

## A NEW COBRA (ELAPIDAE: NAJA) FROM MYANMAR (BURMA)

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**ABSTRACT:** We describe a new species of spitting cobra of the genus *Naja* from central Myanmar. Multivariate analyses of morphological characters and analyses of mtDNA sequences confirm the distinctiveness of the new species. Phylogenetic analysis of the mtDNA data indicate that, among the cobra species of the southeast Asian mainland, the new species is most closely related to the Thai spitting cobra, *Naja siamensis*. The new species is apparently endemic to an arid region in central Myanmar.

**Key words:** Systematics; Multivariate morphometrics; Molecular phylogenetics; Mitochondrial DNA; Myanmar; *Naja*

UNTIL recently, Asian cobras of the genus *Naja* were generally regarded as belonging to one, widespread species: *N. naja*. However, recent work by Wüster and Thorpe (1989, 1990, 1991, 1992a, 1994) has revealed that *N. naja* (sensu lato) represents at least 10 species. Additionally, Wüster and Thorpe (1992b) noted the existence of spitting cobras from an uncertain locality in Myanmar (Burma; “Rangoon or Mandalay”) and suggested that they might belong to an undescribed species. During the course of herpetological field work in central Myanmar, the senior author encountered cobras that correspond to the specimens noted by Wüster and Thorpe (1992b). One other species of *Naja*, the monocled cobra, *N. kaouthia*, is known to occur in Myanmar, but this new form differs from it mainly in two ways: the hood mark is very faint or absent in adults and spectacle-shaped in juveniles (normally present in both adults and juveniles and monocle-shaped in *Naja kaouthia*), and the new form has a tendency to spit venom when threatened. Morphological and molecular analyses confirm that this new form is an undescribed species of *Naja*.

### MATERIALS AND METHODS

#### *Multivariate Morphometrics*

In order to determine the distinctiveness of the new species, we used multivariate analysis of morphological characters of this new form, together with the

three other species of *Naja* known from mainland southeastern Asia: *N. atra*, *N. kaouthia*, and *N. siamensis*. Specimens of the four species were obtained from a number of museums in Europe, the United States, Europe, and India, as well as from private sources in Thailand. The specimens of the new species examined in this study are listed below in the Results [Museum abbreviations follow Leviton et al. (1985)]; a list of specimens of the other species was published in Wüster et al. (1995). A large number of morphological characters relating to scalation, color pattern, and body proportions were recorded from each specimen. The characters used correspond to characters 1, 4–12, and 33–43 of Wüster et al. (1995), with five additional characters (Table 1).

We counted ventral scales according to Dowling (1951) and recorded the position of color pattern characters as the number of the ventral scale opposite which they are situated. In order to correct for variation in the number of ventral scales in different specimens, this was then converted into percent ventral scale (%VS) position (Thorpe, 1975). Similarly, the subcaudals were numbered from the vent to the tail tip (not including the tail spine), and the positions of scale reductions along the tail were recorded as percent caudal scale (%CS) position. The snout–vent length (SVL) was recorded with a piece of string to the nearest 1 mm, and other body pro-

TABLE 1.—Additional characters used for multivariate analyses relative to Wüster et al. (1995).

1. %VS position of last ventral of light throat area;
2. %VS position of anterior edge of paired dark lateral spots on throat;
3. %VS length of paired dark spots on throat;
4. %DS height of highest scale row encroached on by paired lateral throat spots;
5. %VS width of first dark band across throat.

portion characters to the nearest 0.1 mm with the aid of a digital caliper.

Because the localities of most museum specimens examined were widely scattered, it was necessary to pool specimens from several localities into one operational taxonomic unit (OTU) in order to obtain statistically representative samples. In such situations, it is important to avoid the formation of compound localities with geographic variation within them. We there-

fore defined OTUs a priori on the basis of collecting gaps and potential physiographic distribution barriers. These groups were then tested for geographic heterogeneity or the presence of several taxa by means of principal components analysis (PCA). All PCAs were run separately for each sex to avoid difficulties due to sexual dimorphism, and all characters were normalized to zero mean and unit standard deviation. Where such an analysis revealed more than one taxon, or geographic heterogeneity of the sample, the proposed OTUs were split into separate units for each phenotype. The OTUs established are listed in Table 2.

Because this study involves a mixture of meristic and mensural measurements, the effect of ontogenetic growth was removed from the mensural measurements by regressing them to the mean SVL of 665

TABLE 2.—Operational taxonomic units (OTUs) and their sample sizes used in this study. Numbering corresponds to Wüster et al. (1995), other numbers follow in sequence.

		Males	Females
<i>Naja kaouthia</i>			
19.	Central Thailand.	19	17
20.	Phuket Island, Thailand	6	0
21.	Eastern slope of Malayan Peninsula.	3	5
22.	Western slope of Malayan Peninsula.	5	6
23.	Northern India and Bangladesh.	17	14
24.	Yangon area, southern Myanmar.	6	2
25.	Southern Vietnam.	2	2
26.	Northern Myanmar.	5	5
27.	Sikkim area, India.	4	0
28.	Hue area, Vietnam.	3	0
29.	Assam, India	0	3
30.	Yongde, Yunnan, China.	0	1
31.	Central Cambodia	0	1
<i>Naja atra</i>			
34.	Northern Vietnam	9	6
35.	Hong Kong and Guangzhou area, China	9	6
36.	Eastern China	7	5
37.	Hainan Island, China	2	3
38.	Taiwan	7	2
40.	Chusan Island, Fujian, China	1	0
<i>Naja siamensis</i>			
41.	Central plain of Thailand	3	6
42.	Northeastern Thailand	12	2
43.	Northern Thailand	2	6
44.	Southeastern Thailand	5	1
45.	Khok Samrong, Lop Buri Prov., Thailand	3	2
46.	Southern Vietnam	2	2
47.	Trapeang Chan, Cambodia	1	4
<i>Naja mandalayensis</i>			
48.	Central plain of Myanmar	12	6

TABLE 3.—Specimens used for molecular analyses, with GenBank accession numbers of the sequences.

