMW 28160.4

NMW 28160.3

NMW 28160.2

NMW 28160.1

NMW 28157.1

NMW 28156.1

NMW 23909.2

NMW 28159.4

NMW 23909.3

NMW 28159.2

NMW 23909.4

NMW 28159.1

NMW 28158.1

NMW 28158.2

BMNH 1936.9.12.3

BMNH 1884.1.8.47

BMNH 1884.1.8.46

BMNH 1884.12.31.13

BMNH 1884.12.31.14

BMNH 1977.1237

BMNH 1979.267

BMNH 1979.268

BMNH 1978.1879

BMNH 1880.9.10.7

BMNH 1936.9.12.5

BMNH 1967.2290

KSP 04361

KSP 04362

IMR 103649

IMR 104270

IMR 104271

IMR 105684

IMR 95995

ZRC 2.2938

ZRC 2.2937

ZRC 2.2936

KLS 01129

KLS 01119

KLS 0008

KLS 0199

AFS 9815

AFS 97b20

AFS 0005

Tenom, Sabah

Tenom, Sabah

Tenom, Sabah

Tenom, Sabah

Kota Mardu, Sabah

Kapit District, Sarawak

Nias, Indonesia

Palembang, Sumatra

Pangna, Thailand

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Medan, Sumatra

Medan, Sumatra

Medan, Sumatra

Padang, Sumatra

Padang, Sumatra

Padang, Sumatra

Kedah, W. Malaysia

Kedah, W. Malaysia

Betong, Thailand

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Nias, Indonesia

Siberut, Indonesia

Siberut, Indonesia

Siberut, Indonesia

G.Mulu, Sarawak

Singapore

Kuala Teku, W. Malaysia

G.Benom, W. Malaysia

Kota Mardu, Sabah

Bukit Tawau, Sabah

Bukit Lakai, W. Malaysia

Simpang, W. Malaysia

Ulu Gombak, W. Malaysia

Ulu Gombak, W. Malaysia

Simpang, W. Malaysia

Siberut, Indonesia

Siberut, Indonesia

Siberut, Indonesia

Siberut, Indonesia

Poring, Sabah

Poring, Sabah

Endau Rompin NP, W. Malaysia

Perak, W. Malaysia

Songkhla, Thailand

Satun, Thailand

Kuala Lumpur, W. Malaysia

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AFS/KLS indicate wild caught specimens examined under anaesthesia.