Species	Locality	GenBank accession number
<i>Naja mandalayensis</i>	Myanmar: Mandalay Division: 96 km south of Mandalay along Highway 1 (CAS 204375-6)	AF155211
<i>Naja siamensis</i>	Thailand: Lop Buri Province: Khok Samrong (WW 23); Thailand: Chainat Province: Manorom (Thai National Research Council Collection, unnumbered)	AF155214
<i>Naja kaouthia</i>	Thailand: Chumphon Province: Route 4139, 15 km W. Route 41 (roadkill, not collected)	AF155212
<i>Naja atra</i>	Chinese mainland near Hong Kong (2 specimens, not vouchered)	AF155213
<i>Naja annulifera anchietae</i>	Namibia: Okahandja (gift from H.-W. Herrmann)	AF155216
<i>Naja mossambica</i>	South Africa: KwaZulu-Natal: Durban (gift from H.-W. Herrmann)	AF155215

mm, using the pooled within-group regression coefficient obtained by means of analysis of covariance.

The population affinities of the OTU's were investigated by means of canonical variate analysis (CVA). This technique maximizes the separation between groups relative to the within-group variance, taking into account the within-group correlation between characters (Thorpe, 1976, 1980). The following four CVA's were run: the first pair used male and female specimens of all four species and the second pair used male and female specimens of all species except *N. atra*.

#### Molecular Phylogenetic Methods

Tissue samples were obtained for the new species and the other three species of cobras known to inhabit mainland southeast Asia: *N. atra*, *N. kaouthia*, and *N. siamensis*. As outgroup species, we chose the African species *N. annulifera anchietae* (previously *N. haje anchietae*: see Broadley, 1995) and *N. mossambica*. The choice of outgroup was based on morphological data (Szyndlar and Rage, 1990) and unpublished mtDNA sequence analysis (Wüster, in preparation), both of which support the monophyly of the Asiatic species of *Naja*, contradicting previous speculation that Asiatic spitting and non-spitting cobras may be derived from separate spitting and non-spitting lineages of African *Naja* (Ineich, 1995; Minton, 1986). Table 3 lists the specimens from which tissues were sampled.

We purified DNA from the samples by means of RNase and proteinase k digestion, phenol, phenol-chloroform, and chloroform centrifugation and ethanol precipitation, or through the use of various commercial kits. A 767 bp fragment of the cytochrome *b* gene was amplified through use of the polymerase chain reaction (PCR). PCR was carried out in 50  $\mu$ l of a solution of 20 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.52  $\mu$ M of each primer, 0.4 mM dNTP, and 2.0 units Taq polymerase. Typical thermal cycle parameters were 4 min at 94 C, then 35 cycles of 1 min at 94 C, 1 min at 50 C, 2 min at 72 C, and finally 3 min at 72 C and 10 s at 28 C. Primer sequences were 5'-GCT TCC ATC CAA CAT CTC AGC ATG ATG-3' and 5'-GGC AAA TAG GAA GTA TCA TTC TG-3'. Sequencing was carried out through a commercial automated sequencing service (PNAOL, University of Leicester, UK).

Phylogenetic trees were inferred using the methods of maximum likelihood (ML) and maximum parsimony (MP), implemented with PAUP<sup>o</sup> 4.0 (Swofford, 1999). The low number of taxa allowed the use of branch and bound searches, thereby guaranteeing that the optimal trees would be found. For the maximum likelihood searches, it was first necessary to choose an appropriate model of sequence evolution (Swofford et al., 1996). This was done using likelihood ratio tests (Huelsenbeck and Rannala, 1997) comparing nested, successively more-parameter-rich models.

Three models were compared using PAUP\* 4.0: the F81, HKY85, and GTR models (Swofford et al., 1996). Because it is well understood that the three codon sites in protein-coding genes experience different substitution rates, rate heterogeneity was assumed and rate variation was modeled as a discrete gamma distribution (Yang, 1993, 1996) with 4 rate categories. Fixing the maximum likelihood tree from the F81 search as the tree for the remaining tests, the gamma HKY85 model was found to be superior to the gamma F81 model ( $\chi^2 = 293.95$ ,  $df = 1$ ,  $P \ll 0.001$ ), but the gamma GTR model did not provide a significantly improved fit over the gamma HKY85. This indicates that among substitution types, there is a strong preponderance of transitions over transversions, but that within transitions or transversions there is no strong bias among the possible substitutions. A maximum likelihood branch and bound search was then run under the gamma HKY85 model using the estimated transition/transversion (Ti/Tv) ratio (10.782) and the estimated gamma parameter (0.181).

Two types of MP searches were conducted: all substitutions weighted equally and with transversions weighted more heavily than transitions. The latter analyses were appropriate given that the ML analyses demonstrate that there is a strong preponderance of transitions over transversions, creating the possibility of transitional saturation. Failure to deal with substitutional saturation can diminish the accuracy of a MP search (e.g., Knight and Mindell, 1993). Weights were determined separately for the three codon sites as follows: the gamma HKY85 model was used to determine the Ti/Tv ratio based on the ML tree; parsimony weights were then generated directly from the estimated ratio, with transitions receiving a base weight of 10 and transversions a weight of  $10 \times \text{Ti/Tv}$ . This resulted in the following transversion weightings: first positions, 59; second positions, 76; third positions, 114. Using these weights, the most parsimonious trees were found with a branch and bound search.

The phylogenetic signal in the data was

assessed with the  $g_i$  statistic that measures the skewness of the tree length distribution (Hillis and Huelsenbeck, 1992). The statistic was estimated from a random sample of 10,000 trees. Maximum likelihood and maximum parsimony (with transversions weighted as described above) bootstrapping (Felsenstein, 1985) was performed with 1000 replicates analysed by heuristic searches to assess the support for individual clades.

## RESULTS

### Description

*Naja mandalayensis* sp. nov.  
The Burmese Spitting Cobra

*Holotype*.—CAS 207097 (Fig. 1), a male collected near Monywa (N 22 13'; E 95 20'), Sagaing Division, Myanmar; collected by the senior author on 6 May 1998.

*Paratypes*.—CAS 204375–76, from 96 km south of Mandalay along Highway 1 (N 21 20'; E 96 04'), Mandalay Division, Myanmar; purchased from snake collectors by the senior author on 20 February 1998. CAS 207098–106, near Kyauk Se (no coordinates), Mandalay Division, Myanmar; purchased from snake collectors by the senior author on 7 May 1998. USNM 524075, from Chatthin Wildlife Sanctuary (N 23 34', E 95 44'), Sagaing Division, Myanmar; collected by Htun Win, Than Zaw Min, and Wi Lhon on 28 May 1998. CAS 208974, from near Pakokku (N 21 24', E 95 07'), Magwe Division, Myanmar; collected by the senior author on 14 May 1999. NHRM MAL. 1935.809.3321 A–F, from "Rangoon or Mandalay" [note: these specimens are almost certainly from somewhere near Mandalay, rather than Yangon (Rangoon)]; from extensive interviews with snake collectors and villagers, the senior author has determined that *N. mandalayensis* is endemic to the central dry zone of Myanmar, encompassing the Mandalay, Magwe, and Sagaing Divisions].

*Diagnosis*.—**Myanmar cobra species:** The only cobra in the genus *Naja* known to cooccur with *N. mandalayensis* in Myanmar is *N. kaouthia*. The two species differ from each other as follows: (1) fangs:

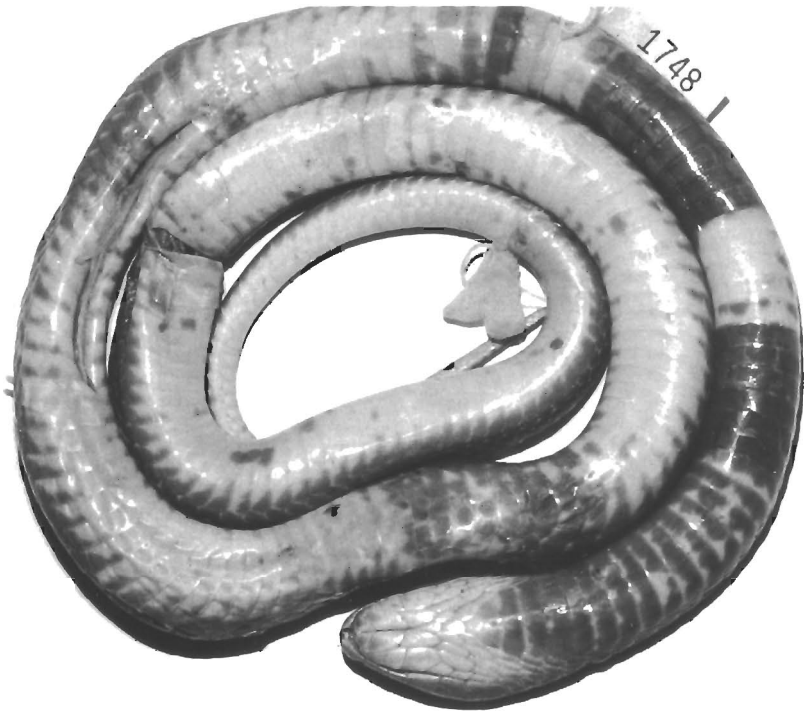
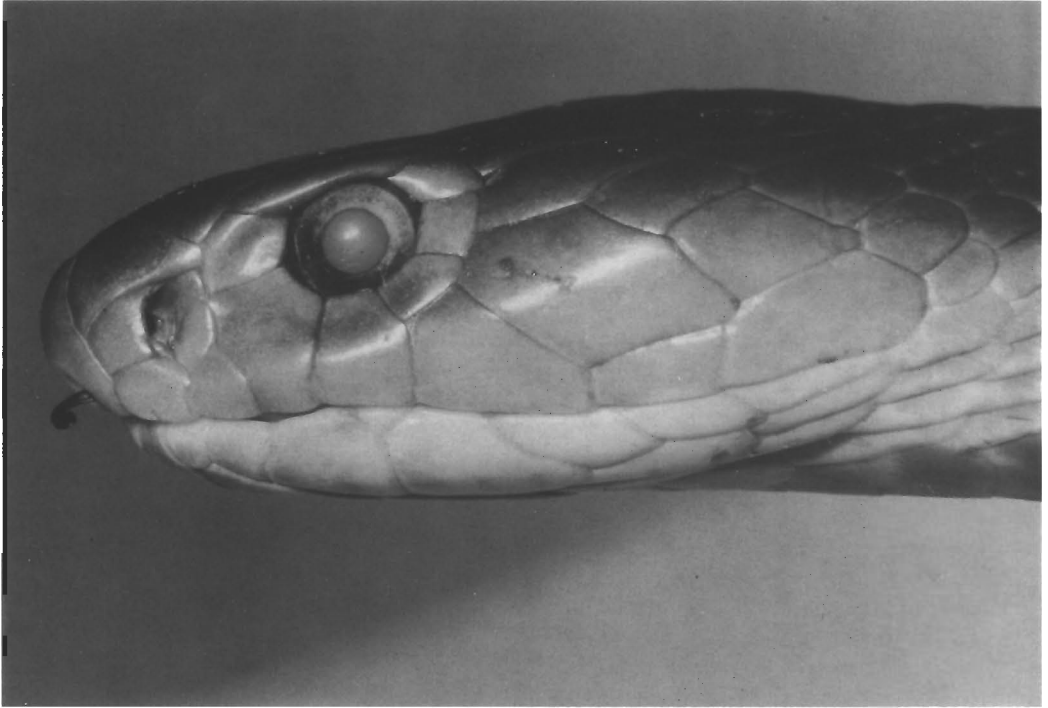


FIG. 1.—Holotype of *Naja mandalayensis*, CAS 207097. Photo by W. Wüster.

*Naja mandalayensis* has fangs adapted for spitting (as evidenced by a smaller venom discharge orifice), whereas the fangs of *N. kaouthia* are much less modified, and the latter species only rarely spits (W. Wüster, personal observation, anecdotal reports summarized in Wüster and Thorpe, 1992). (2) Hood mark: none of the 15 adults (SVL > 650 mm) of *Naja mandalayensis* examined by us have more than a very poorly defined hood mark (Fig. 2). Two juveniles, with SVLs of 298 mm and 415 mm, display a spectacle-shaped hood mark, but three other juveniles, with SVLs of between 284 and 335 mm, have no discernible hood mark. Most (130 out of 143 specimens examined in this study) specimens of *N. kaouthia* retain their hood marks throughout life. A spectacle-shaped mark is exceedingly rare in *N. kaouthia*, and the common pattern consists of a pale, dark-centered, O-shaped mark; further dark markings may be included in the pale field of the mark, giving a mask-shaped appearance. An O- or mask-shaped hood mark was seen in all 15 Burmese specimens of *N. kaouthia* examined in this study that possessed distinct hood marks. (3) Throat pattern: all but the smallest (SVL < 350 mm) specimens of *N. mandalayensis* display extensive dark mottling under the throat, which often covers the entire throat area anterior to the first dark throat band, obscuring the dark lateral throat spots characteristic of Asian *Naja*; *N. kaouthia* invariably has a pale, creamy throat, in most cases with a pair of well-defined lateral throat spots. (4) Ventral banding and coloration: specimens of *Naja mandalayensis* have at least two dark bands on the throat and anterior ventral side; the remainder of the venter is pale, with some dark mottling. Of 20 Burmese specimens of *N. kaouthia* examined, 13 (65%) have only a single dark band under the throat; the remainder of the venter is either pale or increasingly clouded with dark pigment towards the rear, the posterior half of the venter often being totally dark. **Other Asiatic cobra species:** *Naja naja* and *N. oxiana* can be distinguished from *N. mandalayensis* by the fact that their fangs lack spitting modifications. In addition, *N. oxi-*

*ana* has consistently higher ventral scale counts (191–210) and usually higher subcaudal scale counts (63–71 in males, 57–70 in females). *Naja siamensis* has fewer than 175 ventral scales and all but three out of 68 specimens examined had fewer than 172 ventral scales. In *N. mandalayensis*, males have 172 or more ventral scales (11 out of 13 specimens had 178 or more) and females have 182 ventral scales or more. In *N. siamensis*, males have 47–54 subcaudals and females 41–51 subcaudals, whereas in *N. mandalayensis*, the corresponding figures are 56–58 and 50–56. It should be stressed, however, that due to the unusually high frequency of tail breakage in our sample of *N. mandalayensis*, our sample sizes for subcaudal scale counts are small (six males and five females) and the actual range of variation encountered in this species is likely to be greater than reported here. Out of 79 specimens of *Naja atra* examined, all had a light throat and only four lacked a hood mark (hood mark faint or absent in adult *N. mandalayensis*). Additionally, 52 of 79 specimens of *N. atra* had extensive pigmentation (clouded or totally dark) on the ventral side, and 41 of 49 specimens had only a single dark band under the throat (ventral side pale with at least two dark bands present in *N. mandalayensis*). Also, *N. atra* has lower ventral and subcaudal scale counts (161–179 ventrals and 42–52 subcaudals in males, 169–180 ventrals and 37–51 subcaudals in females) than *N. mandalayensis*; all known females of *N. mandalayensis* have higher ventral scale counts than any recorded in *N. atra*, and all known males of *N. mandalayensis* have 56 or more subcaudals. *Naja sumatrana* and *N. samarensis* have fewer than 27 scale rows around the hood and fewer than 21 scale rows around the anterior half of the body. Additionally, *N. samarensis* has a characteristic speckled pattern and a single, very wide dark band under the throat. In *N. sputatrix*, the ventral and dorsal pattern of adults is indistinct and this species has, on average, fewer subcaudals (44–54 in males, 42–52 in females) than *N. mandalayensis*. *Naja philippinensis* has 46 or fewer subcaudals (50 or more in *N. man-*

*dalayensis*) and lacks a well-defined ventral pattern. *Naja sagittifera* has more than 60 subcaudals.

*Description of holotype.*—Body dimensions: SVL 676 mm; tail length 152 mm; total length 828 mm. Body scalation: 183 ventrals; 58 subcaudals, all divided; anal entire. Dorsal scale row reduction formula: 29 8+9(10), 28 8+9(13), 26 7+8(15), 25 7+8(17), 24 5+6(19), 23 6+7(22/24), 21 6+7(116)/4+5(116), 19 4+5(126/129), 17 4+5(149/154), 15. Caudal scale row reduction formula: 12 1+2(3/3), 10 1+2(6)/2+3(6), 8 3+4(8/9), 6 2+3(23/26), 4 1+2(56/58), 2. Head scalation: 7/7 supralabials, third and fourth enter orbit; third also contacts nasal; 8/8 infralabials (excluding cuneates), first four contact anterior chin shields; on left side, one small triangular cuneate between mouth edge and infralabials 4 and 5; on right side, two small cuneates between infralabials 4 and 6 debar infralabial 5 from contact with mouth edge; anterior chin shields slightly wider than posterior chin shields, of similar length; nasal almost completely divided by large, vertically oval nostril; 1/1 preoculars, in contact with nasal and internasal; 3/3 postoculars; prefrontals larger than internasals, frontal longer than broad (6.9 × 5.2 mm), longer than distance from rostral (6.1 mm) and the interparietal suture (6.5 mm); posterior tip of frontal forms acute angle; 2/2 anterior temporals, 4/4 posterior temporals; right lower anterior temporal partly fused with lowest posterior temporal, an individual aberration; seven temporals and nuchals contact the posterior and lateral edges of the parietal shields.

*Coloration of holotype in preservative.*—Head pattern (Fig. 1): dorsal surface of head uniformly dark brown; sides of head above mouth edge slightly paler, grayish brown; anterior edge of supralabial 7 slightly paler; underside of head, including infralabials, mostly cream, with some grayish-brown smudging, especially on the first four infralabials and anterior chin shields; on the gulars and the scales on the side of the throat below the mouth, the cream-colored area extends back to approximately the angle of the mouth, before grading into the dark grayish-brown of the

lower flanks. Dorsal pattern: dorsal surface uniformly dark grayish-brown; lowest two scale rows on each side slightly paler; skin between scales cream; dorsal side of tail of same color as body; no other pattern. Ventral pattern (Fig. 1): first 15 ventrals dark brown (similar to dorsal ground color), with cream mottling near bases; ventrals 16–21 blackish brown, without paler areas, forming a dark throat band clearly set off from the throat area; ventrals 22–26 cream, with dark brown outer tips, and a few dark brown mottles on ventral 22; ventrals 27–34 very dark brown, without paler markings; ventral 35 covered with this pigment on its right half; ventrals 36–38 cream with dark brown outer tips; ventral 39 almost entirely dark brown; remaining ventrals largely cream, with dark brown outer tips (dark pigment on these is contiguous with the dorsal ground color) and occasional small dark spots on the ventral surface; a zone of intensive dark mottling covers ventrals 89–98; lower surface of the tail cream, the outer edges of the subcaudals pigmented like the dorsal side. Markings of bluish-black pigment are perceptible in the interstitial skin where ventrals 11 and 12 contact the first dorsal scale row on the right side; the corresponding area near the lateral tips of ventrals 10 and 11 is distinctly darker on the left side; these inconspicuous dark marks are homologous to the often obvious lateral throat spots seen in many other Asiatic cobra species.

*Coloration of holotype in life.*—Essentially the same as above, except that the pale areas of the interstitial skin and ventral scales were yellowish-cream.

*Variation.*—From the limited material available, *Naja mandalayensis* does not appear to be very variable, compared to other cobra species. Size: the largest specimen (CAS 207102, a male) measures 1018 mm SVL; the tail is broken, but the intact tail could be expected to have measured approximately 240–250 mm. Scalation: variation in scalation (as well as several other characteristics) is compiled in Table 4. Pattern: all adult specimens examined correspond quite closely to the pattern described for the holotype. Juveniles differ in lacking the dark pigmentation of the

TABLE 4.—Variation in scalation and other characters in *Naja mandalayensis* (exceptional values in parentheses). The last four characters are not sexually dimorphic.

	Males	Females
Sample size	13	7
Ventrals	173–185	182–185
Subcaudals	56–58	50–56
Dorsal rows at 10th ventral	27–31	27
Dorsal rows at midbody	19–22	19–21
Dorsal rows at 80% VS length	15–17	15
Palatine teeth		6–7
Pterygoid teeth	14–18	
Last ventral of light throat area	(11) 14–18	
No. of ventrals in first throat band	4–9	

throat that is present in adults. Two of the juveniles have spectacle-shaped hood marks. In one of these juveniles (NHRM MAL. 1935.809.3321E; SVL 415 mm), the hood mark was connected to the pale throat area on both sides, in the other (NHRM MAL. 1935.809.3321C; SVL 298 mm), this connection was absent. In adults, U- or spectacle-shaped hood marks are often present, but faint and confined to the interstitial skin (Fig. 2). Some adults possess indistinct dorsal dark markings on the otherwise yellowish-cream interstitial skin; in some individuals (e.g., CAS 207105; Fig. 2), these markings form weak crossbands.

*Etymology*.—The specific name is an adjective referring to Mandalay, a major city within the range of *N. mandalayensis* (see below).

*Distribution*.—*Naja mandalayensis* is apparently endemic to central Myanmar, spanning the Mandalay, Magwe, and Sagaing Divisions (Fig. 3). Two cobra specimens [BNHM (Bombay Natural History Museum) 2249–50] from Bihar in northeast India must be mentioned here. The specimens were briefly discussed by Wüster and Thorpe (1992a), who noted that they do not seem to correspond to either *N. naja* or *N. kaouthia*. Wüster recently re-examined the data on these specimens and found them to be similar in many ways to *N. mandalayensis*, including having fangs modified for spitting. However, without

tissues for molecular analyses and further specimens, it is not possible at this time to allocate them to *N. mandalayensis*, or to any other named *Naja*. Spitting in spectacled cobras from northeast India was also reported by Whitaker (1978). *Naja naja* lacks fang modifications for spitting and is almost certainly incapable of doing so. It therefore appears that an undescribed spitting cobra, possibly closely related to or conspecific with *N. mandalayensis*, occurs in northeast India.

The range of *N. mandalayensis* corresponds to the dry zone of Myanmar, an area generally receiving <1000 mm of rain annually. Originally, this region was characterized by acacia savanna and, in wetter areas, indaing, a stunted dipterocarp savanna. But being an area that is intensively used for agriculture, very little of the original vegetation remains. However, *N. mandalayensis* has adapted quite well, thriving in agricultural fields and in and around villages. In fact, the holotype (CAS 207097) was captured by the senior author inside a hut in a small village. Although *N. kaouthia* is widely distributed within Myanmar, it appears to be rare or absent from the central dry zone occupied by *N. mandalayensis*. This is indicated by interviews conducted by the senior author with snake collectors, who consistently stated that cobras with a monocle-shaped hood mark and without any tendency to spit could only be found south or north of the dry zone. This situation may be similar to that in Thailand, where *N. kaouthia* tends to be more common in wet areas, whereas *N. siamensis* is more common in drier areas.

#### Multivariate Morphometrics

The upper plots in Fig. 4 show the samples of *N. mandalayensis* occupying a somewhat intermediate position between *N. kaouthia* and *N. siamensis*, but highly distinct from *N. atra*. In each case, high first canonical variate scores are most heavily associated with high ventral scale counts and, to a lesser extent, with a short head and high dorsal scale row counts at 40% VS length. High second canonical variate scores in males are associated with a high dorsal scale row count at the level



FIG. 2.—Living individuals of *Naja mandalayensis*. Top: specimen (CAS 207104) with hood spread: a very faint U- or spectacle-shaped hood mark is just visible on the interstitial skin. Bottom: specimen (CAS 207105) showing dorsal coloration: faint bands due to dark interstitial skin markings are just visible posteriorly. Photos by J. Slowinski.

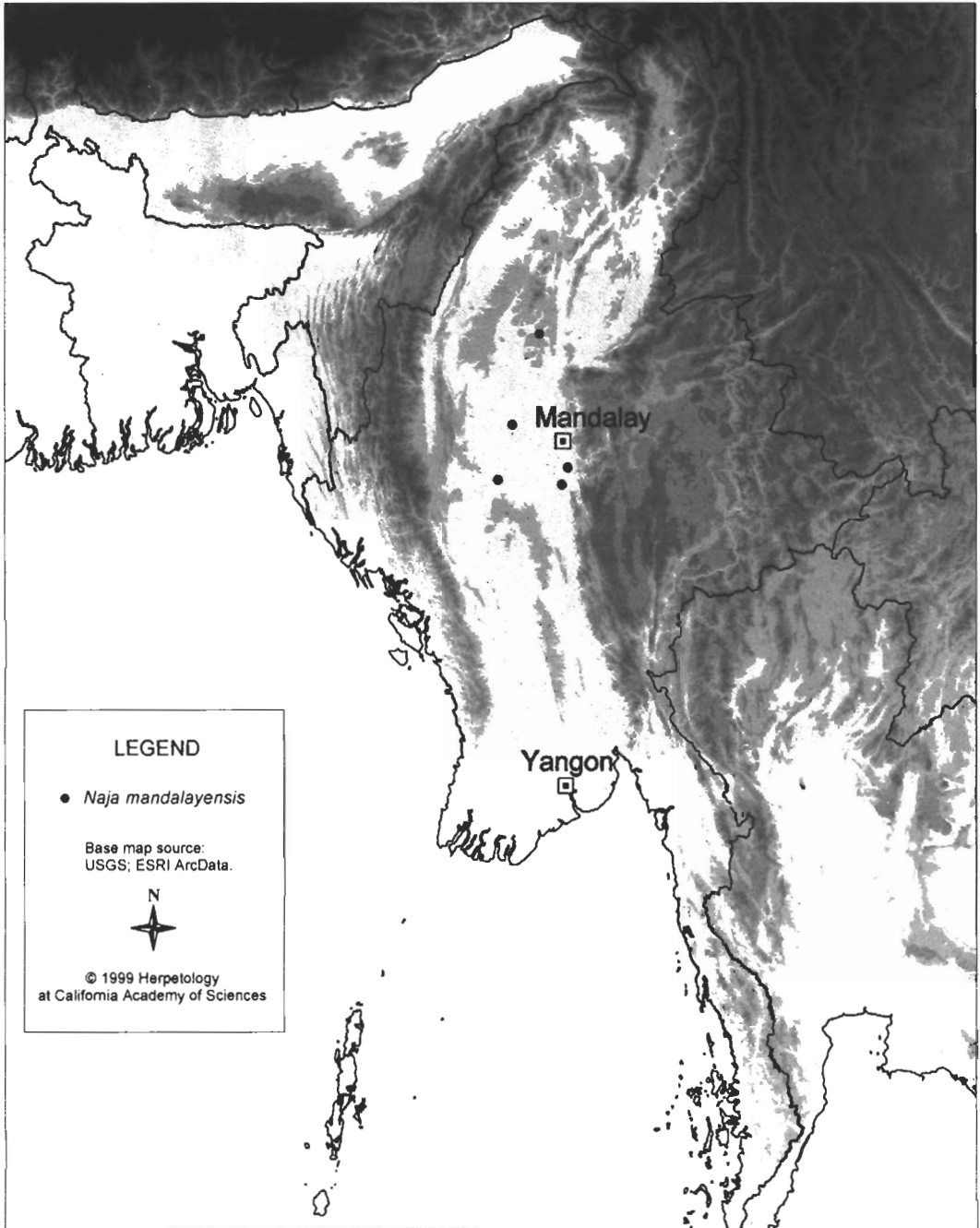


FIG. 3.—Range of *Naja mandalayensis* in central Myanmar.

of the 10<sup>th</sup> ventral, numerous posterior temporal shields, relatively posterior lateral throat spots, a low dorsal scale row count at 20% VS length, an anterior end of the pale throat area and a narrow head.

In the females, high second canonical variate scores are associated with numerous posterior temporals, a high dorsal scale row count at the level of the 10<sup>th</sup> ventral, posterior and long lateral throat spots,

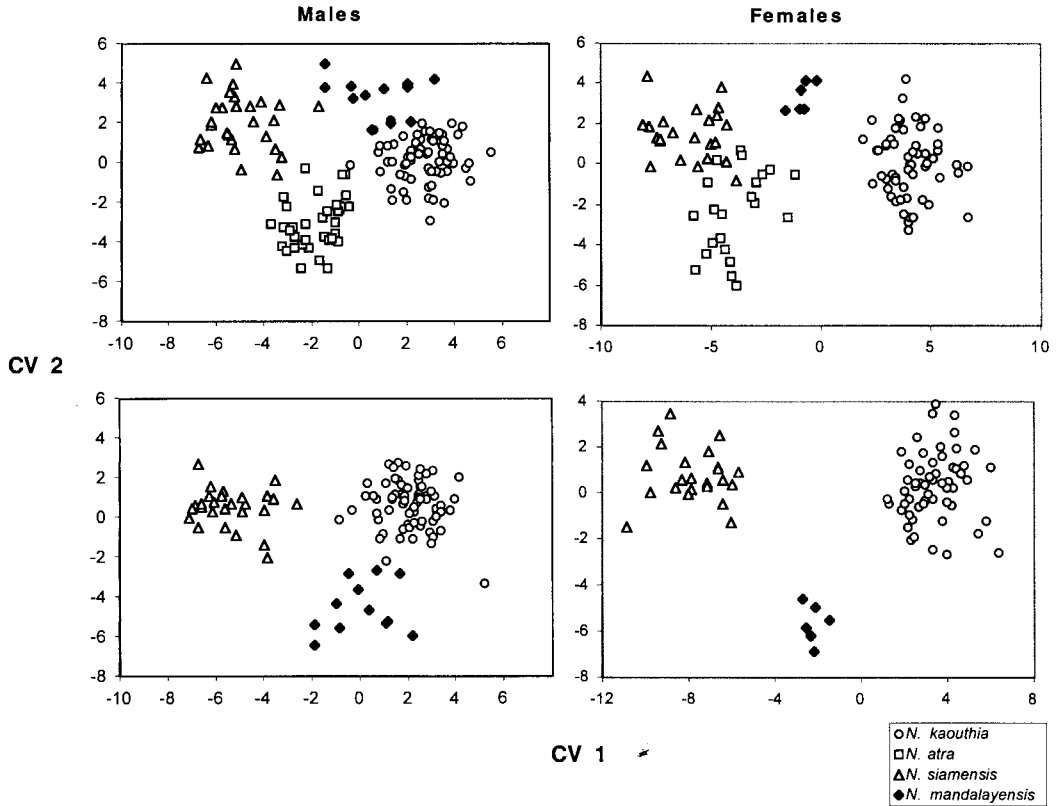


FIG. 4.—Top: ordination of male and female specimens of *N. kaouthia*, *N. atra*, *N. siamensis*, and *N. mandalayensis* from Myanmar along the first two canonical variates of CVAs 1 and 2. The distinctness of *N. mandalayensis* from *N. atra* is clearly evident. The first and second canonical variates summarize respectively 44.0% and 21.0% of the total variation of the data of CVA 1, and 54.2% and 15.7% of the total variation of the data of CVA 2. Bottom: ordination of male (left) and female (right) specimens of *N. kaouthia*, *N. siamensis*, and *N. mandalayensis* along the first two canonical variates of CVAs 3 and 4; *N. mandalayensis* is clearly distinct from the other two species. The first and second canonical variates summarize respectively 59.2% and 11.7% of the total variation of the data of CVA 3, and 66.2% and 11.2% of the total variation of the data of CVA 2.

light throat area ending relatively far forward, and a short distance between the frontal and rostral scales.

Because the spatial relationship among the four taxa may not necessarily be adequately expressed in a two-dimensional plot, the analyses were repeated after the exclusion of *N. atra* (lower plots in Fig. 4). Both of these CVAs showed *N. mandalayensis* to be highly distinct from both *N. kaouthia* and *N. siamensis*. In males, first canonical variate scores are strongly associated with high numbers of ventral scales and, to a lesser extent, with high dorsal scale row counts at 20% VS length, and relatively anterior lateral throat spots. In

females, first canonical variate scores are strongly associated with high ventral scale counts, a relatively wide first dark throat band, a greater distance between the frontal and rostral scales, long parietal scales, and relatively anterior lateral throat spots. Second canonical variate scores in males were very strongly associated with a great distance between frontal and rostral, and, to a lesser extent, with low dorsal scale row counts at 80% VS length, a short interpre-frontal suture, and a short head. In females, second canonical variate scores were most strongly associated with relatively anterior lateral throat spots and a great distance between the frontal and ros-

tral scales, and, to a lesser extent, with low ventral scale counts, low numbers of posterior temporals, short and low lateral throat spots, and a narrow frontal scale.

### Molecular Phylogenetic Analysis

Of the 767 bp PCR fragment amplified, 646 contiguous codon positions, corresponding to codon positions 15,028–15,673 of the full mtDNA sequence of *Dinodon semicarinatus* (Kumazawa et al., 1998), were reliably sequenced and thus selected for analysis. Within these 646 positions, uncorrected pairwise p-distances ranged from 0.050 (between *N. mandalayensis* and *N. siamensis*; gamma HKY85-corrected distance = 0.069 substitutions per site) to 0.171 (between *N. siamensis* and *N. annulifera*; gamma HKY85-corrected distance = 0.498 substitutions per site). *Naja mandalayensis* shows a p-distance of 0.082 from *N. kaouthia* (gamma HKY85-corrected distance = 0.158 substitutions per site) and of 0.076 from *N. atra* (gamma HKY85-corrected distance = 0.131 substitutions per site). Of the 646 base pairs, 176 (27.2%) were variable, and 78 (12.1%) were parsimony-informative. There was no significant nucleotide compositional bias among the sequences ( $\chi^2 = 2.42$ ,  $df = 15$ ,  $P = 0.999$ ).

The ML analysis resulted in a single tree with a  $-\log$  likelihood of 1,848.922 (Fig. 5). The equally weighted MP analysis ( $L = 237$  steps,  $CI = 0.827$ ,  $RI = 0.586$ ) and MP analysis with transversions weighted as described above ( $L = 6,153$ ) both found the same shortest tree, which was identical to the ML tree (Fig. 5). The  $g_i$  statistic was  $-1.234$ , indicating very strong parsimony signal in the data (Hillis and Huelsenbeck, 1992). The ML and MP phylogenetic analyses both strongly support a sister-taxon relationship between *Naja mandalayensis* and *N. siamensis*. Both analyses also supported a sister-taxon relationship between *N. atra* and *N. kaouthia*, but the ML bootstrapping failed to provide strong support for this clustering. Both analyses support the monophyly of the Asian species sampled relative to the African outgroups.

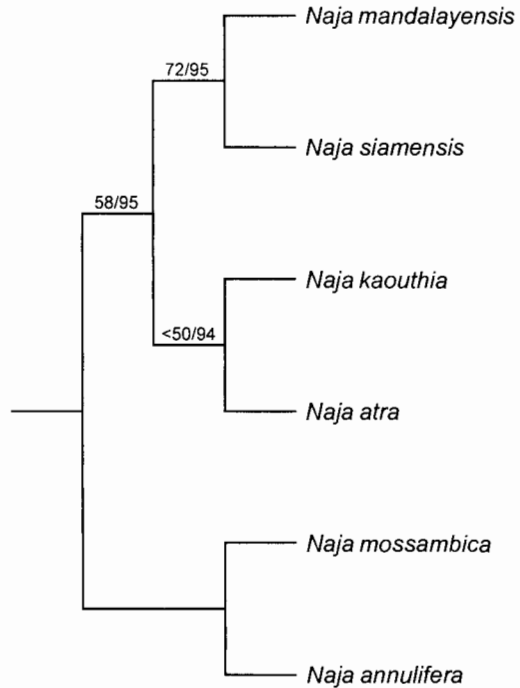


FIG. 5.—Optimal tree resulting from both the ML and MP analyses. Numbers on the internodes correspond to bootstrap proportions; lefthand numbers correspond to the ML bootstrap proportions; righthand numbers correspond to the MP bootstrap proportions.

### DISCUSSION

The morphometric and molecular data clearly establish the distinctiveness of *Naja mandalayensis* from other southeastern Asian species of *Naja*. It is perhaps surprising that this form has gone unnamed for so long, given that previous workers were clearly aware of its existence. For example, Goring Jones (1900) reported an incident wherein a British officer was sprayed in the eye by a “small black cobra” near Mandalay. Bogert (1943) cited this report in his classic tome on elapid fangs. And Wüster and Thorpe (1992b: 432) reported six specimens (NHRM MAL. 1935.809.3321A–F) of uncertain affinities from “Rangoon or Mandalay” with fangs highly modified for spitting, but did not describe this form as a new species because of the ambiguous locality. However, the same situation has occurred elsewhere within this problematic genus. Thus, de-

spite the existence of conspicuously different coloration and behavior from *Naja kaouthia*, *N. siamensis* was not diagnosed as a species distinct from the latter species until recently (Wüster and Thorpe, 1994).

Except for *Naja naja* and *N. oxiana*, Asian species of *Naja* are capable, to varying extents, of spitting their venom when threatened (Wüster and Thorpe, 1992b). This is reflected in modifications to the fang morphology, specifically a small venom discharge orifice allowing venom to be expelled in streams (Bogert, 1943; Wüster and Thorpe, 1992b). With respect to the size of the discharge orifice, the fangs of *N. mandalayensis* are comparable to those of *N. siamensis*, its putative sister species. Rasmussen et al. (1995) analyzed the spitting behavior of several African and one Asian species (*N. siamensis*) of cobras using cinematography. They found that it was difficult to evoke spitting in *N. siamensis*, and that when individuals of the species did spit, the quantity of venom expelled was low and that the venom travelled more as a mist than a stream. Further, they reported that the species' spitting range was approximately 1 m, the lowest for any of the species that they examined. The experiences of the second author with this species contradict the observations of Rasmussen et al. (1995). Wüster (unpublished) has found that *N. siamensis* readily spits, that the venom is expelled in streams consisting of tiny droplets, and that the spitting range exceeds 1 m.

Wüster's anecdotal observations of the spitting behavior of *N. siamensis* match those of the senior author's experience with newly captured *N. mandalayensis*. Little provocation is required to trigger spitting, and the venom is expelled as two streams of tiny droplets, with an effective range of at least 2 m. Spitting is generally accompanied by a forward lunge and a clearly audible exhalant hiss of the reared and hooded animal (this is also true of *N. siamensis*; Rasmussen et al., 1995).

Villagers within the range of *Naja mandalayensis* are well aware of its propensity for spitting venom. When a villager suffers a spitting injury to the eyes, leaves of the tamarind tree (*Tamarindus indica*) are

used as a folk remedy to treat the injured eyes. The leaves are first chewed—either by the afflicted or by bystanders—and the juice dripped into the injured eyes. Whether this treatment really works will require experiments conducted with suitable controls.

Nothing is known about the venom of *N. mandalayensis*. There is considerable variation in venom composition among the different species of Asiatic *Naja*, and in some cases, an antivenom raised from the venom of one species can be ineffective in neutralizing the venom of another (Warrell, 1986; Wüster and Thorpe, 1991). At present, only venom of *N. kaouthia* appears to be used in the production of antivenom in Myanmar (Warrell, personal communication). Studies assessing the ability of this antivenom to neutralize the antivenom of *N. mandalayensis* are urgently required.

Several snake species (e.g., *Oligodon splendidus*, *Bungarus magnimaculatus*) are endemic (Smith, 1943) to the central dry zone of Myanmar; this study adds another species. The herpetofauna of Myanmar has been under-studied, and it is likely that additional field work will reveal that the central dry zone is a area of significant herpetological endemism.

*Acknowledgments.*—Financial support for the senior author's field work in Myanmar was provided by NSF grant DEB-9971861 and the Lindsay Field Research Fund of the California Academy of Sciences (CAS), the CAS Department of Herpetology's Duffelbag Fund, as well as other funds from CAS. We thank the following donors to the Duffelbag Fund: Drs. M. and J. Miller, Dr. D. L. Jameson, Mr. E. Wilke, Mrs. E. Date, Mr. and Mrs. R. Dunke, Drs. M. and M. Aldrich, and Mr. and Mrs. J. E. C. Nielsen. Collecting permits and various other forms of assistance were generously provided by U Uga, Director of the Myanmar Nature and Wildlife Conservation Division. Invaluable assistance in Myanmar was provided by U Win Aung. For assistance in the field, the senior author thanks H. Robeck, C. Spencer, J. Vindum, and K. Wiseman. For other forms of assistance, the senior author thanks B. Drewes, M. Koo, and J. Vindum. The manuscript was read and criticized by A. Leviton and J. Vindum. We thank H.-W. Herrmann for providing the blood samples of *Naja mossambica* and *N. annulifera* used in this study. During this study, the junior author was funded by a Research Career Development Fellowship of the Wellcome Trust; previous work on mtDNA evolution in *Naja* was funded by the Natural Environment Re-

search Council (NERC) and the Leverhulme Trust. The junior author wishes to thank G. S. Allen (Royal Ontario Museum, Toronto) for recording color pattern and scalation data from a number of cobra specimens in the ROM.

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Accepted: 1 July 1999

Associate Editor: John Wiens